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EFFECT OF *BETULA UTILIS* BARK EXTRACT ON CAFETERIA DIET-INDUCED OBESITY IN RATS

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ABSTRACT
To evaluate the anti-obesity activity of *Betula utilis* bark against Cafeteria diet Induced obesity in rats. **Materials and Methods:** Obesity was induced in albino rats by feeding them a CD daily for 28 days, in addition to a normal diet. Body weight and food intake was measured initially and then every week thereafter. On day 28, the serum biochemical parameters were estimated and the animals were sacrificed with an overdose of ether. Orlistat was served as standard drug in the model. Evaluation of body weight, serum lipid profile and glucose level, liver weight and its TG level was carried out CD experimental model. Body temperature and locomotor activity was also evaluated. **Results:** Reported activity for the experimental models showed dose dependent significant anti-obesity activity for *Betula utilis* bark extract in terms of decrease in serum TG, TC, LDL-C, VLDL-C, liver TG and increased of serum HDL-C, locomotors activity in cafeteria diet induced obesity model rats. Body weight, liver weight and food intake also decreased. The result indicate that EBEB (200mg/kg) is more significant ($P<0.05$) than EBEB (100mg/kg) animals. **Conclusion:** In conclution, the findings of the study suggest that ethanolic bark extract of *Betula utilis* produces inhibitory effects on cafeteria diet-induced obesity in rats.

KEYWORDS: *Betula utilis*, Antiobesity, Cafeteria diet.

INTRODUCTION
Obesity means too much body fat; it is different from being overweight, which means weighing too much. Both terms mean that a person's weight is greater than what's considered healthy for his or her height. The balance between calories-in and calories-out differs for each person. Factors affecting weight include genetic makeup, overeating, eating high-fat foods, and not being physically active.[1]

Overweight and obesity are the fifth leading risk for global deaths. At least, 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity. WHO global estimates for the year 2008, reported 1.5 billion people were overweight, of these, over 200 million men and nearly 300 million women were obese. Overall, more than one in ten of the world's adult population was obese.[2]

At present only two drugs, sibutramine and orlistat have been approved for long-term use in the treatment of obesity. Each of these promotes 5 to 10% loss of body weight and has their own limitations and side effects.

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. Furthermore, the bark of *Betula utilis* plant are claimed in Ayurveda to control obesity.[3] However, there is no known scientific data are available to prove this claim. Hence in the present study, the ethnolic extract of the bark of *Betula utilis* is selected for antiobesity activity. It has been reported to possess Anti-inflammatory.[4], Anti oxidant[4], anti HIV.[5], Anti bacterial.[6], Mild Antihyperglycaemic[7] activity.

Hence, the objective of the present study is to investigate the effect of the ethanolic extract of *Betula utilis* bark on cafeteria diet induced obese rats. In addition, the possible mechanism of antiobese action of the extract was investigated by Evaluating of body weight, serum lipid profile and glucose, orlistat was used as the reference standard drug.

PLANT MATERIAL AND EXTRACTION[8]
The fresh *Betula utilis* bark used for the present studies was collected from Himachal pradesh, in July 2017. It was authenticated by Mr. Jayaprakash P.M, M.sc Botany, B.Ed HSST Botany Gvt. HSS Mappila. Koyilandy, Kozhikkode Kerala.

The bark of *Betula utilis* (Bhojpatra) was dried in shade. The dried bark of *Betula utilis* was cut into small pieces and then powdered with the help of mixer grinder. The powdered bark of *Betula utilis* (45 gm) was defatted with petroleum ether (1.25 L) and then the defatted powder of bark of *Betula utilis* was extracted with 95% ethanol (1.25 L) in the soxhlet apparatus at 40ºc for about 72 hours.

After the completion of extraction, the extract was concentrated at vacuum rotary evaporator to get a yellowish brown residue which will be placed in vacuum desiccator for 4-5 days for drying and then used for subsequent experiments.

