“INVESTIGATION INTO THE EFFECTS OF ALSTONIA SCHOLARIS IN EXPERIMENTAL MODELS OF DIABETES”

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

By

Ms. Sonam Mishra

Enrolment No.179999901009

Under supervision of

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GUJARAT TECHNOLOGICAL UNIVERSITY

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ABSTRACT

Diabetes mellitus stands as the foremost prevalent metabolic affliction globally, prompting exploration in conventional medicine towards the utilization of various medicinal plants for its treatment. *Alstonia scholaris*, a plant deeply rooted in Ayurveda and belonging to the Apocynaceae family, is ascribed with multifaceted therapeutic properties. This study endeavors to delineate the characteristics of different extracts derived from *Alstonia scholaris* leaves and evaluate their potential efficacy in combating diabetes. Three distinct extracts (chloroform, ethyl acetate, and ethanol) underwent scrutiny for their glucose homeostasis activity employing an oral glucose tolerance test (OGTT) in male Wistar rats across three dosage levels (200, 400, and 800 mg/kg). Notably, the ethanol extract at 400 mg/kg demonstrated significant benefits in regulating glucose levels. Subsequent investigations in models utilizing low-dose streptozotocin and high-fat diet-induced type 2 diabetes unveiled substantial enhancements in various biochemical parameters, blood pressure, electrocardiogram (ECG) readings, and increased urinary glucose excretion. Conclusively, the ethanol extract at 400 mg/kg exhibited a noteworthy protective effect against type 2 diabetes mellitus and its associated metabolic, renal, and cardiovascular complications. Moreover, the isolation and characterization of alkaloid and flavonoid fractions from the ethanol extract aimed to assess their anti-diabetic and antioxidant activities. These fractions, evaluated at four different doses (15, 30, 60, and 90 mg/kg), exhibited positive effects on glucose regulation, particularly at a dose of 30 mg/kg. Furthermore, assessment in the aforementioned diabetes models showcased a reduction in fasting blood glucose levels and an amelioration in biochemical parameters, accompanied by an inverse modulation in histology of the heart, kidney, pancreas, and liver tissues. The plausible mechanism underlying these protective effects involves an increase in urinary glucose excretion and antioxidant potential, contributing to an improvement in metabolic activity. However, future endeavors should delve into elucidating the molecular mechanisms of action.

Keywords: *Alstonia scholaris*, Anti-diabetes, Streptozotocin, Oral Glucose Tolerance Test (OGTT)

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List of Abbreviations

IDF	International Diabetic Federation
IHA	The Indian Heart Association
T2DM	Type 2 diabetes mellitus
WHO	World Health Organization
IDF	International diabetes federation
SGLT	Sodium glucose linked transporters
GLP	Glucagon-like peptide-1 amide agonists
OGTT	Oral glucose tolerance test
CVD	Cardiovascular diseases
IGT	Impaired glucose tolerance
LMIC	Low and middle-income countries
CMNND	Communicable, maternal, neonatal and diet-related diseases
T1DM	Type I diabetes mellitus
Th1	T helper 1
IL-2	Interleukin-2
TNF	Tumor necrosis factor
ICAs	Islet cell auto antibodies
Anti GAD65	Anti-glutamic acid decarboxylase
HHNS	Hyperosmolar Hyperglycemic Nonketotic Syndrome
FPG	Fasting plasma glucose
RPGT	Random plasma glucose test
HDL	High-density lipoprotein
ATPase	Adenosine triphosphatase
GFR	Glomerular filtration rate
RTG	Renal glucose excretion threshold
DN	Diabetic nephropathy
AMPK	AMP activated protein kinase
FDA	Food and Drug Administration
EEAS	Ethanol extract of *Alstonia scholaris* leaves
EAEAS	Ethyl acetate extract of *Alstonia scholaris* leaves

CHEAS	Chloroform extract of *Alstonia scholaris* leaves
PEEAS	Petroleum ether extract of *Alstonia scholaris* leaves
TLC	Thin Layer Chromatography
STZ	Streptozotocin
CANA	Canagliflozin
MET	Metformin
EDTA	Ethylenediamine tetraacetic acid
NBT	Nitro blue tetrazolium
DTNB	1, 2-dithiol-bis nitro benzoic acid
Na_2HPO_4	Disodium hydrogen phosphate
CMC	Carboxymethyl cellulose
GOD-POD	Glucose oxidase and peroxidase
ALT	Alanine aminotransferase
FBG	Fasting blood glucose level
T-CH	Total cholesterol
TG	Triglyceride
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
VLDL	Very low-density lipoprotein
AST	Aspartate aminotransferase
LDH	Lactate dehydrogenase
CK-MB	Creatinine kinase MB
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
Et-AF	Alkaloid fraction of ethanol extract of *A. scholaris* leaves
Et-FF	Flavonoid fraction of ethanol extract of *A. scholaris* leaves
SOD	Superoxide Dismutase
PBS	Phosphate buffered saline
CAT	Catalase
GSH	Glutathione
API	Ayurvedic pharmacopeia limits
HFD	High fat diet

List of symbols

Symbols	Names
%	Percentage
µ	Micro
µl	Microliter
A	Absorbance
b.w.	body weight
C	Celsius
dl	Decilitre
g	Gram
IU	International Unit
Kg	Kilogram
mg	Milligram
min	Minute
ml	Millilitre
nm	Nanometre
º	Degree
w/v	Weight by volume
w/w	Weight by weight
α	Alpha
β	Beta

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CHAPTER- 1

Introduction

1.1 Description on the state of art of the research

Glucose homeostasis is an essential part of physiological function, which depends on hormonal control (1). The rise in plasma glucose levels is regulated by the secretion of β-cells from the pancreas, which promote the uptake of glucose and its conversion into glycogen or triglycerides by muscles and adipocytes, resulting in a fall in glucose levels. Therefore, impaired glucose homeostasis, metabolic disorders and constant pressure on β-cells (insulin resistance) lead to type 2 diabetes (T2DM) (2). Most of pathological conditions of diabetes mellitus can be attributed to one of the following three main effects of insulin resistance: (i) Decreased glucose utilization by the body's cells with an increase in blood glucose concentration (ii) Disturbed lipid metabolism, which leads to the deposition of fat in the vascular walls causes atherosclerosis and increased formation of ketone bodies (iii) Protein deficiency in body tissues. These mechanisms can lead to a metabolic imbalance that is responsible for the development of the disease.

Epidemiological data show alarming levels predicting worrying future prospects for T2DM. According to the 2021 International Diabetic Federation (IDF) report, approximately 537 million adults (20-79 years) are living with diabetes worldwide (3, 4). The Indian Heart Association (IHA) reportedthat the total number of people with diabetes is expected to increase by 643 million by 2030 and 783 million by 2045. Three quarters of adults with diabetes live in low and middle-income countries. Almost 1 million Indians die from diabetes every year. These data show that the number of patients with diabetes is increasing rapidly and has become a human health challenge. In addition, approximately 95% of all diabetics suffer from type 2 diabetes, resulting in significant human and economic damage to health. Therefore, the main purpose of treating T2DM is to prevent or delay the onset of complications and to keep blood glucose levels within the normal range. Organ dysfunctions in the liver, heart, kidneys, blood vessels, urogenital tract and eyes are a common complication of type 2 diabetes mellitus (5). Free radicals can damage cell molecules, DNA, proteins and lipids and lead to altered cell functions.

Many recent studies show that antioxidants are effective in neutralizing free radicals (6) to prevent experimentally induced diabetes in animal models (7, 8). Currently, treatment of T2DMdepends mainly on lifestyle interventions (nutrition therapy and physical activity), oral drugs (such as biguanides, sulfonylureas, and glucoside inhibitors), and injected drugs (such as insulin), and glucagon-like peptide-1 amide agonists (GLP-1) (9). There are many side effects of these available anti-hyperglycemic drugs as they act by increasing insulin sensitivity, increasing insulin secretion and decreasing glucose absorption, including one or more undesirable side effects such as hypoglycemia, hematological problems, obesity, osteoporosis, hepatotoxicity and lactic acidosis (10). Diabetics are 25 times more likely to develop blindness, have an increased risk of heart attack and suffer more often from peripheral vascular diseases such as gangrene (11). They are also more prone to kidney failure and peripheral neuropathy than non-diabetics. These assessments of diabetics are the reason for the transition from symptomatic and allopathic therapy to natural therapy. Therefore, it is important to find an effective drug with fewer side effects against diabetes, giving preference to traditional medicinal plants.

Diabetes has been treated with herbal medicines since ancient times. Recent scientific research has confirmed the effectiveness of many antidiabetic herbal supplements, some of which are very effective and relatively non-toxic. A study by the World Health Organization (WHO) shows that 80% of the world's population rely solely on medicinal plants for their primary health care (12). Before the introduction of insulin in 1922, the management of diabetes mellitus relied primarily on dietary measures, including traditional herbal medicines (13). Over the past 10 to 20 years, scientific research has confirmed the effectiveness of many of these supplements, some of which are remarkably effective. As an alternative approach, medicinal herbs with anti-hyperglycemic effects are increasing in demand by diabetics and healthcare professionals. Many diabetic patients use herbal formulations together with hypoglycemic drugs to achieve an anti-diabetic effect and prevent complications (14). Most plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., which are often attributed to have antidiabetic effect (15). Therefore, in light of these shreds of evidence and reports, we have been encouraged to develop herbal medicines that are considered as potential sources for the treatment of diabetes mellitus.

1.2 Rational of the project

Therapies for type 2 diabetes have improved over the past few decades, but still there is need for new insights to slow the progression of this disease. According to the International diabetes federation (IDF) 10th Edition report, diabetes is becoming a constant burden worldwide (16). The total number of people with diabetes is projected to increase to 643 million by 2030 and 783 million by 2045 (17). In addition, the WHO predicts that diabetes will be the seventh leading cause of death worldwide by 2030 (18). These data suggest that diabetes needs to be diagnosed and reduced with appropriate treatment. Oral anti hyperglycemic drugs for controlling blood glucose levels with different mechanisms of action are available on the market, but their clinical use is limited by their side effects (19). There is an unmet need for a novel treatment strategy for type 2 diabetes that lowers plasma glucose levels without hypoglycemia by increasing urinary glucose excretion (20). SGLTs (Sodium glucose linked transporters) are responsible for active glucose and galactose absorption in the gut and glucose reabsorption in the kidney (21). SGLT-1 is responsible for most of the dietary glucose uptake in the gut and reabsorption of the rest of the filtered glucose in the renal tubular system with SGLT-2 (22). Sodium glucose cotransporter-2 (SGLT-2) inhibitors are a class of anti-hyperglycemic agents that act on the SGLT-2 proteins expressed in the proximal convoluted tubules of the kidney (23). It exerts its action by preventing the reabsorption of filtered glucose from the tubular lumen have proven to be extremely effective and reliable in reducing hyperglycemia (24). Thus, SGLT-2 inhibitor raised a new perspective for patients with diabetes mellitus. Therefore, they are currently one of the most important targets in the treatment of T2DM and diabetes-related complications. The clinical effects of SGLT2 inhibitors, including reducing HbA1c levels, when combined with a healthy lifestyle and diet, result in weight loss, a reduction in systolic blood pressure, a reduction in fasting blood glucose levels, and a reduction in uric acid, which indicates a reduction in cardiovascular events (25). Their mechanism of action is independent of insulin and therefore are expected to be effective at any stage of progressive cellular failure and insulin resistance, provided the kidneys are functioning adequately. In recent years, there has been rapid growth in herbal medicine and, interestingly, these drugs are gaining acceptance in both developing and developed countries (26). Herbal medicines are believed to be the first line of defense to keep people healthy by preventing disease and its complications (27). In addition, a study by the WHO shows that 80% of the world's population rely solely on medicinal plants to cover their basic medical needs (28). It has compiled a list of 21,000 medicinal plants used worldwide, including communicable and

non-communicable diseases (29). Among them, there are 2500 species in India, of which 150 species are used commercially on an industrial scale. India is the largest producer of medicinal herbs and is called the botanical garden of the world (30). Medicinal plant research has also contributed to the search for new SGLT-2 inhibitor therapeutics to treat diabetes mellitus by demonstrating the pharmacological efficacy of natural drugs as viable SGLT-2 inhibitors in various animal models (31). Therefore, herbal medicines with high potency and minimal side effects can be developed to replace or substitute for existing SGLT2 inhibitors for the treatment of type 2 diabetes. Researchers and scientists around the world are looking for natural sources to avoid side effects and the immense potential of their conventional use (32). The hypoglycemic and antidiabetic effects of several traditionally used plants have been demonstrated. *Alstonia. scholaris R. Br*. (Apocynaceae) is the medicinal plant described in Ayurveda with its therapeutic importance. It is also known as Saptaparna, with Sapta meaning seven and Parna meaning leaves. In Ayurveda, *A. scholaris* is used in Jwara, Shwas, Kushtha, Gulma, Vrana, etc (33). It is widely distributed and used in India, Thailand, the Philippines, Africa, China and Australia. It has various pharmacological effects including antibacterial, anxiolytic, antiulcer, antimicrobial, antioxidant and antidiarrheal effects and is also used in wound healing (34). Traditionally the plant is used for whooping cough, chronic bronchitis, asthma, and other respiratory infections (35). Plant products possess rich sources of antioxidant activity. Antioxidants combat oxidative stress in tissues by indirectly strengthening the cells' natural defense mechanisms and directly scavenging oxidative free radicals (36). Various experimental studies indicate that antioxidants reduce the oxidative stress caused by diabetes and alleviate its complications. Many researchers indicate that the anti-hyperglycemic effect of medicinal plants is due to the presence of various phytochemical components such as terpenoids, flavonoids, alkaloids, etc. Literature search revealed that these phytochemical components are also present in *A. scholaris* (37). There appeared to be insufficient data on the antihyperglycemic activity of *A. scholaris*. Therefore, it was examined for the presence of active phytochemicals and investigated for its pharmacological effect in the treatment of diabetes mellitus and its complications. In this current study, different extracts (ethanol, ethyl acetate and chloroform extracts) and their active fractions from *A. scholaris* leaves were examined. Their identification, characterization, and utility provided evidence to their potential antihyperglycemic effects. In addition, an in vitro method using goat liver tissue was used to estimate the antioxidant potential of the active extract and its fractions. The development of the traditional healthcare system relies heavily on these culturally familiar, technically simple, and financially affordable approaches to meeting healthcare needs.

1.3 Objectives

Based on the rationale of the study, we have aimed the following objectives:

1. To isolate and characterize different extracts of *Alstonia scholaris* leaves by successive extraction technique.
2. To evaluate the effects of different extracts on glucose homeostasis using oral glucose tolerance test (OGTT).
3. To study the effects of effective extract/s of *A. scholaris* in type 2 diabetes mellitus and its complications.
4. To isolate the alkaloid and flavonoid rich fractions from effective extract of *A. scholaris.*
5. To evaluate the effects of the alkaloid and flavonoid rich fractions on glucose homeostasisusing OGTT (efficacy and dose selection study).
6. To investigate effects of alkaloid and flavonoid rich fractions on type 2 diabetes mellitusand its complications.
7. To assess the antioxidant potential of alkaloid and flavonoid rich fractions.

CHAPTER- 2

Literature Review

2.1 Diabetes

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia) resulting from impaired insulin secretion, insulin action, or both, causing disorders of carbohydrate, protein, and lipid metabolism (38). The word diabetes comes from the Greek word means “to pass through” and the Latin word mellitus means “honey-sweet”. Aretaeus in Cappadocia gave a complete description of diabetes in the 1st century AD. The word diabetes was first mentioned in a medical text in 1425 (39). In 1675, Thomas Willis added mellitus to the disease word when he observed that diabetic urine had a sweet taste (glycosuria) (40).

2.1.1 Epidemiology of diabetes

In the 21st century, diabetes is emerging as one of the global health emergencies and 9th leading cause of death along with cardiovascular diseases (CVD), cancer and respiratory diseases (41, 42). According to the 2019, World Health Organization (WHO) report, 74% of deaths worldwide are attributed. The International Diabetes Federation (IDF) estimated that 536.6 million People are suffering from diabetes (diagnosed or undiagnosed) in 2021, and thatnumber is expected to increase by 46%, reaching 783.2 million by 2045 (43). As previous IDFestimates and other studies have shown, approximately 50% of all individuals with diabetics are unaware of their condition (44). From a clinical perspective, earlier detection in the asymptomatic stage is important to allow earlier treatment and prevent or delay the development of micro and macro vascular complications. Recently, the IDF reported on a rapidly growing diabetes in low and middle economic country. This rapid change was due to urbanization and industrialization associated with many risk factor such as unhealthy diet sedentary lifestyle and increase in populations (44). The uncontrollable rise in diabetes create a burden of type 2 diabetes due to macro vascular (cardiovascular), cerebrovascular, and peripheral artery disease) and micro vascular (diabetic retinopathy, nephropathy, and neuropathy) complications and vascular disease (45).

A). Global Burden of diabetes

Susceptibility to type 2 diabetes varies widely around the world, with Pacific Islanders, Asian Indians, and Native Americans all at significantly higher risk of developing the disease. The number of people with type 2 diabetes began to increase worldwide in the 1990s, and since 2000 there has been a dramatic increase in the number of people with diabetes worldwide (46). According to the International Diabetes Federation (IDF), 8.8% of the adult population has diabetes, with a higher rate in males (14.6%) than females (9.1%) (47). Current global statistics show that 536 million and 541 million people have diabetes and impaired glucose tolerance (IGT), a prediabetic condition. It is estimated that by 2045, this numbers will increase to 783 million people with diabetes and 730 million people with IGT (47). Among the IDF regions, the Western Pacific has the highest number of people with diabetes (173.1 million), followed by Southeast Asia (103.3 million), Europe (67 million), the Middle East and North Africa (44.8 million) and North America and the Caribbean (36.1 million). The lowest numbers are currently in South and Central America (34.1 million) and Africa (52.7 million) (47). So it turns out that not only the prosperous regions of Europe as well North America are affected by the diabetes epidemic. China is the country with the most diabetics worldwide, around 141 million peoples suffering from the disease (48). By 2045, around 174 million people in China are expected to have diabetes. The burden of diabetes is growing faster in low and middle-income countries than in high-income countries (49). In low and middle-income countries (LMICs), three out of four adults are living with diabetes. In addition, modifiable metabolic, environmental, and behavioral factors have been found to be the major risk factors for diabetic burden. According to these assessments, there is an urgent need for improved diabetes screening. They also emphasize the importance of recognizing undiagnosed diabetes and providing appropriate and timely treatment, as undiagnosed diabetes can have negative consequences. Global diabetes-related healthcare expenditure for adults aged 20 to 79 years has been estimated at US$966 billion.



Figure 2.1: Epidemiology of diabetes around the world in 2021. Adapted from *International Diabetes Federation (IDF)* (47).

B). Burden of Diabetes in India

Diabetes has been steadily increasing in India and around the world over the past three decades, with India accounting for a significant portion of the global burden. India's disease patterns have changed due to an epidemiological transition: mortality from communicable, maternal, neonatal and diet-related diseases (CMNNDs) has declined significantly, while non-communicable diseases and injuries have significantly rises their contribution to the overall burden of disease and mortality (50). The collaborators of the India State-Level Disease Burden Initiative Diabetes (ISLDBID) studyreported that the prevalence and number of people suffering from diabetes in India increased from 5.5%, 26.0 million in 1990 to 7.7%, 65 million in 2016 and 74 million in 2021. Tamil Nadu was reported to have had the highest prevalence in 2021, followed by Kerala (diabetic capital of India), Delhi, Punjab, Goa and Karnataka (47). India is called the diabetes capital of the world because it is home to 17% of all diabetics in the world (51). In India, the average healthcare expenditure for diabetes is US$209 per year and the total number of deaths directly related to diabetes is 1.5 million (52).

2.2 Classification of Diabetes Mellitus

According to the latest American Diabetes Association (ADA) classification, diabetes can be classified into the following general categories (53):

2.2.1 Type 1 Diabetes Mellitus

Type I diabetes mellitus (T1DM) is an autoimmune disease that leads to destruction of the insulin-producing beta cells in the pancreas. The incidence and prevalence of T1DM has steadily increased, affecting approximately 5 to 10% diabetic people (54). It is further classified into two types: a). Idiopathic, b). Immune-mediated. It occurs genetically, but the actual cause of the disease is still unknown. It is also called "Juvenile diabetes" because it typically occurs in children and young adults.

2.2.2 Type 2 Diabetes Mellitus

Type II diabetes mellitus (T2DM) is characterized by two main defects: insulin resistance, in which many cells become less responsive to insulin; and a deterioration in beta cells, resulting in sluggish insulin production by the pancreas. This leads to an increase in fasting blood glucose levels in the bloodstream and an imbalance in metabolism. Approximately 90-95% of diabetics suffer from T2DM. It develops slowly and typically occurs in older adults, but children can also be affected.

2.2.3 Gestational Diabetes

Gestational diabetes mellitus (GDM) is a temporary condition that occurs during pregnancy. About 2-4% of all pregnancies are affected and the risk of developing diabetes is increased for both mother and child. GDM is a heterogeneous disease that develops in the third trimester of pregnancy. It involve inadequate insulin secretion might get better or disappear once the baby is delivered. According to the reports about 50% of women with gestational diabetes tends to develop type 2 diabetes after several years.

2.2.4 Secondary types of Diabetes:

It is further classified into the following:

A). Maturity onset diabetes of the young (MODY)

MODY is a group of inherited disorders of non-autoimmune diabetes mellitus (54). It is also known as neonatal diabetes and the maturity-onset of the young. DNA methylation defects of genes (responsible for cell development) have been observed in the pancreas. Failure of pancreatic β-cells within the first six months leads to an increase in blood glycemic levels. It is also called as "neonatal diabetes" and the maturity-onset of the young.

B). Cystic fibrosis-related diabetes

People suffering from cystic fibrosis are more susceptible to this type of diabetes. The syndrome is caused by impaired insulin secretion due to partial fibrotic reduction of β-cells in the pancreas (55). It also leads to pancreatitis, pancreatic neoplastic, and hereditary hemochromatosis.

C). Drugs associated with diabetes

Synthetic drugs affects the physiological system and leads to alterations in the secretion and function of insulin (56). This leads to insulin resistance and diabetes in susceptible individuals. For example, glucocorticoids have been reported to cause insulin resistance and hyperglycemia, and also interfere with the various phases of insulin signaling (57). It is known that antiretroviral drugs induce insulin resistance and inhibit glucose disposal via GLUT4 in the cell system by impairing glucose tolerance (58).

2.3 Pathophysiology

2.3.1 Type 1 Diabetes Mellitus

The deficiency of insulin secretion causes autoimmune damage to the cells of the pancreas, leading to metabolic irrationalities associated with type 1 diabetes. Environmental and genetic factors generate auto antigens which are ingested by antigen-presenting cells. They activate T helper 1 (Th1) and T helper 2 (Th2) lymphocytes. Activated Th1 lymphocytes secrete interleukin-2 (IL-2) and interferon. IL-2 activates auto-antigen specific T cytotoxic lymphocytes that destroy islet cells by secreting toxic perforins and granzymes. Interferon activates macrophages and stimulates the release of inflammatory cytokines (including IL-1 and tumor necrosis factor [TNF]) that further destroy beta cells (59). Activated Th2 lymphocytes produce IL-4, which stimulates B lymphocytes to proliferate and produce islet cell auto antibodies (ICAs) and anti-glutamic acid decarboxylase (anti GAD65) antibodies. Anti GAD65 is an enzyme that helps control the release of insulin from beta cells and can be used to determine the cause of diabetes.

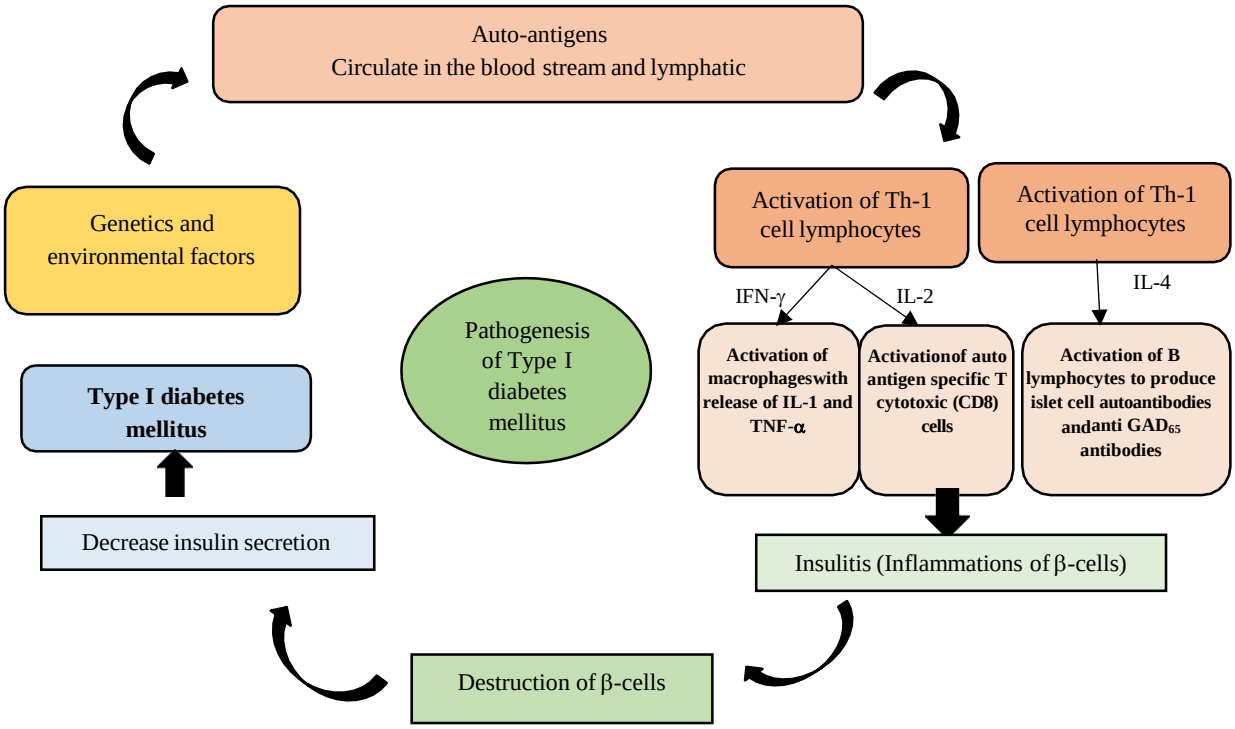


Figure 2.2: Pathophysiology of Type I Diabetes Mellitus (59).

2.3.2 Type 2 Diabetes Mellitus

In type 2 diabetes mellitus (T2DM), a combination of genetic factors related to impaired insulin secretion and insulin resistance, as well as environmental factors such as obesity, overeating, lack of exercise and stress, and aging results in a metabolic imbalance responsible for the development of the pathophysiological condition.

i). Impaired insulin secretion, a decrease in early-phase glucose-responsive insulin secretion, and decreased post meal insulin secretion lead to postprandial hyperglycemia.

ii). Insulin resistance is a condition in which the insulin in the body does not have an adequateeffect that is proportional to its blood concentration.

It contributes to increased glucose production in the liver and decreased glucose uptake in both muscle and liver and adipose tissue. Even when both processes occur early in pathogenesis, hyperglycemia is amplified, leading to the progression of T2DM.

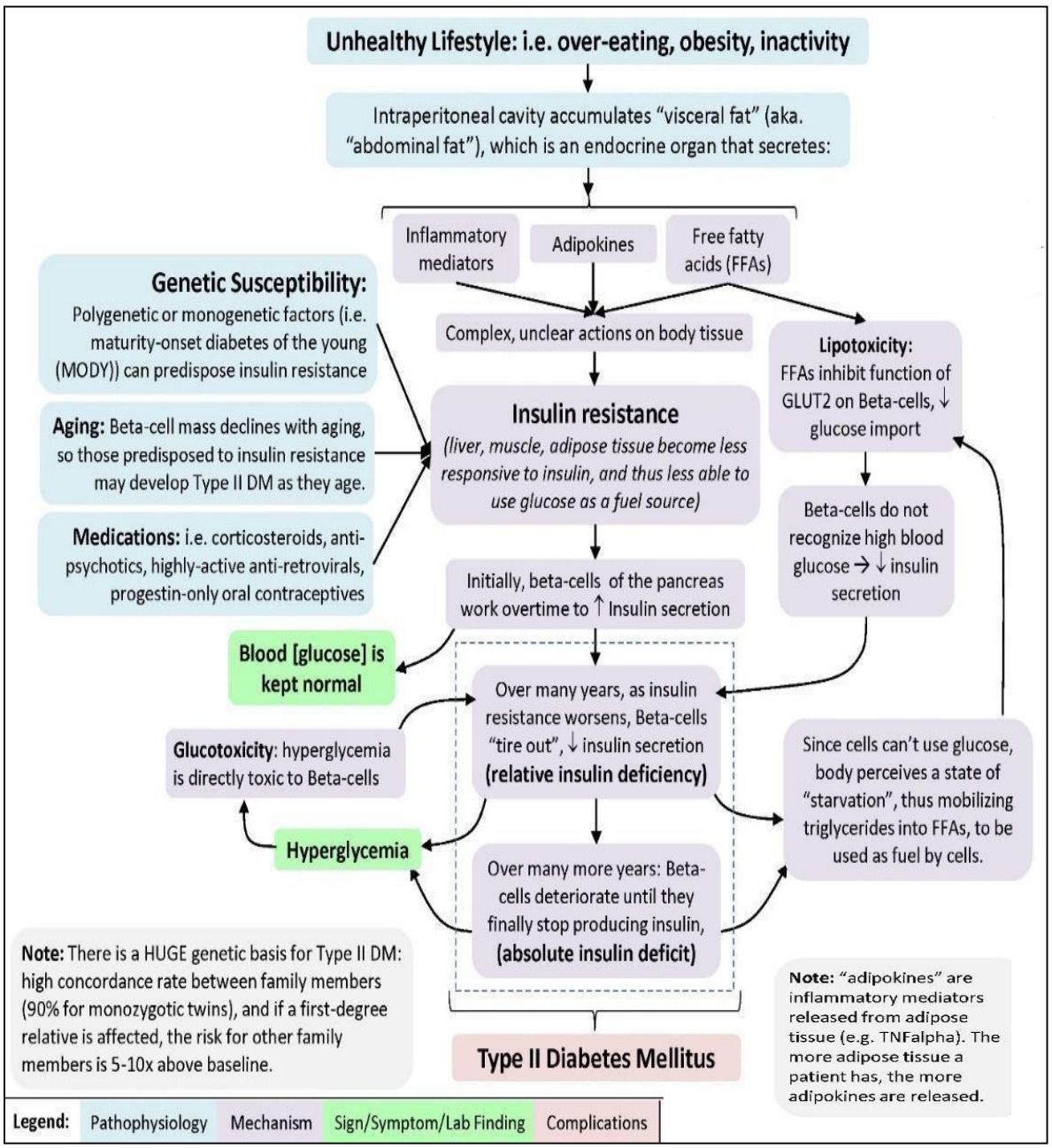


Figure 2.3: Pathophysiology of Type II Diabetes Mellitus (60).

2.4 Symptoms of Type 2 Diabetes Mellitus

The main symptoms of type 2 diabetes include increased thirst (polydipsia), increased hunger (polyphagia), frequent urination (polyuria) and weight loss. Other symptoms include peripheral neuropathy, vaginal infections, fatigue, blurred vision, and itching. Patients with type 2 diabetes rarely have hyperosmolar hyperglycemia (high blood sugar accompanied by low blood pressure and decreased consciousness).

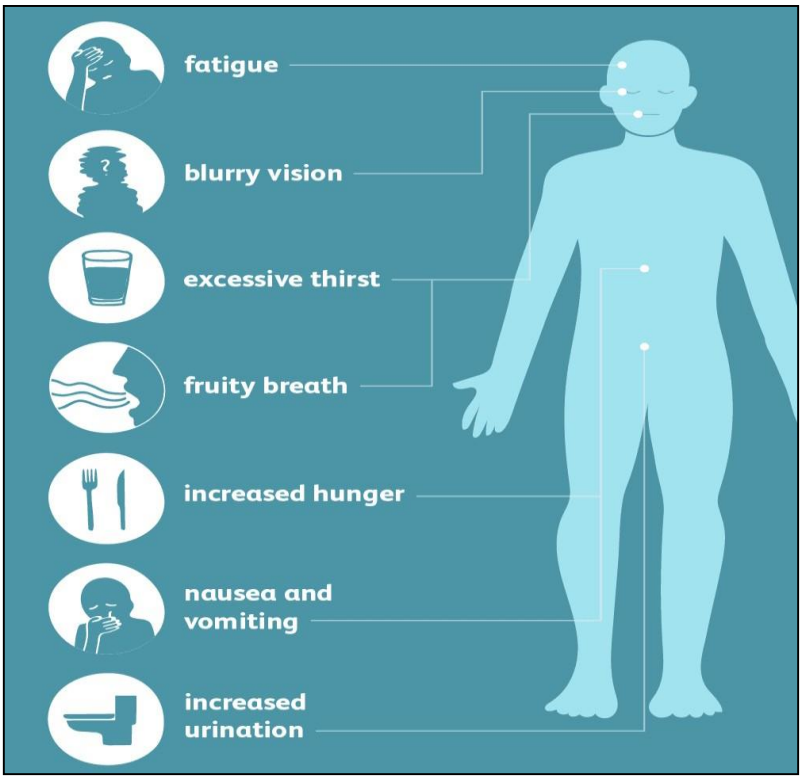


Figure 2.4: Common symptoms of type II diabetes mellitus (61).

2.5 Complications

Diabetes mellitus is known to cause long-term complications, the consequences of which are due to uncontrolled hyperglycemia causing either acute or chronic complications (62).

Acute Complications - Require Immediate Medical Intervention

- *Ketoacidosis*: Despite of hyperglycemia, there can be a lack of glucose in the body cells (muscle and lipid cells) due to improper insulin function. Cells begin to use fats as a source of energy. Liver cells produce ketone bodies from fatty acids for energy production. High levels of ketones can acidify urine and cause fruity-smelling breath.
- *Hyperosmolar Hyperglycemic Nonketotic Syndrome (HHNS)*: High blood glucose levels lead to increased urination and severe dehydration.

Chronic Complications - Long-term Side Effects of Diabetes

- *Microvascular complications:* The smaller blood vessels, such as in the eyes (leading to retinopathy), kidneys (leading to nephropathy), and neurons (leading to neuropathy).
- *Macrovascular complications*: The larger blood vessels, such as those supplying the heart, brain, and extremities. The causes of these complications are narrowing of blood vessels due to inflammation, lipid deposition and other factors.

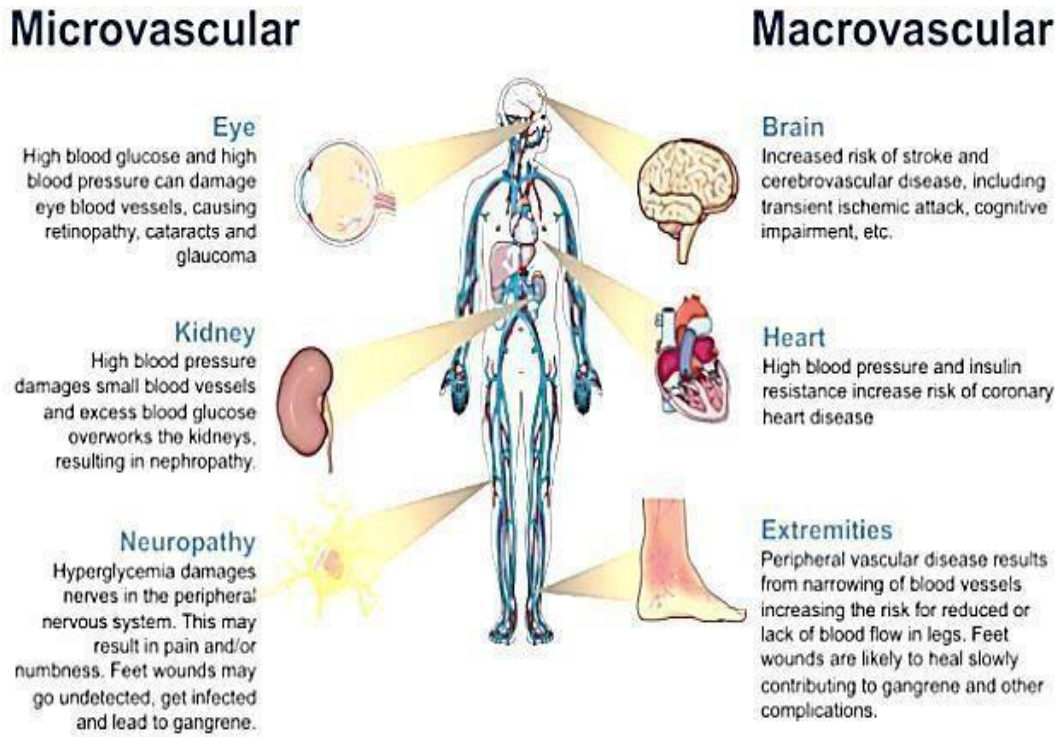


Figure 2.5: Major microvascular and macrovascular complications associated with T2DM (62).

2.6 Diagnosis assessment of diabetes mellitus

1. **Glycated hemoglobin (A1C) test**

   The A1C test is used to detect type 2 diabetes and prediabetes.it reflects the average of a person's blood glucose levels over the past three months and shows no daily fluctuations.

2. **Fasting plasma glucose (FPG) test**

   The FPG test is used to detect diabetes and pre-diabetes. It is the most commonly used test to diagnose diabetes. It measures a person's blood sugar level after at least eight hours of fasting.

3. **Oral glucose tolerance test (OGTT)**

   The OGTT can be used to diagnose diabetes, pre-diabetes and gestational diabetes. It measures fasting blood glucose levels for at least 2 and 8 hours after administration of 75 grams of loading glucose.

4. **Random plasma glucose test (RPGT)**

   The RPGT can be used to diagnose diabetes. It measures blood glucose without regard to when the person being tested last ate.

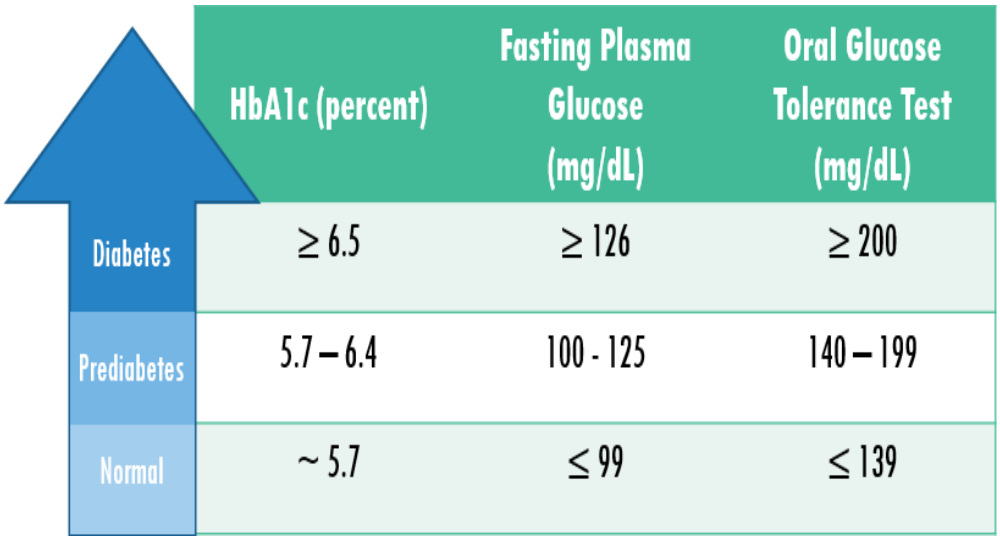


Figure 2.6: Blood test levels for Diagnosis of Diabetes and Pre-diabetes.

2.7 Prevention and management of diabetes mellitus

T2DM can be prevented by maintaining normal body weight and exercise, which has beneficial effects on the heart and lungs by supporting insulin function and maintain body glycemic level. Physically active people improve high-density lipoprotein (HDL), lower triglyceride levels, and maintain blood pressure. Dietary changes have been shown to be effective in controlling blood sugar levels, eating a healthy diet, and choosing good fats like polyunsaturated fats found in nuts, fish, and vegetable oils. Foods that are low in salt, sugar, and high in fiber such as beans, fruit, and grains, helps in maintaining body weight. Limit the consumption of sugary drinks, less red meat and foods with saturated fats. Active smoking can lead to the development of diabetes, so preventive measures should also be taken. There are presently eight classes of oral anti-hyperglycemic agents are available in market to control the blood glucose levels with different mechanisms, including sulfonylureas, meglitinides, biguanides, thiazolidinediones, DPP-4 inhibitors, alpha-glucosidase inhibitors, GLP1 analogs, and SGLT 2 inhibitors (Table 2.1).

Table 2.1: Conventional oral anti-diabetic drugs and their major adverse effects (63).

Drug Class	Example	Mechanism	Adverse effect
Biguanides	**Metformin**	↓ Hepatic glucose production, improvement in glucose uptake and insulin sensitivity	Gastrointestinal upset, lactic acidosis (rare), B-12 deficiency, contraindication: kidney and liver dysfunction.
Sulfonylureas	**Glipizide**	Closes K_{ATP} channels, ↑ insulin secretion	Hypoglycemia, ↑ weight
Meglitinides	**Repaglinide**	Closes K_{ATP} channels, ↑ insulin secretion	Hypoglycemia,↑ weight
Thiazolidinediones	**Pioglitazone**	PPAR-γ activator, ↑ insulin sensitivity	↑ Weight, ↑ LDL (rosi),↑ MI (rosi), edema/heart failure
α-Glucosidase Inhibitors	**Acarbose**	Inhibits α-glucosidase, slows down the digestion/absorption of carbohydrates	Gastrointestinal upset, dosing frequency, ↓ A1c
DPP-4 Inhibitors	**Sitagliptin**	Inhibits DPP-4, increases incretin (GLP-1, GIP) levels	Angioedema / urticarial, pancreatitis, ↑heart failure
GLP 1 analogue	**Semaglutide**	Bind to GLP-1 receptor, ↑ insulin release	Headache, muscle weakness
SGLT2 inhibitors	**Canagliflozin**	Inhibits SGLT2 in proximal nephron, increases glucosuria	Genitourinary tract infections, polyuria

The novel oral glucose-lowering agents include the sodium-glucose co-transporter inhibitors (SGLTi), which have the potential to improve glycemic control but also through glucose also have renal hemodynamic effects, including a reduction in glomerular hyper filtration, as well as inhibitory effects on the inflammatory and fibrotic responses of proximal tubular cells to hyperglycemia.

Diabetes was well established as a metabolic disorder and the possible role of the kidney in the pathogenesis of diabetes was ignored. Interestingly, attention has once again focused on the kidney, not only as an organ that can be affected by diabetes, but also as an important role in glucose homeostasis and a potential target for the treatment of hyperglycemia.

2.7 Role of kidney in glucose homeostasis

The involvement of the kidneys in glucose homeostasis was first described in the 1930s (64). The mammalian kidney has three distinct mechanisms for regulating glucose homeostasis:

(i) Release of glucose into the circulation via gluconeogenesis

(ii) Uptake of glucose from the circulation to satisfy its energy needs

(iii) Reabsorption of glucose from the glomerular filtrate into the circulation.

2.8.1 Renal gluconeogenesis

A). Post-absorptive state

In humans, the liver and kidneys are the only organs that contain significant amounts of glucose-6- phosphatase enzyme and therefore they are only capable of gluconeogenesis (65). About 75–80% of glucose is obtained from the liver and 20–25% from the kidneys and released into the circulation at a rate of about 10 mol/(kg min) after an overnight fast (66, 67). Regarding the hormonal effect, insulin suppresses the distribution in these organs (68). Indirectly both liver and kidney glucose release were affected by glucagon and catecholamine by rising the presence of gluconeogenic substrates and suppressing insulin secretion (69).

B). Post-prandial state

Postprandial plasma glucose levels are significantly affected by the levels of glucagon and insulin. After glucose uptake, rise in the concentration of plasma glucose triggers insulin release, which stimulates peripheral glucose uptake and suppresses endogenous glucose production. Hepatic gluconeogenesis decreases by about 82% while renal gluconeogenesis rises by about 60% of endogenous glucose release in the postprandial phase (70).

2.8.2 Renal glucose utilization

Renal glucose utilizes about 10% of the total glucose used by the human body. In postprandial, about 45% of the ingested glucose is normally converted to glycogen in the liver, about 30%, 15%, 10%, and 5% of the glucose is used by skeletal muscle, brain, kidneys, and adipose tissue (70). The kidneys have different glucose metabolism pathways in different regions. The metabolic pathwayof glucose is different in different regions of the kidney. Due to its low oxygen content and oxidative enzymes, the renal medulla is an obligate consumer of glucose for its energy needs (anaerobic).

2.8.3 Renal glucose reabsorption

The kidney influences glucose homeostasis by recycling glucose into the circulation via the reabsorption of glucose from the glomerular filtrate. On average, the entire blood volume of a human being is filtered through the kidney more than 50 times per day, with approximately 160-180 g of glucose per day being filtered from plasma by glomeruli. The glucose filtration rate per healthy person increases with increasing glycemic value. Prospectively, 15-55 g of glucose will be produced by gluconeogenesis and 25-35 g of glucose will be metabolized.

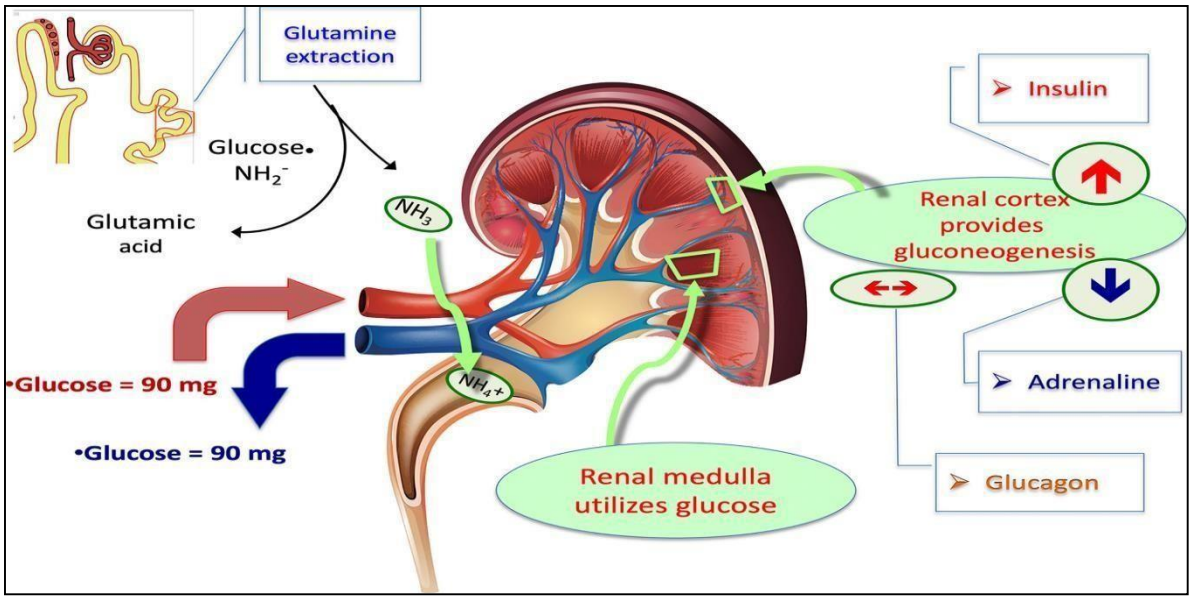


Figure 2.7: The role of the kidney in glucose metabolism (71).
In the kidney, gluconeogenesis occurs in the cortical cells while the marrow cells metabolize glucose. Insulin inhibits production of glucose in the kidney while adrenaline stimulates it. Renal glucose production is not affected by glucagon. Renal glucose metabolism supports glutamine extraction from tubularcells to produce glutamic acid and ammonia (NH3). This energy-based metabolic pathway plays a crucial role in the elimination of uric acid (NH4+). Ultimately, the kidney's glucose metabolism maintains a neutral balance between the amount of glucose entering with arterial blood and leaving with venous blood.

2.8.4 Renal glucose transport

Glucose reabsorption from the glomerular filtrate occurs via both sodium glucose co-transporters (SGLTs) and GLUTs. The energy for the SGLT-mediated active transport of glucose across the cell membrane is obtained from the electrochemical potential gradient of sodium. This is maintained by the transport of intracellular sodium ions into the blood via sodium-potassium adenosine triphosphatase (ATPase) pumps in the basolateral membrane. GLUTs bind glucose and induce a conformational change, and glucose is passively transported across the cell membrane from the intracellular compartment to the plasma (72).

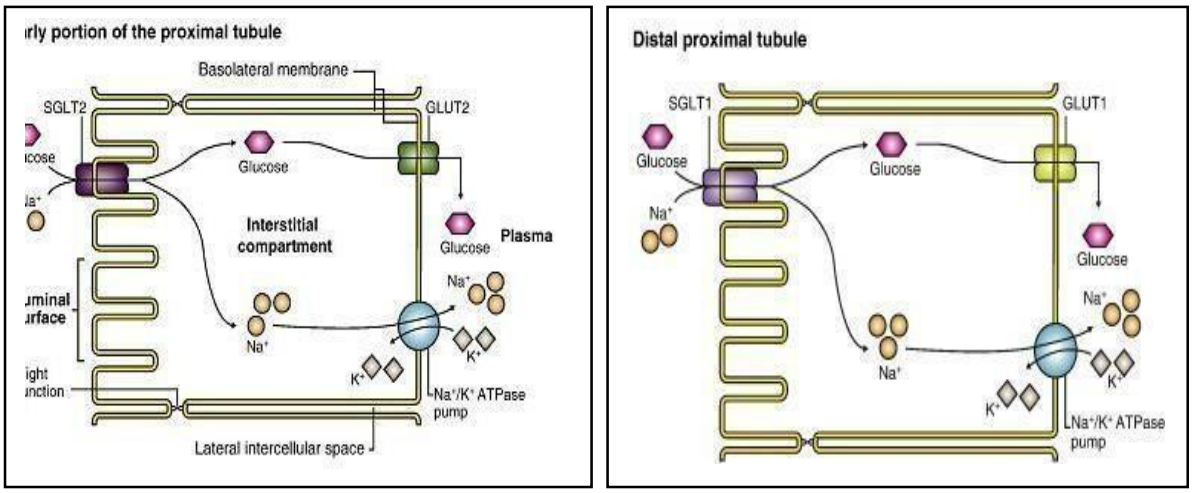


Figure 2.8: Renal glucose transport in the early part of the proximal tubule and distal proximal tubule (72)**.**

Proximal renal tubules have both SGLTs and GLUTs which are responsible for glucose transport and reabsorption and are expressed at the luminal brush border and basolateral membrane on epithelial cells. SGLT2 is a high-capacity, low-affinity co-transporter responsible for reabsorption of renal glucose and couples the active transport of glucose and sodium in a 1:1 ratiowithin the early proximal tubule. Glucose is reabsorbed into the blood stream via GLUT2. Theremaining glucose is resorbed by SGLT1, a high-affinity transporter expressed in the distal proximal tubule (2:1, sodium: glucose), and then resorbed into the blood via GLUT1.

2.8.5 Renal threshold for glucose excretion

The physiological relationship between plasma glucose concentration and renal glucose flux (i.e. filtration, reabsorption and excretion) has typically been described as a threshold relationship. The amount of glucose filtered by the kidneys increases linearly with increasing plasma glucose concentration and decreases with decreasing glomerular filtration rate (GFR). Renal glucose reabsorption rises until a certain concentration of plasma glucose is present. However, there is a distinct deviation from this linear relationship as renal glucose- recovery capacity approaches saturation, presumably due to inter-nephron variability in peak absorptive capacity (73).

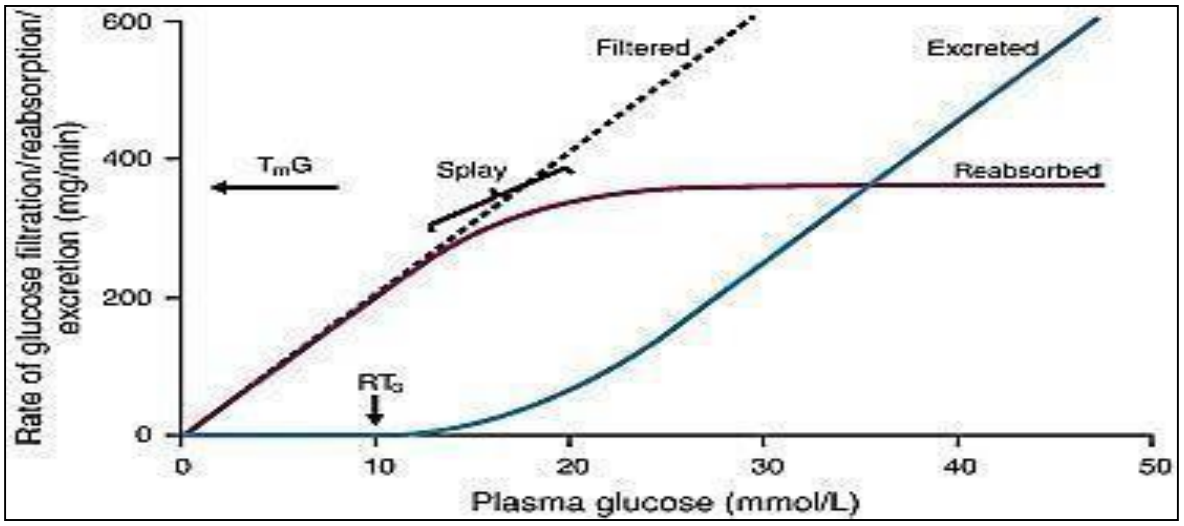
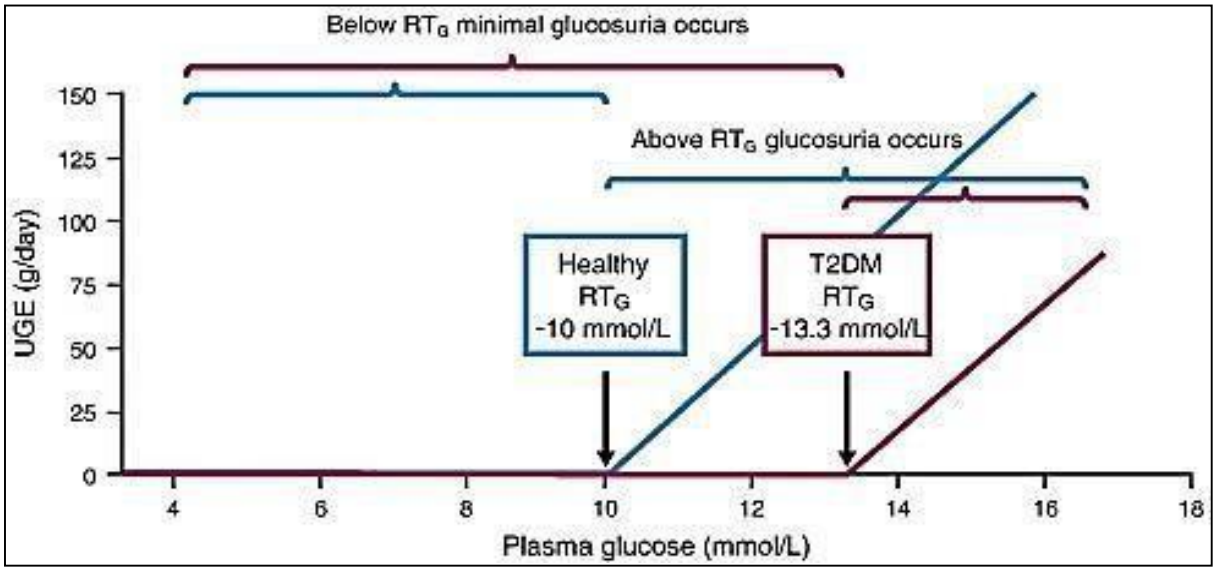


Figure 2.9: Relationship between plasma glucose concentration and renal glucose reabsorption.

TmG, tubular maximum glucose reabsorption; $RT_{G,}$ Renal threshold for glucose excretion.

Under normal conditions, in healthy individuals, total filtered glucose reabsorbed in the renal tubules. However, when the filtered glucose load exceeds the maximal glucose reabsorption capacity of the tubule, excess glucose excreted through urine. The renal glucose excretion threshold (RTG) is the plasma glucose concentration at which TmG is exceeded;below this concentration, glycosuria is minimal, while RTG is elevated in T2DM patients.While studies examining RTG in T2DM patients indicate some inter-individual variability, many patients have elevated levels that are above the normal range, with values between 112 and 240 mg/dL.

Figure 2.10: Relationship between plasma glucose concentration and renal glucose excretion in healthy



individual and T2DM patients.

TmG may be elevated in diabetic patients and contribute to worsening hyperglycemia. Increased tubular reabsorption may be due to rise in GLUT expression or activity, with upregulation of SGLT2 and GLUT2 being a possible mechanism for the increase in glucose reabsorption. In hyperglycemia, both SGLT2 and GLUT2 mRNA levels were significantly rises in the T2DM group compared to controls. Rise in tubular reabsorption in individuals withT2DM leads to an increase in the flow of glucose into the blood and hyperglycemia. Based on observations, the mean RTG in T2DM is approximately 40 mg/dL (2.2 mmol/L) higher than the commonly reported values of 180 to 200 mg/dL (10-11 mmol/L).

In healthy subjects, and using a mean GFR of 100 mL/min, calculations indicate that an increased RTG results in an average of approximately 50 to 70 mg/min of additional glucose entering the circulation when the plasma glucose level is higher the RTG. Compared to glucose reabsorption when RTG was not increased. For comparison, it is estimated that increased hepatic glucose production in a 100 kg type 2 diabetes patients results in approximately 24 mg/min of extra glucose. Thus, both the kidney and the liver are instrumental in the hyperglycemia seen in T2DM. However, it should be noted that the additional renal glucose reabsorption may be significantly lower in patients with renal impairment because their GFR is below 100 mL/min.

2.8 Sodium glucose co-transporter (SGLT)

In August 1960, Robert K. Crane in Prague discovered SGLT as a mechanism for glucose absorption in the gut (74). In biology, crane's discovery of co-transport was the first proposal for flux coupling. SGLT are responsible for the active glucose that enables the absorption of glucose and galactose in the gut and reabsorption of all filtered glucose by nephron. In the kidneys, when the plasma glucose level is high (hyperglycemia), the glucose is excreted in the urine (glucosuria) because the SGLT is saturated with the filtrate glucose.

2.8.1 SGLT family

In humans, there are seven different transport proteins in the SGLT family. SGLT1 and SGLT2 are known members of the SGLT family of the SLC5A gene family. In which SGLT 1 is the first member of the SLC5 family to be cloned. SLC5A4/SGLT3 (SAAT1) has low affinity for the glucose transporter, while SLC5A3/SGLT2 has the highest capacity to mediate

Na+/glucose transport. Members of the SGLT family transporter proteins are mentioned as follows:

Table 2.2: SGLT family transporter proteins.

S.no	Gene	Proteins	Transport type	Human tissue distribution
1	**SLC5A1**	**SGLT1**	Co-transport of glucose or galactose with sodium and hydrogen uniport of water and urea	Kidney, small intestine, brain, testis, prostate, trachea, heart,
2	**SLC5A3**	**SGLT2**	Co-transport of glucose with sodium	Kidney, thyroid, brain, heart, muscle, liver, salivary gland
3	**SLC5A3**	**SMIT1**	Co-transport of myoinositol glucose with sodium	Brain, heart, kidney, lung
4	**SLC5A4**	**SGLT3**	Acts as a glucose sensor and activated sodium channel	Kidney, small intestine, thyroid, uterus, skeletal muscle, testis, lung, brain
5	**SLC5A9**	**SGLT4**	Co-transport of mannose, fructose or glucose with sodium	Kidney, small intestine, brain, liver, heart, lung, trachea, uterus, pancreas
6	**SLC5A10**	**SGLT5**	Co-transport of mannose ,fructose or glucose with sodium	Kidney cortex
7	**SLC5A11**	**SGLT6**	Co-transport of myoinositol with sodium	Thyroid, brain, heart muscle, spleen, liver, lung

2.9.2 Mode of action of SGLT1 and SGLT2

SGLT2 is a low-affinity, high-capacity glucose/sodium transporter, located on the luminal membrane of cells in the S1 and S2 segments and is responsible for 90% of total glucose reuptake. The SGLT-1 transporters, located downstream in the S3 segment of the proximal tubules, are responsible for the remaining 10% of the glucose load that is reabsorbed into the renal interstitial fluid. SGLT1 is a high affinity, low capacity glucose/galactose transporter found in the gut, brain, skeletal muscle, liver, lungs and kidneys. SGLT1 couples the absorption of 2 molecules of sodium with 1 molecule of glucose at the intestinal brush border membrane (75).

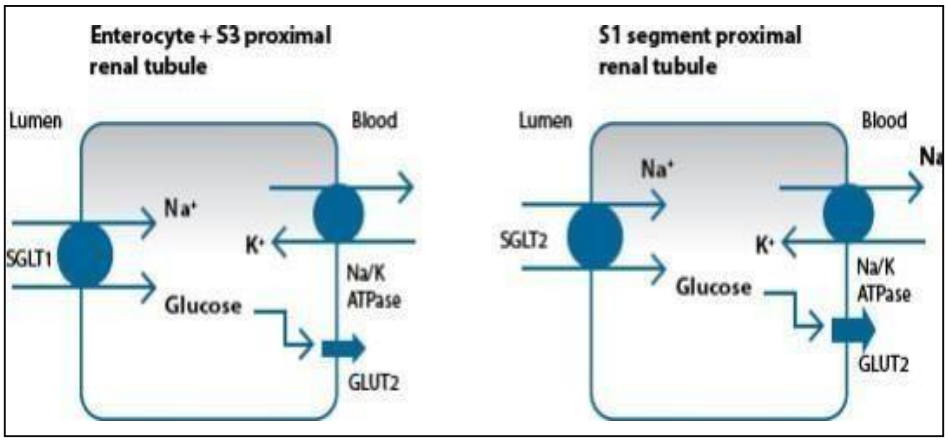


Figure 2.11: Mode of action of SGLT1 and SGLT2.

SGLT is responsible for urinary glucose despite normal glucose levels (76). Therefore, SGLT2 is being widely studied by the pharmaceutical industry as a therapeutic target to control blood glucose levels in diabetics. In addition, SGLT2 target drugs increased attention due to their cardioprotective effects (77).

2.10 SGLT -2 inhibitors

SGLT-2 inhibitors depend on renal excretion and prevent glucose from entering the bloodstream by promoting glycosuria. Gliflozines are a class of drugs used to treat type 2 diabetes mellitus that work by inhibiting SGLT-2.

2.10.1 Mechanism of action

The mechanism of action of SGLT-2 inhibitors is independent of insulin secretion or action. It decreases the reabsorption of glucose from the proximal convoluted tubule in the kidneys, resulting in increased urinary glucose excretion and there-by a reduction in plasma glucose levels and weight loss. It also increases insulin sensitivity in muscle and liver, while a reduction in gluconeogenesis leads to an improvement in pancreatic beta cell function (78).

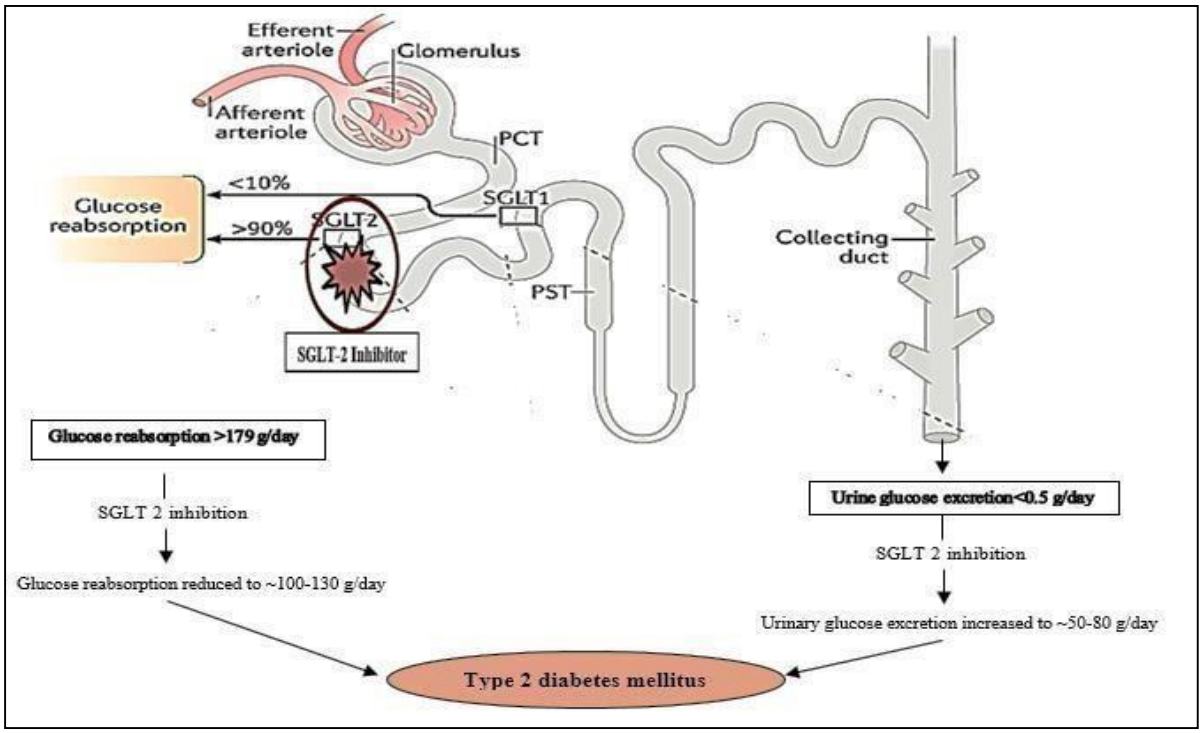


Figure 2.12: Mechanism of action of SGLT-2 inhibitors.

2.10.2 Pleiotropic effects

SGLT-2 inhibitors (SGLT2i) lower blood glucose levels by excreting excess glucose in the urine (79). The reduction in lipid profile leads to a reduction in body weight of around 1 to 5 kg. It also lowers systolic and diastolic blood pressure (BP) significantly. These changes are relatively more pronounced in systolic blood pressure and are not dose-dependent. The reduction in blood pressure in SGLT2i is initially due to osmotic diuresis and later to local inhibition of the rennin-angiotensin system. Uric acid levels, a marker of metabolic disorders, are significantly reduced (59-17.8%) by SGLT2i, with effects lasting for two years.

