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EVALUATION OF ETHANOLIC EXTRACT OF *BAUHINIA VARIEGATA* LINN. IN HIGH FAT DIET INDUCED OBESITY IN RATS

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Abstract

Bauhinia variegata Linn. (Family- Leguminosae) bark is traditionally used for treat obesity and diarrheoa. Therefore, the present investigation was carried out to investigate the effect of ethanol extract of *Bauhinia variegata* (EEBV). Hence this study is emphasized to explore the effect of EEBV 200 & 400mg/kg, p.o on energy balance disorders like, obesity, hyperphagia, hyperglycaemia and hyperlipidemia. From the observations of the study performed, it could be predicted that *Bauhinia variegata* root extract exerted significant anti-obese activity due to its hypophagic, hypoglycaemic and hypolipidemic effect in rats fed on high fat diet. Further investigation need to measures the enzymes in lipid pathways and hormones would ascertain the exact mechanism of anti-obese effect and to figure out the therapeutic potential of *Bauhinia variegata* root in the treatment of obesity.

Key words: *Bauhinia variegata* Linn., Anti-obesity, Hypophagic, Hypoglycaemic and Hypolipidemic

INTRODUCTION

Obesity is a serious health problem, among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, and lack of exercise, psychological factors and the consumption of energy rich diets are the major causes. Due to obscure etiology, the treatment of obesity is difficult and challenging. Further, the cause of concern is the non availability of drugs for its treatment and the short term efficacy and the limiting side effects of available drugs (Rang HP *et al*., 2003).

Bauhinia variegata Linn. is a species of flowering plant in the family Leguminosae, native to southeastern Asia, from southern China west to India. Decoction of bark is a useful wash in ulcers and skin diseases and a remedy in obesity and diarrheoa. Dried buds are useful in diarrheoa, worms, piles, dysentery (Nadkarni AK, 1996). The claim for the utility of this plant in treatment of obesity has not been scientifically evaluated. Hence this study is emphasized to explore the effect of root on energy balance disorders like, obesity, hyperphagia, hyperglycaemia and hyperlipidemia. Therefore, the present investigation was carried out to investigate the constituents and anti-obesity activity of the ethanolic extract of *Bauhinia variegata* is being reported here.

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MATERIALS AND METHODS

Collection and authentication of plant material

The root part of *Bauhinia variegata* was collected from Tirunelveli district, Tamil Nadu in the month of July 2007. The plant material was identified and authenticated by V.Chelladurai Research officer botany C.C.R.A.S Govt of India. Specimen was submitted at C.L Baid Metha College of pharmacy.

Preparation of ethanol extract of *Bauhinia variegata*

Freshly collected root of *Bauhinia variegata* was dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (1200g) was passed through sieve number 40 and subjected to hot solvent extraction in a soxhlet apparatus using ethanol, at a temperature range of 55^0C to 65^0C. Before and after every extraction the marc was completely dried and weighed. The filtrate was evaporated to dryness at 40^0C under reduced pressure in a rotary vacuum evaporator. The percentage yield of ethanolic extract was 8.83% w/w.

Experimental animals

Colony inbred strains of wistar rats female weighing 150-180g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 p.m, 22 ± 1^0C room temperature, in polypropylene cages. The animals were feed on standard pelleted diet (Pranav Agro industries, Sangli) and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. It was randomly distributed into five different groups with six animals in each group under identical conditions throughout the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals). IAEC Reference number: IAEC/XI/13/CLBMCP/2007-2008.

Pharmacological studies
High fat diet Induced obesity in experimental rats

Preparation of diet

High fat diet is a hyper caloric diet and was prepared by mixing the above constituents in fixed percentage. The above mentioned percentage is for 100g diet. The feed was prepared, dried, powdered and administered every day in morning to animals with water *ad libitum*. Diet was administered and weight gain was observed in rats on third day, therefore confirming the development of obesity in rats. Study was continued for 40 days.