Phytochemical study

The ethanolic bark extract of *Betula utilis* was subjected to standard phytochemical screening tests for various phytoconstituents.

EXPERIMENTAL ANIMALS

Healthy Wistar albino rats (150–200g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2^0C, Relative humidity 60 ± 5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (Approval no **SCP/IAEC/F150/P121/2017).** All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

Composition of Cafeteria diet[9]

The CD consisted of three diets

1. Condensed Milk (8g) + Bread (8 g).
2. Chocolate (3 g) + Biscuits (6 g) + Dried Coconut (6 g).
3. Cheese (8 g) + Boiled Potato (10 g).

The three diets were presented to the individual rats on days one, two, and three, respectively, and then repeated for 28 days in the same succession.

EXPERIMENTAL METHOD

The animals were divided into five groups of six animals each and individually housed in cages. The normal control group continued to be fed a laboratory pellet chow ad libitum (calorie value = 280 kcal/100 g). The cafeteria diet-control group received the CD in addition to the normal diet. The remaining two groups were fed with the CD along with the normal diet and received orlistat (10mg/kg/day i.p.) and ethanolic bark extract of *Betula utilis* (100 mg/kg/day & 200mg/kg/day p.o.), respectively. The treatment was continued for four weeks. The animals were weighed at the start of the experiment and then every week thereafter.

EVALUATION

Body Weight and Food Intake: The body weight and food intake was recorded every week till completion of the experiment.

Body Temperature: The body temperature was measured using a rectal thermometer on the last day of experiment with a contact time of one minute.

Biochemical Estimations: After completion of the experiment, blood was collected by retro-orbital puncture from the ether-anesthetized rats and serum was separated for estimation of biochemical parameters.

Estimation of liver weight and liver triglyceride content: After completion of the experiment, the animals were sacrificed with an overdose of diethyl ether. The liver was quickly removed, weighed and used for estimation of triglyceride.

Table 1: Effect of EBEB on body weight and serum lipid profile in rats fed with cafeteria diet induced obesity.

Sl No.	Groups	Body weight difference(g)	Serum TG (mg/dl)	Serum TC (mg/dl)	Serum HDL-C (mg/dl)
1	Normal Control group	17.50±0.28	82.28±0.23	98.05±0.14	60.44±0.15
2	CD Obese control group	37.25±0.21	160.5±0.98	210.5±0.18	36.38±0.16
3	CD and Orlistat treated group	21.08±0.23***	104.1±0.14***	120.3±0.23***	53.66±0.16***
4	CD and EBEB (100mg/kg) treated group	31.08±0.23**	135.3±0.72**	162.2±0.18**	44.28±0.14**
5	CD and EBEB (200mg/kg) treated group	25±0.31***	115.8±0.33**	132.2±0.21***	50.42±0.17***

Values are expressed as mean ±SEM. n=6 for each group. Significance between various groups at *P<0.05 by one way ANOVA followed by Dunnet's t-test using GraphPad Prism 5.01.

Table 2: Effect of EBEB on serum lipid profile, glucose levels & liver TG level in rats with cafeteria diet induced obesity.

Sl no	Groups	Serum LDL-C (mg/dl)	Serum VLDL-C (mg/dl)	Serum glucose (mg/dl)	Liver TG (mg/g)
1	Normal Control goup	55.40±0.25	17.01±0.16	88.65±0.26	87.86±0.09
2	CD obese control group	205.2±0.30	33.64±0.20	137.0±0.17	165.6±0.14
3	CD and Orlistat treated group	87.94±0.31***	21.58±0.16***	100.5±0.19***	107.6±0.12***
4	CD and EBEB (100mg/kg) treated group	144.4±0.18**	26.87±0.10*	122.6±0.14***	136.1±0.24**
5	CD and EBEB (200mg/kg) treated group	105.4±0.16***	23.04±0.64**	111.6±0.15***	116.5±0.20***

Values are expressed as mean ±SEM. n=6 for each group. Significance between various groups at *P<0.05 by one way ANOVA followed by Dunnet's t-test using GraphPad Prism 5.01.

