Pharmacological Evaluation of Dopamine containing plants in obesity

A Synopsis Submitted to

Gujarat Technological University

Ahmedabad

For The Award

Of

Doctor of Philosophy

In

Pharmacy (Pharmacology)

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June 2017

Title: Pharmacological evaluation of Dopamine containing plants in obesity.

ABSTRACT

Objective: To evaluate anti-obesity effect of Dopamine containing plants in experimentally induced obesity in rats.

Methods: Male Sprague Dawley (SD) rats were subjected to high fat diet (HFD) for 12 weeks. L-DOPA (12.5 mg/kg, p. o.) as standard drug and aqueous extract of *Mucuna pruriens* seeds (AEMP 200 mg/kg, p. o. and 400 mg/kg, p. o.), aqueous extract of *Vicia faba* seeds *(* AEVF 300 mg/kg, p. o. and 600 mg/kg, p. o.), and aqueous extract of *Bauhinia purpurea* seeds (AEBP 300 mg/kg, p. o. and 600 mg/kg, p. o.) as test drugs were administered in last 4 weeks along with HFD. Body weight, food intake, body mass index (BMI), serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) levels were measured at the end of fourth , eighth and twelfth weeks, while white adipose tissue (WAT) mass and brain dopamine levels were measured at the end of the twelfth week.

Results: All treated groups with test drugs showed a significant decrease in food intake and weight gain without altering BMI. Moreover TG levels were lower in treated groups as compared to the HFD group, but no significant changes were observed in TC and HDL levels. L-DOPA treated group showed a significant decrease in body weight, food intake, BMI and WAT. All three plants extracts and L-DOPA treated groups showed an increase in brain dopamine levels as compared to disease control group ($p<0.05$).

Conclusion:L-DOPA and all test compounds showed anti-obesity activity by reducing body weight gain, food intake and WAT weights. This accompanied by modulation in TG levels and increased brain dopamine levels correlates to the inhibitory action of dopamine on reward mechanism.

Keywords :*Mucuna pruriens*, *Vicia faba, Bauhinia purpurea*, Dopamine.

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

Obesity is a medical condition in which life is hindered by excess body fat. The generally accepted benchmark of obesity is the BMI. The world health organization (WHO) classifies population with a BMI of <18.5 kg/m^2 as underweight, and those with a BMI of 18.5-24.9 kg/m^2 as normal weight. A BMI in the range of 25.0-29.9 $kg/m^2$is considered as grade 1 overweight. If it is between 30.0-39.9 kg/m^2, the patient is said to be obese or grade 2 overweight, while those with a BMI of 40 kg/m^2 are deemed to be grade 3 overweight or morbidly obese. The prevalence of obesity is increasing not only in adults, but also among children and adolescents. The prevalence of obesity has increased steadily over the past five decades, and may have a significant impact on the quality adjusted life years [1]. The causes of obesity may include dietary, exercise, social, cultural and financial factors. Sibutramine was widely marketed and prescribed until 2010, when it was withdrawn after a large study showed that it increased the risk of cardiovascular events and strokes and had minimal efficacy. An endocannabinoid receptor antagonist, rimonabant was withdrawn from the market due to concerns about its safety, including risk of seizures and suicidal tendencies. At present only one drug, Orlistat has been approved for long-term use in the treatment of obesity. Orlistat promotes 5 to 10% loss of body weight and has its own limitations and side effects. This currently licensed drug is best, when used in combination with diet, exercise, and behaviour change regimens. However, it does not cure obesity and weight rebounds when discontinued. Some drugs employed to treat clinical obesity are associated with adverse effects such as nausea, insomnia, constipation, gastrointestinal problems, and potential adverse cardiovascular effects. Thus, there is a great demand for the search of new and safer anti-obesity medicines [2].

In a brain, nucleus accumbens is an important component of reward circuitry [3] and the dopaminergic system is integral to reward-induced feeding behaviour [4].The influence of central dopamine signalling on feeding is thought to be mediated by the D2 receptors [5].Drugs that block dopamine D2 receptor increase appetite whereas drugs that increase brain dopamine concentration are anorexigenic [6]. This proves that there is an important role of dopamine in weight management.

