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Preliminary phytochemical, acute toxicity study & Anti-obesity activity of roots of *bauhinia tomentosa*

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ABSTRACT

Obesity is a global health problem. It is also known to be a risk factor for the development of metabolic disorders, type 2 diabetes, systemic hypertension, cardiovascular disease, dyslipidemia, and atherosclerosis. In this study we evaluated the ethanolic & aqueousextract of roots of *Bauhinia Tomentosa* (EEBT & AEBT) for its phytoconstituents & antiobesity activity. The plant material was extracted and subjected to preliminary phytochemical screening. Acute toxicity study was performed to evaluate the toxicity profile. High fat diet was prepared and the 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 aqueous and ethanolic root extracts of *Bauhinia tomentosa* AEBT and EEBT respectively.After 40 daysthe anti-obesity parameters were evaluated.In preliminary phytochemical analysis, the EEBT & AEBT showed presence of Alkaloids, flavonoids, glycoside sterols, proteins, fixed oils, saponins, tannins. Gums and mucilage, terpenes, steroids, carbohydrates and flavones were absent. The EEBT and AEBT were found to be free from toxicity upto dose of 2000 mg/kg/p.o. Both the extracts at 200 mg/kg and 400 mg/kg significantly reduced body weight, feed intake in the animals. Thereby it could be concluded that *Bauhinia tomentosa* root extracts exerted significant anti-obese activity

Keywords: *Bauhinia Tomentosa,* Obesity, Alkaloids, saponins, body weight.

INTRODUCTION

Obesity has been linked with an uptick in morbidity, mortality, and decreased life expectancy [1], and is a significant issue in the world. It is caused by an energy imbalance between imbalance between energy intake consumption, which causes an increase in blood lipid concentration and an increase in fat mass [2]. While fat is important for good health, a huge proportion of fat accumulation is associated with a number of health hazards, such as dyslipidemia, osteoarthritis, hypertension, diabetes mellitus, disease of the fatty liver, asthma, cancer and obesity[3, 4].

Worldwide, the prevalence of obesity is increasingly growing. 300 million people are reportedly medically obese, while over 1 billion

adults are overweight [5]. WHO also projected that by 2030, this number will rise to 3.3 billion. Including sedentary lifestyles such as white collar occupations, lack of physical work, rise in calorie intake, endocrine disorders, and psychiatric problems, this disease has several factors that lead to its etiology [6, 7].

Past studies also show that when stressed, people increase their consumption of high-energy snack foods, contributing to obesity [8]. In addition, labor-saving technologies such as elevators, vehicles, remote controls, personal computers, and sedentary leisure practices such as watching TV, surfing the Internet, and playing video games have made a significant contribution to the world's obesity [9, 10]. Despite the urgent need for successful and safe therapeutics and the likely size of the anti-obesity drug market, the latest attempts to develop these drugs remain unsatisfactory [11]. That was due to these drugs-related adverse side effects. More existing methods have centered on natural sources for treating obesity and hyperlipidemia and reducing weight gain with less side effects reported [12]. The possible use of natural agents for obesity treatment is currently not thoroughly explored and may be an excellent alternative method for the production of safe and successful anti-obesity drugs.

Bauhinia Tomentosa belongs to the family of caesalpiniaceae, commonly known as yellow Bauhinia (Eng) medium to large shrub with its attractive, light green, two-lobed leaves produces beautiful, bright yellow flowers with black to maroon colored centers in summer. Medium to large shrub to a small tree, up to 4m in height. Leaves are divided into two lobes, light green in colour, with a leathery texture, carried on branches that are often drooping. It produces large bell-shaped, bright yellow flowers with a black to deep maroon coloured centre in mid to late summer (from December to March). The fruits are pea like pods, slender and velvety. They are light green, turning a pale brown with age and are produced from January to June or even later. Bark is gray or brown [13].The wood is used to make rafters for huts and the dried leaves and flower buds, and the roots and bark are used in traditional medicine in Africa and India. Three other species of *Bauhinia* are also used medicinally for everything from coughs, convulsions and constipation to pneumonia and venereal diseases. (*Bauhinia galpinii, Bauhinia thonningii, Bauhinia petersiana}*[14].

