ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Evaluation of Antiobesity Activity of *Bryophyllum pinnatum* (Lam) Oken Leaves Extract in Cafeteria Diet Induced Obesity in Rats

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Abstract

Present study was aimed to scrutinize the antiobesity activity of *Bryophyllum pinnatum* (Lam) Oken leaves aqueous extract (AEBP) in the cafeteria diet-induced obesity in rats. Female Wistar rats weighing between 100-150 g were allocated in five groups (n=6) i.e. normal control, obesity control, standard drug (Orlistat) treated group, test drug (200 mg/kg and 400 mg/kg AEBP) treated groups. The result revealed that *B. pinnatum* leaves prevented the increase in BMI, food intake, body weight, glucose, triglycerides and cholesterol level without affecting water intake. This study provides evidence for the antiobesity activity of *Bryophyllum pinnatum* (Lam) Oken leaves.

Keywords: Antiobesity, *Bryophyllum pinnatum*, cafeteria diet-induced obesity, lipase inhibitory activity, obesity

Asian Pac. J. Health Sci., (2021); DOI: 10.21276/apjhs.2021.8.3.35

Introduction

Obesity is a chronic, stigmatized and exorbitant disease and its pervasiveness have increased throughout the world. It arises due to inactive lifestyles, consumption of high-calorie diet, absence of workout, environmental variations, emotional and genetic factors[1,2]. According to 2016 National Institute of Health data, 1.9 billion adults (≤18 years) were overweight (BMI more than 25 kg/m^2) and out of these 650 million were found to be obese (BMI more than 30 kg/m^2). 41 million children (>5 years) and 340 million children (<5 years) were having BMI of more than 25kg/m^2. Due to the effects of obesity nearly 2.8 million deaths has been reported[3]. Obesity is commencing to swap with under-nutrition and infectious diseases as the supreme contributor to ill health. Obesity is a multifactorial ailment of energy equilibrium wherein energy intake surpasses energy expenditure[4]. Surplus energy is warehoused in adipose tissue and leads to adiposity (increase levels of triglycerides in adipocytes)[5]. The term obesity means fat or plump and stout which had been coined from the Latin word obesitas. A surrogate measure of body fatness is elevated body mass index (BMI), which expresses weight (in kilograms) as a function of body weight (in meters^2)[6]. According to the World health organization (WHO) adults with a body mass index of more than 25 kg/m^2 and 30 kg/m^2 are classified as being overweight and obese singly.

Bryophyllum pinnatum (Lamark) Oken (synonyms: *Bryophyllum calycium, Kalanchoe pinnata*; generally known as life plant, air plant [Mexican], love plant, miracle leaf, canterbury bells, Cathedral bells, Parnbija, Patharchatta, Zakhm-e-hyat [Hindi], Goodluck or resurrection plant is extensively dispersed in tropical Africa, America, Hawaii, China, India, Australia and Madagascar and has been used as folk medicine[7,8].

Its leaves seem to be the choicest part of the plant due to its medicinal purposes. Researchers have reported a variety of medicinal properties in leaves of *Bryophyllum pinnatum* including anti-tumora[9,10], antiulcer[11], antimicrobial[12], hepatoprotective[13], wound healing[14], tocolytic, neurosedative and muscle relaxant[17,18], antinociceptive, anti-inflammatory, antidiabetic[19], analgesic[20] and renal protective activity[21].

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How to cite this article: Chandra K, Kumar P, Kaur V, Kumar Y, Kumar T. Evaluation of Antiobesity Activity of *Bryophyllum pinnatum* (Lam) Oken Leaves Extract in Cafeteria Diet Induced Obesity in Rats. Asian Pac. J. Health Sci., 2021;8(3):194-200.

Source of support: Nil

Conflicts of interest: None.