Mucuna pruriens(L.) DC. (Leguminosae) known as ‘‘velvet bean” and ‘‘atmagupta’’ is a climbing legume, endemic in India and in other parts of the tropics including Central and South America. *Mucuna pruriens* seed powder contains a large amount of L-DOPA (1.5%), which is a dopamine precursor and effective remedy for the relief in parkinson’s disease [7]. *Mucuna pruriens* seeds in addition to L-DOPA contain 5-hydroxytryptamine (5-HT), tryptamine, mucunine and mucunadine. Ethanolic extract of *Mucuna pruriens* shows protection against haloperidol induced tardive dyskinesia in rats [8]. *Mucuna pruriens* has been reported to inhibit chlorpromazine-induced hyperprolactinaemia in man [9]. *Mucuna pruriens* has proven to be more effective than L-DOPA in parkinson’s disease in animal model [10].

Vicia faba (Leguminosae) known as ‘‘broad bean” is widely grown and consumed, especially in China, North African countries and parts of Europe and North and South America, and is served in a large variety of forms, mostly based on the immature or mature seed.[11] *Vicia faba* seed powder contains a large amount of L-DOPA (0.75%)[12], which is a dopamine precursor and effective remedy for the relief in parkinson’s disease.[13] *Vicia faba* seeds in addition to L-DOPA contain crude protein (27.5%), vicine and vicicine.[14].Parkinson’s disease patients showed a significant improvement in their motor features after eating *Vicia faba* which was similar to the improvement measured after receiving 125 mg of levodopa plus 12.5 mg of carbidopa. [15]. L-dopa of *V. faba* prolongs “On” period in patients with Parkinson’s disease who have ‘on-off’ effect fluctuation.[16].

Bauhinia purpurea is a shrub or small tree of Fabaceae family. It is found in most types of vegetation ranging from evergreen lowlands, rain forests to mountain forests, up to 2000-3000 m altitude and also in savanna, scrub and dry deciduous forests to swamp forests on various soils. *B. purpurea* is good source of L-DOPA (1.34 %) [17]. The alcoholic seed extracts shows anti-parkinson’s activity [18]. The anti-obesity effect of bark extract is also reported [19].

In light of above observations, current investigation was carried out to study the effect of dopamine containing plant *Mucuna pruriens (L.) DC., V. faba and B. purpurea* in HFD induced obesity on rats.

Definition of the Problem

Several neurotransmitters (dopamine, norepinephrine and serotonin) as well as peptides and hormones like ghrelin are involved in the regulation of food intake [20, 21]. Of particular interest is dopamine, since this neurotransmitter seems to regulate food intake [22] by modulating food reward via the meso-limbic circuitry of the brain [23]. In fact, drugs that block dopamine D2 receptors increase appetite and result in significant weight gain [24, 25] whereas drugs that increase brain dopamine are anorexigenic [26, 27]. Additionally, an increase in body weight is a side effect of many commonly used drugs. Particularly, anti-dopaminergically acting neuroleptics, tricyclic antidepressants, lithium, and some anticonvulsants contribute to weight gain. Similarly, in obesity body mass index is negatively correlated with D2 receptor density in the striatum [28, 29], which might reflect neuroadaptation secondary to over stimulation with palatable food [30, 31]. Thus, increased food intake may be a compensatory behaviour for low dopaminergic drive [32, 33]. Recently it is reported that lower striatal activation in response to food intake was associated with obesity. Furthermore, this relation was modulated by genetically determined D2 receptor availability [34-36].

Plants have been the basis for traditional medicine systems. Numerous preclinical and clinical studies, with various herbal medicines have reported significant improvement in controlling body weight, without any noticeable adverse effects.

In light of above observations, current investigation was carried out to study the effect of dopamine containing plants in HFD induced obesity on rats.

Objectives

1. Evaluation of anti-obesity effect of aqueous extract of *Mucunapruriens* seeds on rats.
2. Evaluation of anti-obesity effect of aqueous extract of *Viciafaba* seeds on rats.
3. Evaluation of anti-obesity effect of aqueous extract of *Bauhinia purpurea* seeds on rats.

