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Antiobesity effect of *Bauhinia variegata* bark extract on female rats fed on hypercaloric diet

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Abstract

The present study was carried out to investigate the antiobesity effect of methanolic extract of stem and root barks of *Bauhinia variegata* Linn in female rats fed with hypercaloric diet. Obesity was induced by administration of hypercaloric diet for 40 days. The plant extract (at the tested doses of 200 and 400 mg/kg body weight) exhibited a significant hypolipidemic effect and thus reduced the obesity. The body weight and feed intake was reduced significantly. Treatment of obese animals with the methanolic extract of *B. variegata* exhibited an increased brain serotonin level and high density lipoprotein with a concomitant decrease in total cholesterol, triglycerides and low density lipoprotein. Thus, the study indicates that the antiobesity activity of methanolic extract of *B. variegata* could be attributed to the presence of P-sitosterol in the stems and the tendency of the extract to reduce lipid profile and elicit the brain serotonin level.

Introduction

Obesity is a growing health problem in many of the nations of the world. With a body mass index (BMI) above 30, increased risk of non-insulin dependent diabetes mellitus, hypertension, hypertriglyceridemia and ischemic heart disease is prominent (Farooqi et al., 2003). Obese subjects also carry an increased risk of colon, breast, prostate, gall bladder, ovary and uterine cancer.

Since many factors influence energy balance, and they interact at many levels, determining the pathophysiology of obesity is difficult. The main determinant is a disturbance of the homeostatic mechanisms that control energy balance. Other factors such as food intake and lack of physical activity also contribute.

Bauhinia variegata Linn has been extensively used as Indian traditional and folklore medicine to cure various human ailments. The stem contains some important phytoconstituents like P-sitosterol, lupeol, kaempferol-3 -glucoside and the root bark contains flavones. Traditionally, a decoction is given in piles (also used against tumors), hematuria, and menorrhagia. Root is carminative, used in dyspepsia and flatulence and a decoction is used to prevent obesity (Khare, 2007).

The ethanolic extract of *B. variegata* was reported to possess antitumor effect in Dalton's ascitic lymphomas, antiulcer activity (RajKapoor et al., 2003a), chemo preventive and cytotoxic effect in liver tumor against diethyl nitrosamine (RajKapoor et al., 2006), anti arthritic activity (RajKapoor et al., 2007) and hepatoprotective activity (Bodakhe and Ram, 2007). The methanol extract possess significant antibacterial effect (Parekh et al., 2006). The traditional folklore claim of the plant encouraged us to investigate the antiobesity activity of this plant.

Materials and Methods

Plant material

Root and stem barks of *B. variegata* was collected from Thirunelveli District, Tamil Nadu, India during July 2007. The plant material was identified by Dr. Sasikala Ethirajulu, Research officer, CCRAS, Govt. of India, Chennai. A voucher specimen was deposited at C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India for future reference.

Preparation of extract

Freshly collected root and stem barks of *B. variegata* were dried in shade and pulverized to a coarse powder. Weighed quantity of powder was extracted with methanol in a soxhlet apparatus, the filtrate obtained was evaporated to dryness at 40-50°C in a rotary vacuum evaporator to obtain a dark colored molten mass. The percentage yield was found to be 8.8% w/w.

Experimental animals

In bred female Wistar rats weighing 150-160 g obtained from the animal house of C.L. Baid Metha College of Pharmacy were used in the study. The animals were maintained in well ventilated rooms with 12:12 hours light/dark cycle in polypropylene cages. All animals were acclimatized to the laboratory conditions one week prior to the initiation of the study. The study proposal was approved by Institutional animal Ethical Committee constituted under CPSCEA.

Acute toxicity studies

Wistar rats weighing 200-250 g (n=3) were use in the procedure. Acute oral toxicity was performed as OECD-423 guidelines (Ecobichon, 1997). The animals were fasted overnight, provided only water, after which extract was administered to the animals orally at the dose of 5 mg/kg body weight by gastric intubation and the animals were observed for 24 hours. If mortality was observed in 2 or 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for further 72 hours.