Received: 01/06/2021 **Revised:** 22/06/2021 **Accepted:** 13/07/2021

Material and Methods

Animals

Female Wistar rats weighing between 100-150 g were taken from Departmental Animal house. The animals were housed in animal house according to OECD guideline for animals with natural 12 hour light-dark cycle, humidity (30-70%) and controlled temperature 22ºC (± 3ºC). The animals were familiarized to the environment before the preceding experiment with liberty to water and pellet diet for rats. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) with protocol number KUDOPS/113.

Plant Material

Leaves of *Bryophyllum pinnatum* (Lam) Oken were collected from Kotabag region of Nainital (Uttarakhand), during the month of September. The plant was authenticated from the Botanical Survey of India (BSI) Dehradun, Uttarakhand.

Preparation of Extract

Plant leaves were shade dried at room temperature and blended to powder. The measured amount of powder was then soaked in Distilled water for 72 hours. After 3 days filtrate was filtered using whatman filter paper. Excess water was evaporated using rotary vacuum evaporator at 45°C [21].

Phytochemical Screening

Qualitative analysis was performed of AEBP for identifying chemical constituents present in extract.[22]

Determination of Total Phenolic Contents in AEBP

Total phenolic contents were assessed by the addition of 0.5 ml of Folin-Ciocalteu reagent to a solution comprising of mixture of 1 mL of test sample (1 mg/mL) and 3 mL of distilled water. The solution was then mixed properly and after 3 min, 0.5 mL of 2% sodium carbonate solution was added to it. The mixture was incubated for 90 min at 25°C, then absorbance was measured using UV-spectroscopy at 430 nm. The assay was performed in triplicate for each test sample (AEBP or Orlistat) and gallic acid was taken as standard phenolic compound for comparison. The total phenolic content was calculated by a standard gallic acid graph and the results were expressed in mg of gallic acid equivalents (GAE) per gram of the extract.

Determination of Total Flavonoid Contents in AEPB

The total flavonoid contents were determined by taking Quercetin as standard flavonoid containing compound. 2% aluminum chloride and methanol solution (1 mL) and sample or Orlistat (1 mL) was taken in a test tube. Then samples were incubated for 15 min at 37° C temperature and absorbance was measured using Shimadzu UV-Vis spectrophotometer at 430 nm. Total flavonoids content was calculated by a standard Quercetin graph and the result is expressed in mg of quercetin equivalent (QE) per g of the extract[23].

Lipase Inhibitory Activity *in-vitro*

Samples (25-200 µg/ml) were prepared of AEBP and orlistat in DMSO of concentration 1mg/mL. Porcine pancreas lipase enzyme powder was dissolved in Tris-buffer (50 mM, pH 8) with concentration of 0.1mg/mL and solution was mixed properly. Then 1 mL of a sample (Extract or Orlistat) was taken and to it 0.5 mL lipase solution was added. It was then incubated (30 min at 37°C), after incubation 1mL caprylic acid (substrate) was added into it. Again the mixture was incubated (2 hours at 37°C) and then absorbance was recorded at 410nm against a blank. The percent inhibition (%) of pancreas enzyme (lipase) of samples (AEBP and Orlistat) was calculated using the following formula:

%Activity = [Absorbance of control-Absorbance of sample/ Absorbance of control] × 10[24].

In-vivo Anti-Obesity Activity

Preparation of Cafeteria diet

Supermarket foods were taken as the Cafeteria diet. It was prepared by taking chocolate (10%), butter cookies (20%), dry coconut (5%), butter (5%), boiled potatoes (40%) and white bread (20%), which was then shaped into a ball (sphere shaped)[25].

Induction of obesity and drug treatment[26]

Female Wistar rats were randomly divided into five groups comprising of 6 animals in each. Group I (normal control) was served with a standard pellet diet (300 g) and distilled water (400 mL). Group II (obesity control) was served with a standard pellet diet (300 g), distilled water (400 mL) and cafeteria diet (80 g). Group III (standard group) was served with a standard pellet diet (300 g), distilled water (400 mL), cafeteria diet (80 g) and Orlistat 30 mg/kg/day. Group IV (low dose test group) was served with a standard pellet diet (300 g), distilled water (400 mL), cafeteria diet (80 g) and 200 mg/kg/day AEBP. Group V (high dose test group) was served with a standard pellet diet (300 g), distilled water (400 mL), cafeteria diet (80 g) and 400 mg/kg/day AEBP.

