Title: Investigation into Cardiovascular effects of Regulators of G-protein signaling-2 (RGS2) agonist in animal models of cardiovascular complications

Abstract:
Now a day, cardiovascular diseases (CVDs) are most one of the leading cause of worldwide mortality and morbidity. According to WHO, it was estimated that 17.9 million people died from CVDs in 2016 which representing 31% of all the global deaths. The underlying pathogenesis of cardiovascular diseases suggested the hyperactivity of various stimulatory mediators such as endothelin-1 (ET-1), angiotensin II, adrenaline and noradrenaline as major contributing factor. However, these mediators produce their action via G-protein coupled receptors (GPCRs). GPCRs are one of the largest subfamily of cell surface receptors and largest class of drug target. More than 100 GPCRs are expressed in cardiovascular system and regulate the essential cardiovascular functions. Thus, GPCR signaling has crucial role in the development of cardiovascular complications. The previous reports suggested that chronic hyper-activation of GPCR signaling, especially Gαq signaling leads to development of various cardiovascular abnormalities such as cardiac hypertrophy, fibrosis, and heart failure. GPCR signaling is controlled both at receptor level by agonist and antagonist drugs as well as at intracellular signaling components. Regulator of G-protein signaling (RGS) proteins are major contributor to regulate the GPCRs functioning at the intracellular signaling components. RGS proteins are negative regulator of GPCR signaling. Numerous of evidences ranging from biochemical characterizations to genetic studies demonstrated the RGS protein as potential drug target in pathologies of CNS diseases, cardiovascular diseases and diabetes. Therefore, we have identified the binding affinity of small molecule (Gαq-RGS2 signaling inhibitor) on RGS2, RGS4 and RGS5 by computational study using software. In computational study, 100 confirmations of ligand were obtained on each protein and binding pocket and free binding energy were analyzed using Autodock4, Genetic Algorithm, Lamarckian Analysis. The results of computational study showed that small molecule (RGS2 agonist) has more binding affinity towards the RGS2 as compared to RGS4 and RGS5 proteins.

Further to assess the cardiovascular potential of RGS2 agonist, we have evaluated the effect of RGS2 agonist on the action of various receptor-dependent (angiotesin II, adrenaline, acetylcholine) and –independent (potassium chloride, calcium chloride, sodium nitroprusside) mediators using isolated heart and aorta in-vitro experiment. The results of in-vitro study
showed that RGS2 agonist decreased the action of receptor-dependent agonists; angiotensin II and adrenaline while increased the action of acetylcholine in the isolated heart and aorta. However, the action of receptor-independent mediators did not alter by the RGS2 agonist.

From the results of *in-vitro* study, we have aimed to evaluate the therapeutic potential of RGS2 agonist in the cardiac arrhythmia and myocardial infarction. However, we have established the acute and sub-acute toxicity profile of the RGS2 agonist before exploring the compound in the *in-vivo* efficacy study. The results of acute and sub-acute study indicated that RGS2 agonist did not show any toxic effect on acute and sub-acute administration in the normal rats. The efficacy of RGS2 agonist was evaluated in aminophylline induced cardiac arrhythmia in the rats. Rats were divided into four groups; normal rats, arrhythmic rats (DC), RGS2 agonist (1 and 10 mg/kg, p.o.) treated arrhythmic rats. The treatment of RGS2 agonist (1 and 10 mg/kg/d, p.o., 7 days) and aminophylline (100 mg/kg/d, i.p., 7 days) were given from 1st day. RGS2 agonist was administered 1 hr prior the administration of aminophylline. At the end of study, heart rate (HR), QRS complex, QT interval and RR interval were measured from the electrocardiogram (ECG) of anesthetized rats. Systolic and diastolic blood pressure (SBP and DBP) by invasive method and cardiac damage markers in the serum (CK-MB and LDH) were measured. The results of study showed that treatment of RGS2 agonist (10 mg/kg) significantly decreased the aminophylline induced increase in the HR as compared to untreated rats. The PR interval did not significantly alter in the aminophylline treated rats as compared to NC. The treatment of RGS2 agonist (1 and 10 mg/kg) significantly decreased the elevation in the QRS complex while QT interval prolongation and RR interval significantly reduced by RGS2 agonist at 10 mg/kg dose as compared to untreated rats. The treatment of RGS2 agonist (1 and 10 mg/kg) significantly decreased the SBP and DBP in the aminophylline treated rats as compared untreated rats. The treatment of RGS2 agonist (1 and 10 mg/kg) significantly decreased the serum CK-MB and LDH level in the aminophylline treated rats as compared to untreated rats. These data are providing the insight on cardioprotective effects of RGS2 agonist on aminophylline induced cardiac arrhythmia.