Induction of experimental obesity

Basal diet (rodent feed) was obtained from Pranav Agro Industries, Sangli, to which the following constituents were added to prepare hypercaloric diet (HCD): Casein -20%; D-L-methionine- 0.3%; corn starch- 15%; sucrose-27.5%; cellulose powder- 5%; mineral mixture- 3.5%; vitamin mixture- 1%; choline bitartarate- 0.2%; coconut oil-9.9% and lard oil-17.6% (Vasselli et al., 2005).

Animal grouping

The animals were divided into five groups containing six animals each. Group I served as control; Group II received HCD; Group III received

HCD and methanolic extract of *B. variegata* 200 mg/kg body weight; Group IV HCD and methanolic extract of *B. variegata* 400 mg/kg body weight and Group V received HCD and sibutramine 5 mg/kg body weight for 40 days. Replenishing a known quantity of fresh food daily at 10.30 a.m. and thereby measuring the food intake of the previous day carried out measurement of daily food consumption. Body weight of rats was recorded weekly to assess percentage of weight gain in each group. The daily feed intake for groups of animals were measured everyday for 40 days and expressed as mean daily feed intake in gram. General well being and behavior of the animals were observed daily throughout the period of study. The litter in the cage was renewed twice a week to ensure maximum comfort for the animals. The body rectal temperature were recorded post-treatment on day 41 in order to measure thermogenesis.

Estimation of lipid profile

On day 41, the animals were sacrificed by cervical dislocation and blood samples were collected by carotid bleeding separately into sterilized dry centrifuge tubes and allowed to stand for 30 min at 37°C. The clear serum was separated at 2,500 rpm for 10 min and was used for the estimation of total cholesterol by CHOD-PAP method (Siedel et al., 1983), High density lipoprotein (HDL) cholesterol by PEG-CHOD-PAP method (Warnick and Wood, 1995) and triglycerides by GPO-PAP method (McGowan et al., 1983) using standard kits. LDL-cholesterol, VLDL-cholesterol and the atherogenic index was calculated using standard equations (Friedwald et al., 1972). The percentage of lipid lowering effect was calculated according to the equation mentioned elsewhere (Puri et al., 2007):

Estimation of serotonin

Serotonin level in rat brain was carried out by spectrofluorimetry. The whole brain was dissected out and the striatum and hippocampus was separated from the brain and homogenized in 3 mL HCl-butanol (0.9 mL of 37% hydrochloric acid in 1L *n*-butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged at 2,000 rpm for 10 min. Supernatant phase 0.8 mL was removed and added to an Eppendorf reagent tube containing 2 mL of heptane and 0.3 mL of 0.1 M HCl. After 10 min of vigorous shaking, the tube was centrifuged to separate two phases. Upper organic phase was discarded and the aqueous phase was used for serotonin assay. 0.5 mL of tissue and 0.6 mL of ortho-pthalaldihyde was heated to 100°C for 10 min to develop fluorophore. After the

Table I

Effect of *B. variegata* on serum lipid parameters

Group	Total cholesterol	Triglycerides	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol
I	110.4 ± 1.6	173.0 ± 2.2	43.2 ± 0.9	84.4 ± 0.3	34.6 ± 2.0
II	214.1 ± 2.4^{a***}	250.2 ± 4.6^{a***}	32.4 ± 1.1^{a***}	170.5 ± 0.5^{a***}	50.0 ± 2.1^{a***}
III	167.4 ± 0.9^{b***}	223.7 ± 2.2^{b***}	34.5 ± 0.4^{bNS}	129.5 ± 0.2^{b***}	44.7 ± 1.1^{bNS}
IV	143.5 ± 0.7^{b***}	208.8 ± 2.7^{b***}	37.4 ± 0.4 ^{b***}	109.2 ± 0.1^{b***}	41.8 ± 1.2^{b**}
V	124.4 ± 0.5^{b***}	192.2 ± 1.8^{b***}	40.4 ± 0.6 ^{b***}	94.0 ± 0.2^{b***}	38.4 ± 0.4^{b***}

Values are expressed as mean ± SEM, n = 6; Comparisons: ^{a}Gp I Vs Gp II, ^{b}Gp II Vs Gp III & IV; NS; Non significant; ***p<0.001; One-way ANOVA followed by Dunnet's "t" Test