Parameters of Evaluation

Percentage of change in body weight (%)

Calculated by using formula "Percentage of change in body weight (%) = [Body weight at the end of 'n' week (g) – Body weight on day 1 (g)/Body weight on day 1] × 100".

Food consumption

All animals were retained individually in polypropylene cages. A measured quantity of food was kept in each cage every day. Then on the next day, the leftover food was weighed. Cafeteria diet is provided as a supplement to the standard pellet diet. Food consumption per 100g of body weight of animals was calculated as:

Diet consumed (g) by animal = total diet provided (g) – total diet remained (g)

Diet consumed per 100 g body weight (g) = (Diet consumed in 'n' week/mean body weight in that week) × 100

Water intake

Animals were provided with a measured volume of water every day. Next morning leftover volume was pen down to calculate water intake per 100 g of body weight.

Water consumed (mL) = Water provided (mL) – Water remaining (mL)

Water intake per 100 g of body weight (mL) = (Water consumed in 'n' week/mean body weight in that week) × 100

Body mass index

BMI of the animal was calculated by using formula "BMI = Weight of body (kg)/square of height (m^2)" before starting experiment and then on last day (98 day).

Behavioral parameters - Locomotor activity test

Locomotor activity was assessed by actophotometer. The number of cut-offs of the photoelectric cells was counted for each rat for 10 min for the measure of locomotor activity.

Rotarod test

Motor coordination in rats was evaluated via rota-rod by recording the fall of time of rat by placing each animal on accelerating rod of the instrument at 20 rpm of speed.

Biochemical parameters

Blood was collected from retro-orbital plexus in anesthetized rats on day 1 and day 98 of each animal in all five groups. Collected blood was subjected to centrifugation to obtain serum. Changes in glucose, total cholesterol, triglycerides, SGPT, and SGOT levels were measured using semi-auto analyser[27].

Statistical Analysis

Results of different parameters are expressed as mean ± SD and mean ± SEM of 6 rats per group. Statistical comparison of the data was performed using One way analysis of variance (ANOVA) followed by Turkey's test. The *P* values less than 0.05 ($P<0.05$), 0.01 ($P<0.001$) and 0.01($P<0.01$), were considered significant.

Results

Preliminary qualitative analysis (Table 1) revealed the presence of flavonoids, tannins, phenols, glycosides, steroids, carbohydrates, proteins and absence of alkaloids in prepared AEBP.

Total Phenolic and Flavonoid Content

The total phenolic content (Figure 1) was calculated using the gallic acid standard curve (y=0.003x+0.4821: R^2 =1). The total phenolic content of AEBP was 0.74±0.20 mg/g GAE. The total flavonoid content (Figure 2) was calculated using a calibration curve of Quercetin standard curve (y=0.0061x+0.042: R^2 = 0.9996). The total flavonoid content of aqueous extract of *B.pinnatum* was found to be 1.586667±0.2554602 mg/g Quercetin equivalent/g of extract.

Table 1: phytochemical screening of aqueous extract of *B. pinnatum* leaves

S.No	*Phytochemicals*	*AEBP*
1	Flavonoids	+
2	Tannins	+
3	Phenols	+
4	Saponins	+
5	Carbohydrates	+
6	Steroids	+
7	Proteins	+
8	Alkaloids	-