Further, we evaluated the effect of RGS2 agonist in doxorubicin and isoproterenol induced myocardial infarction in rats. The preventive treatment of RGS2 agonist was given for 10 days prior the administration of isoproterenol and doxorubicin, respective groups. In the isoproterenol induced myocardial infarction, the isoproterenol (85 mg/kg, s.c.) was
administered for two consecutive days (10th and 11th days) in all the groups except normal rats. In the doxorubicin induced myocardial infarction, doxorubicin (20 mg/kg, s.c.) was administered on 10th day of study in all the groups except normal rats. At the end of study, ECG and various biochemical parameters such as cardiac damage markers (CK-MB, LDH), kidney damage markers (urea, creatinine) nitrite level were measured in the serum of all the rats. The western blot analysis of RGS2 protein, cAMP, cGMP level and endogenous antioxidant status (superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH)) in cardiac tissue were measured. Histological examination of left ventricular cardiac tissue was performed. The prior treatment of RGS2 agonist showed the protective action against both doxorubicin and isoproterenol induced myocardial infarction in rats. The treatment of RGS2 agonist significantly decreased the serum cardiac damage markers (CK-MB, LDH) in both doxorubicin and isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly reversed the increase in heart rate, QRS prolongation and QT interval prolongation in doxorubicin and isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly improved the endogenous antioxidant status (SOD, Catalase, Glutathione) in cardiac tissue of doxorubicin and isoproterenol induced myocardial infarcted rats as compared to untreated rats. The RGS2 agonist treatment significantly increased the serum nitrite level in the serum of doxorubicin and isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly increased the cGMP level in the cardiac tissue of doxorubicin induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly decreased the cAMP level in the cardiac tissue of isoproterenol induced myocardial infarcted rats as compared to untreated rats. In conclusion, RGS2 agonist showed the protective action against the aminophylline induced cardiac arrhythmia and doxorubicin as well as isoproterenol induced myocardial infarction in rats. This protective action of RGS2 agonist might be due to modulation of intracellular concentration of cAMP and cGMP level and antioxidant activity.

**Brief description on the state of the art of the research topic:**

Cardiovascular disease (CVD) is a group of diseases that include coronary artery disease (CAD) and coronary heart disease (CHD). CAD is a pathologic process affecting the coronary arteries while CHD includes angina pectoris, myocardial ischemia and myocardial infarction (MI) (1). In recent years, cardiovascular diseases are one of the leading causes of mortality and morbidity. According to WHO fact sheet, it was estimated that 17.9 million
people died from CVDs in 2016, representing 31% of all global deaths. Among them, an estimated 7.4 million were died due to coronary heart disease and 6.7 million due to stroke (2). These data the prevalence of cardiovascular disease is rapidly increasing in developed as well as developing countries and is one of the leading causes of death and disability. The epidemiological figures are showed that significant number of patients of cardiovascular disease exists in India including Gujarat. As per the Registrar General of India and Million Death Study investigators, cardiovascular disease is the leading cause of deaths, about 2 million deaths annually. Cardiovascular disease has a major impact on the physical, social, psychological and occupational life of the patient and also affects a patient’s quality of life. The case-control INTERHEART and INTERSTROKE studies reported that hypertension, lipid abnormalities, smoking, obesity, diabetes, sedentary lifestyle, low fruit and vegetable intake and psychosocial stress are important etiological factors in Indian populations as in other populations of the world. Individual studies have reported that there are substantial regional variations in risk factors in India. At a macro-level these regional variations in risk factors explain some of the regional differences in CVD mortality. Tobacco addiction, inadequate sleep, diabetes, obesity and aging are major risk factors for the cardiovascular disease. Tobacco addiction, obesity and diabetes are highly prevalent in Gujarat population which increases the risk of cardiovascular diseases in Gujarat population. The epidemiological figure suggested that cardiovascular disease is the most prevalent non-communicable disease in Gujarat and increases the mortality, morbidity and economic burden of populations.