MATERIALS AND METHODS

Collection and authentication of plant material

The root part of *Bauhinia tomentosa* was collected from Salem district, Tamil Nadu. The plant material was identified and authenticated by Dr. P.Jayaraman, Plant anatomy research centre, Chennai.

Preparation of alcoholic plant extract

Freshly collected root of *Bauhinia tomentosa* 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 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.

Preparation of aqueous plant extract

Freshly collected root of *Bauhinia tomentosa* was dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (200g) was passed through sieve number 40 and subjected to cold maceration extraction in a simple apparatus using 1000ml water kept in refrigerator. Compound was immersed in cold water and stirred occasionallyduring a period of 48 hours. After 48 hours filtered the extract and pressed the mark. Pressed extract was added to previous extract. The filtrate was evaporated to dryness at 40^0C under reduced pressure in a rotary vacuum evaporator. The percentage yield of aqueous extract was 12% w/w.

Experimental animal

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 □ 2^0C room temperature, in polypropylene cages. The animals were feed on standard pelleted diet (Pranav Agro industries, Sangli) and tap water *ad libitum*. 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)

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents using different chemical tests [15,16]

Acute oral toxicity study

The acute oral toxicity procedure was followed by using OECD 423 guidelines. Adult female wistar rats weighing 150-200 g were used for the study. The starting dose level of ethanolic and aqueous extracts of *Bauhinia tomentosa* was 2000 mg/kg/body weight p.o as most of the crude extracts posses LD_{50} value more than 2000 mg/kg/ p.o. So the starting dose used was 2000 mg/kg/ p.o. Dose volume was administered 1 ml/100 gm body weight to the rat, which were fasted over night with water *ad libitum*. Food was with held for a further 3-4 hours after administration (p.o) of drugs and observed for the signs of toxicity. Body weight of the rats before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes, salivation, nasal discharge, urination and behavioural (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular, lethargy, sleep and coma were noted. The animals were kept under observation for 14 days. [17], [18].

ANTI-OBESITY ACTIVITY

High fat diet formula: [19-23]

The high fat diet contains the casein, D, L methionine, corn starch, sucrose, cellulose powder, mineral mixture, vitamin mixture, choline bitartrate, corn oil, lard oil. The composition is given in the Table-1.

Table-1: High Fat Diet Formula

S.No	Nutrient	Percentage
1	Casein	20%
2	D,L methionine	0.3%
3	corn starch	15%
4	Sucrose	27.5%
5	cellulose powder	5%
6	mineral mixture	3.5%
7	vitamin mixture	1%
8	choline bitartrate	0.2%
9	corn oil	9.9%
10	lard oil	17.6%

Mineral mixture

The mineral mixture contains Calcium Phosphate-500 gm/kg, Sodium chloride 74 gm/kg, Potassium sulphate - 52 gm/kg, Potassium citrate - 220 gm/kg, Magnesium oxide - 24 gm/kg, Ferric citrate - 66 gm/kg, Zinc carbonate - 1.6 gm/kg, Cupric carbonate - 0.3 gm/kg, Potassium iodate - 0.01 gm/kg, Sodium selenite - 0.01, Chromium potassium sulphate - 0.55 gm/kg, Sucrose finely powdered - 118.03 gm/kg

Vitamin mixture

The vitamin mixture contains Thiamine HCL- 0.6 gm/kg, Riboflavin - 0.6 gm/kg, Pyridoxine - 0.7 gm/kg, Cyanocobalamin - 1.0 gm/kg, Sucrose fine powder - 981.01 gm/kg. This 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.

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*.

Female wistar rats (150-180g) were given high fat diet for 40 days. Forty two rats were randomly divided into7 groups of six animals each. The following schedule of dose, diet administration in

experimental groups was followed: Group: I animals received 0.9% saline (5ml/kg/p.o) and served as normal control. Group: II animals received only high fat diet and served as negative control. Group: III animals received high fat diet and treated with AEBT (200mg/kg/ p.o) suspended in in 0.9% saline. Group: IV animals received high fat diet and treated with AEBT (400mg/kg/ p.o) suspended in in 0.9% saline. Group: V animals received high fat diet and treated with EEBT (200mg/kg p.o) suspended in 0.9% saline. Group: VI animals received high fat diet and treated with EEBT (400mg/kg /p.o) suspended in 0.9% saline. Group: VII animals received sibutramine (5mg/kg/p.o) suspended in 0.9% saline and high fat diet.