+ Tests positive; - Tests negative

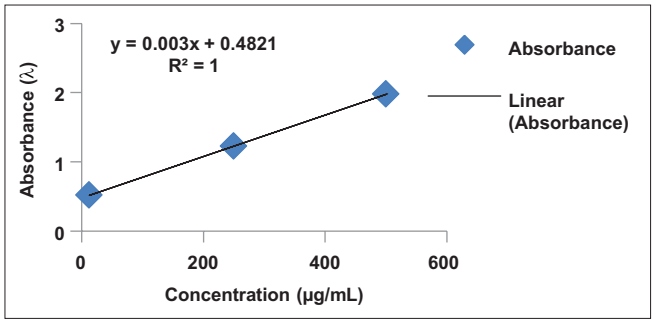

Figure1: Calibration curve of Gallic acid

Pancreatic lipase inhibitory activity of Orlistat (standard drug) showed higher inhibition of pancreatic lipase at a concentration 200 µg/ml (86.78 %). It's IC50 (µg/ml) values was 62.445, 66.12 and 73.47. The highest inhibition due to Bryophyllum pinnatum leaves extract was at a concentration 200 µg/ml (72.18%). IC50 (µg/ml) values of AEBP was 45.895, 49.92, 57.97 and 74.07. Table 2 showed concentration dependent inhibition of lipase enzyme.

Effect of *Bryophyllum pinnatum* Extract on Food and Water Intake

No significant difference (P>0.05) was found in water intake (Figure 3), while there was a significant (P<0.5) difference in food intake before and after study (Figure 4). Obesity control group showed an increase in daily food intake and there was significant difference (*P<0.05) when compared to the normal control group. AEBP 200 mg/kg, 400 mg/kg and standard drug (Orlistat 30mg/kg) caused a significant decrease (***P<0.001) in food intake when compared to obesity control group.

Effect on Body Weight

The cafeteria diet significantly increased (***P<0.001) body weight in obesity control group compared to normal control group in 14 weeks of study (Figure 5). AEBP treated groups significantly (*P<0.05) prevented the increase in % change in body weight as compared to the obesity control group.

Effect on Body Mass Index (BMI)

On the last day of animal study, it was found that there was a significant increase in BMI in the obesity control group (***P<0.001) when compared with the normal group (Figure 6).

Table 2: Pancreatic lipase inhibitory activity

Samples	*Concentration (ug/mL)*	*% Inhibition*	*IC50 value*
Orlistat (Standard drug)	25	59.24	62.445
	50	68.01	66.12
	100	76.35	73.47
	200	86.78	88.17
Aqueous extract of *B.pinnatum*(AEBP)	25	43.25	45.895
	50	50.06	49.92
	100	62.44	57.97
	200	72.18	74.07