**Definition of the Problem:**

G-protein coupled receptor (GPCRs) regulates the essential cardiovascular functions. In cardiovascular system, adrenoceptors, angiotensin II receptors, muscarinic receptors and endothelin-1 receptors are expressed and mediate their biological actions via \( G_\alpha q \) signaling (1). Previous evidences suggest that increase in \( G_\alpha q \) signaling implicated in development of cardiac remodeling, cardiac hypertrophy, endothelial dysfunction and hypertension. It reported that inhibition of \( G_\alpha q \) signaling decreased the increase in blood pressure in renal artery stenosis in mice and genetic vascular smooth muscle-derived models of hypertension (2). The activation of \( G_\alpha q \) signaling in cultured cardiomyocyte and transgenic mice showed the phenotypes of cardiac remodeling. Similar to this, in neonatal cardiomyocyte and transgenic mice activation of \( G_\alpha q \) signaling showed the malfunction of Ca\(^{++}\) regulated protein kinase and increased the insitol triphosphate mediated Ca\(^{++}\) release (3). Regulator of G-
protein signaling-2 (RGS2) is negative regulator of this Gαq signaling. RGS2 deficient mice showed the severe hypertension with endothelial dysfunction and increased peripheral resistance (4). Vascular reactivity assay on mesenteric artery of RGS\(^{−/−}\) and showed increase in % constriction with phenyl epinephrine compared to normal. All the evidences are demonstrating that hyperactivity of Gαq signaling has major role in the development of cardiovascular diseases. Therefore, till date RGS/Gαq is important therapeutic target for novel drug discovery in the area of cardiovascular diseases.

**Objectives:**

1. To identify the binding affinity and site of small molecule on the RGS2, RGS4 and RGS5 protein by computational study.
2. To synthesize and characterize the compound RGS2 agonist.
3. To assess the acute and sub-acute safety profile of RGS2 agonist.
4. To study the effect of RGS2 agonist on activity of receptor dependent and independent agonists in isolated heart and aorta.
5. To study the effect of RGS2 agonist on aminophylline induced cardiac arrhythmia.
6. To investigate the effect and possible mechanism of RGS2 agonist on doxorubicin and isoproteronol induced myocardial infarction.

**Scope of study:**
The current study identified the binding affinity and site of ligand on the RGS2 protein. Further, this research generated the preclinical data related to the therapeutic potential of RGS2 agonist in cardiac arrhythmia and myocardial infarction in the rats. The development of appropriate formulation and exploration of compound either at the dose of 10 mg/kg or higher dose for long time at the clinical level is the great interest of study.

**Original contribution by the thesis:**
The contributions of our studies include;

1. The current study showed the binding site and affinity of small molecule on the RGS2, RGS4 and RGS5 proteins.
2. Synthesis and characterization of the RGS2 agonist.
3. Evaluated the acute and sub-acute safety profile of RGS2 agonist in the rats.
4. Assessed an anti-oxidant activity of RGS2 agonist showed against organic free radical, superoxide radical, cationic radical, peroxyl radical, ferric reduction and chelation capacity which is compared to trolox.
5. Evaluated the effect of RGS2 agonist on the activity of receptor-dependent and – independent agonists.

6. This study showed a protective effect of RGS2 agonist against the aminophylline induced cardiac arrhythmia.

7. The present study showed the protective action of RGS2 agonist against the doxorubicin and isoproterenol induced myocardial infarction.

**Methodology of Research and Results:**

**Animal husbandry and feeds:**

The albino wistar rats (200-250 g) and Swiss albino mice (20-30 g) of either sex were were procured from Zydus research center (ZRC), Ahmedabad, India at 1 wk before the study. They were housed 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 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/COLOGY/16/12, LMCP/COLOGY/18/04) 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.

1. **Computational study:**

In computational study, binding efficacy of ligand on RGS2, RGS4 and RGS5 proteins was evaluated using Autodock4, Genetic Algorithm and Lamarckian Analysis. The structure of proteins; 2AF0 (RGS2), 1EZT (RGS4) and 2CRP (RGS5) were selected from the protein databank for computational study. 100 binding confirmations of ligand on each protein were obtained. Binding affinity of ligand was confirmed from the binding pocket formation and free binding energy. The results of computational study indicated that small molecule has high binding affinity on RGS2 protein as compared to RGS4 and RGS5 proteins. The probable binding site of small molecule on RGS2 protein is tryptophan 113 and lysine 120 by forming hydrogen bond with help first nitrogen of triazolone ring and oxygen of hydroxyl group present on secondary ring.