Table 3: Effect of EBEB on Liver weight, food intake body temperature & locomotor activity in rats with cafeteria diet induced obesity.

Sl no	Groups	Liver weight (g)	Food intake (g/day/rat)	Body temperature(0C)	Locomotor activity
1	Normal Control group	5.28±0.12	6.80±0.09	36.02±0.004	167.6±0.11
2	CD obese control group	10.27±0.09	12.81±0.09	36.13±0.007	106.5±0.11
3	CD and Orlistat treated group	6.21±0.08***	8.70±0.09***	36.28±0.008	150.5±0.13***
4	CD and EBEB (100mg/kg) treated group	8.44±0.06*	11.26±0.06***	36.54±0.007	132.2±0.19**
5	CD and EBEB (200mg/kg) treated group	6.72±0.08***	10.18±0.08***	36.67±0.04	144.5±0.10***

Values are expressed as mean ±SEM. n=6 for each group. Significance between various groups at *P<0.05 by one way ANOVA followed by Dunnet's t-test using GraphPad Prism 5.01.

RESULTS

Preliminary phytochemical screening

Phytochemical screening revealed the presence of, Flavonoids, Alkaloids, and Carbohydrates compounds in EBEB.

Effect on serum triglyceride (TG) level

Serum TG level significantly increased in the cafeteria diet induced obese animals compared to control animals (P<0.05). Both Orlistat (10mg/kg) and EBEB (100mg/kg), (200mg/kg) treated animals significantly decreased serum TG level compared to cafeteria diet induced obese animals as shown in table 1.

Effect on serum total cholesterol (TC) level

There was significant increase in the serum total cholesterol of cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals significantly decreased serum total cholesterol compared to cafeteria diet induced obese animals as shown in the table 1.

Effect on serum HDL cholesterol (HDL-C) level

The serum HDL cholesterol significantly decreased in cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals significantly increased serum HDL, cholesterol compared to cafeteria diet induced obese animals as shown in table 1.

Effect on serum LDL cholesterol (LDL-C) level

The serum LDL cholesterol significantly increased in cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals significantly decreased serum LDL cholesterol compared to cafeteria diet induced obese animals as shown in table 2.

Effect on serum VLDL cholesterol (VLDL-C) level

There was significant increase in the serum VLDL cholesterol level in cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10 mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals significantly decreased serum VLDL cholesterol compared to cafeteria diet induced obese animals as shown in table 2.

Effect on serum glucose level

The serum glucose level significantly increased in cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) significantly decreased serum glucose level compared to cafeteria diet induced obese rat as shown in table 2. Both EBEB (100mg/kg) and (200mg/kg) treated animals significantly decreased serum glucose compared to cafeteria diet induced obese animals.

Effect of EBEB on Liver TG level in rats with cafeteria diet induced obesity

Liver TG level significantly increased in the cafeteria diet induced obese animals compared to control animals (P<0.05). Both Orlistat (10mg/kg) and EBEB (100mg/kg), (200mg/kg) treated animals significantly decreased liver TG level compared to cafeteria diet induced obese animals as shown in table 2.

Effect of EBEB on Liver weight in rats with cafeteria diet induced obesity

There was significant increase in the liver weight of

cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) and both EBEB (100mg/kg) & (200mg/kg) treated animals significantly reduced liver weight compared to cafeteria diet induced obese animals as shown in table 3.

Effect of EBEB on food intake in rats with cafeteria diet induced obesity

There was significant increase in the food intake of cafeteria diet induced obese animals compared to control animals (P<0.05). Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals significantly reduced food intake compared to cafeteria diet induced obese animals as shown in table 3.

Effect of EBEB on body temperature in rats with cafeteria diet induced obesity

There was significant increase in body temperature of cafeteria diet induced obese animals compared to control animals (P<0.05). A little but significant ($p < 0.05$) increase in body temperature was also observed in Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) treated animals as shown in table 3.