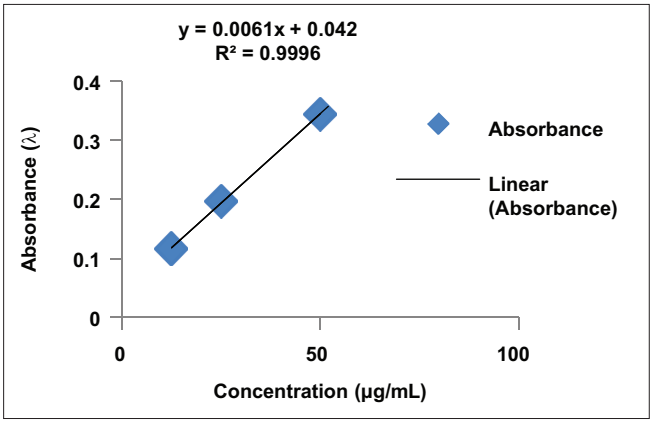

Figure 2: Calibration curve of Quercetin

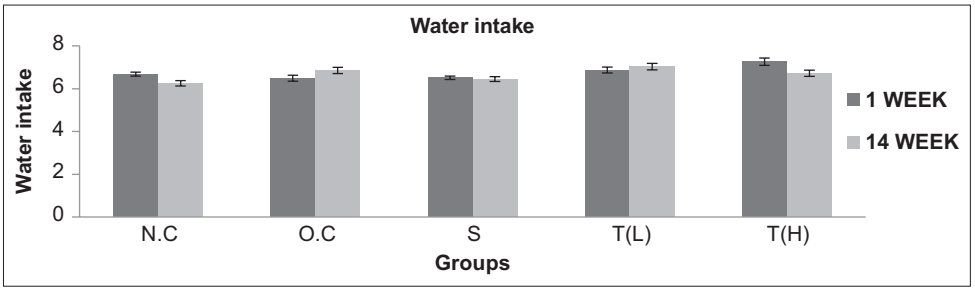

Figure 3: depicts comparison of food intake among groups by rats before and after 14 weeks of study, all values are expressed as Mean ± SEM. After study there was significant difference P<0.5 in food intake when compared to food intake before study, which was analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test. Where, N.C-Normal control, O.C-Obesity control, STD-Standard drug (Orlistat 30mg/kg) treated group, T(l)-200 mg/kg AEBP and T(h)-400 mg/kg AEBP

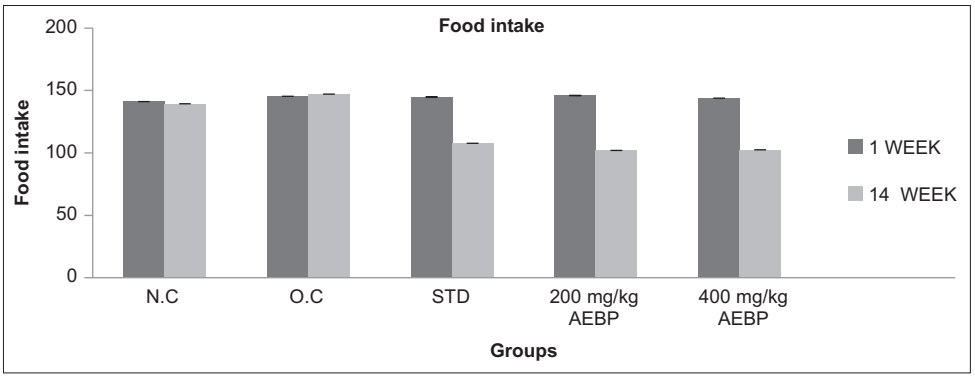

Figure 4: depicts water intake comparison among groups before and after 14 week of study, all values are expressed as Mean ± SEM, there was no significant difference P>0.001 among groups before and after study which was analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test. Where, N.C-Normal control, O.C-Obesity control, STD-Standard drug (Orlistat 30mg/kg) treated group, 200 mg/kg AEBP and 400 mg/kg AEBP

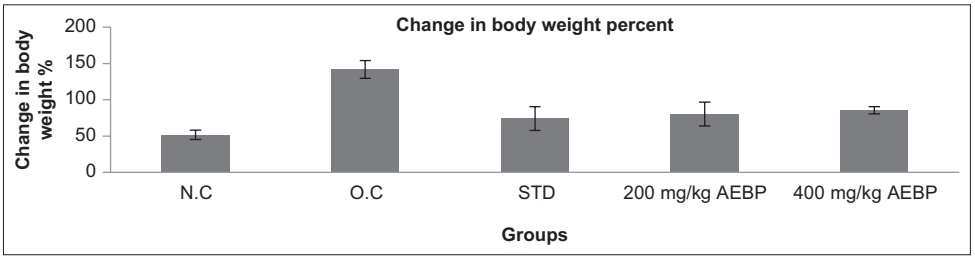

Figure 5: depicted change in body weight intake among groups during 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference ***P<0.001 among groups was found out when analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test. Where, N.C-Normal control, O.C-Obesity control, STD-Standard drug (Orlistat 30mg/kg) treated group, 200 mg/kg AEBP and 400 mg/kg AEBP

Administration of AEBP orally daily for the 14 weeks significantly prevented the increase in BMI in 200mg/kg AEBP group (**P<0.01) and 400mg/kg group (**P<0.01) as compared to obesity control group.

Effect of AEBP in Biochemical Parameters

Bryophyllum pinnatum aqueous extract treated animals showed significantly less increase in serum glucose (***P<0.001), cholesterol (***P<0.001), triglycerides (***P<0.001), SGPT (***P<0.001) and SGOT (***P<0.001) levels when compared to cafeteria diet fed group as shown in Figure 07,08,09,10,11 respectively.