The 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 aqueous and ethanolic root extracts of *Bauhinia tomentosa.* The anti-obesity parameters were evaluated as follows

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. [24, 25].

RESULT AND DISCUSSION

Preliminary Phytochemical screening

The Aqueous and Ethanolic extracts of *Bauhinia tomentose*root were subjected to preliminary phytochemical screening. The result of phytochemical analysis of *Bauhinia tomentosa* root is shown in Tab:2.The ethanolic extractshowed the presence of various phytochemical constituents like alkaloids, proteins, carbohydrates, tannins, sterols, glycoside, flavonoids, saponins, fixed oils. Steroid, gum mucilage, flavones were found absent. The aqueous extractshowed the presence of various phytochemical constituents like alkaloids, carbohydrates, terpenes, tannins, sterols, glycoside, flavonoids and saponins. Steroid, proteins, gum mucilage, fixed oils, flavones were found absent.

Table: 2 Phytochemical Screening of *Bauhinia tomentosa* ethanolic and aqueous extracts

S. No.	Constituents	Ethanolic Extract	Aqueous Extract
1.	Alkaloids	+	+
2.	Carbohydrates	-	-
3.	Protein	+	-
4.	Steroids	-	-
6.	Sterols	+	+
6.	Tannins	+	+
7.	Flavonoids	+	+
8.	Gums and Mucilage	-	-
9.	Glycosides	+	+
11.	Saponins	+	+
12.	Terpenes	-	-
13.	Fixed oil	+	-

14.	Flavones	-	-

Acute oral toxicity study

The acute oral toxicity study was done according to OECD guidelines 423 (acute toxic class method). A single dose of 2000 mg/kg body weight / p.o of the AEBT and EEBT was administered to 3 female rats each and observed for 3 days. Animals were observed for signs of toxicity for first 3 hours at 30 min time interval. Thereafter animals were observed for 24 hours with continuous monitoring. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed. LD_{50} cut off dose per kilogram body weight was categorized as X (unclassified) and Globally Harmonized system (GHS) classes also comes under X (unclassified). The results are shown in Table: 3.

Table: 3 acute toxic class methods (OECD 423 guideline)

S. No	Treatment group	Dose	Weight-of animal in gms		Signs-of toxicity	Onset of Toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	EEBT	2g/kg	172	166	No signs of toxicity	Nil	Nil	14 days
2.	EEBT		169	165	No signs of toxicity	Nil	Nil	14 days
3.	EEBT		175	170	No signs of toxicity	Nil	Nil	14 days
4.	AEBT		170	166	No signs of toxicity	Nil	Nil	14 days
5.	AEBT		176	169	No signs of toxicity	Nil	Nil	14 days
6.	AEBT		173	170	No signs of toxicity	Nil	Nil	14 days

Effect on body temperature

Group II animals exhibited significant ($p<0.01$, $p<0.05$) increase in body temperature at 60, 120, 180 minutes as compared to group I animals. Group III when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 60,120,180 minutes. Group IV when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60,120,180 minutes. Group V when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60, 90,180 minutes. Group VI when compared with group II animals exhibited significant ($p<0.01$, $p<0.05$) increase at 30, 60, 90,120,180 minutes. Results are shown in Table: 4, Figure : 1.