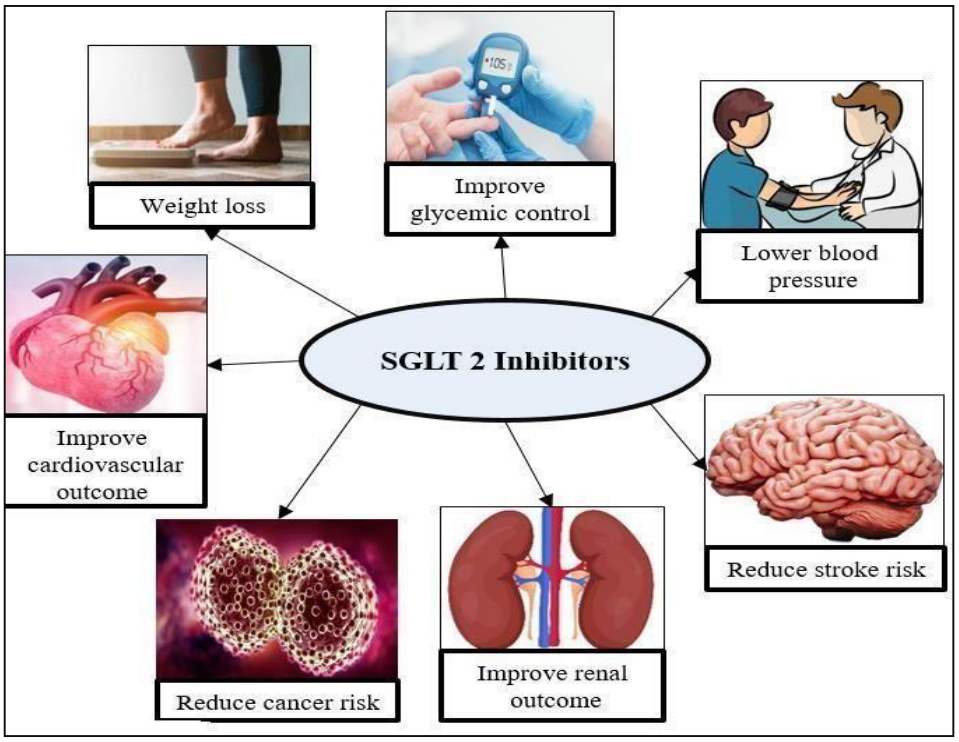


Figure 2.13: Beneficial effects of SGLT 2 inhibitors.

Renal hyperfiltration has been found to be closely associated with the development of diabetic nephropathy (DN). SGLT2 is an important determinant of glomerular hyperfiltration, and SGLT2i suppresses reactive oxygen species production, reduces glomerulosclerosis, and produces nephroprotective effects in the tubular interstitial space (80). Its anticancer effects suppress proliferation through indirect activation of AMPK (AMP-activated protein kinase) in cancer cells (81). It inhibits oxidative phosphorylation, decreases ATP production and increases AMP/ATP, leading to a significant increase in AMPK activity. It also reduces the risk of hemorrhagic stroke by about 50% (82).

2.10.3 SGLT-2 inhibitors for type 2 diabetes mellitus

SGLT 2 inhibitors, a new group of oral drugs are used to treat type 2 diabetes mellitus. According to the American Diabetes Association (ADA), SGLT2 inhibitors as initial pharmacological therapy for type 2 diabetes (usually with metformin) will become the standard care for diabetes by 2022, particularly in patients with chronic kidney disease and cardiovascular disease (83). There are currently three selective SGLT2 inhibitors approved by the Food and Drug Administration (FDA) for mono, dual, and triple therapy: canagliflozin (Invokana), dapagliflozin (Farxiga), and empagliflozin (Jardiance), as shown in Table 2.3. In addition, bexagliflozin was approved in the US under the brand name Brenzavvy in January

2023 and remogliflozin etabonate was first commercially launched in India by Glenmark in May 2019 (84).

Table 2.3: List of current SGLT 2 inhibitors.

Drug	Brand name	Company name	Current status	Months, Years
Canagliflozine	Invokana	Mitsubishi, Tanabae Pharma, Janseen	US FDA Approved, Approved in Europe (EMA)	March, 2013
Dapagliflozine	Farxiga	Bristol-Myers Squibb, Astra Zeneca	US FDA Approved, Approved in Europe (EMA)	January, 2014
Empagliflozine	Jardiance	Boehringer inelheim, eli lilly	US FDA Approved, Approved in Europe (EMA)	August, 2014
Ertugliflozine	Steglaro	Pfizer, Merck	US FDA Approved, Approved in Europe (EMA)	December, 2017
Ipragliflozine	Suglat	Astellas, Kotobuki pharmaceutical	Approved in Japan (MHLW)	January, 2014
Bexaglifozine	Brenzavvy	Elanco	US FDA Approved	January, 2023
Luseogliflozine	Lusefi	Taisho, Novartis	Approved in Japan (MHLW)	March, 2014
Tofogliflozine	Deberza	Chugai, Kowa, sanofi	Approved in Japan (MHLW)	March, 2014

FDA, Food drug administration; EMA, European medicines agency; MHLW, Ministry of health and labour welfare.

2.11 Medicinal plants

Medicinal plants are plants that have therapeutic properties in certain parts and contain substances against certain diseases or serve as a source of useful medicines. Herbal substances have received attention as they remain the basis for a large proportion of commercial medicines. The World Health Organization (WHO) has compiled a list of 21,000 medicinal plants used worldwide including communicable and non-communicable diseases. There are 2500 species in India, 150 of which are endemic to the country (85). Herbal medicine has experienced rapid growth in recent years and these medicines are gaining acceptance in both developing and developed countries. According to World Ethanobotanical, 800 medicinal plants are used to prevent diabetes mellitus. It has been clinically proven that only 450 medicinal plants have anti-diabetic properties, of which 109 medicinal plants have a provenmode of action (86).

2.12 Natural SGLT inhibitors

The first natural SGLT inhibitor, phlorizin, and its glucoside analogues were isolated from apple tree bark in 1835 (50). Although many SGLT2 inhibitors are now available and widely used, great efforts are still being made to find new drugs derived from natural products as viable candidates for novel SGLT2 inhibitors. Some important natural SGLT 2 inhibitors are described in Table 2.4.

Table 2.4: Natural SGLT inhibitors.

Plant	Family	Plant parts	Extract	Study	Chemical constituent	Reported
Malus pumila	**Rosaceae**	Bark	Ethanol extract	Currents induced by α-MDG in *Xenopus* oocytes expressing hSGLT1	Phlorizine Quercetin Kaempferol	Phlorizin inhibits both SGLT1 and SGLT2 Quercetin &Kaempferol-Only SGLT 1 inhibitory activity (87)
Sophora flavescens	**Fabeceae**	Dried root	Methanol extract	Methyl-a-D-gluco pyranoside uptake in cultured cells expressing SGLT1 or SGLT2	Pterocarpin ,Macakiain,Variabilin Formononetin e , (-)-kurinone, Sophra flavonone G	Macakiain, Variabilin Formononetine – Inhibited only SGLT 2 kurinone & Sophra flavonone G - inhibited both SGLTs. Also they were most potent SGLT2 inhibitor (88)
Acer nikoense	**Aceraceae**	Stem bark	Methanol extract	Methyl-α-D glucopyranoside uptake in COS-1 cells expressing hSGLT1 or hSGLT2 were evaluated	Acerogenin A, B, and C	Acerogenin A & B - marked inhibition for both SGLTS (89)
Gnetum gnemonoides	**Gnetaceae**	Dried bark	Ethanol extract	Methyl-α-D glucopyranoside Uptake in Cultured Cells (expressing SGLT1 or SGLT2).	Gneyalin A and B	Non selective SGLT1 and SGLT2 inhibitory activity Both shows higher SGLT 1 inhibitory activity (90)
Allium cepa	**Amaryllidaceae**	Bulb onion	Ethanol extract	Currents induced by α-MDG in *Xenopus* oocytes	4'-O-glycosylated quercetin derivative	Only SGLT 1 inhibitory activity (91)
Grapevine	**Vitaceae**	Shoot	Water extract	Currents induced by α-MDG in *Xenopus* oocytes expressing SGLT1	Trans resveratrol ε-viniferin and hophenol a dimer of resveratrol	Polyphenols shows Non selective SGLT1 and SGLT2 inhibitory activity (92)
Brassica oleracea	**Brassicaceae**	Leaves	Ethanol extract	Currents induced by α-MDG in *Xenopus* oocytes expressing SGLT1	Kaempferol and quercetin	Polyphenols shows only SGLT 1 inhibitory activity (93)
Schisandra chinensis	**Schisandraceae**	Schisandrae Chinensis Fructus (SCF)	Ethanol extract Fraction	^{14}[C]-α-methyl-d-glucopyranoside (^{14}[C]-AMG) uptake assay	Fractions of ethanolic extract of (SCF) F9 AND F8	Ethanolic extract (1 mg/mL) Only two fractions (F8 and F9) showed significant SGLT2-selective inhibition (94)
Alstonia macrophylla	**Apocynaceae**	Leaves	Ethanol extract	Inhibition of up taking of methyl-a-D-glucopyranoside in cultured cells expressing SGLT1 or SGLT2	10-Methoxy-*N*(1)-methylburnamine-17-*O*-veratrate, Alstiphyllanine D	Non selective SGLT1 and SGLT2 inhibitory activity Highest in 10-Methoxy-*N*(1)-methylburnamine-17-*O*-veratrate (95).

2.13. Alstonia scholaris

2.13.1 Introduction

Alstonia scholaris (L.) R. Br. is an evergreen plant of the Apocynaceae family used for medicinal purposes. *'Alstonia'* is named after Dr. C. Alston (1685-1760), a professor of botany at Edinburgh University. The specific name scholaris derives from the use of the wood for school blackboards in Myanmar (96). It is popularly known as "Saptaparni" and is used in Ayurvedic, homeopathic and folk medicine of India to treat various types of ailments. It is attracting the attention of researchers around the world due to its pharmacological effects ranging from antimalarial to anticancer activities.

A).Taxonomical classification of *A. scholaris* (97)

Taxonomy: *Alstonia scholaris*
Domain: Eukaryotes (nucleus within a membrane)
Kingdom: Plantae (plant)
Subkingdom: Tracheobionta (vascular plants)
Phylum: Spermatophytes (seed-bearing plant)
Sub phylum: Angiosperm (seeds in a closed ovary)
Class: Dicotyledon (two cotyledons)
Subclass: Asteridae
Order: Gentianales

B). Native Distribution

A. scholaris is an evergreen tropical tree native to the India, Sri Lanka, Pakistan, Nepal, Thailand, Burma, Malaysia, Africa, northern Australia, the Solomon Islands and southern China (98).



Figure 2.14: ***Alstonia scholaris*** **Tree.**



Figure 2.15: ***Alstonia scholaris*** **Leaves.**

C). Profile of *Alstonia scholaris* (99)

Local names Bengali (Satiani, Chattin, Chatium); Burmese (lettok); English (White Cheesewood, Birrba, Milkwood Pine, Milk Wood, Milky Pine, Black Board Tree, Devil's Tree, Dita Bark); Filipino (dita, dalipoen); Gujarati (satuparni); Hindi (Chatian, Satni, Satwin); Indonesian (rite, pulai, pule); Sanskrit (saptaparna); Tamil (Elalaipalai, Palegaruda, Pala); Thai (Sattaban, Teenpet, Teenpethasaban); trade name (pulai, shaitan wood, chatiyan wood, white cheese wood); Urdu (Chatiana); Vietnamese (caay suwxa).

D). Botanic Description

Alstonia scholaris is an evergreen tree up to 40 m tall with cork gray to off-white bark. The outer blaze is cream to yellowish in color and contains copious amounts of milky sap that flows rapidly after cutting. Leaves in whorls of 4–8 in upper leaf axils; Petioles 1–1.5 cm long, the lamina oviate to elliptic or elliptic-lanceolate, glabrous or sparsely hairy, tapering to the base, 11.5–23 x 4–7.5 cm. The inflorescence is a strongly branched terminal panicle up to 120 cm long; flowers 7-10 mm long white, cream or green; the tube hairy; lobes sparsely or densely hairy, 1.5–4 mm long, overlapping left margins; heavily perfumed and bloom in the month of October. Seeds of *A. scholaris* are oblong, have ciliated margins, and end in 1.5 to 2 cm tufts of hair.

2.13.1 Phytochemical constituents of *Alstonia scholaris* leaves

Phytocomponents such as alkaloids, steroids, triterpenes and triterpenoids, flavonoids, phenols and steroid are present in the leaves of *A. scholaris.* Different phytocomponents are characterized from the different parts of *A. scholaris* is considered a rich source of alkaloids and is used for therapeutic purposes. Alkaloids are a class of great importance for the development of new drugs because alkaloids have a wide variety of chemical constituents and are responsible for the pharmacological properties (Table 2.5).

Table 2.5: Phytochemical constituents present in *Alstonia scholaris* leaves (100).

Class of compounds	Compounds
Alkaloids	Scholaricine, Alstonamine , Alschomine, Alstoscholarine, Akuammidine, Angustilobine , Echitamine, Echitamidine, 19-Epischolaricine, Lagunamine, Losbanine , Rhazimanine, Manilamine, Angustilobine, Picrinin, Methylscholaricine, Methoxyaspidophylline, 5a-Methoxystrictamine, 5-Methoxystrictamine, Nareline, Picralinal, Picraline, Scholaricine, Tubotaiwine oxide, Vallesamine.
Triterpenes and triterpenoids	Alstonic acids, Betulin, Betulinic acid, n-Tetracosane, Ursolic acid.
Flavonoids	Kaempferol, Quercetin, Isorhamnetin.
Phenols	4-hydroxy-3-methoxybenzoic acid and 2,3-dihydroxybenzoic acid
Steroids	β-Sitosterol and β-sitosterol glucoside

2.13.2 Pharmacological activity of *A. scholaris* leaves

The plant has been reported to have anti-malarial, anti-cancer, anti-cough, anti-inflammatory, antispasmodic, analgesic, antipyretic, antipsychotic, antioxidant, anthelmintic, anti-asthmatic, and antihypertensive effects (101). The pharmacological activities of *A. scholaris* leaves are listed in Table 2.6. They have been scientifically studied in animal models to confirm the plant's potential to cure various diseases.

Table 2.6: Pharmacological activity of *Alstonia scholaris* leaves.

Biological activity	Active extract or fractions	Effective concentration /dose	Study model
Antimicrobial	Butanol fraction	4 mg/well	In vitro (102)
	Methanol extract	5 mg/well	In vitro (103)
Antioxidant	Ethanol extract	50 µg/ml	In vitro (104)
	Dichloromethane and ethyl acetate fraction of ethanol extract	250 µg/ml	In vitro (105)
	Methanol extract	200 µg/ml	In vitro (106)
	Ethanol extract	100 mg/kg	In vivo (107)
	Aqueous extract	2.21 mg/kg	In vivo (102)
Analgesic and anti-inflammatory	Alkaloid fraction and alkaloids Picrinine, Vallesamine, Scholaricine	100 mg/kg ,10 mg/kg , 8 mg/kg, 5 mg/kg	In vivo (mice)
	Dichloromethane	30 mg/kg	In vivo (mice)
Anticancer	80% Methanol	4% Extract	In vitro
CNS activity	Ethyl acetate fraction of ethanol extract	30 mg/kg	in vivo (rat)
Wound healing activity	Ethanol and aqueous extracts	Tropical application (5%) and oral administration (50 mg/ml of suspension)	In vitro and in vivo (rats)
Bronchodilatory activity	Ethanol extract	0.008 mg/kg	In vivo (rat)

2.14 Overview of *Alstonia scholaris* as hypoglycemic activity

Several in vitro and in vivo studies have been performed by researchers to evaluate the traditional and local antihyperglycemic effects of *A. scholaris*. The antidiabetic potential of *A. scholaris* was studied in terms of its alpha-glucosidase inhibitory activity (108). Ragasa et al studied the hypoglycemic potential of triterpenes (betulin and lupeolus acetate) isolated from the dichloromethane extract of A. scholaris bark (109). Bandawane et al. reported hypoglycemic and antihyperlipidemic effects in diabetics indced by streptozotocin on rats by ethanolic extract of A. scholaris bark (110). Deepti et al studied the antihyperglycemic and antihyperlipidemic effect of aqueous extract of *A. scholaris* bark on streptozotocin (STZ) induced diabetes in rats (111). John Prosper et al studied aqueous and methanolic extracts of *A. scholaris* studied blood glucose levels in normal and alloxan-treated diabetic rabbits.

CHAPTER –3

Materials and methodology

3.1 Pharmacognostic studies

3.1.1 Plant collection and Identification

Alstonia scholaris leaves were collected in the month of September from Naroda, Ahmedabad, and Gujarat. Authentication of the plant material was verified by a taxonomist from Gujarat University (GU), Ahmedabad. The voucher specimen (AS-1) has been deposited in the GU herbarium of botanical survey of India, Ahmedabad.

3.1.2 Physicochemical analysis

A. scholaris leaves powder was analyzed by using various parameters including foreign matter, total ash, acid insoluble ash, and water and alcohol soluble extract. It was compared to its standard API limits (112) to assess purity and quality.

A). Determination of foreign matter

Depending on the specification, this test was performed on the leaves of the plant material (113). 100 g of test material was spread in a thin layer on a clean surface and examined with the naked eye or under a 6x lens microscope. The foreign matter was removed, weighed, and the foreign matter content was calculated using the following formula:

$$\text{Foreign matter (\% w/w)} = \frac{\text{Weight of foreign matter} \times 100}{\text{Weight of sample}}$$

B). Determination of total ash

Two grams of dry powder were placed in a quartz crucible and gradually heated to 500°C until white, indicating the absence of carbon (114). The ash was cooled in a desiccator and immediately weighed. The total ash percentage value was calculated as follows:

$$\text{Total ash (\% w/w)} = \frac{\text{Ash weight} \times 100}{\text{Weight of sample}}$$

C). Determination of acid-insoluble ash

A shallow thin fused silica porcelain was weighed and ignited and 2 g of powdered drug was added to the furnace until carbon-free ash formed (115). Then, 25 ml of diluted hydrochloric acid was mixed to the ash and left in the water bath for 8-10 minutes after covering with a watchglass. The contents were then allowed to cool and filtered through #42 Whatman filter paper and then placed in the oven at 100°C for about 3 hours. It was ignited in the muffle furnace forone hour. The bowl was heated for 30 minutes, dried, cooled and weighed. This process was repeated for 30 minutes till the difference between two consecutive weighing become less than 1 mg. lowest weight was recorded. The percentage of acid insoluble ash was obtained using following formula:

$$\text{Acid-insoluble ash (\% w/w)} = \frac{\text{Acid insoluble ash weight X 100}}{\text{Weight of sample}}$$

D). Determination of ethanol soluble extractive

5 g of air-dried drug were macerated with ethanol (100 ml) for 24 hours (115). After filtration, filtrate (25 ml) was evaporated to dryness at 105°C. Calculate the percentage of ethanol soluble extract based on the air-dried sample.

$$\text{Ethanol soluble extractive value (\% w/w)} = \frac{[(\text{Final weight} - \text{Initial weight}) \text{ X } 4] \text{ X } 100}{\text{Weight of sample}}$$

E). Determination of water soluble extractive

5 g of powdered air-dried medicinal product was macerated with chloroform water (100 ml) for twenty-four hours, shaken frequently for six hours, and kept for eighteen hours (116). It was then quickly filtered taking precautions against solvent loss. Filtrate (25 ml) was evaporated to dryness. The percentage of water soluble extract was calculated as:

$$\text{Water soluble extractive value (\% w/w)} = \frac{[(\text{Final weight} - \text{Initial weight}) \text{ X } 4] \text{ X } 100}{\text{Weight of sample}}$$

3.1.3 Extraction of dried powder of *A. scholaris* leaves using successive method

Coarsely powdered material of the *A. scholaris* leaves were subjected to successive solvent extraction in a soxhlet apparatus using different solvents in increasing order of polarity, starting

with petroleum ether (PE), chloroform (CH), ethyl acetate (EA) and ethanol (E) (117). After completion of extraction, the solvent was distilled off and the residue was concentrated and finally dried. The marc remained after extraction with each solvent was dried completely in air before subjecting to next solvent. The vacuum dried extracts were then subjected to a chemical test to determine the presence of various phytocomponents (118).

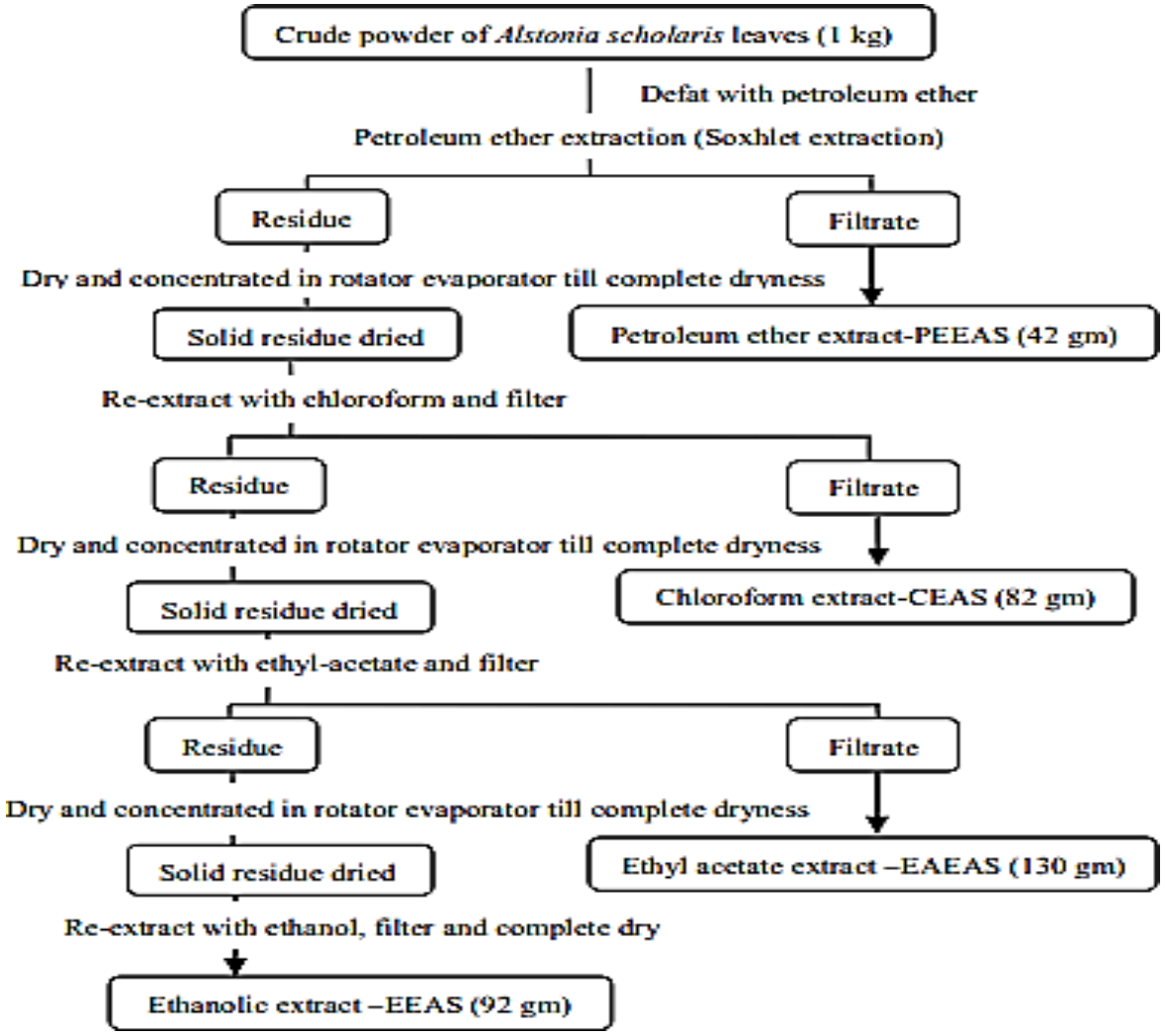


Figure 3.1: Schematic sequence of successive extraction of dried powdered *Alstonia scholaris* leaves. Ethanolic extract of *Alstonia scholaris* leaves (EEAS), Ethyl acetate extract of *Alstonia scholaris* leaves (EAEAS), Chloroform extract of *Alstonia scholaris* leaves (CHEAS), Petroleum ether extract of *Alstonia scholaris* leaves (PEEAS).

A). Percentage yield

The percent yield of the different extracts was determined using the following formula (119).

$$\% \text{ yield (w/w)} = \frac{\text{Final weight of sample extract} \times 100}{\text{Initial weight of sample}}$$

B). Phytochemical analysis

Phytochemical analysis are preliminary tests performed to detect the presence of both primary and secondary metabolites in an extract such as alkaloid flavonoids, steroids, phenols, tannins, saponins, terpenoids and glycosides (118). All extracts underwent phytochemical screening to determine the presence or absence of primary and secondary metabolites.

i).Test for alkaloids

Dragendorff's test: 1 ml extract was taken and placed in a test tube. Then 1 ml of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange-red precipitate that forms indicates the presence of alkaloids

ii). Test for flavonoid

Ammonia test: Diluted ammonia (5 ml) was added to a part of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was added. A yellow colour that disappears on standing indicates the presence of flavonoids.

iii). Test for tannins

Ferric chloride test: 2 ml of the aqueous solution of the extract was added to a few drops of 10% ferric chloride solution (light yellow). The presence of a black-blue colour indicated the presence of gallic tannins and a green-black colour indicated the presence of catechol tannins.

iv). Test for phenolic

Lead acetate test: 1 ml solution of an extract was taken and placed in a test tube. Then 1 ml of alcoholic solution was added and then diluted with 20% sulphuric acid. Finally, a sodium hydroxide solution was added. The formation of a red to blue colour indicates the presence of phenols.

v). Test for saponine

Foam test: 3 ml of the aqueous solution of the extract was mixed with 10 ml of distilled water in a test tube. The test tube was shaken vigorously for about 5 minutes. It was allowed to stand for 30 minutes and examined for honeycomb foam indicating the presence of saponins.

vi). Test for terpenoid

The Salkowski test: Extract (5ml) was mixed with chloroform (2ml) and concentrated sulphuric acid (3ml) added cautiously to form a layer. A reddish-brown discoloration interface that formed

indicated positive results for the presence of terpenoids.

C). Thin Layer Chromatography (TLC)

Alstonia scholaris leaves extracts were subjected to TLC analysis to determine the chemical components present (120).

i). Preparation of the plates

The precoated TLC plates (Merk, Germany) consisting of silica gel G as adsorbent were activated in an oven at 110°C for 30 minutes. Test samples (1 mg/ml of all extracts in the respective solvents) were applied in the form of bands.

ii). Development of solvent system Two mobile phases suitable for detecting the presence of alkaloids were identified i). First solvent system:-toluene: ethyl acetate: methanol in the ratio 7:2:1 and ii). Second solvent system:-chloroform: ethyl acetate: ammonia in the ratio 7:3:0.1. Orange bands were visualized by spraying with different spray reagents to find different compounds present in Dragendorff's reagent for alkaloids in the extract and the R_f value was recorded.

3.2 Pharmacological studies

3.2.1 Experimental Animals and Ethical Approval

Male albino wistar rats (200-250 g) were procured from Zydus research center (ZRC), Ahmedabad, India at, 1 week before the study. They were acclimatized in a room maintained at 22 ± 1°C with a relative humidity of 55 ± 5% and a 12 h light-dark cycle. Animals had free access to a standard pellet diet (certified Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water. All experiments were carried out with strict adherence to ethical guidelines and were conducted as per protocol (LMCP/P'cology/19/10) approved by the Institutional Animal Ethics Committee (IAEC) and as per Indian norms laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

3.2 Drugs and Chemicals

Streptozotocin (STZ) was procured from ANJ biomedical, USA. The standard drugs are Metformin procured from Apollo, Knoll Pharmaceuticals Ltd., Canagliflozin (INVOKANA) was purchased from Johnson & Johnson's Private Limited of Maharashtra, India. Sodium carbonate (Na_2CO_3), ethylenediamine tetraacetic acid (EDTA), and hydrogen peroxide (H_2O_2) procured from Merck. Nitro blue tetrazolium (NBT), 1, 2-dithiol-bis nitro benzoic acid (DTNB), disodium hydrogen phosphate (Na_2HPO_4), and sodium dihydrogen phosphate

(NaHPO4) Procured from Sigma Aldrich. The chemical and solvent used in the experiments were of analytical grade.

Normal and high fat diet

- Normal diet was containing 3% fat, 20% protein, 55% carbohydrate with 310 kJ/kg total calorific value.
- High-fat food was containing 43% fat, 15% protein, 42% carbohydrate providing 412 kJ/kg calories for animals.

Table 3.1: Composition of high fat diet (121).

S. No.	Ingredients	g (%)	KJ
1.	Butter	1.5 g (15%)	43
2.	Egg	1 g (10%)	12.50
3.	Coconut oil	0.2 g (2%)	7.53
4.	Mixture of flours =Corn (2 g)+wheat (2 g)+soya (2.1 g)+ barely (2 g) flour	7.1 g (73%)	67
Total		10 g	130.03 KJ

3.3 Preliminary study

3.3.1 Glucose tolerance test (dose optimization)

Male wistar rats were divided into three main groups; Normal control (NC), test and standard (STD) group. In addition, it was divided into twelve groups (G) of three animals each, as shown in figure 3.2. In this present study, fasted rats were fed 2 g/kg glucose (loading dose) overnight (122). Three test doses (200, 400 and 800 mg/kg, o.p.) of chloroform extract, ethyl acetate extract, ethanol extract of *Alstonia scholaris* leaves, canagliflozin (10 mg/kg, o.p.) and metformin (200 mg/kg, o.p.) were administered. Fasting blood glucose levels were measured with a glucometerby pricking the tail vein at 0, 30, 60, 90, 120 and 150 minutes after the glucose loading dose.

The three test doses 200 mg/kg, 400 mg/kg and 800 mg/kg were selected with reference to acute toxicity studies (123). All of these extracts were dissolved in 1% sodium carboxymethyl cellulose (CMC).

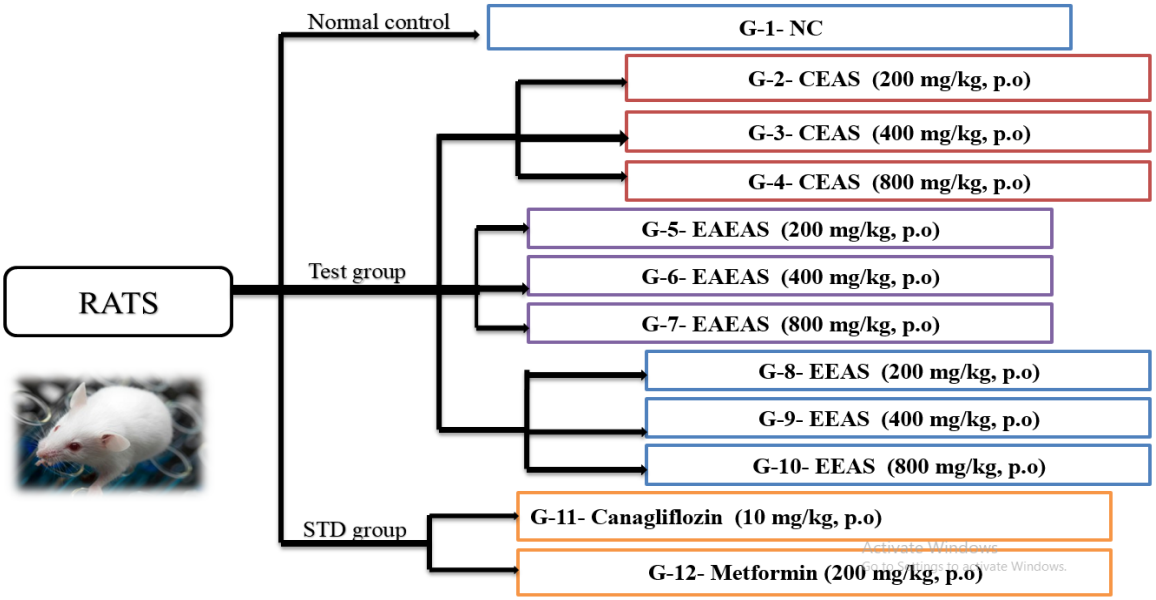


Figure 3.2: Experimental design for oral glucose tolerance testing of various *Alstonia scholaris* leaves extracts. Normal control (NC), Chloroform extract of *Alstonia scholaris* leaves (CHEAS), Ethyl acetate extract of *Alstonia scholaris* leaves (EAEAS), Ethanol extract of *Alstonia scholaris* leaves (EEAS).

3.3.2 Statistical analysis

All data obtained from the experiments were expressed as the mean± standard error of the mean (SEM). Statistical analysis was performed by unpaired Student's t-test using Graphpad Prism 8.1 Software (Graphpad, San Di go, CA). P<0.05 was considered statistically significant.

3.4 Study of active extracts in rats with HFD and STZ-induced type 2 diabetes mellitus

3.4.1 Experimental design

Male wistar rats received their respective diet (normal pellet diet or high-fat diet) for 4 week. A single dose of STZ (45 mg/kg/i.p.) in chilled 0.1 M sodium citrate buffer adjusted to pH 4.3 was administered. Drinking water was replaced with a 5% sucrose solution before and after the administration of STZ to overcome drug-induced hypoglycaemia. Moderately diabetic range rats (250–400 mg/dl blood glucose level) were included in the study. Thereafter, the animals were divided into six groups, each group consistingof six animals: Group I: Normal control (NC) treated with 0.9% saline, Group II: Diabetic control (DC) treated with STZ (45 mg/kg, i.p.), Group III: EAEAS (Ethyl acetate extract from *A. scholaris* leaves, 400 mg/kg, p.o.) Group IV: EEAS (Ethanol extract from *A. scholaris* leaves, 400 mg/kg, p.o.) Group V: Metformin (200 mg /kg) Group VI: Canagliflogin (10 mg/kg). The dose of both extracts was selected based on the previously performed OGTT analysis.

Then, the diabetic rats were kept for four weeks for the induction of complications. Treatment was given for four weeks. The food intake, water intake and weight of the rats were measured weekly. Urinalysis and fasting blood glucose were measured weekly after STZ administration. All other parameters were measured at the end of the study (Table 3.2). All animals were fasted overnight, blood samples were collected and serum separated for analysis of biochemical parameters. After blood collection, the animals were sacrificed for collecting organs. Histopathological examination of the liver, pancreas, heart, kidney and liver organs was performed. All samples were fixed in 10% phosphate buffered formaldehyde and embedded in paraffin. They were sectioned and stained with hematoxylin and eosin stain. Images of the selection were taken at 10x magnification witha zoom digital camera (MLX, Magnus, and New Delhi, India).

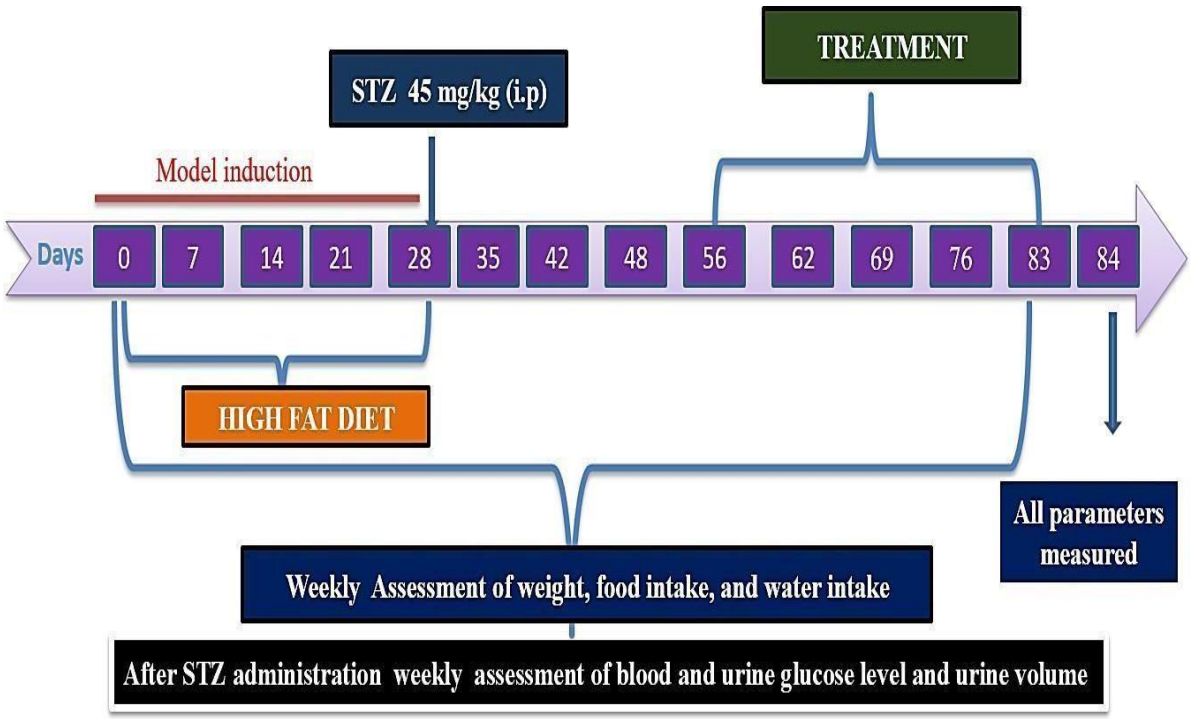


Figure 3.3: Experimental design for a high-fat diet and low dose of streptozotocin-induced type 2 diabetes mellitus.

3.4.2 Statistical analysis

Results expressed as the mean standard error of the mean (SEM). Statistical analysis was performed by one-way ANOVA after Tukey's multiple comparisons using Graph Pad Prism 8.01 software (Graph pad, San Diego, CA). In comparison to the respective controls, a $p<0.05$ (p-value) was identified as statistically significant.

3.4.3 Evaluating parameters

A). General parameters

Weekly assessment of weight of rats, food intake and water intake.

B). Urinalysis

Post-streptozotocin administration, weekly assessment of urinary output and urinary glucose excretion (124).

C) Biochemical parameters

i). Estimation of fasting blood glucose level (god/pod method)

Principle: Glucose is oxidized by the enzyme glucose oxidase (GOD) and converted into gluconic acid and hydrogen peroxide. In the presence of the enzyme peroxidase (POD), hydrogen peroxide and chromogen 4-aminoantipyrine/phenolic compound covert into a red coloured compound. Concentration of glucose was measured at 505 nm. Finally, Intensity of colour is stable for 30 min.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{POD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{P-Hydroxybenzoic acid} + \text{4-aminoantipyrine} \xrightarrow{\text{GOD}} \text{Quinoneimine dye} + 2H_2O$$

Procedure: 1.0 mL of the working solution was mixed with 1.0 mL of serum sample. Similarly, standard (STD) and blank were prepared using 1.0 mL of glucose standard (included in the kit) and distilled water, respectively. These solutions were then mixed and incubated at room temperature for 30 minutes (end point reaction). The absorbance of the test and STD was measured at 505 nm compared to the blank.

Calculations: The glucose concentration in test samples was calculated using the following formula:-

$$\text{Serum glucose level (mg /dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

ii). Total cholesterol estimation method (enzymatic method)

Principal: The red complex (red quinone) formed during the reaction is directly proportional to the cholesterol concentration in the sample and is measured at 500 nm.

Cholesterol Ester + O_2 $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Dehydroacetone phosphate + H_2O_2

$2H_2O_2$ + Phenol + 4-aminoantipyrine $\xrightarrow{\text{Peroxides}}$ Red quinone + $4H_2O$

Procedure: Reagents were reconstituted as described in the leaflet insert included with the kit. 1.0 mL of serum samples, distilled water as a control and standard triglyceride (200 mg/dL) as a STD were added to 1.0 mL of reconstituted reagent. They were incubated at 37°C for one minute and the absorbance was read against the blank at 500 nm.

Calculation: The total cholesterol content was calculated using following formula:-

$$\text{Serum total-cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 200$$

iii) Estimation of the serum triglyceride (enzymatic method)

Principle: Triglycerides are enzymatically hydrolyzed to glycerol according to the following reactions .The intensity of the red complex (red quinone) complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and recorded at 546 nm. The final color is stable for at least 30 minutes.

Triglycerides + H_2O $\xrightarrow{\text{Lipoprotein lipase}}$ Glycerol + free fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 $\xrightarrow{\text{GPO}}$ Dehydroacetone phosphate + H_2O_2

2 H_2O_2 + 4-aminoantipyrine + ADPS $\xrightarrow{\text{peroxidase}}$ Red quinone + 4 H_2O

GPO: Glycerol-3-Phosphate Oxidase; ADPS: N-Ethyl-N-Sulfopropyl-n-anisidine

Procedure: The microtiter plates were arranged as described with the package insert included in the kit and the reagents were reconstituted. The serum samples, distilled water as a control and standard triglyceride (200 mg/dl) as a standard were added as follows:

REAGENT	BLANK (B)	STANDERD (S)	TEST (T)
Triglyceride	1.0 ml	1.0 ml	1.0 ml
Triglyceride standard	-	10µl	-
Serum sample	-	-	10 µl

They were then incubated at 37°C for 5 minutes and the absorbance was read in comparison to the absorbance at 546 nm.

Calculation: The serum triglyceride content was calculated using following formula:-

$$\text{Serum triglyceride (mg/dl)} = \frac{\text{Absorbance of test X Concentration of standard (mg/dl)}}{\text{Absorbance of standard}}$$

(V) Estimation of serum LDL (low-density lipoproteins) level

Principle: LDL Reagent is manufactured using a combination of detergents and phosphorus compounds that specifically bind HDL, VLDL, and chylomicron (CM), but not LDL. The combination protects HDL, VLDL and CM from reaction by cholesterol esterase and cholesterol oxidase. This selectively exposes LDL cholesterol to a reaction with both enzymes.

$$\text{LDL (ester-cholesterol)} + H2O \xrightarrow{\text{CEH}} \text{Cholesterol + Fatty Acids}$$

$$\text{Free Cholesterol} + O2 \xrightarrow{\text{CHOD}} \text{Delta 4 Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{HDAOS} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye}$$

HDAOS = N-(2-hydroxy-3-sulfopropyl)-3, 5 dimethoxyaniline

Procedure: The microplates were arranged and the reagents reconstituted as described in the package insert included with the kit. The serum samples, distilled water as a control and standard LDL as a standard were added as follows:

PIPETTE INTO TEST TUBES	BLANK (B)	STANDERD (S)	TEST (T)
R_1 Cholesterol (µl)	750 µl	750 µl	750 µl
Calibrator (µl)	-	10 µl	-
Sample (µl)	-	-	10 µl

Mix well and Incubate at 370 C for 5 minutes.

R_2 Cholesterol	250 µl	250 µl	250 µl

After incubation, take the first reading at 600 nm or 578 nm. (A1) R2 LDL-Cholesterol were added and incubated again at 370°C for 5 minutes and read the final reading. (A2) at 600 nm or 578 nm. Determine the mean absorbance change according to: A= (A2-A1) value.

Calculation: The serum LDL level in the sample was calculated using following formula:-

LDL Concentration (mg/dl) = $\frac{\text{Absorbance of sample X Concentration of standard (mg/dl)}}{\text{Absorbance of standard}}$

v). Estimation of serum VLDL (very low-density lipoproteins) level

VLDL can be determined from the measured triglycerides using the Friedewald formula:-

$$\text{VLDL (mg/dl)} = \frac{\text{Triglyceride}}{5}$$

vi). Estimation of serum HDL (high-density lipoproteins) level

Principle: The test is based on a classic precipitation method coupled with modified polyvinyl sulfonic acid (PVS) and polyethylene glycol methyl ether (PEGME). LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results LDL, VLDL and CM inaccessible to cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes react selectively with HDL and produce H_2O_2, which is detected by a Trinder reaction.

HDL +LDL + VLDL+ CM $\xrightarrow{\text{PVS, PEGME}}$ HDL + (LDL + VLDL+ CM) + PVS/PEGME

HDL $\xrightarrow{\text{CHOD, CHER}}$ Fatty acid+ H_2O_2

$2H_2O_2$ + 4-AA+TODB $\xrightarrow{\text{Peroxidase}}$ $4H_2O$ + Quinone (Red dye)

TODB- N, N-Bis (4-sulfobutyl)-3-methylaniline

Procedure: The micro titer plates were arranged, then serum samples, distilled water as a control and standard HDL as a standard are added as follows:

PIPETTE INTO TEST TUBES	BLANK (B)	STANDERD (S)	TEST (T)
R_1 Cholesterol (µl)	750 µl	750 µl	750 µl
Calibrator (µl)	-	10 µl	-
Sample (µl)	-	-	10 µl

Mix well and Incubate at 37° C for 5 minutes.

R_2 Cholesterol	250 µl	250 µl	250 µl

After the incubation, first reading was taken at 600 nm or 578 nm. (A1) R2 HDL-cholesterol were added and incubated again at 37° C for 5 minutes and final reading were taken. (A2) at 600 nm or 578 nm. Mean absorbance change was determined according to: A= (A2-A1) value.

Calculation: The serum HDL level in the sample was calculated using following formula:-

HDL Concentration (mg/dl) = $\frac{\text{Absorbance of sample X Concentration of standard (mg/dl)}}{\text{Absorbance of standard}}$

vii). Estimation of serum urea level

Principle: Samples are compared to a known concentration of a urea standard in a 96-well micro titer plate format. Samples and standards were kept for 10 minutes with the enzyme urease, which hydrolyzes urea into ammonia and CO_2. The ammonia further reacts with a chromogen in alkaline solution and produces a blue-green colour product. After 30 minutes, the plate is read with a STD 96-well spectrophotometric microplate reader at an optical density between 580 nm and 630 nm. Higher OD (optical density) values correlate with high urea concentrations. The urea concentrations of the samples are determined by comparison with known urea standards.

Procedure: The kit contained three reagents: 1 (Enzyme Reagent), 2 (Chromogenic Reagent), and 3 (Urea Standard). Working Reagent 1 was prepared by adding enzyme 1 in deionized water according to the amount listed on the vial. Prepared reagents 1 and 2 were mixed to microplate wells as follows:

REAGENT	BLANK (B)	STANDERD (S)	TEST (T)
Working reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Serum sample	-	-	10 µl
Reagent 2	1.0 ml	1.0 ml	1.0 ml

It was mixed well and kept at 37°C for 5 minutes. Sample absorption and the standard was then compared with the blank at 580 nm.

Calculation: The serum urea level in the sample was calculated using following formula:-

$$\text{Urea concentration (mg/dl)} = \frac{\text{Test sample absorbance x}}{\text{Standard absorbance}} \text{ Standard concentration (mg/dl)}$$

viii). Estimation of serum creatinine level

Principle: Creatinine (2-amino-1-methyl-5H-imadazol-4-one) is a metabolite of phosphocreatine (p-creatinine), a molecule that serves as a storehouse for high-energy phosphate used by tissues for ATP production 1. Creatinine either present in food or is synthesized from the amino acids glycine, methionine and arginine. 2. Creatinine and p-creatinine are converted non-enzymatically into the metabolite creatinine, which diffuses into the blood and is excreted via the kidneys.

Procedure: The kit contained three reagents: 1 (1-picric acid), 2 (0.75 N sodium hydroxide), and 3 (stock creatinine standard). Reagents 1 and 2 were mixed to form working reagent to use and filled the microtiter plate as follows:

REAGENT	BLANK (B)	STANDERD (S)	TEST (T)
Working reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	10 µl	-
Serum sample	-	-	10 µl

After mixing, the initial absorbance (A1) is absorbed for 20 seconds and the final absorbance for 80 seconds (A2).

Calculation: The serum urea level was calculated as follows:

Absorbance of test = A1-A2

$$\text{Creatinine concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dl)}$$

ix). Estimation of serum CK-MB (Creatinine kinase MB) level

Principle: CK-MB is based on the principle of specific immune inhibition by a blend of monoclonal antibody which completely inhibit CK-MM activity and 50 % of CK-MB activity. While not affecting the B Subunit activity of CK-MB and CK-BB, the CK-B activity is measured. The CK-MB activity is obtained by multiplying the CK-B activity by two. Increased levels may be found due to severe exercise and by large multiple intra-muscular injuries. With other symptoms and suggestive history, serum CK estimation is an important parameter of choice for myocardial infarction.

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CK-B}} \text{Creatine} + \text{ATP}$$

$$\text{ATP} + \text{Glucose} \xrightarrow{\text{HK}} \text{G-6 PO4} + \text{ADP}$$

$$\text{G-6 PO4} + \text{NADP} \xrightarrow{\text{G-6 PDH}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}$$

Procedure: The kit contained two reagents: 1 (enzyme reagent) and 2 (starter reagent). The working reagent was prepared by mixing 4 parts Reagent 1:1 part Reagent 2. Leave for 60 minutes. The working reagent was stable for 30 days at 2-8°C. 50 L of sample was added to the micro titer plates and 1mL of working reagent was added to each test tube. The contents were mixed and incubated at 37°C for 10 minutes. Then the absorbance was recorded at a wavelength of 340 nm.

Calculation: The serum CK-MB level was calculated using following formula:-

CK-MB activity (IU/L) = Δ A/min x F

= Δ A/min x 6752

Where F = 1 X T.V X 2 x1000 6.22 S.V

Where Δ A/min = Change in absorbance per minute.

T.V. = Total volume = 1.05 ml.

S.V. = Sample volume = 0.05 ml.

2 = Express CK-MB activity

1000 = Express CK-MB activity per litre.

6.22 = Milimolar absorption coefficient of NADPH at 340 nm.

F = 6752

ix). Estimation of LDH (lactate dehydrogenase) level

Principle: LDH catalyses the oxidation of lactate to pyruvate with the simultaneous reduction of NAD to NADH. LDH activity in serum is proportional to the increase in absorbance due to NADH reduction.

$$\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH}$$

Procedure: The kit contained two reagents: 1 (buffer reagent) and 2 (starter reagent).Working reagent was prepared by mixing 4 parts of reagent 1:1 part of reagent 2. To each test tube was added 20 µl of the sample collected in the micro titer plate and 1 mL of the reconstituted reagents. The contents were mixed and the absorbance at a wavelength of 340 nm was measured.

Calculation: The serum LDH level was calculated as follows:-

LDH activity = ΔA/min X F

Where F = 1 X T.V. X 1000 = 3376 6.22 S.V.

T.V. = Total Volume = 1.05 ml.

S.V. = Sample Volume = 0.05 ml.

6.22 = Milli molar Extinction Coefficient of NADH at 340 nm.

xi). Estimation of serum AST (aspartate aminotransferase) level

Principle: 1. Amino group was sifted from L-aspartate to 2-oxoglutarate and formed oxaloacetate and L-glutamate after catalysis by AST present in the sample. 2. Oxaloacetate is reduced to L-malate in the existence of NADH and malate dehydrogenase (MDH). In this reaction, NADH is oxidized to NAD. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm due to oxidation of NADH to NAD. 3. The addition of lactate dehydrogenase (LDH) to the reagent is required to achieve rapid and complete reduction of endogenous pyruvate so that it does not interfere with the assay.

L-Aspartate + 2-oxoglutarate $\xrightarrow{\text{AST}}$ Oxaloacetate + L-Glutamate

Oxaloacetate + NADH $\xrightarrow{\text{MDH}}$ Malate + NAD+ LDH

Sample pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-lactate + NAD

Procedure: For the two-reagent method, 1.0 ml of substrate reagent 1 (buffer) and 0.1 ml of sample were mixed well and incubated at 37 °C for 5 minutes. Then, 0.25 ml of reagent 2 (substrate) was added and incubated at 37°C for 1 minute. The initial absorbance of the calibrator and sample was recorded versus the reagent blank. Measure the absorbance change exactly after 1, 2 and 3 minutes. Thereafter, 1 minute absorbance change (ΔA/min) was calculated.

Calculation: The serum AST level was calculated as follows:-

$$\text{AST activity (IU/L)} = \Delta\ \text{A/min} \times F$$
$$= \Delta\ \text{A/min} \times 1768$$

Where $F = 1 \times T.V \times 2 \times 1000 \over 6.22 \times S.V$

Where Δ A/min = Change in absorbance per minute.

T.V. = Total volume = 1.05 ml.

S.V. = Sample volume = 0.05 ml; 2 = Express AST activity; 1000 = Express AST activity per litre; 6.22 = Milimolar absorption coefficient of NADPH at 340 nm; F = 1768

x). Estimation of serum ALT (alanine aminotransferase) level

Principle: The enzymatic rate method is used to measure ALT activity in serum or plasma. In the reaction, ALT catalyses the reversible transamination of L-alanine and L-ketoglutarate to pyruvate and L-glutamate. Subsequently, the pyruvate is reduced to lactate in the existence of lactate dehydrogenase (LDH), at the same time NADH is oxidized to NAD. The system

monitors the rate of change of absorbance (340 nm) over a fixed time interval. ALT measurement used in the diagnosis and treatment of liver and heart disease.

L-Alanine + 2-oxoglutarate $\xrightarrow{\text{ALT}}$ Oxaloacetate + L-Glutamate

Sample pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-lactate + NAD

Procedure: For the two-reagent method, 1.0 ml of substrate reagent 1 (buffer) and 0.1 ml of sample are mixed well and incubated at 37 °C for 5 minutes. Then, 0.25 ml of reagent 2 (substrate) was mixed and incubated at 37°C for 1 minute. The initial absorbance of the calibrator and sample was recorded versus the reagent blank. Measure the absorbance change exactly after 1, 2 and 3 minutes. Thereafter, 1 minute absorbance change (ΔA/min) was calculated.

Calculation: The serum ALT level was calculated as follows:-

ALT activity (IU/L) = Δ A/min x F

= Δ A/min x 1768

Where F = 1 X T.V X 2 x1000 6.22 S.V

Where Δ A/min = Change in absorbance per minute.

T.V. = Total volume = 1.05 ml.

S.V. = Sample volume = 0.05 ml; 2 = Express ALT activity; 1000 = Express ALT activity per litre; 6.22 = Milimolar absorption coefficient of NADPH at 340 nm; F = 1768.

D) Cardiac parameters

At the end of the experiment, blood pressure (125) and electrocardiogram (ECG) (126) were measured.

E) Histopathology

All samples (liver, kidney, pancreas and heart) were fixed in 10% phosphate buffered formaldehyde. They were sectioned and stained with haematoxylin and eosin stain. Images of the selection were taken at 10x magnification with a zoom digital camera (MLX, Magnus and New Delhi, India).

Table 3.2: Evaluating parameters.					
Parameters			**Days**	**Method**	**Diagnostic kit**
General	Weight of rats, food intake, and water intake		Weekly assessment	Weighing machine	-
Urine analysis	Urine glucose level		After STZ administration weekly assessment from day 28 to83 days	GOD-POD method End point assay	Autospan
	Urine output			Measuring cylinder	-
Biochemical parameters	Fasting blood glucose level			GOD-POD method End point assay	Autospan
	Lipid parameters	Total cholesterol	83rd day (end of thestudy)	Trinder's method (end pont)	ERBA Diagnostics
		Triglyceride		Trinder's method (end pont)	
		LDL		Direct homogenous	Beacon diagnostic
		HDL		CHOD-POD method	ERBA diagnostics
		VLDL		Calculated by formula	
	Cardiac markers	CK-MB		IFCC method	ERBA diagnostics
		LDH		DGKC, kinetic	ERBA diagnostics
	Liver markers	Alanine aminotransferase (ALT)		IFCC, Kinetic Method	ALT KIT Erba manhiem
		Aspartate aminotransferase (AST)		IFCC, Kinetic Method	AST KIT Erba manhiem
	Kidney markers	Serum Urea		Urease-GLDH	Urea (bun) kit (Erba Manheim)
		Serum creatinine level		Jaffe's method	Creatinine kit (Erba M.)
Cardiac parameters		Blood pressure		Invasive method	BIOPAC MP-150
		ECG			
Histology	Liver, kidney, pancreas and heart		83rd day (end of the study	Hematoxylin and eosin stain	Digital microscope (MLX-B plus,Magnus microscope)

3.5 Isolation of rich fractions from effective extract of *Alstonia scholaris*

Several studies have examined the pharmacological effects of active phytochemical components of herbal products, which mainly include phytochemicals such as alkaloids and flavonoids (120). Therefore, the alkaloid fraction (Et-AF) (127) and the flavonoid fraction (Et-FF) (128) were fractionated from active ethanol extract of *A. scholaris* leaves. Three steps followed to determine the most active dose of the alkaloid and flavonoid fraction of the ethanolextract: 1). Alkaloid fraction and flavonoid fraction were fractionated from ethanol extract of *A. scholaris*, 2). Phytochemical analysis were performed to confirm the chemical components,3). Efficacy study of active fractions of EEAS on glucose homeostasis using.

3.5.1 Preparation of the alkaloid fraction and the flavonoid fraction were fractionated from ethanol extract of *A. scholaris*

A). Preparation of Et-AF:

The ethanol extract (5 g) was mixed in 0.3% HCl and the solid residue was identified as non-alkaloid fraction. Finally, 10% ammonium hydroxide (NH4OH) was used to bring the pH of the acidic aqueous layer to 9 for basification. Then it was extracted with ethyl acetate (EtOAc) to obtain the alkaloid fraction (Et-AF).

B). Preparation of Et-FF preparation (Liquid-liquid partition)

The ethanol extract (10g) was mixed with distilled water (20 ml). It was partitioned with dichloromethane (30ml X 3x). After separation, the dichloromethane layer was evaporated at 50°C. Finally, ethyl acetate was used to create partitions in the aqueous layer (3 times 300 mL). The flavonoid fraction (Et-FF) was obtained by evaporating the ethyl acetate layer at 60°C. To facilitate oral administration to rats, both Et-AF and Et-FF were dissolved in 1% carboxymethyl cellulose (CMC).

3.5.2 Phytochemical analysis were performed to confirm the chemical components

A). Qualitative phytochemicals determination

To approximate the existence of secondary metabolites, standard procedures were used for the phytochemical examination of Et-AF and Et-FF (129).

B). Quantitative phytochemicals determination

i). Et-AF content

An adaptation of the Harborne (1973) methods was used to carry out the study (130). In a 250 mL beaker was placed 5 g powder sample and then 10% acetic acid (200 ml) in ethanol was added. The reaction mixture was left at room temperature for 4 hours. This reaction mixture was filtered and concentrated to a quarter of the original volume using a water bath. Then ammonium hydroxide was added until precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and filtered again. The resulting alkaline residue (alkaloid) was evaporated to dryness and weighed. The formula for calculating percentage of alkaloid content in the ethanol extract of *A. scholaris*:

$$\% \text{ Alkaloid} = \frac{\text{Final weight of sample}}{\text{Initial weight of extract}} \times 100$$

ii). Et-FF content

The Et-FF content was determined by a Harborne (1998) method (131). 5g of sample powder was put in 50 ml of water and 2 ml of HCl solution was placed in an Erlenmeyer flask. After boiling for 30 minutes, the solution was allowed to cool before filtering through Whatman filter paper (#42). The filtrate residue was collected and placed in an oven at 60°C to dry while discarding the aqueous layer. The formula for calculating percentage of flavonoids in the ethanol extract of *A. scholaris*:

$$\% \text{ Flavonoid} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Here, W1= Weight of empty filter paper W2= Weight of paper + Flavonoid extract

3.5.3 Dose optimization study of active fractions of EEAS on glucose homeostasis (132)

i). Experimental design

Male wistar rats were divided into eleven groups with three animals in each group as Group I: Normal control (NC) treated with 0.9% saline p.o.; Group II: Et-AF (15 mg/kg, p.o.); Group III: Et-AF (30 mg/kg, p.o.); Group IV: Et-AF (60 mg/kg, p.o.); Group V: Et-AF (90 mg/kg, p.o.); Group VI: Et-FF (15 mg/kg, p.o.); Group VII: Et-FF (30 mg/kg, p.o.); Group VIII: Et-FF (60 mg/kg, p.o.); Group IX: FF- Et-FF (90 mg/kg, p.o); Group X: EEAS (400 mg/kg, p.o.); Group XI: Standard drug canagliflozin (10 mg/kg, p.o). The Four test doses 15, 30, 60, and 90 mg/kg were selected with reference to acute toxicity studies (133, 134). Here, Et-AF

represents alkaloid fraction of ethanol extract of *A. scholaris* and Et-FF stand for flavonoid fraction of ethanol extract of *A. scholaris.* All of these fractions and extract were dissolved in 1% sodium carboxymethyl cellulose (CMC). Blood glucose levels were measured at 0, 30, 60, 90, 120, and 150 minutes after the glucose loading dose by pricking the tail vein.

ii). Statistical analysis

Results were expressed as the mean± standard error of the mean (SEM). GraphPad Prism 8.01 (GraphPad, San Diego, CA) was used for statistical analysis, specifically an unpaired Student's t-test. Statistical significance was accepted at $p<0.05$.

3.6 Study of active fractions in rats with HFD and STZ-induced type 2 diabetes mellitus

i) Experimental design

Animals were freely fed a high-fat diet for four weeks except for the vehicle control group. A single dose of STZ (45 mg/kg/i.p.) in chilled 0.1 M sodium citrate buffer adjusted to pH 4.3 was administered. Drinking water was replaced with a 5% sucrose solution before and after the administration of STZ to overcome drug-induced hypoglycemia. Moderately diabetic range rats (250–400 mg/dl blood glucose level) were included in the study. Thereafter, theanimals were divided into six groups, each group consisting of six animals: Group I: Normal control (NC) treated with 0.9% saline, Group II: Diabetic control (DC) treated with STZ (45 mg/kg, i.p.), Group III: Et-AF (30 mg/kg, p.o.); Group IV: Et-FF (30 mg/kg, p.o.) Group V: EEAS (400 mg/kg) Group VI: Canagliflogin (10 mg/kg). The dose of both extracts was selected based on the previously performed OGTT analysis. Then, the diabetic rats were kept for four weeks for the induction of complications. The treatment was carried out for four weeks and general parameters (food intake, water intake and weight of the rats), urinalysis and fasting blood glucose (after STZ administration) were measured weekly (Figure 3.4). At the end of the study, biochemical parameters, blood pressure and ECG parameters were measured (Table 3.1). At the end of the experimental period, fasting blood samples were taken for analysis of biochemical parameters. After blood collection, the animals were sacrificed for collecting organs. Histological examinations of the liver, pancreas, kidney and heart were performed. All specimens were fixed in the 10% phosphate-buffered formaldehyde. They were sectioned and stained with hematoxylin and eosin stain. Images of the selections were capturedat 10x magnification with a zoom digital camera (MLX, Magnus, and New Delhi, India).

ii) Statistical analysis

Results were expressed as the mean ± standard error of mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test using Graphpad Prism 8.1 software (Graphpad, San Diego, CA). $P<0.05$ was considered statistically significant.

3.7 *In-vitro* antioxidant study of active fractions

The antioxidants generally scavenge free radicals is important to measure the free radical scavenging activity. Therefore, antioxidant analysis of EEAS, Et-AF (alkaloid fraction of EEAS), and Et-FF (flavonoid fraction of EEAS) was done by using following parameters:-

- Superoxide Dismutase (SOD)
- Catalase (CAT)
- Glutathione (GSH)

3.7.1 Preparation of goat liver slices

The goat was slaughtered immediately, so fresh liver could be obtained from the nearby butcher shop (135) .Goat liver tissue was stored in cold (4°C) phosphate buffered saline (PBS). A sterile scalpel was used to cut the tissue into 1 mm thick pieces yielding 250 mg. Use large flat-bottomed bottles and discard 1.0 mL of sterile PBS. H_2O_2 and samples were added and the mixture was incubated for 1 hour at 37°C with gentle shaking. In addition, it was necessary to form corresponding normal control group. The usual oxidizing agent, H_2O_2, was used at a tissue concentration of 2 ml/kg. After incubation, cell debris was removed by homogenizing the tissue and PBS buffer in the same aliquot of the homogenizer. Antioxidant potential was evaluated by estimating the number of parameters from the supernatant.

3.7.2 Preparation of Plant extracts and fractions

Active plant extract and there fractions were dissolved in distilled water to reach a concentration of 1 mg/ml and diluted to obtain 100, 200, 400, 800 and 1600 µg/ml. The samples were divided into three groups: 1. negative control (untreated tissue homogenate), 2. Vehicle

control (tissue homogenate exposed with H_2O_2) and 3. Treatment group (exposed tissue treated with Et-AF, Et-FF and EEAS). The mixtures were incubated for 1 hour at 37°C with gentle shaking. After the incubation, a homogenizer was used to homogenize liver tissue with PBS buffer and centrifuged to collect the supernatant. The absorbance was measured at 570 nm (SOD), 240 nm (CAT), and 412 nm (GSH) in a UV spectrophotometer.

3.7.3 Procedure for enzymatic antioxidants assay

In this experiment, homogenized tissue (goat liver) was used for enzymatic analysis of antioxidants including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). The antioxidant assay for SOD, CAT and GSH was perform by using following procedure:

1). Superoxide dismutase (SOD)

The SOD was estimated using the method of *Misra and Fridovich (1972)* method (136).

Principle:

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2). In this auto-oxidation method, superoxide ions (O_2^-) generated by the conversion of xanthine to uric acid and hydrogen peroxide by xanthine oxidase (XOD). Convert NBT to NBT-diformazan, which absorbs light at 570 nm. SOD reduces the superoxide ion concentration and thereby decreases the rate of NBT-diformazan formation. The extent of reduction in the appearance of NBT diformazan is a measure of the SOD activity present in a test sample.

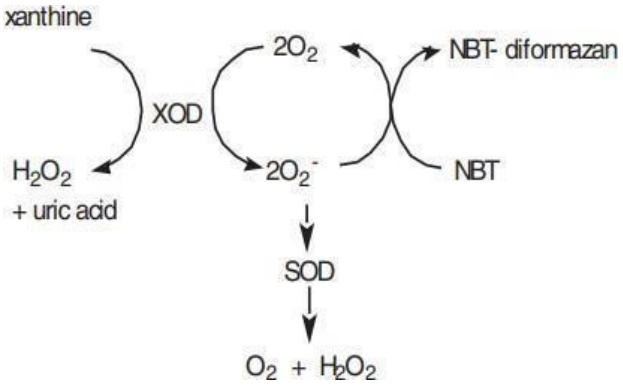


Figure 3.4: Relationship between XOD, SOD, substrates, products, and the superoxide radical.

Procedure:

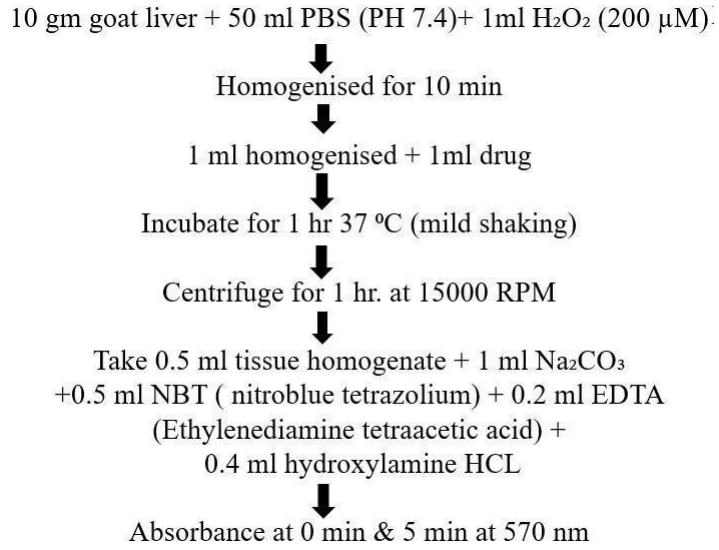


Figure 3.5: Schematic procedure for the SOD antioxidant assay.