2. **Synthesis and characterization of compound:**

Chemical moiety (RGS2 agonist) was synthesized using reported chemical scheme and characterized by infrared (IR) and mass spectra in our laboratory.
3. Effect of RGS2 agonist on action of various agonists in isolated heart and aorta:

3.1. Isolated perfused rat heart preparation:
Rats were heparinized (500 IU heparin/rat) and sacrificed. Heart was rapidly isolated and placed in ice-cold Krebs–Henseleit (K-H) buffer. Heart was cannulated via aorta and perfused with non-recirculating K–H buffer (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 11 mM glucose, pH 7.4) at constant perfusion pressure 70 mmHg. The perfusate was equilibrated with 95% O2 and 5% CO2 and maintained at a temperature of 37 °C. A fluid-filled latex balloon was inserted into the left ventricle to measure the left ventricular systolic pressure (LVSP). Balloon was connected to a pressure transducer (Biopac-MP 100; Biopac, Santa Barbara, CA, USA) and inflated to achieve left ventricular end-diastolic pressure (LVEDP) of about 10 mm Hg. The biopac data acquisition software was used to record the left ventricular systolic pressure (10). The effect of various receptor-dependent (acetylcholine, angiotensin II and adrenaline) and independent (calcium chloride and sodium nitroprusside) agonists in absence and presence of RGS2 agonist on LVSP were evaluated. The results of study showed that RGS2 agonist (100 µM) significantly attenuated the adrenaline and angiotensin II induced increase in LVSP in isolated heart. However, the effect calcium chloride did not significantly alter by RGS2 agonist. The effects of acetylcholine and sodium nitroprusside were significantly increased by RGS2 agonist in the isolated heart. Ultimately, the RGS2 agonist modulated only the effect of receptor-dependent agonists in the isolated heart.

3.2. Isolated rat aorta preparation:
Thoracic aorta was isolated and spirally cut strip (3-5mm width, 20-30mm length) was prepared. The strip was mounted in 35-ml organ tube containing Krebs-Henseleit buffer maintained at 37°C and oxygenated with 95% O2, CO2 mixture. The preparations were suspended under 1 g resting tension which was determined in the baseline studies and equilibrated for 60 min, with changes of bathing fluid every 15 min. Isometric tension studies were performed using Iworx data acquisition system. The effect of various receptor-dependent (acetylcholine, angiotensin II and adrenaline) and independent (calcium chloride and sodium nitroprusside) agonists in absence and presence of RGS2 agonist were evaluated on the isolated aorta. RGS2 agonist (100 µM) significantly attenuated the adrenaline and angiotensin II mediated contractile response on the aorta. However, effects calcium chloride and sodium nitroprusside did not significantly alter by RGS2 agonist. The effect of acetylcholine was significantly increased by RGS2 agonist on the isolated aorta. Ultimately,
the RGS2 agonist modulated only the effect of receptor-dependent agonists in the isolated aorta.

4. Total antioxidant activity assays:
The following assays were performed to evaluate the *in-vitro* total antioxidant activity of RGS2 agonist.

1. Diphenylpicrylhydrazyl (DPPH) assay
2. Azobis-ethylbenzthiazoline sulfonic acid (ABTS) assay
3. Superoxide scavenging assay
4. Iron chelating assay
5. Ferric reducing anti-oxidant power (FRAP) assay
6. Nitrite scavenging assay

RGS2 agonist showed an anti-oxidant activity against organic free radical, superoxide radical, cationic radical, peroxyl radical, ferric reduction and chelation capacity that is comparable to trolox. This anti-oxidant activity may be due to electron, nitric oxide donating and free radical capturing properties in lipophilic-hydrophilic environment.

5. Acute and sub-acute toxicity study:

5.1 Acute toxicity study:
Acute toxicity study of chemical moiety (RGS2 agonist) was performed as per the OECD guideline 425. A total three male and female albino mice (25-30g) were selected per group. RGS2 agonist at doses of 5, 50, 300 and 2000 mg/kg was orally administered in respective groups and 1% DMSO was orally administered in vehicle control group. All animals were observed for clinical signs (time of toxic symptom onset and recovery) and mortality at 30 min, 1, 2, 4, and 6 hrs after dosing and twice a day thereafter for the 14-day experimental period. The treatment of RGS2 agonist in mice up to the dose 2000 mg/kg of did not show the mortality in 24 hrs and within 14 days. Other than this, RGS2 agonist treatment in mice up to 2000 mg/kg did not show the alteration in muscle activity (Locomotion, muscle coordination, catatonia, convulsive episode), reflex activity (Visual place response, writhing response, Tail pinch response, piloerection) and secretory activity (Lacrimation, salivation, sniffing and defecation). These data suggested that single dose administration of RGS2 agonist up to 2000 mg/kg is safe in the mice.