High Fat Diet Formula

Casein -20%, D,L methionine-0.3%, corn starch-15%, sucrose-27.5%, cellulose powder-5%, mineral mixture-3.5%, vitamin mixture-1%, choline bitartrate-0.2%, corn oil -9.9%, lard oil-17.6% (Augusti KT and Mattew BC, 1996; Vasselli JR *et al*., 2005; Shih MF and Cherny JY, 2005)

Anti-Obesity Studies

Female wistar rats (150-180g) were given high fat diet for 40 days. Thirty rats were randomly divided into 5 groups of six animals each. The following schedule of dose, diet administration in experimental groups was followed:
Group: I- The animals received 0.9% saline (5ml/kg/p.o) and served as normal control.
Group: II- The animals received only high fat diet and served as negative control.
Group: III- The animals received high fat diet and treatedwith EEBV (200mg/kg/ p.o) suspended in in 0.9% saline.
Group: IV- The animals received high fat diet and treated with EEBV (400mg/kg/ p.o) suspended in in 0.9% saline.
Group: V-The animals received sibutramine (5mg/kg/p.o) suspended in 0.9% saline and high fat diet.

The above mentioned treatment schedule was followed for the respective group of animals for 40 days. Daily all the animals were given high fat diet with drug treatment of ethanol root extract of *Bauhinia variegata* (Tripathi SN *et al*., 1969; Augusti KT *et al*., 1996)

In vivo pharmacological evaluation

Body Weight

The body weight weight (gm) was recorded on day one and then on alternate days for 40 days using digital weighing balance.

Food Intake

The daily food intake for group of 6 rats was measured daily for 40 days and expressed as mean daily food intake for group of 6 rats.

Body Temperature

The body temperature was recorded on day 39 using rectal telethermometer before and after drug administration at 30, 60, 90, 120, 180 minutes with a contact time of 1 minute.

Biochemical studies

On day 41 of experiment the animals were sacrificed by cervical dislocation and blood samples were collected by carotid bleeding separately into sterilized dry centrifugation tubes and allowed to stand for 30 minute at 37^{o}c. The clear serum was separated at 2500 rpm for 10min using micro centrifuge and biochemical investigation such as Cholesterol, HDL-C, Triglycerides, LDL-C, VLDL-C, Atherogenic index, percentage protection, SGOT, SGPT, Blood glucose and Total protein were carried out.

Statistical Analysis

The Statistical Analysis was carried our using analyses of variance (ANOVA) followed by Dunnet's test. p values <0.05 were considered as significant.

Results

Effect On body Weight:

Group II animals fed on high fat diet (HFD) exhibited significant ($p<0.05$) increase in body weight between day 1and day 40 as compared to group I animals.
Treatment with EEBV (200 and 400 mg/Kg/p.o) showed a significant ($p<0.05$) decrease in body weight as compared with group II animals. EEBV (200 and 400 mg/Kg/p.o) dose dependently decrease body weight. Results are shown in Table: 1.

Effect on Feed intake

Group II animals fed on HFD fed rats showed significant ($p<0.01$) increase in daily food intake when compared with group I animals. Treatment with EEBV (200 and 400 mg/kg/p.o) showed significant ($p<0.01$) decrease daily food intake as compared with group II animals. Results are shown in Table: 2.

Effect on body temperature

Group II animals exhibited significant ($p<0.01$, $p<0.05$) increase in body temperature at 60, 120, 180 minute as compared to group I animals. Group III when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60, 90,180 minute. Group IV when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60, 90,120,180 Minute. Group V when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60, 90,180 minute. Results are shown in Table: 3.

Lipid Profile

Group II animals fed with HFD exhibited significant ($p<0.01$) increase in total cholesterol, TG, LDL, and VLDL when compared with group I animals. Group III to group V animals exhibited a significant ($p<0.01$) decrease total cholesterol, TG, LDL, and VLDL when compared with group II animals. The group II animals exhibited significant ($p<0.01$) reduction in HDL cholesterol when compared with group I animals. Group III to V animals exhibited significant ($p<0.01$) increase HDL when compared to Group II animals. Result are shown Table: 4.

Atherogenic index and percentage protection

There was decrease in atherogenic index in all the treated groups. Percentage protection for III (19.5%) and Group IV (33.64%). Results are shown in Table: 5.