Formula:

Free radical scavenging activity was calculated using the following formula:

Percentage (%) SOD scavenging activity = [(Absorbance of control-Absorbance of test Sample) / (Absorbance of control) × 100

2). Catalase (hydrogen peroxidase: hydrogen peroxide oxido-reductase)

The CAT was estimated using the method of *Jhansyrani and Sujata* (2019) and modified by different authors for specific purposes (137).

Principle:

In the UV range, H_2O_2 shows a continuous increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The absorbance difference per unit time as a measure of catalase activity.

$H_2O_2 \longrightarrow 2H_2+O_2$

RDOH $\longrightarrow$ $H_2O+ROH+A$

Procedure:

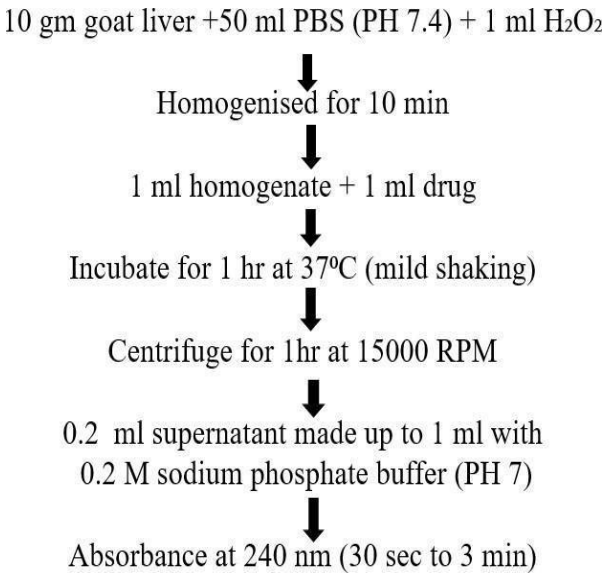


Figure 3.6: Schematic procedure for the CAT antioxidant assay.

Formula:

Free radical scavenging activity was calculated using the following formula:

Percentage (%) CAT scavenging activity = [(Absorbance of control-Absorbance of test Sample) / (Absorbance of control) × 100

3). Reduced glutathione (GSH)

Reduced glutathione was estimated by the method of Moron et al. (138)

Principle:

The entire non-protein sulfhydryl group in the form of GSH-DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid) is a disulfide bond, which is easily reduced by the sulfhydryl compound, forming a bright yellow anion. A compound measured optically forms a bright yellow anion measured optically at 412 nm.

Procedure:

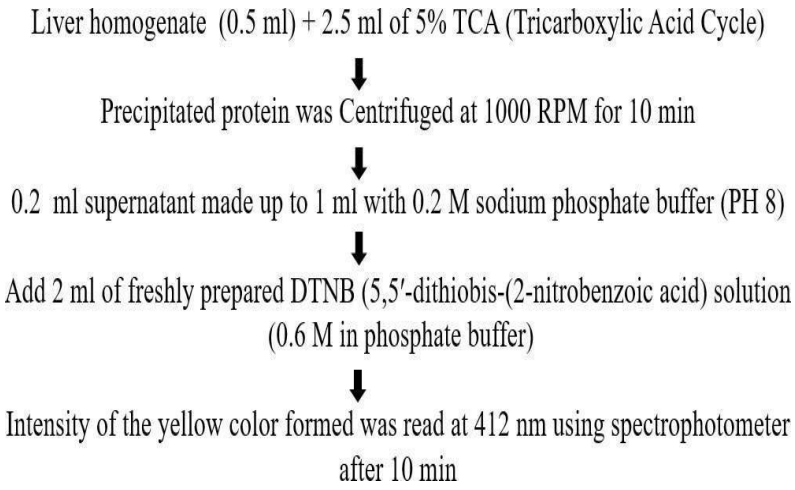


Figure 3.7: Schematic procedure for the GSH antioxidant test.

Formula:

Free radical scavenging activity was calculated using the following formula:

Percentage (%) GSH scavenging activity = [(Absorbance of control-Absorbance of test Sample) / (Absorbance of control) × 100

CHAPTER –4

Results

The present research examines the phytochemical analysis of *Alstonia scholaris* leaves and itseffect on type 2 diabetes associated with its complications. The observations and results of this present study have been divided into seven parts. The first part includes the physicochemical and phytochemical properties of various extracts from *A. scholaris* leaves. In the second part, the effect of different extracts on glucose homeostasis was examined using OGTT. The third part examines the effect of active extracts of *A. scholaris* on type 2 diabetes mellitus and its complications. The fourth part involves the isolation of the alkaloid and flavonoid-rich fractions from the active extract of *A. scholaris* leaves. In the fifth part, the effects of the alkaloid and flavonoid-rich fractions on glucose homeostasis were assessed using the OGTT. In the sixth part, the effects of alkaloid and flavonoid-rich fractions on type 2 diabetes mellitus and its complications were examined. The seventh part evaluates the antioxidant activity of fractions rich in alkaloids and flavonoids.

4.1 Results

4.1.1. Physiochemical and phytochemical physiochemical evaluation of leaves of ethanol extract of *Alstonia scholaris*

A). Physiochemical evaluation

Table 4.1: Physicochemical evaluation of *A. scholaris* leaves.

S.no	Physicochemical parameter	Obtained Value %(w/w)	API limit (139)
1.	Foreign matter	0.62	NMT 2%
2.	Total ash	6.85	NMT 11%
3.	Acid -insoluble ash	2.2	NMT 3%
4.	Water soluble extractive	18.5	NLT 12%
5.	Alcohol soluble extractive	11.25	NLT 4%

(API- Ayurvedic pharmacopeia limits NMT-Not more than, NLT –Not less than)

The physicochemical analysis of medicinal products is an essential parameter for detecting adulteration or improper handling of medicinal products. The physicochemical properties of *A. scholaris* leaves powder, such as foreign matter, total ash, acid-insoluble ash, water-soluble extractive substance, alcohol-soluble extractive substance, are shown in Table 4.1. The testedforeign matter content in the *A. scholaris* leaves powder was not more than 2% (w/w). This suggests that they were not content foreign bodies. The total ash value does not exceed the API limit of 11%, indicating the preferred quality and purity of the raw drug. The acid-insoluble ash, which does not exceed the API limit of 3%, indicates that there is less silica present in the powder. Water soluble and alcohol soluble extract values were recorded (18.5 and 11.25%), which are not less than 12 and 4% of the API limit.

B). Percentage yield

Table 4.2: The percentage yield of different extracts of *Alstonia scholaris* leaves.

S.No	Extracts	Colour	Physical Nature	Yield (%w/w)
1.	Petroleum ether	Dark Green/Sticky mass	Waxy semisolid	4.2%
2.	Chloroform	Dark Green/Solid mass	Solid	8.2%
3.	Ethyl acetate	Green/ Thick solid mass	Solid	13%
4.	Ethanol	Dark green/Thick solid	Solid	9.3%

The yields of all four extracts obtained from crude powder of *A. scholaris* leaves using petroleum ether, chloroform, ethyl acetate and ethanol as solvents are shown in Table 4.2.

Yield value of the extracts is expressed in % yield (w/w) and determined with the Soxhlet extraction method.

C). Phytochemical screening (140)

Table 4.3: Phytochemical screening of the various extracts of *A. scholaris.*

Plant constituents	Petroleum ether Extract	Chloroform Extract	Ethyl acetate Extract	Ethanol Extract
Alkaloid	-	-	+	+
Flavonoid	-	-	+	+
Tannins	-	-	-	+
Phenolic compound	-	+	-	+
Saponins	-	-	-	+
Terpenoids	-	+	-	+
Carbohydrate	-	-	+	+
Steroid	-	+	+	+

+ = Present and - = Absent

The observations and results of the qualitative phytochemical analysis of various extracts from *A. scholaris* leaves are shown in Table 4.3. Chloroform extracts indicate the presence of phenols, terpenoids and steroids. Ethyl acetate extract exhibited alkaloids, flavonoids, carbohydrates and steroids, while ethanol extracts showed the existence of alkaloids, flavonoids, tannins, saponins, phenolic, terpenoids, carbohydrates and steroids. Apart from fatty oils and fats, PEEAS does not shows any other components. Therefore, only three extracts chloroform, ethyl acetate and ethanol extracts were analysed for further investigation.

D). Phytochemical screening pictures

i). Ethanol extract of *A. scholaris* leaves (EEAS)



Froth test



Dragendroff test



Flavonoid test



Molisch test



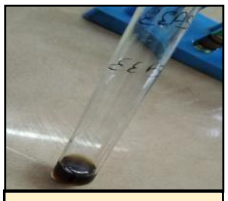
Lead acetate test



Salkowaski test

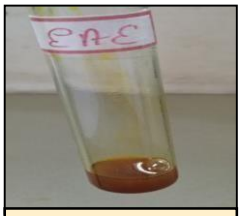


Tannin test

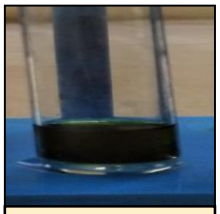


Steroid test

ii). Ethyl acetate extract of *A. scholaris* leaves (EAEAS)



Dragendroff test



Molisch test



Flavovoid test



Steroid test

iii). Chloroform extract of *A. scholaris* leaves (CHEAS)



Lead acetate test



Terpenoid test



Steroid test

The phytochemical study images showed the existence of various phytochemical components such as orange precipitate, indicating alkaloids in EAEAS and EEAS. The ammonium test revealed a yellow coloration of the solution, indicating the presence of flavonoids in EAEAS and EEAS. The observation of the purple ring in the Molisch's test indicated the presence of carbohydrate in both EAEAS and EEAS. In the lead acetate test, voluminous white appears phenols form in CHEAS and EEAS. A dark blue or green-grey colour of the solution indicates the existence of tannins in the ethanol extract. A reddish-brown coloration of the solution indicated the presence of terpenoids in CHEAS and EEAS. Foaming indicates steroids in CHEAS, EAEAS, and EEAS.

E). TLC with dragendroff's reagent (Alkaloid test)

The analysis was performed using the TLC method with emphasis on the detection of the alkaloid content in the extracts visualized by the dragendorff spray reagents (141).

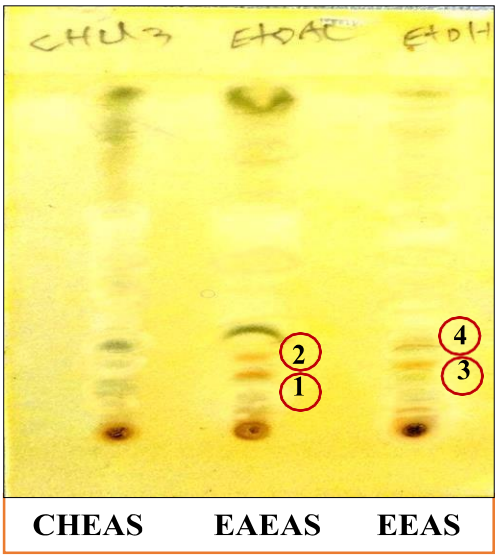


Figure 4.1: TLC profile of CHEAS, EAEAS and EEAS using mobile phase toluene: ethyl acetate: methanol (7:2:1).

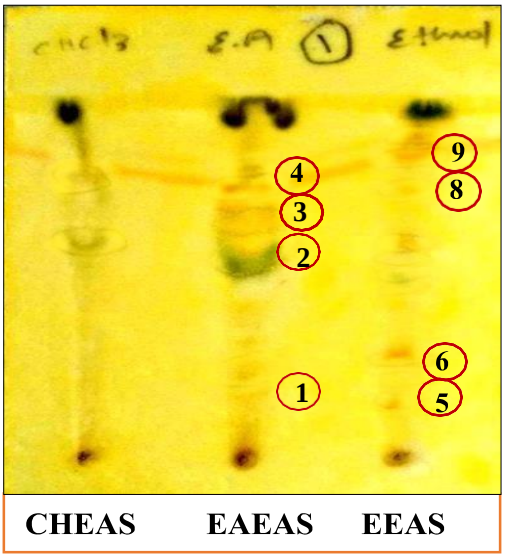


Figure 4.2: TLC profile of CHEAS, EAEAS and EEAS using mobile phase chloroform: ethyl acetate: ammonia (7:3:0.1).

Table 4.4: R_f values of the presence of alkaloids in different extracts of *A. scholaris.*

S.no.	Extracts	Mobile phase	R_f values (Bands no.)
1.	EAEAS	Toluene: ethyl acetate: methanol (7:2:1)	0.17 (1), 0.21(2)
2.	EEAS		0.20 (4), 0.05 (3)
3.	EAEAS	Chloroform: ethyl acetate: ammonia (7:3:0.1)	0.28 (1), 0.59 (2), 0.64 (3), 0.77 (4)
4.	EEAS		0.22 (5), 0.33 (6), 0.51 (7) 0.84 (8), 0.88 (9)

A TLC study using two different mobile phases was performed to confirm the presence of secondary metabolites in CHEAS, EAEAS, and EEAS as shown in Figure 4.1 and 4.2. The corresponding R_f values of secondary metabolites in the presence of dragendroff's reagents is given in Table 4.4. Two mobile phases suitable for detecting the presence of alkaloids (orange bands) have been identified, for example the combination toluene: ethyl acetate: methanol in the ratio 7:2:1 and chloroform: ethyl acetate: ammonia in the ratio 7:3:0.1. Ethyl acetate and ethanol extract from *A. scholaris* leaves confirm the presence of alkaloid components.

4.1.2 Effect of different extracts on glucose homeostasis using OGTT

A). Effect of CHEAS on glucose homeostasis

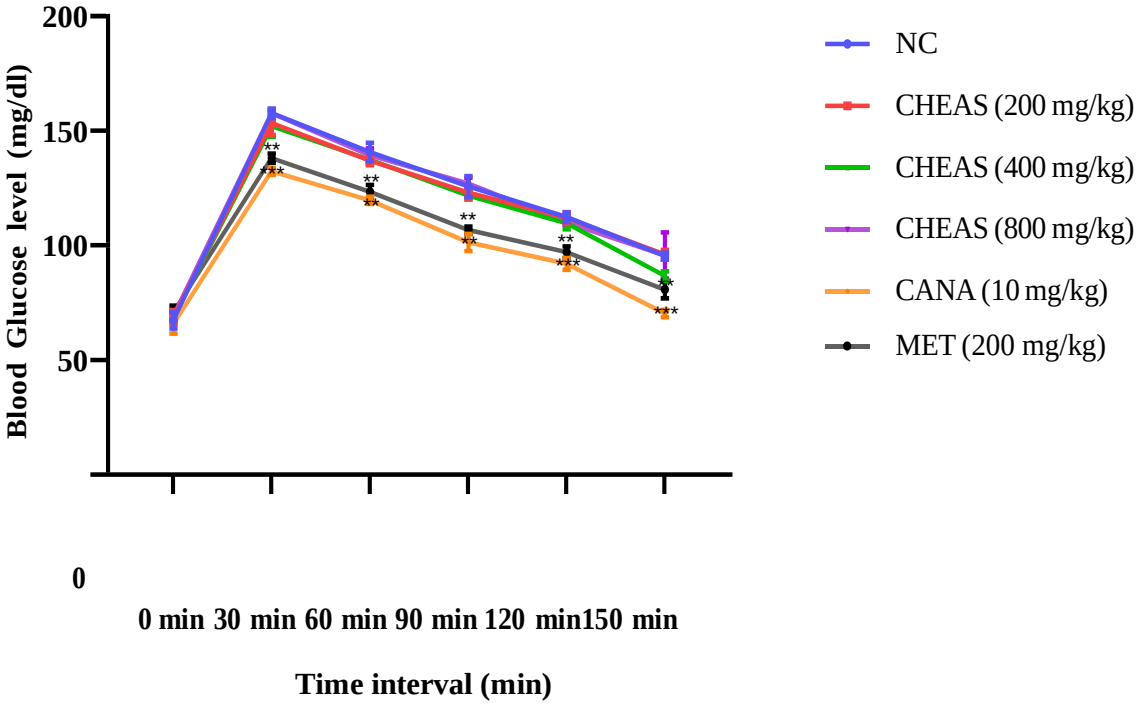


Figure 4.3: Effect of CHEAS on glucose homeostasis.
The results are expressed in Mean ± SEM. Each group comprised of three animals. Normal control group, standard group received 10 mg/kg canagliflozin and 200 mg/kg metformin. Treatment group is Chloroform extract of *A. scholaris* (CHEAS). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to NC. Data were analyzed by unpaired t-test for comparison.

The results of OGTT showed a significant increase in glucose levels after administration of 2 g/kg glucose (Figure 4.3). The effect of chloroform extracts on glucose homeostasis was studied at a dose of 200, 400, 800 mg/kg. CHEAS shows no significant results compared to the normal control. Treatment with canagliflozin (10 mg/kg) and metformin (200 mg/kg) shows a significant reduction in blood glucose levels at 30, 60, 90, 120 and 150 minutes.

B). Effect of EAEAS on glucose homeostasis

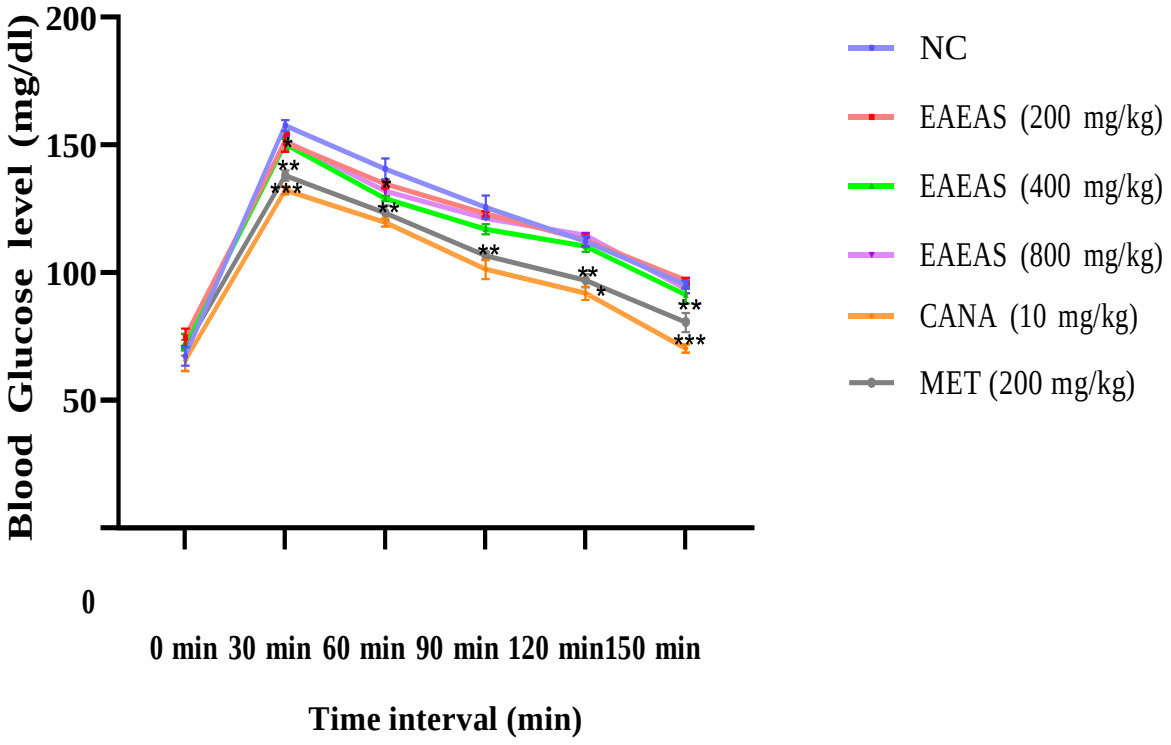


Figure 4.4: Effect of EAEAS on glucose homeostasis.
The results are expressed in Mean ± SEM. Each group comprised of three animals. Normal control group, standard group received 10 mg/kg canagliflozin and 200 mg/kg metformin. Treatment group is ethyl acetate extract of *Alstonia scholaris* (EAEAS). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to NC. Data were analyzed by unpaired t-test for comparison.

In the EAEAS treatment groups, blood glucose levels decreased significantly ($p<0.05$) at a dose of 200 mg/kg after 60 minutes. The group treated with EAEAS (400 mg/kg) showed that the glucose level was significantly reduced after 30 ($p<0.05$) and 60 minutes ($p<0.01$). Canagliflozin (10 mg/kg) and metformin (200 mg/kg) showed a significant reduction in blood glucose levels at 30, 60, 90, 120 and 150 minutes.

C). Effect of EEAS on glucose homeostasis

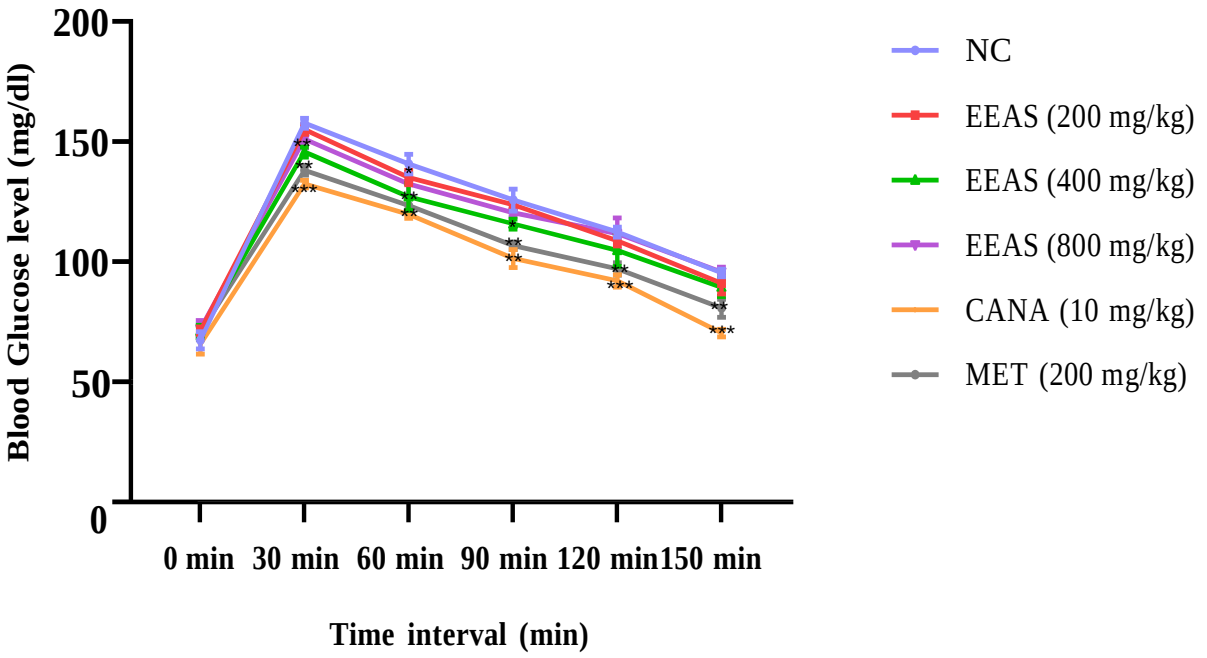


Figure 4.5 Effect of EEAS on glucose homeostasis.
The results are expressed in Mean ± SEM. Each group comprised of three animals. Normal control group, standard group received 10 mg/kg Canagliflozin and 200 mg/kg Metformin. Treatment group is ethanol extract of *Alstonia scholaris* (EEAS). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to NC. Data were analyzed by unpaired t-test for comparison.

In the EEAS treatment groups, glucose levels were significantly reduced ($p<0.05$) at a dose of 200 mg/kg at 60 minutes. The group treated with EEAS (400 mg/kg) shows that the blood glucose level drops significantly by 30% ($p<0.01$).), 60 minutes ($p<0.01$) and 90 minutes ($p<0.05$). Canagliflozin (10 mg/kg) and metformin (200 mg/kg) showed a significant reduction in blood glucose levels at 30, 60, 90, 120 and 150 minutes.

D). Effect of different extracts on glucose homeostasis

Table 4.5: Effect of different extracts on glucose homeostasis.

Experimental Groups	Blood glucose level (mg/dl)					
	0 min	30 min	60 min	90 min	120 min	150 min
Normal control	67.3±2.0	157.6±1.2	140.6±2.4	125.66±2.6	112.3±1.2	95.0±0.8
CHEAS (200 mg/kg)	68.6±1.7	153.3±2.9	137±1.1	123± 1.5	112.0±1.5	96.0±1.1
CHEAS (400 mg/kg)	68.3±1.2	152.0±2.6	137.33±1.20	121.66±0.8	109.7±1.4	86.6±1.2
CHEAS (800 mg/kg)	69.3±1.4	157.6±0.8	139.3±1.76	127.0±1.5	110±0.57	95.67±5.7
EAEAS (200 mg/kg)	74.6±2.0	151±2.08	134.6±0.88	123.0±0.5	112.6±1.2	97.0±0.5
EAEAS (400 mg/kg)	73.1±1.7	150.3±1.2*	129.0±0.5**	117.0±1.1	110.3±1.2	91.3±1.8
EAEAS (800 mg/kg)	73±1.7	151.6±1.5	132.0±2.0*	121.3±0.5	114.7±1.5	93.6±1.5
EEAS (200 mg/kg)	71.3±0.8	155.0±1.1	135.0±1.73*	123.7±0.8	108.7±1.2	91±2.5
EEAS (400 mg/kg)	71.0±1.5	145.6±1.2**	127.0±3.2**	115.7±1.2*	104.7±3.7	89.3±2.1
EEAS (800mg/kg)	72.0±2.1	151.0±2.0	132.3±2.8*	120.3±0.8	111.6±3.8	95.6±1.2
CANA (10 mg/kg)	65.6±2.3	132.3±0.8***	119.6±0.8**	101.3±2.2**	92.0±1.5**	70.3±.1***
MET (200 mg/kg)	70.6±1.7	138.0±1.1**	123.31.76**	106.7±0.8**	97.0±1.5**	80.7±2.2**

The results are expressed in Mean ± SEM. Each group comprised of three animals. Chloroform extract of *A. scholaris* (CHEAS), Ethyl acetate extract of *A. scholaris* (EAEAS), and Ethanol extract of *A. scholaris* (EEAS).

In the normal control group, an increase in glucose levels was observed after 30, 60 and 90 minutes (Table 4.5). In the treatment groups, there was a significant decrease in blood glucose levels in EAEAS (200 mg/kg), EAEAS (400 mg/kg), EEAS (200 mg/kg) and EEAS (400 mg) at 30, 60, 90 minutes. In addition, the canagliflozin and metformin treated groups significantly reduced blood glucose levels at 30, 60, 90, 120 and 150 minutes compared to the normal control group.

Based on the results of the glucose tolerance test, two active extracts were selected for further studies in streptozotocin (STZ) and high-fat diet-induced type 2 diabetes mellitus, namely ethyl acetate extract of *A. scholaris* leaves (EAEAS) and ethanol extract of *A. scholaris* leaves (EEAS).

4.1.3 The effects of two active EAEAS and EEAS in type 2 diabetes mellitus

A). Effect of EAEAS and EEAS on body weight

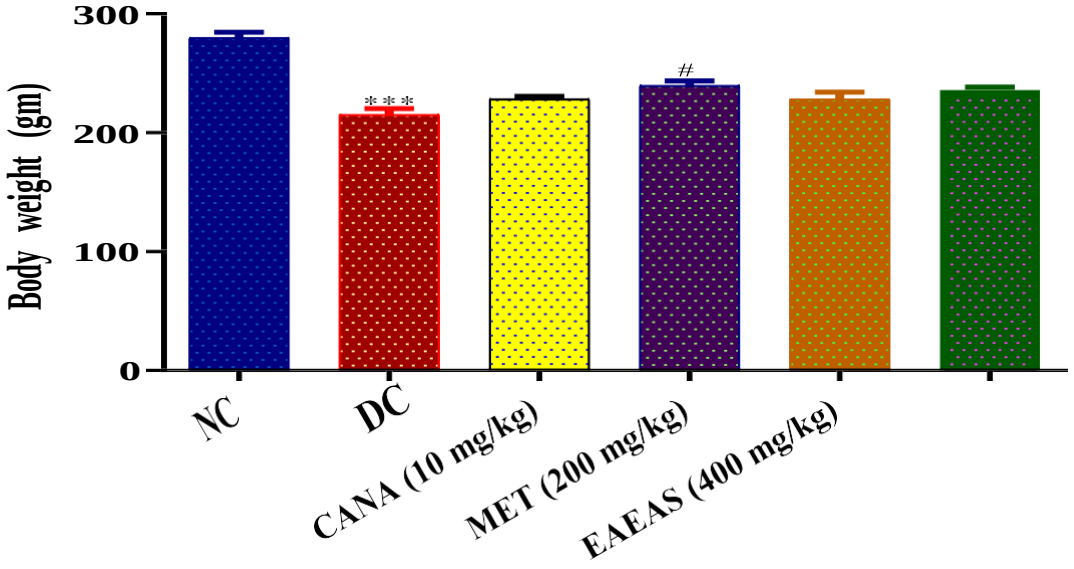


Figure 4.6: Effect of EAEAS and EEAS on body weight.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The body weight of male wistar rats on the last day of the experiment is shown in Figure 4.6. A significant increase in body weight was observed in the HFD group compared to the normal control (NC) group. Diabetic control (DC) Significant decrease in body weight compared to the NC group (Figure 4.6). Groups treated with EAEAS and EEAS improved body weight compared to DC after 4 weeks (83rd day) of treatment. Metformin shows a significant increase in body weight compared to DC. Treatment with canagliflozin also improves the weight of rats compared to diabetic rats.

B). Effect of EAEAS and EEAS on food intake

Table 4.6: Food intake of rats.

Week	NC	DC	CANA (10 mg/kg)	MET (200 mg/kg)	EAEAS (400 mg/kg)	EEAS (400 mg/kg)
0	25.5±7.5	29.8±8.2	32.0±3.2	27.7±7.0	29.2±3.0	29.6±6.5
1	25.1±4.5	33.9±10.8*	28.83±5.6	29.5±6.5	28.66±4.5	30.2±4.0
2	21.6±4.0	36.2±11.6**	28.6±7.8	28.6±2.0	26±1.0	28.0±2.0
3	22.2±1.5	32.4±12.3**	29.6±8.9	30.2±2.5	29.6±1.5	28.8±9.0
4	22.6±6.0	34.4±14.1*	28.8±5.8	30.3±5.0	28.8±2.5	30.8±8.5
5	21.2±6.5	31.4±16.1*	25.5±6.8	24.8±3.5	26.8±8.5	25.8±2.5
6	25.0±7.0	32.8±11.0*	27.2±3.5	30.2±5.5	29.3±1.0	32±2.6.0
7	25.6±5.0	31.5±7.0	31.0±.2.0	28.6±5.0	33.3±4.0	33.3±3.0
8	22.0±4.0	27.0±9.3	31.8±5.6	26.2±1.5	30.3±0.5	27.5±5.0
9	22.8±32.5	25.7±11.2	29.6±7.0	26.0±1.0	30.8±2.0	27.3±2.5
10	18.5±0.5	27.7±8.2*	31.3±4.0	23.2±0.5	32.3±6.0	25.3±3.6
11	20.7±9.0	22.2±5.0	32.6±5.5$^{\#}$	28.83±9.5	28.8±2.5	24.8±1.5
12	22.1±3.5	21.8±11.5	32.3±3$^{\#\#}$	26.67±7.8	28.2±2.6	29.5±5.5$^{\#}$

NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The food intake results of NC, DC and all treatment groups are summarized in Table 4.6. Compared to the normal control group, there is a significant increase in food intake in the HFD group up to the 4th week. Significant increases in food intake were observed with canagliflozin and metformin. EAEAS and EEAS groups also increased food intake compared to standards.

C). Effect of EAEAS and EEAS on water intake

Table 4.7: Water intake of rats.

Weeks	NC	DC	CANA (10 mg/kg)	MET (200 mg/kg)	EAEAS (400 mg/kg)	EEAS (400 mg/kg)
0	28.33±8.8	32.0±9.3	30.0±2.9	28.3±2.1	32.5±9.8	33.3±5.6
1	30.0±6.2	35.0±5.8	38.3±4.6	36.7±5.6	38.0±5.7	40.0±7.8
2	36. 8±3.1	34.0±3.6	40.0±4.8	40.0±9.2	42. 5±6.1	45.0±4.2
3	38.3±6.5	45.0±5.3	40.0±9.8	45.0±8.6	47.5±11.1	43.3±5.4
4	38.3±7.2	49.0±6.6	43.3±6.4	46.7±5.4	52.4±7.4	40.0±6.1
5	35.0±5.8	52.0±7.2***	52.0±7.8	50.0±11.4	45.6±8.2	48.3±5.8
6	35.0±3.2	53.0±7.8***	53.0±4.9	53.0±6.4	51.2±5.6	46.7±9.6
7	38.0±9.1	57.0±6.5***	56.4±5.5	48.0±7.4	43.3±4.6	48.3±6.0
8	40.0±6.0	57.0±10***	54.0±7.5	45.0±8.3$^{\#\#}$	48.3±7.2$^{\#\#}$	48.3±9.8$^{\#\#}$
9	36.6±7.4	60.0±9.8***	59.0±6.3	48.0±11.1$^{\#\#}$	54.0±8.4$^{\#}$	51.7±5.2$^{\#}$
10	40.0±2.4	61.0±5.6***	65.0±5.2$^{\#}$	47.0±5.7$^{\#\#}$	58.0±6.3	55.0±7.4$^{\#}$
11	40.0±5.1	59.0±7.2***	66.0±7.4$^{\#\#}$	43.3±4.4$^{\#\#\#}$	57.0±5.6	58.0±9.2
12	41.7±8.2	61.0±8.4***	68.0±6.8$^{\#\#}$	45.0±8.6$^{\#\#\#}$	54.0±12.4$^{\#}$	63.0±3.7

NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The water intake outcome data for all treatment groups are summarized in Table 4.7. Compared to the normal control group, there was a significant ($p<0.001$) increase in water intake in the DC group (after 4th week). A significant increase in water intake was observed with canagliflozin ($p<0.01$), while EEAS treatment had no significant effect on water intake compared to DC (12th week). Treatment with metformin ($p<0.001$) and EAEAS ($p<0.05$) showed a significant declination in water intake compared to DC.

D). Effect of EAEAS and EEAS on urine output

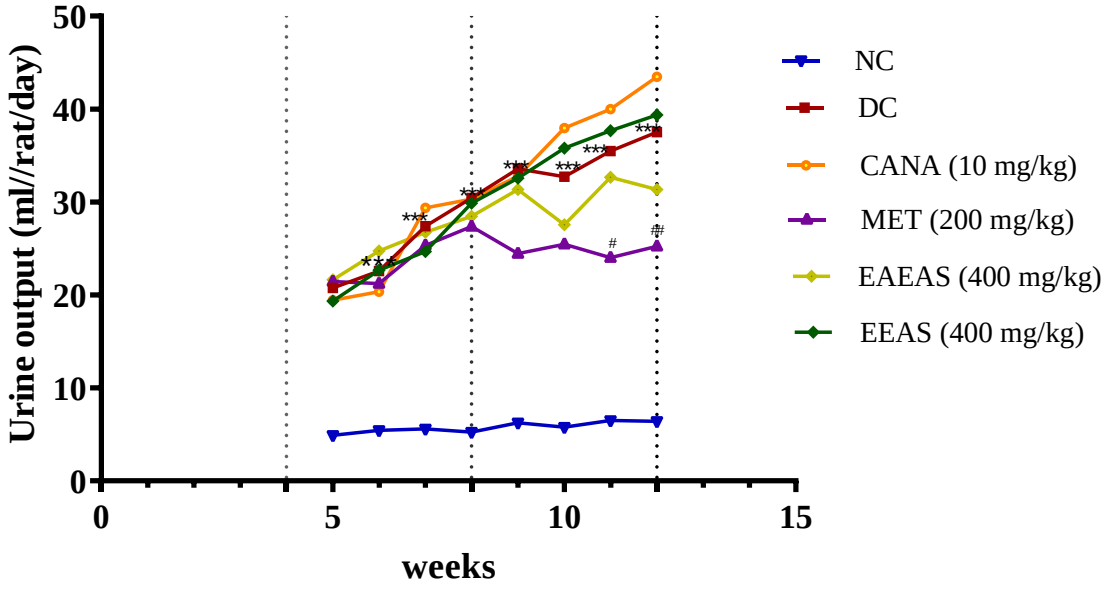


Figure 4.7: Effect of EAEAS and EEAS on urine output.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Urine output data for all DC and treatment groups from week 5 (administered with STZ) to week 12 are shown in Figure 4.7. In the urinalysis, there was an increase in urine output after STZ induction in the DC group of the type 2 diabetic model (after 4th week). EAEAS decreases urine output compared to DC. The EEAS and canagliflozin treatment group showed increase in urinary output, while metformin significantly ($p<0.01$) decreased urinary output compared to diabetic rats (at week 12).

E). Effect of EAEAS and EEAS on urine glucose level

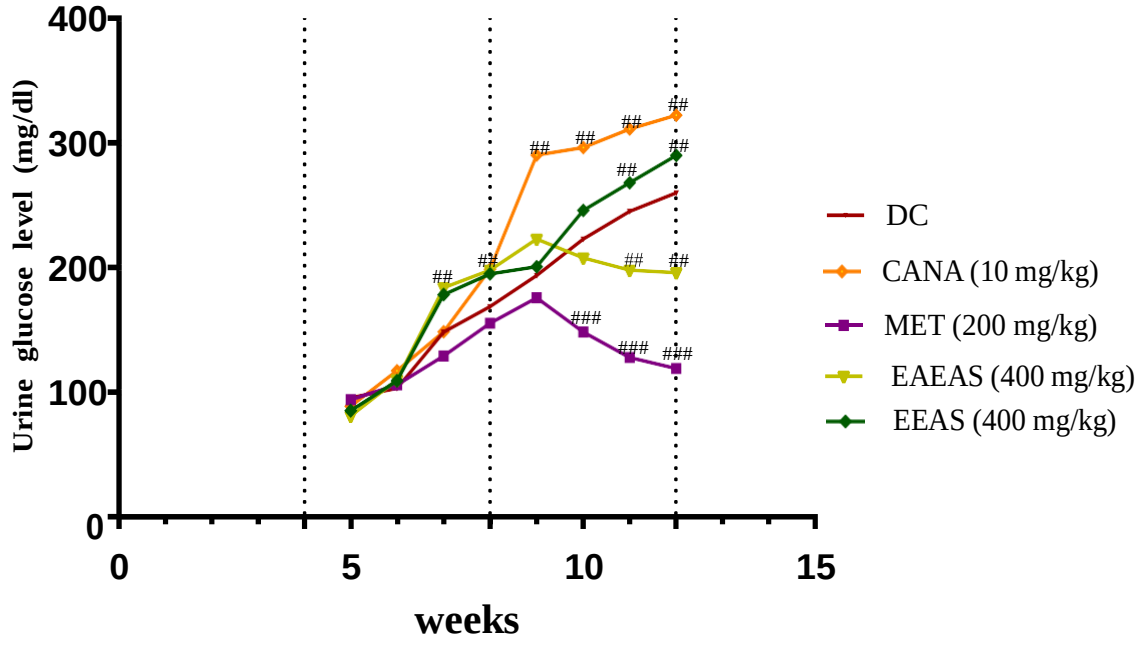


Figure 4.8: Effect of EAEAS and EEAS on urine glucose level.
DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n =6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The data of the urine glucose levels of all DC and treatment groups from week 5 (administered with STZ) to week 12 are shown in Figure 4.8. In the DC group, the urine glucose level increased (after 4th week). Significant decreases in urine glucose levels were observed in the EAEAS ($p<0.01$) and metformin ($p<0.001$) treatment groups compared to the DC group. On the other hand, EEAS ($p<0.01$) and canagliflozin ($p<0.01$) significantly increased urine glucose levels compared to the DC group (at week 12).

F). Effect of EAEAS and EEAS on fasting blood glucose level

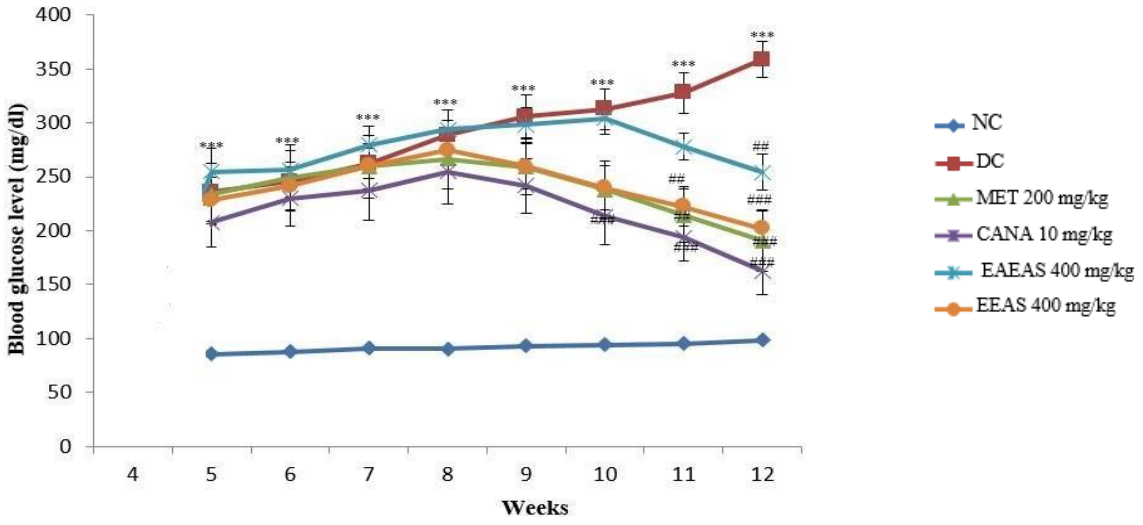


Figure 4.9: Effect of EAEAS and EEAS on fasting blood glucose level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC. Data were analyzed by one-way Analysis of Variance (Tukey's multiple tests for comparison).

The result of the fasting blood glucose (FBG) level of all NC, DC and treatment groups from week 5 (administered with STZ) to week 12 is shown in Figure 4.9. In the DC group, the blood sugar level increased (after 4th week). A significant decrease in FBG values was observed in the EAEAS ($p<0.01$), EEAS ($p<0.001$), metformin ($p<0.001$) and canagliflozin ($p<0.001$), group compared to the DC group (at week 12).

G). Effect of EAEAS and EEAS on serum total cholesterol level

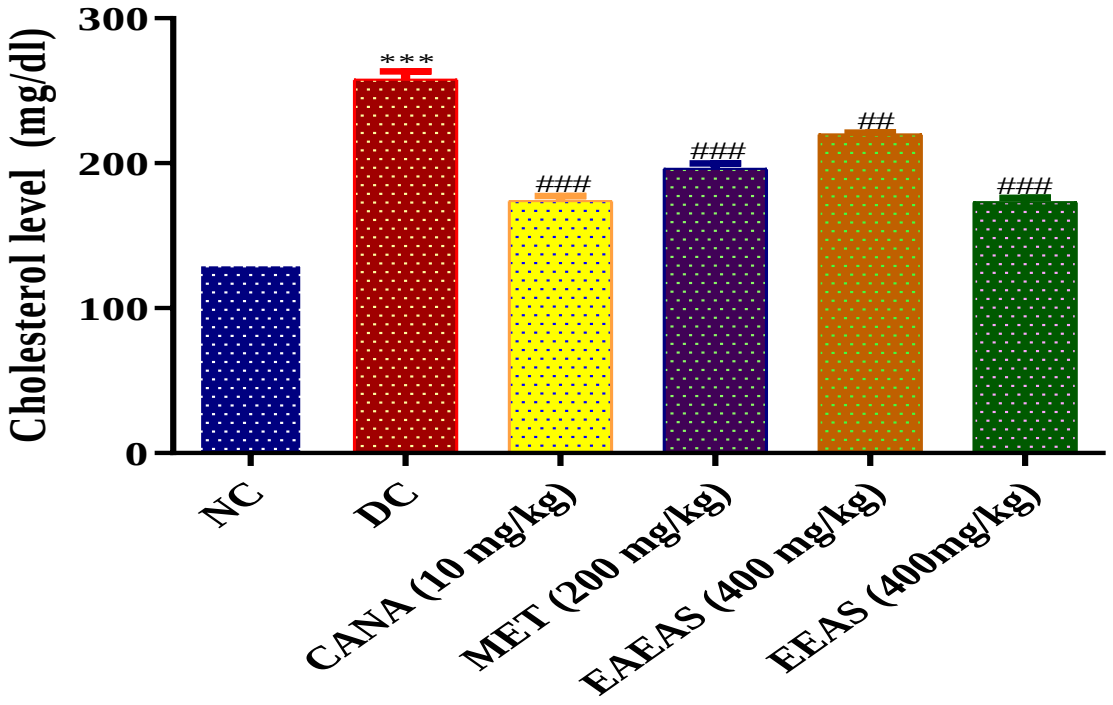


Figure 4.10: Effect of EAEAS and EEAS on serum total cholesterol level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Total cholesterol (T-CH) results from normal control rats, diabetic rats and all treatment groups are summarized in Figure 4.10 (at week 12). In diabetic rats, T-CH was significantly increased ($p<0.001$) compared to normal controls. EAEAS ($p<0.01$) and EEAS ($p<0.001$) showed a significant reduction in total cholesterol compared to the DC group. The standard group (canagliflozin and metformin) reduced T-CH significantly ($p<0.001$) compared to the diabetic group.

H). Effect of EAEAS and EEAS on serum triglyceride level

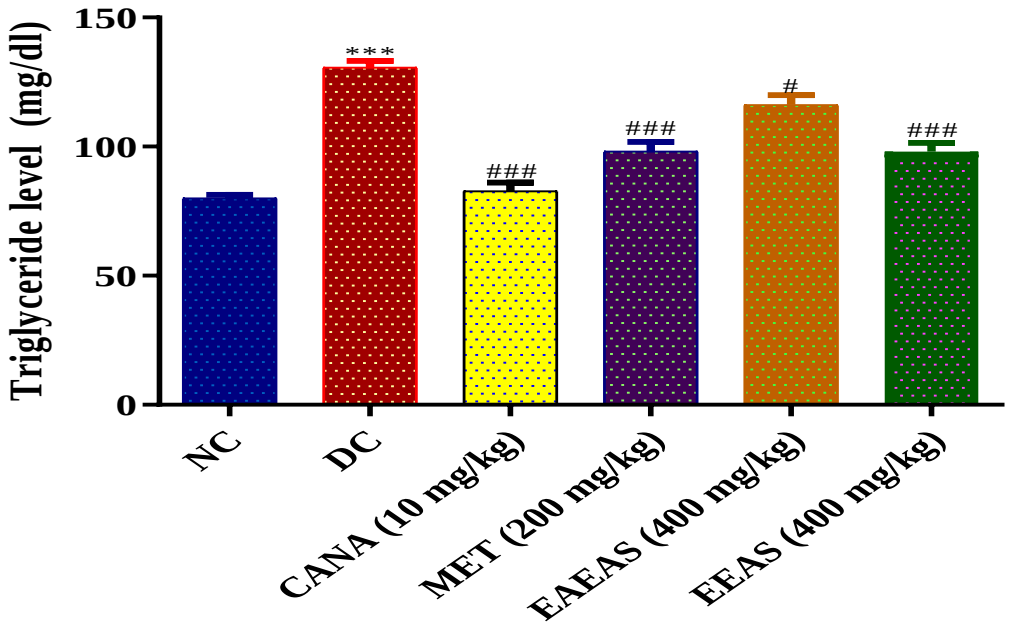


Figure 4.11: Effect of EAEAS and EEAS on serum triglyceride level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Data on serum triglyceride (TG) levels from normal control rats, diabetic rats and all treatment groups are summarized in Figure 4.11 (at week 12). Triglycerides were significantly increased in diabetic rats compared to normal controls ($p<0.001$). EAEAS ($p<0.05$) and EEAS ($p<0.001$) treatment group reflects a significant reduction in triglyceride levels compared to the DC group. The standard group (canagliflozin and metformin) reduced TG significantly ($p<0.001$) compared to the diabetic rat group.

I). Effect of EAEAS and EEAS on serum LDL level

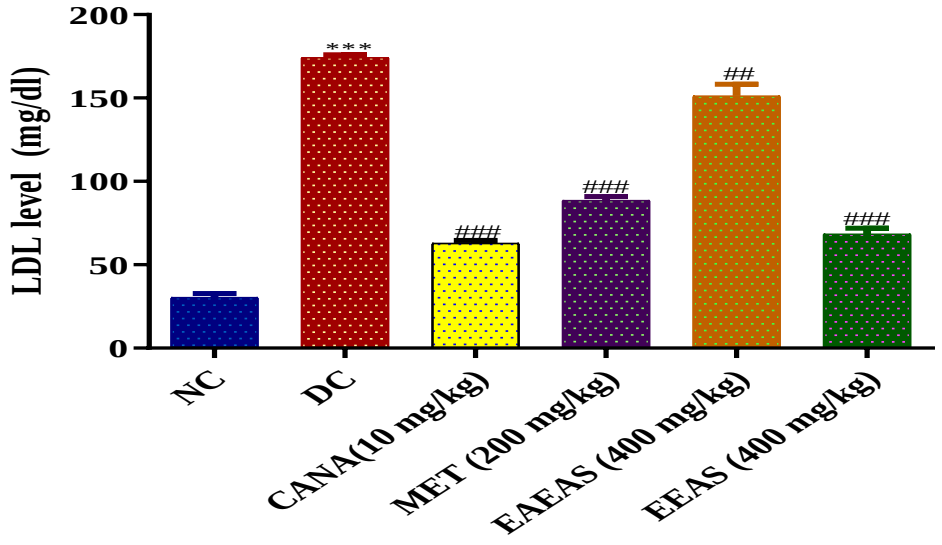


Figure 4.12: Effect of EAEAS and EEAS on serum LDL level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

At the end of the study, Figure 4.12 shows data on serum LDL (low-density lipoprotein) levels for all treatment and non-treatment groups. Diabetic rats significantly increase serum LDL levels compared to normal controls ($p<0.001$). EAEAS ($p<0.01$) and EEAS ($p<0.001$) treatment group significantly reduced LDL levels compared to the DC group. The standard group (canagliflozin and metformin) reduced LDL levels significantly ($p<0.001$) compared to the diabetic rat group.

J). Effect of EAEAS and EEAS on serum VLDL level

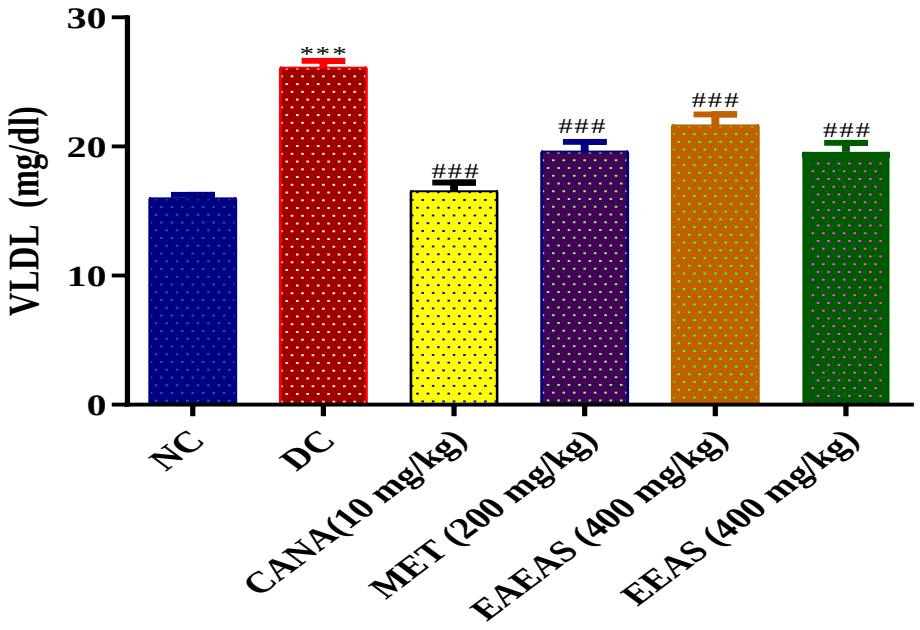


Figure 4.13: Effect of EAEAS and EEAS on serum VLDL level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Data on serum very low lipid lipoproteins (VLDL) in all treatment groups and non-treatment groups are presented in Figure 4.13 (at week 12). Serum VLDL levels were significantly ($p<0.001$) rise in diabetic rats compared to normal controls. Both the EAEAS and EEAS treatment groups significantly reduced VLDL values compared to the DC group ($p<0.001$). The standard group (canagliflozin and metformin) reduced VLDL significantly ($p<0.001$) compared to the diabetic rat group.

K). Effect of EAEAS and EEAS on serum HDL level

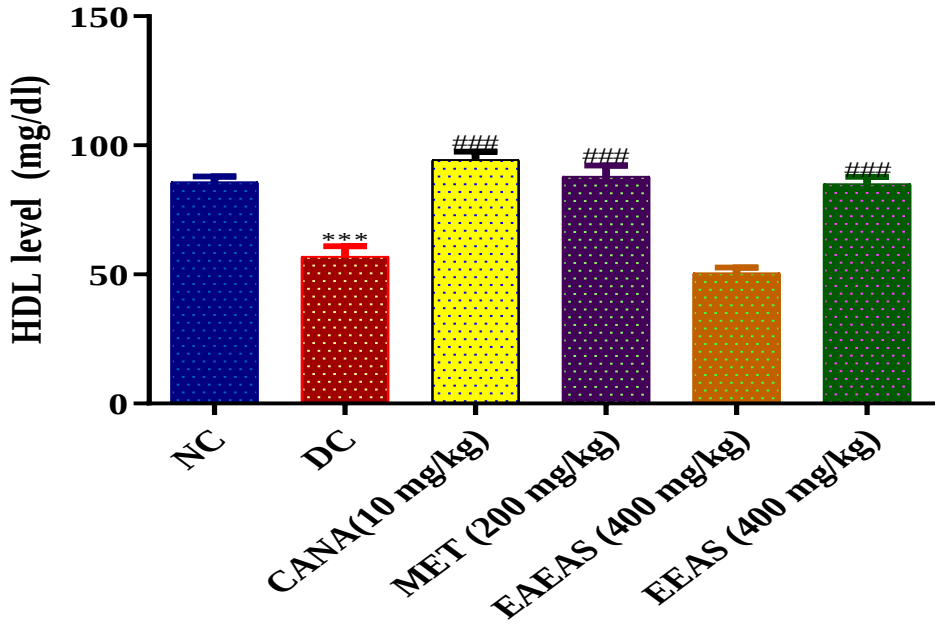


Figure 4.14: Effect of EAEAS and EEAS on serum HDL level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

In the lipid profile, the serum high density lipoprotein (HDL) level of all groups were summarized in Figure 4.14 (at 12 weeks). Serum HDL levels were significantly reduced ($p<0.001$) in diabetic rats compared to the NC group. EEAS treatment group significantly improve HDL levels compared to diabetic rats ($p<0.001$). While the EAEAS treatment group shows a non-significant reduction in HDL levels compared to the DC groups. On the other hand, HDL was significantly increased in both canagliflozin and metformin ($p<0.001$).

L). Effect of EAEAS and EEAS on serum urea level

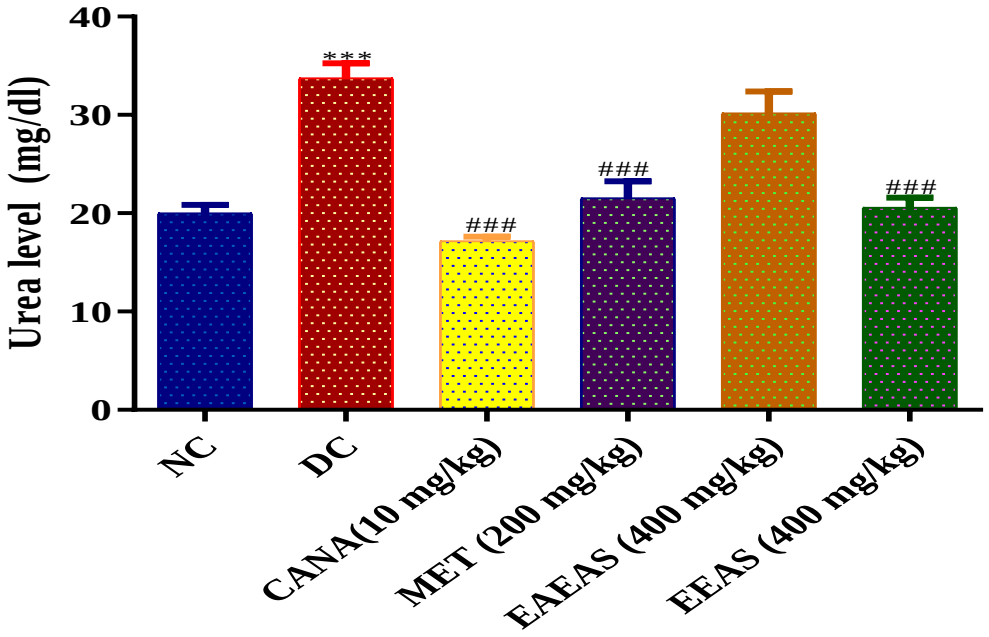


Figure 4.15: Effect of EAEAS and EEAS on serum urea level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The results of renal damage markers such as serum urea levels of all groups are shown in Figure 4.15 (at 12 week). Serum urea levels were significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, urea levels decreased significantly ($p<0.001$) compared to diabetic rats. While the EAEAS treatment group shows a non- significant reduction in urea levels compared to the DC groups. Furthermore, a significant reduction ($p<0.001$) in urea levels is reflected in the canagliflozin and metformin treated groups compared to the diabetic rats.

M). Effect of EAEAS and EEAS on serum creatinine level

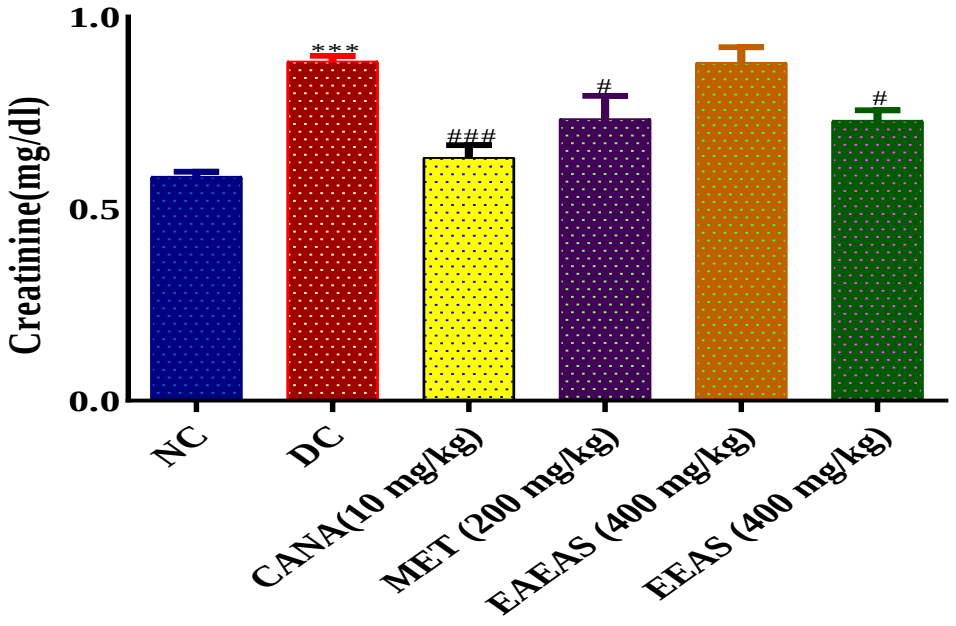


Figure 4.16: Effect of EAEAS and EEAS on serum creatinine level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$and $^{\#\#\#}p < 0.001$ as compared to DC.

The data on renal damage markers such as serum creatinine levels of all groups are shown in Figure 4.16 (at 12 weeks). The serum creatinine level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, creatinine levels decreased significantly ($p<0.05$) compared to diabetic rats. While the EAEAS treatment group shows no change in creatinine levels compared to the DC groups. In addition, there was a significant reduction in serum creatinine in the canagliflozin ($p<0.001$) and metformin ($p<0.05$) treated groups compared to the diabetic rats.

N). Effect of EAEAS and EEAS on serum AST level

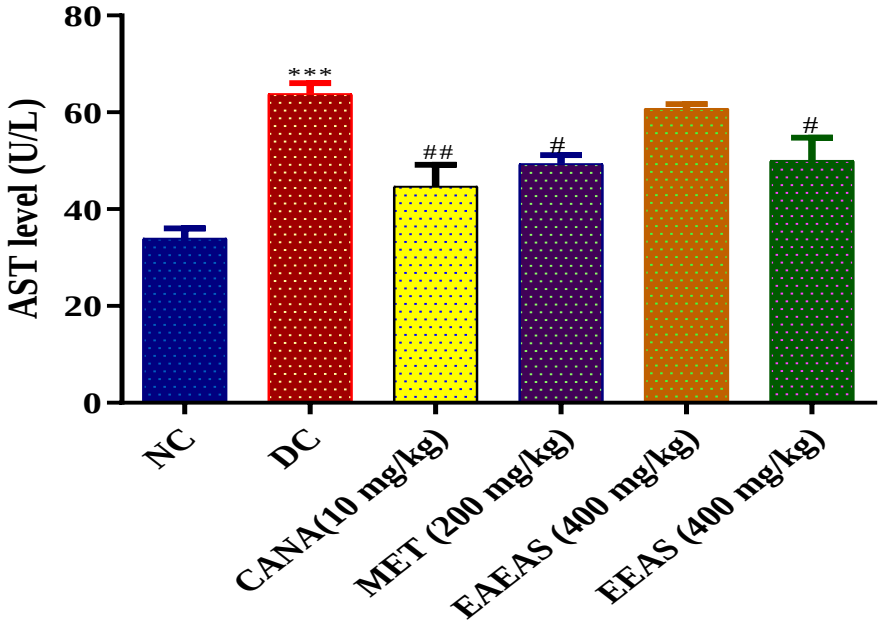


Figure 4.17: Effect of EAEAS and EEAS on serum AST level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The data on markers of liver damage such as serum AST levels of all groups are shown in Figure 4.17 (at week 12). The serum creatinine level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, AST levels decreased significantly ($p<0.05$) compared to diabetic rats. While EAEAS treatment group shows no significant change in AST levels compared to the DC groups. There was a significant reduction in serum AST levels in the canagliflozin ($p<0.01$) and metformin ($p<0.05$) treated groups compared to the diabetic rats.

O). Effect of EAEAS and EEAS on ALT level

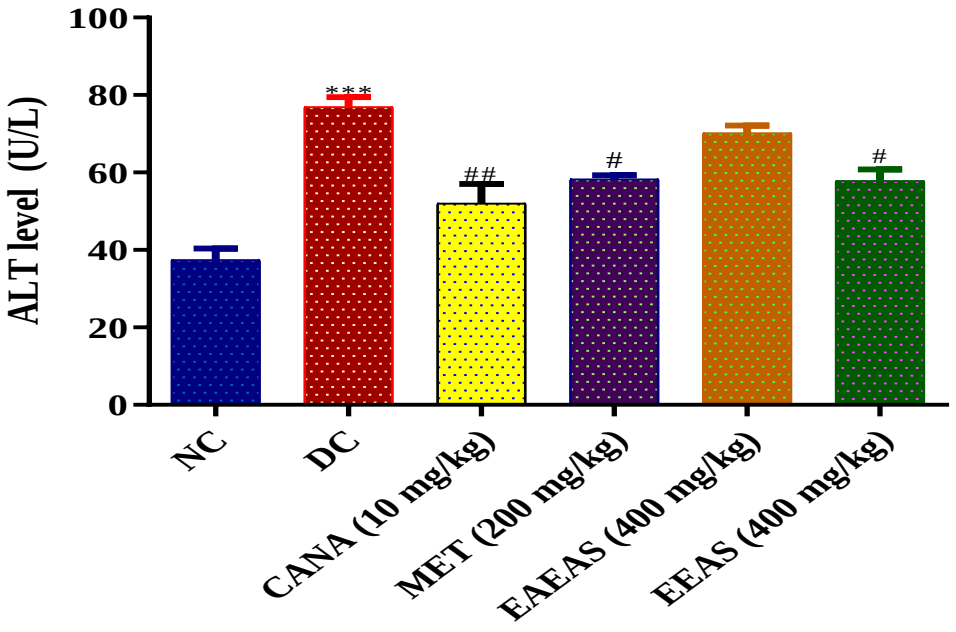


Figure 4.18: Effect of EAEAS and EEAS on ALT level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The effect of serum ALT levels of all groups are shown in Figure 4.18 (at week 12). The serum ALT level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, ALT levels decreased significantly ($p<0.05$) compared to diabeticrats. On the other hand, the EAEAS treatment group shows non-significant decrease in ALT levels compared to the DC groups. Significant reductions in serum ALT levels in thecanagliflozin ($p<0.01$) and metformin ($p<0.05$) treated groups compared to the diabetic rats.

P). Effect of EAEAS and EEAS on serum CK-MB level

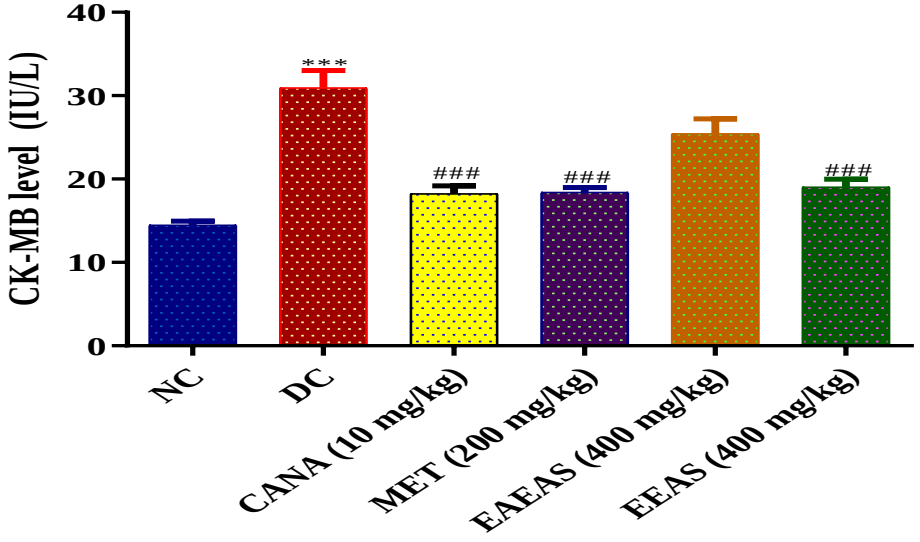


Figure 4.19: Effect of EAEAS and EEAS on serum CK-MB level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The effect of cardiac damage marker such as serum CK-MB levels of the treated and non-treated groups is shown in Figure 4.19 (at week 12). The data show a significantly increased level of CK-MB in diabetic rats compared to normal rats. The CK-MB level was significantly decreased in the EEAS treatment group compared to the DC group ($p<0.001$). The EAEAS treatment group showed a non-significant reduction in cardiac damage compared to the DC groups. Both standard treatment groups showed a significant ($p<0.001$) reduction in CK-MB compared to the diabetic rats.