Effect of EBEB on locomotor activity in rats with cafeteria diet induced obesity

There was significant decrease in locomotor activity in cafeteria diet induced obese animals as compared to control group ($p < 0.05$). Treatment with Orlistat (10mg/kg) and both EBEB (100mg/kg), (200mg/kg) resulted in significant increase in locomotor activity in cafeteria diet animals as shown in Table 3.

DISCUSSION

Obesity is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure. Among the multiple factors contributing to its etiology, the sedentary life styles, white collar jobs, lack of exercise, psychological factors and the consumption of energy rich diets are the major ones. It is characterized by enlarged fat mass and elevated lipid concentration in blood.

Various animal models of obesity have been used to emulate obesity-like condition in humans, in order to develop effective antiobesity treatments. Among the animal models of obesity, rats that are fed a high-fat diet are considered useful; a high percentage of fat in their diet is considered to be an important factor in the development of obesity, leading to the accumulation of body fat, even in the absence of an increase in calorie intake.[10]

The present study showed that the effects of ethanolic bark extracts of *Betula utilis* for 28 consecutive days, by p.o. administration, on body weight changes, locomotor activity, change in body temperature, blood lipid levels, liver weight, liver TG level and food intake in normal and CD fed rats and in fructose fed rats. In our study, initially a pharmacognostic and phytochemical analysis was carried out in the phytochemical investigation. It was clear that *Betula utilis* contained carbohydrates, flavonoids and alkaloids. Some chemical constituents like alkaloids and flavonoids, have been noted for their anti-obesity effect in various plants.[11]

The socalled 'cafeteria diet' involves feeding experimental animals a mixture of palatable commercially available supermarket foods, to stimulate energy intake.[12]

Characteristic for such diets is the combination of the high fat content with high carbohydrate content. Furthermore, the components of the cafeteria diet are a variety of foods high in fat and sugar, but usually low in protein, vitamins, and minerals. Such diets have pronounced implications in the development of obesity, leading to significant body weight gain, fat deposition, and also insulin resistance, resembling that in human beings.[13] It has been suggested that rats become more obese with cafeteria diets than with pure high-fat diets and normal chow diet, indicating a greater hyperphagia arising from the food variety, texture, and palatability.[13]

Orlistat was most significant in this case. Several studies show that an increase in HDL cholesterol is associated with a decrease in cardiovascular risk, which is a major complication of obesity-associated dyslipidemia, and most of the drugs that decrease total cholesterol also decrease HDL cholesterol.[14] However, in the present study the extract decreased the total cholesterol and LDL cholesterol, and enhanced the HDL cholesterol significantly. This is an important advantage in the treatment of hypercholesterolemia, especially among Indians, where low HDL cholesterol is the prevalent lipoprotein abnormality.[15]

The decrease in serum TG level is an important finding of this experiment. Recent studies have shown that triglycerides are independently related to obesity-induced cardiovascular complications.[16,17] and most of the antihypercholesterolemic drugs (can also be used to correct obesity-associated dyslipidemia) do not decrease the triglyceride levels, but EBEB had lowered it significantly when compared to the obese control group. flavonoids are the bioactive phytoconstituents in *Betula utilis* which may be responsible for its anti- obesity activity.

Administration of EBEB and standard Orlistat have shown a significant increase in locomotor activity compared to obese control rats. The increases in rectal temperature by EBEB may be attributed to the thermogenic property of the phytoconstituents in the extracts. In cafeteria diet induced obese rats showed elevated levels of serum glucose, Triglycerides (TG), Total cholesterol (TC), and Low density lipoprotein cholesterol (LDL-C), and a fall in the HDL-C levels as compared to normal control animals, which was

significantly decreased by the administration of EBEB 100 and 200 mg/kg.

The present study confirms the rational basis for its use in traditional medicine for the treatment of obesity. However, further studies are under progress to isolate and characterize the phytoconstituents responsible for the antiobese activity.

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