Liver Function Tests

The levels of SGOT, SGPT, Blood Glucose and Total Proteins in Group II animals were significantly ($p<0.01$) increased when compared with group I animals. Group III animals exhibited a significant ($p<0.01$) decrease when compared with group II animals. Group V exhibited a significant ($p<0.01$) increase when compared with group II animals. Results are shown in Table: 6.

Table: 1. Effect of EEBV on weight gain (g) in rats

S.No	Groups	Treatment	Weight on day 1	Weight on day 40	Weight gain
1.	I	Control	185.83±3.5620	168.33±3.1545	10.0 ± 0.4174
2.	II	Diet control	161.33±3.0948	229.00±4.5240	67.67 ± 1.1492*
3.	III	EEBV(200mg)	156.33±2.3758	187.66±2.0270	31.32±0.7692*
4.	IV	EEBV(400mg)	161.33±3.6390	187.84±1.8700	26.00±0.4852*
5.	V	Sibutramine (5mg)	159.33± 3.4897	183.16±3.3600	23.83 ± 0.1121*

Table: 2. Effect of EEBV on daily feed intake(g) in rats

S.No	Group	Treatment	Feed intake
1.	I	Control	136.58±0.6982
2.	II	Diet control	155.53±2.294**
3.	III	EEBV(200mg)	122.14±1.087**
4.	IV	EEBV(400mg)	109.74±2.428**
5.	V	Sibutramine (5mg)	94.25±2.428**

Values are mean ± SEM of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. Comparisons were made between: a) Group I vs Group II. b) Group III, IV, V vs Group II. **p value< 0.01, *p value <0.05, ns non-significant

Table: 3. Effect of EEBV on Body Temperature in rats

Groups	Treatment	Body temperature (min)					
		0	30	60	90	120	180
I	Control	37.03±0.33	36.28±0.08	35.43±0.37	36.80±0.33	36.15±0.23	36.08±0.15
II	Diet control	36.73 ±0.33^{ns}	36.73±0.19^{ns}	36.83±0.07**	36.93±0.15^{ns}	37.63±0.36*	37.37±0.32**
III	EEBV(200mg)	36.55±0.07^{ns}	37.16±0.19**	37.05±0.23**	37.71±0.30*	36.58±0.07^{ns}	36.46±0.08^{ns}
IV	EEBV(400mg)	36.88± 0.23^{ns}	37.30±0.19**	37.21±0.20**	37.81±0.09*	37.43±0.39*	37.50±0.17**
V	Sibutramine(5 mg)	36.7±0.22^{ns}	37.34±0.16**	37.28±0.21**	37.84±0.09*	37.40±0.39^{ns}	37.48±0.36**

Table: 4. Effect of EEBV on Serum lipid profile in rats

Groups	Treatment	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C(mg/dl)	VLDL-C(mg/dl)	HDL-C(mg/dl)
I	Control	66.37±1.58	173±2.21	25.48±0.51	34.61±0.44	43.17±0.94
II	Diet control	154.08±2.35**	250.2±4.61**	43.12±0.90**	50.08±0.91**	32.44±1.11**
III	EEBV (200mg)	104.37±0.92**	223.7±219**	37.29±0.47**	44.74±0.43**	37.27±0.42**
IV	EEBV (400mg)	94.47±0.71**	208.8±2.67**	33.68±0.53**	41.76±0.53**	40.39±0.62**
V	Sibutramine(5mg)	77.36±1.45**	192.2±1.92**	29.37±0.39**	38.46±0.39**	45.18±0.41**

Values are mean ± SEM of 6 animals. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. Comparisons were made between: a) Group I vs Group II, III, IV, V. **p value< 0.01, *p value <0.05, ns non-significant.