Table:4 Effect of EEBV and AEBV on Body Temperature in rats

Treatment	Body temperature(min)					
	0	30	60	90	120	180
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
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**
AEBT (200mg)	36.41±0.09^{ns}	36.34±0.07^{ns}	37.15±0.22**	37.25±0.17^{ns}	37.48±0.32*	36.96±0.19*

AEBT (400mg)	36.86± 0.22^{ns}	37.10±0.21**	37.30±0.20**	37.18±0.26^{ns}	37.68±0.36*	37.15±0.15**
EEBT(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}
EEBT (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**
Sibutramine (5mg)	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**

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

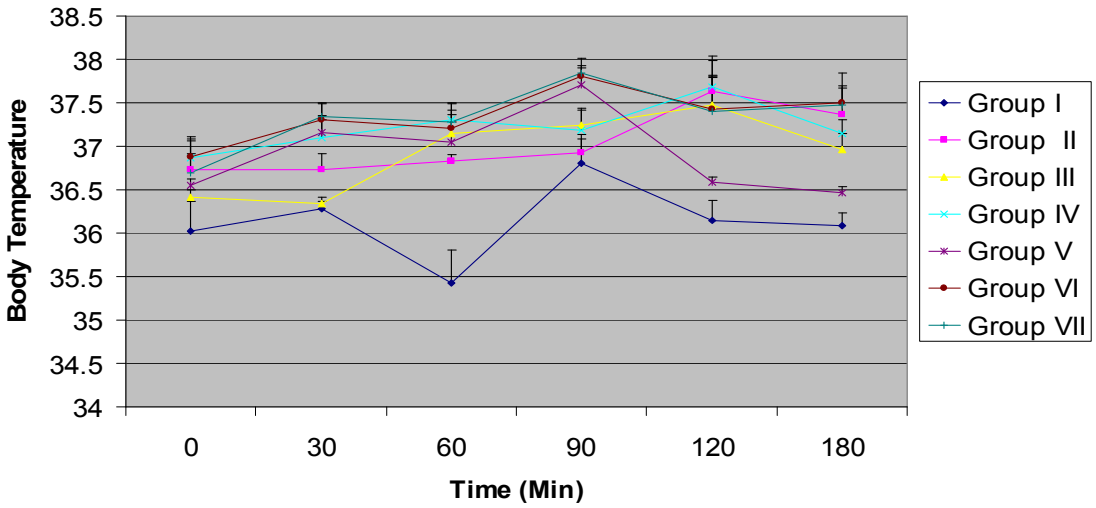

Figure:1 Effect of EEBT and AEBT on Body Temperature in rats

Effect on body Weight

Group II animals fed on high fat diet (HFD) exhibited significant ($p<0.01$) increase in body weight between day 1and day 40 as compared to group I animals. Treatment with AEBT and EEBT (200 and 400 mg/Kg/p.o) showed a significant ($p<0.01$, $p<0.05$) decrease in body weight as compared with group II animals. The two extracts at two dose levels resulted in dose dependent decrease body weight. EEBT was active when compared to AEBT. Results are shown in Table 5 & 6, Figure 2&3.

Table:5 Effect of EEBT and AEBT on weight gain (g) in rats

Groups	Treatment	Day 1	Day 40	Weight gain
I	Control	185.83±3.5620	168.33±3.1545	10.0 ± 0.4174
II	Diet control	161.33±3.0948	229.00±4.5240	67.67 ± 1.1492*
III	AEBT(200mg)	157.00± 2.3520	205.33±2.6540	47.50 ± 0.1077*
IV	AEBT(400mg)	157.00± 2.3520	190.50±3.6120	33.50 ± 1.2061*
V	EEBT(200mg)	156.33± 2.3758	187.66±2.0270	31.32 ± 0.7692*
VI	EEBT(400mg)	161.33±3.6390	187.84±1.8700	26.00 ± 0.4852*

VII	Sibutramine (5mg)	159.33± 3.4897	183.16±3.3600	23.83 ± 0.1121*

Values are mean □ SEM of six animals Statistical significance test for comparisons was done by ANOVA, followed by Dunnett's test Comparisons were made between: a) Group I vs Group II. b) Group III, IV, V, VI, VII vs Group II *p value < 0.05.