Scope of study

Plants have been the basis for traditional medicine systems. Numerous preclinical and clinical studies, with various herbal medicines have reported significant improvement in controlling body weight, without any noticeable adverse effects. The research involves evaluation of ability of the drug to reduce weight gain and food intake and its effect on various parameters

like TG, white adipose tissue and brain dopamine level. The plant extractswas evaluated for presence of L-DOPA.

Original contribution by the thesis

The effect of dopamine containing plants have not yet been evaluated for their effect on obesity, thusthis thesis will provide a scientific basis for studying the role of dopamine in food intake and obesity and for the development of effective treatment for obesity.

Methodology

Preparation of extracts

Seeds of *Mucuna pruriens (L.) DC., V. faba and B. purpurea* were purchased from the authorised dealer and authentication was done by Botany department of MS University, Vadodara, India. For the extraction, freshly collected seeds were dried in shade and pulverized to get a coarse powder. One kg seed powder was initially defatted with 750 ml of petroleum ether and then aqueous extract was prepared by cold maceration method. After 24 h, the extract was filtered using whatman filter paper (No. 1) and then concentrated under reduced pressure (bath temp. 50 °C) and finally dried in a vacuum desiccator [37].

HPTLC analysis of extracts was performed using following parameters

- Stationary phase: Merck Silica gel 60F254 TLC precoated aluminum plates.
- Sample Volume: 20µl freshly prepared
- Mobile phase: n-butanol: acetic acid: water (4:1:1, v/v/v)

Experimental animals

Male SD rats weighing 250-300 g were used in the study. The animals were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22±2 °C), humidity (55±5%) and light-dark cycles (12:12h). They were maintained under standard environmental conditions and were fed a standard rat chow diet with water given ad libitum. The study was approved by Institutional Animal Ethical Committee, Parul institute of pharmacy, Parul University, Vadodara, Gujarat, India (PIPH 19/12, PIPH 12/14, PIPH 01/15).

Experimental Design

Male SD rats were acclimatized for 1 week. Obesity in the rats was induced by giving a HFD for 8weeks and then continuing it for12 weeks. The composition of HFD is mentioned in table 1 [38, 39]. The rats were divided into nine groups consisting of six rats in each as follows:

Group I: Normal protein diet (NPD)

Group II: HFD

Group III: HFD + L-DOPA (12.5 mg/kg, p. o.)

Group IV: HFD + AEMP (200 mg/kg, p. o.)

Group V: HFD + AEMP (400 mg/kg, p. o.)

Group VI: HFD + AEVF (300 mg/kg, p. o.)

Group VII: HFD + AEVF (600 mg/kg, p. o.)

Group VIII: HFD + AEBP (300 mg/kg, p. o.)

Group IX: HFD + AEBP (600 mg/kg, p. o.)

Group I was fed NPD, while groups II to IX were fed HFD for 12 weeks, that is throughout the study. At the end of 8 weeks, groups II to IX were treated with test extract or standard drug for 4 weeks along with HFD. Body weight, food intake, BMI and serum TC, TG and HDL levels were measured at the end of 4, 8 and 12 weeks. The epididymal WAT mass [40] and brain dopamine levels were measured at the end of 12 weeks[41, 42].

Table 1: Composition of HFD

Ingredients	Weight (g)	Energy (Kcal)
Powdered NPD	50	0.21
Animal fat	20	0.18
Sucrose	29.8	0.11
Sodium chloride	0.2	-
	100 g	0.5 Kcal/100 g

Fat pad analysis

At the end of the 12 weeks, animals were decapitated between 09:00 and 12:00 h. After sacrificing by decapitation, the epididymal WAT was dissected out. The collected fat was weighed immediately and compared with the other groups.