5.2 Sub-acute toxicity study:
Rats were randomly divided into four groups; normal, vehicle control (1% DMSO treated), RGS2 agonist treated (10 mg/kg) and RGS2 agonist treated (100 mg/kg). Each group contained six animals. The dose levels were selected based on the result of efficacy study. Safety profile of RGS2 agonist was assessed at effective dose (10 mg/kg) and 10 times higher dose (100 mg/kg). The treatment of RGS2 agonist 10 mg/kg/d, 100 mg/kg/d and vehicle (1% DMSO) 1 ml/kg/d were given in respective groups for 28 days. All the animals were observed once daily for clinical signs, mortality and morbidity throughout the study. Body weights of rats were measured on day 1 and then once a weekly during study. At the end of study, estimation of hematological parameters, various organ damage markers and histological examination of essential organs were performed. The repeated administration of RGS2 agonist at the dose of 10 mg/kg and 100 mg/kg did not show the significant alteration in hematological parameters (RBC, WBC and Platelets count), cardiac damage markers (CK-MD, LDH), metabolic parameters (glucose, cholesterol, triglyceride), kidney damage markers (urea, creatinine) and liver damage markers (ALT, AST) as compared to control rats. Histological examination of various organs did not show any significant damage in RGS2 agonist treated rats (10 and 100 mg/kg) as compared to control rats.

6. Effect of RGS2 agonist on aminophylline induced cardiac arrhythmia:
Rats were divided into four groups; normal, disease control, RGS2 agonist treated (10 mg/kg) and RGS2 agonist treated (100 mg/kg). Arrhythmia was induced by treatment of aminophylline (100 mg/kg/d, i.p.) for seven days. Treatments of RGS2 agonist (1 and 10 mg/kg/d, p.o.) were started with initiation of aminophylline treatment in respective groups for seven days. RGS2 agonist was administered one hour before the administration of aminophylline. At the end of study, cardiac parameters such as PR interval, QRS complex, QT interval were measured from electrocardiogram (ECG). Cardiac damage markers (CK-MB and LDH) were measured in serum using spectrophotometer based kits. The endogenous antioxidant enzymes and cAMP level were measured in the cardiac homogenate. Arterial blood pressure was measured by invasive method using biopac data acquisition system. The results of study showed that treatment of RGS2 agonist (10 mg/kg) significantly decreased the aminophylline induced increase in the HR as compared to untreated rats. The PR interval did not significantly alter in the aminophylline treated rats as compared to NC. The treatment of RGS2 agonist (1 and 10 mg/kg) significantly decreased the elevation in the QRS complex while QT interval prolongation and RR interval significantly reduced by RGS2 agonist at 10 mg/kg dose as compared to untreated rats. The treatment of RGS2 agonist (1 and 10 mg/kg)
significantly decreased the SBP and DBP in the aminophylline treated rats as compared to untreated rats. The treatment of RGS2 agonist (1 and 10 mg/kg) significantly decreased the serum CK-MB and LDH level in the aminophylline treated rats as compared to untreated rats. The antioxidant enzymes significantly decreased in the cardiac tissue of aminophylline treated rats as compared to NC. The treatment of RGS2 agonist (10 mg/kg) significantly increased the SOD, catalase, glutathione antioxidant enzymes as compared to untreated rats. The cAMP level significantly increased in the homogenate of cardiac tissue in the aminophylline treated rats as compared to NC. The treatment of RGS2 agonist (10 mg/kg) significantly decreased the cAMP level as compared to untreated rats.