Table: 5. Atherogenic index and percentage protection in various groups of rats

Groups	Treatment	Atherogenic index	Percentage protection (%)
Group I	Control (Normal saline)	4.00	-
Group II	Diet Control	7.71	-
Group III	EEBV (200mg)	6.23	19.15
Group IV	EEBV (400mg)	5.12	33.64
Group V	Sibutramine (5mg)	4.25	44.90

Table: 6. Effects of EEBV on Liver function test and Serum glucose in rats

Groups	Treatment	SGOT Levels(Iu/L)	SGPT Levels(Iu/L)	Blood glucose(mg/dl)	Total protein(mg/dl)
I	Control	30.00±0.58	12.00±0.57	61.74±0.79	5.43±0.07
II	Diet control	46.00±1.86**	25.33±1.40**	92.90±0.86**	12.45±0.12**
III	EEBV(200mg)	63.17±1.10**	20.00±0.57*	80.80±0.42**	5.73±0.08**
IV	EEBV(400mg)	66.00±1.12**	22.33±0.76^{ns}	81.07±0.42**	4.49±0.07**
V	Sibutramine(5mg)	80.83±1.64**	44.55±2.21**	72.09±0.43**	4.25±0.06**

Values are mean ± SEM of six animals. Statistical significance test for comparisons was done by ANOVA, followed by Dunnet's test. Comparisons were made between: a) Group I vs Group II. b) Group III, IV, V vs Group II.
**p value< 0.01, *p value <0.05, ns non-significant

DISCUSSION AND CONCLUSION

Dietary obesity can be induced readily in laboratory rodents by giving high fat diets or cafeteria diets. Obesity also occurs in rodents given a palatable sugar solution in additional to laboratory chow. These animals consume only about half of as much chow as animals not given sugar, additional calories from sugar solution generally results in greater total dietary energy intake and development of profound obesity (Chen MD and Linn PY, 2000).

In the present study, the anti-obese activity of *Bauhinia variegata* ethanol extract was studied using dietary animal's model of Obesity. The present pharmacological investigation revealed that HFD elicited significant increase in body weight, food intake, serum levels of glucose, protein, total cholesterol, LDL Cholesterol, VLDL cholesterol, Triglycerides, SGOT, SGPT. Treatment with EEBV resulted in reduction of body weight in HFD fed rats indicating that the extract possess weight reducing property. Since obesity is associated with hyperphagia, HFD fed rats consumed more food than normal diet fed rats. EEBV effective in decreasing daily food intake in HFD fed rats, indicating that it possess hypophagic property. The increase in rectal body temperature may be attributed to the overall stimulant and thermogenic property of phytoconstituents of the extract.

Lipids are mostly consumed in the form of neutral fats, which are also known as triglycerides. The triglycerides are made up of a glycerol nucleus and free fatty acids. Triglycerides form major constituents in food of animal origin and much less in food of plant origin. Saturated fats increase blood cholesterol and thereby increase risk of atherosclerosis and coronary heart disease. Monounsaturated and polyunsaturated fats decrease blood cholesterol and reduce blood pressure. There is risk of obesity. Tran's fats increase LDL and increase risk of atherosclerosis and coronary heart disease (Rajalakshmi D and Sharma DK, 2004).

EEBV showed significant reduction in serum levels of total cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides along with significant increase in serum HDL cholesterol levels in HFD fed rats. Considering the enhancement of cardioprotective lipid HDL, it can be concluded that root of *Bauhinia variegata* is a potent cardioprotective agent (Choudhary MI *et al*., 2005).

Blood glucose levels were also significantly decreased in both doses of alcoholic extract. Total protein levels also decreased significantly in both doses but effect was more observed at higher dose levels of 400 mg/kg /p.o. The Enzyme SGOT, SGPT increased in group of animals treated with HFD and of EEBV (400 mg/kg/p.o) decreases the SGOT, SGPT levels was observed. There was no significant change at 200mg/kg/p.o of EEBV.

From the observations of the study performed, it could be predicted that *Bauhinia variegata* root extract exerted significant anti-obese activity due to its hypophagic, hypoglycaemic and hypolipidemic effect in rats fed on high fat diet. The long history of use of *Bauhinia variegata* may have therapeutic and protective applications in the treatment of these disorders. Further investigation involving measure of enzymes in lipid pathways and hormones would ascertain the exact mechanism of anti-obese effect and to figure out the therapeutic potential of *Bauhinia variegata* root in the treatment of obesity. This ensures an understanding of the mechanism involved in the treatment of these disorders. Further there is need to identify exact phytoconstiuents responsible for the activity at brain level and to formulate poly herbal anti-obese preparation containing *Bauhinia variegata* extract as main ingredient along with other novel weight reducing and hypolipidemic herbal drugs.

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