Collection of blood samples and Estimation of TC, TG and HDL levels

At the end of fourth, eighth and twelfth weeks, blood was collected under inhalation anaesthesia by retro-orbital puncture from overnight fasted animals. Blood was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 4000-5000 rpm for 15 min and analysed for serum TC, TG and HDL levels. TC, TG and HDL were estimated by using a Bayer diagnostic kit (Bayer Diagnostic India Ltd.)

Brain dopamine levels

Preparation of tissue extract

On the last day of experiment, rats were sacrificed and whole brain was dissected out, weighed and was homogenized in 3 ml HCl butanol in a cool environment. The sample was subsequently centrifuged for 10 min at 2000 rpm. 0.8 ml of the supernatant phase was removed and added to an eppendorf reagent tube containing 2 ml of heptane and 0.25 ml 0.1 M HCl. After 10 min, the tube was shaken and centrifuged under the same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay.

Dopamine assay

0.02 ml of the HCL phase+0.005 ml 0.4 M HCL+0.01 ml EDTA

↓

1.01 ml iodine solution was added for oxidation

↓

After 2 min, 1 ml sodium thiosulphate in 5 M sodium hydroxide was added to stop the reaction

↓

10 M acetic acid was added 1.5 min later

↓

Solution was then heated to 100 ^{o}C for 6 min

↓

Excitation and emission spectra were read (330 to 375 nm) in a spectrofluorophotometer(Shimadzu RF-5301 PC) when the samples again reached room temperature.

Tissue values (fluorescence of tissue extract minus fluorescence of tissue blank) were compared with an internal reagent standard (fluorescence of internal reagent standard minus fluorescence of internal reagent blank).Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reversed order (sodium thiosulphate before iodine). Internal reagent standards were obtained by adding 0.005 ml bidistilled water and 0.1 ml HClbutanol to 20 ng of dopamine standard.

Evaluation of anti-obesity effect using MSG induced obesity model

Female wistar rats weighing 150-200 g were used in the study. The animals were housed in a group of 6 rats per cage under well-controlled conditions of temperature (22±2 °C), humidity (55±5%) and light-dark cycles (12:12h). They were maintained under standard environmental conditions and were fed a standard rat chow diet with water given ad libitum. The study was approved by Institutional Animal Ethical Committee, Parul institute of pharmacy, Parul University, Vadodara, Gujarat, India (PIPH 02/15).

Experimental Design

Female Wistar rats were acclimatized for 1 week. Obesity in the rats was induced by administration of Monosodium glutamate (MSG) 5 mg/g body weight for 15 days. The rats were divided into nine groups consisting of six rats in each as follows:

Group I: Normal

Group II: Disease control (MSG 5 mg/kg body weight).)

Group III: MSG + L-DOPA (12.5 mg/kg, p. o.)

Group IV: MSG + AEMP (200 mg/kg, p. o.)

Group V: MSG + AEMP (400 mg/kg, p. o.)

Group VI: MSG + AEVF (300 mg/kg, p. o.)

Group VII: MSG + AEVF (600 mg/kg, p. o.)

Group VIII: MSG + AEBP (300 mg/kg, p. o.)

Group IX: MSG + AEBP (600 mg/kg, p. o.)

Changes in body weight and food intake was recorded for all test groups and compared with the disease control group.

Phytochemical evaluation

HPTLC of extracts

Optimization of mobile phase was done using various mobile phases to get optimum separation of bands. The Mobile phase n-butanol: acetic acid: water (4:1:1, v/v/v) was used for present study. Chromatography was performed on Merck Silica gel 60F254 TLC precoated aluminium plates. 20 μ l of freshly prepared samples were applied on the plate as a band of 10 mm width with the help of LINOMAT V^{R} Automatic sample spotter at the distance of 10 mm from edge of the plate. The plate was developed to a distance 80 mm in a CAMAG twin trough chamber (10*10 cm) previously equilibrated with mobile phase for 20 minutes. After development, densitometric evaluation of plate was performed at 254 nm in absorption mode using TLC scanner 3 linked to WinCats Software (CAMAG).

Statistical Analysis

All data were presented as mean±standard error of means (SEM). One-way analysis of variance (ANOVA) followed by Tukey's test was used for statistical analysis to compare more than two groups, while two way ANOVA were used to compare values of different time period of the same group. P values of less than 0.05 were considered significant. Regression analysis was also performed to check correlation between brain dopamine levels and weight loss between the groups.