Q). Effect of EAEAS and EEAS on serum LDH level

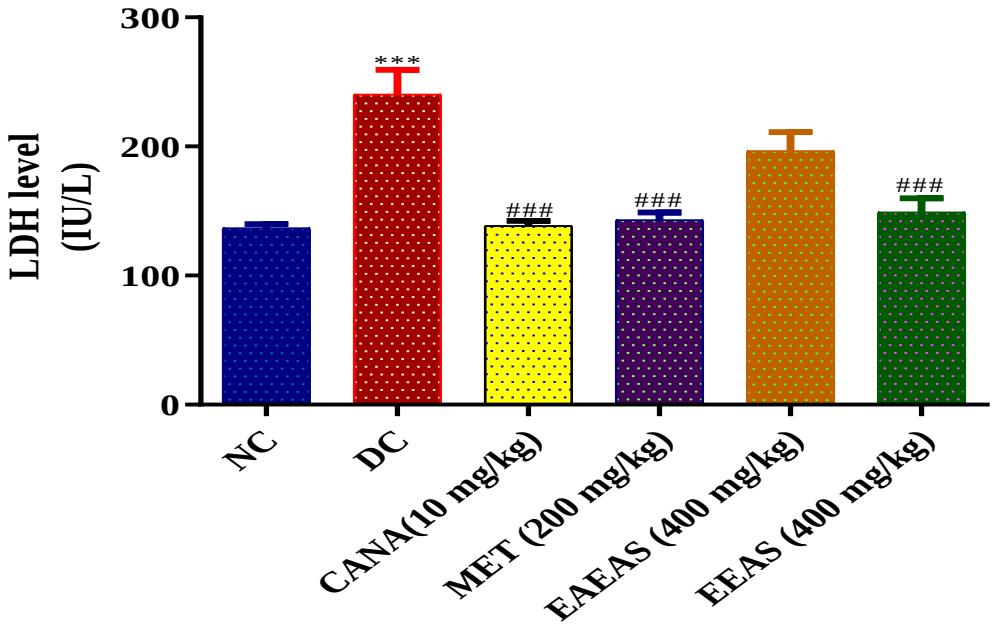


Figure 4.20: Effect of EAEAS and EEAS on serum LDH level.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The data on the serum LDH levels of all groups are presented in Figure 4.20 (at week 12). The serum LDH level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, LDH levels decreased significantly ($p<0.001$) compared to diabetic rats. While the EAEAS treatment group shows no change in LDH levels compared to the DC groups. There was a significant reduction ($p<0.001$) in serum LDH levels in the canagliflozin and metformin treated groups compared to the diabetic rats.

R). Effect of EAEAS and EEAS on systolic blood pressure

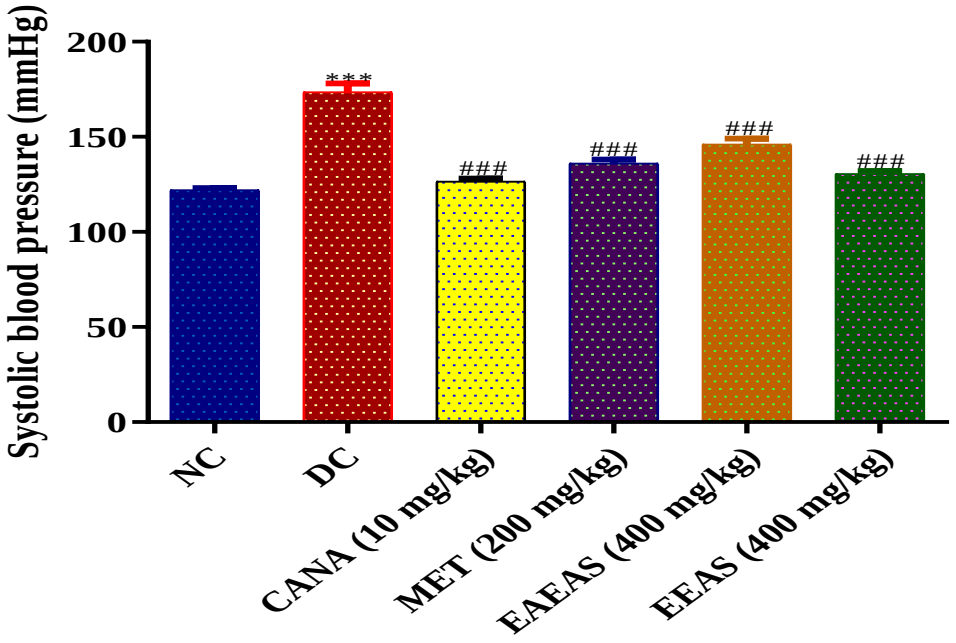


Figure 4.21: Effect of EAEAS and EEAS on systolic blood pressure.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

At the end of the study, systolic blood pressure (SBP) was significantly increased in diabetic rats compared to the NC group ($p<0.001$) (Figure 4.21). Both the EEAS and EAEAS treatment groups significantly improve systolic pressure compared to the DC group. In addition, the groups treated with canagliflozin and metformin significantly reduced SPB compared to the diabetic rats ($p<0.001$).

S). Effect of EAEAS and EEAS on diastolic blood pressure

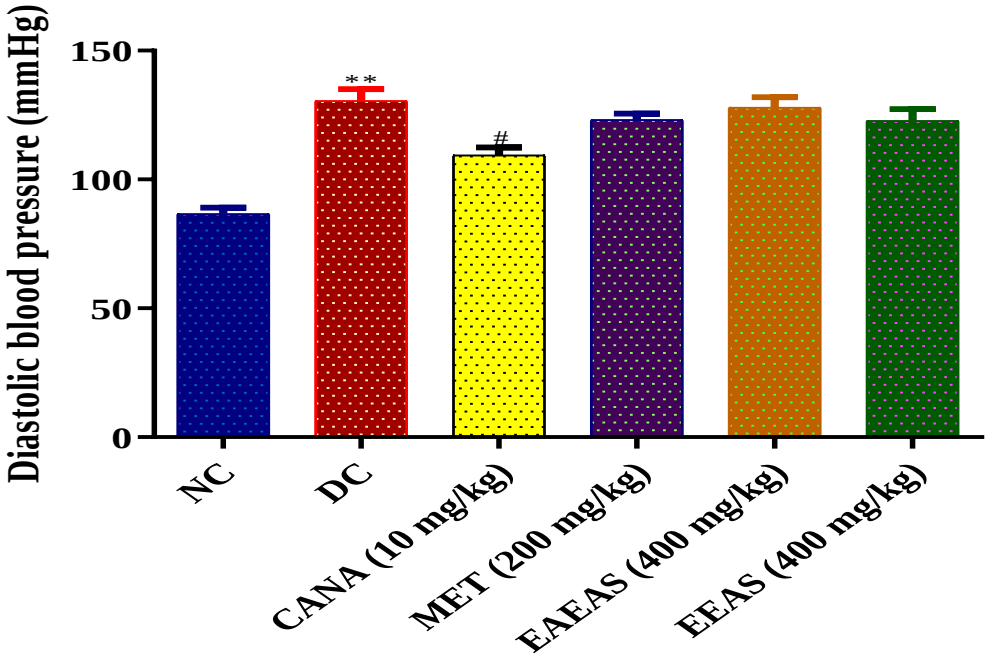


Figure 4.22: Effect of EAEAS and EEAS on diastolic blood pressure.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Diastolic blood pressure (DBP) was significantly increased in diabetic rats compared to the NC group ($p<0.01$) (Figure 4.22). An improvement in diastolic blood pressure was observed in both the EEAS and EAEAS treatment groups compared to the diabetic rats. The group treated with canagliflozin ($p<0.001$) and metformin reduced DPB compared to DC group.

T). Effect of EAEAS and EEAS on heart rate

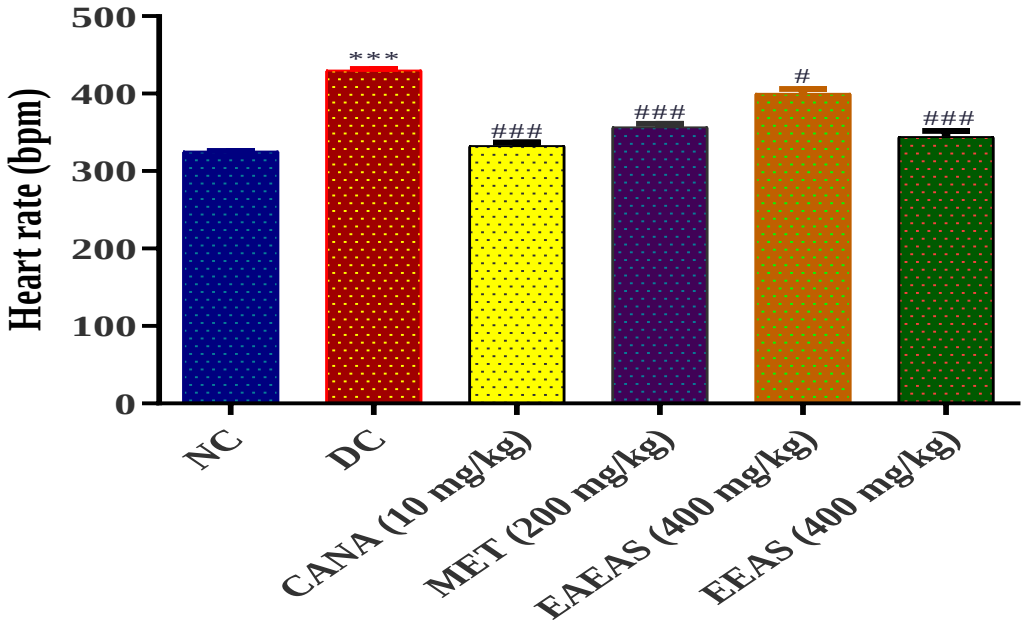


Figure 4.23: Effect of EAEAS and EEAS on heart rate.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Heart rate (HR) data from all groups are shown in Figure 4.23 (at week 12). Heart rate was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the EEAS treatment group, HR decreased significantly ($p<0.001$) compared to diabetic rats. A significant ($p<0.05$) decrease in heart rate was observed in the EAEAS treatment group. Significant ($p<0.001$) improvement in HR of the canagliflozin and metformin treated groups compared to the diabetic rats.

U). Effect of EAEAS and EEAS on PR Interval

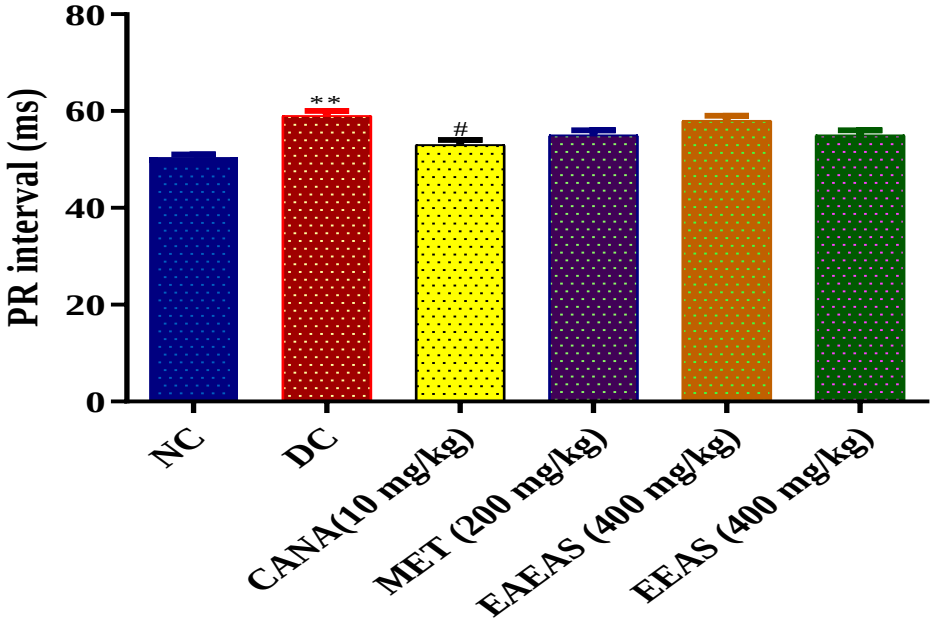


Figure 4.24: Effect of EAEAS and EEAS on PR Interval.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

Data on PR intervals of all groups are presented in Figure 4.24 (at week 12). A prolongation of the PR interval was observed in diabetic rats. EEAS and EAEAS treatment groups improve the PR interval compared to diabetic rats. A significant shortening in the PR interval was observed in the canagliflozin-treated groups compared to the diabetic rats ($p<0.01$). Metformin also improves the PR interval compared to the DC group.

V). Effect of EAEAS and EEAS on QRS complex

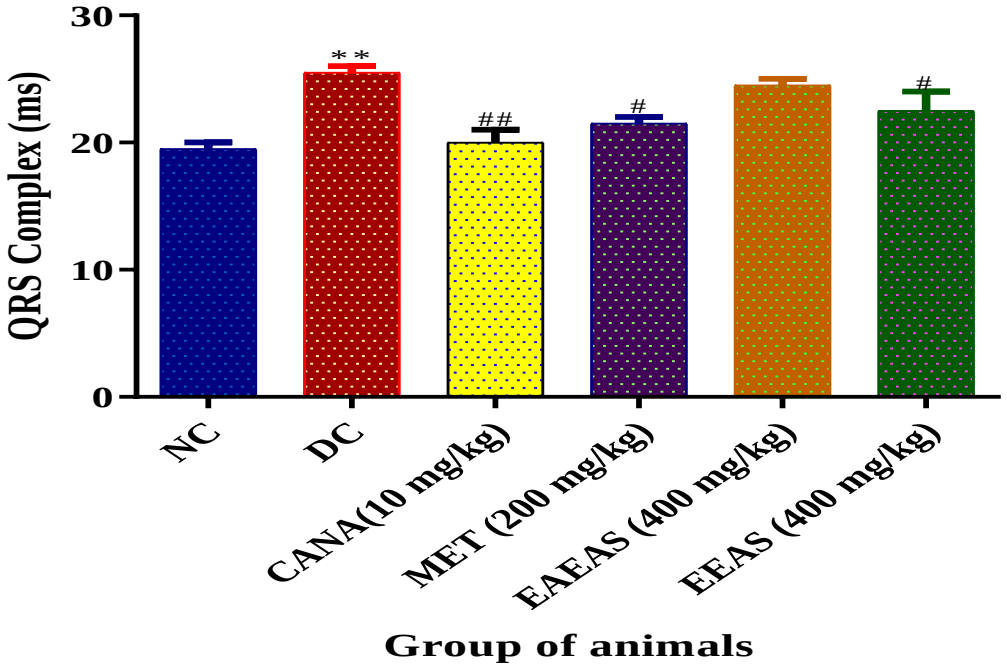


Figure 4.25: Effect of EAEAS and EEAS on QRS complex.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to NC; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ as compared to DC.

The QRS complex results of all treatment groups are shown in Figure 4.25 (at week 12). The QRS complex was significantly increased in diabetic rats compared to the NC group ($p<0.01$). In the EEAS treatment group, the QRS complex decreased significantly ($p<0.05$) compared to diabetic rats. While the EAEAS treatment group shows no change in the QRS complex. There was a significant reduction in the QRS complex in the canagliflozin ($p<0.01$) and metformin ($p<0.05$) treated groups compared to the diabetic rats.

W). Effect of EAEAS and EEAS on QT interval

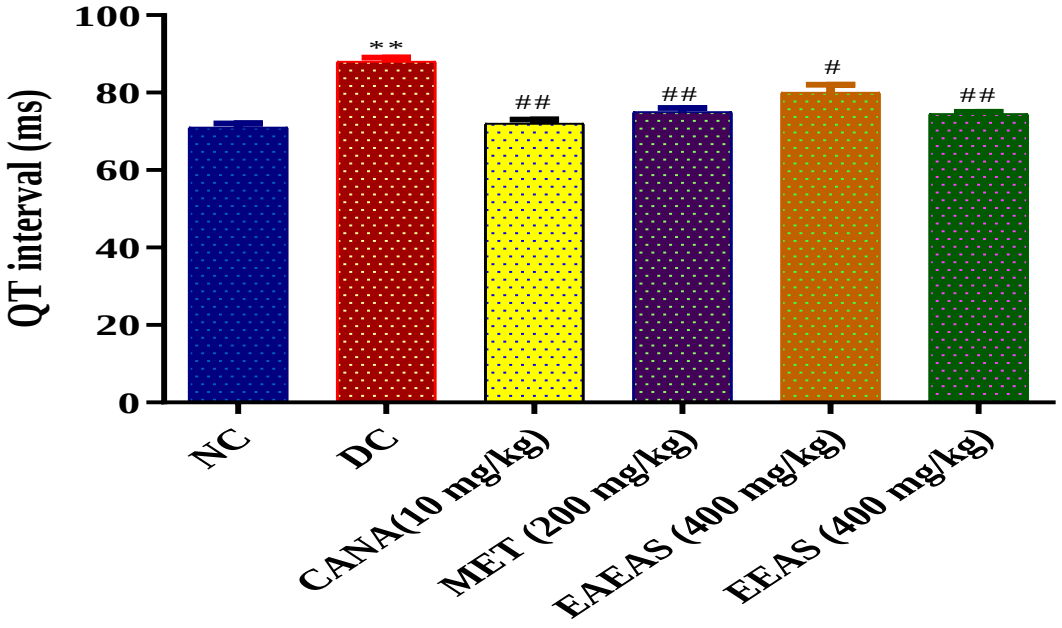


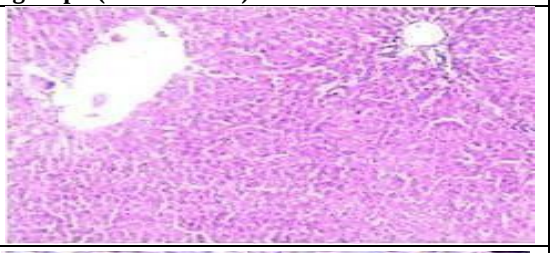
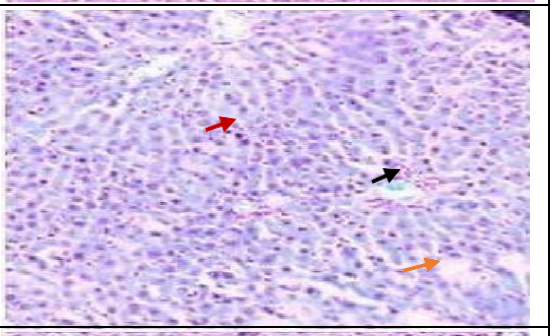
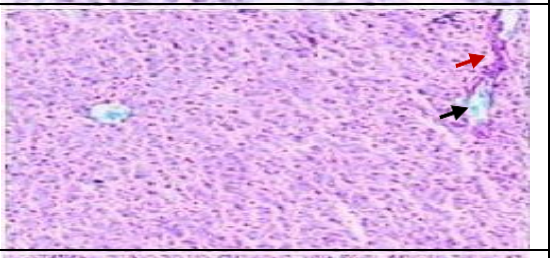
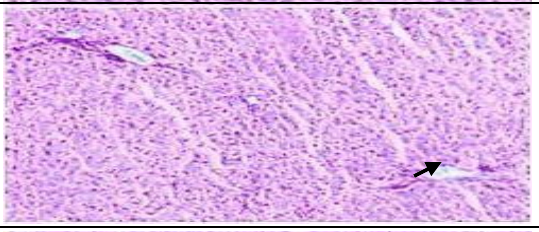
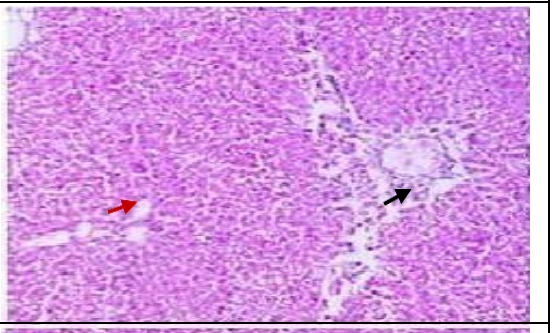
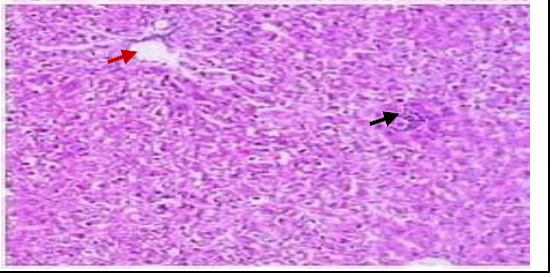
Figure 4.26: Effect of EAEAS and EEAS on QT interval.
NC, normal control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanol extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean ± S.E.M. (n = 6). $^{*}p < 0.05$, $^{**}p< 0.01$ and $^{***}p< 0.001$ as compared to NC; $^{\#}p< 0.05$, $^{\#\#}p< 0.01$ and $^{\#\#\#}p< 0.001$ as compared to DC.

The QT interval results of all groups are shown in Figure 4.26 (at week 12). Significantly increased QT interval in diabetic rats compared to NC group ($p<0.01$). The EEAS treatment group significantly improved QT intervals compared to diabetic rats ($p<0.01$). In addition, the EAEAS treatment group shows a significant ($p<0.05$) shortening of QT intervals compared to the DC groups. The standard group (canagliflozin and metformin) significantly shortened the QT intervals in the treated groups compared to the diabetic rats ($p<0.01$).

X). Histopathology

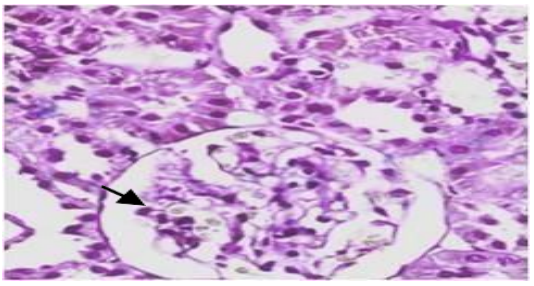
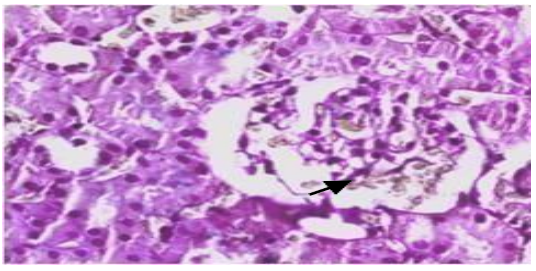
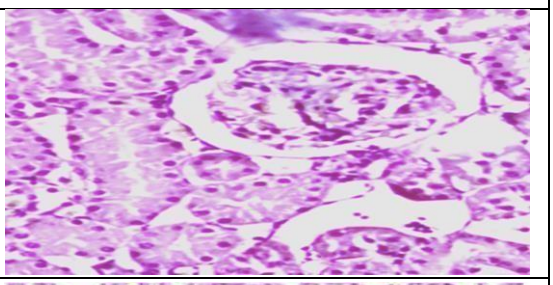
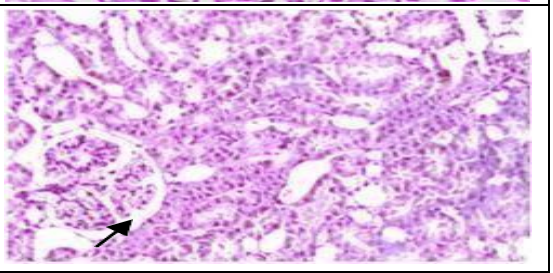
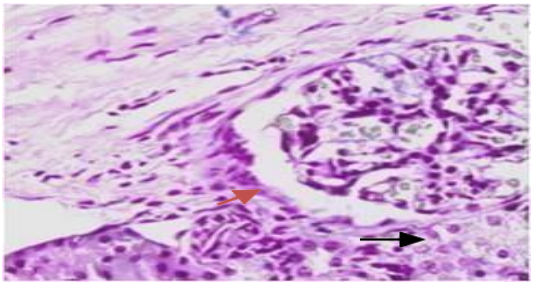
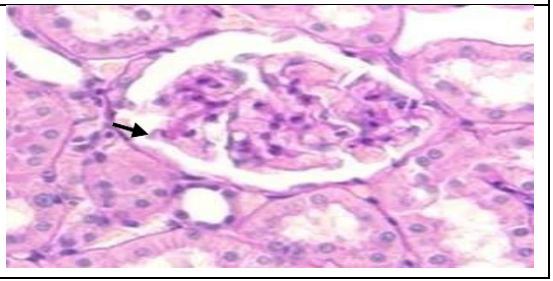
i). Effect of different groups on the histological profile of the liver of a STZ-induced diabetic treated Wister rat.

Table 4.8: Histological sections of rat liver from different groups (H and Ex10).

A.	**NC group** showing normal hepatic cords and portal triad.	
B.	**DC group** representing increased cytoplasmic vacuolation of hepatocytes (Orange arrow), mononuclear cell infiltration of liver tissue (black arrow) and activation of Kupffer cells (red arrow).	
C.	**CANA group** shows areas of leukocyte aggregations (red arrow) and an area of portal tract with dilated obstructed portal vein (black arrow).	
D.	**MET group** shows congestion of some central veins (black arrow).	
E.	**EAEAS treated group** showing hepatocytes associated with mononuclear cell infiltration (black arrow), and cytoplasmic vacuolation of hepatocytes (red arrow) and portal vein arrays.	
F.	**EEAS treated group** showing improved portal vein triad, central vein, mild hydropic degeneration (black arrow) and mild hepatic sinusoid congestion (red arrow).	

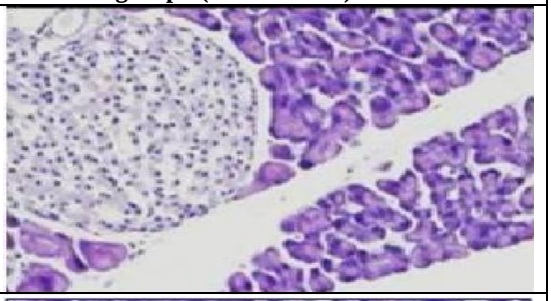
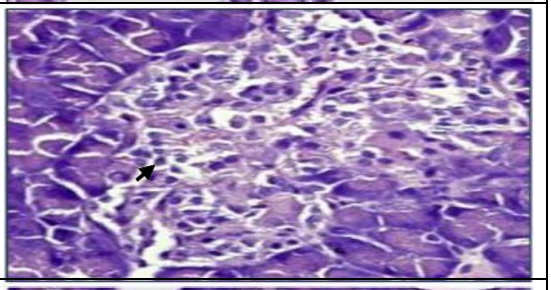
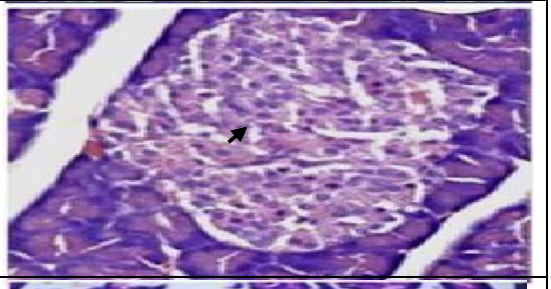
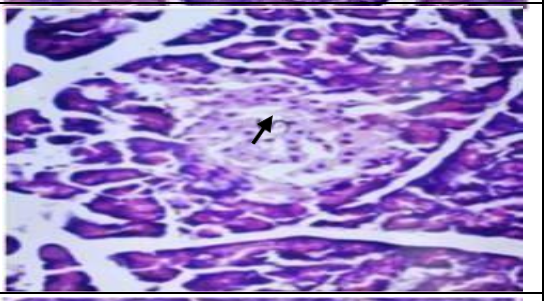
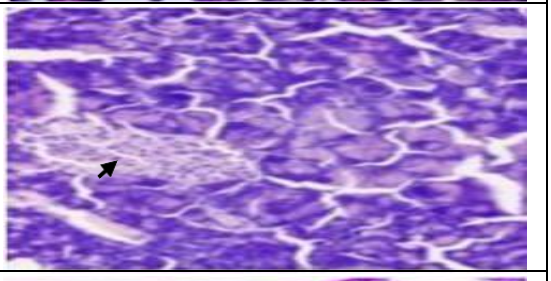
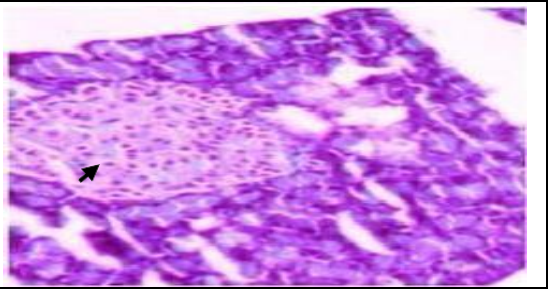
ii). Effect of different groups on the histological profile of the kidney of a STZ-induced diabetic treated wistar rat.

Table 4.9: Histological sections of rat kidney from different groups (H and E x10)

A.	**NC group** showing normal glomeruli with normal baseline and tubules (black arrow); DC group representing disrupted glomeruli with baseline fat deposition along with infiltration of lymphocytes (black arrow).	
B.	**DC group** representing disrupted glomeruli with baseline fat deposition along with infiltration of lymphocytes (black arrow).	
C.	**CANA group** with improved vascular system and less intestinal infiltration.	
D.	**MET group** with improved glomeruli with normal baseline and tubules (black arrow).	
E.	**EAEAS group** with glomerular lymphocytic infiltration (black arrow) thickening of basement membrane (red arrow).	
F.	**EEAS group** showing improved vasculature and glomeruli (black arrow).	

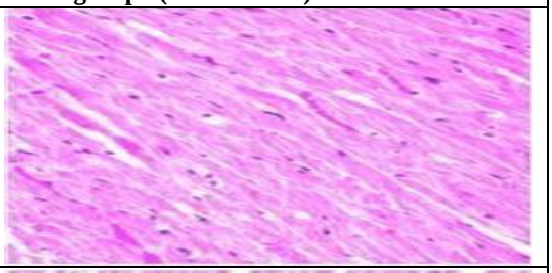
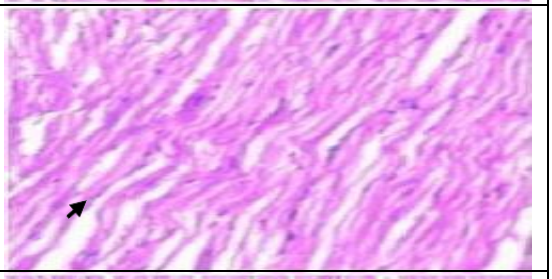
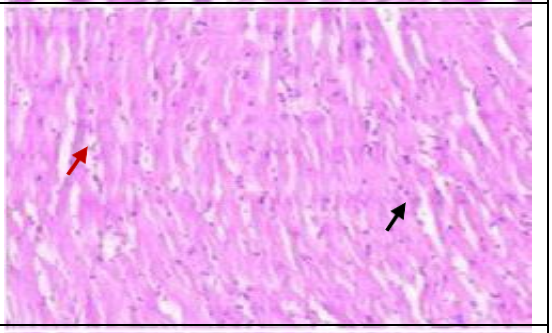
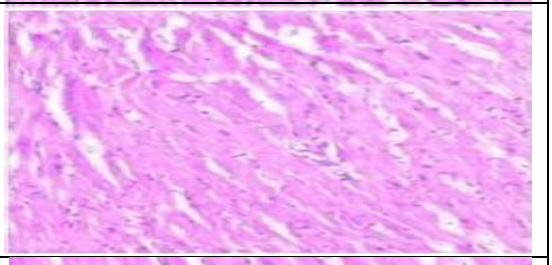
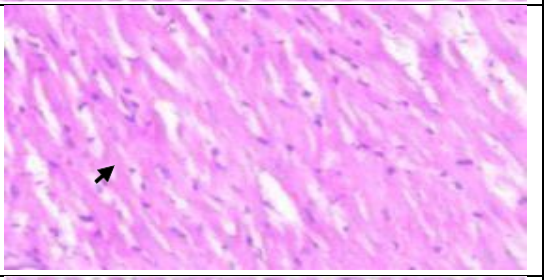
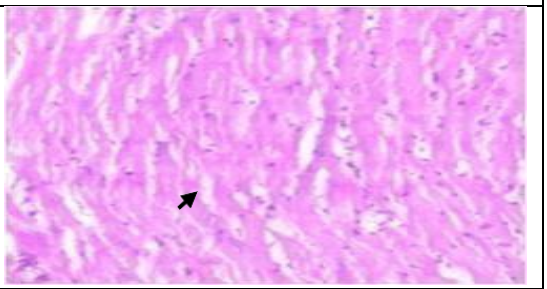
iii). Effect of different groups on the histological profile of the pancreas of a STZ-induced diabetic treated wistar rat.

Table 4.10: Histological sections of rat pancreas from different groups (H and Ex10).

A.	**NC group** representing normal islet of Langerhans.	
B.	**DC group** showing extensive degranulation, destruction of cells, presence of necrotic cells and inflammatory cells (black arrow).	
C.	**CANA group** showing improved pancreatic islets of Langerhans with beta cell enhancement (black arrow).	
D.	**MET group** showing recovery of normal Langerhans islet cell population (black arrow).	
E.	**EAEAS group** representing abnormal islets of Langerhans (black arrow).	
F.	**EEAS group** showing improvement in islet of Langerhans morphology with increasing number of beta cells (black arrow).	

iv). Effect of different groups on the histological profile of the heart of a STZ-induced diabetic treated wistar rat.

Table 4.11: Histological sections of rat heart from different groups (H and Ex10).

A.	**NC group** showing well-arranged cardiomyocytes and normal streaks.	
B.	**DC group** depicting disordered cardiac myocytes with disrupted streaks, enlarged interstitial space (red arrow) and distorted intercalated disc (black arrow).	
C.	**CANA group** showing normal heart tissue with enhanced features of myocytes, less visualization of the interstitial space (red arrow) and distortion of the intercalated disc (black arrow).	
D.	**MET group** with some distorted intercalated discs.	
E.	**EAEAS group** showing stronger evidence of interstitial space (black arrow).	
F.	**EEAS group** showing the mild imaging of the interstitial space (black arrow) and distorting the intercalated disc.	

4.1.4 Phytochemical study

i). **Qualitative chemical analysis of the phytoconstituents alkaloid and flavonoid fractions ethanol extracts of A. scholaris leaves.**

- Et-AF –Dragendroff's test- Orange precipitation
- Et-FF- Ammonium test- Yellow colour

Observations of the phytochemical analysis of both fractions of ethanol extract from *A. scholaris* leaves confirmed the presence of secondary metabolites like alkaloids and flavonoids.

ii). Quantitative chemical analysis of the phytoconstituents alkaloid fraction and flavonoid of ethanol extracts of *A. scholaris* leaves.

The quantitative results show that Et-AF has the most abundant compound in EEAS at 1.6%(16.2 mg/g extract), while Et-FF contains 0.6% (6.5 mg/g extract).

4.1.5 Effect of Et-AF and Et-FF on glucose homeostasis using OGTT

i). Effect of Et-FF on glucose homeostasis

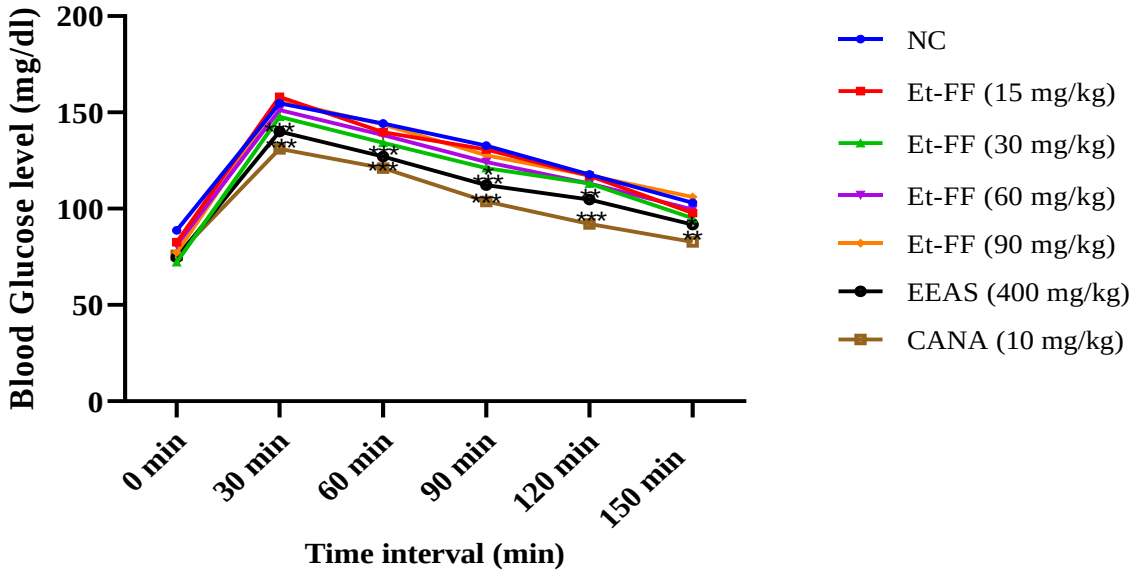


Figure 4.27: Effect of Et-FF on glucose homeostasis.

The results are expressed in Mean ± SEM. Each group comprised of three animals. NC: Normal control, EEAS: Ethanol extract of *Alstonia scholaris,* Et-AF: Alkaloid fraction of ethanol extract of *Alstonia scholaris,* Et-FF: Flavonoid fraction of ethanol extract of *Alstonia scholaris,* CANA: Canagliflozin. Data were analyzed by unpaired t-test for comparison.

The results of the OGTT showed a significant increase in glucose levels after administration of 2 g/kg glucose compared to the normal control (Figure 4.27). The effect of the flavonoid fraction of ethanol extracts (Et-FF) on glucose homeostasis was studied at a dose of 15, 30, 60 and90 mg/kg. EEAS shows a significant reduction in blood glucose levels at 30, 60, 90 and 120 minutes compared to the normal control. Treatment with canagliflozin (10 mg/kg) significantly reduces blood glucose levels at 30, 60, 90, 120 and 150 minutes.

ii). Effect of Et-AF on glucose homeostasis

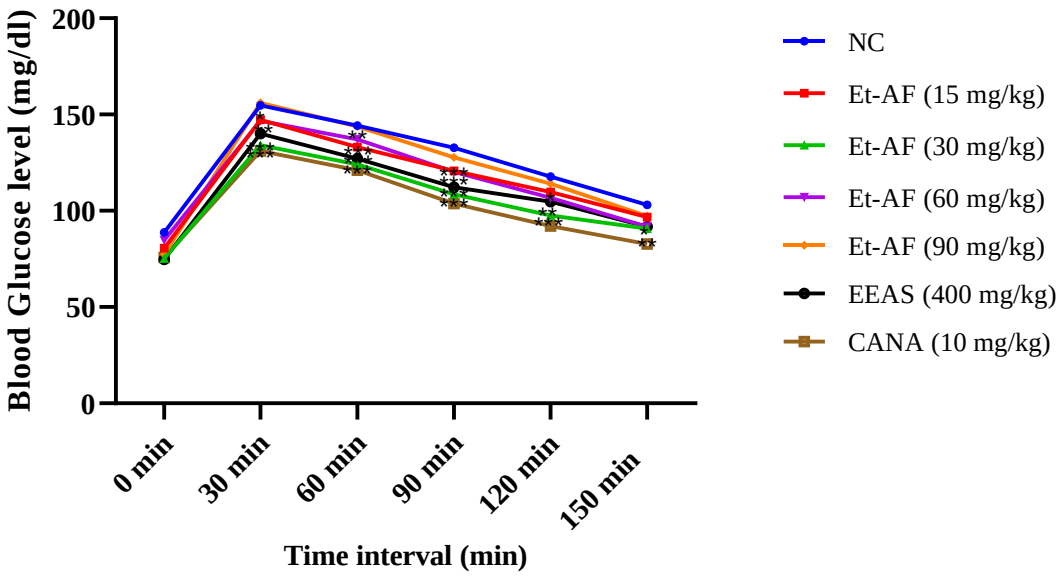


Figure 4.28: Effect of Et-AF on glucose homeostasis.
The results are expressed in Mean ± SEM. Each group comprised of three animals. NC: Normal control, EEAS: Ethanol extract of *Alstonia scholaris*, Et-AF: Alkaloid fraction of Ethanol extract of *Alstonia scholaris*, Et-FF: Flavonoid fraction of ethanol extract of *Alstonia scholaris*, CANA: Canagliflozin. Data were analyzed by unpaired t-test for comparison.

In the alkaloid fraction of ethanol extract (Et-AF) treatment group, glucose levels were significantly reduced ($p<0.01$) at a dose of 15 mg/kg after 60 minutes (Figure 4.28). The group treated with Et-AF (30 mg/kg) showed that blood glucose levels increased after 30 minutes ($p<0.01$), 60 minutes ($p<0.001$), 90 minutes ($p<0.001$) and 120 minutes ($p< 0.05$) decreases significantly. EEAS reflects a significant reduction in blood glucose levels at 30, 60, 90 and 120 minutes compared to the normal control. Canagliflozin (10 mg/kg) showed a significant reduction in blood glucose levels at 30, 60, 90, 120 and 150 minutes.

iii). Effect of Et-AF and Et-FF on glucose homeostasis using OGTT

Table 4.12: Effect of Et-AF and Et-FF on glucose homeostasis using OGTT.

Treatment/ Groups	Blood glucose level (mg/dl)					
	Initial	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**
Normal control	84.3±3.38	159.3±1.45	149.3±2.40	132.6±3.28	117.6±3.17	103±3.78
Et- AF (15 mg/kg)	80.5 ±1.40	147.3±2.55*	133± 2.03**	120.6±1.74**	109.8±2.18	96.8±2.86
Et- AF (30mg/kg)	75.1±3.13	134±1.80***	124±1.59***	108.5±2.14***	100.3±2.63**	90.8±1.75*
Et -AF (60 mg/kg)	84.6±3.75	146.66±2.40	137±3.05	120.3±0.88	106.6±2.72	91.6±2.33
Et -AF (90 mg/kg)	77.3±5.60	156±5.03	143.6±3.38	127.6±3.75	114±1.73	97.3±3.92
Et- FF (15 mg/kg)	82.6±5.23	158±6.55	139.6±1.85	130.6±4.48	117±2.08	97.6±4.17
Et- FF (30 mg/kg)	72.3±5.20	147.66±2.90	134.3±3.38	121±2.30*	113±1.52	95±20
Et- FF (60 mg/kg)	81±3.03	151.33±3.08	138.3±2.62	124±3.03	113.3±2.55	99.6±2.04
Et- FF (90 mg/kg)	77.3±5.60	156±5.03	143.6 ±3.38	127.6±3.75	117.3±2.60	106±2.64
EEAS (400 mg/kg)	74.7±3.25	140±2.51**	127±3.21***	112.3±2.33***	104.6±3.75*	91.6±3.75
CANA (10 mg/kg)	75.66±2.33	131±0.57***	121±0.57***	103.6±0.66**	92±4.50***	82.6±2.18**

In the normal control group, an increase in glucose levels was observed after 30, 60 and 90 minutes (Table 4.12). There was a significant reduction in blood glucose levels in the Et-FF (30 mg/kg), Et-AF (15 mg/kg), Et-AF (30 mg/kg) and EEAS (400 mg) treatment groups at 30, 60, 90 and 120 minutes. In addition, the canagliflozin-treated groups significantly reduced blood glucose levels at 30, 60, 90, 120, and 150 minutes compared to the normal control group.

Based on the results of the glucose tolerance test, two active fractions were selected for further studies on high-fat diet and streptozotocin (STZ)-induced type 2 diabetes mellitus, namely the alkaloid fraction and the flavonoid fraction of the ethanol extract of *A. scholaris* leaves.

4.1.6 : Effects of alkaloid and flavonoid rich fractions on type 2 diabetes mellitus and its complications.

A). Effect of Et-AF and Et-FF on body weight

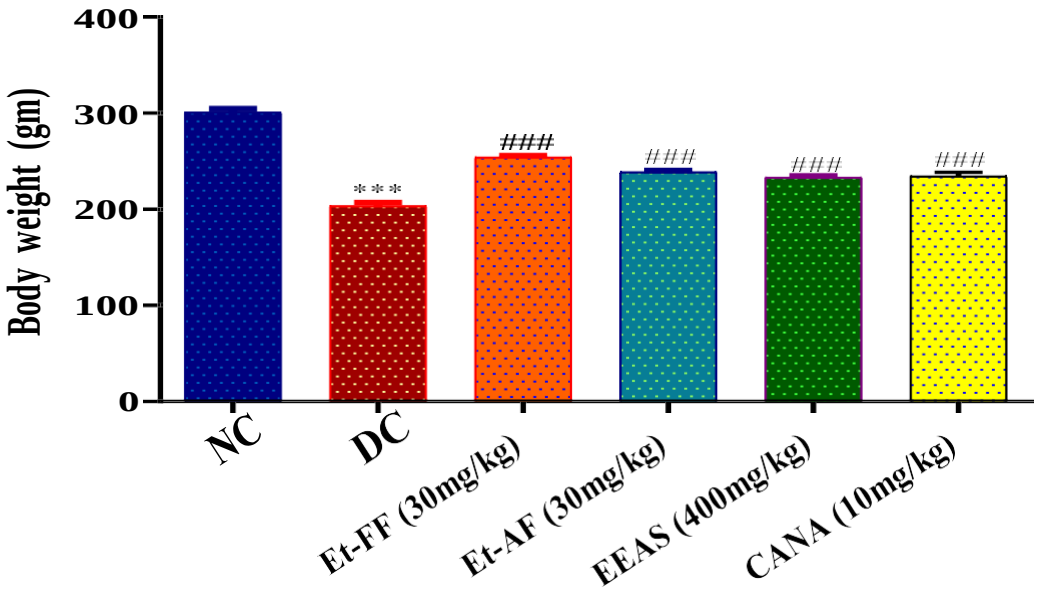


Figure 4.29: Effect of Et-AF and Et-FF on body weight.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Figure 4.29 shows the body weight of male wistar rats from the last day of the experiment. A significant increase in body weight was observed in the HFD group compared to the normal control (NC) group. Diabetic control group (DC) significant (p<0.001) decreased in the body weight compared to the NC group. The Et-AF treated group significantly improved the rats' weight compared to the normal control (p<0.01). Groups treated with Et-FF significantly (p<0.05) improved body weight compared to DC after 4 weeks (day 83) of treatment. EEAS shows a significant (p<0.01) increase in body weight compared to DC. Treatment with canagliflozinalso improves the weight of rats compared to diabetic rats.

B). Effect of Et-AF and Et-FF on food intake

Table 4.13: Food intake of rats.

Week	NC	DC	Et-AF (30 mg/kg)	Et-FF (30 mg/kg)	EEAS (400 mg/kg)	CANA (10 mg/kg)
0	25.5±7.4	27±8.6	26.67±4.5	26.67±5.8	29.17±10.5	28.8±9.2
1	25.17±8.2	28.83±5.2	27.33±5.6	27.17±2.5	28.67±6.5	27.9±8.5
2	21.67±5.3	28.67±7.5	27.67±3.2	28±3.5	28±8.2	26.2±2.14
3	22.17±8.4	29.67±4.8*	28.33±2.8	28.13±5.5	29.67±5.5	28.4±9.8
4	22.67±4.5	28.83±9.1*	27.5±9.1	27.83±7.8	28.83±6.2	27.4±7.1
5	21.17±7.2	30.5±11.5***	30.67±9.6	28.83±8.2	26.83±3.4	26.4±5.6
6	22±2.0	31.17±7.9***	29.5±14.6	32±6.5	29.33±9.8	32.8±11.5
7	24.67±8.5	31±12.5***	32.83±6.5	33.33±7.8	30.33±7.9	31.5±14
8	22±6.0	31.83±8.8***	30.5±8.2	31±4.5	29.33±11.5$^{\#}$	27±5$^{\#}$
9	22.83±3.8	32.67±9.1***	28.33±6.5	30±10	28.83±6.8$^{\#}$	25.7±.7.1$^{\#\#}$
10	22.5±7.6	31.33±8.2***	27.33±5.2$^{\#\#}$	29.67±2.3$^{\#}$	29.33±9.2$^{\#}$	26.7±8.4$^{\#\#}$
11	20.67±5.5	32.67±6.1***	27.83±9$^{\#\#}$	29.33±5.6$^{\#}$	28.83±11$^{\#}$	26.2±5.5$^{\#\#}$
12	22.17±1.9	32.33±11.2***	26.5±12.5$^{\#\#}$	28±8.2$^{\#}$	27.17±2.6$^{\#\#}$	25.8±8.7$^{\#\#}$

NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The food intake results of NC, DC and all treatment groups are summarized in Table 4.13. Compared to the normal control group, there is a significant increase in food intake in the HFD group up to the 4th week. A significant ($p<0.01$) reduction in food intake was observed in the Et-AF, EEAS and canagliflozin treated group compared to DC. In addition, treatment with the flavonoid fraction significantly reduced food intake in diabetics ($p<0.05$).

C). Effect of Et-AF and Et-FF on water intake

Table 4.14: Water intake of rats.

Weeks	NC	DC	Et-AF (30 mg/kg)	Et-FF (30 mg/kg)	EEAS (400 mg/kg)	CANA (10 mg/kg)
0	17.5±8.4	18.8±2.51	18.7±4.9	20.7±8.0	22.2±6.4	20.0±8.9
1	21.2±5.5	22.9±5.7	21.3±6.7	21.2±9.3	23.7±9.3	21.8±4.6
2	21.7±6.8	23.2±11.4	22.7±7.5	22.0±12.4	21.0±5.2	22.7±6.3
3	22.2±9.6	23.4±9.2	20.3±9.2	24.8±5.6	23.7±4.5	24.7±5.7
4	22.7±6.2	25.0±8.0	24.5±6.1	23.8±5.8	22.8±3.8	24.8±3.5
5	21.2±8.7	52.0±10	47.7±9.4	53.8±6.8	46.8±9.6	55.5±6.8
6	25.0±5.2	53.0±5.3	49.5±8.6	56.0±9.1	49.3±7.8	57.2±7.05
7	25.6±4.8	57.0±14.2	52.8±7.4	53.3±7.6	53.3±12.3	56.0±8.9
8	22.0±9.1	65.0±6.8	49.0±9.8$^{\#\#}$	47.5±5.0$^{\#\#}$	50.3±5.6$^{\#\#}$	51.8±5.6$^{\#\#}$
9	22.8±6.8	67.0±9.4	47.0±4.6$^{\#\#}$	42.3±7.4$^{\#\#\#}$	48.8±9.4$^{\#\#}$	49.7±7.4$^{\#\#}$
10	18.5±5.7	60.0±7.2	48.7±3.1$^{\#\#}$	37.3±11.5$^{\#\#\#}$	45.3±8.6$^{\#\#}$	51.3±8.2$^{\#\#}$
11	20.7±3.2	59.0±8.1	50.3±4.8$^{\#}$	34.8±9.1$^{\#\#\#}$	48.8±5.9$^{\#\#}$	52.7±5.5$^{\#}$
12	22.2±6.3	52.0±6.3	55.0±6.7	35.5±12.9$^{\#\#\#}$	51.2±6.4$^{\#\#}$	59.3±8.2

NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p$<0.05, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The water intake results for all treatment groups are summarized in Table 4.14. Compared to the normal control group, there was a significant (p<0.001) increase in water intake in the DC group (after week 4). No significant change in water intake was observed in the canagliflozin and Et-AF treatment group compared to the DC group (at 12th week). At the end of the study, treatment with Et-FF (p<0.001) and EEAS (p<0.01) showed a significant decrease in water intake compared to diabetic rats.

D). Effect of Et-AF and Et-FF on fasting blood glucose level

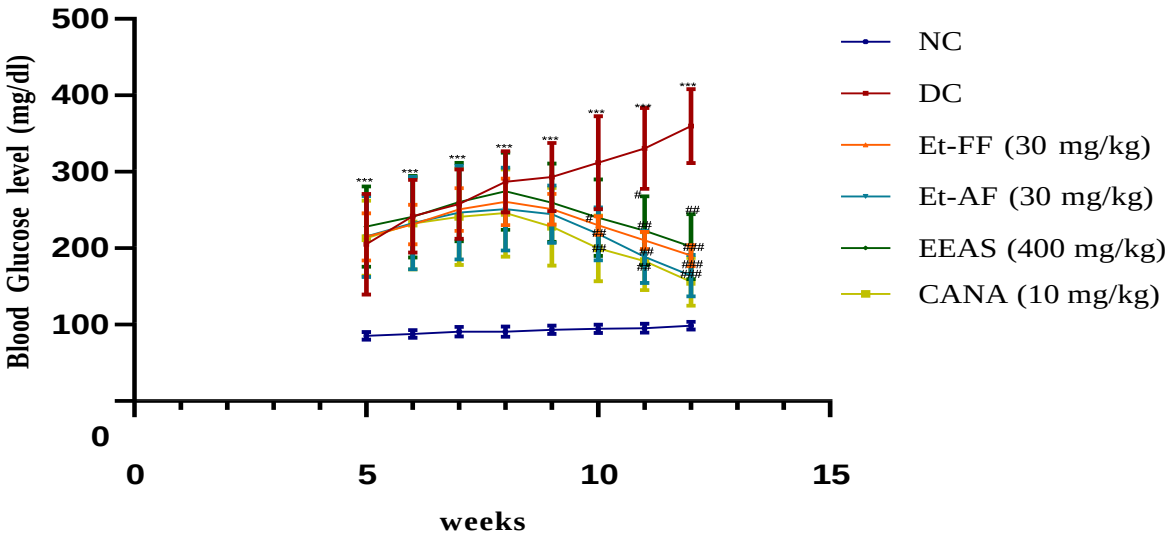


Figure 4.30: Effect of Et-AF and Et-FF on fasting blood glucose level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p$<0.05, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The FBG result of all NC, DC and treatment groups from week 5 (administered with STZ) to week 12 is shown in Figure 4.30. In the DC group, the blood glucose level was significantly increased compared to normal rats (after 4th week). A significant ($p<0.01$) decrease in FBG values was observed in the EEAS group compared to the DC group. The Et-FF treatment group significantly reduced FBG compared to diabetic rats. The Et-AF and canagliflozin treatment group reduced FBG levels significantly ($p<0.001$) compared to the DC group (at week 12).

E). Effect of Et-AF and Et-FF on urine output

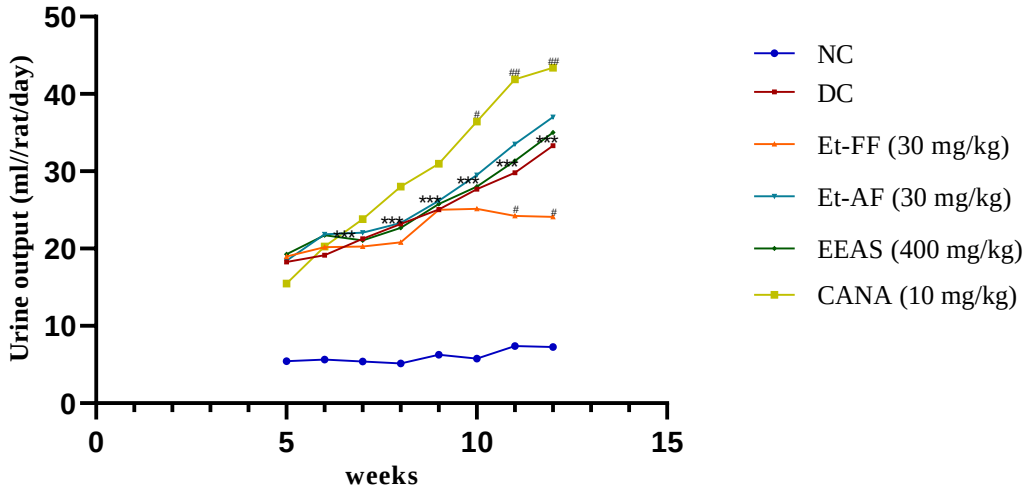


Figure 4.31: Effect of Et-AF and Et-FF on urine output.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p$<0.05, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Urinary output data for all DC and treatment groups from week 5 (administered with STZ) to week 12 are shown in Figure 4.31. In the urinalysis, there was an increase in urine output after STZ induction in the DC group of the type 2 diabetic model (after the 4th week). The Et-FF treatment group significantly reduced urinary output compared to the DC group ($p<0.05$). The EEAS and Et-AF treatment group showed an insignificant increase in urinary output, while canagliflozin showed a significant ($p<0.01$) increase in urinary output compared to diabetic rats (at week 12).

F). Effect of Et-AF and Et-FF on urine glucose level

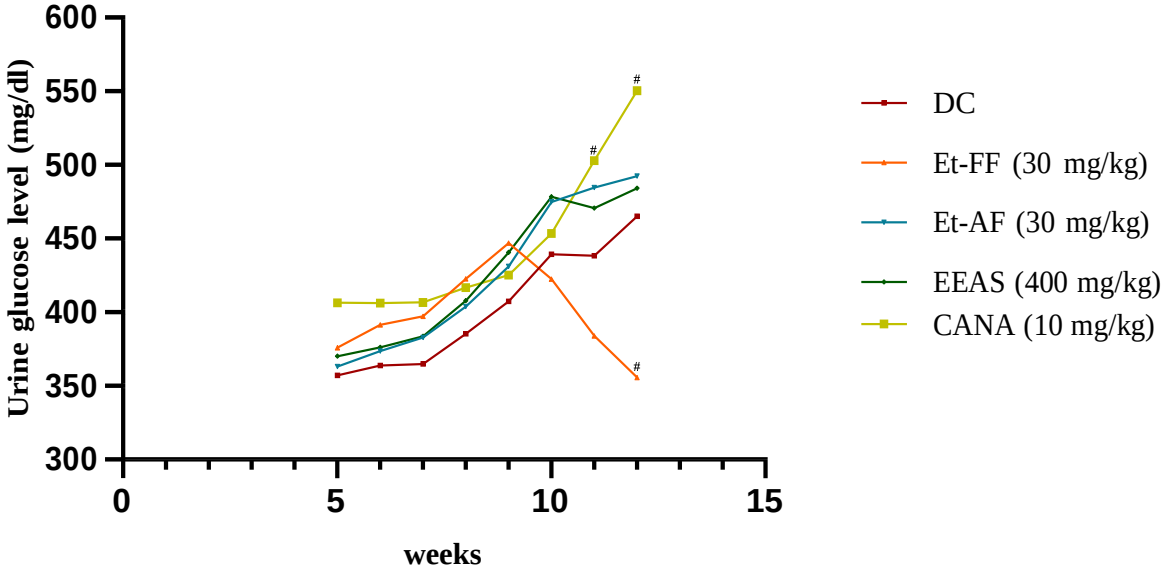


Figure 4.32: Effect of Et-AF and Et-FF on urine glucose level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The data of the urine glucose levels of all DC and treatment groups from week 5 (administered with STZ) to week 12 are presented in Figure 4.32. In the DC group, the urine glucose level increased (after the 4th week). A significant declination in urine glucose levels was recorded in the Et-FF treatment groups ($p<0.01$) compared to the DC group. The EEAS and Et-AF treatment group showed an increase in urinary glucose excretion. On the other hand, canagliflozin ($p<0.01$) resulted in a significant rise in urine glucose levels compared to the diabetic rats (at week 12).

G). Effect of Et-AF and Et-FF on serum cholesterol level

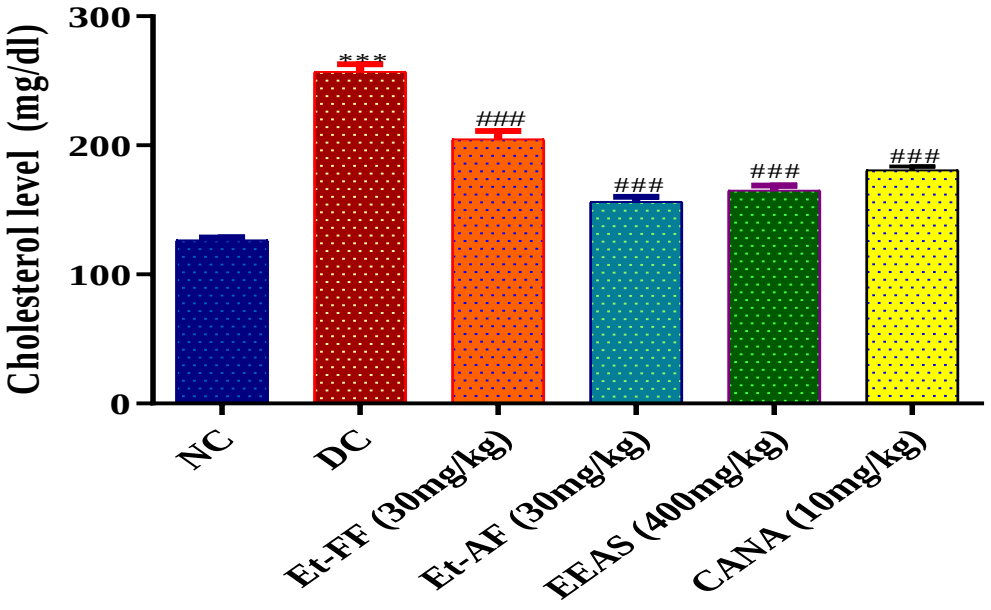


Figure 4.33: Effect of Et-AF and Et-FF on serum cholesterol level.
NC, Normal control (n=6); DC=Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

In the lipid profile, the serum total cholesterol (T-CH) level of all groups was summarized in Figure 4.33 (after 12 weeks). Serum T-CH levels were significantly increased in diabetic rats compared to the NC group ($p<0.001$). In both the Et-AF and Et-FF treatment groups, T-CH levels decrease significantly ($p<0.001$) compared to diabetic rats. Also, the EEAS and canagliflozin treatment group shows a significant ($p<0.001$) decrease in T-CH levels compared tothe DC groups.

H). Effect of Et-AF and Et-FF on serum triglyceride level

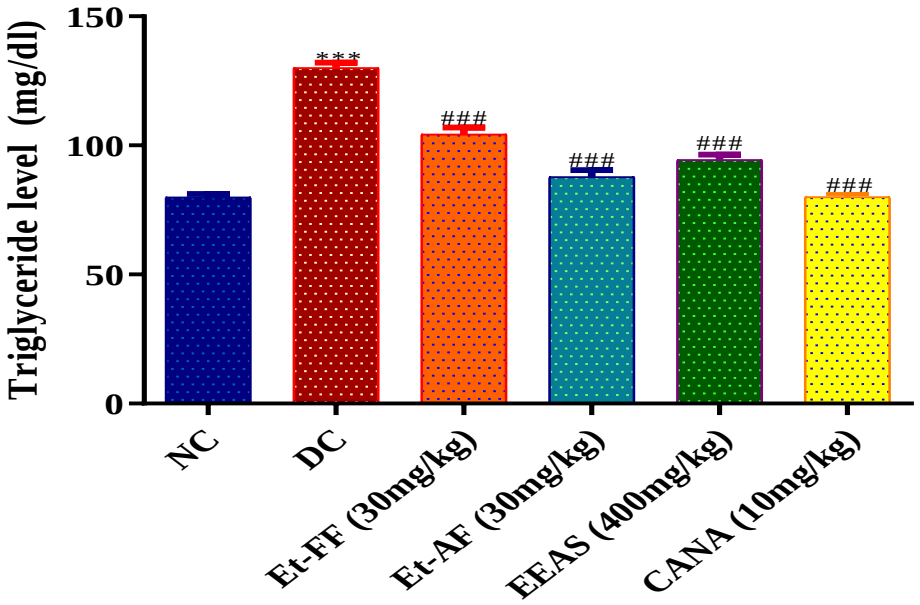


Figure 4.34: Effect of Et-AF and Et-FF on serum triglyceride level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Triglyceride (TG) level data from all groups are summarized in Figure 4.34 (after 12 weeks). Serum TG levels were significantly increased ($p<0.001$) in diabetic rats compared to normal rats. In both the Et-AF and Et-FF treatment groups, TG levels decrease significantly ($p<0.001$) compared to DC. In addition, the EEAS and canagliflozin treatment group shows a significant ($p<0.001$) reduction in TG levels compared to the DC groups.

I). Effect of Et-AF and Et-FF on serum LDL level

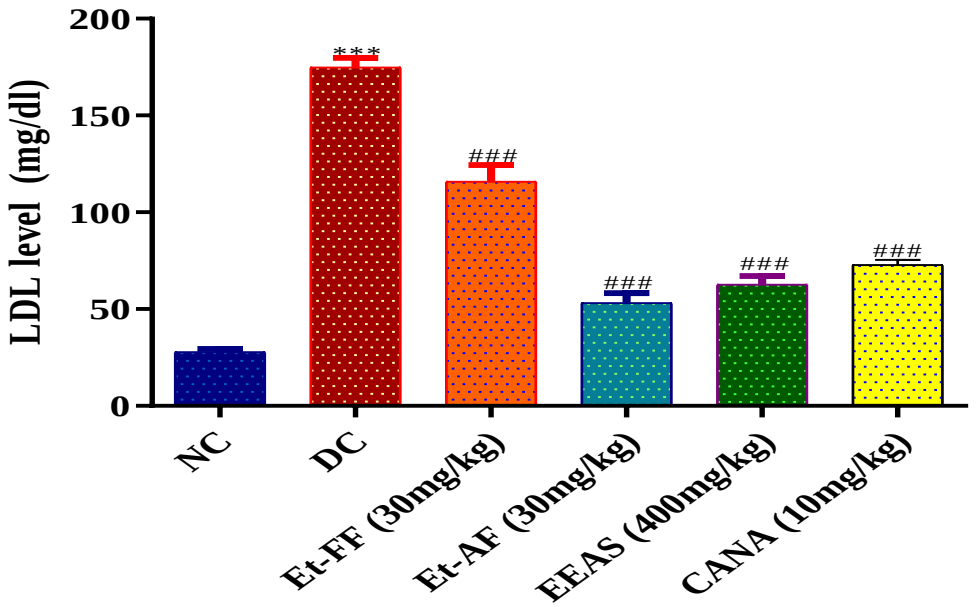


Figure 4.35: Effect of Et-AF and Et-FF on serum LDL level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The results of the LDL values of all treatment groups are summarized in figure 4.35 (after 12 weeks). Serum LDL levels were significantly increased in diabetic rats compared to normal rats ($p<0.001$). Compared to diabetic rats, there was a significant ($p<0.001$) reduction in LDL levels in the Et-AF, Et-FF and EEAS treatment groups. In addition, the canagliflozin treatment group shows a significant ($p<0.001$) reduction in LDL levels compared to the DC groups.