7. Effect of RGS2 agonist on doxorubicin induced myocardial infarction:

The rats were divided into three groups; normal, disease control and RGS2 agonist treated (10 mg/kg/d, p.o.). Myocardial infarction in rats was induced by single bolus injection of doxorubicin (20 mg/kg, s.c.) in all the groups except normal rats. Treatment of RGS2 agonist (10 mg/kg) and vehicle (1% DMSO) was given for 10 days in DR and DC groups respectively. An injection of doxorubicin was given on 10th day of study in all the groups except normal rats. At the end of study, ECG and various biochemical parameters such as cardiac damage markers (CK-MB, LDH), kidney damage markers (urea, creatinine) nitrite level were measured in the serum of all the rats. The western blot analysis of RGS2 protein, cAMP, cGMP level and endogenous antioxidant status (superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH)) in cardiac tissue were measured. Histological examination of left ventricular cardiac tissue was performed. The treatment of RGS2 agonist significantly decreased the serum cardiac damage markers (CK-MB, LDH) in the doxorubicin induced myocardial infarcted rats as compared to control rats. The treatment of RGS2 agonist significantly reversed the increase in heart rate, QRS prolongation and QT interval prolongation in the doxorubicin induced myocardial infarcted rats as compared to control rats. The treatment of RGS2 agonist significantly improved the endogenous antioxidant status (SOD, Catalase and Glutathione) in cardiac tissue of doxorubicin induced myocardial infarcted rats as compared to control rats. The RGS2 agonist treatment significantly increased the serum nitrite level in the serum of doxorubicin induced myocardial infarcted rats as compared to control rats. The treatment of RGS2 agonist significantly increased the cGMP level in the cardiac tissue of doxorubicin induced myocardial infarcted rats as compared to control rats.
8. Effect of RGS2 agonist on isoproterenol induced myocardial infarction:
The rats were divided into three groups; normal (NC), disease control (SC) and RGS2 agonist treated (SR, 10 mg/kg/d, p.o.). Myocardial infarction in rats was induced by administration of single bolus injection of isoproterenol (85 mg/kg, s.c.) for two consecutive days in all the groups except normal rats. Treatment of RGS2 agonist (10 mg/kg) and vehicle (1% DMSO) was given for 10 days in SR and SC groups respectively. An injection of isoproterenol was given on 10th and 11th days of study in all the groups except normal rats. At the end of study, ECG and various biochemical parameters such as cardiac damage markers (CK-MB, LDH), kidney damage markers (urea, creatinine) nitrite level were measured in the serum of all the rats. The western blot analysis of RGS2 protein, cAMP, cGMP level and endogenous antioxidant status (superoxide dismutase (SOD), Catalase (CAT) and reduced glutathione (GSH)) in cardiac tissue were measured. Histological examination of left ventricular cardiac tissue was performed. The treatment of RGS2 agonist significantly decreased the serum cardiac damage markers (CK-MB, LDH) in the isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly reversed the increase in heart rate, QRS prolongation and QT interval prolongation in the isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly improved the endogenous antioxidant status (SOD, Catalase, and Glutathione) in cardiac tissue of isoproterenol induced myocardial infarcted rats as compared to untreated rats. The RGS2 agonist treatment significantly increased the serum nitrite level in the serum of isoproterenol induced myocardial infarcted rats as compared to untreated rats. The treatment of RGS2 agonist significantly decreased the cAMP level in the cardiac tissue of isoproterenol induced myocardial infarcted rats as compared to untreated rats.

Achievements with respect to objectives:

1. The current study identified the binding affinity and site of ligand on the RGS2, RGS4 and RGS5 proteins.
2. Established the effect of RGS2 agonist on action of receptor-dependent and – independent mediators.
3. Preclinical acute and sub-acute safety profile of RGS2 agonist
4. Therapeutic potential of RGS2 agonist in the cardiac arrhythmia and myocardial infarction.

Conclusion:
The small compound has more binding affinity towards the RGS2 as compared to RGS4 and RGS5. It showed the binding affinity on two amino acids; TRYPTOPHAN 113 and LYSINE 120 of RGS2 protein by forming hydrogen bond with help first nitrogen of triazolone ring and oxygen of hydroxyl group present on secondary ring. The RGS2 agonist attenuated the activity of receptor-dependent mediators (angiotensin II and adrenaline) while potentiated the activity of acetylcholine in isolated heart and aorta. Further, RGS2 agonist showed the protective action against the aminophylline induced cardiac arrhythmia and doxorubicin as well as isoproterenol induced myocardial infarction. This protective action of RGS2 agonist might be due to modulation of RGS2, cAMP and cGMP level in the cardiac tissue and antioxidant activity of compound.

Publications:
References:


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