



Original Research Paper

PHARMACOLOGICAL SCREENING OF ANTI-OBESITY POTENTIAL OF *LEAVES OF BRYOPHYLLUM PINNATUM* IN HIGH-FAT DIET-INDUCED MALE WISTAR RAT MODEL

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ABSTRACT

Introduction: Obesity is a chronic, complicated metabolic disease characterized by excessive body fat accumulation that has detrimental effects on health. It has become a major global public health concern due to its rapidly increasing prevalence and association with multiple co-morbidities, including type 2 diabetes mellitus, cardiovascular diseases, hypertension, dyslipidemia, and some forms of cancer. Obesity develops as a result of a complex interplay between genetic, environmental, behavioral, and socioeconomic factors. Because obesity has significant effects on morbidity, mortality, and healthcare costs, effective prevention and treatment strategies are essential to battling the growing obesity pandemic.

Objective: To evaluate the anti-obesity effects of the *Bryophyllum pinnatum* leaves extracts (BPeth., BPAq.) in a male Wistar rat model of a high-fat diet.

Method: Male Wistar rats were fed a diet high in fat to cause obesity for the anti-obesity experiment. 42 male rats were used in the experiment and divided into groups I, II, III, IV, V, VI, and VII. Group I is made up of 6 rats (normal control) that were given a normal diet and no medication for 84 days. For 84 days, Group II (the obese control) was treated with a diet high in fat without the use of any medications. For 84 days, Group III received treatment with a standard medication (orlistat) and a diet high in fat.

Bryophyllum pinnatum leaves were administered orally to Group IV rats at a dose of 200 mg per day for 42 days while they were fed a diet high in fat. *Bryophyllum pinnatum* leaves were administered to the rats in Group V. Rats were given an oral dose of 400 mg of aqueous extract each day for 42 days after being fed a diet high in fat. Group VI rats were fed a diet high in fat for 42 days before receiving an oral dose of 200 mg of ethanolic extract from *Bryophyllum pinnatum* leaves. *Bryophyllum pinnatum* leaf ethanolic extract was administered orally to Group VII rats at a dose of 400 mg per day. These animals were placed in the treatment group for *Bryophyllum pinnatum*. Daily food intake and body weight were regularly monitored during the trial. The various adipose pads were weighed after 84 days, and serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and glucose were measured. The results of an acute toxicity (LD50) study were used to determine the extract dose.

Results: The results showed that feeding rats a high-fat diet resulted in a significant increase in both body mass index (BMI) and waist-hip ratio (WHR) in the obese group. Conversely, when *Bryophyllum pinnatum* leaf extract (aqueous and ethanolic) was administered in a single dose to groups IV, V, VI, and VII, the WH ratio and BMI significantly decreased, as did body weight, tissue weight, and lipid accumulation in the liver when compared to the obese control group. Additionally, serum triglyceride, total cholesterol, and leptin concentrations decreased.

The presence of flavonoid compounds with anti-obesity properties in *Bryophyllum pinnatum* leaves was found using LC-MS/MS. This study suggests that *Bryophyllum pinnatum* may have a significant anti-obesity effect.

Conclusions: Experimental proof for the anti-obesity activity of *Bryophyllum pinnatum* aqueous and ethanolic extract doses (200 mg/kg and 400 mg/kg, respectively) is provided by the current study's findings. *Bryophyllum pinnatum* leaves extracts (200 mg/kg and 400 mg/kg of ethanolic and aqueous extract, respectively reduced body weight, body mass index, waist-hip ratio, glucose, lipid, and plasma leptin levels, as well as fat deposits in the kidney, organs, and liver. Therefore, current research supports the recommendation that *Bryophyllum pinnatum* be consumed to lower obesity.

Keywords: Obesity, High-fat diet, Leptin, Adiponectin, *Bryophyllum pinnatum* Linn., Orlistat.

INTRODUCTION

Obesity is a medical disease characterised by an excessive amount of body fat. This metabolic state is brought on by a persistent discrepancy between energy expenditure and consumption [1]. Obesity in children has spread throughout the world. According to studies,

80% of obese teenagers will continue to be obese throughout adulthood, and almost 55% of obese children will continue to be obese throughout their adolescent years. Numerous health concerns are brought on by this growth, including heart disease, type 2 diabetes (T2D), and other metabolic problems [2]. Because healthcare professionals spend less time and

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engage with patients less to establish rapport, patients who are obese may receive lower-quality care. A clinic's physical environment, such as an ill-fitting gown, can create stigma and cause patients to put off or forego primary treatment. In order to address the unpleasant experiences that people with obesity face, patient-centered care is crucial [3]. In addition to promoting the development of obesity, eating a high-fat diet (HFD) that is heavy in saturated fats damages the integrity and structure of several tissues and interferes with intestinal chemo-sensing [4]. Excessive consumption of saturated fatty acids mostly affects the stomach since it is the initial site of extended interaction [5]. Epithelial deficiencies are caused by diet-derived saturated fatty acids: The stomach epithelium is lipotoxically affected by excess dietary fat, which compromises tissue integrity [6].