J). Effect of Et-AF and Et-FF on serum VLDL level

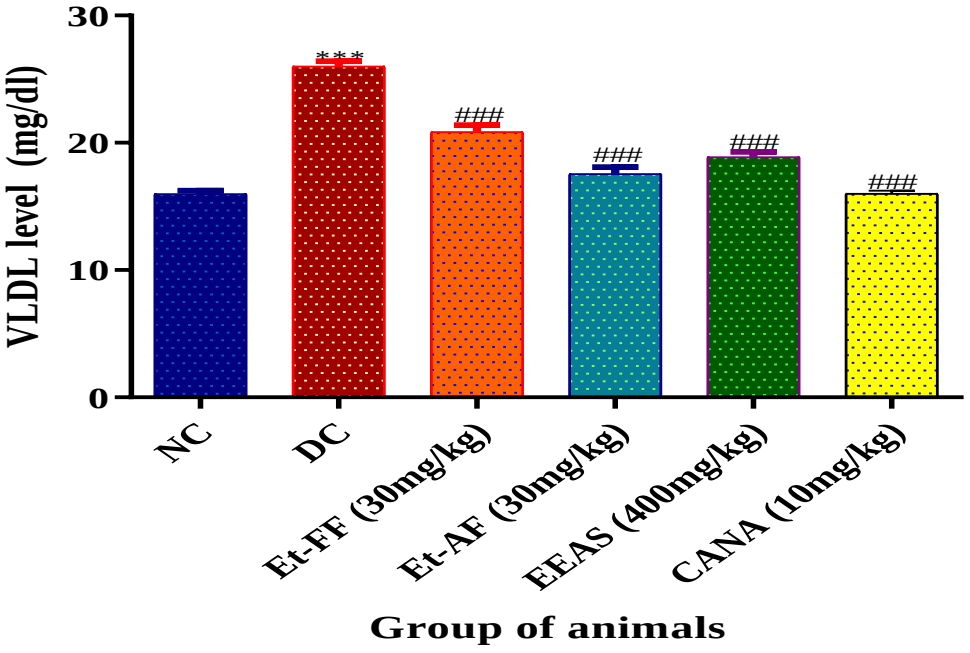


Figure 4.36: Effect of Et-AF and Et-FF on serum VLDL level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The results of the LDL values of all treatment groups are shown in Figure 4.36 (at week 12). LDL levels were significantly increased in diabetic rats compared to normal controls ($p<0.001$). The effect of the Et-AF, Et-FF and EEAS treatment group reflects a significant ($p<0.001$) reduction in LDL levels compared to the DC group. The standard group (canagliflozin) reduced LDL significantly ($p<0.001$) compared to the diabetic rat group.

K). Effect of Et-AF and Et-FF on serum HDL level

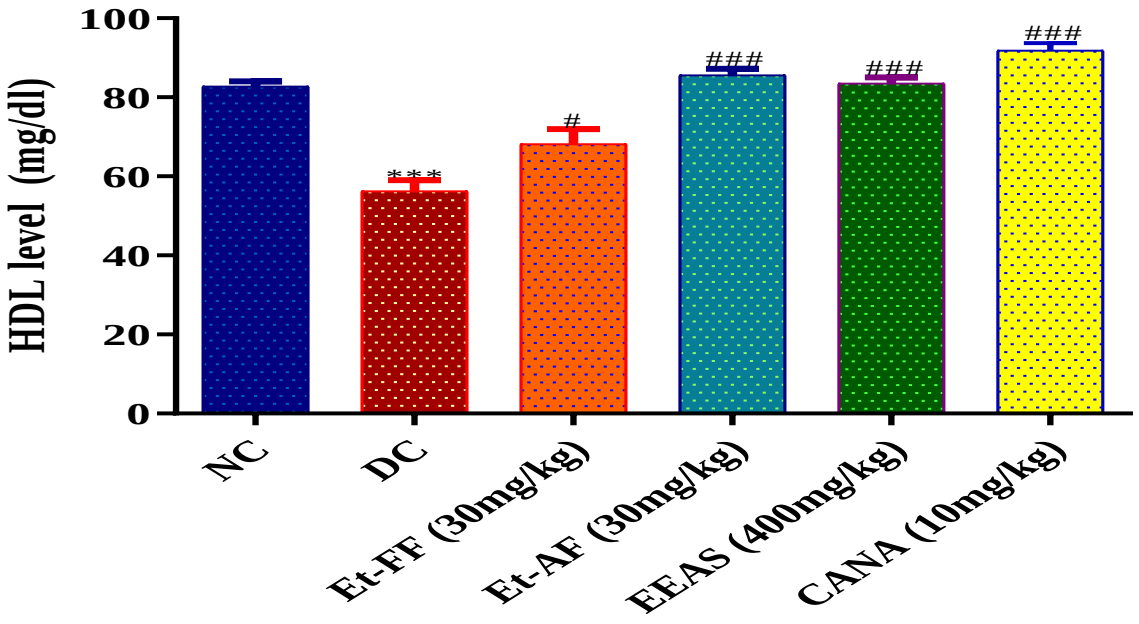


Figure 4.37: Effect of Et-AF and Et-FF on serum HDL level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p$<0.05, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

In the lipid profile, the serum levels of high density lipoprotein (HDL) of all groups were summarized in Figure 4.37 (after 12 weeks). Serum HDL levels were significantly reduced (p<0.001) in diabetic rats compared to normal rats. Treatment groups Et-AF (p<0.001), EEAS (p<0.001) and Et-FF (p<0.05) significantly improved HDL levels compared to diabetic rats. Significantly increased HDL levels (p<0.001) were observed with canagliflozin compared to diabetic rats.

L). Effect of Et-AF and Et-FF on serum urea level

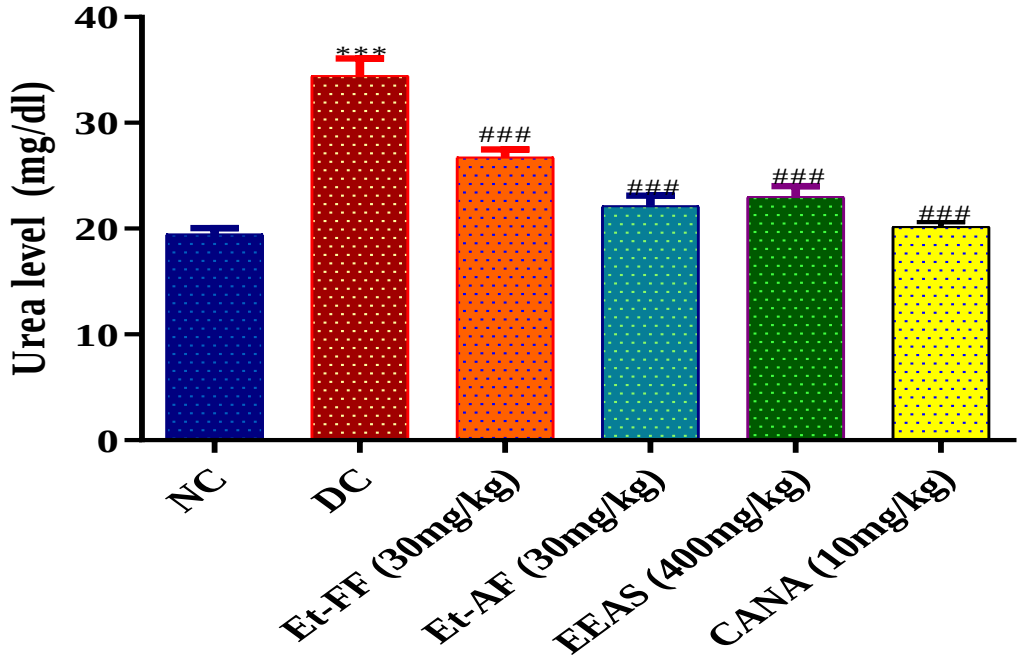


Figure 4.38: Effect of Et-AF and Et-FF on serum urea level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The results of the renal damage markers such as serum urea levels of all groups are shown in Figure 4.38 (at 12 week). Serum urea levels were significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the Et-AF and Et-FF treatment group, urea levels decreased significantly ($p<0.001$) compared to diabetic rats. In addition, there was a significant reduction ($p<0.001$) in urea levels in the EEAS and canagliflozin treated groups compared to the diabetic rats.

M). Effect of Et-AF and Et-FF on serum creatinine level

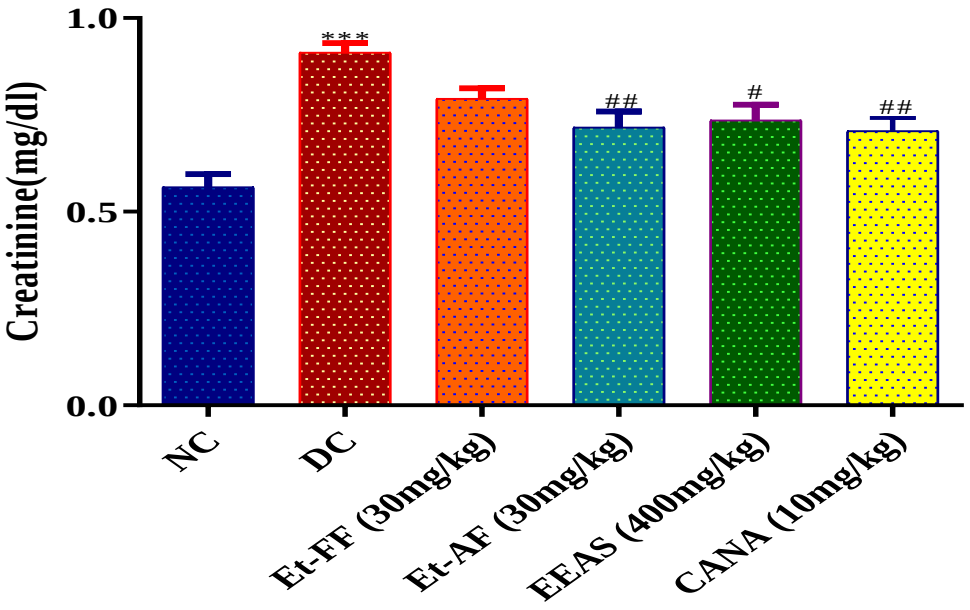


Figure 4.39: Effect of Et-AF and Et-FF on serum creatinine level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 wmg/kg) (n=6). Data represent the mean
± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The results of the serum creatinine levels of all groups are shown in figure 4.39 (after 12 weeks). The serum creatinine level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the Et-AF ($p<0.01$) and EEAS ($p<0.05$) treatment group, creatinine levels decreased significantly compared to diabetic rats. While the Et-FF treatment group showed no change in creatinine levels. There was a significant reduction in serum creatinine in the canagliflozin treated groups compared to the diabetic rats ($p<0.001$).

N). Effect of Et-AF and Et-FF on serum AST level

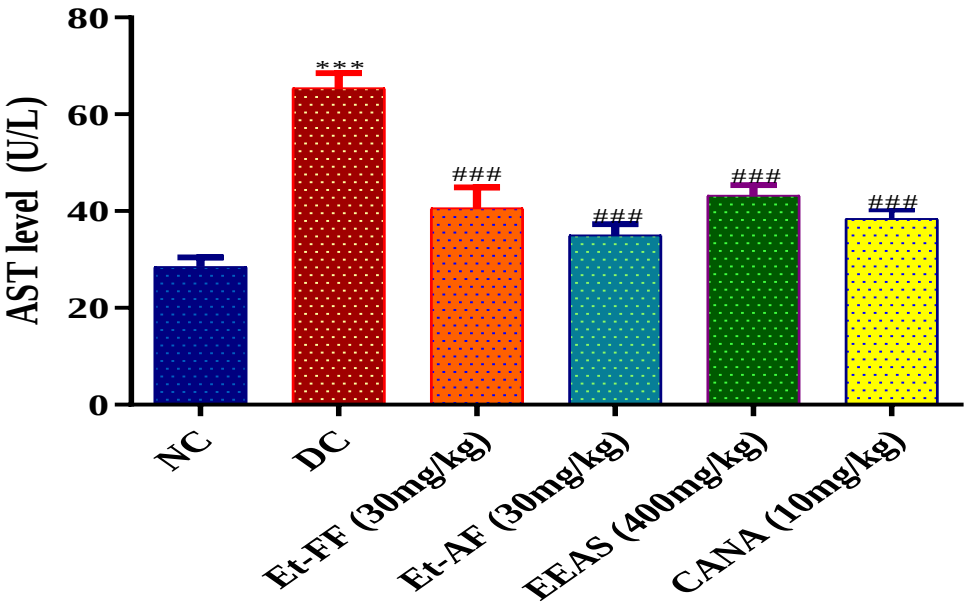


Figure 4.40: Effect of Et-AF and Et-FF on serum AST level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The data on markers of liver damage such as serum AST levels of all groups are shown in Figure 4.40 (at week 12). The serum AST level was significantly increased in diabetic rats compared to the NC group (p<0.001). In the Et-AF, Et-FF and EEAS treatment groups, AST levels decreased significantly (p<0.001) compared to diabetic rats. In the standard group canagliflozin significantly decreases serum AST levels compared to the DC group.

O). Effect of Et-AF and Et-FF on serum ALT level

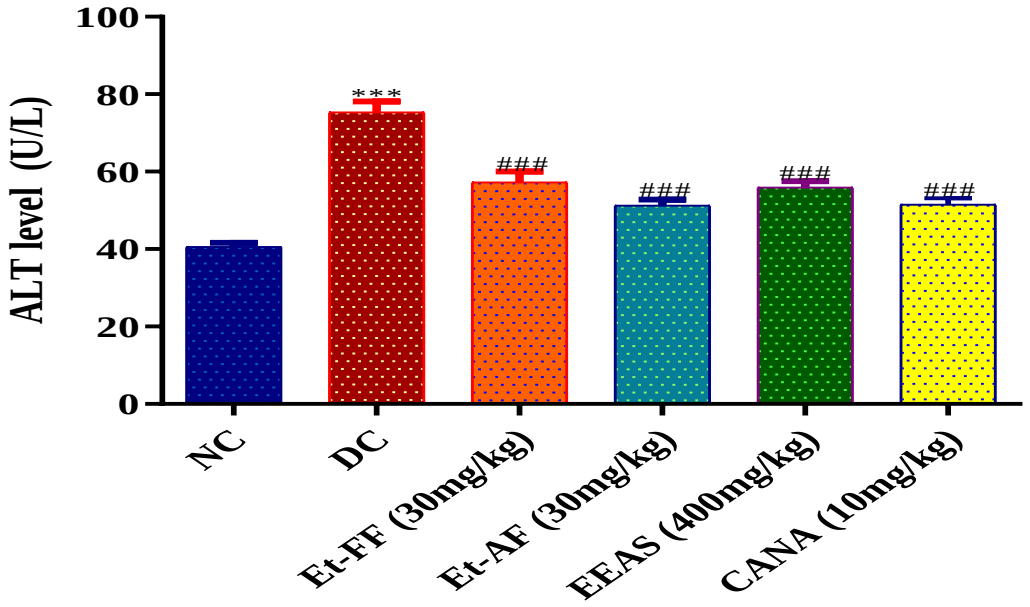


Figure 4.41: Effect of Et-AF and Et-FF on serum ALT level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The results of the ALT values of all groups are presented in Figure 4.41 (at week 12). The serum ALT level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). Significant ($p<0.001$) reduction in ALT levels in the Et-AF, Et-FF, and EEAS treatment groups compared to diabetic rats. In the standard group, canagliflozin significantly reduced serum ALT levels compared to the DC group.

P). Effect of Et-AF and Et-FF on serum CK-MB level

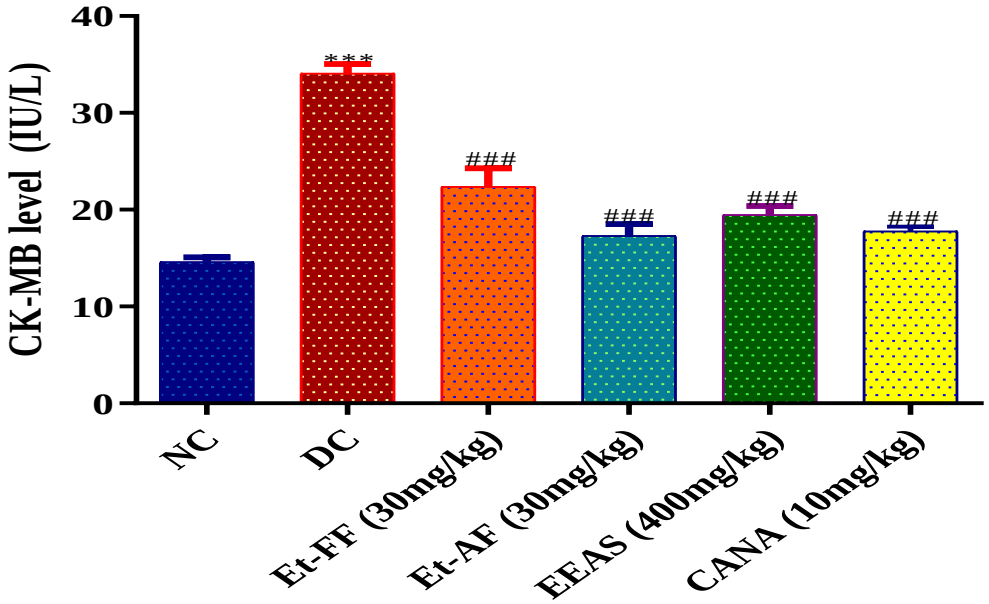


Figure 4.42: Effect of Et-AF and Et-FF on serum CK-MB level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Data on cardiac damage markers such as serum CK-MB levels of all groups are shown in Figure 4.42 (after 12 weeks). The serum creatinine level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the Et-AF and Et-FF treatment groups, CK-MB levels decreased significantly ($p<0.05$) compared to diabetic rats. Also, CK-MB levels decreased significantly in the EEAS treatment group compared to the DC groups. In addition, there was a significant reduction in serum CK-MB in the canagliflozin-treated groups compared to the diabetic rats ($p<0.001$).

Q). Effect of Et-AF and Et-FF on serum LDH level

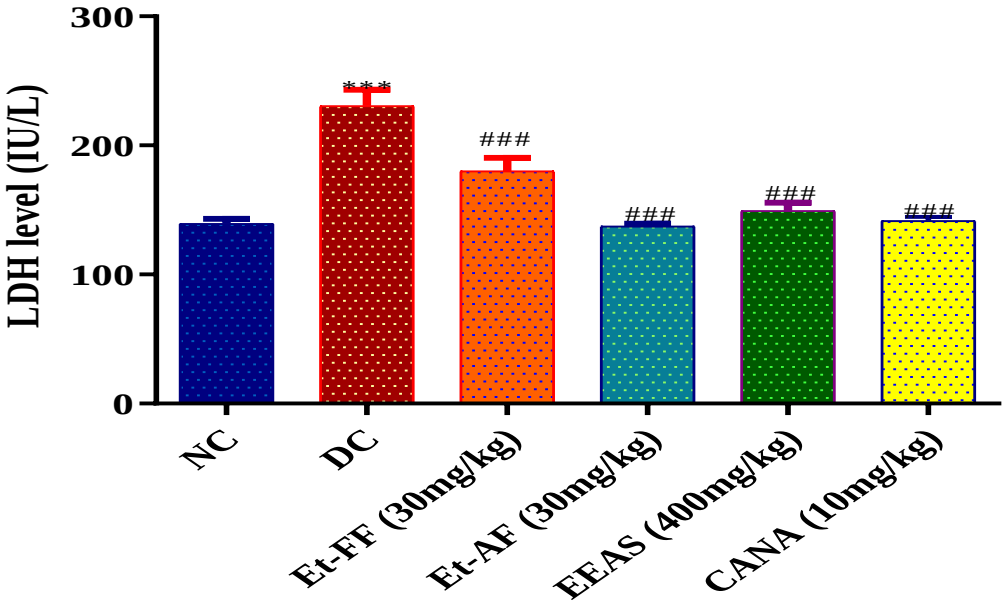


Figure 4.43: Effect of Et-AF and Et-FF on serum LDH level.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The data on the serum LDH levels of all groups are presented in Figure 4.43 (at week 12). The serum LDH level was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In the Et-AF, Et-FF and EEAS treatment group, LDH levels decreased significantly ($p<0.001$) compared to diabetic rats. There was a significant reduction ($p<0.001$) in serum LDH levels in the canagliflozin treated groups compared to the DC group.

R). Effect of Et-AF and Et-FF on systolic blood pressures

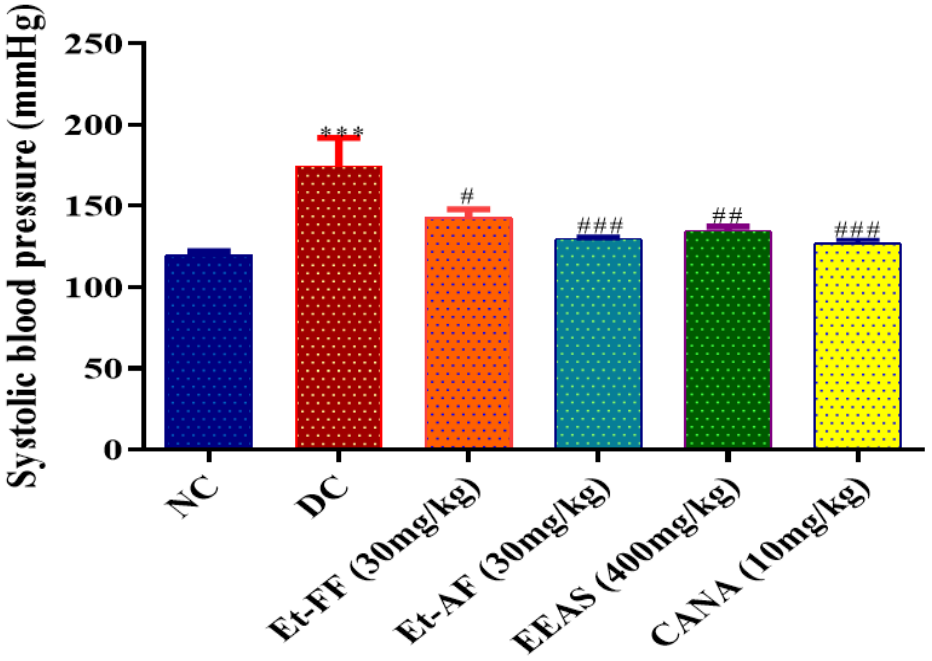


Figure 4.44: Effect of Et-AF and Et-FF on systolic blood pressures.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

At the end of the study, systolic blood pressure (SBP) was significantly increased in diabetic rats compared to the NC group ($p<0.001$) (Figure 4.44). The Et-AF ($p<0.001$), Et-FF ($p<0.05$), and EEAS ($p<0.01$) treatment groups significantly improved systolic pressure compared to the DC group. In addition, the canagliflozin-treated groups significantly reduced SPB compared to the diabetic rats ($p<0.001$).

S). Effect of Et-AF and Et-FF on diastolic blood pressures

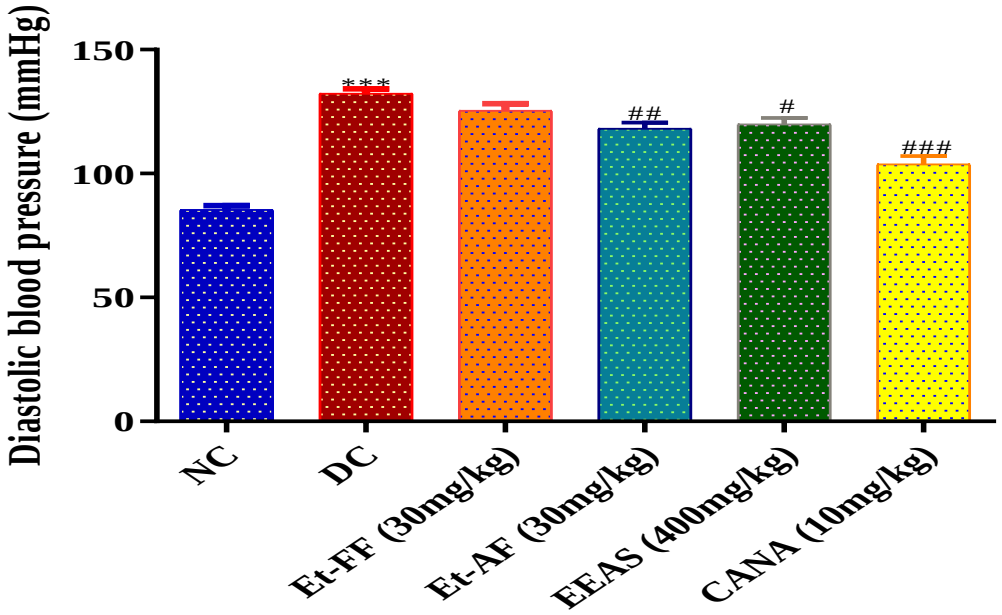


Figure 4.45: Effect of Et-AF and Et-FF on diastolic blood pressures.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Diastolic blood pressure (DBP) was significantly increased in diabetic rats compared to the NC group ($p<0.001$) (Figure 4.45). An improvement in diastolic blood pressure was observed in the Et-AF treatment groups compared to the diabetic rats. The EEAS treated group significantly ($p<0.05$) reduced the DBP, while Et-FF showed no significant changes compared to diabetic rats. The canagliflozin treated group ($p<0.001$) reduced DPB compared to the DC group.

T). Effect of Et-AF and Et-FF on heart rate

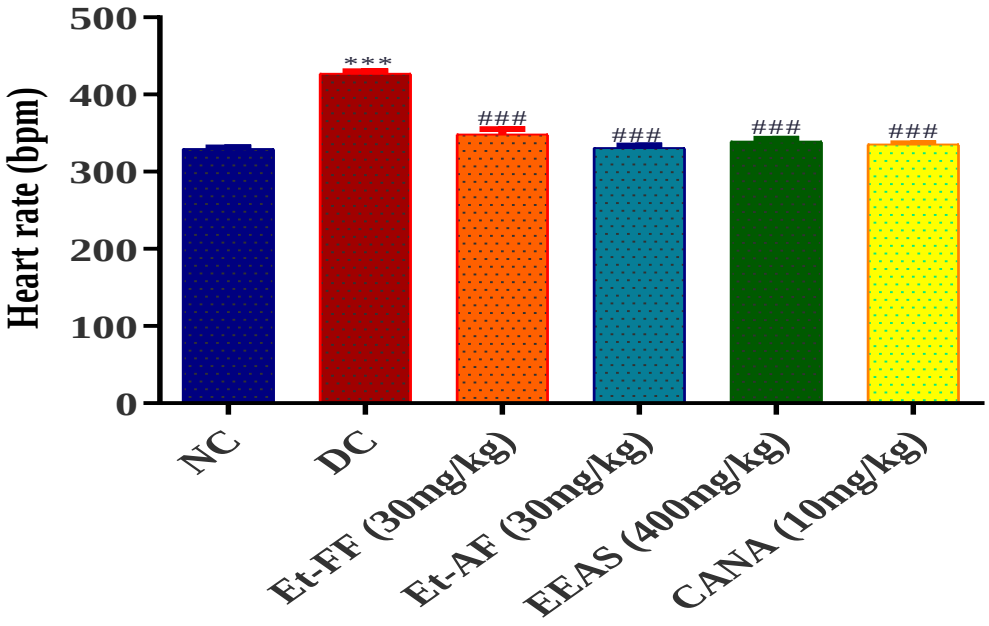


Figure 4.46: Effect of Et-AF and Et-FF on heart rate.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozine (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p< 0.001$ vs. DC.

Heart rate (HR) data from all groups are shown in Figure 4.46 (at week 12). Heart rate was significantly increased in diabetic rats compared to the NC group ($p<0.001$). In both Et-AF and Et-FF treatment group, HR decreased significantly ($p<0.001$) compared to diabetic rats. A significant ($p<0.001$) decrease in heart rate was observed in the EEAS treatment group. Significant ($p<0.001$) improvement in HR of the canagliflozin treated groups compared to the diabetic rats.

U). Effect of Et-AF and Et-FF on PR Interval

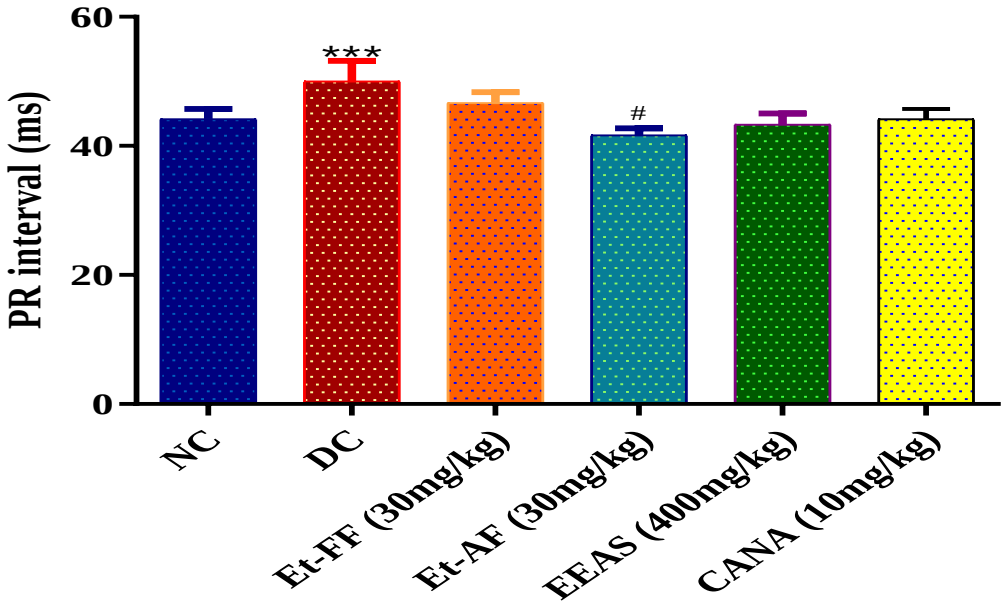


Figure 4.47: Effect of Et-AF and Et-FF on PR Interval.
NC, Normal control (n=6); DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6); Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6); Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

Data on the effect of PR intervals on all treated and non-treated groups are presented in Figure 4.47 (at week 12). A prolongation of the PR interval was observed in diabetic rats. Et-FF and EEAS treatment groups improve the PR interval compared to diabetic rats. A significant shortening of the PR interval was observed in the Et-AF treated group compared to the diabetic rats ($p<0.05$). Canagliflozin treatment also improves the PR interval compared to the DC group.

V). Effect of Et-AF and Et-FF on QRS complex

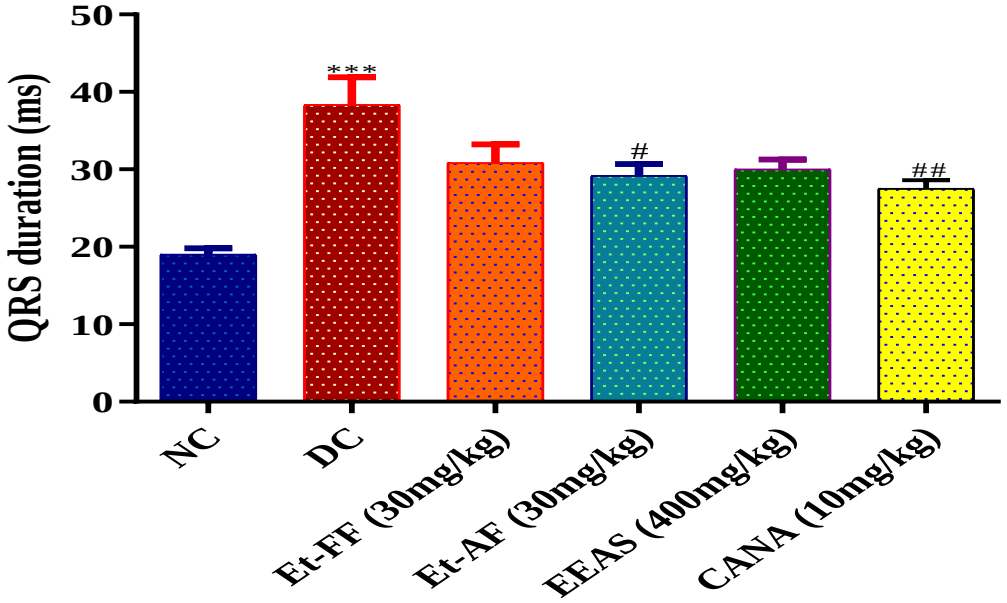


Figure 4.48: Effect of Et-AF and Et-FF on QRS complex.
NC Normal control (n=6), DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6), Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400 mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The QRS complex results of all treatment and non-treatment groups are shown in Figure 4.48 (at week 12). The QRS complex was significantly increased in diabetic rats compared to the NC group ($p<0.01$). In the Et-AF treatment group, QRS duration decreased significantly ($p<0.05$) compared to diabetic rats. In addition, the Et-FF and EEAS treatment groups showed an improvement in the amplitude of the QRS complex. The amplitude of the QRS complex was significantly ($p<0.01$) improved in the canagliflozin treated group compared to the diabetic rats.

W). Effect of Et-AF and Et-FF on QT interval

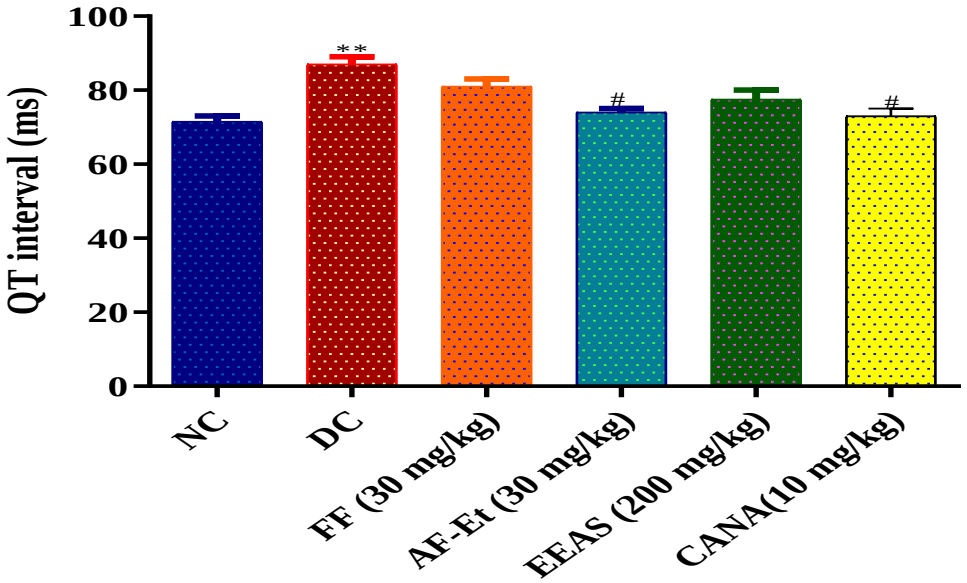


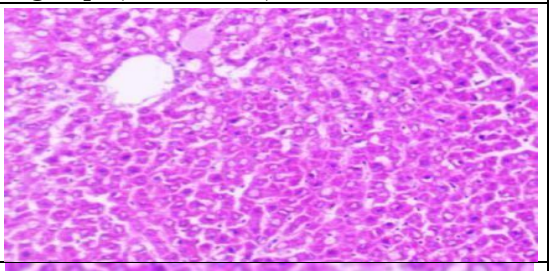
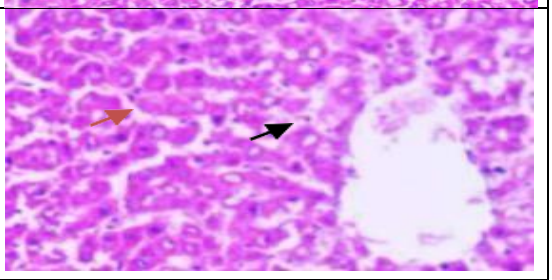
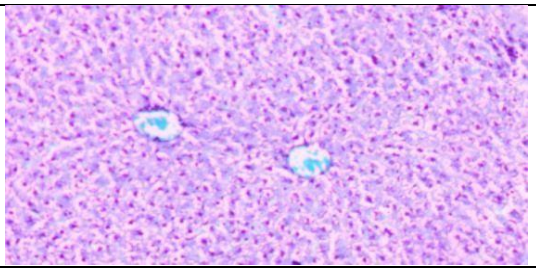
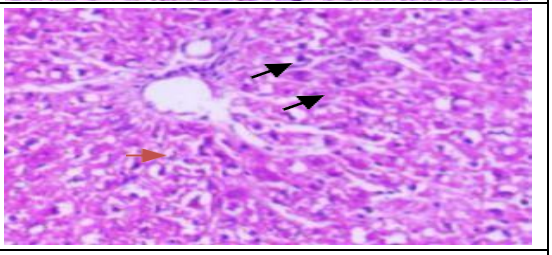
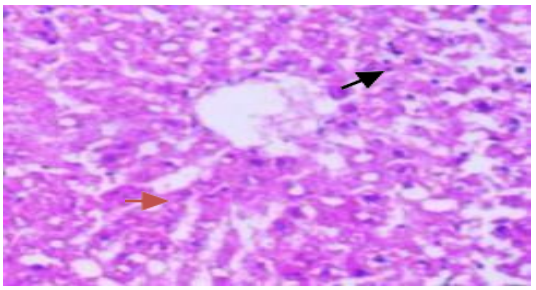
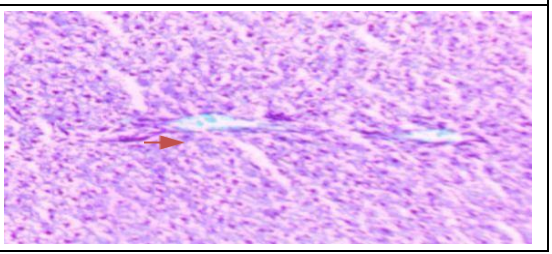
Figure 4.49: Effect of Et-AF and Et-FF on QT interval.
NC Normal control (n=6), DC, Disease control (n=10), CANA, Cangliflozin (10 mg/kg) (n=6), Et-AF, Alkaloid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), Et-FF, Flavonoid fraction of Ethanol extract of *A. scholaris* (30 mg/kg) (n=6), and EEAS, Ethanol extract of *A. scholaris* (400mg/kg) (n=6). Data represent the mean ± SEM. $^{*}p<0.05$, **p<0.01 and ***p<0.001 vs. NC, $^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p< 0.001 vs. DC.

The QT interval results of all groups are shown in Figure 4.49 (at week 12). Significantly increased QT interval in diabetic rats compared to NC group (p<0.01). The Et-AF treatment group significantly improved QT intervals compared to diabetic rats (p<0.05). In addition, the EEAS and Et-FF treatment group shows a shortening of the QT intervals. The standard group canagliflozin significantly shortened the QT intervals in the treated groups compared to the diabetic rats (p<0.05).

X). Histopathology

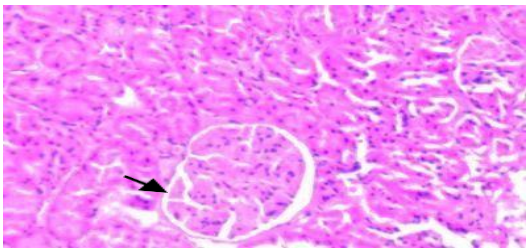
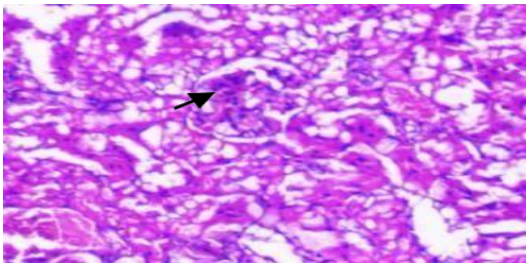
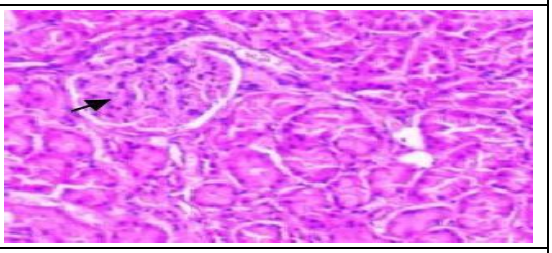
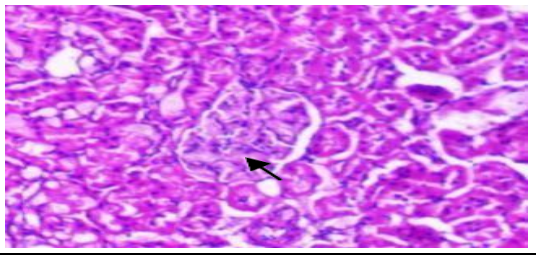
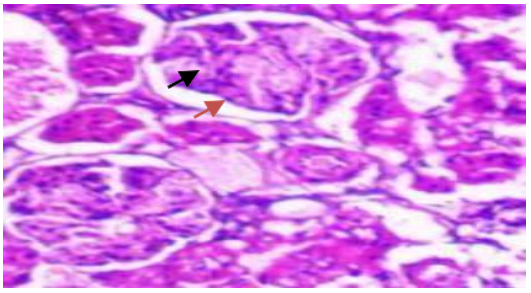
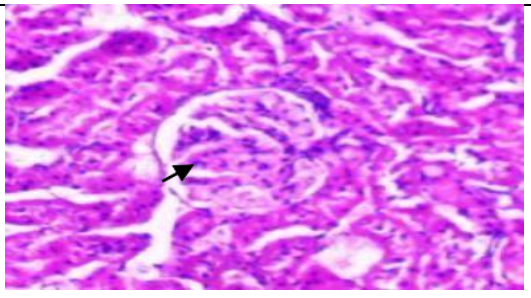
i).Effect of different groups on the histological profile of the Liver of a STZ-induced diabetic treated wistar rat.

Table 4.15: Histological sections of rat liver from different groups (H and E x10).

A.	**NC group** showed normal hepatic cords and portal triad.	
B.	**DC group** representing increase vacuolation in the cytoplasm of hepatocytes appeared as indistinct clear vacuoles (black arrow) and activation of Kupffer cells (red arrow).	
C.	**CANA group** shows improved portal vein triad, central vein.	
D.	**EEAS group** shows mild hydropic degeneration (black arrow) and mild hepatic sinusoid congestion (red arrow).	
E.	**Et-FF group** shows mononuclear cellinfiltration (black arrow), and cytoplasmic vacuolation of hepatocytes (red arrow).	
F.	**Et- AF** treated group showing areas of leukocyte aggregations (red arrow) and an area of portal tract with dilated obstructed portal vein (black arrow)	

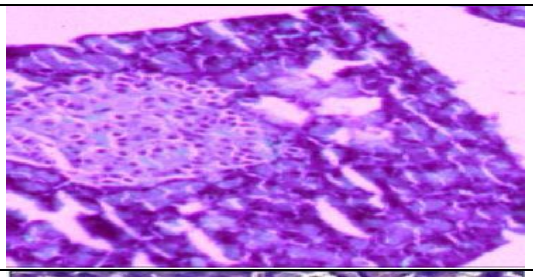
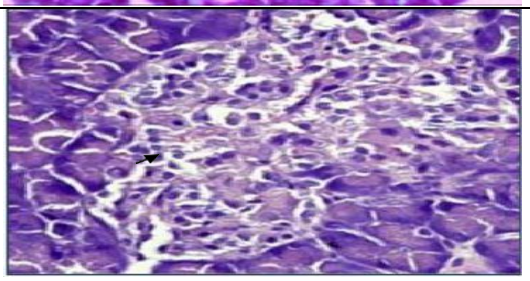
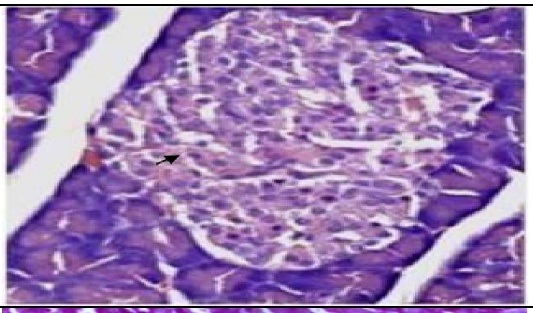
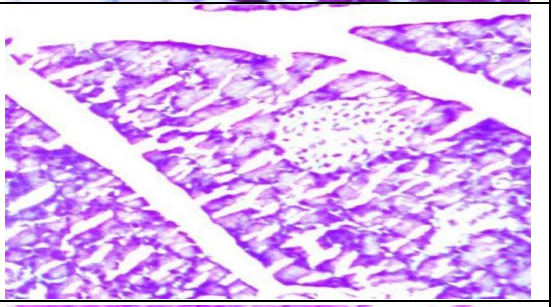
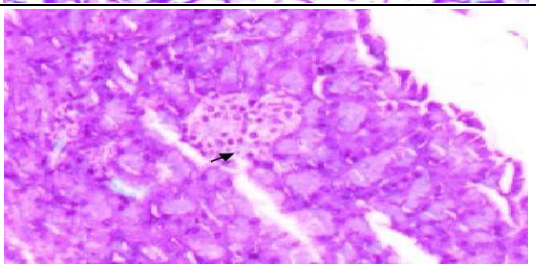
ii). Effect of different groups on the histological profile of the kidney of a STZ-induced diabetic treated wistar rat.

Table 4.16: Histological sections of rat kidney from different groups (H and E x10).

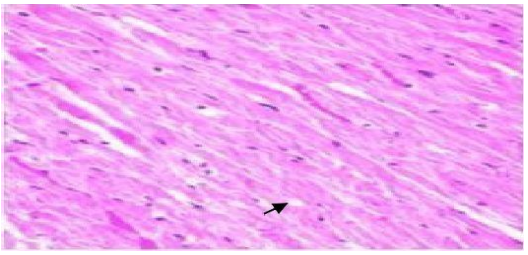
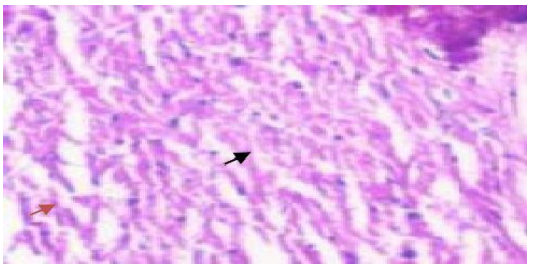
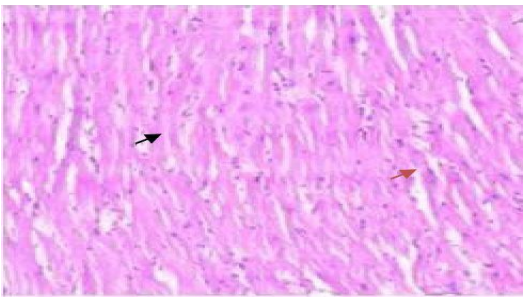
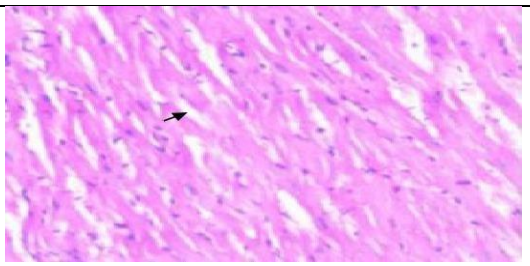
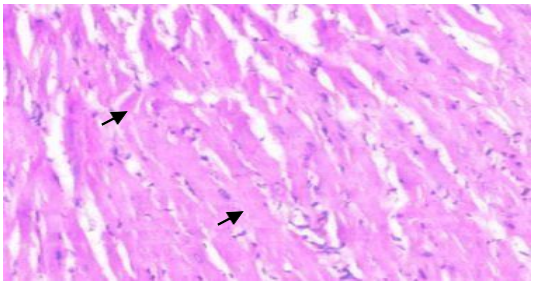
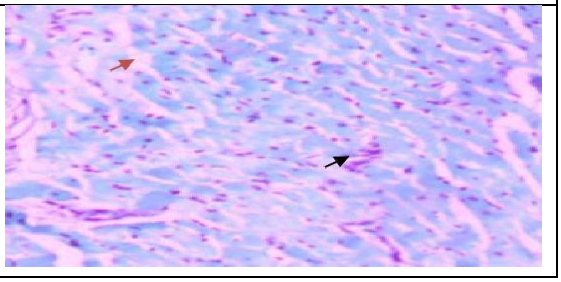
A.	**NC group** showing normal glomeruli with normal baseline and tubules (black arrow).	
B.	**DC group** representing disrupted glomeruli with baseline fat deposition along with infiltration of lymphocytes (black arrow).	
C.	**CANA group** with improved vascular system (black arrow) and less intestinal infiltration.	
D.	**EEAS group** with improved glomeruli with normal baseline (black arrow).	
E.	**Et-FF group** with glomerular lymphocytic infiltration (black arrow) thickening of basement membrane (red arrow).	
F.	**Et-AF** group showing improved vasculature and glomeruli (black arrow).	

iii) Effect of different groups on the histological profile of the pancreas of a STZ-induced diabetic treated wistar rat.

Table 4.17: Histological sections of rat pancreas from different groups (H and E x10).

A.	**NC group** representing normal islet of Langerhans	
B.	**DC group** showing extensive degranulation, destruction of cells, presence of necrotic cells and inflammatory cells (black arrow).	
C.	**CANA group** showing improved pancreatic islets of Langerhans with beta cell enhancement (black arrow).	
D.	**EEAS group** showing recovery of normal Langerhans islet cell.	
E.	**Et-FF group** representing abnormal islets of Langerhans.	
F.	**Et-AF group** showing improvement in islet of Langerhans morphology with increasing number of beta cells (black arrow).	

iv). Effect of different groups on the histological profile of the heart of a STZ-induced diabetic treated wistar rat.

Table 4.18: Histological sections of rat heart from different groups (H and E x10)		
A.	**NC group** showing well-arranged cardiomyocytes and normal streaks.	
B.	**DC group** depicting disordered cardiac myocytes with enlarged interstitial space (red arrow) and extensive degeneration in cardiac muscle (black arrow);	
C.	**CANA group** showing normal heart tissue with enhanced features of myocytes, lessvisualization of the interstitial space (red arrow)and distortion of the intercalated disc (black arrow).	
D.	**EEAS display** mild space in intercalated disc (black arrow).	
E.	**Et-FF** Display less interstitial space and intercalated disc (black arrow).	
F.	**Et-AF group** showing the mild interstitial space (black arrow) and few mayocardial cell degeneration (red arrow).	

4.1.7 Antioxidant analysis

A). Effect of Et-AF, Et-FF and EEAS on SOD antioxidant activity

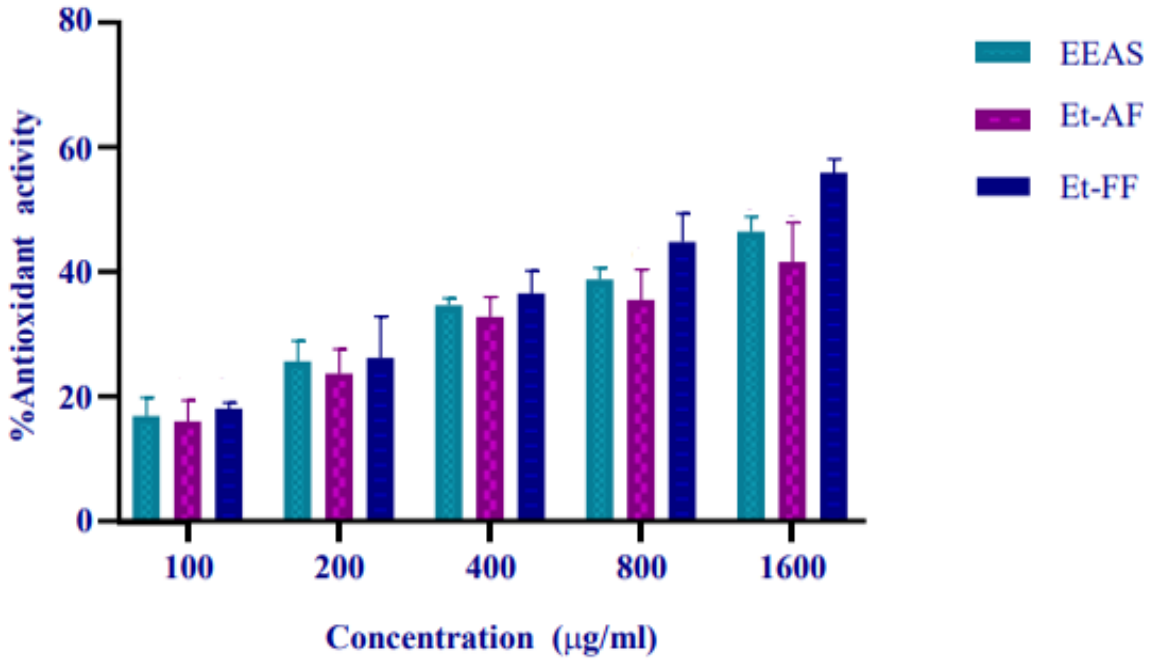


Figure 4.50: Effect of Et-AF, Et-FF and EEAS on SOD antioxidant activity.
Results are expressed in Mean ± SEM for three treatment groups at each concentration

The results of superoxide dismutase (SOD) scavenging activity of Et-AF, Et-FF and EEAS are shown in Figure 4.50. The percent antioxidant activity of these three drugs was analyzed at a dose of 100,200, 400, 800, 1,600 g/ml goat liver. There was a dose-dependent increase in percent antioxidant activity at all concentrations tested. It was observed that the Et-AF, Et-FF and EEAS treatment group had significant SOD scavenging property.

B). Effect of Et-AF, Et-FF and EEAS on catalase antioxidant activity

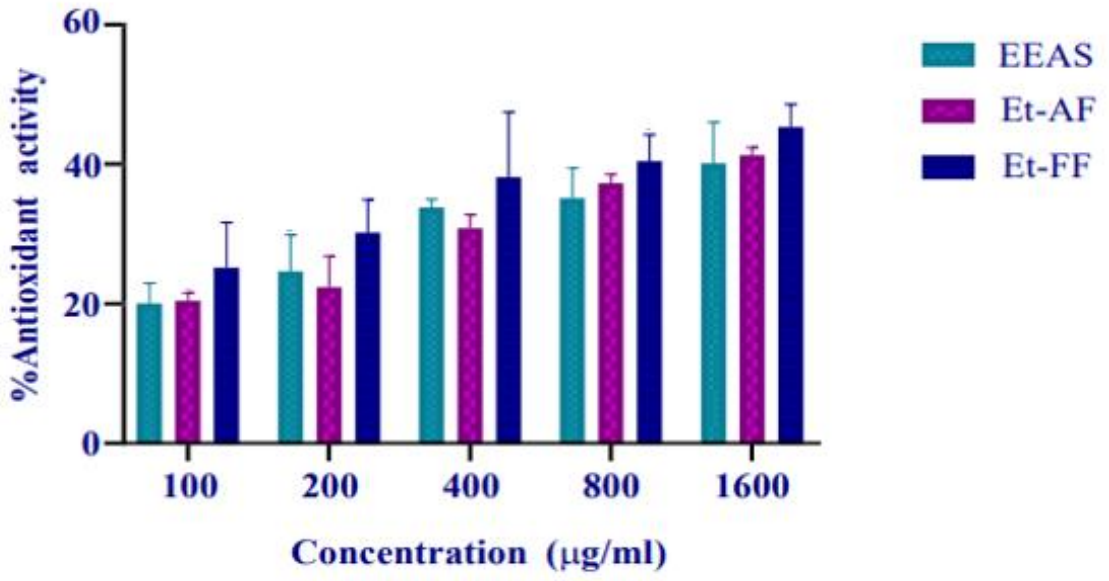


Figure 4.51: Effect of Et-AF, Et-FF and EEAS on catalase antioxidant activity.
Results are expressed in Mean ± SEM for three treatment groups at each concentration.

The results of catalase (CAT) scavenging activity from all three treatment groups are summarized in Figure 4.51. The percent antioxidant activity of these treatment groups was analyzed at a dose of 100,200, 400, 800, 1,600 µg/ml goat liver. The results show that the Et-AF, Et-FF and EEAS treatment group had significantly increased CAT scavenging property with increasing doses.

C). Effect of Et-AF, Et-FF and EEAS on glutathione antioxidant activity

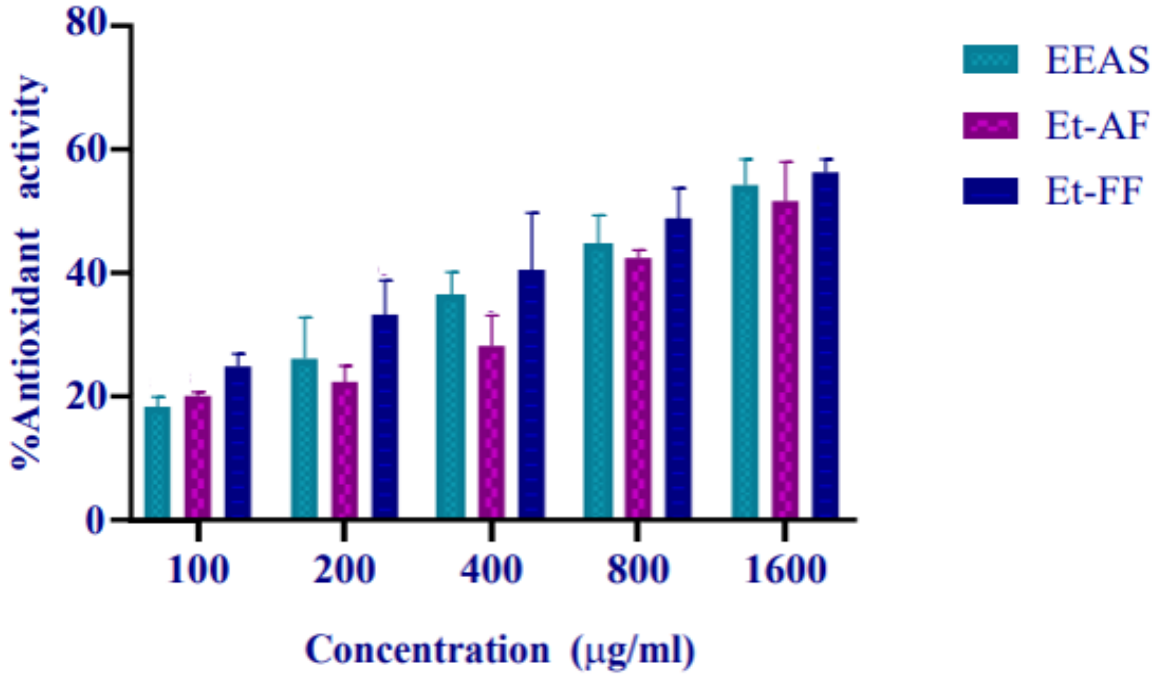


Figure 4.52: Effect of Et-AF, Et-FF and EEAS on glutathione antioxidant activity.
Results are expressed in Mean ± SEM for three treatment groups at each concentration.

All glutathione (GTH) scavenging activity data for all three treatment groups are summarized in Figure 4.52. The results show that the Et-AF, Et-FF and EEAS treatment group had significantly increased glutathione antioxidant activity with increasing doses.

These results suggest that both alkaloid and flavonoid fractions and ethanol extract from *A. scholaris* leaves have antioxidant potential by reducing the oxidative degradation of superoxide dismutase, catalase and glutathione.

4.1.8 Comparative study between EEAS, Et-AF and Et-FF parameters

Table 4.17: Comparison between EEAS, Et-AF and Et-FF parameters.

S.no.	PARAMETERS	DC	Et-FF	Et-AF	EEAS
1.	Weight of rats (gm)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
2.	Food intake (gm/rat/day)	↑↑↑	↓↓	↓↓	↓
3.	Water intake (ml/rat/day)	↑↑↑	↓↓↓	↓↓	↓↓
4.	FBG (mg/dl)	↑↑↑	↓↓↓	↓↓↓	↓↓↓
5.	Urine output (ml/rat/day)	↑↑↑	↓	↑↑↑	↑↑↑
6.	Urine glucose level (mg/dl)	↑↑↑	↓	↑↑↑	↑↑↑
7.	Cholesterol (mg/dl)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
8.	Triglyceride (mg/dl)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
9.	LDL (mg/dl)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
10.	VLDL (mg/dl)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
11.	HDL (mg/dl)	↓↓↓	↑	↑↑↑	↑↑↑
12.	Urea (mg/dl)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
13.	Creatinine (mg/dl)	↓↓↓	↑	↑↑	↑↑↑
14.	AST (IU/L)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
15.	ALT (IU/L)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
16.	CK-MB(IU/L)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
17.	LDH (IU/L)	↓↓↓	↑↑↑	↑↑↑	↑↑↑
18.	SBP (mmhg)	↑↑↑	↓	↓↓↓	↓↓
19.	DBP (mmhg)	↑↑↑	↓	↓↓↓	↓
20.	HR (beats per min)	↑↑↑	↓↓↓	↓↓↓	↓↓↓
21.	PR interval (ms)	↑	↓	↓	↓
22.	QRS complex (ms)	↑	↓	↓	↓

Et-AF=Alkaloid fraction of ethanolic extract of *A. scholaris* (30mg/kg), Et-FF=Flavonoid fraction of ethanolic extract of *A. scholaris* (30mg/kg), and EEAS=Ethanol extract of *A. scholaris* (400mg/kg). Data represent the mean ± SEM. ↓ $p<0.05$, ↓↓ $p<0.01$ and ↓↓↓ $p< 0.001$ vs. DC.

- Et-AF, Et-FF and EEAS demonstrated significant anti-hyperglycemic effect.
- Et-AF and EEAS showed an improvement in HDL level and also an increase in urinary glucose excretion (compared to Et-FF).
- Improvement in renal and cardiac functions (compared to Et-FF and EEAS).

CHAPTER –5

Discussion

The discovery of the new natural antidiabetic drugs could hold promise for the world currently searching for better treatments for diabetes, as the efficacy and safety concerns of current antidiabetic drugs are minimal (142). In this context, research into the phytochemical substance responsible for the antidiabetic effect has made progress in recent decades. The antidiabetic effect of herbal substances is attributed to the secondary metabolites of plant extracts. Our present study aimed to investigate the effect of *Alstonia scholaris* leaves on type 2 diabetes mellitus related to its complications in rats. Current research, demonstrated the antidiabetic effects of the active extract and its fractions from *A. scholaris* leaves and maintain glucose homeostasis using a primary test (OGTT) and a pilot model i.e. High Fat Diet (HFD) and low-dose STZ type 2 diabetes model on male wistar rats.

Phytochemical study of herbal medicines, both qualitative and quantitative analyses support differentiation and stability assessment (143). Qualitative phytochemical analysis of four extracts of *Alstonia scholaris* leaves (PEEAS, CHEAS, EAEAS and EEAS) was performed. EEAS showed presence of alkaloids, flavonoids, tannins, terpenoid phenols and steroids. While EAEAS showed the presence of alkaloids, flavonoids, and steroids, CHEAS includes phenols, terpenoids, and steroids, but PEEAS does not contain any other constituents except fatty oils and fats. The presence of these components in the extracts of the plants studied strongly supports their beneficial effect in the treatment of diabetes (144).

Taking into account the phytochemical analysis, three extracts (CHEAS, EAEAS, and EEAS) wereselected for the primary test i.e. oral glucose tolerance test (OGTT). It is a test of the body's ability to metabolize glucose and determine the level of glucose in the blood at various time intervals (145). Blood sugar-lowering drugs work by directly altering the action of glucagon and insulin. Comparisons of glucose values during the glucose tolerance test at different time points, with and without drugs, lead to a better understanding of the benefit of the study drugs in diabetes. The oral glucose tolerance test (OGTT) is a widely used method for diagnosing diabetes and intermediate stages of hyperglycaemia (146). In this test, the blood glucose level and the insulin level provide information about the insulin secretion ability of the pancreatic cells and the insulin sensitivity of the tissue (147). Therefore, OGTT can also be used to assess

cell function and insulin resistance (148). OGTT is a rapid, convenient, inexpensive, and reproducible method for assessing the hypoglycaemic effect of drugs in normal animals. It helps in selecting an effective drug dose for chronic model studies. It was observed that EEAS and EAEAS at a dose of 400 mg/kg are effective as anti-hyperglycemic agents in the primary OGTT test, supporting the potential of these extracts for further pilot studies.

In order to compare the anti-hyperglycaemic effects of both extracts, the model with HFD-fed rodents injected with a low dose of STZ-induced type 2 diabetes mellitus was preferred for the study. This model accelerates the time course of type 2 diabetes by inducing a state of obesity-associated insulin resistance over a period of HFD feeding followed by injection of STZ to deplete pancreatic beta cells. A few days after STZ administration, animals showed slightly decreased plasma insulin levels, slightly increased plasma glucose levels and decreased pancreatic insulin levels (149, 150). The β-cells of STZ rats show similarity to the insulin secretion properties of type 2 diabetics (151). Therefore, this model is valuable for evaluating T2DM treatments. It is estimated that the global prevalence of diabetes will reach 9% by 2030 (152), with the world population projected to be 8.5 billion, suggesting that around 690 million people will suffer from T2DM (153). Although treatment of T2DM is essential for management of microvascular complications and is also reduced if done shortly after diagnosis of diabetes (154, 155). Here, our current approach is to treat hyperglycaemia in long-term diabetes with diabetes-associated complications or multiple risk factors for cardiovascular disease (CVD) and other associated complications. Glucose transport is known to play a crucial role in maintaining glucose homeostasis (156), and several natural SGLT2 inhibitors have been characterized as having utility in the management of diabetes (157). SGLT2 inhibition decreases renal glucose reabsorption and improves plasma glucose concentration (158). In addition, this class of drugs has the unique property of inducing weight loss, which may also be effective against hyperlipidaemia and metabolic disorders (159). There is growing interest in natural products that can be used to inhibit SGLT2. Therefore, there is a drive to develop therapeutic agents with novel mechanisms of action that are expected to fill unmet needs of available therapies and improve their outcomes.

In this T2DM model experiment, diabetic rats steadily lose weight due to an increase in blood glucose levels, loss of tissue proteins (gluconeogenesis), and increased muscle wasting (160). EAEAS improved weight loss in diabetic rats because it offers opportunities to restore protein metabolism. Similarly, the insignificant improvement in weight loss of EEAS compared to diabetic rats could be due to mild osmatic diuresis and a reduction in fat mass attributed to

energy loss from urinary glucose excretion. High protein catabolism and hyperosmolarity due to high blood glucose levels promote polyphagia and polyuria in diabetic rats (161). EEAS improve polyphagia and polydipsia due to improved glycaemic control. In urinalysis, EEAS treatment showed no significant effect on urinary output, while urinary glucose concentration increased, which is close to that of canagliflozin in diabetic rats. EAEAS lowers urinary glucose levels by lowering FBG levels. Ethanol extract has been observed to significantly lower blood sugar levels and improve glucose homeostasis. Consequently, the anti-hyperglycaemic effect of EEAS can be attributed to increased urinary glucose excretion. Irregularities in lipid metabolism lead to atherosclerosis, which is more common in people with diabetes mellitus (162). In this present experiment, EEAS significantly decreased T-CH, TG, LDL and VLDL levels and improved HDL levels compared to diabetic rats. The improved lipid profiles of the ethanol extract reflect its effectiveness in preventing cardiovascular problems that can result from diabetes (163). EEAS have an anti-hyperlipidemic effects based on an increase in lipoprotein lipase and decreased HMG-Co-A reductase activity (164). While in EAEAS, a slight improvement in lipid parameters was observed. This suggests that ethanol extract of *A. scholaris* leaves supports the lipid-lowering property. In addition, a significant improvement in general and biochemical parameters was observed with the standard drugs metformin and canagliflozin.T2DM accelerates liver fibrosis and inflammation, leading to more severe liver failure (165). Long-term high blood sugar levels damage the millions of tiny filter units in each kidney, leading to kidney failure (166). Excessive use of certain herbs can be harmful to the liver and kidneys (167). Alanine aminotransferase and aspartate aminotransferase are two reliable liver indicators. Streptozotocin induces liver toxicity in diabetic control rats due to the leakage of these protein enzymes from the liver cytosol into the blood flow region (168). Therefore, we studied the effect of both extracts on kidney and liver function in diabetic rats. EEAS restores levels of these enzymes damaged by STZ. Creatinine and urea are the marker of kidney damage, which were significantly reduced by EEAS treatment in diabetic rats. It also significantly improves blood pressure and heart rate. A previous study on type 2 diabetes reported an increased prevalence of minor (from7.5% to 12.5%) and major (from 23% to 37.0%) ECG abnormalities (169). ECG abnormalities in people with T2DM, resulting in ventricular conduction abnormalities, arrhythmias, and unrecognized ischemic heart disease lead to diabetic cardiomyopathy (170, 171). The PR interval represents the conduction time between atrial excitation to ventricular excitation while the QT interval is the time between ventricular depolarization to ventricular repolarization (172).

