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Possible Anti-Obesity Activity of Methanol Extract of *Byttneria pilosa* Roxb. Leaves

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Abstract: The purpose of this study was to investigate the possible anti-obesity activity of methanol extract of *Byttneria pilosa* Roxb. (MEBP) on Swiss albino mice by inducing anorexia. 18 h fasted mice were administered with different doses of MEBP (200 and 400mg/kg, orally) and the amount of food intake was measured hourly for a period of 5 h. Weight of mice of each groups was determined prior to the experiment and 48 h after the experiment. In another experiment, percentage of gastric emptying at 4th h was ascertained after the administration of MEBP (200 and 400mg/kg, orally) in different groups of mice which had free access to pre-weighed food for 1, 2 or 4 h. MEBP significantly reduced the cumulative food intake over a 5 h period in dose dependent manner. The percentage reduction of cumulative food intake at the end of 5th h in MEBP treated mice was 12.7% (200mg/kg) and 14.34% (400mg/kg). Moreover, there was a significant weight reduction in MEBP treated mice compared to control. This study reveals for the first time, a possible anorectic activity of *Byttneria pilosa*, most probably mediated through the CNS without affecting the gastric emptying rate. However, to be established as an anti-obesity agent, further investigation can be done to find out the possible mechanism and specific chemical agent.

Key words: *Byttneria pilosa* · Anti-obesity · Gastric emptying · Anorexia

INTRODUCTION

Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide, resulting into a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke and cancers [1-3]. Prevention and treatment of this problem are an important deal for health systems, whose aim is to reduce the obesity and overweight prevalence and related complications over the world [4]. Both lifestyle and pharmacotherapy interventions have been considered by physicians and other health care professionals as obesity treatment modalities. Studies show that only 5-10 % subjects can maintain their weight loss over the years [5]. The complex pathogenesis of obesity indicates the need of different intervention strategies to confront this problem with a simple drug therapy which is more acceptable to patients [4]. Disappointing results, after cessation the lifestyle modification or pharmacotherapy indicated the need of other treatment modalities to produce better and long lasting results, in terms of weight loss [6]. Herbal supplements and diet-based therapies for weight loss are among the most common complementary and alternative medicine [CAM] modalities [7]. A vast range of these natural products and medicinal plants, including crude extracts and isolated compounds from plants can be used to induce weight loss and prevent diet-induced obesity. In the recent decades, these have been vastly used in management of obesity [4,8] due to containing a large variety of several components with different anti-obesity and anti-oxidant effects on body metabolism and fat oxidation. Medicinal plants have been investigated and reported to be useful in treatment of obesity, diabetes and other chronic diseases [9,10].

Byttneria pilosa Roxb. (Family: Sterculiaceae) is a large woody climber with grooved, strigose, branchlets. Leaves are suborbicular, palmately 3-lobed, pilose on both surfaces. Flowers are minute campanulate, in a lax much branched inflorescence. Capsule is globose, size of a large

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chery, studded with subulate barbed prickles. Locally it is called 'Harjora' as it is used in the treatment of fractured bones. The crushed stems are applied to affected areas for the treatment of boils; an infusion of the leaves used in baths for the treatment of scabies by the Chakma people. Paste prepared from tender stem with leaves is tied to around limbs for the treatment of fractured bones by the Khumi community. Roots are used to prepare a paste, which is applied to affected areas for the treatment of elephantiasis in Tripura community. It is found in Forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Srimongal, Gajni (Sherpur) and Habiganj of Bangladesh [11].

In this report, we investigated the presence of different chemical constituents and possible anti-obesity activity of *Byttneria pilosa* on Swiss albino mice.

MATERIALS AND METHODS

Collection and Authentication of Plant Material: The leaves of *Byttneria pilosa* were collected from Chittagong hill tracts area specifically from the area of Department of forestry, University of Chittagong in May, 2014 and it was authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist & Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh. Voucher specimen of the plant was deposited in the herbarium of Department of Botany, University of Chittagong.

Methanol Extract Preparation: The collected plant was washed thoroughly with water and air dried for a week at 35 to 40°C and pulverized in electric grinder. The obtained powder was successively added to methanol with vigorous shaking in rotary shaker machine for 7 days. Then filtered by muslin cloth and no.1 Whatman filter paper successively. The Methanol was evaporated at a temperature below 45°C and concentrated extract was weighed and stored at 4°C.

Phytochemical Screening: The phytochemical examination of methanol extract of *Byttneria pilosa* was performed by the standard methods [12].