Table II

Effect of B. variegata on brain serotonin levels

Group	Serotonin
I	375 ± 10.8
II	175 ± 5.4a**
III	225 ± 7.9^{b**}
IV	257 ± 7.7^{b**}
V	328 ± 7.5^{b**}

Values are expressed as mean ± SEM, n = 6; Comparisons: ^{a}Gp I Vs Gp II, ^{b}Gp II Vs Gp III & IV; NS; Non significant; **p<0.01; One-way ANOVA followed by Dunnet's "t" Test

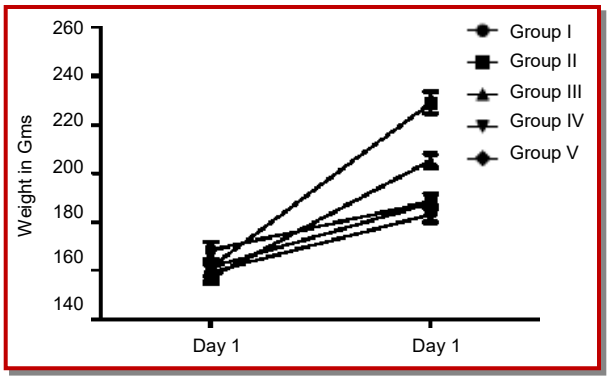

Figure 1: Body weight variation between animal groups

samples reached equilibrium with ambient temperature, excitation/emission spectra readings were taken at a wavelength 360-470 nm in a spectrofluorometer (Schlumpf et al., 1974).

Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnet's t-test for determining the statistical significance of difference between experimental groups. The minimum level of significance was set at p<0.05.

Results

Methanolic extract of *B. variegata* (MEBV) did not produce any toxic symptom or mortality upto the dose level of 2,000 mg/kg body weight orally in rats and hence the extract was considered safe for further pharmacological screening. According to OECD-423 guidelines for acute oral toxicity, the LD 50 dose of 2,000 mg/kg is categorized as unclassified. Group II animals fed on hypercaloric diet exhibited a non-significant rise in body weight between day 1 and 40 as compared to Group I animals. Treatment with MEBV (200 and 400 mg/kg body weight) exhibited a insignificant decrease in body weight of the animals in comparison with Group II animals. The standard drug sibutramine (5 mg/kg body weight) produced a reduction in body weight of the animals but statistically not significant (Figure 1). The body rectal temperature were recorded post treatment on day 41 and was found to be elevated in Groups III, IV and V, but was statistically not significant (Figure 2). Similarly the animals fed on HCD showed an increased feed seeking behavior when compared to all the other group of animals. Animals treated with MEBV (200 and 400 mg/ kg body weight) and sibutramine (5 mg/kg) had a decreased feed consumption (p<0.001) than Group II animals (Figure 3). Administration of MEBV at 200 mg/ kg orally to obesity induced animals resulted in a decreased total cholesterol (21.8%), triglycerides (10.6%), LDL-c (24.1%), VLDL-c (10.6%) and a increased HDL-c (5.9%). With a dose of 400 mg/kg MEBV, a further reduction occurred in total cholesterol (33.0%), triglycerides (16.5%), LDL-c (36.0%), VLDL-c (16.6%) and an increased HDL-c (15.2%).

Sibutramine produced 41.9% reduction in total cholesterol and 23.2% reduction in triglyceride levels (Table I). Animals fed on HCD had a reduced level of serotonin in brain tissues (p<0.01) than the Group I animals. Treatment with MEBV restored the decreased level of serotonin in a dose-dependent manner (p<0.01) when compared to Group II animals. Sibutramine produced an increased serotonin level comparable to control animals (p<0.01; Table II).