QT interval prolongation is afundamental manifestation of autonomic neuropathy and helps diagnosis of diabetics who are at risk of cardiac death (173). In the current experiment, aprolongation of the PR and QT intervalwas observed in diabetic rats due to myocardial damage, which returns to normal after EEAS treatment. In addition, the QRS duration indicates the rate of rapid depolarization. Diabetic animals show a shortening of the QRS duration due to damage to the cardiomyocytes (oxidative stress) (174). Ethanol extract improves these conditions significantly better than ethyl acetate extract. Histopathological examination of the liver showed improvement in portal vein triad andmild hydropic degeneration. EEAS treatment also improved the vasculature and glomeruli. It restored damage, increased beta cells and improved islet of langerhans morphology. Cardiac histology shows small interstitial spaces and a distorted disc. It suggest that ethanol extract at a dose of 400 mg/kg has a protective effect on type 2 diabetes associated with its complications ascompared to EAEAS groups.

The anti-hyperglycemic effects of EEAS may be due to the presence of alkaloids and flavonoids. Literature-Based Analysis: The most abundant and diverse group of secondary metabolites are alkaloids. They show antidiabetic activity by inhibiting enzymes (α-amylase, α-glucosidase, aldose reductase, dipeptidyl peptidase-IV and protein tyrosine phosphatase-1B); inhibition of advanced glycation end products; Increase in insulin secretion and its sensitivity; improving glucose uptake; and its antioxidant activity (175). Also, flavonoids can have a beneficial effectin diabetes by increasing insulin secretion, reducing apoptosis and promoting pancreatic cell proliferation, inflammation and oxidative stress in muscles and fat, improving hyperglycaemia, regulating glucose metabolism in hepatocytes, reducing insulin resistance and increase glucose uptake in skeletal muscle and white adipose tissue (176).

Several studies have examined the anti-hyperglycemic effects of active phytochemicals from plant products, which mainly include phytochemicals such as alkaloids and flavonoids (141, 177). Here, the leaves of *A. scholaris* reflected the presence of these components. Therefore, the alkaloid fraction (Et-AF) and the flavonoid fraction (Et-FF) were fractionated from active ethanol extract of *A. scholaris* leaves (178).

In the dose optimization study (OGTT), both fractions were administered at doses of 15, 30, 60, and 90 mg/kg p.o. the result of this study showed marked hypoglycemic activity of Et-AF (30 mg/kg) and Et-FF (30 mg/kg) by improving the glucose tolerance test in normoglycemic rats.

As no significant benefit was observed in these fractions with dose increases above 30 mg/kg, therefore, both fractions at a dose of 30 mg/kg were selected for further pilot study.

Subsequently, a study was conducted to evaluate the anti-diabetic activity of alkaloid and flavonoid fractions of ethanol extract from *A. scholaris* leaves in HFD+STZ-induced T2DM rats. A high-fat diet leads to increasing hyperinsulinemia, indicating a progressive worsening of insulin resistance (179). The results of the study showed a significant reduction in food intake and improved water intake in both treated fractions and canagliflozin compared to the diabetic rats. They also showed an improvement in body weight in diabetic rats and the results were comparable to those of the standard drug canagliflozin. After 4 weeks of Et-AF and Et-FF treatment, a significant decrease in fasting blood glucose was observed compared to STZ-induced diabetic rats. In urinalysis, Et-AF treatment showed an insignificant effect on urinary output, while urinary glucose concentration increased significantly compared to diabetic rats. The possible mechanism of the antidiabetic effect of Et-AF could be that the excretion of glucose in the urine is increased because the reuptake of glucose from the nephron is inhibited. A marked decrease in triglycerides, total cholesterol, LDL and VLDL was observed, while an increase in HDL cholesterol was observed in both fractions of the treated diabetic rats. Both liver (ALT and AST) and renal damage marker enzymes (urea and creatinine) were elevated by these fractions in the vicinity of canagliflozin. In blood pressure and ECG reports, the alkaloid fraction showed a better effect compared to flavonoid fractions. Histopathological examination of the liver showed improvement in portal vein triad and mild hydropic degeneration. Et-AF treatment improved the vasculature and glomeruli. It restores damage and improves the morphology of the islets of Langerhans as the number of beta cells increases. Cardiac histology shows less interstitial space and less degeneration of myocardial cells. The group treated with Et-FF showed mononuclear cell infiltration and cytoplasmic vacuolation of hepatocytes. A slight glomerular lymph infiltration is reflected in the kidney and a slight improvement in the morphology of the islets of langerhans was observed in the pancreas. In addition, less displacement in the interstitial space and in the intervertebral disc was observed in cardiological histology. These experimental data suggest that Et-AF and Et-FF exert an anti-hyperglycaemic effect at a dose of 30 mg/kg. They also have a protective effect against liver, kidney and cardiovascular complications. Further research into these alkaloid and flavonoid fractions will help to advance a new drug for mankind.

Goat liver slices are described as a valuable tool for in vitro metabolism studies of potential drugs (180). In the current study, the protective effects of *A. scholaris* in vitro against hydrogen peroxide-induced oxidative stress were evaluated using precision-cut goat liver slices as

adjuvants. Enzymatic and non-enzymatic antioxidants were studied in liver slices exposed to oxidative stress in the presence of *A. scholaris* leaves. It has several defence systems consisting of enzymes and radical scavengers. They are referred to as the first-line antioxidant defence system. As an antioxidant, superoxide dismutase helps protect against cell destruction by superoxide radical anions, which are formed by the transfer of an electron to molecular oxygen (181). It is responsible for both direct damage to biological macromolecules and the generation of other reactive oxygen species. SOD keeps the concentration of superoxide radicals at a low level and therefore plays an important role in defence against oxidative stress (182). It was found that the SOD value was increased in the treatment group compared to the toxic group. Catalase is a heme-containing enzyme found in most cells that catalyzes the breakdown of hydrogen peroxide into water and oxygen. Cytosolic catalase was found to be important for the inactivation of many environmental mutagens (183). As in the positive control group, catalase levels were elevated to a near-normal range. Glutathione peroxidase is a well-known first line of defence against oxidative stress, which in turn requires glutathione as a cofactor (184). It catalyzes the oxidation of glutathione (GSH) to glutathione disulphide (GSSG) at the expense of H_2O_2. It is found in both the cytosol (70%) and mitochondria (30%) of various tissues. The level of this enzyme was reduced in the toxic group compared to the vehicle control (185). It was observed that the concentrations of enzymatic antioxidants were increased in the treatment groups Et-AF, Et-FF and EEAS compared to vehicle control. Our reports indicate the protective effect of *A. scholaris* leaves against H_2O_2-induced oxidative stress. It is responsible for both direct damage to biological macromolecules and the generation of other reactive oxygen species.

In summary, our results demonstrated anti-hyperglycemic activity of selected plant extracts, alkaloid and flavonoid fractions of ethanol extract of *A. scholaris* leaves, against both the primary and pilot experimental models and demonstrated cardiovascular protection. It can clearly be seen that the presence of bioactive components such as alkaloids and flavonoids are responsible for lowering blood sugar levels and alleviating the abnormalities associated with them. In addition, it has been observed that Et-AF shows a significant reduction in hyperglycemia, urinary glucose excretion, lipid levels, kidney and liver damage, marker and improvement in blood pressure (ECG) compared to Et-FF and EEAS. Et-AF also reverses the modulation of liver, kidney, pancreas and heart histology. It also improves antioxidant status in goat liver slices exposed to oxidative stress in vitro. The mechanism of action of the anti-hyperglycemic activity could be due to an increased urinary glucose excretion and an antioxidant effect. Therefore, the beneficial anti-hyperglycemic effect is largely attributed to the

presence of secondary plant metabolites, namely alkaloids and flavonoids in the selected ethanol extract of *A. scholaris* leaves.

CHAPTER- 6

Conclusions

6.1 Summary

1. *Alstonia scholaris* plants were authenticated. Various extracts from *A. scholaris* leaves were isolated and characterized. The chloroform, ethyl acetate and ethanol extracts showed the presence of pharmacologically active components.
2. Ethanol and ethyl acetate extracts showed beneficial effects on the regulation of glucose homeostasis in the oral glucose tolerance test at a dose of 400 mg/kg.
3. Ethanol extract (400 mg/kg) showed a significant protective effect in T2DM and its metabolic, renal and cardiovascular complications compared to the disease group.
4. Alkaloid and flavonoid rich fractions were isolated from active ethanol extract of *A. scholaris* leaves and showed a positive effect on improving glucose tolerance and regulating glucose metabolism.
5. The Et-AF fraction showed anti-hyperglycaemic potential in diabetes mellitus associated with metabolic complications at a dose of 30 mg/kg compared to disease group.
6. Et-AF (30 mg/kg) showed a significant reduction in fasting blood glucose levels, an increase in urinary glucose excretion and an improvement in biochemical parameters with inverse modulation of the histology of heart, kidney, pancreas and liver tissues compared to the DC group.
7. Et-AF, Et-FF and ethanol extract also showed significant antioxidant potential in superoxide dismutase, catalase and glutathione antioxidant assay.

6.2 Conclusions

Ethanol extract, Et-AF and Et-FF showed prominent anti-hyperglycemic effect and exerted a protective effect against metabolic, renal and cardiovascular complications. The possible mechanism considered for these protective effects might be an increase in urinary glucose and antioxidant potential, which could lead to an improvement in metabolic activities. However, it is of great interest to explore the mechanism of action at the molecular level in the future.

List of references

1. Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & molecular medicine*, 48(3), e219-e219. https://doi.org/10.1038%2Femm.2016.6
2. Hiriart, M., Velasco, M., Larqué, C., & Diaz-Garcia, C. M. (2014). Metabolic syndrome and ionic channels in pancreatic beta cells. *Vitamins & Hormones*, 95, 87-114. https://doi.org/10.1016/b978-0-12-800174-5.00004-1
3. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B. & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, 183, 109-119. https://doi.org/10.1016/j.diabres.2021.109119
4. Ogurtsova, K., Guariguata, L., Barengo, N. C., Ruiz, P. L. D., Sacre, J. W., Karuranga, S., & Magliano, D. J. (2022). IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. *Diabetes research and clinical practice*, 183, 109-118. https://doi.org/10.1016/j.diabres.2021.109118
5. Liang, Y., Arakawa, K., Ueta, K., Matsushita, Y., Kuriyama, C., Martin, T. & Demarest, K. (2012). Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PloS one*, 7(2), e30555. https://doi.org/10.1371/journal.pone.0030555
6. Matteucci, E., & Giampietro, O. (2000). Oxidative stress in families of type 1 diabetic patients. *Diabetes care*, 23(8), 1182-1186. https://doi.org/10.2337/diacare.23.8.1182
7. Oberley, L. W. (1988). Free radicals and diabetes. *Free radical biology and medicine*, 5(2), 113-124. https://doi.org/10.1016/0891-5849(88)90036-6
8. Lipinski, B. (2001). Pathophysiology of oxidative stress in diabetes mellitus. *Journal of Diabetes and its Complications*, 15(4), 203-210. https://doi.org/10.1016/s1056-8727(01)00143-x
9. Weng, J., Ji, L., Jia, W., Lu, J., Zhou, Z., Zou, D. & Chinese Diabetes Society. (2016). Standards of care for type 2 diabetes in China. *Diabetes/metabolism research and reviews*, 32(5), 442. https://doi.org/10.1002/dmrr.2827
10. Vickers, N. J. (2017). Animal communication: when i'm calling you, will you answer too? *Current biology*, 27(14), R713-R715. https://doi.org/10.1016/j.cub.2017.05.064
11. Gibbons, G. W., & Shaw, P. M. (2012, June). Diabetic vascular disease: characteristics of

vascular disease unique to the diabetic patient. *In Seminars in vascular surgery*. 25 (2), 89-92. WB Saunders. https://doi.org/10.1053/j.semvascsurg.2012.04.005

12. Elujoba, A. A., Odeleye, O. M., & Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *African journal of traditional, complementary and alternative medicines*, 2(1), 46-61. https://doi.org/10.4314/ajtcam.v2i1.31103
13. Swanston-Flatt, S. K., Flatt, P. R., Day, C., & Bailey, C. J. (1991). Traditional dietary adjuncts for the treatment of diabetes mellitus. *Proceedings of the Nutrition Society*, 50(3), 641-651. https://doi.org/10.1079/pns19910077
14. Abubakar, U. S., Abdullahi, S., Ayuba, V., Kaigama, S., Halidu, U. S., & Ayuba, M. K. (2017). Medicinal plants used for the management of diabetes mellitus in Zaria, Kaduna state, Nigeria. *Journal of Pharmacy & Pharmacognosy Research*, 5(3), 156-164.
15. Malviya, N., Jain, S., & Malviya, S. A. P. N. A. (2010). Antidiabetic potential of medicinal plants. *Acta pol pharm*, 67(2), 113-118.
16. Wang, H., Li, N., Chivese, T., Werfalli, M., Sun, H., Yuen, L. & Yang, X. (2022). IDF diabetes atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. *Diabetes research and clinical practice*, 183, 109050. https://doi.org/10.1016/j.diabres.2021.109050
17. Farooq, M. D., Tak, F. A., Ara, F., Rashid, S., & Mir, I. A. (2022). Vitamin B12 deficiency and clinical neuropathy with metformin use in type 2 diabetes. *Journal of Xenobiotics*, 12(2), 122-130. https://doi.org/10.3390/jox12020011
18. Booth, F. W., & Hawley, J. A. (2015). The erosion of physical activity in Western societies: an economic death march. *Diabetologia*, 58, 1730-1734. https://doi.org/10.1007/s00125-015-3617-5
19. Cheng, A. Y., & Fantus, I. G. (2005). Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Cmaj*, 172(2), 213-226. https://doi.org/10.1503/cmaj.1031414
20. Jung, C. H., Jang, J. E., & Park, J. Y. (2014). A novel therapeutic agent for type 2 diabetes mellitus: SGLT2 inhibitor. *Diabetes & metabolism journal*, 38(4), 261-273. https://doi.org/10.4093/dmj.2014.38.4.261
21. Han, S., Hagan, D.L., Taylor, J.R., Xin, L., Meng, W., Biller, S.A., Wetterau, J.R., Washburn, W.N. and Whaley, J.M., 2008. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. *Diabetes*, 57(6), pp.1723-1729.

https://doi.org/10.2337/db07-1472

22. Zhang, Y., Xie, P., Li, Y., Chen, Z., & Shi, A. (2023). Mechanistic evaluation of the inhibitory effect of four SGLT-2 inhibitors on SGLT 1 and SGLT 2 using physiologically based pharmacokinetic (PBPK) modeling approaches. *Frontiers in Pharmacology*, 14, 1142003. https://doi.org/10.3389%2Ffphar.2023.1142003
23. Zeidan Jr, B. S., Boadu, C., Hernandez, A., Frunzi, J., & Adetula, I. (2020). Adverse side effects: empagliflozin-related acute pancreatitis case report. *Cureus*, 12(12). https://doi.org/10.7759/cureus.12325
24. Ravindran, S., & Munusamy, S. (2022). Renoprotective mechanisms of sodium-glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. *Journal of cellular physiology*, 237(2), 1182-1205. https://doi.org/10.1002/jcp.30621
25. Abdel-Wahab, A. F., Bamagous, G. A., Al-Harizy, R. M., ElSawy, N. A., Shahzad, N., Ibrahim, I. A., & Al Ghamdi, S. S. (2018). Renal protective effect of SGLT2 inhibitor dapagliflozin alone and in combination with irbesartan in a rat model of diabetic nephropathy. *Biomedicine & Pharmacotherapy*, 103, 59-66. https://doi.org/10.1016/j.biopha.2018.03.176
26. Gilani, A. H. (2005). Trends in ethnopharmacology. Journal of ethnopharmacology, 100(1-2), 43-49. https://doi.org/10.1016/j.jep.2005.06.001
27. Rahman, M. M., Islam, M. R., Shohag, S., Hossain, M. E., Rahaman, M. S., Islam, F. & Cavalu, S. (2022). The multifunctional role of herbal products in the management of diabetes and obesity: a comprehensive review. *Molecules*, 27(5), 1713. https://doi.org/10.3390/molecules27051713
28 Chakraborty, P. (2018). Herbal genomics as tools for dissecting new metabolic pathways of unexplored medicinal plants and drug discovery. *Biochimie open*, 6, 9-16. https://doi.org/10.1016/j.biopen.2017.12.003
29. Mpelangwa, E. M., Makindara, J. R., Sorensen, O. J., Bengesi, K. M. K., & Mabiki, F. P. (2022). Effect of changes in business environments on traded medicinal plants products in Tanzania: An explorative study. *Heliyon*, 8(9), e10426. https://doi.org/10.1016/j.heliyon.2022.e10426
30. Shukla, P., Jain, S. D., Agrawal, A., & Gupta, A. K. (2019). Indian Herbal Plants used as Antipyretic: A review. International Journal of Pharmacy & Life *Sciences*, 10. https://doi.org/10.3389%2Ffphar.2020.578970
31. Da Silva, P. N., da Conceição, R. A., do Couto Maia, R., & de Castro Barbosa, M. L. (2018).

Sodium–glucose cotransporter 2 (SGLT-2) inhibitors: a new antidiabetic drug class. *MedChemComm*, 9(8), 1273-1281. https://doi.org/10.1039%2Fc8md00183a

32 Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21(5), 559. https://doi.org/10.3390/molecules21050559

33. Arora, A., & Rai, Y. (2015). A review: phytochemictry, ethanobotanical and pharmacological activities of Alstonia Scholaris R. Br.(Apocynaceae)[J]. *Int J Adv Res*, 3(8), 584-590.

34. Zhao YL, Gou ZP, Shang JH, Li WY, Kuang Y, Li MY, Luo XD, (2021). Anti-microbial effects invitro and in vivo of Alstonia scholaris. *Natural Products and Bioprospecting*. 11 (1):127-35. https://doi.org/10.1007/s13659-020-00294-6.

35. Zhao, Y. L., Yang, Z. F., Shang, J. H., Huang, W. Y., Wang, B., Wei, X., & Luo, X. D. (2018). Effects of indole alkaloids from leaf of Alstonia scholaris on post-infectious cough in mice. *Journal of ethnopharmacology*, 218, 69-75. https://doi.org/10.1016%2Fj.jep.2018.02.040

36. Matough, F. A., Budin, S. B., Hamid, Z. A., Alwahaibi, N., & Mohamed, J. (2012). The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos university medical journal*, 12(1), 5-18. https://doi.org/10.12816%2F0003082

37. Kanase, V., & Mane, D. J. (2018). A pharmacognostic and pharmacological review on Alstonia scholaris. *Asian J Pharm Clin Res*, 11(12), 22-6. http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28124

38. Shivashankar, M., & Mani, D. (2011). A brief overview of diabetes. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(4), 22-27.

39. Nwaneri, C. (2015). Diabetes mellitus: a complete ancient and modern historical perspective. 1-26. http://dx.doi.org/10.9754/journal.wmc.2015.004831

40. Ahmed, A. M. (2002). History of diabetes mellitus. *Saudi medical journal*, 23(4), 373-378.

41. Atlas, D. (2019). IDF diabetes atlas. *International Diabetes Federation (9th editio)*.

42. World Health Organization. 'Available online: https://www. who. int/oral_health/action/services/en.' Accessed May (2021).

43. Aschner, P., Karuranga, S., James, S., Simmons, D., Basit, A., Shaw, J. E.,Ogurtsova, K. & Saeedi, P. (2021). The International Diabetes Federation's guide for diabetes epidemiological studies. *Diabetes research and clinical practice*, 172.

44. Beagley, J., Guariguata, L., Weil, C., & Motala, A. A. (2014). Global estimates of undiagnosed diabetes in adults. *Diabetes research and clinical practice*, 103(2), 150-160.

https://doi.org/10.1016/j.diabres.2013.11.001

45. Pradeepa, R., & Mohan, V. (2017). Prevalence of type 2 diabetes and its complications in India and economic costs to the nation. *European journal of clinical nutrition*, 71(7), 816-824. https://doi.org/10.1038/ejcn.2017.40

46. Susan van, D., Beulens, J. W., Yvonne T. van der, S., Grobbee, D. E., & Nealb, B. (2010). The global burden of diabetes and its complications: an emerging pandemic. *European Journal of Cardiovascular Prevention & Rehabilitation*, 17(1_suppl), s3-s8. https://doi.org/10.1097/01.hjr.0000368191.86614.5a

47. Mohan, V., & Pradeepa, R. (2017). The global burden of diabetes and its vascular complications. *Mechanisms of Vascular Defects in Diabetes Mellitus*, 3-23. https://doi.org/10.4103%2Fijo.IJO_1627_21

48. Federation, I. D. (2021). IDF Diabetes Atlas, tenth. *International Diabetes*.

49. Lam, A. A., Lepe, A., Wild, S. H., & Jackson, C. (2021). Diabetes comorbidities in low-and middle-income countries: an umbrella review. *Journal of global health*, 11. https://doi.org/10.7189%2Fjogh.11.04040

50. Tandon, N., Anjana, R. M., Mohan, V., Kaur, T., Afshin, A., Ong, K., & Dandona, L. (2018). The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990–2016. *The Lancet Global Health*, 6(12), e1352-e1362. https://doi.org/10.1016/s2214-109x(18)30387-5

51. Saboo, B., & Parikh, R. M. (2021). RSSDI's Defeat Diabetes Campaign: India takes a major leap in the direction of diabetes care capital of the world. *International Journal of Diabetes in Developing Countries*, 41(4), 523-525. https://doi.org/10.1007%2Fs13410-021-01036-1

52. Sathyanath, S., Kundapur, R., Deepthi, R., Poojary, S. N., Rai, S., Modi, B., & Saxena, D. (2022). An economic evaluation of diabetes mellitus in India: A systematic review. Diabetes & Metabolic Syndrome: *Clinical Research & Reviews*, 102641. https://doi.org/10.1016/j.dsx.2022.102641

53 American Diabetes Association. (2021). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes 2021. *Diabetes Care*, 44, S15-S33.

54 Naylor, R., Knight Johnson, A., & del Gaudio, D. (2019). Maturity-Onset Diabetes of the Young Overview. 2018 May 24. GeneReviews®[Internet]. Seattle (WA): University of Washington, Seattle. https://doi.org/10.1016/j.mcna.2014.08.015

55 Coderre, L., Debieche, L., Plourde, J., Rabasa-Lhoret, R., & Lesage, S. (2021). The potential causes of cystic fibrosis-related diabetes. *Frontiers in Endocrinology*, 885.

56. Thomas, C. C., & Philipson, L. H. (2015). Update on diabetes classification. Medical Clinics, 99(1), 1-16. https://doi.org/10.1016/j.mcna.2014.08.015

57. Ferris, H. A., & Kahn, C. R. (2012). New mechanisms of glucocorticoid-induced insulin resistance: make no bones about it. *The Journal of clinical investigation*, 122(11), 3854-3857.

58. Koster, J. C., Remedi, M. S., Qiu, H., Nichols, C. G., & Hruz, P. W. (2003). HIV protease inhibitors acutely impair glucose-stimulated insulin release. *Diabetes*, 52(7), 1695-1700. https://doi.org/10.2337/diabetes.52.7.1695

59. Huether, S. E., McCance, K. L., Brashers, V. L., & Rote, N. S. (2014) Pathophysiology: The Biologic Basis for Disease in Adults and Children, *Elsevier* 6 (a).

60. Yan Yu (2013), Endocrinology Pathogenesis of Diabetes Mellitus (DM), Type II, the Calgary guide to understand disease.

61. Barbie C.MS, RD, CDCES, CDN (2022), Type 2 Diabetes Symptoms, *verywell health*.

62. Monnier, L., Mas, E., Ginet, C., Michel, F., Villon, L., Cristol, J. P., & Colette, C. (2006). Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Jama*, 295(14), 1681-1687. https://doi.org/10.1001/jama.295.14.1681

63. Rendell, M. (2013). Diabetes: new drug options and old choices. *Consultant*, 53(4), 217-227.

64. Bergman, H., & Drury, D. R. (1938). The relationship of kidney function to the glucose utilization of the extra abdominal tissues. *American Journal of Physiology-Legacy Content*, 124(2), 279-284. https://doi.org/10.4239%2Fwjd.v12.i5.556

65. Gerich, J. E. (2010). Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. *Diabetic Medicine*, 27(2), 136-142. https://doi.org/10.1111/j.1464-5491.2009.02894.x

66. Gerich, J. E. (2000). Physiology of glucose homeostasis. *Diabetes, Obesity and Metabolism*, 2(6), 345-350. https://doi.org/10.1046/j.1463-1326.2000.00085.x

67. Landau, B. R., Wahren, J., Chandramouli, V., Schumann, W. C., Ekberg, K., & Kalhan, S. C. (1996). Contributions of gluconeogenesis to glucose production in the fasted state. *The Journal of clinical investigation*, 98(2), 378-385. https://doi.org/10.1172%2FJCI118803

68. Cersosimo, E., Garlick, P., & Ferretti, J. (1999). Insulin regulation of renal glucose metabolism in humans. *American Journal of Physiology-Endocrinology and Metabolism*, 276(1), E78-E84. https://doi.org/10.1152/ajpendo.1999.276.1.e78

69. Meyer, C., Stumvoll, M., Welle, S., Woerle, H. J., Haymond, M., & Gerich, J. (2003). Relative importance of liver, kidney, and substrates in epinephrine-induced increased gluconeogenesis in humans. *American Journal of Physiology-Endocrinology and Metabolism*, 285(4), E819-E826. https://doi.org/10.1152/ajpendo.00145.2003

70. Meyer, C., Dostou, J. M., Welle, S. L., & Gerich, J. E. (2002). Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *American Journal of Physiology-Endocrinology And Metabolism*, 282(2), E419-E427. https://doi.org/10.1152/ajpendo.00032.2001

71. Gronda, E., Jessup, M., Iacoviello, M., Palazzuoli, A., & Napoli, C. (2020). Glucose metabolism in the kidney: neurohormonal activation and heart failure development. *Journal of the American Heart Association*, 9(23), e018889. https://doi.org/10.1161/jaha.120.018889

72. Hummel, C. S., Lu, C., Loo, D. D., Hirayama, B. A., Voss, A. A., & Wright, E. M. (2011). Glucose transport by human renal Na+/D-glucose cotransporters SGLT1 and SGLT2. A*merican Journal of Physiology-Cell Physiology*, 300(1), C14-C21. https://doi.org/10.1152/ajpcell.00388.2010

73. Yale, J. F., Bakris, G., Cariou, B., Yue, D., David-Neto, E., Xi, L. & Meininger, G. (2013). Efficacy and safety of canagliflozin in subjects with type 2 diabetes and chronic kidney disease. *Diabetes, Obesity and Metabolism*, 15(5), 463-473. https://doi.org/10.1111/dom.12090

74. Hamilton, K. L. (2013). Robert K. Crane—Na+-glucose cotransporter to cure?. *Frontiers in Physiology*, 4, 53. https://doi.org/10.3389/fphys.2013.00053

75. Lee, W. S., Kanai, Y., Wells, R. G., & Hediger, M. A. (1994). The high affinity Na+/glucose cotransporter. Re-evaluation of function and distribution of expression. *Journal of Biological Chemistry*, 269(16), 12032-12039. https://doi.org/10.1016/S0021-9258(17)32677-7

76. Gallo, L. A., Wright, E. M., & Vallon, V. (2015). Probing SGLT2 as a therapeutic target for diabetes: basic physiology and consequences. *Diabetes and Vascular Disease Research*, 12(2), 78-89. https://doi.org/10.1177/1479164114561992

77. Packer, M. (2020). Autophagy stimulation and intracellular sodium reduction as mediators of the cardioprotective effect of sodium–glucose cotransporter 2 inhibitors. *European Journal of Heart Failure*, 22(4), 618-628. https://doi.org/10.1002/ejhf.1732

78. Garofalo, C., Borrelli, S., Liberti, M. E., Andreucci, M., Conte, G., Minutolo, R & De Nicola, L. (2019). SGLT2 inhibitors: nephroprotective efficacy and side effects. *Medicina*, 55(6),

268. https://doi.org/10.3390/medicina55060268

79. J. F., & Whaley, J. M. (2011). Glucose dynamics and mechanistic implications of SGLT2 inhibitors in animals and humans. *Kidney International*, 79, S20-S27. https://doi.org/10.1038/ki.2010.512

80. Barutta, F., Bernardi, S., Gargiulo, G., Durazzo, M., & Gruden, G. (2019). SGLT2 inhibition to address the unmet needs in diabetic nephropathy. *Diabetes/metabolism research and reviews*, 35(7), e3171. https://doi.org/10.1002%2Fdmrr.3171

81. Dutka, M., Bobiński, R., Francuz, T., Garczorz, W., Zimmer, K., Ilczak, T., & Hajduga, M. B. (2022). SGLT-2 Inhibitors in Cancer Treatment—Mechanisms of Action and Emerging New Perspectives. *Cancers*, 14(23), 5811. https://doi.org/10.3390/cancers14235811

82. Tsai, W. H., Chuang, S. M., Liu, S. C., Lee, C. C., Chien, M. N., Leung, C. H., ... & Shih, H. M. (2021). Effects of SGLT2 inhibitors on stroke and its subtypes in patients with type 2 diabetes: a systematic review and meta-analysis. *Scientific Reports*, 11(1), 15364.

83. American Diabetes Association Professional Practice Committee, & American Diabetes Association Professional Practice Committee: (2022). 11. Chronic kidney disease and risk management: Standards of Medical Care in Diabetes—2022. *Diabetes Care*, 45(Supplement_1), S175-S184. https://doi.org/10.2337/dc22-S011

84. Satyadev, T. N. V. S. S., Gayatri, M., Venkatesh, M., & Krishnaveni, (2022). Bio Analytical method for the estimation of remogliflozin using LC-MS. 21(8), 988-998. http://dx.doi.org/10.37896/YMER21.08/85

85. Suthari, S., Kota, S., Kanneboyena, O., Gul, M. Z., & Abbagani, S. (2021). Ethnobotanical perspectives in the treatment of communicable and noncommunicable diseases. *In Phytomedicine* (pp. 251-289). Academic Press.

86. Verma, S., Gupta, M., Popli, H., & Aggarwal, G. (2018). Diabetes mellitus treatment using herbal drugs. *International Journal of Phytomedicine*, 10(1), 1-10. http://dx.doi.org/10.5138/09750185.2181

87. Blaschek, W. (2017). Natural products as lead compounds for sodium glucose cotransporter (SGLT) inhibitors. Planta medica, 83(12/13), 985-993. https://doi.org/10.1055/s-0043-106050

88. Sato, S., Takeo, J., Aoyama, C., & Kawahara, H. (2007). Na+-glucose cotransporter (SGLT) inhibitory flavonoids from the roots of Sophora flavescens. *Bioorganic & medicinal chemistry*, 15(10), 3445-3449. https://doi.org/10.1016/j.bmc.2007.03.011

89. Morita, H., Deguchi, J., Motegi, Y., Sato, S., Aoyama, C., Takeo, J. & Hirasawa, Y. (2010). Cyclic diarylheptanoids as Na+-glucose cotransporter (SGLT) inhibitors from Acer

nikoense. *Bioorganic & medicinal chemistry letters*, 20(3), 1070-1074. https://doi.org/10.1016/j.bmcl.2009.12.036

90. Choi, C. I. (2016). Sodium-glucose cotransporter 2 (SGLT2) inhibitors from natural products: discovery of next-generation antihyperglycemic agents. *Molecules*, 21(9), 1136. https://doi.org/10.3390/molecules21091136
91. Schulze, C., Bangert, A., Schwanck, B., Vollert, H., Blaschek, W., & Daniel, H. (2015). Extracts and flavonoids from onion inhibit the intestinal sodium-coupled glucose transporter 1 (SGLT1) in vitro but show no anti-hyperglycaemic effects in vivo in normoglycaemic mice and human volunteers. *Journal of Functional Foods*, 18, 117-128. https://doi.org/10.1002/mnfr.201400016
92. Wang, X. F., & Yao, C. S. (2016). Naturally active oligostilbenes. *Journal of Asian natural products research*, 18(4), 376-407. https://doi.org/10.1002/med.21536
93. Solayman, M., Ali, Y., Alam, F., Islam, A., Alam, N., Khalil, I., & Hua Gan, S. (2016). Polyphenols: potential future arsenals in the treatment of diabetes. *Current pharmaceutical design*, 22(5), 549-565. https://doi.org/10.2174/1381612822666151125001111
94. Nowak, A., Zakłos-Szyda, M., Błasiak, J., Nowak, A., Zhang, Z., & Zhang, B. (2019). Potential of Schisandra chinensis (Turcz.) Baill. in human health and nutrition: a review of current knowledge and therapeutic perspectives. *Nutrients*, 11(2), 333. https://doi.org/10.3390%2Fnu11020333
95. Arai, H., Hirasawa, Y., Rahman, A., Kusumawati, I., Zaini, N. C., Sato, S., & Morita, H. (2010). Alstiphyllanines E–H, picraline and ajmaline-type alkaloids from Alstonia macrophylla inhibiting sodium glucose cotransporter. *Bioorganic & medicinal chemistry*, 18(6), 2152-2158. https://doi.org/10.1016/j.bmc.2010.01.077
96. Oktavia, R., Misfadhila, S., & Rivai, H. (2020). Overview of traditional, phytochemical, and pharmacological uses of pulai (Alstonia scholaris). *World Journal of Pharmacy and Pharmaceutical Sciences*, 9(8), 334-354. http://dx.doi.org/10.20959/wjpps20208-16892
97. Srivastava, A., Shankar, M. B., & Sharma, R. K. (2015). Phytochemistry and chromatographic analysis of Alstonia scholaris (l.) r. br. used as a traditional medicine: a review. *World journal of pharmaceutical research*, 5(01), 1503-1519.
98. Kanase, V. A. N. I. T. A., & Mane, D. J. (2018). A pharmacognostic and pharmacological review on Alstonia scholaris. *Asian J Pharm Clin Res*, 11(12), 22-26. http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28124
99. Pratap, B., Chakraborthy, G. S., & Mogha, N. (2013). Complete aspects of Alstonia scholaris. *Int J Pharm Tech Res*, 5(1), 17-26.

100. Shang, Jian-Hua, Xiang-Hai Cai, Tao Feng, Yun-Li Zhao, Jing-Kun Wang, Lu-Yong Zhang, Ming Yan, and Xiao-Dong Luo (2010). 'Pharmacological evaluation of Alstonia scholaris: anti- inflammatory and analgesic effects.' *Journal of ethnopharmacology* 129(2), 174-181. https://doi.org/10.1016/j.jep.2010.02.011

101. Dey, A. (2011). Alstonia scholaris R. Br.(Apocynaceae): Phytochemistry and pharmacology: A concise review. *Journal of Applied Pharmaceutical Science*, 1(6), 51-57.

102. Khyade, M.S., Kasote, D.M. and Vaikos, N.P., 2014. Alstonia scholaris (L.) R. Br. and Alstonia macrophylla Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, 153(1), pp.1-18. http://dx.doi.org/10.1016/j.jep.2014.01.025

103. Singh, B., & Sangwan, P. (2011). Taxonomy, ethnobotany and antimicrobial activity of Alstonia scholaris (L.) R. Br., Carissa carandas L. and Catharanthus roseus (L.) G. Don. *Int J Biotechnol Biosci*, 1(1), 102-112.

104. Sinnathambi, A., Mazumder, P. M., Ashok, P., & Narayanan, L. S. (2007). In vitro antioxidant and free radical scavenging activity of Alstonia scholaris Linn. R. Br. *Iranian Journal of Pharmacology and Therapeutics*, 6(2), 191-196.

105. Thakurdesai, P. A., Narayanan, L. S., & Mazumder, P. M. (2010). In vitro antioxidant and free radical scavenging activity of fractions from Alstonia scholaris Linn. R. Br. *American International Journal of Pharmaceutical Technology Research*, 2(1), 18-25.

106. Kumar, A., Kaur, R., & Arora, S. (2010). Free radical scavenging potential of some Indian medicinal plants. *J. Med. Plants Res*, 4(19), 2034-2042. https://doi.org/10.5897/JMPR10.165

107. Sinnathambi, A., Papiya, M. M., Lohidasan, S., & Purnima, A. (2011). Anti-arthritic and antioxidant activity of leaves of Alstonia scholaris Linn. *Eur J Integr Med*, 3(2), 83-90. https://doi.org/10.1016/J.EUJIM.2011.04.019

108. Jong-Anurakkun, N., Bhandari, M. R., & Kawabata, J. (2007). α-Glucosidase inhibitors from Devil tree (Alstonia scholaris). *Food Chemistry*, 103(4), 1319-1323. https://doi.org/10.1016/J.FOODCHEM.2006.10.043

109. Ragasa, C. Y., Lim, K. F., Shen, C. C., & Raga, D. D. (2015). Hypoglycemic potential of triterpenes from Alstonia scholaris. *Pharmaceutical Chemistry Journal*, 49, 30-33. http://dx.doi.org/10.1007/s11094-015-1217-9

110. Bandawane, D., Juvekar, A. and Juvekar, M., 2011. Antidiabetic and antihyperlipidemic effect of Alstonia scholaris Linn bark in streptozotocin induced diabetic rats. *Indian Journal*

of Pharmaceutical Education and Research, 45(2), pp.114-120.

111. Rahmatullah, M., Azam, N. K., Khatun, Z., Seraj, S., Islam, F., Rahman, A& Aziz, S. (2012). Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines*, *9*(3), 380-385. https://doi.org/10.4314/ajtcam.v9i3.12
112. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J biol Chem*, 226(1), 497-509. http://dx.doi.org/10.1016/S0021-9258(18)64849-5
113. World Health Organization. (1998). Quality control methods for medicinal plant materials. *World Health Organization.*
114. Marshall, M. R. (2010). Ash analysis. *Food analysis*, 4, 105-116.
115. Bora, K. S. (2016). Pharmacognostic evaluation and physico-chemical analysis of Ulmus wallichiana Planch. *Journal of Applied Pharmaceutical Research*, 4(3), 01-07.
116. Chauhan, S., Pundir, V., & Sharma, A. K. (2013). Pharmacopeial standardization of mahasudarshan churna: a polyherbal formulation. *Journal of Medicinal Plants*, 1(2).
117. Kanase, V. A. N. I. T. A., & Mane, D. J. (2018). A pharmacognostic and pharmacological review on Alstonia scholaris. *Asian J Pharm Clin Res*, 11(12), 22-6. http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28124
118. Hussain, A., Zaman, M. K., & Ramteke, A. (2013). Preliminary Phytochemical Screening and Proximate Analysis of the trunk bark of Alstonia scholaris (L.) R. Br. *Journal of Pharmacognosy and Phytochemistry*, 1(5), 13-17.
119. Tanvir, E.M., Hossen, M., Hossain, M., Afroz, R., Gan, S.H., Khalil, M. and Karim, N., 2017. Antioxidant properties of popular turmeric (Curcuma longa) varieties from Bangladesh. *Journal of Food Quality*, 2017. http://dx.doi.org/10.1155/2017/8471785
120. Reddy, D. S. (2016). Phytochemical analysis of active constituents of *Alstonia scholaris* and their cytotoxicity in vitro. *International Journal of Pharmaceutical Sciences and Research*, 7(8), 3262. http://dx.doi.org/10.13040/IJPSR.0975-8232.7(8).3262-73
121. Xiao-Ying, L., Fu-Cheng, L., Chun-Yu, D., Meng-Zhen, Z., Yang, M., Ding-Zhang, X. & Xi-Yong, Y. (2016). Left ventricular deformation associated with cardiomyocyte Ca2+ transients delay in early stage of low-dose of STZ and high-fat diet induced type 2 diabetic rats. *BMC Cardiovascular Disorders*, 16, 1-12. https://doi.org/10.1186/s12872-016-0220-8
122. Chaimum-aom, N., Chomko, S., & Talubmook, C. (2017). Toxicology and oral glucose

tolerance test (OGTT) of Thai medicinal plant used for diabetes controls, Phyllanthus acidus L.(Euphorbiaceae). *Pharmacognosy Journal*, 9(1), 58-61. http://dx.doi.org/10.5530/pj.2017.1.11

123. Baliga, M. S., Jagetia, G. C., Ulloor, J. N., Baliga, M. P., Venkatesh, P., Reddy, R. & Bairy, K. L. (2004). The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (Alstonia scholaris) in mice and rats. Toxicology letters, 151(2), 317-326. https://doi.org/10.1016/j.toxlet.2004.01.015

124. Ansary, T. M., Fujisawa, Y., Rahman, A., Nakano, D., Hitomi, H., Kobara, H. & Nishiyama, A. (2017). Responses of renal hemodynamics and tubular functions to acute sodium–glucose cotransporter 2 inhibitor administration in non-diabetic anesthetized rats. Scientific Reports, 7(1), 1-9. https://doi.org/10.1038/s41598-017-09352-5

125. Soni, H. M., Patel, P. P., Patel, S., Rath, A. C., Acharya, A., Trivedi, H. D., & Jain, M. R. (2015). Effects of combination of aliskiren and pentoxyfylline on renal function in the rat remnant kidney model of chronic renal failure. *Indian Journal of Pharmacology*, 47(1), 80. https://doi.org/10.4103/0253-7613.150351

126. Barış, V. Ö, Dinçsoy, B., Gedikli, E., & Erdemb, A. (2021). Empagliflozin significantly attenuates sotalol-induced QTc prolongation in rats. *Kardiologia Polska (Polish Heart Journal)*, 79(1), 53-57. https://doi.org/10.33963/kp.15666

127. Njeh, F., Feki, H., Koubaa, I., Hamed, N., Damak, M., Ayadi, A. & Mezghani-Jarraya, R. (2016). Molluscicidal activity of Solanum elaeagnifolium seeds against Galba truncatula intermediate host of Fasciola hepatica: Identification of β-solamarine. *Pharmaceutical Biology*, 54(4), 726-731. https://doi.org/10.3109/13880209.2015.1073332

128. Diniatik, A. K., Siswanto, A., & Doti, D. M. (2020). Antioxidant Activities and Identification of Compounds in Ethyl Acetate Fraction, Dichloromethane Fraction and Ethanol Extract of Garcinia mangostana L. Leaves. *International Journal of Pharmaceutical Research*, 12(2). http://dx.doi.org/10.31838/ijpr/2020.12.02.0010

129. Kamonlakorn, K., Supon, C., Riankrasin, A., Pekthong, D. and Parhira, S., (2020). Quantitative Phytochemicals Determination of the Extracts from the Flowers of Alstonia scholaris and their Anti-Lipoxygenase Activities. In Key Engineering Materials. 859, 94-99. Trans Tech Publications Ltd. http://dx.doi.org/10.4028/www.scientific.net/KEM.859.94

130. Harbone, J.B., 1973. Phytochemical Methods London. Chapman and Hall1973.

131. Harborne, A.J., 1998. Phytochemical methods a guide to modern techniques of plant analysis. *Springer science & business media*.

132. Han, S., Hagan, D.L., Taylor, J.R., Xin, L., Meng, W., Biller, S.A., Wetterau, J.R., Washburn, W.N. and Whaley, J.M., 2008. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. *Diabetes*, 57(6), 1723-1729. https://doi.org/10.2337/db07-1472

133. Gou, Z. P., Zhao, Y. L., Zou, L. L., Wang, Y., Shu, S. Q., Zhu, X. H., ... & Feng, P. (2021). The safety and tolerability of alkaloids from Alstonia scholaris leaves in healthy Chinese volunteers: a single-centre, randomized, double-blind, placebo-controlled phase I clinical trial. *Pharmaceutical Biology*, 59(1), 482-491. https://doi.org/10.1080%2F13880209.2021.1893349

134. Soni, P., Choudhary, R., & Bodakhe, S. H. (2019). Effects of a novel isoflavonoid from the stem bark of Alstonia scholaris against fructose-induced experimental cataract. *Journal of Integrative Medicine*, 17(5), 374-382.https://doi.org/10.1016/j.joim.2019.06.002

135. Vidya, R., Kalaivani, K. and Venkatesh, R., (2014). Antioxidant activity of goat liver slices treated with cucumis melo (L). Fruit extract. *International Journal of Pharmaceutical Sciences and Research*, 5(11), 4846. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4846-50

136. Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*, 247(10), 3170-3175.

137. Eldamaty, H. S. E. (2020). Protective effects of barley and wheat grasses on nephrotoxicity in rats and some biochemical parameters induced by tramadol. *Egyptian Journal of Nutrition and Health*, 15(1), 67-83. https://doi.org/10.21608/ejnh.2020.117339

138. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta (BBA)-general subjects*, 582(1), 67-78. https://doi.org/10.1016/0304-4165(79)90289-7

139. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J biol Chem*, 226(1), 497-509. http://dx.doi.org/10.1016/S0021-9258(18)64849-5

140. Hussain, A., Zaman, M. K., & Ramteke, A. (2013). Preliminary Phytochemical Screening and Proximate Analysis of the trunk bark of Alstonia scholaris (L.) R. Br. *Journal of Pharmacognosy and Phytochemistry*, 1(5), 13-17.

141. Reddy, D. S. (2016). Phytochemical analysis of active constituents of Alstonia scholaris and

their cytotoxicity in vitro. *International Journal of Pharmaceutical Sciences and Research*, 7(8), 3262. http://dx.doi.org/10.13040/IJPSR.0975-8232.7(8).3262-73

142. Salehi B, Ata A, V. Anil Kumar N, Sharopov F, Ramirez-Alarcon K, Ruiz-Ortega A, Abdulmajid Ayatollahi S, Valere Tsouh Fokou P, Kobarfard F, Amiruddin Zakaria Z, Iriti M (2019). Antidiabetic potential of medicinal plants and their active components. *Biomolecules*. 9 (10):551. https://doi.org/10.3390/biom9100551
143. Li, S., Han, Q., Qiao, C., Song, J., Lung Cheng, C., & Xu, H. (2008). Chemical markers for the quality control of herbal medicines: an overview. *Chinese medicine*, 3(1), 1-16.
144. Aba, P. E., & Asuzu, I. U. (2018). Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review. *Indian Journal of Natural Products and Resources*, 9(2), 85-96.
145. Nagy, C., & Einwallner, E. (2018). Study of in vivo glucose metabolism in high-fat diet-fed mice using oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). *JoVE* (Journal of Visualized Experiments), (131), e56672. https://doi.org/10.3791%2F56672
146. American Diabetes Association Professional Practice Committee, & American Diabetes Association Professional Practice Committee: (2022). Chronic kidney disease and risk management: Standards of Medical Care in Diabetes—2022. *Diabetes Care*, 45(Supplement_1), S175-S184. https://doi.org/10.2337/dc22-s011
147. Malin, S. K., Rynders, C. A., Weltman, J. Y., Barrett, E. J., & Weltman, A. (2016). Exercise intensity modulates glucose-stimulated insulin secretion when adjusted for adipose, liver and skeletal muscle insulin resistance. *PLoS One*, 11(4), e0154063. https://doi.org/10.1371/journal.pone.0154063
148. Cheng, X., Yang, N., Li, Y., Sun, Q., Qiu, L., Xu, L. & Zhang, H. (2019). The shape of the glucose response curve during an oral glucose tolerance test herald's β–cell function in a large Chinese population. *BMC Endocrine Disorders*, 19(1), 1-11. https://doi.org/10.2337/diab.36.5.654
149. Fantus, I. G., Chayoth, R., O'Dea, L., Marliss, E. B., Yale, J. F., & Grose, M. (1987). Insulin binding and glucose transport in adipocytes in neonatal streptozocin-injected rat model of diabetes mellitus. *Diabetes*, 36(5), 654-660.
150. Arulmozhi, D. K., Veeranjaneyulu, A., & Bodhankar, S. L. (2004). Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: A glance. Indian Journal of Pharmacology, 36(4), 217.
151. Kergoat, M., & Portha, B. (1997). Glucagon-like peptide-1 (7-36)-amide confers glucose sensitivity to previously glucose-incompetent beta-cells in diabetic rats: in vivo and in vitro

studies. *J. Endocrinol*, 155, 369-376. https://doi.org/10.1677/joe.0.1550369

152. Wou, C., Unwin, N., Huang, Y., & Roglic, G. (2019). Implications of the growing burden of diabetes for premature cardiovascular disease mortality and the attainment of the Sustainable Development Goal target, *Cardiovascular Diagnosis and Therapy*, 9(2), 140. https://doi.org/10.21037/cdt.2018.09.04
153. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B. & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes research and clinical practice*, 183, 109-119. https://doi.org/10.1016/j.diabres.2021.109119
154. King, P., Peacock, I., & Donnelly, R. (1999). The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. *British journal of clinical pharmacology*, 48(5), 643. https://doi.org/10.1046%2Fj.1365-2125.1999.00092.x
155. Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R., & Neil, H. A. W. (2008). 10-year follow-up of intensive glucose control in type 2 diabetes. *New England journal of medicine*, 359(15), 1577-1589. https://doi.org/10.1056/nejmoa0806470
156. Sohn, K., Wende, A. R., Abel, E. D., Moreno, A. P., Sachse, F. B., & Punske, B. B. (2013). Absence of glucose transporter 4 diminishes electrical activity of mouse hearts during hypoxia. *Experimental physiology*, 98(3), 746-757. https://doi.org/10.1113/expphysiol.2012.070235
157. Shriwas, P., Chen, X., Kinghorn, A. D., & Ren, Y. (2020). Plant-derived glucose transport inhibitors with potential antitumor activity. *Phytotherapy research*, 34(5), 1027-1040. https://doi.org/10.1002/ptr.6587
158. Bailey, C. J. (2011). Renal glucose reabsorption inhibitors to treat diabetes. *Trends in pharmacological sciences*, 32(2), 63-71. https://doi.org/10.1016/j.tips.2010.11.011
159. Ghosh, R. K., Ghosh, S. M., Chawla, S., & Jasdanwala, S. A. (2012). SGLT2 inhibitors: a new emerging therapeutic class in the treatment of type 2 diabetes mellitus. *The Journal of Clinical Pharmacology*, 52(4), 457-463. https://doi.org/10.1177/0091270011400604
160. Poongothai, K., Ponmurugan, P., Ahmed, K. S. Z., Kumar, B. S., & Sheriff, S. A. (2011). Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. *Asian Pacific Journal of Tropical Medicine*, 4(10), 778-785. https://doi.org/10.1016/s1995-7645(11)60193-4
161. Garau, C., Cummings, E., Phoenix, D. A., & Singh, J. (2003). Beneficial effect and mechanism of action of Momordica charantia in the treatment of diabetes mellitus: a mini

review. *Dubai Diabetes and Endocrinology Journal*, 11, 46-55.

162. Soumya, D., & Srilatha, B. (2011). Late stage complications of diabetes and insulin resistance. *J Diabetes Metab*, 2(9), 1000167. https://doi.org/10.4172/2155-6156.1000167
163. De Rosa, S., Arcidiacono, B., Chiefari, E., Brunetti, A., Indolfi, C., & Foti, D. P. (2018). Type 2 diabetes mellitus and cardiovascular disease: genetic and epigenetic links. *Frontiers in endocrinology*, 9, 2. https://doi.org/10.3389/fendo.2018.00002
164. Muhammed Thwayyib, P. K. (2017). Study of Antihyperlipidaemic Activity of Alstonia Scholaris Leaves (Doctoral dissertation, Karpagam College of Pharmacy, Coimbatore).
165. Thomas, M. C. (2022). The clustering of cardiovascular, renal, adipo-metabolic eye and liver disease with type 2 diabetes. *Metabolism*, 128, 154961. https://doi.org/10.1016/j.metabol.2021.154961
166. Mittal, A., Sathian, B., Kumar, A., Chandrasekharan, N., & Sunka, A. (2010). Diabetes mellitus as a potential risk factor for renal disease among Nepalese: A hospital based case control study. *Nepal journal of epidemiology*, 1(1), 22-25. https://doi.org/10.3126/nje.v1i1.4108
167. Pal, S. K., & Shukla, Y. (2003). Herbal medicine: current status and the future. *Asian pacific journal of cancer prevention*, 4(4), 281-288.
168. Gandhi, G. R., Ignacimuthu, S., & Paulraj, M. G. (2011). Solanum torvum Swartz. Fruit containing phenolic compounds shows antidiabetic and antioxidant effects in streptozotocin induced diabetic rats. *Food and Chemical Toxicology*, 49(11), 2725-2733. https://doi.org/10.1016/j.fct.2011.08.005
169. Harms, P. P., van der Heijden, A. A., Rutters, F., Tan, H. L., Beulens, J. W., Nijpels, G., & Elders, P. (2021). Prevalence of ECG abnormalities in people with type 2 diabetes: The Hoorn Diabetes Care System cohort. *Journal of Diabetes and its Complications*, 35(2), 107810. https://doi.org/10.1016/j.jdiacomp.2020.107810
170. Bugger, H., & Abel, E. D. (2014). Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia*, 57, 660-671. https://doi.org/10.1007%2Fs00125-014-3171-6
171. Parim, B., Sathibabu Uddandrao, V. V., & Saravanan, G. (2019). Diabetic cardiomyopathy: molecular mechanisms, detrimental effects of conventional treatment, and beneficial effects of natural therapy. *Heart Failure Reviews*, 24, 279-299. https://doi.org/10.1007/s10741-018-9749-1
172. Dupre, A., Vincent, S., & Iaizzo, P. A. (2005). Basic ECG theory, recordings, and interpretation. *Handbook of cardiac anatomy, physiology, and devices*, 191-201.

http://dx.doi.org/10.1007/978-1-59259-835-9_15

173. Ukpabi, O. J., & Onwubere, B. J. (2017). QTc prolongation in Black diabetic subjects with cardiac autonomic neuropathy. *African health sciences*, 17(4), 1092-1100. https://doi.org/10.4314%2Fahs.v17i4.17

174. Laddha, A. P., & Kulkarni, Y. A. (2021). Daidzein mitigates myocardial injury in streptozotocin-induced diabetes in rats. *Life Sciences*, 284, 119664. https://doi.org/10.1016/j.lfs.2021.119664

175. Adhikari, B. (2021). Roles of alkaloids from medicinal plants in the management of diabetes mellitus. *Journal of Chemistry*, 2021, 1-10. http://dx.doi.org/10.1155/2021/2691525

176. Babu, P. V. A., Liu, D., & Gilbert, E. R. (2013). Recent advances in understanding the anti-diabetic actions of dietary flavonoids. *The Journal of nutritional biochemistry*, 24(11), 1777-1789. https://doi.org/10.1016/j.jnutbio.2013.06.003

177. Shanmugapriya, D., & Jayanthi, G. (2019). Phytochemical and antioxidant activity of methanolic leaf extract of Alstonia scholaris Linn. *Journal of Pharmacognosy and Phytochemistry*, 8(2S), 466-471.

178. DINIATIK, A. K., SISWANTO, A., & DOTI, D. M. (2020). Antioxidant Activities and Identification of Compounds in Ethyl Acetate Fraction, Dichloromethane Fraction and Ethanol Extract of Garcinia mangostana L. Leaves. *International Journal of Pharmaceutical Research*, 12(2), 22-28. http://doi.org/10.31838/ijpr/2020.12.02.0010

179. Mosser, R. E., Maulis, M. F., Moullé, V. S., Dunn, J. C., Carboneau, B. A., Arasi, K., ... & Gannon, M. (2015). High-fat diet-induced β-cell proliferation occurs prior to insulin resistance in C57Bl/6J male mice. *American Journal of Physiology-Endocrinology and Metabolism*, *308*(7), E573-E582. https://doi.org/10.1152/ajpendo.00460.2014

180. Vidya, R., Kalaivani, K. and Venkatesh, R., 2014. Antioxidant activity of goat liver slices treated with cucumis melo (L). Fruit extract. *International Journal of Pharmaceutical Sciences and Research*, 5(11), 4846-. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4846-50

181. Fujii, J., Homma, T., & Osaki, T. (2022). Superoxide radicals in the execution of cell death. *Antioxidants*, *11*(3), 501. https://doi.org/10.3390/antiox11030501

182. Fang, Y. Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, 18(10), 872-879. https://doi.org/10.1016/s0899-9007(02)00916-4

183. Janssen, R., van der Straaten, T., van Diepen, A., & van Dissel, J. T. (2003). Responses to reactive oxygen intermediates and virulence of Salmonella typhimurium. *Microbes and*

infection, 5(6), 527-534. https://doi.org/10.1016/s1286-4579(03)00069-8

184. Rekha, V. R., Sunil, S., & Rathy, R. (2017). Evaluation of oxidative stress markers in oral lichen planus. *Journal of oral and maxillofacial pathology: JOMFP*, 21(3), 387. https://doi.org/10.4103%2Fjomfp.JOMFP_19_17

185. Roveri, A., Casasco, A., Maiorino, M., Dalan, P., Calligaro, A., & Ursini, F. (1992). Phospholipid hydroperoxide glutathione peroxidase of rat testis. Gonadotropin dependence and immunocytochemical identification. *Journal of Biological Chemistry*, 267(9), 6142-6.

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APPENDIX-I

Authentication certificates for the study plants

DEPARTMENT OF BOTANY
UNIVERSITY SCHOOL OF SCIENCES
GUJARAT UNIVERSITY
AHMEDABAD-380009
GUJARAT, INDIA
Phone: 91 – 079 -26302578

REF: GU/Bot/2018 / *Date: 05/12/18*

Certificate

The Herbarium specimen prepared by Sonam Mishra is

Sr. No	Family	Botanical Name	Authentication no.
1	Apocynaceae	Alstonia *scholaris* (L) R.Br	GU/BOT/A40

Dr. Hitesh A. Solanki
Professor
University School of Sciences
Gujarat University
Ahmedabad-380 009

APPENDIX-II

Certificate of approval for experiment- IAEC

Certificate

This is certifying that the project title **"Investigation into the effects of Alstonia scholaris in experimental models of diabetes"** of **Sonam Mishra** (PhD scholar, GTU Enrollment No. 179999901009) to conduct the study with wide protocol no. **LMCP/P'cology/19/10** has been approved by the IAEC.

Name of Chairman/ Member Secretary IAEC: Name of CPCSEA nominee:

Signature with date

16/10/19 16/10/19

Chairman/ Member Secretary of IAEC: CPCSEA nominee:

APPENDIX-III

List of Poster Presentation

- Participated in **POSTER PRESENTATION** in GUJCOST Sponsored International e-Conference on 16th and 17th July 2021 at L.J Institute of pharmacy, Ahmedabad, Gujarat. Topic: **Effect of *Alstonia scholaris* leaf extracts on glucose homeostasis in normal rats**.
- Participated in **POSTER PRESENTATION** in GUJCOST & DST Sponsored International e-Conference on 12th and 13th August, 2021 at Arihant school of pharmacy & Bio-research institute, Adalaj, Gandhinagar, Gujarat. Topic: **Evaluation of anti-diabetic potential of ethanolic extract of *Alstonia scholaris* in streptozotocine-induced diabetic rats**.

LJ University

DST
Department of Science & Technology
Government of Gujarat

GUJCOST Sponsored International e-Conference on

"Regulatory Trends of Biologics and Medical Devices in Covid-19 Pandemic"

Certificate of Particpation

This certificate is awarded to

Dr./Mr./Mrs./Ms. **Sonam Mishra**

For presented e-Presentation in **GUJCOST sponsored International e-Conference on "Regulatory Trends of Biologics and Medical Devices in Covid-19 Pandemic"**

organized by L.J Institute of Pharmacy, L.J.K University, Ahmedabad on **16th and 17th July 2021.** **RCON_189**

Coordinator
Dr. Dilip Maheshwari

Convener
Dr. Shreeraj Shah

Co-Coordinator
Mr. Darshil Shah

Emcure
SUCCESS THROUGH INNOVATION

Dst
Department of Science & Technology (Government of Gujarat)

GUJARAT COUNCIL ON SCIENCE AND TECHNOLOGY • GOVERNMENT OF GUJARAT

Certificate of Participation

This certificate is presented to

SONAM MISHRA

For Participation in e-Poster Presentation Competition on the Topic of
EVALUATION OF ANTI- DIABETIC POTENTIAL OF ETHANOLIC EXTRACT OF ALSTONIA SCHOLARIS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS
in GUJCOST & DST sponsored Two Days International e-Conference on
"Revolutionary Applications of 3D-Printing Technology in Pharma and Healthcare System"
on 12th & 13th August, 2021
Organized by Arihant School of Pharmacy & Bio-Research Institute, Adalaj, Gandhinagar.
Co Author/s: Jayesh V. Beladiya, Anita A. Mehta

Principal & Convener

Co-Ordinator

APPENDIX-IV

List of Publications

1. Mishra S, Beladiya JV, Mehta AA. Evaluation of the Antioxidant, Antihyperglycemic and Hypolipidemic Potential of *Alstonia scholaris* Leaves Extracts in Streptozotocin-Induced Diabetic Rats: Journal of Natural Remedies: April 2023; 23 (2): ISSN: 2320-3358. [Scopus]

2. Mishra S, Beladiya JV, Mehta AA. Phytochemical screening and evaluation of various *Alstonia scholaris* (L.) R. Br. Leaves extracts on glucose homeostasis in normal rats: International Journal of Biology, Pharmacy and Allied Sciences: May 2023; 12 (5), pp. 2059-2069: ISSN: 2277–4998. [Web of Science]

3. Mishra S, Beladiya JV, Mehta AA. *Alstonia scholaris* Leaves Fractions and Ethanolic Extract on Glucose Homeostasis in Normal Rats: Antioxidant, Inhibits Renal Glucose Absorption, and Anti-diabetic Potential: Journal of Xidian University. 2022; 16 (11), pp. 473-481: ISSN: 1001-2400. [Scopus]

4. Mishra S, Mehta AA. Cardioprotective effect of *Alstonia scholaris* Linn. R. Br: A review: YMER: October 2022; 21 (10), pp. 1000-1011: ISSN: 0044-0477. [Scopus]

JOURNAL OF NATURAL REMEDIES
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RESEARCH ARTICLE

Evaluation of the Antioxidant, Antihyperglycemic and Hypolipidemic Potential of *Alstonia scholaris* Leaves Extracts in Streptozotocin-Induced Diabetic Rats

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Abstract

The evergreen tree *Alstonia scholaris* (L) R. Br. (Family: Apocynaceae) is native to Australasia, southern China and the tropics of Asia. Despite its importance as a medicinal plant, little is known about its potential role in complementing standard methods of treating diabetes and its associated consequences. Therefore, the present study scientifically investigated extracts from the leaves of *A. scholaris* for their antioxidant (*in vitro*), anti-diabetic, and hypolipidemic effects in rats with type 2 diabetes mellitus. Male Wistar rats were administered streptozotocin (45 mg/kg, i.p.) and fed a high-fat diet to induce type 2 diabetes mellitus. They were treated with 400 mg/kg of an ethyl acetate (EAEAS) and ethanolic (EAAS) extract of *A. scholaris* leaves after complications persisted. Typical drugs were metformin (200 mg/kg) and canagliflozin (10 mg/kg). In the end, blood was drawn to determine various biochemical parameters such as fasting blood sugar, lipid profile and markers of heart, liver and kidney damage. In addition, the rat's weight, urinary glucose concentration, urine volume, blood pressure, Electrocardiogram (ECG), and antioxidant potential of EEAS were measured. The pancreas, heart, kidneys, and liver were all subjected to histopathological analysis. A wide range of biochemical and physiological markers, including blood and urine glucose, lipid profile, markers of heart, kidney and liver damage, antioxidant levels and blood pressure, showed significant improvement in response to EEAS. Histopathology illustrates the reverse modulation in heart, kidney, and liver tissue compared to disease control. Based on the data obtained, the EAEAS achieved is far inferior to that required to treat diabetes mellitus. In summary, this present study demonstrates that EEAS (400 mg/kg) can lower blood sugar levels, fight free radicals, and lower bad cholesterol levels in rats with diabetes and complications. Further investigations can be undertaken to explore its mechanism of action at the molecular level.

Keywords: *Alstonia scholaris*, Anti-hyperglycemia, Antioxidant, Hypolipidemia, Streptozotocin

1. Introduction

Insulin resistance and persistent hyperglycemia arethe hallmarks of diabetes mellitus[1]. According to the International Diabetes Federation (IDF) report, 10th edition 2021, 537 million adults aged 20 to 75 are living with diabetes and approximately 6.7 million deaths are attributed worldwide[2]. Organ dysfunction in the liver, heart, kidneys, blood vessels, urogenital tract system, and eyes is a common complication of type 2 diabetes mellitus[3]. Chronic hyperglycemia is associated with a decrease in antioxidant status, an increase in reactive oxygen species formation, and dyslipidemia[4]. The rate of diabetes and its complications evolve most directly with oxidative stress. Although several scientific and pharmacological arsenals are available, includin

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many potent hypoglycemic drugs, approximately half of the patients receive the glycolic treatment that is recommended based on our expertise. Side effects of currently available anti-hyperglycemic drugs include hypoglycemia, haematological problems, obesity, osteoporosis, hepatotoxicity, and lactic acidosis. These assessments of diabetics are the reason for the transition from symptomatic and allopathic therapy to natural therapy[5]. Therefore, there is a need to identify effective drugs for diabetes with fewer side effects, and traditional medicinal plants are preferred for this purpose. Globally, Herbal medicines are gaining popularity as a viable option for treating diabetes due to their low cost and low risk of side effects[6,7]. Many medicinal plants are currently available that also reduce the risk of cardiovascular disease due to hyperlipidemia in diabetics[8,9]. *Alstonia scholaris R.Br.* (Apocynaceae) is the medicinal plant described in Ayurveda with its therapeutic importance. Sapta means seven, and Parna means leaves; hence, another name for this plant is Saptaparna. It is widely distributed in India, Thailand, the Philippines, Africa, China, and Australia[10]. Plant-based products possess rich sources of antioxidant activity[11]. Tissue oxidative stress is managed by antioxidants by indirectly enhancing the cell's natural defence mechanisms and directly scavenging oxidative free radicals[12]. Many experimental studies indicate that antioxidants reduce oxidative stress caused by diabetes and manage its complications[13]. Therefore, medicinal plants are gradually gaining attentionforthe treatmentof diabetes with complication management. Antibacterial, anxiolytic, antiulcer, antimicrobial, antioxidant, and antidiarrheal are just a few of its pharmacological effects[14]. Traditionally, the plant has been used to treat whooping cough, chronic bronchitis, asthma and other respiratory infections[15]. Phyto components such as alkaloids, steroids, reducing sugars, and flavonoids are present in the plant. Previous studies only reported the hypoglycemic effect of *A. scholaris* leaves in Wistar rats[16]. However, the anti-hyperglycemic and antioxidant activities of the various active extracts of *A. scholaris* have not yet been recognized, nor has the mechanism involved in its action. Herbal remedies cannot be used therapeutically in the modern era without supporting scientific data. Therefore, the effects of ethyl acetate and ethanolic extract of *A. scholaris* leaves on antioxidant activity, anti-hyperglycemic activity, and lipid-lowering STZ-induced diabetic rats were examined.