Experimental Animal: Colony inbred strains of Swiss albino mice weighing 30-35g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm at 22 ± 10°C room temperature, in polypropylene cages. The animals were fed on standard pelleted diet and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. All experiments involving animals were conducted according to the UK Home Office regulations (UK Animals Scientific Procedures Act 1986) and the 'Principles of Laboratory Animal Care' (National Institutes of Health publication no. 86-23, revised 1985).

Acute Toxicity Test: The acute toxicity of MEBP was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the mice even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [13].

Measurement of Food Intake in Mice: Mice fasted for 18 h (with free access to water) were placed in individual cages. One hour later mice received vehicle (distilled water) or MEBP (200 and 400mg/kg). 30 min after the administration of either vehicle or MEBP, pre-weighed food (5gm) was placed and at every hour the food was replaced by removing all the solid pellets and also the spill. The intake was determined by the difference between the pre-weighed food and the weight of food and spill left at the end of each hour for a period of 5 h [14]. After 48 h of the experiment, the weight of each mouse was taken and compared with the weights taken before the experiment to ensure the weight reduction.

Food Intake and Gastric Emptying of Solid Meal in Mice: Mice divided into 9 groups of 3 mice each were fasted for 18 h but had access to water before and during the experiment. Fasted mice were placed in individual cages 1 h before and then received either vehicle or MEBP (200 and 400mg/kg). Immediately after the administration of extract, the mice had free access to pre-weighed (1gm for each mouse) food for either 1, 2 or 4 h. The animals were sacrificed at the end of 4^{th} h by chloroform. The stomach was identified and the pyloric and the cardiac ends were ligated. The isolated stomach was cut open, the contents were removed and wet contents were weighed. The gastric emptying was calculated [14] according to the formula:

% of Gastric emptying (GE) = (1- weight of wet food recovered from stomach/ Weight of intake food) ×100

Statistical Analysis: Data analysis was done using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. $P<0.001$(first experiment) and $P<0.05$(second experiment) were considered statistically significant. The statistical analysis was carried out by GraphPad Prism ver.5.04.

RESULTS

The methanol extract of *Byttneria pilosa* significantly reduced the cumulative food intake over a 5h period in mice, in a dose-dependent manner (Table 1). For MEBP 200 and 400mg/kg, from the first hour, there was a significant reduction in cumulative food intake when compared to control. At the fifth hour the percentage reduction of food intake was 12.7% and 14.34% with 200 and 400mg/kg of MEBP respectively (Table 1). The total intake of food was also significantly ($P<0.001$) reduced with all doses of MEBP-treated mice.

There was a significant weight reduction in the MEBP treated mice in comparison to control mice after 48 h. The control group mice gained the weight slightly as usual (Fig. 1).

The fourth hour gastric emptying by MEBP (200 and 400mg/kg) was also statistically significant when compared to control (Table 2). Control animals exposed to food for 1 h had %GE of 91.67±2.028 while 200 and 400mg/kg of MEBP-treated animals had 82±2.646, 75±4.359 of 4th h % GE respectively ($P<0.05$). Similarly, the MEBP (200 and 400mg/kg) treated animals exposed to food for 4 h had 4^{th} h % GE of 68.67±7.265, 51.33±1.33 respectively which were also significant when compared to control, 71.00±2.646.

The phytochemical screening revealed the presence of terpenoids, phenols, tannins, flavonoids, alkaloids, carbohydrates, proteins, saponins, cardiac glycosides, steroids (Table 3).

DISCUSSION

Obesity is a severe metabolic disorder, Characterized with increase in energy intake and a decrease in energy output concerning body weight and glucose metabolism. The present study was carried out to investigate the anorectic activity of methanol extract of *Byttneria pilosa* on Swiss albino mice. The study reveals that MEBP produces reduction of food intake in mice which suggests the anorectic action of MEBP. However, the exact mechanism through which MEBP causes this effect was not a concern of this study and needed to be established. Although the role of hypothalamus in feeding is well known, peripheral factors also play an important role in modifying the feeding behavior. Hence, it was of interest to see whether gastric emptying could cause reduction in food intake as this also plays an important regulatory role in food intake [15,16]. Rapid gastric emptying of food has a causal relationship with overeating and obesity [15] whereas delayed gastric emptying reduces food intake or produces satiety [16,17]. Although, the result from the 4 h gastric emptying study in mice revealed significant change in gastric emptying in the extract-treated animals compared to the control, decision cannot be made whether the anorectic effect is due to gastric emptying or not. In the present study the stomach contents were lesser in quantity in MEBP-treated mice than the vehicle-treated group, probably due to decreased food