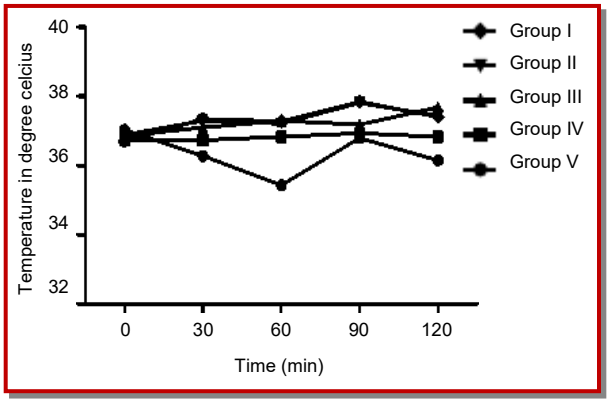

Figure 2: Body temperature of animals post-treatment

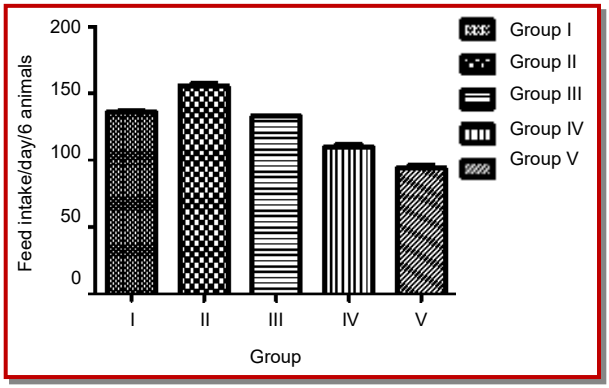

Figure 3: Feed intake per day of animal groups

Discussion

In the present study, the antiobesity effect of barks of *B. variegata* was studied using dietary animal models of obesity as they have been reported to bear close resemblance to human obesity (Sclafani and Springer, 1976). The results of our study showed that rats fed with a HCD elicited significant increase in body weight. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans (Bull, 1988) and in animals (Rothwellet et al., 1983). Further the composition and variety of cafeteria foods also exert synergistic effects on the development of obesity. In our study, hypercaloric diet exhibited an increased body weight along with corresponding rise in cholesterol levels which was promptly reduced by the administration of MEBV at the respective dosage levels. The weight reducing effect may be attributed to its potential to inhibit lipogenesis and enhanced thermogenesis, since obesity is associated with defective thermogenesis (Pasquali and Casimirri, 1993). The increased rectal body temperature may be attributed to the overall stimulant and thermogenic property of the phytoconstituents present in the extract.

MEBV may offer enormous therapeutic potential for the treatment of obesity at the tested doses, as evident from the reduced levels of total cholesterol and triglycerides. Inclusion of saturated fatty acids in the diet has been shown to induce hypercholesterolemic effect in rats (Zulet et al., 1999). The coconut oil included in the diet in this study contains saturated fatty acids and this could account for the difference in increase in the accumulation of cholesterol in the liver in this study. It is possible that the normal catabolism of liver lipids was impaired in the rats fed with modified diet with consequent accumulation of lipids in liver. The presence of phytoconstituents such as oleic acid, β-sitosterol is reported to reduce the hyperlipidemic states (Boppanna et al., 1997) and such components are previously reported in *B. variegata* (Khare, 2007).

Obesity is linked to high intake of diet and dietary fats. Microinjection of serotonergic agents directly into paraventricular nucleus reduces the intake of carbohydrates and fats. This phenomenon of diet induced obesity in rats is a reliable model in which daily energy intake is markedly increased largely through an increase in the meal size. The standard drug sibutramine used in our study is a noradrenergic/serotonergic agent, which acts primarily by affecting appetite centre and satiety centre respectively. It affects food intake by enhancing serotonergic transmission in the hypothalamus and reduced food seeking behavior as well as decreases quantity of food consumed at any meal (Tripathi, 2004), thus the role of serotonin in controlling appetite is evident. Estimation of brain tissue serotonin in our study evidenced increased level of it and thus attributable to the reduced feed seeking behavior and reduction in body weight.

Also, 5-HT modulators are reported to possess significant beneficial effects on dyslipidemia in conditions of syndrome X (James et al., 2000). Sibutramine was reported to possess significant lipid lowering activity associated with hyperglycemia and hyperinsulinemia (James et al., 2000; Finer et al., 2000) and was also reported to reduce body weight in conditions of syndrome X (McLaughlin et al., 2001). Similarly it appears that the beneficial effect obtained by *B. variegata* is similar to 5-HT receptor modulator sibutramine.

Conclusion

B. variegata may be useful in conditions of hyperlipidemia and obesity as it can reduce the elevated cholesterol, triglyceride, VLDL-cholesterol levels and body weight.

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Conflict of Interest

Authors declare no conflict of interest

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