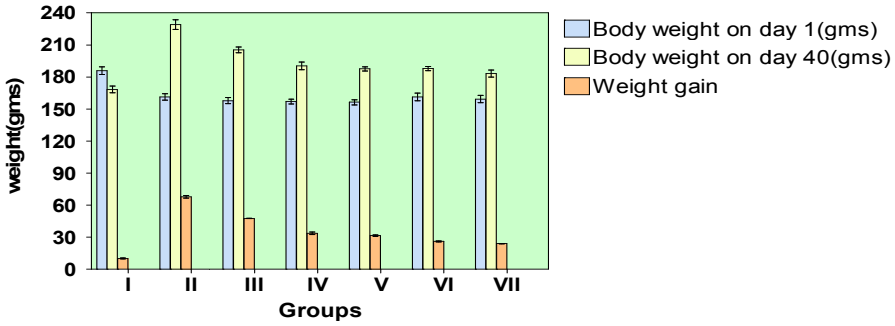

Figure:2 Effect of EEBT and AEBT on weight gain in rats

Table:6 Effect of EEBT and AEBT on Body weight (g) in rats

Groups	Treatment	Body weight
I	Control	163.51±0.7587
II	Diet control	192.37± 4.444**
III	AEBT (200mg)	178.75±3.311**
IV	AEBT (400mg)	171.19±2.243**
V	EEBT (200mg)	169.68±2.258**
VI	EEBT (400mg)	169.75±1.316**
VII	Sibutramine(5mg)	172.30±1.521**

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. b) Group III, IV, V, VI, VII vs Group II ** p value< 0.01.

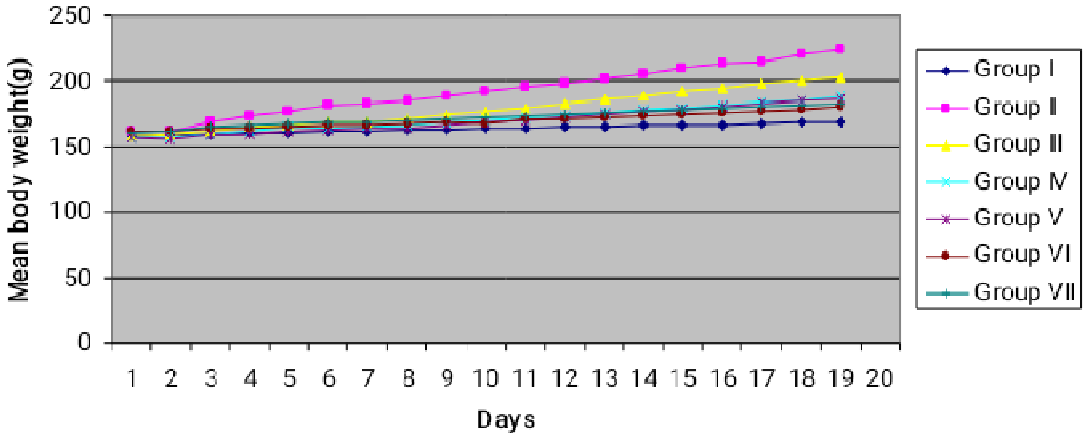

Figure 3: Effect of AEBT and EEBT on body weight (g) in rats

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 AEBT (200 and 400 mg/kg/p.o) and EEBT (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:7, Figure:4.

Table: 7Effect of EEBT and AEBT on daily feed intake(g) in rats

Group	Treatment	Feed intake
I	Control	136.58±0.6982
II	Diet control	155.53±2.294**
III	AEBT(200mg)	142.73±0.6229**
IV	AEBT(400mg)	133.25±0.4716**
V	EEBT(200mg)	122.14±1.087**
VI	EEBT(400mg)	109.74±2.428**
VII	Sibutramine(5mg)	94.25±2.428**

Values are food intake for group of 6 rats per day and mean ± SEM of 40 observations. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test. Comparisons were made between: a) Group I vs Group II. b) Group III, IV, V, VI, VII vs Group II ** p value< 0.01.