RESULTS

Body weight

Body weight was measured every week till twelve weeks. The body weights of all groups (II, III, IV & V) were significantly increased compared to the control group (group I) for first 8

weeks. After 12 weeks the L-DOPA and AEMP (400 mg/kg) groups had significantly ($p<0.05$) lower mean body weights than HFD group. The mean body weight in the HFD group increased by 46.67 g after 12 weeks of experimental period, whereas L-DOPA and AEMP (400 mg/kg) group lost 23.3 g and 30.0 g respectively. In normal and AEMP (200 mg/kg) treated groups mean body weight gain was found to be 9.66 g and 6.66 g respectively.The AEVF (600 mg/kg) group lost 23.03 g body weight. In AEVF (300 mg/kg) treated groups mean body weight gain was found to be 16.67 g. The AEBP 300 mg/kg & 600 mg/kg treated groups lost 6.67 g and 30.0 g respectively.

Food intake

There was a significant increase in food intake among the HFD treated rats as compared to the normal diet-fed rats up to 8 weeks. The rats treated with all extracts showed a significant decrease in food intake as compared to the HFD group. L-DOPA treated group also exhibited a significant reduction in food intake as compared to the HFD group.

Body mass index

HFD treated rats showed significant increase in BMI as compared to control group. L-DOPA treated group showed significant lower BMI whereas AEMP (200 mg/kg and 400 mg/kg) treated group showed no significant change in the BMI. Also, AEVF (300 mg/kg and 600 mg/kg) and AEBP (300 mg/kg and 600 mg/kg) treated groups showed no significant change in the BMI. However, all the plantextracts (AEMP, AEVF and AEBP) treated groups showed preventive effect against increase in BMI with HFD.

White adipose tissue

Feeding a high-fat diet for 12 weeks produced a significant ($p<0.05$) increase in epididymal WAT weight of HFD treated group as compared to normal diet fed rats. There was a significant reduction in the epididymal fat mass in the L-DOPA , AEMP (400 mg/kg) , AEVF (600 mg/kg) and AEBP (600 mg/kg) treated groups while no significant alteration were observed in AEMP 200 mg/kg, AEVF 300 mg/kg and AEBP 300 mg/kg groups as compared to HFD.

Serum lipid profile

Feeding of HFD caused a significant ($p<0.05$) increase in serum levels of TG as compared to normal diet fed rats. All treated groups showed significantly lower serum TG levels than in

HFD group. However, no alteration was found in TC and HDL levels by treatment with either drug.

Brain dopamine levels

Brain dopamine levels of HFD group weresignificantly reduced compared with the control group. However, groups administered with L–DOPA and all plant extracts showed significant increase in brain dopamine levels as compared to the HFD group.

Regression analysis

The F calculated value (5.41) between the groups was found to be greater than F tabulated value (2.51) which correlates significant effectiveness between brain dopamine and body weight loss. Also, P value was less than 0.05.

Result of MSG induced obesity

No significant body weight gain was observed by administration of MSG for 15 days in disease control group. Hence the anti-obesity effect of test and standard drugs couldn't be evaluated in MSG induced obesity model. However, further studies are being carried out to evaluate the anti-obesity effect in MSG induced obesity.

Achievement of objectives

1. The aqueous extracts of all plant seeds were found to exert antiobesity effect on High fat diet induced obesity model.
2. In addition to this, they also had favourable effect on other factors involved in progression of obesity like reduction of food intake, TG levels and white adipose tissue mass.
3. All the extracts increased the brain dopamine levels.
4. Significant correlation was observed between brain dopamine levels and prevention of weight gain by HFD.
5. L-DOPA was found to be the major constituent of plant seeds selected for the study.

Conclusion

The present study revealed that all plant seeds had weight lowering effect. The brain dopamine assay indicated that the presence of L-DOPA (dopamine precursor) in the seed extracts might be the constituent responsible for these activities.

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