2. Materials and Methods

2.1 Plant Authentication and Collection

In September 2020, we collected the leaves of *Alstonia scholaris* from Ahmedabad, Gujarat, India. The botanical identity of the plant material was verified by a taxonomist from Gujarat University (GU), Ahmedabad. The AS-1 specimen has been deposited in the GU herbarium in Ahmedabad for further reference.

2.2 Chemicals

Streptozotocin (STZ) was procured from ANJ biomedical, USA. The standard drugs are metformin from Apollo, Knoll Pharmaceuticals Ltd., and canagliflozin from INVOKANA, Johnson & Johnson. The chemicals and solvents used in the experiments were the entire analytical grade.

2.3 Preparation of Various Extracts

The leaves of *A. scholaris* were thoroughly washed with water and shaded at room temperature for seven days. Subsequently, they were reduced to a powder. The leaves of A. scholars were extracted using a Soxhlet apparatus with Petroleum Ether (PE), Chloroform (CH), Ethyl Acetate (EA), and Ethanol (Et) as solvents. The pulverized leaves of *A. scholaris* were packedin a Soxhlet apparatus and extracted with these four solvents by successive methods for 48 hours. A rotary evaporator was then used to concentrate the extracts. The percentage yields of PE, CH, EA, and Et were 4.2 ± 0.33 %, 8.2 ± 0.54 %, 13 ± 0.36 %, and 9.3 ± 0.71 % (w/w), respectively. For future research purposes, the powdered extracts were stored in a cool and dark place.

2.4 Preliminary Phytochemical Screening

The photochemical approach was used to perform a qualitative screening for bioactive components in four different *A. scholaris* preparations[17].

2.5 Animal

Male albino Wistar rats (230-250 g) were considered for this present experiment. All animals were maintained at 22°C temperature, 12–12 hour light-dark cycle, and 55% relative humidity. The rats were allowed to consume water ad libitum and SPD Standard Pellet Diet with 3% fat, 55% carbohydrates and 20% protein with 310 kJ/kg total calories. High-fat content with 15% butter, 10% egg yolk, 2% coconut oil, and 73% standard feed provides 412 kJ/kg calories for animals[18].

The study protocol with reference number: LMCP/Cology/17/09 was sanctioned by the Institutional Animal Ethics Committee (IAEC) according to Committee for Control and Supervision of Experiment on Animals (CPCSEA) guidelines, Animal Welfare Division, India[19].

2.6 Assessment of the Anti-Diabetic Effect of the EAEAS and EEAS

2.6.1 Induced Experimental T2DM

Animals were freely fed a high-fat diet for four weeks except for the vehicle control group. A single dose of STZ (45 mg/kg/i.p.) in chilled 0.1 M sodium citrate buffer adjusted to pH 4.3 was administered[20]. Drinking water was replaced with a 5% sucrose solution before and after the administration of STZ to overcome drug-induced hypoglycemia[21]. Moderately diabetic range rats (250–400 mg/dl blood glucose level) were included in the study. Then, the diabetic rats were kept for four weeks for the induction of complications.

2.6.2 Experimental Design and Treatment Protocol

Thirty-six rats were randomized into six different groups (G). Each group carried six animals.

G I: Vehicle Control (VC) treated with 0.9% saline

G II: Diabetes Control (DC) treated with STZ (45 mg/kg, i.p)

G III: EAEAS (400 mg/kg, PO bw)

G IV: EEAS (400 mg/kg, PO bw)

G V: MET (200 mg/kg, PO bw)

G VI: CANA (10 mg/kg, PO bw)

The dose of both extracts was selected based on the previously performed OGTT analysis. Both extracts and standard drugs were dissolved in 1% sodium Carboxymethylcellulose (CMC).

2.7 Bodyweight, Food, and Water Intake

The weight, water, and food intake of the rats were measured every week during the experiment.

2.8 Fasting Blood Glucose (FBG)

Throughout the experiment, FBG readings were recorded weekly. They were tested by pricking the tail veins of the rats with a glucometer (Accu Check, Germany). Estimates of fasting serum glucose levels were made on the last day of the experiment using a diagnostic kit and spectrometry (Span Diagnostic Ltd., Surat, and Gujarat, India).

2.9 Urine Output and Glucose Concentration

A metabolic cage was used to collect urine from animals. The urinary output was measured in ml using a glass cylinder. Glucose concentrations in urine were determined using a urine glucose assay kit (Oxidase method, India).

2.10 Analysis of Biochemical Parameters

In the end, the biochemical parameters were determined by collecting blood from the retro-orbital of Wistar rats. They were kept at room temperature for 30 min before centrifugation. They were centrifuged at 2500 rpm for 15 min at 4°C to isolate the serum. The appropriate spectrometric diagnostic kit was used to examine all biochemical variables.

2.10.1 Lipid Profile

Serum Total Cholesterol (T-CH), Triglyceride (TG), and High-Density Lipoprotein Cholesterol (HDL-CH) were determined with diagnostic kits (ERBA Diagnostics, Gujarat, India). Friedewald's formula[22] was used to calculate Low-Density Lipoprotein Cholesterol (LDL-CH) and very–low-density lipoprotein cholesterol (VLDL-CH).

$$VLDL\text{-}CH = TG\ 5\ LDL\text{-}CH = T\text{-}CH - (HDL\text{-}CH + VLDL\text{-}CH)$$

2.10.2 Cardiac Function Parameters

Standard diagnostic kit instructions were followed to measure cardiac injury markers Lactate Dehydrogenase (LDH) and Creatine Phosphokinase-MB (CK-MB) (Crest Biosystems, Goa, India). An automated plate reader was used to analyze the microplates (Lab System Multi-scan 51118220, Thermo Bioanalysis, Helsinki, Finland).

2.10.3 Liver Function Parameters

At the end of the study, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated with standard diagnostic kits (Crest Biosystems, Goa, India). Microplates were stored for further analysis using a plate reader.

2.10.4 Kidney Function Parameters

The levels of urea and creatinine were measured using standard test kits (Span Diagnostics, India).

2.11 *In Vitro* Liver Antioxidant Activity

2.11.1 Goat Liver Slices Preparation

Goat liver was chosen as the mammalian tissue to analyze the antioxidant effects of EEAS[23]. Fresh livers were collected from the local slaughterhouse immediately after the animal was sacrificed. The tissue was quickly immersed in a cold sterile (4°C) Phosphate Buffer Saline (PBS) solution. Liver tissue (1mm) was cut with a sterile scalpel. 250 mg tissue was stored in sterile PBS (1.0 ml). Both H_2O_2 and EEAS were mixed to incubate for 1 hour at 37°C with gentle shaking. Suitable Normal Control (NC) groups were established and H_2O_2 (2 ml/kg tissue) was used as the standard oxidant. After the incubation, a homogenizer was used to homogenize liver tissue with PBS buffer and centrifuged to collect the supernatant. It was used to estimate various parameters for evaluating the antioxidant potential.

2.11.2 Assessment of Enzymatic Antioxidants

Homogenized tissue (goat liver) was used for enzymatic antioxidant analysis, including Superoxide Dismutase (SOD) was estimated by Misra, *et al.*,[24] methods, reduced Glutathione (GSH) activity was estimated by the method of Moron, *et al.*,[25] and Catalase (CAT) was measured according to the procedure of Jhansyrani, *et al*[26].

2.12 Blood Pressure and Electrocardiogram (ECG)

Rats were given urethane i.p. at a dose of 1.3–1.5 g/kg in a solution of 1.5 g/5 ml. They were cannulated carotid arteries for monitoring systemic blood pressure and heart rate using the BIOPAC MP-150 data acquisition system (Biopac Systems Inc. Camino Goleta, CA, USA). ECG signals were also recorded using the appropriate three-needle electrode method for the limb lead at position II[27]. Three-needle electrodes were attached to theleft arm, right arm, and left hind leg. A negative electrode was placed under the skin of the frontpaw of the right arm and the positive electrode was placed on the left hind paw. Analysis and documentation of changes in the P wave, QT interval, and QRS complex in ECG were performed.

2.13 Histopathology

In the end, animals were sacrificed from which the heart, pancreas, kidney, and liver were isolated for histopathological examination. Formalin (10% v/v) was used to preserve all tissues of these organs after they had been irrigated with normal saline. Hematoxylin and eosin were used to stain each part of the organ (H and E). Digital microscope photos (MLX-B plus, Magnus microscope, China) were captured at 40x or 10x magnification to record histological details.

2.14 Statistical Analysis of Data

All data were presented in mean±SEM (Standard Error Mean) form. All data information was obtained from a one-way ANOVA following Tukey's multiple comparisons with Graph pad Prism 8.01 software. Compared to the respective controls, $P<0.05$ (p-value) was identified as statistically significant.

3. Results

3.1 Qualitative Phytochemical Screening

The phytochemical screening of EAEAS and EEAS reveals the presence of flavonoids, alkaloids, and steroids as the main chemical constituents, followed by a standard protocol[28].

3.2 Bodyweight Water and Food Intake

Table 1 summarizes the weight, water intake, and food intake data of male Wistar rats during the experiments. DC participants lost more weight compared to those in the vehicle control group. After four weeks of treatment, the body weights of the rats in the EEAS group improved significantly compared to those in the DC group. The diabetic group consumed significantly ($p<0.05$) more water than the VC group. There was no discernible difference in water consumption between the EEAS-treated group and the diabetic rat group. Comparing the DC to the VC, we find that the DC significantly $p<0.05$ increases its food consumption. At the end of the treatment period, the EEAS groups showed a statistical ($p<0.05$) reduction in food intake compared to the DC group. The water intake of the DC was significantly ($p<0.05$) increased compared with the VC. As a final result, the EEAS treatment group showed no significant difference in water intake comparedwith diabetic rats. A significant ($p<0.05$) increase in food intake was observed in the DC compared to the VC. The EEAS groups showed a significant ($p<0.05$) reduction in food intake compared to the DC group on the 28th day of the treatment period.

3.3 Fasting Blood Glucose

Figure 1 summarizes the data on the FBG levels of the various groups. A reduction in fasting blood glucose levels was observed after treatment with EAEAS and EEAS compared to the DC group. EAEAS and EEAS, reduced fasting blood glucose by 70.94% and 56.14% in diabetic rats. Canagliflozin and metformin, two common diabetes drugs, have been shown to reduce fasting blood glucose levels by 45.25% and 53.07%, respectively.

Table 1. Effect of EAEAS and EEAS on body weight, food and water intake in STZ-induced diabetic

Experimental groups	Body weight (gm)		Food intake (gm/rat/day)		Water intake (ml/rat/day)	
	Initial	Final	Initial	Final	Initial	Final
VC	240.7±3.81	309.7±13.65	20.9±0.6	24.68±0.51	24.01±1.683	38.49±1.831
DC	246.8±2.27	196.3±8.25***	22.45±0.35	30.7±1.1*	23.8±0.19	63.15±2.15**
EAEAS	248.16±5.24	197±6.97***	22.88±0.28	28±0.5	21.9±0.6	56.2±2.2**
EEAS	246±6.40	228.7±13.29$^{\#\#}$	22.83±0.16	25.78±0.61$^{\#}$	22.31±1.01	63.65±0.64***
CANA	244.67±1.88	240.7±1.88$^{\#\#\#}$	24.4±0.4	25.56±0.76$^{\#}$	25.47±0.13	69.11±1.11***
MET	208.5±3.56	257.7±4.49$^{\#\#}$	23.63±0.03	21.58±1.03$^{\#\#}$	25.31±0.98	48.2±3.2$^{\#}$

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 and $^{\#\#\#}$p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

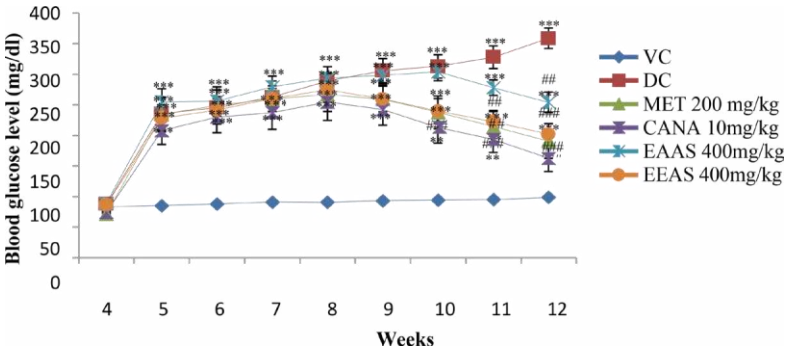


Figure 1. Effects of EAEAS and EEAS on blood glucose levels in STZ-induced diabetic rats. The results are expressed in Mean ± SEM. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 and $^{\#\#\#}$p < 0.001as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

3.4 Urine Output and Glucose Concentration

There was a statistically significant ($p<0.001$) increase in urination in diabetic rats compared to VC (Table 2). In diabetic rats, urine output was significantly ($p<0.001$) increased compared to VC. Compared to DC, EEAS treatment showed no significant effect on urinary output. Based on the results, the data of the EEAS-treated group showed no significant difference in urinary glucose concentration compared to diabetic rats.

3.5 Lipid Profile

Table 3 compiles the lipid profile changes caused by EAEAS and EEAS. The effects of EAEAS and EEAS on lipid profile. The STZ-induced diabetic rats reflected a significant increase in TG, T-CH, LDL-CH, and VLDL-CH while HDL-CH decreased compared to VC. EAEAS and EEAS (400 mg/kg) showed a significant decrease in lipid profile, while both treated groups significantly improved HDL-CH levels.

3.6 Cardiac, Liver, and Kidney Parameters

The results of the heart, liver, and kidney parameters are summarized in Table 3. The EEAS treatment group significantly ($p<0.001$) improved CK-MB and LDH levels compared to diabetic rats. Treatment with EEAS significantly ($p<0.001$) reduced ALT and AST levels in diabetic rats. Oral administration of EEAS lowers serum urea and creatinine levels. Standard drugs significantly improved markers of cardiac, renal, and hepatic damage in diabetic rats.

Table 2. Effect of EAEAS and EEAS in urine profile in STZ-induced diabetic rats

Parameters	VC	DC	EAEAS	EEAS	CANA	MET
Urine output (ml/rat/day)	16.38± 0.36	37.52±0.69***	31.34±1.41***	39.35±1.08***	43.78±0.57***	25.24±1.20##
Urine glucose level (mg/dl)	6.76±0.70	465.23±16.5***	407.63±11.1#	484±10.2	550.46±10.13###	374.29±10###

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6*p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison

Table 3. Effect of EAEAS and EEAS on biochemical parameters in STZ-induced diabetic rats

Parameters		VC	DC	EAEAS	EEAS	CANA	MET
Lipid profile	CH (mg/dl)	129.90±3.183	257.00±5.55***	220.00±1.06###	173.40±2.78###	174.20±3.23###	196.00±3.68###
	TG (mg/dl)	80.04±1.23	130.00±0.49***	108.3±4.11###	98.00±3.46###	82.77±3.29###	98.23±3.52###
	HDL (mg/dl)	85.82±2.09	56.95±3.9***	50.55±2.15#	85.07±2.74###	94.47±3.06###	87.93±4.22###
	LDL (mg/dl)	30.45.±2.24	174.00±1.81***	148.30±3.93##	68.40±3.48###	62.86±1.79###	88.54±4.48###
	VLDL (mg/dl)	16.01±0.24	26.14±0.49***	21.67±0.82###	19.60±0.69###	16.55±0.65###	19.65±0.65###
Liver function test	ALT (IU/L)	37.48±2.87	76.96±2.51***	70.20±1.90	57.83±2.93#	52.00±5.02##	58.30±0.95#
	AST (IU/L)	33.95±2.10	63.82±2.22***	60.75±0.95	50.03±4.71#	44.73±4.43##	49.33±1.81#
Kidney function test	Creatinine (mg/dl)	0.58±0.01	0.88±0.01***	0.89±0.02	0.73±0.03#	0.63±0.03###	0.73±0.06#
	UREA (mg/dl)	20.00±0.86	33.75±1.49***	30.19±2.19	20.57±1.01###	17.17±0.46###	21.54±1.70###
Cardiac function test	CK-MB (IU/L)	14.47±0.48	30.96±2.06***	25.45±1.75	19.03±0.93###	18.23±0.93###	18.40±0.58###
	LDH (IU/L)	136.80±3.06	240.00±19.10***	196.50±14.50	149.00±10.97###	138.70±3.75###	143.00±5.85###

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; #p < 0.05, ##p < 0.01 and ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey multiple tests for comparison.

3.7 Liver Oxidative Stress of Enzymatic and Non-Enzymatic Biomarkers

Figure 2 illustrates SOD, CAT, and GSH antioxidant activity performed on goat liver tissue reflecting a significant ($p<0.001$) declination in the toxic (H_2O_2 exposed) group compared to the untreated-Normal Control (NC). The EEAS treatment group has significantly ($p<0.001$) increased SOD, GSH, and CAT levels compared with the Toxic Group (TG).

3.8 Effect of EEAS on Blood Pressure and ECG

At the end of the study, Systolic (SBP) and Diastolic Blood Pressure (DBP) were elevated in the DC group (Table 4). Compared to vehicle control, EEAS significantly ($p>0.05$) reduces SBP but does not affect DBP. STZ-induced diabetic rats had a lower heartrate while they improved in the EEAS-treated group. Table 4 shows the relationship between PR interval, QT interval, and QRS duration. Both the PR interval and the QRS duration were significantly longer in the STZ-induced diabetic rats. Diabetic rats treated with EEAS observed a reduction in prolonged intervals.

3.9 Histology Study of Liver, Pancreas, Kidney, and Heart

Figure 3 shows a histological section of the liver. No obvious microscopic or morphological changes were found in the tissue sample of the VC group. Hepatocytes with significant vacuolation in their cytoplasm, a disrupted portal connection, disorganized hepatocytes,

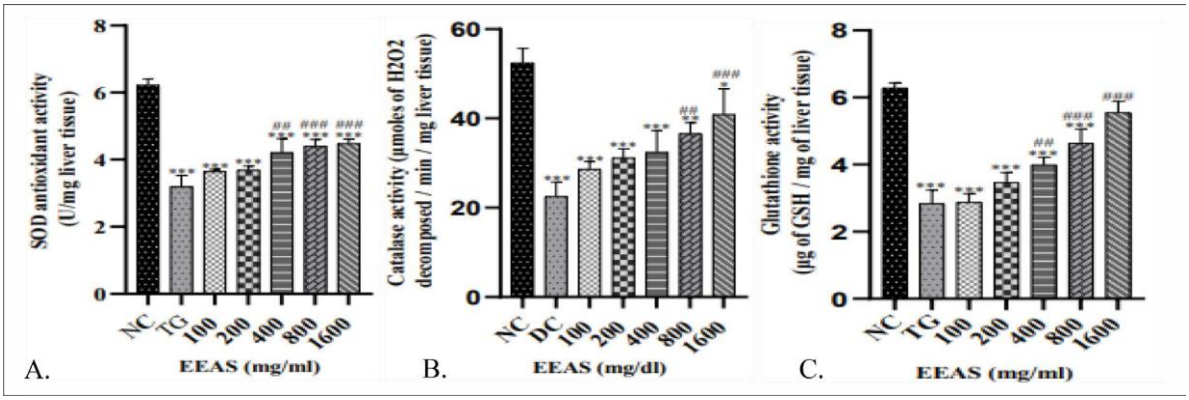


Figure 2. Antioxidant enzyme activity of EEAS on goat liver homogenate. **(A)** SOD. **(B)** CAT and **(C)** GSH. NC, untreated normal control; TG, Toxic group; EEAS, ethanolic extract of *A. scholaris* leaves. The results are expressed in Mean ± SEM. Each group comprised triplicates. *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to NC; $^{\#}$p < 0.05, $^{\#\#}$p <0.01 and $^{\#\#\#}$p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

Table 4. Effect of EAEAS and EEAS on blood pressure and ECG on STZ-induced diabetic rats

Parameters	VC	DC	EAEAS	EEAS	CANA	MET
Systolic BP (mmhg)	122.00± 1.0	150.00±2.0***	144.50± 1.50	130.50± 1.50$^{\#\#}$	126.50± 1.5$^{\#\#\#}$	122.70± 0.85$^{\#\#}$
Diastolic BP (mmhg)	102.60± 5.9	140.50±18.69***	137.50± 5.56	122.90± 4.45$^{\#\#}$	109.40± 3.0$^{\#\#\#}$	116.40± 2.96$^{\#\#}$
Heart rate (beats/min)	325.50± 0.5	430.00±2.0***	400.0± 6.0$^{\#}$	344.50±7.5$^{\#\#\#}$	332.50± 4.50$^{\#\#\#}$	357.00± 4.0$^{\#\#\#}$
PR interval (ms)	50.50±1.53	59.00±1.0**	58.00±1.0	55.50±0.50	53.00±1.0$^{\#}$	55.00±1.0
QT interval (ms)	71.00±1.0	88.00±1.0*	80.00±2.0$^{\#}$	74.50±0.50$^{\#\#}$	72.00±1.00$^{\#\#}$	75.00±1.0$^{\#\#}$
QRS interval (ms)	19.50±0.50	25.50±0.5**	24.50±0.5	21.50±0.50$^{\#}$	20.00±1.0$^{\#\#}$	22.50±1.50$^{\#}$

VC, Vehicle control; DC, diabetic control; EAEAS, ethyl acetate extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin; MET, metformin. Data are expressed as mean±S.E.M. (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 as compared to VC; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 and $^{\#\#\#}$p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

and a central vein were seen in the DC group. The microvasculature of hepatocytes was found to be moderately enhanced in the CANA group. Hepatocyte, portal vein, and central vein damage in STZ-induced diabetic rats were alleviated in the EEAS therapy group. Figure 4 depicts a microscopic histological segment of the pancreas. Massive degranulation, cell death, and the presence of necrotic and inflammatory cells were all seen in the DC group. EEAS restores the damage, increases B cells, and improves islets of Langerhans morphology. A histological kidney section is shown in Figure 5. Damaged glomeruli and early lipid deposition, lymphocyte infiltration, and renal deformation from inflammation were all observed in the diabetic rat. EEAS treatment improved the vasculature and glomeruli. Figure 6 shows a heart section under a microscope. Disorganized cardiomyocytes were observed in diabetic rats, characterized by a lack of striations, a large interstitial space, and a deformed intercalated disc. The EEAS group exhibits slight displacement of the gap and few numbers of the degenerated cell.

4. Discussion

The World Health Organization (WHO) ranked type 2 diabetes mellitus (T2DM) as the ninth leading cause of death worldwide in 2019[29]. Despite the widespread availability of various anti-diabetic drugs, natural products have always had an advantage in the management of diabetes. In the present study, the fasting blood glucose level of the EEAS group (400 mg/kg) in diabetic rats was significantly lower after four weeks of treatment in this study. Both EAEAS (400 mg/kg) and EEAS (400 mg/kg) significantly reduced

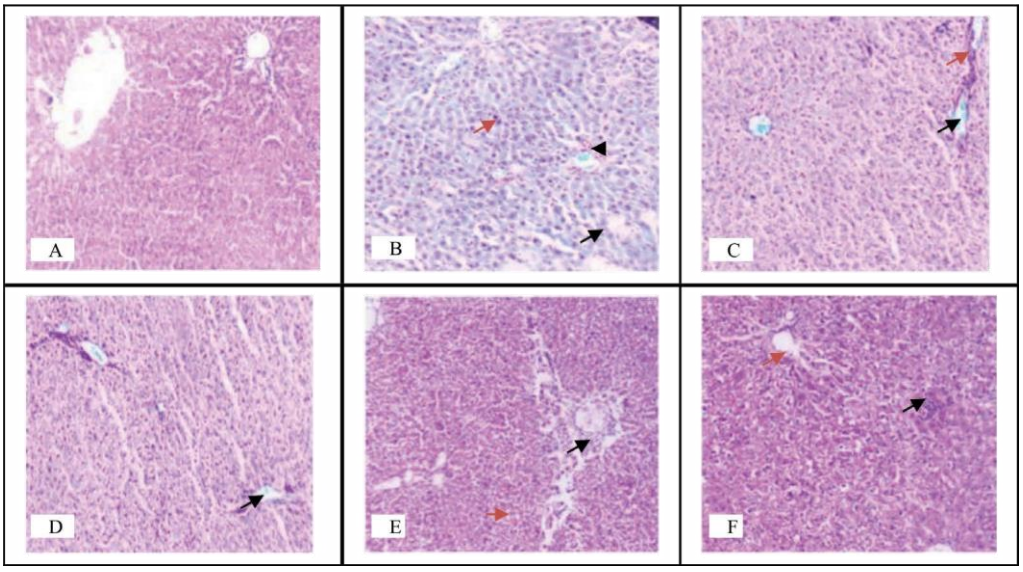


Figure 3. Histological sections of rat liver from different groups (H and E x10). **(A).** VC group showing normal hepatic cords and portal triad; **(B).** DC group representing increased cytoplasmic vacuolation of hepatocytes (black arrowhead), mononuclear cell infiltration of liver tissue (black arrow) and activation of Kupffer cells (red arrow); **(C).** CANA group shows areas of leukocyte aggregations (red arrow) and an area of the portal tract with dilated obstructed portal vein (black arrow); **(D).** MET group shows congestion of some central veins (black arrow); **(E).** EAEAS group showing hepatocytes associated with mononuclear cell infiltration (black arrow), and cytoplasmic vacuolation of hepatocytes (red arrow) and portal vein arrays; **(F).** EEAS treated group showing improved portal vein triad, central vein, mild hydropic degeneration (black arrow) and mild hepatic sinusoid congestion (red arrow).

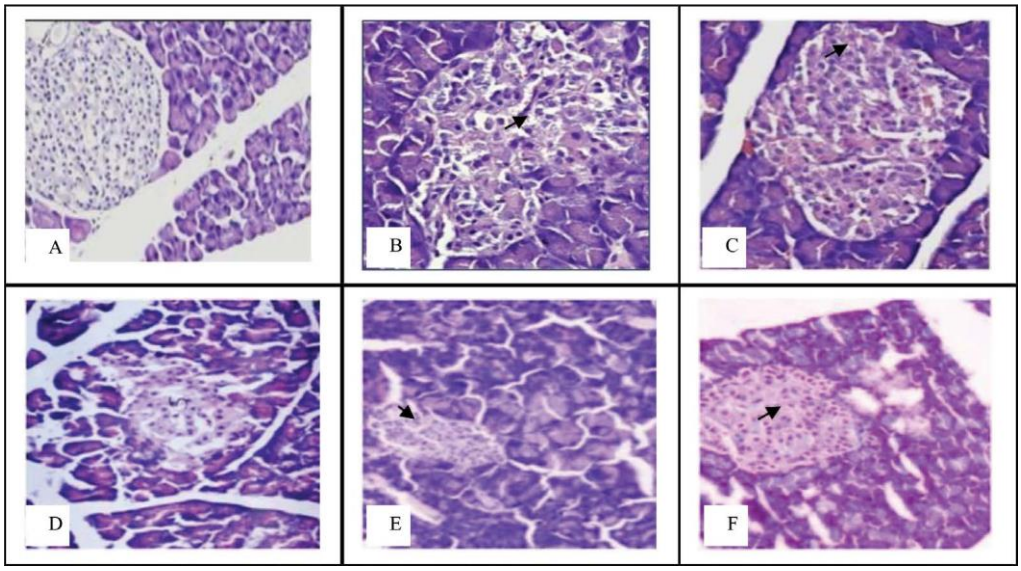


Figure 4. Histological sections of rat pancreas from different groups (H and E x40). **(A).** VC group representing normal islet of Langerhans; **(B).** DC group showing extensive degranulation, destruction of cells, presence of necrotic cells and inflammatory cells (black arrow); **(C).** CANA group showing improved pancreatic islets of Langerhans with beta cell enhancement (black arrow); D: MET group showing recovery of normal Langerhans islet cell population; **(E).** EAEAS group representing abnormal islets of Langerhans; **(F).** EEAS group showing improvement in the islet of Langerhans morphology with an increasing number of beta cells (black arrow).

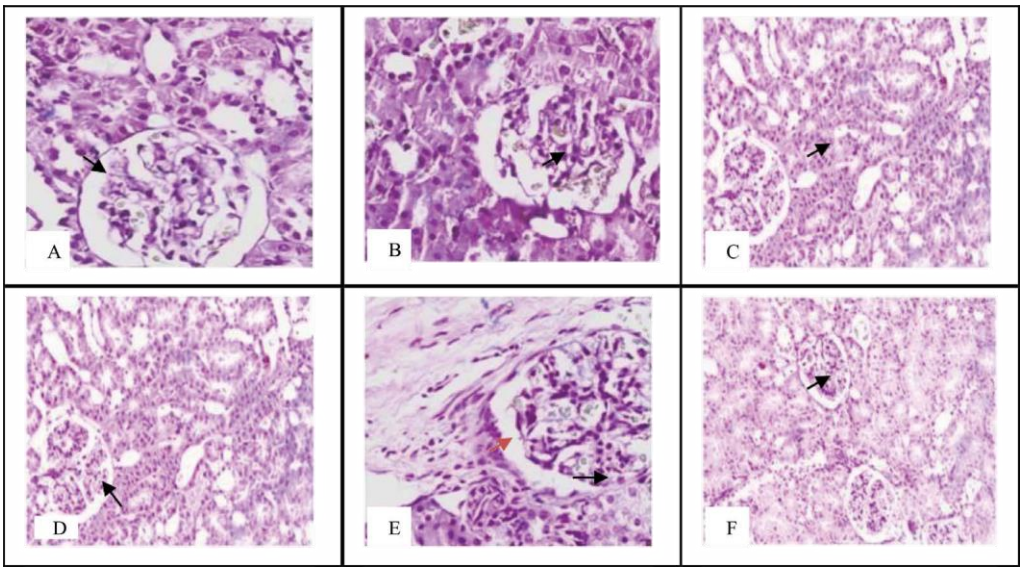


Figure 5. Histological sections of rat kidneys from different groups (H and E x40). **(A).** VC group showing normal glomeruli with normal baseline and tubules (black arrow); **(B).** DC group representing disrupted glomeruli with baseline fat deposition along with infiltration of lymphocytes (black arrow); **(C).** CANA with improved vascular system (black arrow) and less intestinal infiltration; **(D).** MET group with improved glomeruli with normal baseline and tubules (black arrow); **(E).** EAEAS group with glomerular lymphocytic infiltration (black arrow) thickening of basement membrane (red arrow); **(F).** EEAS group showing improved vasculature and glomeruli (black arrow).

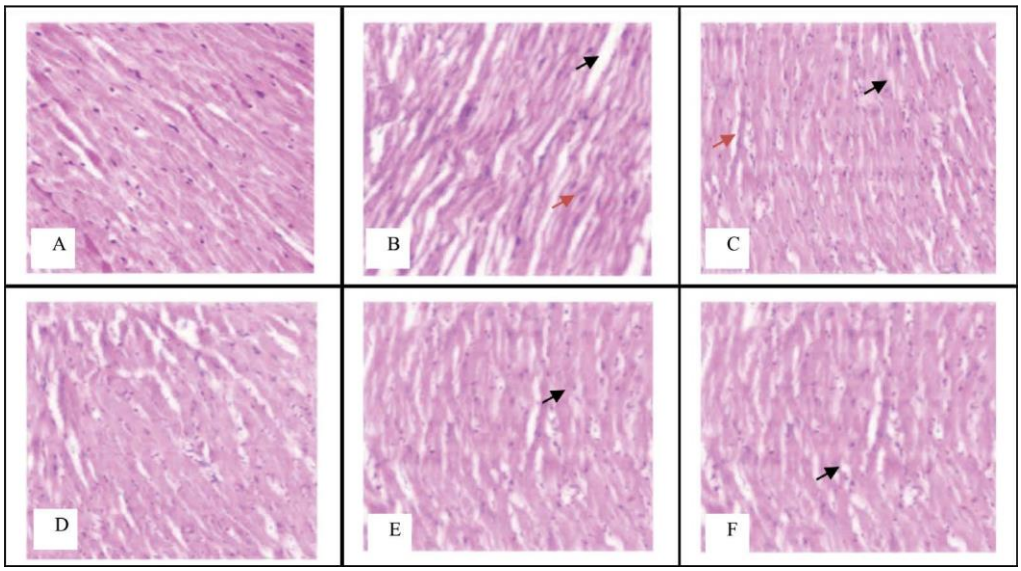


Figure 6. Histological sections of rat hearts from different groups (H and E x40). **(A).** VC group showing well-arranged cardiomyocytes and normal streaks; **(B).** DC group depicting disordered cardiac myocytes with disrupted streaks, enlarged interstitial space (red arrow) and distorted intercalated disc (black arrow); **(C).** CANA group showing normal heart tissue with enhanced features of myocytes, less visualization of the interstitial space (red arrow) and distortion of the intercalateddisc (black arrow); **(D).** MET group with some distorted intercalated discs; **(E).** EAEAS group showing stronger evidenceof interstitial space (black arrow); **(F).** EEAS group showing the mild imaging of the interstitial space (black arrow) and distorting the intercalated disc.

FBG levels in normoglycemic rats, as reported in our previous Oral Glucose Tolerance Test (OGTT) results. The anti-diabetic effects of the ethanolic extract of *A. scholaris* were more pronounced. Diabetic rats lose weight steadily due to an increase in blood sugar levels, loss of tissue proteins (gluconeogenesis), and increased muscle wasting[30]. EEAS improved weight loss in diabetic rats because it offers opportunities to restore protein metabolism. High protein catabolism and hyperosmolarity from high blood glucose levels promote polyphagia and polyuria in diabeticrats. The result of the present study indicates that increased glucose consumption is associated with decreased food and water intake. Metformin improves treatment by increasing hepatic glucose metabolism, while canagliflozin causes excess glucose excretionin the urine. To investigate the potential mechanism underlying the anti-hyperglycemic effect, we analyzed several biochemical markers and urinalysis results. EEAS (400 mg/kg) increases both urine production and urine glucose concentration, leading to a decrease in high blood glucose levels. Consequently, the anti-hyperglycemic effect of EEAS can be attributed to increased urinary glucose excretion. Irregularitiesin lipid metabolism lead to atherosclerosis, which is more common in people with diabetes mellitus[31]. Oral administration of EEAS improved lipid profiles and HDL-CH levels. EEAS's ability to lower blood cholesterol levels reflects its effect in preventing cardiovascular problems that can arise from diabetes. Long-term use of certain herbal plants can be harmful to the liver and kidneys. Therefore, we evaluated EEAS activity in diabetic rats to examine its long- term impact on kidney and liver function. Alanine aminotransferase and aspartate aminotransferase are two reliable liver indicators. This STZ induces liver toxicity in diabetic control rats due to leakage of these protein enzymes from the liver cytosol into the blood flow region[32]. EEAS restores levels of these enzymes that have been damaged by STZ. Creatinine and urea are the marker functions of kidney damage reduced by EEAS treatment in diabetic rats. Reactive oxygen species are unstable chemicals that have negative effects on many biological components, including lipids, proteins, and DNA, through their oxidative action. In our study (*in vitro*), EEAS showed significant

SOD, CAT, and GSH activity, suggesting that it also has antioxidant potential. Antioxidants present in the body provide resistance to oxidative stress by scavenging free radicals and suppressing both oxidation and lipid peroxidation to prevent disease[33]. Diabetic rats showed morphological abnormalities and microscopicchanges in the liver, pancreas, kidneys, and heart as compared to VC animals. Extensive vacuolation in the cytoplasm of the hepatocytes, a damaged portal specimen, disorganized hepatocytes, and a central vein were observed in a histological segment of the liver. The rate of morphological damage in the EEAS treatment group decreased to levels similar to the VC group. Microscopy of a pancreatic tissue segment of diabetic rats showing severe degranulation, cell death, and the presence of necrotic and inflammatory cells. The shape of the islets of Langerhans and the number of beta cells improved during EEAS treatment. Histological analysis of a kidney tissue sample reveals that glomeruli have been damaged, with fat depositing at baseline and lymphocytes infiltrating the area. Diabetic rats induced by Streptozotocin (STZ) show severe damage and morphological abnormalities, although EEAS therapy successfully mitigates these effects. The cardiac tissue of diabetic rats showed significant interstitial space and myocardial cell dysfunction with disrupted streaksupon histological examination. Minor interstitial space movement, intercalated disc distortion, and a few deteriorated cells were seen in the EEAS therapy group. For any interpretation of the pharmacological action, it is necessary to evaluate the phytochemical compositions of various extracts of each herbal drug. Qualitative phytochemical screening identified the primary bioactive components of EEAS were identified as alkaloids, flavonoids, and phenols. Bioactive components such as alkaloids, flavonoids, and phenols are discussed for their role in the treatment of diabetes[34]. Fortunately, all of these bioactive components are found in *A. scholaris* in moderate concentrations. Therefore, phytochemical components of *A. scholaris* leaves can be considered a significant approach to enhancing the antioxidant, hypolipidemic, and anti-hyperglycemic potential for treating type two diabetes mellitus. Future developments in experimental design are intended to provide its mechanism of action, which will make an innovative contribution to research communities.

5. Conclusion

This research leads to a valid conclusion and supports the ethanolic extract of *A. scholaris* at a dose of 400 mg/kg for the reduction of common symptoms of type 2 diabetes mellitus including polydipsia, polyphagia, and hyperlipidemia in diabetic rats. It significantly reduces markers of heart, kidney and liver damage. It also reflects the improvement in blood pressure, ECG, antioxidant levels, and reverse modulation in the histopathology of various organs. Consequently, it can exert anti-hyperglycemic, antioxidant and hypolipidemic effects in diabetes with its attendant complications. In the future, studying its effect on intestinal absorption at the molecular level and *in vitro* research could shed light on the primary mechanism of its anti-diabetic efficacy.

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7. References

1. Mellitus D. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2005; 28(S37):S5-10. https://doi.org/10.2337/diacare.28.suppl_1.S37 PMid:15618111
2. Aschner P, Karuranga S, James S, Simmons D, Basit A, Shaw JE, Wild SH, Ogurtsova K, Saeedi P. The International Diabetes Federation's guide for diabetes epidemiological studies. Diabetes research and clinical practice. 2021; 172. https://doi.org/10.1016/j.diabres.2020.108630
3. Alabi TD, Brooks NL, Oguntibeju OO. Antioxidant sta- tus and hepato-protective role of *Anchomanes difformis* in streptozotocin-induced diabetes in male Wistar rats. Herba Polonica. 2020; 66(1). https://doi.org/10.2478/hepo-2020-0005
4. Vickers NJ. Animal communication: when I'm calling you, will you answer too? Current Biology. 2017; 27(14):R713-5. https://doi.org/10.1016/j.cub.2017.05.064 PMid:28743020
5. Chandrashekar CN, Madhyastha S, Benjamin S, Gopalakrishna K, Srinivasan KK. Effect of *Salacia reticulata* Wight extracts on drug induced diabetes mellitus in rats. Herba Polonica. 2008; 54(2).
6. Kaushik G, Satya S, Khandelwal RK, Naik SN. Commonly consumed Indian plant food materials in the management

of diabetes mellitus. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2010; 4(1):21-40. https://doi.org/10.1016/j.dsx.2008.02.006

7. Hnatyszyn O, Mino J, Ferraro G, Acevedo C. The hypoglycemic effect of *Phyllanthus sellowianus* fractions in streptozotocin-induced diabetic mice. Phytomedicine. 2002; 9(6):556-9. https://doi.org/10.1078/09447110260573209 PMid:12403166
8. Bhatnagar D. Lipid-lowering drugs in the managementof hyperlipidaemia. Pharmacology and Therapeutics. 1998; 79(3):205-30. https://doi.org/10.1016/S0163-7258(98)00018-7 PMid:9776377
9. Rani A, Kumar S, Khar RK. Murraya koenigii extract loaded phytosomes prepared using antisolvent precipitation technique for improved antidiabetic and hypolidemic activity. Indian Journal of Pharmaceutical Education and Research. 2022; 56 (2):326-38. https://doi.org/10.5530/ijper.56.2s.103
10. Zhao YL, Gou ZP, Shang JH, Li WY, Kuang Y, Li MY, Luo XD. Anti-microbial effects *in vitro* and *in vivo* of *Alstonia scholaris*. Natural Products and Bioprospecting. 2021; 11(1):127-35. https://doi.org/10.1007/s13659-020-00294-6 PMid:33389714 PMCid:PMC7778864
11. Craig WJ. Health-promoting properties of common herbs. The American Journal of Clinical Nutrition. 1999; 70(3):491s-499s. https://doi.org/10.1093/ajcn/70.3.491s PMid:10479221
12. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *Invitro* and *in vivo* antioxidant properties of different frac- tions of *Moringa oleifera* leaves. Food and Chemical Toxicology. 2009; 47(9):2196-201. https://doi.org/10.1016/j.fct.2009.06.005 PMid:19520138
13. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos University Medical Journal. 2012; 12(1):5. https://doi.org/10.12816/0003082 PMid:22375253 PMCid:PMC3286717
14. Anubha A, Rai Y. A Review: Phytochemictry, Ethanobotanical and Pharmacological Activities of *Alstonia scholaris* R. Br (Apocynaceae). Int J Adv Res. 2015; 3:584-90.
15. Zhao YL, Yang ZF, Shang JH, Huang WY, Wang B, Wei X, Khan A, Yuan ZW, Liu YP, Wang YF, Wang XH. Effects of indole alkaloids from leaf of *Alstonia scholaris* on post-infectious cough in mice. Journal of Ethnopharmacology. 2018; 218:69-75. https://doi.org/10.1016/j.jep.2018.02.040 PMid:29496577 PMCid:PMC7126965
16. Arulmozhi S, Mazumder PM, Lohidasan S, Thakurdesai P. Antidiabetic and antihyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R. Br. European Journal of Integrative Medicine. 2010; 2(1):23-32. https://doi.org/10.1016/j.eujim.2009.12.001
17. Hussain AI, Anwar F, Chatha SA, Latif S, Sherazi ST, Ahmad A, Worthington J, Sarker SD. Chemical composition and bioactivity studies of the essential oils from two Thymus species from the Pakistani flora. LWT-Food Science and Technology. 2013; 50(1):185-92. https://doi.org/10.1016/j.lwt.2012.06.003
18. Liu XY, Liu FC, Deng CY, Zhang MZ, Yang M, Xiao DZ, Lin QX, Cai ST, Kuang SJ, Chen J, Chen SX. Left ventricular deformation associated with cardiomyocyte Ca2+ transients delay in early stage of low-dose of STZ and high-fat diet induced type 2 diabetic rats. BMC Cardiovascular Disorders. 2016; 16(1):1-2. https://doi.org/10.1186/s12872-016-0220-8 PMid:26879576 PMCid:PMC4754853
19. Pereira S, Veeraraghavan P, Ghosh S, Gandhi M. Animal experimentation and ethics in India: the CPCSEA makes a difference. Alternatives to Laboratory Animals. 2004; 32(1_suppl):411-5. https://doi.org/10.1177/026119290403201s67 PMid:23581110
20. Bhandari U, Kumar V, Kumar P, Tripathi CD, Khanna G. Protective effect of pioglitazone on cardiomyocyte apoptosis in low-dose streptozotocin and high-fat diet-induced type-2 diabetes in rats. The Indian Journal of Medical Research. 2015; 142(5):598. https://doi.org/10.4103/0971-5916.171290 PMid:26658596 PMCid:PMC4743348
21. Rani AN, Mahmud R, Amran N, Asmawi MZ, Mohamed N, Perumal S. *In vivo* hypoglycemic investigation, antihyperglycemic and antihyperlipidemic potentials of Pereskia bleo Kunth. in normal and streptozotocin-induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2019; 9(2):73. https://doi.org/10.4103/2221-1691.250858
22. Freidewald WT. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of a centrifuge. Clin Chem. 1972; 18:449-502. https://doi.org/10.1093/clinchem/18.6.499 PMid:4337382
23. Vidya R, Kalaivani K, Venkatesh R. Antioxidant activity of goat liver slices treated with *Cucumis melo* (L). Fruit extract. International Journal of Pharmaceutical Sciences and Research. 2014; 5(11):4846.
24. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972; 247(10):3170-5. https://doi.org/10.1016/S0021-9258(19)45228-9 PMid:4623845
25. Moron MS, Depierre JN and Mannervik VC: Levels of glutathione, glutathione reductase and glutathione Stransferase activities in rat lung and liver. Biochem Biophys Acta. 1979; 582:67-8. https://doi.org/10.1016/0304-4165(79)90289-7 PMid:760819
26. Jhansyrani T, Sujatha D, Rupasree P, Bharathi K, Kvsrg P. *In-vitro* antioxidant potential of selected nutraceuticals. International Journal of Pharmaceutical Sciences and Research. 2019; 10(3):1426-32.

27. Fontaine AK, Futia GL, Rajendran PS, Littich SF, Mizoguchi N, Shivkumar K, Ardell JL, Restrepo D, Caldwell JH, Gibson EA. Optical vagus nerve modulation of heart and respiration via heart-injected retrograde AAV. Scientific Reports. 2021; 11(1):1-2. https://doi.org/10.1038/s41598-021-83280-3 PMid:33574459 PMCid:PMC7878800
28. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pacific Journal of Tropical Biomedicine. 2014; 4:S359-67. https://doi.org/10.12980/APJTB.4.2014C1030 PMid:25183112 PMCid:PMC4025295
29. World Health Organization; 2019.
30. Poongothai K, Ponmurugan P, Ahmed KS, Kumar BS, Sheriff SA. Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown and *in vitro* raised) extracts on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine. 2011; 4(10):778-85. https://doi.org/10.1016/S1995-7645(11)60193-4 PMid: 22014732
31. De Rosa S, Arcidiacono B, Chiefari E, Brunetti A, Indolfi C, Foti DP. Type 2 diabetes mellitus and cardiovascular disease: Genetic and epigenetic links. Frontiers in Endocrinology. 2018; 9:2. https://doi.org/10.3389/fendo.2018.00002 PMid:29387042 PMCid:PMC5776102
32. Hosseini SA, Ahmadipour A, Soltani M, Mehdipour M, Mandegary A, Karami-Mohajeri S. Malathion- increased hepatotoxicity in diabetic rats. Pharmaceutical and Biomedical Research. 2020; 6 (1):53-60. https://doi.org/10.18502/pbr.v6i1.3428
33. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition Journal. 2015; 15(1):1-22. https://doi.org/10.1186/s12937-016-0186-5 PMid:27456681 PMCid:PMC4960740
34. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 2020; 9(9):252. https://doi.org/10.3390/biology9090252 PMid:32872226 PMCid:PMC7563488

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PHYTOCHEMICAL SCREENING AND EVALUATION OF VARIOUS *ALSTONIA SCHOLARIS* (*L.) R. Br.* LEAVES EXTRACTS ON GLUCOSE HOMEOSTASIS IN NORMAL RATS

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ABSTRACT

Alstonia scholaris (L.) R. Br. is medicinal plant species (Family: Apocynaceae). *A. scholaris* has several drug applications for the treatment of various chronic diseases andit has limited information regarding its traditional use in treating diabetes from a scientific perspective. To determine the phytochemical compositions of three different extracts of *A. scholaris*; chloroform extract (CEAS), ethyl acetate extract (EAEAS), and ethanolic extract (EEAS) of *A. scholaris* leave. In addition, we evaluated the effects ofthese three extracts on glucose homeostasis in normal rats using the oral glucose tolerance test (OGTT). Male Wistar rats (250-300 g) were randomly divided into twelve groups (n=3/group). Normal saline-treated group, three different doses of all the three extracts (200, 400, 800 mg/kg, p.o.) treated groups, canagliflozin (CANA) 1mg/kg, p.o treated group, and metformin (MET) 200 mg/kg, p.o standard treated group. After 30 minutes of drug administration, all groups were given a glucose solution (2g/kg). At 0,30, 60, 90, 120, and 150 minutes, blood glucose levels were measured by pricking tail veins with a glucometer. The phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, terpenoids, phenols, and saponins. At doses of 400mg/kg, EAEAS and EEAS significantly reduced blood glucose levels ($p<0.05$) compared to the vehicle control and the standard drugs. However, CEAS did not show any

significant changes compared to vehicle control at any dose. EAEAS and EEAS at 400mg/kg dose have phytochemical constituents that improve the anti-hyperglycemic effect and maintain glucose homeostasis in hyperglycemia.

Keywords: *Alstonia scholaris*, Anti-hyperglycemic, Diabetes, Glucose Homeostasis, Oral Glucose Tolerance Test (OGTT)

INTRODUCTION :

An important part of the physiological function is glucose homeostasis, which is regulated by two hormones, insulin, and glucagon **[1]**. By increasing lipogenesis, and glycogen synthesis, insulin increases glucose disposition in peripheral tissues, while glucagon produces the opposite effect. Diabetes mellitus, orhyperglycemia, is the result of anabnormality in glucose homeostasis thatis usually caused by an inability to secrete insulin or by insulin resistance. Furthermore, long-term high blood glucose levels may lead to further macro or microvascular complications. Therefore, improving insulin secretion and resistance is very important for normalizing glucose homeostasis and preventing chronic complications. Numerous research projects are being conducted all over the world, and several pharmacological products containeffective anti-hyperglycemic medications, but few patients achieve the glycemic treatment set by our experts **[2]**. The Oral Glucose Tolerance Test (OGTT) is a test of the body's ability to metabolize glucose that involves the administration of a measured dose of glucose to the fasting stomach and the determination of glucose levels in the blood at measured intervals thereafter and that is used especially to evaluate specific values for normal and disease condition(diabetes mellitus). Currently approved anti-hyperglycemic agents act by enhancing insulin sensitivity, insulin secretion increase, and glucose absorption reduction but available agents have one or more undesirable side effects like hypoglycemia, hematological disorders, obesity, osteoporosis, hepatotoxicity, and lactic acidosis **[3]**. Therefore, it is important to find out an effective medication with fewer side effects for diabetes in which traditional medicinal plants are more preferred. World health organization (WHO) reported that traditional plant medicinesare used for the treatment of diabetes with fewer side effects and are cost- effective **[4]**. Many medicinal plants are currently available that also reduce the risk of cardiovascular disease that arises

due to hyperlipidemia in diabetics **[5]**. Many diabetic patients have consumed extracts, whole plants, and herbal formulations along with hypoglycemic drugs to accomplish the anti-diabetic effect and prevent its complications **[6]**. Hence, in light of these shreds of evidence, the development of plantsources and drugs are considered potential sources for the treatments. *Alstonia. scholaris* R.Br. (Apocynaceae) is the medicinal plant described in Ayurveda with its therapeuticimportance. It is also known as saptaparna in which "Sapta" means seven and "Parna" means leaves. In Ayurveda, *A. scholaris* is used in Jwara, Shwas, Kushtha, Gulma, Vranaetc **[7]**. It is widely distributed and used in India, Thailand, Philipines, Africa, China, Australia **[8]**, etc. It has various pharmacological activities also as antibacterial, anti-anxiety, antiulcer, anti- microbial, antioxidant, anti-diarrheal, and is also used in wound healing **[9]**, etc. Traditionally the plant is used in whooping cough, chronic bronchitis, asthma, and other respiratory tract infections **[10]**. On the other hand, some phytoconstituents such as alkaloids, steroids, reducing sugars, and flavonoids showed anti-diabetic activity **[11]**. Since the major phytochemical constituent of *A. scholaris* may cause anti- hyperglycemic activity. Anti-hyperlipidemic and anti-hyperglycemic activity of *A. scholaris* leaves have been reported on rats **[12]**. There is not enough data on the investigation of different extracts of *A. scholaris* and their anti-hyperglycemic activity. Therefore, to further identify the beneficial glucose-lowering effect of petroleum ether, ethyl acetate, chloroform, and ethanolic extract of *A. scholaris* leaves was investigated and maintaining glucose homeostasis in normal rats.

MATERIALS AND METHODS:

The chemicals and solvents used in the experiments were the entire analytical grade. Metformin (Apollo, Knoll Pharmaceuticals Ltd.), canagliflozin (INVOKANA, Johnson & Johnson's), and Streptozotocin were procured from (ANJ biomedical, USA).

Plant material collection and authentication

The *Alstonia scholaris* (Leaves) specimens were collected in September from Krishna Nagar, Nava Naroda, Ahmedabad, and Gujarat. It was authenticated by the Department of Botanyat Gujarat University.

Extraction method

The *A. scholaris* leaves were thoroughly cleaned in distilled water and shade dried

for seven days at room temperature. 1kg of powdered form of *A. scholaris* leaves was extracted by soxhlet extraction. The powdered *A. scholaris* leaves were packed in a soxhlet apparatus and extracted using petroleum ether, chloroform, ethyl acetate, and ethanol solvents followed by successive methods **[13]** for 48 hours **(Figure 1)**. Extracts were then concentrated in a rotary evaporator and vacuum oven used for complete dryness. The concentrated extracts were subjected to phytochemical screening and Oral Glucose Tolerance Test.

Phytochemical screening

The preliminary phytochemical screening of bioactive compounds, including carbohydrates, alkaloids, flavonoids, steroids, phenols, tannins, saponins, terpenoids, glycosides, and proteins were carried out using standard protocols **[14]**.

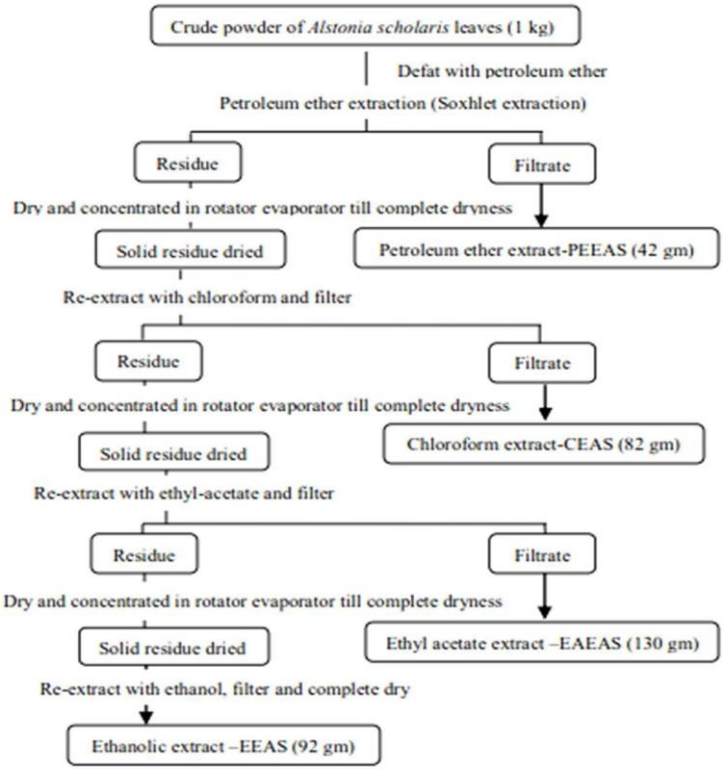


Figure 1: Flow chart of extraction process

In-vitro Oral glucose tolerance test (OGTT)

Experimental animals

Wistar albino rats (200–250 g) of either sex were procured from Zydus researchcenter (ZRC), Ahmedabad, India for the experiment. The protocol (LMCP/P'Cology/19/10) of theexperiment was accepted by the institutional animal ethical committee (IAEC), and these experiments were regulated as per the guidance of the

Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, and New Delhi, India. Rats were supplied water ad libitum and a standard pellet diet according to their weight of rats. They were kept at a constant temperature of 22 ± 1 °C, relative humidity of 55 ± 5%, and 12/12 h light/dark cycle.

Experimental design

Male Wistar rats were divided into twelve groups containing three animals in each group as Group I: Vehicle control (VC) treated with 0.9% saline (p.o.)**;** Group II: CEAS 200 mg/kg (p.o.)**;** Group III: CEAS 400 mg/kg (p.o.)**;** Group IV: CEAS 800 mg/kg (p.o.)**;** Group V:EAEAS 200 mg/kg (p.o.)**;** Group VI: EAEAS 400 mg/kg (p.o.)**;** Group VII: EAEAS 800 mg/kg (p.o.)**;** VIII: EEAS

200 mg/kg (p.o.)**;** Group IX: EEAS 400 mg/kg (p.o.)**;** Group X: EEAS 800mg/kg (p.o.)**;** Group XI: Standard drug Metformin (MET) 200 mg/kg (p.o.)**;** Group XII: Standard drug Canagliflozin (CANA) 1mg/kg (p.o.). The three test doses 200mg/kg, 400mg/kg, and 800mg/kg were selected with the reference of acute toxicity studies **[15]**. All these extracts were dissolved in sodium carboxymethycellulose (CMC).

Study Protocol

OGTT was performed according to the standard method **[16]**. Rats were starved for 16 hours before being tested for OGTT. The baseline glucose level was measured using a glucometer (OneTouch Select plus Simple, Lifescan medical devices India Pvt Ltd). The differentextracts (Group II, III, IV, V, VI, VII, VIII, IX, and X), and standard drugs (Group XI, and XII) were then orally administered to the respective groups. After one hour of drug administration, the rats were orally fed with glucose of 2g/kg, b.wt. At 30, 60, 90, 120, and 150 minutes, the serum glucose level of blood from tail veins was assessed with a glucometer.

Statistical analysis

All the data of fasting blood glucose levels were expressed as the Mean ± SEM (Standard error of the mean). Statistical analysis was performed by student unpaired t-test comparison using Graphpad Prism 8.01 software (Graphpad, San Diego, CA). $P < 0.05$ was considered statistically significant.

RESULT:

Physiochemical assessment

The physicochemical assessment of raw crude drugs is essential to identify their purity. It revealed the presence of foreign organic matter, total ash, acid insoluble

ash, water-soluble extractives, and alcohol extractive of plant drugs, within the Ayurvedic pharmacopeia limits **[17] (Table 1)**.

Percentage yield

The yield values of all four extracts of *A. scholaris* leaves are expressed in %yield (w/w) **(Table 2)**. Yields of extracts were achieved by the soxhlet extraction method.

Phytochemical screening

Phytochemical screening of PEEAS, CEAS, EAEAS, and EEAS was performed according to the standard method. The results of the preliminary phytochemical screening are presented in tabular form **(Table 3)**. Phytochemical constituents were predominant in EAEAS and EEAS, including alkaloids,flavonoids, steroids, and carbohydrates. However, PEEAS does not contain any constituents other than fixed oils and fats. Thus, only three extracts (CEAS, EAEAS, and EEAS) were analyzed for glucose homeostasis via OGTT in normal Wistar rats.

Effect on oral glucose tolerance test (OGTT)

The OGTT results after single oral administration of CEAS, EAEAS, and EEAS at a dose of 200, 400, 800 mg/kg, p.o were represented as mean±SEM depicted in **(Table 4)**. Blood glucoselevel was represented in mg/dl. EAEAS and EEAS at a dose of 400 mg/kg hadproduced the maximum anti- hyperglycemic effect. EAEAS showed a significant ($p<0.05$) reduction in the blood glucose level (BGL) at 30, and 60 min. EEAS showed a significant($p<0.05$) BGL reduction at 30, 60, and 90 min as compared to vehicle control. The standard drugs (Canagliflozin and metformin) prevent a drastic rise in BGL after 60 min of glucose administration. On the other hand, CEAS did not show any significant changes at any doses compared to vehicle control. The fasting blood glucose level of the vehicle control group reached a peak at 30 min and subsequently fell to the normal level. As compared to vehicle control, all the treatment groups showed similar anti-hyperglycemic effects at 120 min deferring significantly.

Table 1: Physicochemical assessment of *A. scholaris* leaves

S. No.	Physicochemical parameter	Obtained Value % (w/w)	API limit
1	Foreign matter	0.62	NMT 2%
2	Total ash	6.85	NMT 11%
3	Acid -insoluble ash	2.2	NMT 3%
4	Water soluble extractive	18.5	NLT 12%
5	Alcohol soluble extractive	11.25	NLT 4%

(NMT-Not more than, NLT –Not less than)

Table 2: The percentage yield of different extracts of *A. scholaris* (leaves)

S. No.	Extracts	Colour	Physical nature	%Yield (w/w)
1.	PEEAS	Dark Green/Sticky mass	Waxy greasy semisolid	4.2±0.33%
2.	CEAS	Dark Green/ Solid mass	Solid	8.2±0.54%
3.	EAEAS	Green/ Thick solid mass	Solid	13±0.36%
4.	EEAS	Dark green/ Thick solid	Solid	9.3±0.71%

PEEAS Petroleum ether extract of *A. scholaris* ; CEAS Chloroform extract of *A. scholaris* ; EAEAS Ethyl acetate extract of *A. scholaris* ; EEAS Ethanol extract of *A. scholaris* . All the values of % yield (w/w) were expressed in terms of mean±standard deviation

Table 3: Qualitative chemical analysis of phytoconstituents of the various extracts of *A. scholaris* leaves

Plant constituents	PEEAS	CEAS	EAEAS	EEAS
Alkaloid	-	-	+	+
Flavonoid	-	-	+	+
Tannins	-	-	-	+
Phenolic compound	-	+	-	+
Saponins	-	-	-	+
Terpenoids	-	+	-	+
Carbohydrate	-	-	+	+
Steroid	-	+	+	+
Fixed oil and fat	+	-	-	-

+ = Present; - = absent

Table 4: Effects of CEAS, EAEAS, and EEAS on oral glucose tolerance test in normal rats

Treatment Groups	Blood glucose level (mg/dl)					
	Initial	30 min	60 min	90 min	120 min	150 min
Vehicle Control	77.67±4.81	155.34±2.72	145.34±1.76	128.34±1.45	114.67±1.23	99.67±3.71
CEAS (200mg/kg)	78.66 ±1.76	150.67±1.45	136± 2.64	129.34±3.52	116.67±1.2	92±2.31
CEAS (400mg/kg)	74.34±3.38	152±2.64	136.34±1.76	125±1.52	114.34±1.21	91.67±2.96
CEAS (800mg/kg)	77.67±4.91	150±1.15	141±2.08	128±2.08	114±2.8	99±5.68
EAEAS(200mg/kg)	80.67±1.45	149.33±2.84	136±1.15	123±0.57	111±0.57	89.34±3.48
EAEAS (400mg/kg)	74.77±3.25	145.33±2.18*	130.34±1.45**	121.67±2.6	112.67±1.76	90.34±2.72
EAEAS (800mg/kg)	79±3.78	149.34±4.05	138.34±2.02	126.23±1.61	114.67±1.76	100.56±0.98
EEAS (200mg/kg)	80±4.58	150.67±2.34	135.34±1.76	124.34±1.26	108.67±1.2	91±2.51
EEAS (400mg/kg)	77.67±3.17	140±2.51**	127±3.21**	116±1.52**	104.67±3.75	89.34±2.18
EEAS (800mg/kg)	77±3.05	148.43±3.36	135.34±0.88	126.67±0.88	115.34±0.88	99.34±3.17
CANA (1mg/kg)	75.67±2.34	131±0.57***	123±2.51***	108.67±3.28***	99±4.51**	84.34±3.84
MET (200mg/kg)	79.67±5.23	139±2.08**	130±2.52**	115.67±1.2**	101.67±2.34*	90.67±4.67

The values are expressed in Mean ± SEM, n=3 in each group. *P<0.05, **p<0.01 and ***p<0.001 significant difference as compared with vehicle control followed by student unpaired t-test comparison

DISCUSSION

Glucose homeostasis is the foundation of energy supply and balance action between insulin and glycogen hormonesto maintain blood glucose levels **[18]**. The pancreas maintains blood glucose levels within the range of 4-6mM **[19]**.Despite its important mechanism of action its vital role and the threshold between physiological requirements that turn to the metabolic disorder remain unknown **[20]**. Diabetes mellitus is a metabolic disorder in which patients suffer from persistent hyperglycemia for the long term. Therefore, the development and investigation of new substances with new strategies always highlights the research. In the traditional system, Ayurveda describes the trustworthy and reliable medicinal system

to use crude drugs as a powerful disease healing property. It is observed that screening of natural products has medicinal benefits from the actions of secondary metabolites that are present in the extracts **[21]**. In this regard, phytochemical screening and OGTT have been shown potential hypoglycemic effects. Phytochemical screening of four different extractions (with solvent petroleum ether, chloroform, ethyl acetate, and ethanol) of leaves of *A. scholaris* was carried out. Evaluation of the anti-diabetic activity of *A. scholaris* was carried out instreptozotocin-induced diabetic rats **[22]**. The determination of qualitative phytochemicals by various chemical tests that consider the presence of alkaloid, flavonoid, terpenoid, phenol, and steroid are major chemical constituents present in CEAS (phenolic, terpenoid, and steroid) EAEAS (alkaloids, flavonoids, and steroids) and EEAS (alkaloids,flavonoids, and steroid) of *A. scholaris* leave. Some of these classes of compounds have been implicated in the anti-diabetic activity of the plants. They also reported alkaloids, flavonoids, and phenolics as bioactive constituents for diabetic treatment **[11]**. Fortunately, *A. scholaris* contains all these bioactive constituents in reasonable quantities.

Therefore, phytochemical constituents o f *A . scholaris* leaves may improve the anti-hyperglycemic potential to manage type two diabetes **[12]**. OGTT is usedas a diagnostic test for type two diabetes **[23]**. It is also extremely used for gestational diabetes. In our present work, we demonstrate the hypoglycemic effect by using OGTT which is an important preliminary test in preclinical study. OGTT is the indication of both insulin secretion and insulin resistance in glucose intolerance. It is the real-time in vivo whole-body test that can be used toidentify the best treatment to prevent and possibly delay to development of diabetes mellitus. The non-toxic dose of *A. scholaris* was up to 200mg/kg **[24]** whenit was administered orally while 100mg/kg showed toxicity with exhibited damage to the major organ of the body when it was administered by the intraperitoneal route. We selected three test doses with the reference of acute toxicity studies is 200mg/kg, 400mg/kg, and 800mg/kg of four different extracts PEEAS, CEAS, EAEAS, and EEAS. EAEAS and EEAS showedhypoglycemic effects following single- dose administration at 400mg/kg body weight in normal rats. EAEAS at 30 min and 60 min while EEAS at 30 min, 60min, and 90 min showed a significant

reduction in blood glucose level after glucose administration in normal rats.Both are favorably competing against standard drugs though 150 min the test lasted. It prevents glucose-induced hyperglycemia better than other extracts. However, these suggest that both EEAS and EAEAS produce anti-hyperglycemiceffects. In the future, long-term protocols and fractionation of highly effective extract may be helpful for the treatment of diabetes mellitus.

CONCLUSION

The ethyl-acetate and ethanol extracts of *A. scholaris* leaves at a dose of 400 mg/kg showed an anti-hyperglycemic effect in the normal rats, which was comparable to metformin and canagliflozin. These results are suggesting that ethyl-acetate and ethanol extract may show beneficial activity in the diabetic condition.

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CONFLICT OF INTEREST

There is no conflict of interest revealed by the author regarding this research.

REFERENCES

[1] Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. Experimental & molecular medicine. 2016 Mar;48(3):e219-e219.

[2] Liang Y, Arakawa K, Ueta K, Matsushita Y, Kuriyama C, MartinT, Du F, Liu Y, Xu J, Conway B, Conway J. Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight innormal and diabetic animal models. PloS one. 2012 Feb 15;7(2):e30555.

[3] Nisha M, Vinod BN, Sunil C. Evaluation of Boerhavia erecta L. for potential antidiabetic and antihyperlipidemic activities in streptozotocin-induced diabetic Wistar rats. Future Journal of Pharmaceutical Sciences. 2018 Dec 1;4(2):150-5.

[4] Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007;40(3):163-73.

