EVALUATION OF ANTI-OBESEITY EFFECT OF AQUEOUS EXTRACT OF MUCUNA PRURIENS SEEDS ON RATS

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ABSTRACT

Objective: Evaluation of the anti-obesity effect of aqueous extract of Mucuna pruriens seeds on rats.

Methods: Male Sprague-Dawley (SD) rats were subjected to high-fat diet (HFD) for 12 wk. L-DOPA (12.5 mg/kg, p. o.) as standard drug and aqueous extract of Mucuna pruriens (AEMP) seeds (200 mg/kg, p. o. and 400 mg/kg, p. o.) as test drugs were administered in last 4 wk 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 wk, while white adipose tissue (WAT) mass and brain dopamine levels were measured at the end of the twelfth wk.

Results: AEMP (200 mg/kg, p. o.) and (400 mg/kg, p. o.) treated groups 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. Both AEMP 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 AEMP showed anti-obesity activity by reducing body weight gains, food intake and WAT weights; modulating TG with increased brain dopamine level which correlates to the inhibitory action of dopamine on reward mechanism.

Keywords: Mucuna pruriens, Dopamine, High fat diet, Body weight, Food intake

INTRODUCTION

Obesity is a medical condition in which life is hindered by excess body fat. The generally accepted benchmark is the BMI. The world health organisation (WHO) classifies population with a BMI of<18.5 kg/m² as underweight, and those with a BMI of 18.5-24.9 kg/m² as normal weight. A BMI in the range of 25.0-29.9 kg/m² as grade 1 overweight. If it is between 30.0-39.9 kg/m², the patient is said to be obese or grade 2 overweight, while those with a BMI of 40 kg/m² and above are termed grade 3 obesity 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 the 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 their own limitations and side effects. This currently licensed drug is best when used in combination with diet, exercise, and behaviour change regimens. However, they do not cure obesity and weight rebound when discontinued. Some drugs are employed to treat clinical obesity is 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].

Several neurotransmitters (dopamine, norepinephrine and serotonin), as well as peptides and hormones like ghrelin, are involved in the regulation of food intake [3, 4]. Of particular interest is dopamine, since this neurotransmitter seems to regulate food intake [5] by modulating food reward via the meso-limbic circuitry of the brain [6]. In fact, drugs that block dopamine D₂ receptors increase appetite and result in significant weight gain [7, 8] whereas drugs that increase brain dopamine are anorexigenic [9-11]. 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 the obesity body mass index is negatively correlated with D₂ receptor density in the striatum [12, 13], which might reflect neuroadaptation secondary to over stimulation with palatable food [14, 15]. Thus, increased food intake may be a compensatory behaviour for low dopaminergic drive [16, 17]. 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 [18, 19].

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.

Mucuna pruriens Linn. 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. In the Ayurvedic system of medicine, Mucuna pruriens was used for the management of male infertility [20], nervous disorders [21] and also as an aphrodisiac [22]. 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 [23]. Mucuna pruriens seeds in addition to L-DOPA contain 5-hydroxytryptamine (5-HT), tryptamine, mucumin and mucunadine. Ethanolic extract of Mucuna pruriens shows protection against haloperidol-induced tardive dyskinesia in rats [24]. Mucuna pruriens has been reported to inhibit chlorpromazine-induced hyperprolactinemia in man [25]. Mucuna pruriens has proven to be more effective than L-DOPA in parkinson’s disease in an animal model [26]. It also shows anti-diabetic [27], anticancer [28], anti-oxidant [29], anti-hyperlipidemic...
and protective effect against nephrotoxicity [31]. These reports led us to hypothesise that high content of dopamine in 
Mucuna pruriens seed could serve as a potent therapeutic agent for the obesity. However, their efficacy needs to be scientifically evaluated in vivo experiments. In light of above observations, current investigation was carried out to study the effect of dopamine-containing plant Mucuna pruriens (L) DC. var. utilis in HFD induced obesity on rats.

**MATERIALS AND METHODS**

**Chemicals and reagents**
L-DOPA was obtained from Torrent Research Centre, Bhat, India; all other reagents used in this study were of analytical grade.

**Preparation of extracts**

Seeds of Mucuna pruriens (L) DC. var. utilis were purchased from the authorised dealer and authenticated by National Institute of Science Communication and Information Resources (voucher specimen no.: NISCAIR/RHMD/consult/2016/3001-28-1), India. For the extraction, freshly collected seeds of Mucuna pruriens were dried in the shade and pulverized to get a coarse powder. One kg seed powder of Mucuna pruriens 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 [31].

**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 (PIPH19/12).

**Experimental design**
Male SD rats were acclimatised for 1 wk. Obesity in the rat was induced by a HFD. The composition of HFD is mentioned in table 1 [32, 33]. The rats were divided into five groups consisting of six rats in each as follows:

| Group   | Diet Description |
|---------|------------------|
| I       | Normal protein diet (NPD) |
| II      | HFD              |
| III     | HFD+AEMP (200 mg/kg, p. o.) |
| IV      | HFD+AEMP (400 mg/kg, p. o.) |
| V       | HFD+L-DOPA (12.5 mg/kg, p. o.) |

Group I was fed NPD, while groups II, III, IV and V were fed HFD for 12 wk that is throughout the study. At the end of 8 wk, group III, IV and V were treated with test extract or standard drug for 4 wk 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 wk. The epididymal WAT mass and brain dopamine level were measured at the end of 12 wk.

**Collection of blood samples**
At the end of fourth, eighth and twelfth wk, blood was collected under inhalation anaesthesia by a 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.