Discussion

Obesity is an excess accrual of fat in the body that often results in significant impairment of wellbeing and longevity. It can be a risk factor for a multitude of other diseases conditions like heart disease, diabetes, a certain type of cancer, osteoarthritis, etc[29]. Prevalence of obesity is rapidly spurting due to sedentary lifestyle, consumption of high calories food, white collar jobs, lack of exercise and psychological factors. We have selected Cafeteria diet model for evaluation of antiobesity activity, as it has been reported well established model for induction of obesity in rats[30]. Cafeteria diet contains mainly supermarket

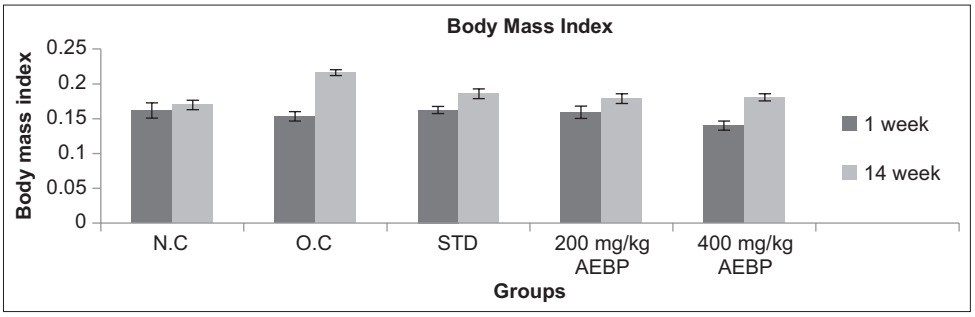

Figure 6: depicted Body Mass Index among groups before and after 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference P<0.001 compared to obesity control group when analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test. Where, N.C-Normal control, O.C-Obesity control, STD- Standard drug (Orlistat 30mg/kg) treated group, 200 mg/kg AEBP and 400 mg/kg AEBP

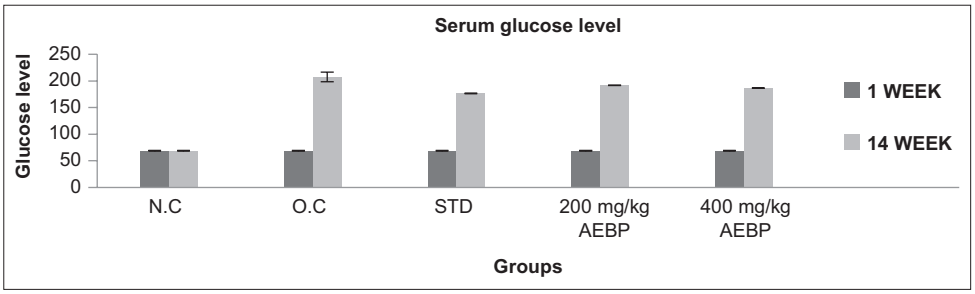

Figure 7: depicted serum glucose level among groups before and after 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference P<0.001 among groups compared to obesity control group, analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test. Where, N.C-Normal control, O.C-Obesity control, STD- Standard drug (Orlistat 30mg/kg) treated group