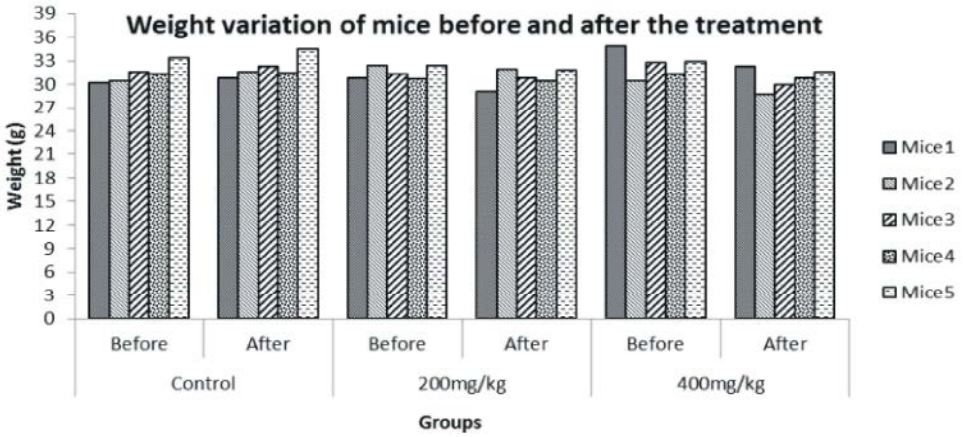

Fig. 1: Weight variation of mice before and 48 h after the treatment with vehicle and MEBP

Table 1: Effect of methanol extract of *Byttneria pilosa* on cumulative food intake in mice

		Cumulative food intake (g) at				
Treatment n=5	Dose (mg/kg)	1st h	2nd h	3rd h	4th h	5th h
Control (water)	0.2ml/10g	2.40±0.0088	3.78±0.0120	4.58±0.0120	4.68±0.0145	4.88±0.0088
MEBP	200	1.41±0.0176* (41.25)	3.11±0.0186* (17.71)	4.13±0.0120* (9.83)	4.20±0.0120* (10.26)	4.26±0.0145* (12.7)
	400	1.01±0.0145* (57.92)	3.04±0.0133* (19.58)	3.19±0.0058* (30.35)	3.30±0.0153* (29.49)	4.18±0.0088* (14.34)
One-way Anova	R square	0.9992	0.9976	0.9992	0.9994	0.9980

Here, MEBP= methanol extract of *Byttneria pilosa*; n= number of mice per group; **P<0.001 Vs. control (Dunnett's multiple comparison).* Values are mean± SEM and those within the parenthesis represent % reduction of food intake

Table 2: Effect of methanol extract of *Byttneria pilosa* (MEBP) on 4h gastric emptying at various duration of exposure to food in mice

Treatment	Dose (mg/kg)	Exposure to food (h)	4h % GE
Control (water)	0.2ml/10g	1	91.67±2.028
		2	93.67±1.453
		4	71.00±2.646
MEBP	200	1	82±2.646
		2	76.33±5.897*
		4	68.67±7.265
	400	1	75±4.359*
		2	73.67±2.603*
		4	51.33±1.33*

All values expressed as mean± SEM. n=3; **P<0.05(Dunnett's multiple comparison)*

Table 3: Phytochemical screening of leaves extract of *Byttneria pilosa*

Constituents	Results
Alkaloids	+
Carbohydrates	+
Cardiac glycosides	+
Steroids	+
Flavanoids	+
Saponins	+
Protein	+
Terpenoids	+
Phenols	+
Tennins	+
Reducing sugar	-

Note: (+): Present; (-): Absent

intake but had similar gastric emptying time. Therefore the data indicates that the MEBP-induced reduction of food intake during the first 4 h is not related to the gastric volume-related satiety signal. Hence, it can be suggested that gastric emptying does not play a major role in producing satiety or reduction of food intake suggesting a central role for MEBP in causing the reduction of food intake. A similar study proposed the serotonin inhibiting mechanism [18]. A significant decrease in appetite was shown in trials by *Trigonella Foenum-graecum* L. [19].

The quantitative phytochemical investigation on the MEBP was found to contain Alkaloids, carbohydrates, protein, steroids, flavanoids, glycosides, saponins, tanins. It has been reported that flavanoids, saponins, tannins lowers the 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 [20, 21].

Although it would be very tempting to suggest further investigation on this mechanism of action of MEBP, its role of serotonin reuptake inhibition in the reduction of food intake [22] cannot be overlooked.

CONCLUSION

From the study, it is apparent that methanol extract of *Byttneria pilosa* possesses anorectic activity. Physiological mechanisms regulating the food intake are comprehensive and body weight is also a considerable factor. So, further studies are needed to determine the mechanism through which MEBP causes reduction in food intake and its therapeutic potential as an anti-obesity agent.

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