[5] Abubakar US, Abdullahi S, AyubaV, Kaigama S, Halidu US, AyubaMK. Medicinal plants used for the management of diabetes mellitus in Zaria, Kaduna state, Nigeria. Journal of Pharmacy & Pharmacognosy Research.2017;5(3):156-164.

[6] Gawali VB, Vyawahare NS. Anti-

Hyperglycemic effect of Polyherbal Formulation in Glucose Loaded and Epinephrine Induced Hyperglycemic Wistar rats. Research Journal of Pharmacy and Technology. 2022 Jan 25;15(1):365-369.

[7] Khatale VL, More DB. A Reviewon Saptaparna (Alstonia Scholaris R. Br). International Ayurvedic Medical Journal. 2016;4(03):334-337.

[8] Zhao YL, Gou ZP, Shang JH, Li WY, Kuang Y, Li MY, Luo XD. Anti-microbial effects in vitro and in vivo of Alstonia scholaris. Natural Products and Bioprospecting. 2021 Feb;11(1):127-135.

[9] Khyade MS, Kasote DM, Vaikos NP. Alstonia scholaris (L.) R. Br. and Alstonia macrophylla Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. Journal of ethnopharmacology. 2014 Apr 11;153(1):1-8.

[10] Misra CS, Pratyush K, Dev L, James J, Veettil AK, Thankamani V. A comparative study on phytochemical screening and antibacterial activity of roots of Alstonia scholaris with the roots, leaves and stem bark. International Journal of Research in Phytochemistry and pharmacology. 2011 Apr 30;1(2):77-82.

[11] Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 2020 Sep; 9(9):252.

[12] Arulmozhi S, Mazumder PM, Lohidasan S, Thakurdesai P. Antidiabetic and antihyperlipidemic activity of leaves of Alstonia scholaris Linn. R. Br. European journal of integrative medicine. 2010 Apr 1;2(1):23-32.

[13] Feng W, Li M, Hao Z, Zhang J. Analytical methods of isolation and identification. InPhytochemicals in human health, IntechOpen, London: 2019 Jul 15, p.45-52.

[14] Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pacific journal of tropical biomedicine. 2014 May 1;4:S359- 67.

[15] Baliga MS, Jagetia GC, Ulloor JN, Baliga MP, Venkatesh P, Reddy R, Rao KM, Baliga BS, Devi S,Raju SK, Veeresh V. Theevaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (Alstonia scholaris) in mice and rats. Toxicology letters. 2004 Jul15;151(2):317-26.

[16] Chaimum-aom N, Chomko S, Talubmook C. Toxicology and oral glucose tolerance test (OGTT) of Thai medicinal plant used for diabetes controls, Phyllanthus acidus L.(Euphorbiaceae). Pharmacognosy Journal, 2017;9(1).

[17] World Health Organization.Quality control methods for herbal materials. Geneva: WHO Press, Malta, 2011, 20.

[18] Acharya KD, Parakoyi AE, Tetel MJ. Endocrine Disruption and the Gut Microbiome. InEndocrine Disruption and Human Health, Academic Press, Wellesley: 2022 Jan 1, p. 355-376.

[19] Ahmed A, Khalique N. Molecular Basis of Blood Glucose Regulation. In Blood Glucose Levels, IntechOpen, London: 2019 Nov 15, p.11-32.

[20] Yager JY. Glucose and perinatal brain injury. Questions and controversies. Neurology. 2012 Mar 2:143-162.

[21] Sahu MK, Singh VK, Rao SP. Development and Evaluation of Antidiabetic Potential ofPolyherbal Formulation in Streptozotocin Induced Animal Model. Int J cell Sci & mol boil. 2018;5(2):29-37.

[22] Bandawane D, Juvekar A, Juvekar M. Antidiabetic and antihyperlipidemic effect of Alstonia scholaris Linn bark in streptozotocin induced diabetic rats. Indian Journal of Pharmaceutical Education and Research. 2011 Apr 1;45(2):114-20.

[23] Darden CM, Farrow AE, Rajan SK, Lakhani M, Lawrence MC, Naziruddin B. Predicting the function of islets after transplantation. InTransplantation, Bioengineering, and Regenerationof the Endocrine Pancreas,Vol.1, Academic Press, Dallas: 2020 Jan 1, p.547-561.

Alstonia scholaris Leaves Fractions and Ethanolic Extract on Glucose Homeostasis in Normal Rats: Antioxidant, Inhibits Intestinal Glucose Absorption, and Anti-diabetic Potential

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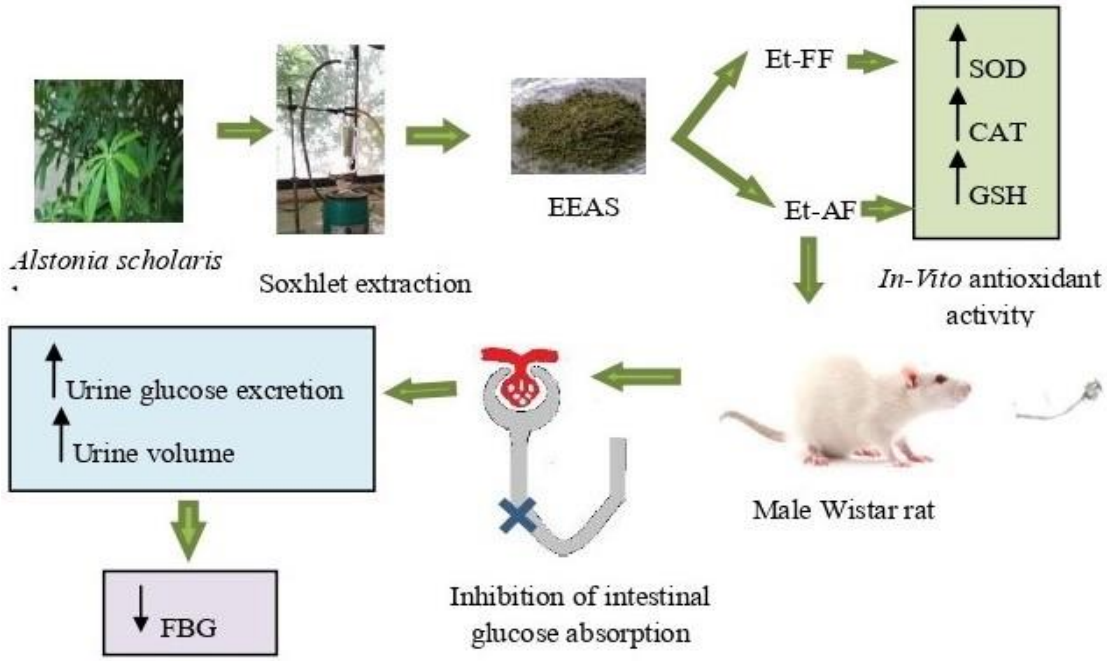
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Abstract

***Alstonia scholaris* (L.) R. Br. is an evergreen plant used for medicinal purposes (Family: Apocynaceae) that has the ability to lower blood sugar levels in rats with type 2 diabetes mellitus. We aimed to characterize the alkaloid (Et-AF) and flavonoid (Et-FF) fractions of the ethanol extract of *A. scholaris* (EEAS) for examining their hypoglycaemic effect and in vitro antioxidant activity in goat liver. In oral glucose tolerance test, Et-AF (15, 30, 60, and 90 mg/kg), Et-FF (15, 30, 60, and 90 mg/kg), EEAS (400 mg/kg), and canagliflozin (10 mg/kg) were administered orally. Et-AF (30 mg/kg) reduced fasting blood glucose level and significantly ($p<0.001$) increased the amount of glucose excreted in urine compared to those observed in vehicle-controls. Hence, 30 mg/kg of Et-AF showed a potent anti-hyperglycaemic effect, possibly due to the reduction in intestinal glucose absorption. However, future studies should explore the corresponding mechanism of action at the molecular level.**

Keywords: ***Alstonia scholaris*, Anti-hyperglycemic, Antioxidant, Oral Glucose Tolerance Test (OGTT)**

Pictorial abstract



1. Introduction

Many people turn to plants as a primary or secondary source for their health care needs. One thousand two hundred plant and animal species are being considered as potential folk remedies for diabetes [1]. Most of these remedies have not been scientifically proven to be effective. *Galega Officinalis* was tested using the standard method for detecting metformin, a drug used to treat diabetes [2]. Diabetes has long been treated with a variety of plants. Researchers and scientists around the world are looking for natural sources to avoid side effects and the immense potential of their conventional use [3]. According to a recent epidemiological study, post-prandial hyperglycemia (PPH) has been associated with increased cardiovascular complications [4]. The goal of treating diabetes is to reduce blood sugar levels after meals. One of the most important ways PPH is inhibited through the digestion and absorption of glucose in the intestines. It is the proximal tubular cells-sodium glucose cotransporter-1 (SGLT-1, 10%) and sodium-glucose cotransporter-2 (SGLT-2, 90%) that are the focus of therapy for type 2 diabetes mellitus [5]. Maintaining constant blood sugar level (a process mediated by insulin and glucagon hormones) is a vital physiological process [6]. The Oral glucose tolerance test (OGTT) assesses the body's glucose metabolism and provides a ballpark estimate of fasting glucose (FBG) level across various parameters [7]. Tissues can be protected from oxidative stress with the help of antioxidants. It improves the body's defences against cell damage and eliminates free radicals [8]. Herbal supplements with active phytochemical components can mitigate the negative effects of oxidative stress. According to scientific research, some photoactive components, including alkaloids, flavonoids, andsteroids, have been shown to help in controlling diabetes [9]. Therefore, its primary phytochemicals can cause hypoglycemic changes. *Alstonia scholaris* is native to Southeast Asia, India, and the tropical regions of China and Australia and is a valuable medicinal plant with a wide distribution [10]. Many studies were conducted over the year on other diseases such as jaundice, microbial infectious diseases, malaria and cancer[11]. Recent research shows that very few have reported anti-diabetic effects. Data on various extracts and fractions of *A. scholaris* and the mechanism behind its hypoglycemic effects are lacking. Consequently, thepresent study aims to determine the beneficial hypoglycemic effect of *A. scholaris* leaves through inhibitionof luminal glucose absorption. In addition, an *in vitro* method using goat liver tissue was used to estimate the antioxidant potential of Et-AF, Et-FF and EEAS. Therefore, in this present research, we determine the beneficial hypoglycemic effect of *A. scholaris* leaves through inhibition of luminal glucose absorption.

2. Material and methods

2.1. Plant collection and authentication

In September 2020, leaves of *Alstonia scholaris* were collected from Ahmedabad, Gujarat. The botanical identity of the plant material was verified by a taxonomist from Gujarat University (GU), Ahmedabad. The #AS-1 specimen has been catalogued in the herbarium for future study.

2.2. Chemicals

All chemicals and solvents used in this study were of the standard variety. The company that provided the canagliflozin (INVOKANA) was Johnson & Johnson's Private Limited of Maharashtra, India. At Merck, I got my supplies of sodium carbonate (Na_2CO_3), ethylenediamine tetraacetic acid (EDTA), and hydrogen peroxide (H_2O_2). Sigma Aldrich supplied nitro blue tetrazolium (NBT), 1, 2-dithiol-bis nitro benzoic acid (DTNB), disodium hydrogen phosphate (Na_2HPO_4), and hydroxylamine dihydrogen phosphate ($NaHPO_4$).

2.3. Preparation of the Ethanolic extract

A. scholaris leaves were washed and dried thoroughly in the shade for seven days. After mechanical grinding, the powder form of *A. scholaris* leaves (4 kg) was extracted with 99% ethanol (20 L × 3X) using Soxhlet extraction for 48 hr. The ethanolic leaves extract of *A. scholaris* was dried under low pressure in a vacuum rotary evaporator (at 10–15 rpm and 40–45°C). Then, they were sealed in a container and stored in the refrigerator.

2.4. Et-AF preparation

The ethanol extract (5 g) was mixed in 0.3% HCl and the solid residue was identified as the non-alkaloid fraction. Finally, 10% ammonium hydroxide (NH_4OH) was used to bring the pH of the acidic aqueous layer to 9 for basification. Then it was extracted with ethyl acetate (EtOAc) to obtain the alkaloid fraction (Et-AF).

2.5. Et-FF preparation (Liquid-liquid partition)

The ethanol extract (10g) was mixed in distilled water (20ml). It was partitioned with dichloromethane (30ml X 3x). Following separation, the dichloromethane layer was evaporated at 50°C. Finally, ethyl acetate was used to create partitions in the aqueous layer (3 times 300 mL). The flavonoid fraction (Et-FF) was obtained by evaporating the ethyl acetate layer at 60°C. To facilitate oral administration to rats, both Et-AF and Et-FF were dissolved in 1% carboxymethyl cellulose (CMC).

2.6. Quanlitative phytochemicals determination

To approximate the existence of secondary metabolites, the following standard procedures were used for the phytochemical examination of Et-AF [12], Et-FF [13] and EEAS [14].

2.7. Quantitative phytochemicals determination

The percent yield of the ethanolic extract of *A. scholaris* was determined using the following formula [15]:

$$\% \text{ yield (w/w)} = \frac{\text{Final weight of sample extract} \times 100}{\text{Initial weight of sample}}$$

2.8. Et-AF content

An adaptation of the *Harborne (1973)* methods was used to conduct the study [16]. In a 250 mL beaker was placed 5 g of powder sample, then 10% acetic acid (200 ml) in ethanol were added. The reaction mixture was left at room temperature for 4 hours. This reaction mixture was filtered and concentrated to one fourth the original volume using a water bath. Then ammonium hydroxide was added until precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and filtered again. The resulting alkaline residue (alkaloid) was evaporated to dryness and weighed. The formula for calculating percentage of alkaloid content in the ethanol extract of *A. scholaris*:

$$\% \text{ Alkaloid} = \frac{\text{Final weight of sample}}{\text{Initial weight of extract}} \times 100$$

2.9. Et-FF content

Et-FF content was determined by a *Harborne (1998)* method [17], which is described following a gravimetric method. 5g of sample powder was put in 50ml of water and 2ml of HCl solution was placed in an Erlenmeyer flask. After boiling the solution for 30 minutes, it was allowed to cool before filtering through Whatman filter paper (#42). The filtrate residue was collected and placed in an oven at 60°C to dry while discarding the aqueous layer. The formula for calculating percentage of flavonoid content in the ethanol extract of *A. scholaris*:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Here, W_1= Weight of empty filter paper W_2= Weight of paper + Flavonoid extract

2.10. Oral glucose tolerance test (OGTT)

2.10.1. Experimental animals

The male Wistar albino rats used in the study weighed between 200 and 250g and were provided by Zydus Research Center (ZRC) in Ahmedabad, India. The Institutional Animal Ethics Committee (IAEC) protocol (# LMCP/PCology/19/10) and the rules established by the Committee for the Protection and Control of Animals Used in Experiments (CPCSEA), Animal Welfare Division in New Delhi, India, were adhered to during these experiments. They were fed a pellet diet that was adjusted based on their body weight and had

free access to water. They were kept at 22°C with a relative humidity of 55± 5%, and subjected to a light/dark cycle of 12 hours each.

2.10.2. Experimental design and Treatment Protocol

Male Wistar rats were divided into eleven groups with three animals in each group as Group I: Vehicle control (VC) treated with 0.9% saline bw PO; Group II: Et-AF 15 mg/kg bw PO; Group III: Et-AF 30 mg/kg bw PO; Group IV: Et-AF 60 mg/kg bw PO; Group V: Et-AF 90 mg/kg bw PO; Group VI: Et-FF 15 mg/kg bw PO; Group VII: Et-FF 30 mg/kg bw PO; VIII: Et-FF 60 mg/kg bw PO; Group IX: Et-FF 90 mg/kg bw PO; Group X: EEAS 400 mg/kg bw PO; Group XI: Standard drug canagliflozin (CANA) 10 mg/kg bw PO. The three doses used in the experiments were chosen by consulting the literature and toxicity studies; these were 15, 30, and 90 mg/kg, respectively.

2.10.3. Study protocol

The OGTT was performed according to the standard method with minor modification in normal rats [18]. Rats were starved for 16 hours before being tested for OGTT. Baseline glucose level was measured using a glucometer (OneTouch Select plus Simple, Lifescan medical devices India Pvt Ltd). All treatment and standard drugs were administered orally. Male Wistar rats were fed glucose (2g/kg bw) after 1 hour of drug administration. After 30, 60, 90, 120, and 150 min, FBG levels were recorded with a glucometer (rat tail vein puncture). Urine samples are collected after 8 hours. It started 1 hour before administration and finished 8 hour after administration.

2.11. In-vitro antioxidant activity

2.11.1 Preparation of goat liver slices

The goat was slaughtered immediately, so fresh liver could be obtained from the nearby butcher shop [19]. Goat liver tissue was stored in cold (4°C) phosphate buffered saline (PBS). A sterile scalpel was used to cut the tissue into 1 mm thick pieces yielding 250 mg. Use large flat-bottomed bottles and discard 1.0 mL of sterile PBS. H_2O_2 and samples were added and the mixture was incubated for 1 hour at 37°C with gentle shaking. It was also necessary to create an appropriate control group. The usual oxidizing agent, H_2O_2, was used at a tissue concentration of 2 ml/kg. After incubation, cell debris was removed by homogenizing the tissue and PBS buffer together in the same aliquot of the homogenizer. The antioxidant potential was evaluated by estimating the number of parameters using the supernatant.

2.11.2 Analysis of antioxidant activity

Homogenized goat liver tissue was used for antioxidant enzymes analysis using the *Misra et al.* method for superoxide dismutase (SOD) [20], the *Jhansyrani et al.* method for catalase preparation [21], and the *Moron et al.* method for measuring glutathione reduction (GSH) activity [22].

2.12. Statistically

The results of our fasting blood glucose measurements were expressed as the mean±SEM (standard error of the mean). GraphPad Prism 8.01 (GraphPad, San Diego, CA) was used for statistical analysis, specifically an unpaired Student's t-test. Statistical significance was accepted at $P<0.05$.

3. Results and discussion

3.1. Phytochemical screening

3.1.1. Qualitative phytochemical analysis

We followed the established protocol to determine which phytochemicals were present in EEAS, Et-AF and Et-FF. The EEAS has detected alkaloids, flavonoids, steroids and carbohydrates. Phytochemical analysis and a re-examination of the archival literature confirmed the high levels of alkaloids and flavonoids in the EEAS.

3.1.2. Quantitative phytochemical analysis

It reported that the percentage yield of ethanolic extract of *A. scholaris* is 9.3±0.71% (w/w) from Soxhlet extraction method. Alkaloids and flavonoids were the most common in the EEAS. The quantitative results show that Et-AF has the most abundant compound 1.56% (16.2 mg/g of extract) while Et-FF has 0.6% (6.5 mg/g of extract). Therefore, the content of flavonoids compared to alkaloids in the EEAS is observed to be the least abundant. The anti-hyperglycemic effect may be due to the presence of alkaloids and flavonoids. Therefore, this work relied on these fractions.

3.2. Effect on oral glucose tolerance test (OGTT)

The results of a single dose of 400, 30, or 30 mg/kg bw of EEAS, Et-AF, or Et-FF were analyzed using the OGTT protocol and expressed as mean±SEM (Table S1). Glucose levels were represented in milligrams per deciliter (mg/dl) of blood. The most potent antihyperglycemic effect of Et-AF was observed at a dose of 30 mg/kg. FBG levels dropped significantly at 30, 60, and 90 min with EEAS ($p < 0.01$). At 30 and 60 min, Et-FF significantly decreased FBG level compared to vehicle control ($p < 0.05$). Taking the standard drug canagliflozin) can prevent a dramatic rise in FBG level after glucose administration for up to 60 min. Et-AF and Et-FF did not differ significantly from VC at 15, 60, or 90 mg/kg. In the vehicle control group, fasting blood glucose levels peaked at 30 min and then returned to normal. At 120 minutes, all treatment groups had similar anti-hyperglycemic effects compared to the VC group. Therefore, Et-AF and Et-FF at doses of 30 mg/kg were selected for further investigation to monitor glucose and urinary excretion.

Table 1: Effects of Et-AF, Et-FF, and EEAS on oral glucose tolerance test in normal rats.

Treatment/Groups	Blood glucose level (mg/dl)					
	Initial	30 min	60 min	90 min	120 min	150 min
Vehicle Control	84.33±3.38	159.33±1.45	149.33±2.40	132.66±3.28	117.66±3.17	103±3.78
Et- AF (15 mg/kg)	80.5 ±1.40	147.33±2.55*	133± 2.03**	120.66±1.74**	109.83±2.18	96.83±2.86
Et- AF (30 mg/kg)	75.16±3.13	134±1.80***	124±1.59***	108.5±2.14***	97.5±2.14**	90.833±1.75*
Et -AF (60 mg/kg)	84.66±3.75	146.66±2.40	137±3.05	120.33±0.88	106.66±2.72	91.66±2.33
Et -AF (90 mg/kg)	77.33±5.60	156±5.03	143.66±3.38	127.66±3.75	114±1.73	97.33±3.92
Et- FF (15 mg/kg)	82.66±5.23	158±6.55	139.66±1.85	130.66±4.48	117±2.08	97.66±4.17
Et- FF (30 mg/kg)	81±3.03	151.33±3.08	138.33±2.62*	124±3.03*	113.33±2.55	99.66±2.04
Et- FF (60 mg/kg)	72.33±5.20	147.66±2.90	134.33±3.38	121±2.30	113±1.52	95±2
Et- FF (90 mg/kg)	77.33±5.60	156±5.03	143.66 ±3.38	127.66±3.75	117.33±2.60	106±2.64
EEAS (400mg/kg)	74.77±3.25	140±2.51**	127±3.21**	112.33±2.33**	104.66±3.75	91.66±3.75
CANA (10 mg/kg)	75.66±2.33	131±0.57***	121±0.57***	103.66±0.66**	99±4.50**	82.66±2.18**

3.3. Determination of Urine volume and glucose excretion

The outcome of this section is shown in Figure 1, where we compare the effect of VC, Et-AF (30mg/kg bw), EEAS (400mg/kg bw) and CANA (10 mg/kg bw) on urine volume and urinary glucose excretion over 8 hours after ingestion. During OGTT estimation, VC, both treatment groups and the standard group were associated with reduction in FBG levels at 1 hour of post-dose in normal rats. Here, this experiment demonstrated that Et-AF, EEAS, and CANA were able to inhibit glucose reabsorption in the gut. From these results it is clear that EEAS significantly ($p<0.001$) increases urinary output more than VC (Figure 1. A). Compared to the VC group, urinary glucose concentration and the amount excreted after administration were significantly higher in Et-AF. Based on the available data, it can be conjectured that Et-AF has a significantly better effect on urinary glucose excretion compared to EEAS (Figure 1. B). At a dose of 10 mg/kg CANA, both urine volume and glucose excretion increased significantly ($p<0.001$) over 8hours of the experiment.

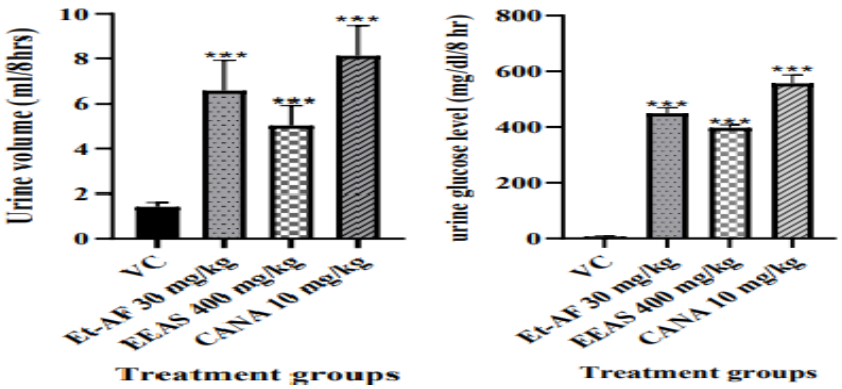


Figure 1. Effect of Et-AF (30mg/kg), EEAS (400mg/kg), and CANA (10mg/kg) on male Wistar rats increases urine volume (A) and urinary glucose excretion (B). The results are expressed in Mean ± SEM. Each group comprised of 3 animals.$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to VC. VC, Vehicle control; Et-AF, a lkaloid fraction of ethanolic extract of *A. scholaris* leaves; EEAS, ethanolic extract of *A. scholaris* leaves; CANA, canagliflozin.

3.4. In vitro determination of antioxidant activity

3.4.1. Super oxidase dismutase scavenging activity

Antioxidant capacity has been tested in three distinct ways to enhanced oxygen radical scavenging. In this experiment, goat liver tissue was used to study the antioxidant activity of EEAS, Et-FF and Et-FF was illustrated in figure 2. A dose-dependent increase in antioxidant activity by these three treatment groups was recorded as the concentration increases (100, 200, 400, 800, and1600 mg/ml). In the case of SOD scavenging activity, a reduction in antioxidant activity was found compared to the toxic group (TG, H_2O_2 exposed slices). Compared to the other two treatment groups, Et-FF shows the highest ($P > 0.001$) dose-dependent rise in antioxidant activity (Figure 2. C).

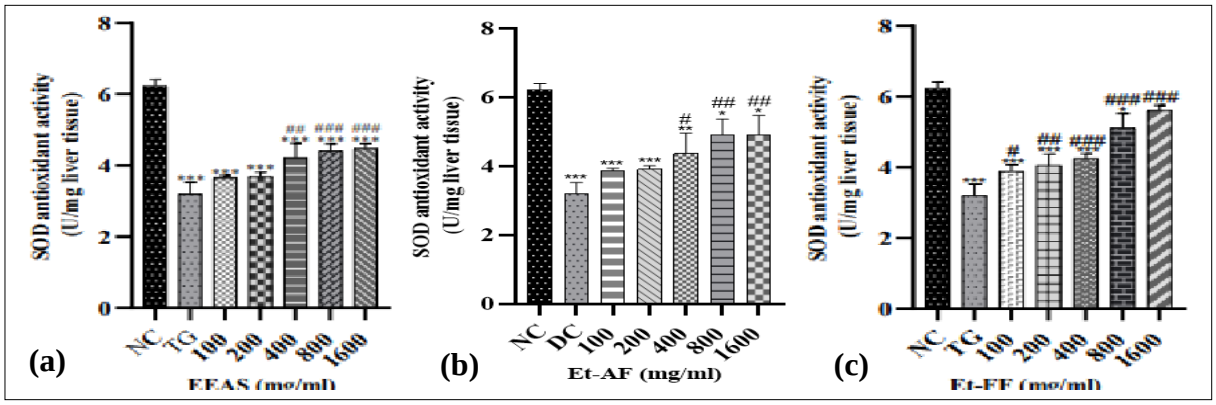


Figure 2. The effect of a). EEAS, b). Et-AF, and c). Et-FF on SOD enzyme activity using goat liver. The results are expressed in Mean ± SEM. Each group comprised of triplicates.$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to NC. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

3.4.2. Catalase scavenging activity

This section summarizes the effect of these three treatment groups on catalase scavenging activity in Figure 3. The results obtained in this experiment demonstrate that incubation of goat liver tissue with H_2O_2 significantly decreases antioxidant activity compared to normal control. Treatment with EEAS, Et-FF, and Et-FF at various concentrations had protection from antioxidant enzyme degradation. Comparative results between three treatment groups illustrate that Et-FF had significantly ($P > 0.001$) high CAT activity (Figure 3.f).

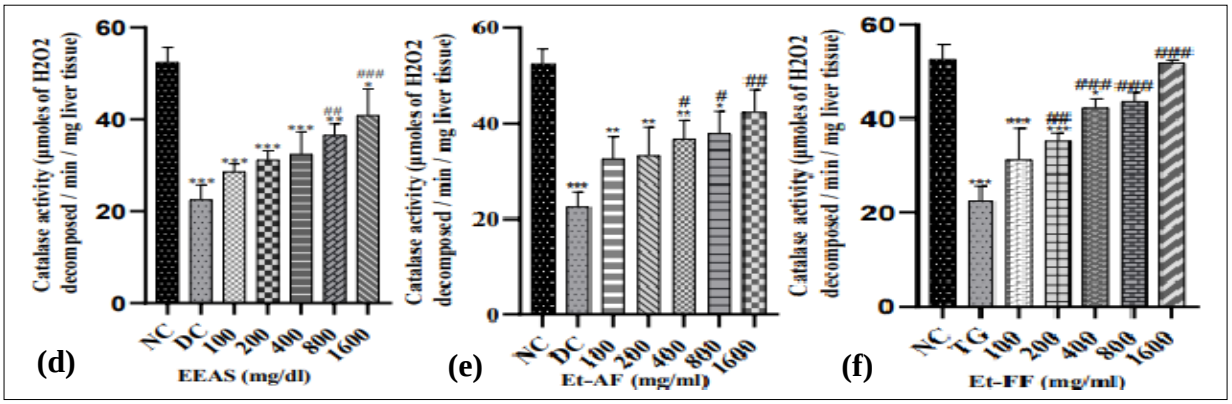


Figure 3. The effect of d). EEAS, e). Et-AF, and f). Et-FF on catalase enzyme activity using goat liver. The results are expressed in Mean ± SEM. Each group comprised of triplicates. .*p < 0.05, **p < 0.01, ***p < 0.001 as compared to NC. #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

3.4. 3. Glutathione scavenging activity

Figure 4 shows the comparative effect of EEAS, Et-FF and Et-FF on the antioxidant enzyme glutathione. It was observed that both EEAS and Et-AF increased in antioxidant activity with increasing concentration. A similar result of SOD and CAT, Et-FF of three treatment groups showed higher antioxidant potential, reflecting significantly ($p<0.001$) increased GSH levels compared to toxic group (Figure 4.i).

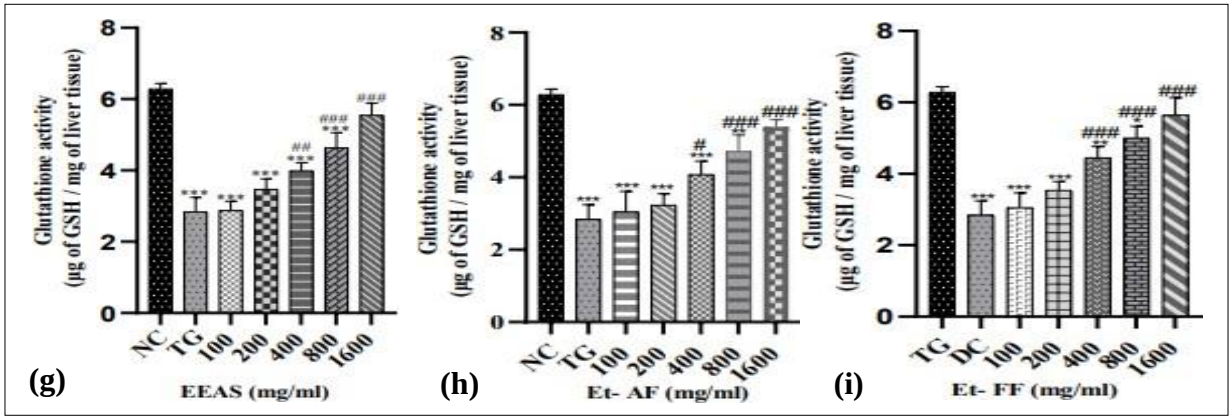


Figure 4. The effect of g). EEAS, h). Et-AF, and i). Et-FF on glutathione enzyme activity using goat liver. The results are expressed in Mean ± SEM. Each group comprised of triplicates.*p < 0.05, **p < 0.01, ***p < 0.001 as compared to NC. #p < 0.05, ##p < 0.01, ###p < 0.001 as compared to DC. Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests for comparison.

3.4.4. Effect of EEAS, Et-AF and Et-FF on glucose homeostasis

Earlier studies reported as anti-diabetic activity of bark and leaves of *Alstonia scholaris* [23], [24]. This provides convincing evidence for the anti-hyperglycemic activity of *A. scholaris*. Moreover, evaluation of its mechanism is yet to be explored. Previous research demonstrate that a dose of 400 mg/kg ethanolic extract from *A. scholaris* in diabetic rats resulted in significantly reduced fasting blood sugar levels. Also, based on our literature review, there is no data on how this affects glucose uptake in the gut. Based on the qualitative analysis, EEAS is rich in alkaloid and floavonoid components.

According to the report obtained from the quantitative analysis, Et-AF is the most abundant secondary metabolite substance compared to Et-FF. Therefore, we investigated the possible mechanism and effects of *in vivo* pretreatment with ethanolic extract of *A. scholaris*, Et-AF and Et-FF on urinary glucose excretion and concentration. Here, every aspect of the study is discussed in the context of the results. Compared to EEAS and Et-FF, Et-AF (30 mg/kg) leads to the most significant increase in urinary output and glucose concentration. In the kidney, sodium-glucose co-transporters (SGLTs) play a major role in maintaining glucose homeostasis through utilization, reabsorption, and gluconeogenesis. A malfunction in renal glucose reabsorption (up regulation) of SGLTs leads to the pathogenesis of type 2 diabetes mellitus [25]. Therefore, inhibition of intestinal glucose absorption to control glycemic status is gaining attention among scientists. It is interesting to note that Et-AF inhibits intestinal luminal glucose absorption at low concentration compared

to EEAS. The overall finding and the technique are very satisfactory and perform well in comparison. Reactive oxygen species are unstable molecules that have a potent oxidative effect on various cellular materials, including lipids, proteins, and DNA. Antioxidants and enzymes such as catalase, superoxide dismutase, and glutathione are naturally produced in the body and protect against disease by scavenging free radicals and dampening oxidation and lipid peroxidation. Goat liver tissue was used to test the antioxidant activity of EEAS and its Et-AF and Et-FF (*in vitro*). Based on the results, we observed that significant SOD, CAT and GSH activity was found in the ethanolic extract and its two fractions. The method presented in this work is sufficiently generalized that all three substances could serve as antioxidants. Type 2 diabetes mellitus can be diagnosed with the help of OGTT. The OGTT was modified in this study to better estimate fasting blood glucose levels, and the results indicated that drug administration might alter the activity of intestinal glucose transporters. Therefore, we use OGTT to examine the effect of EEAS and its two fractions on normal rats. The hypoglycemic effect is reflected in the OGTT report of EEAS, Et-AF, and Et-FF. Compared to EEAS and Et-FF, Et-AF has a more potent anti-diabetic effect. This is an interesting result as it confirms what we know from previous studies on the mechanism. On the other hand, it prevents sugar from being absorbed in the intestine, which leads to lower blood sugar levels, and helps to maintain glucose levels. A novel aspect of this research is that our evidences and findings support 30 mg/kg Et-AF as an anti-hyperglycemic agent by inhibiting intestinal glucose up regulation to control type 2 diabetes mellitus. Several interesting aspects can be further explored through long-term protocols.

4. Conclusion

The current study provide additional support for the use of the alkaloid fraction of ethanolic extract (Et-AF) at a dose of 30 mg/kg marked as a hypoglycemic treatment when taken orally. Furthermore, our results imply that preventing reabsorption of glucose from intestine is essential for lowering blood glucose levels. Clarifying the phytochemical and molecular sites responsible for the intestinal action of the ethanol alkaloid fraction is crucial for future research.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Reference

[1] Willcox ML, Elugbaju C, Al-Anbaki M, Lown M, Graz B. Effectiveness of medicinal plants for glycaemic control in type 2 diabetes: an overview of meta-analyses of clinical trials. *Frontiers in pharmacology*. 12, 2012.
[2] Van Stee MF, de Graaf AA, Groen AK. Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy. *Cardiovascular diabetology*. 17(1):1-22, Dec 2018.
[3] Yeung AW, Tzvetkov NT, Durazzo A, Lucarini M, Souto EB, Santini A, Gan RY, Jozwik A, Grzybek W, Horbańczuk JO, Mocan A. Natural products in diabetes research: Quantitative literature analysis. *Natural Product Research*. 35(24):5813-27, Dec. 2021.
[4] Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat?. *Diabetes*. 54(1):1-7, Jan. 2005.
[5] Abdul-Ghani MA, Norton L, DeFronzo RA. Renal sodium-glucose cotransporter inhibition in the management of type 2 diabetes mellitus. *American Journal of Physiology-Renal Physiology*. 309(11):F889-900, Dec. 2015.
[6] Wilcox, G. Insulin and insulin resistance. Clinical biochemist reviews, 26(2), p.19, 2005.
[7] Chen, M.E., Aguirre, R.S. and Hannon, T.S. Methods for measuring risk for type 2 diabetes in youth: the oral glucose tolerance test (OGTT). *Current diabetes reports*, 18(8), pp.1-9, 2018.
[8] Tangvarasittichai, S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World journal of diabetes*, 6(3), p.456, 2015.
[9] Tran, N., Pham, B. and Le, L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. *Biology*, 9(9), p.252, 2020.
[10] Kaushik P, Kaushik D, Sharma N, Rana A. *Alstonia scholaris*: its Phytochemistry and pharmacology. *Chronicles of young scientists*. 2(2):71, Apr.2011.
[11] Khyade MS, Kasote DM, Vaikos NP. Alstonia scholaris (L.) R. Br. and *Alstonia macrophylla* Wall. ex G. Don: A comparative review on traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*. 11; 153(1):1-8, Apr.2014.
[12] Njeh F, Feki H, Koubaa I, Hamed N, Damak M, Ayadi A, Hammami H, Mezghani-Jarraya R. Molluscicidal activity of *Solanum elaeagnifolium* seeds against Galba truncatula intermediate host of Fasciola hepatica: Identification of β-solamarine. *Pharmaceutical Biology*. 54(4):726-31, Apr.2016.

[13] Kamonlakorn K, Supon C, Riankrasin A, Pekthong D, Parhira S. Quantitative Phytochemicals Determination of the Extracts from the Flowers of *Alstonia scholaris* and their Anti-Lipoxygenase Activities. *In Key Engineering Materials*. Vol. 859, pp. 94-99, 2020. Trans Tech Publications Ltd.
[14] Khandelwal KR. Practical pharmacognosy techniques and experiments. 20th ed. Pune, India: Nirali Prakashan., p. 2.1 -2.6, 20.1-20.5, 23.1-23.17, 2010.
[15] Tanvir EM, Hossen M, Hossain M, Afroz R, Gan SH, Khalil M, Karim N. Antioxidant properties of popular turmeric (*Curcuma longa*) varieties from Bangladesh. *Journal of Food Quality*. May. 2017.
[16] Harbone JB. *Phytochemical Methods London*. Chapman and Hall1973. 1973.
[17] Harborne AJ. *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media; 1998 Apr 30.
[18] Han S, Hagan DL, Taylor JR, Xin L, Meng W, Biller SA, Wetterau JR, Washburn WN, Whaley JM. Dapagliflozin, a selective SGLT2 inhibitor, improves glucose homeostasis in normal and diabetic rats. *Diabetes*. 1;57 (6):1723-9, Jun. 2008.
[19] Vidya R, Kalaivani K, Venkatesh R. Antioxidant activity of goat liver slices treated with *Cucumis melo* (L). Fruit extract. *International Journal of Pharmaceutical Sciences and Research*. 5(11):4846, Nov.2014.
[20] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry*. 25; 247(10):3170-5, May.1972. https://doi.org/10.1016/S0021-9258 (19)45228-9.
[21] Jhansyrani T, Sujatha D, Rupasree P, Bharathi K, Kvsrg P. In-vitro antioxidant potential of selected nutraceuticals. *International Journal of Pharmaceutical Sciences and Research*.10 (3):1426-32, 2010.
[22] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et biophysica acta (BBA)-general subjects*. 582(1):67-78, Jan.1979.
[23] Bandawane D, Juvekar A, Juvekar M. Antidiabetic and antihyperlipidemic effect of Alstonia scholaris Linn bark in streptozotocin induced diabetic rats. *Indian Journal of Pharmaceutical Education and Research*. 45(2):114-20, Apr.2011.
[24] Arulmozhi S, Mazumder PM, Lohidasan S, Thakurdesai P. Antidiabetic and antihyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R. Br. *European journal of integrative medicine*. 2(1):23-32, Apr.2010.
[25] Poudel RR. Renal glucose handling in diabetes and sodium glucose cotransporter 2 inhibition. *Indian journal of endocrinology and metabolism*. 17(4):588, Jul.2013.

Cardioprotective effect of *Alstonia scholaris* Linn. R. Br*:* A review

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Abstract

Cardiovascular diseases (CVD) are becoming a serious threat to human civilization due to their rapidly increasing prevalence. Herbal medicines have been used as cardioprotective medicines to relieve human suffering. Therefore, the purpose of this review is to provide a summary of scientific reports indicating the antihypertensive, anti-hyperlipidemia, and antioxidant effects of Alstonia scholaris R. Br. (Apocynaceae) have therapeutic potential in CVD. Perennial folk medicine focuses on the protective mechanisms against various cardiovascular pathologies. Its phytoactive components, including reducing sugars, flavonoids, alkaloids, tannins, and glycosides, are thought to have vasodilator and antioxidant properties. Antioxidant properties include free radical scavengers, blockage of the cell-dependent Ca2+ channel, increase lipoprotein lipase activity, stimulation of endothelial nitric oxide synthesis, and prevention of thrombosis formation are all proposed mechanisms for cardioprotective effects. As a result, this review demonstrates the therapeutic potential of A. scholaris as a cardioprotective effect with long-term safety. Existing research indicates that clinical trial design and approaches are inadequate. Furthermore, a comprehensive evaluation of its role in primary and secondary coronary prevention has yet to be completed.

Keywords: *Alstonia scholaris, Anti-hypertension, Antioxidant, cardio protective.*

Introduction

For thousands of years, people have used herbal medicines to treat cardiovascular diseases (CVDs) such as atherosclerosis, hypertension, cerebral insufficiency, angina, and arrhythmias [1,2]. In 2021, the World Health Organization (WHO) reported that cardiovascular disease is the number one cause of death worldwide, claiming an estimated 17.9 million lives (31%) each year [3]. CVDs have become the leading cause of increased mortality and morbidity in India. The estimate of the age-standardized cardiovascular death rate of 272 per 100 000 population in India is higher than the global average of 235 per 100 000 population in the global burden of disease [4]. Herbal and alternative medicines are gaining scientific interest as traditional remedies as a new therapy for cardiovascular diseases to alleviate this burden. Plant-derived medicines are important in ensuring adequate public health therapy and disease management. In today's medical world, phytoactive components derived from natural sources reduce the risk of heart disease by scavenging free radicals [5]. Polyphenols reduce the risk of cardiovascular disease by inhibiting the oxidation of low-density lipoproteins [6]. *A. scholaris* is a valuable medicinal herb with various medicinal properties [7]. Saptaparni is another name for it, with Sapta meaning seven and Parna meaning leaves. It is commonly known as devil tree and is found throughout the Indian subcontinent and Southeast Asia [8]. Scientists have discovered a large number of phytoactive components derived from plants. This review provides important data and information about *A. scholaris* to researchers for the management of cardiovascular disease and the development of more effective drugs. Many alkaloids and flavonoids have been shown to reduce blood pressure pharmacologically [9]. Bioflavonoids, such as quercetin and flavonoids, have been shown to have cardiovascular relaxant effects [10]. Antihypertensive, antihyperlipidemic, antioxidant, vasorelaxant, and thrombolytic effects were also reported in this review, which aids in the management of cardiovascular abnormalities. Furthermore, a previous clinical study revealed that A. scholaris worked better at lowering blood pressure. This plant is in high demand due to its therapeutic value in cardiovascular diseases.

Taxonomy

Domain: Eukaryota- nucleus within a membrane; Kingdome: Plantae- plant; Sub kingdom: *Tracheobionta*- vascular plants; Phylum: *Spermatophyta*- seed-bearing plant; Sub phylum: *Angiospermae*- seeds in a closed ovary; Class: Dicotyledonae- two cotyledons; Order: Gentianales; Family: *Apocynaceae*; Genus: *Alstonia*; Species: *Alstonia scholaris.*

Botany

Alstonia scholaris (Apocynaceae) is a tropical ornamental evergreen commonly known as devil tree, milkwood, scholar tree, and blackboard tree. It is widespread in southern China, Australia, Indonesia, Myanmar, Thailand, the Philippines, Vietnam, Germany, India, Nepal, Pakistan, Brunei Darussalam, and New Guinea [11]. This glabrous tree grows up to 130 feet tall. Its young twigs have lenticels and greyish colour of the matured bark. Glossy leaves are arranged in whorls of three to ten with petioles (1-3 cm). Fragrant flowers bloom in the month of October. The elongated seed has a ciliated edge with terminal tufts of hairs (1.5-2 cm).

Phyto active components

The bark and leaves of *A. scholaris* are rich in phytoactive compounds such as *polyphenolic* acids and *triterpenes.* Several previous studies have examined the potential cardioprotective effects of A. *scholaris* by analyzing its major bioactive components, including the alkaloids *(Picrinine, Vallesamines*, and *Vallesamines),* tannins, polyphenols, cardiac glycosides, and flavonoids *(Quarcetine,* and *Quarcitrin).* Table 1 summarizes the primary phytochemical components of *A. scholaris* reported to be responsible for its cardioprotective effects.

Cardiac protective effects

Preclinical studies

Antihypertensive effect

The antihypertensive effect of *A. scholaris* has been documented in reports of traditional *Ayurvedic* treatments [12]. The aim of the study was to determine the underlying therapeutic mechanism. The effects of A. *scholaris* leaves extract on spontaneously hypertensive rats (SHRs) were studied in vivo experiments in 2015. The A. scholars water (ASWE) and methanol (ASME) extracts were given to 16-20 week old SHRs (250-300 g) for two weeks. Daily oral doses of 1000 mg/kg were used for both extracts. The rats' blood pressure was measured using a tail cuff, a non-invasive method. This study result suggests that *methanolic*
A. scholaris extract has a more potent antihypertensive effect when compared to water extract. Also, heart rates of SHRs gradually decrease during ASME, which has a beneficial effect on tachycardia. ASME has an antihypertensive effect similar to that of the standard drug, verapamil (15 mg/kg). As a result of its negative chronotropic and inotropic effect on the heart, ASME can be used to treat hypertension. Antihypertensive drugs with this mechanism of action include angiotensin-converting enzyme inhibitors and calcium channel blockers.

Vasorelaxant activity

An *in vitro* experiment was performed to evaluate the vasorelaxant effect of ASME on an isolated aortic ring [12]. The aortic rings were suspended in the Krebs-Ringer bicarbonate cycle with carbonated oxygen at 37°C. The aortic ring was contracted with phenylephrine (PE, 1M) or potassium chloride (KCL, 80 mM). A force-displacement transducer was used to measure aortic tension. ASME was cumulatively added to the PE solution after the contraction of the aortic ring. The result of this study shows a significant vasorelaxant effect of ASME on the pre-contracted aortic ring. In addition, the author also evaluated the ethyl acetate (EAF-ASME) and n-butanol (NBF-ASME) fraction of the methanolic extract from *A. scholaris* extract bark. NBF-ASME fractions (0.125-4 mg/mL) produced dose-dependent vasorelaxation on endothelium-intact aortic rings pre-contracted with phenylephrine (1 M). Therefore, it seems likely that the vascular effects of NBF-ASME reduce IP3-dependent Ca2+ release from the PE-sensitive sarcoplasmic reticulum (SR).

Anti-hyperlipidaemia effect

The antihyperlipidemic effects of ethanolic extracts from *A. scholaris* leaves in streptozotocin (STZ-65 mg/kg) induced diabetic rats were reported by *S. Arulmozhi et al.* [13]. Ethanolic extract of *A. scholaris* (EAAS) at a dose of 400 mg/kg was orally administered to the diabetic

rats for 6 weeks. Diabetic rats treated with EEAS showed significant reductions in serum triglyceride, low-lipoprotein density (LDL), very low-lipoprotein density (VLDL), and total cholesterol levels. On the other hand, high-density lipoprotein (HDL) levels are significantly elevated.

Another study on the antihyperlipidemic effect of *A. scholaris* Linn bark on streptozotocin-induced diabetic rats was reported by *Deepti Bandawane et al.* [14] reported. In this experiment, STZ (60 mg/kg) induced diabetic rats were treated with an aqueous extract of *A. scholaris* bark (300 mg/kg) for 4 weeks. After treatment, serum triglyceride and serum cholesterol levels decreased, which may play a role in the prevention of diabetes mellitus-related cardiovascular complications.

Author *Muhammed Thwayyib* mentioned earlier, also studied the antihyperlipidemic effect of *A. scholaris* leaves on Triton-induced hyperlipidemia [15]. Methanolic extract of *A. scholaris* leaf at a dose of 300 mg/kg was orally administered to male Wistar rats. After treatment with methanol extract, an antihyperlipidemic effect was observed.

Antioxidant potential

Plants are an excellent source of natural antioxidants that reduce the risk of certain diseases, such as cardiovascular disease and stroke [16,17]. Various extracts (water, hydro-alcohol, and ethanol) from leaves, stems and bark of *A. scholaris* contain many active ingredients that have been studied in many experiments.

The study on phytochemicals and antioxidant activity of *A. scholaris* was published in 2017 by Mistry et al. [18]. They assesses and compares qualitative and quantitative phytochemical components present in leaves, stems and bark of *A. scholaris*. Qualitative phytochemical screening revealed the presence of flavonoids, saponins, alkaloids, phenolic compounds, glycoside steroids and tannins in leaf, stem and bark extracts of *A. scholaris*.

Overall potent antioxidant activity of aqueous and methanolic extracts was observed in an in vitro antioxidant analysis. Terpenoid is the most abundant phytoconstituent in aqueous extracts. Both flavonoids and terpenoids have a defense mechanism against free radicals.

In this research, the percentage of superoxide radicals scavenged by aqueous extracts of *A. scholaris* bark was higher than gallic acid (standard) at each concentration. Similarly, the result of free radical scavenging tests with ascorbic acid and 1, 1-diphenyl-2-picryl-hydrazine(DPPH) at a dose of 200, 250, and 300 g/ml showed that aqueous extracts scavenged free radicals better than the methanolic extract from the bark of *A. scholaris.*

The phytochemical overview of *A. scholaris* was published by *Kanase and Manein* in 2018 [19]. Flavonoids, alkaloids, and tannins played important roles in antioxidant activity. It suggested that as a medicinal plant for degenerative diseases, it was more effective as an antioxidant than those of other species. The methanolic flower extract has been reported to have higher antioxidant activity than the fruit. Phytochemical content of phenols and flavonoidsin flowers and flavonoids in fruits of the methanolic extract of *A. scholaris* [20,21]. The percent inhibition of methanolic *A. scholaris* flower extract was 26.70 and 28.72 at a dose of 250 g/ml. The IC50 (mcg/mL) for *A. scholaris* flower extract is 390.52 and 348.60 by DPPH and nitro blue tetrazolium (NBT) methods, respectively [20].

In another study, the in vitro and in vivo effect of the antioxidant activity was performed by *S. Arulmozhi et al*.[13] The author evaluated the antioxidant potential of ethanolic extract of *A. scholaris* bark using various in vitro tests including DPPH radical scavenger, metal ion chelator, hydrogen peroxide scavenger, superoxide anion radical scavenger, and iron thiocyanate reducing ability. Data obtained from the results indicated that ethanolic extract produced a significant ($p<0.01$) increase in DPPH, free radical scavenging (63%), metal ion chelating agents (74.88%), hydrogen peroxide scavenging (72 .28%), superoxide anion radical shows scavenging (67.66%), while significant ($p<0.05$) iron thiocyanate reducing activity. Free radicals are formed disproportionately and increase oxidative stress in diabetes mellitusthrough glucose breakdown, non-enzymatic glycation of proteins, and subsequent oxidative breakdown [22].

Evidence has shown that glycation induces the formation of oxygen-derived free radicals under diabetic conditions [23]. In vivo investigation of the antioxidant potential of *A. scholaris* leaves was performed on STZ-induced diabetic rats [24]. In this study, the oxidative stress marker malondialdehyde (MDA) increased significantly ($p<0.001$) higher in the diabetic control animals, while treatment with EEAS decreased its level significantly ($p<0.001$). Treatment with EEAS 400 mg/kg significantly ($p<0.001$) increased superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels. EEAS increases enzymatic activity to prevent free radicles. Flavonoids and triterpenoids are reported to be the most abundant bioactive components in EEAS with antioxidant activity.

Clinical study

Thrombolytic effect

Myocardial infarction (MI) is one of the leading causes of death worldwide. MI associated with hypoxia causes myocardial necrosis [25] and an increase in oxidative stress leads to heart failure [26]. Thrombolytic activity helps in the reduction of myocardial infarction [27].

One of the experiments was conducted to evaluate the thrombolytic effect of *A. scholaris* leaves *in vitro* [28]. The ethanolic extract of A. *scholaris* demonstrated potent thrombolytic activity in this investigation. Compared to the thrombolytic effects of n-hexane, ethyl acetate, dichloromethane, and water-soluble extract, the ethanolic extract (1 mg/100 µl water) had the highest effect, with a percentage of 55±330.08%, almost identical to streptokinase (62±070.33%).

The investigators also examined the membrane-stabilizing activity of *A. scholaris* ethanolic extract, presented in Table 2. In the case of hypotonic solution-induced hemolysis, crude ethanolic extract (83.47±0.15%) had the highest RBC membrane protective capacity, which is close to that of standard acetylsalicylic acid (85.09±0.8%). In addition, an ethanolic extract shows the strongest activity (79.82±0.71%) as the standard shows 80.98±1.34% in heat-induced hemolysis analysis. Observed results state that Observed results indicate that *A. scholaris* might inhibit erythrocyte membrane rupture due to its anti-inflammatory activity.

Antihypertensive effect

Clinical research on A. scholars began with Bhogayata K. et al. [29]. Thirty people with essential hypertension participated in the study (EHT). There were two groups of patients:

Group 1, who received a decoction of the bark of A. *scholaris* at a dose of 15 g twice a day for 21 days (15 patients), and Group 2, who received a dose of 250 mg of Sarpagandha (Rauwolfia serpentina) Ghanavati twice a day. It was found that the Saptaparna group was more effective at alleviating the symptoms of Udarshool (colic*), Vibandha Pindikodweshtan* (constipation), Hrud dravatva (cardiac fluid), Hrdaya Daurbalya (cardiac insufficiency), and Aalsya (sluggish symptoms).

In contrast, the two groups had nearly the same observation for *Sweda Pravriti* (tendency to sweat). Systolic and diastolic blood pressure in the supine and seated positions was significantly lower in the study's intervention group compared to the control group. The diastolic blood pressure results were better for Saptaparna in both positions, while the systolic blood pressure result was better for the Sarpagandha group. From this study, the hypothesis emerged that the simultaneous treatment of hypertension in its chronic form and cardiovascular diseases with these two drugs could be more successful with the combination.

Perspective of Future Research

Screening of *A. scholaris* should be performed to investigate its phytoconstituents with cardioprotective effect. Characterization of specific isolated components from this medicinal plant is helpful in new drug development and design for CVDs treatment. This review conclude that *A. scholaris* has cardio protective potential based on non-clinical (in vitro and in vivo models) and clinical studies. In addition, long-term clinical trials should be encouraged to investigate its cardiovascular potential alternative to existing pharmaceutical drug regimens. The quality of research can be improved with government funding.

Conclusion

This review provides important information as it summarizes the preclinical and clinical application of *Alstonia scholaris* and its possible mechanisms in various cardiovascular diseases. There is an urgent need to clarify the mechanism of action behind its cardioprotective potential and to study its pharmacokinetic action at the cellular level, despite the large body of preclinical evidence supporting the plant's cardiovascular benefits. Clinical studies have also observed improved cardiac metabolic markers, confirming its beneficial effects. This suggests that the leaves and bark of A. *scholaris* may have cardioprotective effects, which could help those suffering from cardiovascular disease. Additionally, the issue of A. *scholaris* interaction with other available cardio-protective drugs should be resolved, for which there is currently insufficient data. Further studies are needed to determine the specific pathological mechanism(s) by inhibiting inflammation and preventing cardiovascular diseases.

Reference

[1] Mashour, Nick H., George I. Lin, and William H. Frishman. “Herbal medicine for the treatment of cardiovascular disease: clinical considerations.” Archives of internal Medicine 158, no. 20 (1998): 2225-2234.

[2] Wang, Chong-Zhi, Sangeeta R. Mehendale, and Chun-Su Yuan. "Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness." The American journal of Chinese medicine 35, no. 04 (2007): 543-558.
[3] World Health Organization. "Available online: https://www. who. int/oral_health/action/services/en." Accessed May (2021).
[4] Prabhakaran D, Jeemon P, Roy A. Cardiovascular diseases in India: current epidemiology and future directions. Circulation. 2016;133(16):1605-20.
[5] Wang CZ, Mehendale SR, Yuan CS. Commonly used antioxidant botanicals: active constituents and their potential role in cardiovascular illness. Am J Chin Med. 2007;35(04):543–558.
[6] Zern, Tosca L., and Maria Luz Fernandez. "Cardioprotective effects of dietary polyphenols." The Journal of nutrition 135, no. 10 (2005): 2291-2294.
[7] Baliga, Manjeshwar Shrinath. "Review of the phytochemical, pharmacological and toxicological properties of *Alstonia scholaris* Linn. R. Br (Saptaparna)." Chinese journal of integrative medicine (2012): 1-14.
[8] Khatale, Vaishali Laxman, and D. B. More. "A Review on Saptaparna (*Alstonia Scholaris R. Br*)." International Ayurvedic Medical Journal 4, no. 03 (2016): 334-337.
[9] Cassidy, Aedín, Éilis J. O'Reilly, Colin Kay, Laura Sampson, Mary Franz, J. P. Forman, Gary Curhan, and Eric B. Rimm. "Habitual intake of flavonoid subclasses and incident hypertension in adults." The American journal of clinical nutrition 93, no. 2 (2011): 338-347.
[10] Chen, Cora Ke, and Cecil R. Pace-Asciak. "Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta." General pharmacology 27, no. 2 (1996): 363-366.
[11] Akbar, Shahid. "*Alstonia scholaris* (L.) R. Br.(Apocynaceae)." In Handbook of 200 Medicinal Plants, pp. 225-234. Springer, Cham, 2020.
[12] Idris, Bello, Mohammad Zaini Asmawi, Usman Salisu Nasiba, Roziahanim Mahmud, and Kabiru Abubakar. "Antihypertensive and vasorelaxant effect of *Alstonia scholaris* stem bark extracts and fractions." International Journal of Pharmacology 11, no. 4 (2015): 327-334.
[13] Arulmozhi, S., Papiya Mitra Mazumder, Purnima Ashok, and L. SATHIYA Narayanan. "In vitro antioxidant and free radical scavenging activity of Alstonia scholaris Linn. R. Br." Iranian Journal of Pharmacology and Therapeutics 6, no. 2 (2007): 191-196.
[14] Bandawane D, Juvekar A, Juvekar M. Antidiabetic and antihyperlipidemic effect of Alstonia scholaris Linn bark in streptozotocin induced diabetic rats. Indian Journal of Pharmaceutical Education and Research. 2011 Apr 1;45(2):114-20.
[15] Muhammed Thwayyib, P. K. "Study of Antihyperlipidaemic Activity of *Alstonia scholaris* Leaves." PhD diss., Karpagam College of Pharmacy, Coimbatore, 2017.
[16] Williams, Robert J., Jeremy PE Spencer, and Catherine Rice-Evans. "Flavonoids: antioxidants or signalling molecules?." Free radical biology and medicine 36, no. 7 (2004): 838-849.
[17] Kasote, D. M. "Flaxseed phenolics as natural antioxidants." International Food Research Journal 20, no. 1 (2013).pp 27-34.
[18] Dhruti, Mistry, Parekh Bhavika, and Pithawala Meonis. "Studies on phytochemical constituents and antioxidant activity of Alstonia scholaris." International Journal of Life Science 4, no. 4 (2016): 529-538.

[19] Kanase, V. A. N. I. T. A., and DEEPALI J. Mane. "A pharmacognostic and pharmacological review on Alstonia scholaris." Asian J Pharm Clin Res 11, no. 12 (2018): 22-6.

[20] Shankar, K. Ravi, K. V. R. N. S. Ramesh, and P. Naveena. "Free radical scavenging activity of the flower and fruit extracts of Alstonia scholaris." Biosciences Biotechnology Research Asia 5, no. 1 (2016): 493-494.

[21] Arvind, Kumar, Kaur Rajbir, and Arora Saroj. "Free radical scavenging potential of some Indian medicinal plants." Journal of Medicinal Plants Research 4, no. 19 (2010): 2034-2042.

[22] Mahboob, M., M. F. Rahman, and P. Grover. "Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients." Singapore medical journal 46, no. 7 (2005): 322.

[23] Kumar, Gandhipuram Periasamy Senthil, Palanisamy Arulselvan, Durairaj Sathish Kumar, and Sorimuthu Pillai Subramanian. "Anti-diabetic activity of fruits of Terminalia chebula on streptozotocin induced diabetic rats." Journal of health science 52, no. 3 (2006): 283-291.

[24] Arulmozhi S, Mazumder PM, Lohidasan S, Thakurdesai P. Antidiabetic and antihyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R. Br. European journal of integrative medicine. 2010 Apr 1;2(1):23-32.

[25] Whellan, David J. "Heart failure disease management: implementation and outcomes." Cardiology in review 13, no. 5 (2005): 231-239., (n.d.).

[26] Hill, Michael F., and Pawan K. Singal. "Antioxidant and oxidative stress changes during heart failure subsequent to myocardial infarction in rats." The American journal of pathology 148, no. 1 (1996): 291.

[27] Sudhakar, P., R. Senthil Kumar, R. Kothai, and B. Arul. "In Vitro Thrombolytic and In Vivo Cardioprotective Activities of Methanolic Extract of Indigofera cassioides Rottl. Ex. DC." Indian Journal of Pharmaceutical Sciences 84, no. 2 (2022): 319-327.

[28] Jahan, Nisrat, Sadia Subrin, and Mohammad Rashedul Haque. "In vitro Study of Different Partitionates of Alstonia scholaris (L.) R. Br. Leaf for Thrombolytic and Membrane Stabilizing Activities." Bangladesh Pharmaceutical Journal 21, no. 2 (2018): 145-149.

[29] Bhogayata, Kamlesh, P. P. Sharma, and B. R. Patel. "A clinical evaluation of Saptaparna (*Alstonia scholaris* L., R. Br.) on essential hypertension." AYU (An international quarterly journal of research in Ayurveda) 30, no. 3 (2009): 318.

[30] Shang, Jian-Hua, Xiang-Hai Cai, Tao Feng, Yun-Li Zhao, Jing-Kun Wang, Lu-Yong Zhang, Ming Yan, and Xiao-Dong Luo. "Pharmacological evaluation of Alstonia scholaris: anti-inflammatory and analgesic effects." Journal of ethnopharmacology 129, no. 2 (2010): 174-181.

[31] Verma, Pawan K., Rajinder Raina, Mudasir Sultana, Shahid Prawez, and Maninder Singh. "Polyphenolic constituents and antioxidant/antiradical activity in different extracts of *Alstonia scholaris* (Linn.)." African Journal of Biotechnology 14, no. 47 (2015): 3190-3197.

[32] Salim, S. M., N. M. Yunos, M. H. Jauri, and Y. Kamisah. "Cardiotonic effects of cardiac glycosides from plants of Apocynaceae family." Chula. Med. J 64, no. 4 (2020): 449-456.

Tables

Table 1. Phytochemicals Responsible for the Cardioprotective Activity of *A. scholaris*

Groups	Phytoconstituents	Resources	Cardiac activity	References
Alkaloid	Picrinine	leaves	Anti-inflammatory	[30]
	Vallesamines	leaves		
	Scholaricine	leaves		
Tannins	*NR	Stem and bark	Antioxidants	[12]
Poly-phenol			Anti-inflammatory and anti-oxidants	[31]
Cardiac glycoside			Cardiac tonicity	[32]
Flavonoid (flavonols)	Quarcetine	leaves	Antioxidant	[12]
	Quarcitrin	leaves		

*NR-Not reported

Table 2. Reported Cardioprotective Activity of *A. scholaris*

Model	Population/material	*A. scholaris* formulation	sources	Dosage	Effect	Mechanism	Reference
In vivo Spontaneous hypertensive rats (SHR)	Wistar Kyoto Rats	Methanolic extract	Bark	1000 mg/kg for 2 weeks	Blood pressure Heart rate	**Control hypertension** - Calcium channel blocker and angiotensive converting enzyme inhibitor.	[12]

In vitro aortic rings pre contracted with phenylephrine or KCL	Wistar Kyoto Rats	n- butanol fraction of methanolic extract	Bark	0.125 -4 mg/mL	Dose dependent Vasorelaxant effect of fraction	**Improve endothelium function** -NO and prostacyclin pathway involvement (EDP), reduce IP3-dependent Ca2+ release (EIP)	[12]
STZ induced diabetic rat	Male wistar albino rats	Ethanolic extract	Leaves	400 mg/kg for 6 weeks	↓CH, ↓LDL, ↓TG, ↓VLDL, ↑and HDL	Increase lipoprotein lipase activity	[13]
STZ induced diabetic rat	Male wistar albino rats	Aqueous extract	Bark	300 mg/kg for 4 weeks	↓CH, ↓LDL, ↓TG, and ↑HDL	Prevent from VSMC alteration	[14]
Triton-induced hyperlipidaemia	Male wistar albino rats	Methanolic extract	Leaves	300 mg/kg for 1 week	↓CH, ↓LDL, ↓TG, ↑VLDL and HDL	Increase lipoprotein lipase activity	[15]
In vitro 1. Total antioxidant activity 2. SOD 3. Free radical scavenging activity	1.Molybdate reagent 2. NBT 3. DPPH	Aqueous extract	Bark	1. 200, 250 and 300 µg/ml 2. 175, 200 and 225 µg/ml 3. 32, 64	Cytoprotection effect	Free radical scavenging activity of Flavonoid and terpenoids	[18]

				and 128 µg/ml			
In vitro 1. SOD 2. Free radical scavenging activity	1. NBT 2. DPPH	Methanolic extract	Flower	250 g/ml	Cytoprotection effect	Free radical scavenging activity of Flavonoid and phenol	[19]
In vivo STZ induced diabetic rat	Wister albino rat	Ethanolic extract	Leaves	400 mg/kg for 6 weeks	Cytoprotection effect	Decrease oxidative stress (MDA, SOD,GSH, and CAT)	[24]
Carrageenan-induced air pouch model *In vitro* COX-1, COX-2 and 5-LOX inhibition	ICR mice Heme (non-protein part of heamoblobine)	Alkaloidal fraction Picrinine , Vallesamine , and Scholaricine	Leaves	20 mg/kg intragastrical for 1 week 10 mg/kg 8 mg/kg 5 mg/kg	Decrease cardiac damage	Control inflamaation, Increased SOD activity and decreased levels of NO, PGE2 and MDA Inhibition of COX-1, COX-2 and 5-LOX	[30]
Prompt and swift method	Healthy Human (n=10)	Ethanolic extract	Leaves	1 mg/100 µl water	Thrombolytic Membrane stabilizing activity	Inhibition of erythrocyte membrane rupture	[28]
Increased Blood	Hypertensive	Coarse powder	Leaves	15 g twice a day	Decrease diastolic pressure	-	[29]

pressure and heart palpitation	Human (n=15)	(Decoction)		for 21 days			

NO Nitric oxide; EDP Endothelium dependent pathway; EIP Endothelium independent pathway; VSMC Vascular smooth muscle cell; DPPH 1, 1-diphenyl-2-picryl-hydrazine; NBT Nitro blue tetrazolium