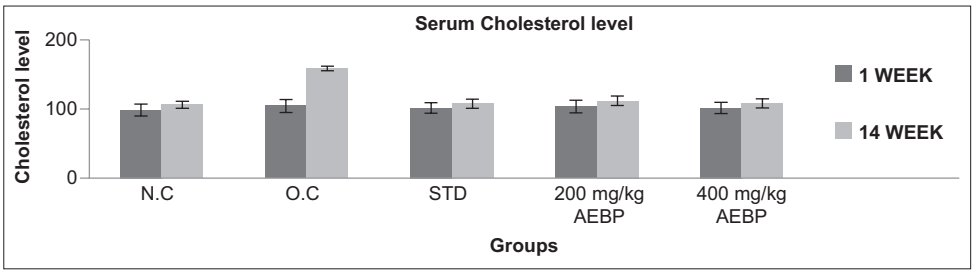

Figure 8: depicted **s**erum cholesterol level among groups before and after 14 weeks of study, results are expressed as mean ± SEM, n=6. Data was analyzed by one-way analysis of variance (ANOVA) followed by Turkey-Kramer multiple comparison test, there was significant difference ***P<0.001 among group when compared to obesity control group. Where, N.C-Normal control, O.C-Obesity control, STD- Standard drug (Orlistat 30mg/kg) treated group, 200 mg/kg AEBP and 400 mg/kg AEBP

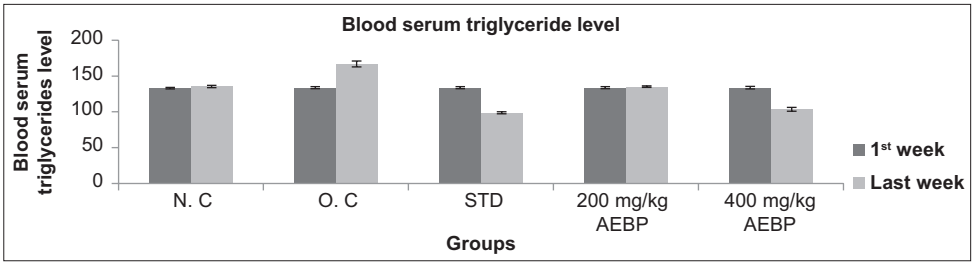

Figure 9: depicted blood **s**erum triglycerides level among groups before and after 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference P<0.001among groups when compared to obesity control group when analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test, per group. Where, N.C-Normal control, O.C-Obesity control group, STD-Standard drug (Orlistat) treated group, 200mg/kg AEBP and 400mg/kg AEBP

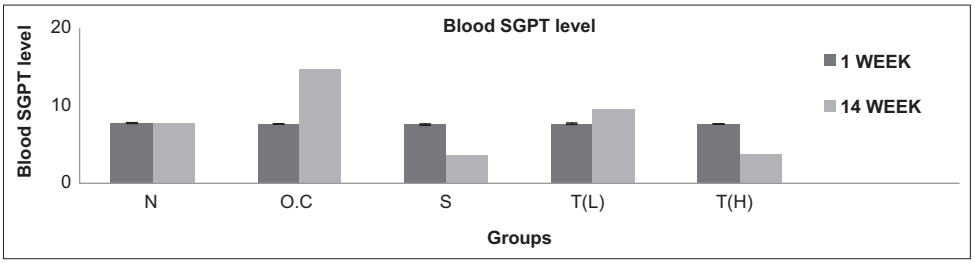

Figure 10: depicted SGPT level among groups before and after 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference P<0.001among groups when compared to obesity control group when analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test, per group. Where N.C-Normal control, O.C-Obesity control group, STD-Standard drug (Orlistat) treated group, T(L) - 200 mg/kg AEBP and T(H) - 400 mg/kg AEBP

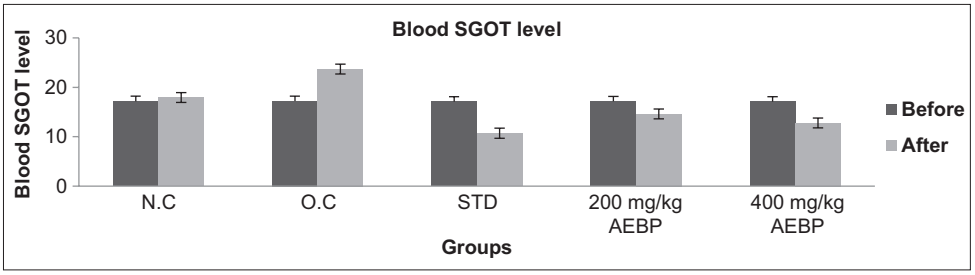

Figure 11: depicts SGOT level among groups before and after 14 weeks of study, all values are expressed as Mean ± SEM, there was significant difference P<0.001among groups when compared to obesity control group when analysed by (one-way analysis of variance) Turkey-Kramer multiple comparison test, per group. Where N.C-Normal control, O.C-Obesity control group, ST-Standard drug (Orlistat) treated group, 200 mg/kg AEBP and 400 mg/kg AEBP