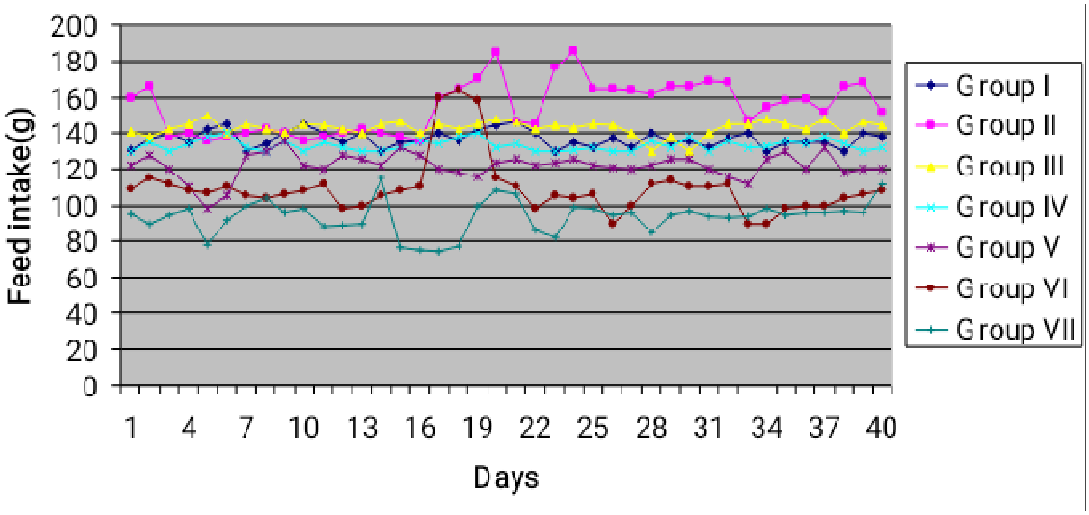

Figure: 4 Effect of AEBT and EEBT on daily feed intake (g) in rats

DISCUSSION

Obesityisa severe metabolic disorder, Characterized with increase in energy intake and a decrease in energy output concerning body weight and glucose metabolism. It may be underlying reason of cancers of breast, endometrium, colon and prostate. It is an increasing problem in modern society, due to the adoption of rapid lifestyles which results in high dietary intake of carbohydrates and fat accompanied by reduced energy consumptions [26].

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 [27].

The quantitative phytochemical investigation on the EEBV and AEBV was found to contain Alkaloids, carbohydrates, protein, sterols, flavanoids, glycosides, saponins, tannins. It has been reported that sterols, flavanoids, saponins, tanins lowers

cholesterol levels and have anti- oxidant and anti-diabetic potentials. Due to these constituents it was found to be useful in treatment of obesity. But the compound which causes weight reduction has to be identified [28]. [29].

Acute oral toxicity studies revealed the non-toxic nature of the ethanolic and aqueous extract of *Bauhinia tomentosa.* There was no lethality observed or any profound toxic reactions found at dose of 2000 mg/kg b.w/p.o and which indirectly pronounced the safety profile of the plant extract.

In the present study, the anti-obese activity of *Bauhinia tomentosa.*Aqueous and ethanolic extracts were studied using dietary animal's model of Obesity. Treatment with AEBT and EEBT resulted in reduction of body weight in HFD fed rats indicating that the extracts possess weight reducing property. Since obesity is associated with hyperphagia, HFD fed rats consumed more food than normal diet fed rats. AEBT and EEBT were 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 extracts.

CONCLUSION

Bauhinia tomentosa a widely known plant all over world and is being traditionally used for the cure and treatment of many ailments. On preliminary phytochemical analysis, the EEBT showed presence of various phytochemical constituents like Alkaloids, flavonoids, glycoside sterols, proteins, fixed oils, saponins, tannins. Gums and mucilage, terpenes, steroids, carbohydrates and flavones were absent. Phytochemical analysis, the AEBT showed presence of various phytochemical constituents like Alkaloids, flavonoids, glycosides, sterols, saponins, tannins. Gums and mucilage, proteins, fixed oils terpenes, steroids, carbohydrates and flavones were absent. The EEBT and AEBT were screened for acute oral toxicity and were found to be free from toxicity at dose of 2000 mg/kg/p.o. The EEBT and AEBT at two dose levels 200 mg/kg and 400 mg/kg and Sibutramine 5 mg/kg body weight were administered orally for 40 days to the HFD fed rats. It significantly reduced body weight, feed intake. From the observations of the study performed, it could be predicted that *Bauhinia tomentosa* root extracts 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 tomentosa* 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 tomentosa* root in the treatment of obesity. This ensures an understanding of the mechanism involved in the treatment of these disorders.

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