**Estimation of TC, TG and HDL levels**
TC, TG and HDL were estimated by using a Bayer diagnostic kit (Bayer Diagnostic India Ltd.)

**Fat pad analysis**
At the end of the 12 wk, 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 [34].

**Brain dopamine levels**

**Preparation of tissue extract**
On the last day of the experiment, rats were sacrificed, and the whole brain was dissected out, weighed and was homogenised 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

↓

0.01 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 °C for 6 min

↓

Excitation and emission spectra were reed (330 to 375 nm) in a spectrophotometer

[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 HCl butanol to 20 ng of dopamine standard [35, 36].

**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 was used to compare values of the different time period of the same group. P values of less than 0.05 were considered significant.

**RESULTS**

**Body weight**
Body weight was measured every wk till twelve wk. The body weights of all groups (II, III, IV and V) were significantly increased.
compared to the control group (group I) for first 8 wk. After 12 wk 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 wk of the experimental period, whereas L-DOPA and AEMP (400 mg/kg) group lost 23 g and 30 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 (table 2).

### Table 2: Body weights at different time interval

|                | Normal       | HFD          | L-DOPA       | AEMP (200 mg/kg) | AEMP (400 mg/kg) |
|----------------|--------------|--------------|--------------|------------------|------------------|
| Body weight    | Initial      | 321.67±11.67 | 268.34±14.24 | 270.00±15.00     | 303.34±3.33      |
|                | 8th wk       | 369.66±15.27 | 420.00±35.11 | 390.00±30.00     | 460.00±25.16     |
|                |              | (152.3)*     | (120.67)*     | (147.3)*         | (140.0)*         |
|                | 12th wk      | 379.33±14.53 | 466.67±38.44 | 366.66±33.33     | 466.67±33.33     |
|                |              | (9.66)       | (46.67)*      | -                | (6.66)           |
|                |              |              |              |                  | (30.00)*         |

Values are expressed as means±SEM, n=6 in each group. Values are significantly different (p<0.05) by one way ANOVA followed by Tukey’s test. *g of body weight gained during the first 8 wk of the experimental period, +g of body weight gained altered after 12 wk experimental period.

### Food intake

There was a significant increase in food intake per wk among the HFD treated rats as compared to the normal diet-fed rats up to 8 wk. The rats treated with AEMP (200 mg/kg, p.o. and 400 mg/kg, p.o.) 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 (fig. 1).

![Fig. 1: Effect of L-DOPA and AEMP on food intake](image)

### Body mass index

HFD treated rats showed a significant increase in BMI every wk till 12 wk 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. AEMP treated groups showed preventive effect (fig. 2).

![Fig. 2: Effect of L-DOPA and AEMP on BMI](image)
Serum lipid profile

Feeding of HFD caused a significant (p<0.05) increase in serum levels of TC as compared to normal diet fed rats. L-DOPA and AEMP (200 mg/kg and 400 mg/kg) 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 (Table 3).

Table 3: Serum levels of TG, TC and HDL of rat (after the 12 wk experimental period)

| Serum level (mg/dl) | Groups                      | HFD             | L-DOPA | AEMP (200 mg/kg) | AEMP (400 mg/kg) |
|---------------------|-----------------------------|-----------------|--------|-----------------|-----------------|
| TG                  | Normal                      | 75.33±7.86      | 118.3±4.41* | 95.3±4.84*      | 94.3±2.196*     |
| TC                  | Normal                      | 58.67±10.37     | 70.67±6.489 | 85.67±3.33      | 63.00±9.07      | 67.3±2.02      |
| HDL                 | Normal                      | 37.93±1.67      | 40.33±4.48 | 48.70±3.2       | 37.47±5.23      | 42.23±1.39     |

Results are presented as means±SEM, n=6. *, Means with different letters in the same row are significantly different (p<0.05) by one-way ANOVA followed by Tukey’s test.

Brain dopamine levels

Brain dopamine levels of HFD group were significantly (p<0.05) reduced compared with the control group. However, groups administered with L-DOPA and AEMP (200 mg/kg and 400 mg/kg) showed significant (p<0.05) increase in brain dopamine levels as compared to the HFD group (fig 3).

DISCUSSION

In the present study, the anti-obesity activity of dopamine and dopamine-containing plant Mucuna pruriens was investigated on HFD induced rat model for obesity.

During the first 8 wk of the experimental period, administration of HFD was found to increase the body weight in all groups. Treatment with L-DOPA and AEMP (200 mg/kg and 400 mg/kg) for subsequent 4 wk attenuated the increase in body weight as compared to non-treatment HFD group. Further, in L-DOPA group and in AEMP (400 mg/kg) group there was a significant reduction in body weight as compared to pre-treatment weights in rats in these groups. The reduction in body weight in treated groups was found to be convergent with a reduction in food intake observed in all treated groups as compared to the HFD group. In L-DOPA group, this was supported further by significant reduction of BMI. Lowering of BMI by AEMP (200 mg/kg and 400 mg/kg) although not statistically significant also supports the anti-obesity activity of Mucuna pruriens.

The results of present study correlate with the previous studies showing the role of brain dopamine and its receptors in reward mechanism and weight gain. In the present study, brain dopamine levels were found to be increased in all treatment groups as compared to HFD groups which confirm the body weight reducing effect of dopamine.

White adipose tissue

Feeding a high-fat diet for 12 wk produced a significant (p<0.05) increase in epididymal WAT weight of HFD treated the group as compared to normal diet fed rats.

There were a significant reduction in the epididymal fat mass in the L-DOPA and AEMP (400 mg/kg) treated groups while no significant alteration was observed in AEMP (200 mg/kg) group as compared to HFD (fig 4).

CONCLUSION

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

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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