products which are rich in energy. Chocolates, dry coconut, butter, white bread, boiled potatoes and butter cookies were chosen as they are commonly consumed products in the modern lifestyles. Female rats are highly susceptibility to obesity, so they are selected for study[31]. Herbal medicines have been gaining wide acceptance in recent times for the treatment of obesity as they have fewer adverse effects. *Bryophyllum pinnatum* is one such herbal medicine that has been widely used as a folk medicine in ancient times for the treatment of various diseases like kidney stones, urinary disease, skin disease, wound healing, cholera and bruises. Extraction of leaves has been done by cold maceration process employing distilled water as solvent. Phytochemical test revealed the presence of flavonoids and saponins which inhibit pancreas lipase enzyme and suppress appetite[32]; phenols which inhibit lipogenesis by down regulation sterol regulatory element binding protein, acetyl-CoA carboxylase-1 and carboxylase-2, stearoyl-CoA desaturase-1 and pyruvate dehydrogenase kinase-4 in the liver[33,34]. They also suppress increase in body weight and fat storage and decrease serum levels of total cholesterol and triacylglycerol. Total phenolic content was 0.74±0.20 mg GAE/g of extract, this implies a rich source of polyphenols. Polyphenols like catechins, egepicatechin, gllate, epigallo catechin, epigallo catechin gllate, gallic acid inhibit pancrease lipase enzyme[35], enhanced energy expenditure, stimulate thermogenesis. Flavonoid content in AEBP extract was found out to be 1.58667±0.23334602 mg Quercetin/g and it reduces weight gain and body fat. Carbohydrates were also present in extract which possess lipase inhibitory activity[36].Natural pancreatic lipase inhibitors are widely explored as pancreas lipase (PL) enzyme, it is a main causative agent of obesity. The highest IC_{50} value was found out to be at the concentration of 200 µg/mL *in-vitro* was 74.07 which was comparably equivalent to Orlistat (standard drug) in inhibiting pancreatic lipase. Increase in food intake and decrease in physical activity leads to increase in body weight and therefore BMI, leaves of *Bryophyllum pinnatum* significantly (***P<0.001) prevented increase in body weight and BMI by decreasing triacylglycerol digestion due to inhibition of pancreas enzyme as Orlistat (standard drug) or suppressing appetite as Sibutramine (Anti-obesity drug). This can be due to polyphenols, flavonoids, carbohydrate, saponins[37] and gallic acid. There was no significant difference (P>0.05) in water intake due to obesity and AEBP. Adipocytes (fat cells) store triacylglycerols and glycogen and due to altered energy metabolism size of fat cells increases which in turn linearly increases cholesterol, triglycerides and glucose. AEBP prevented dose dependent increase in cholesterol, glucose and triglycerides which had again given the evidence of lipase enzyme (key enzyme for digestion) inhibition. Results revealed that there was no significant difference among groups. There is vital need to discover potent or effective newer lipases inhibitors of natural sources as to avoid unwanted side effects of available standard anti-obesity drugs. The results of the present study indicate that *Bryophyllum pinnatum* Lam Oken leaves extract prevented the increase in food intake, body weight, BMI without affecting water intake. It alters lipid profile and prevents an increase in glucose and cholesterol level. These actions suggest a potential antiobesity effect by inhibition of pancreas enzyme of *Bryophyllum pinnatum* due to presence of gallic acid, polyphenols, flavonoids, carbohydrate and saponins. It may be further developed for the management of obesity.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

Acknowledgement

We are thankful to the HOD, Department of Pharmaceutical Sciences, Sir J C Bose Technical Campus, Bhimtal, Nainital for providing research facilities.

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