Many medical diseases are thought to be safely treated by medicinal plants, which are rich in bioactive components such as flavonoids, alkaloids, and phenolic compounds [7]. *Bryophyllum pinnatum*, a member of the Crassulaceae family, is one such plant with incredible potential. It is widely grown in India and is indigenous to Angola, Africa. Numerous *in vitro* and *in vivo* studies have demonstrated the potential health advantages of *Bryophyllum pinnatum* as an antioxidant, anti-inflammatory, anticancer, antidiabetic, antihypertensive, anti-obesity, and antiallergic agent [10]. Many phytoconstituents, especially flavonoids (Anthocyanins), are responsible for its biological effects, which is why it is used in herbal remedies and dietary supplements [11].

Additionally, it has been discovered that *Bryophyllum pinnatum* possesses anti-obesity properties through a number of pathways, including oxidative stress reduction and enzyme inhibition [12].

The goal of the current study is to assess the possible protective effects of *Bryophyllum pinnatum* leaf aqueous and ethanolic extracts against HFD-induced obesity in male Wistar rats. The most important factors linked to obesity—HDL, LDL, total cholesterol, total glycerides, glucose, hormones like leptin and adiponectin, and body weight—have been measured in this study. Histological analyzes were performed on liver and fat samples. In order to validate *Bryophyllum pinnatum* anti-obesity impact in HFD-induced obesity, plasma biomarkers, including those linked to appetite and liver biomarkers, as well as inflammatory biomarkers, were also evaluated [13].

METHODS

Plant Material Collection, Identification, and Authentication

Plant source selected for this study (leaves of *Bryophyllum Pinnatum* Linn.) was collected freshly from Local Market, Bijnor and identified and authenticated from the Central Ayurveda Research Institute, Bengaluru, Ministry of Ayush, Government of India via Ref No. Ref-Authentication/SMPU/CARI/BNG/2021-22/1130, (Ref.RRCBI-7692). The collected leaves were washed

and shade dried. The dried leaves were then grounded into a coarse powder.

Orlistat was acquired from Sun Pharmaceuticals Pvt.Ltd. Gujarat Maharashtra and all other chemicals utilized in this investigation were of analytical quality.

Preparation of Ethanol and Aqueous extracts

The selected plant source was accurately identified, cleaned, allowed to dry in the shade, and then ground into a coarse powder. The leaves of a 100 g powdered sample of *Bryophyllum pinnatum* were placed in a thimble and then placed in a Soxhlet extractor. A 500 mL round-bottom flask equipped with a condenser and attached to the Soxhlet extractor was filled with ethanol and water separately. The extractor was then set up on a heating mantle. The solvent started to evaporate as it moved through the Soxhlet extractor and into the condenser after being heated by the heating mantle. Condensed solvent started to seep from the Soxhlet extractor that was holding the thimble with the plant sample. The extract-containing solvent was recycled back into the round-bottom flask once the solvent level reached the syphon. The extract solution was then allowed to cool to room temperature once this process was completed for the duration of the extraction. After passing the extract through Whatman No. 1 filter paper, a rotary evaporator was used to concentrate it. The extraction yields, TPC and TFC, were subsequently obtained. The extracts were also evaluated for their anti-obesity effects in HFD-induced obesity in Wistar male rats. LC-MS was also performed to find out the constituents responsible for their pharmacological activity. The dried extracts were stored at 4°C [8].

Animals for Experiments

Healthy male Wistar rats weighing between 150 and 200 g were purchased for this study from the Animal House at Vivek College of Technical Education in Bijnor. The rats were housed in polypropylene cages with a 12-hour light/dark cycle, a constant temperature of 23±2°C and 65±5% humidity, unlimited access to water, and regular rat chow pellets (obtained from Asha Medical Store in Meerut, India). The animals were given a week to acclimatize to the lab setting before the testing. All of the research was conducted in compliance with the CPCSEA ethical principles after obtaining the necessary committee approval (Approval No: VCTE/IAEC/22-23/001). All the research was carried out in accordance with the CCSEA ethical guidelines.

Table 1: Composition of HFD

Composition of HFD	
Ingredients	Diet (g/kg)
Powdered NPD	375
Lard	290
Casein	265
Corn oil	10
Cholesterol	10

Vitamin and mineral mix	60
DL-Methionine	03
Yeast powder	01
Sodium chloride	01

Induction of Obesity

To encourage obesity, a diet high in fat was fed to Wistar male rats of normal weight. The components of a diet high in fat include powdered NPD (375 g), lard (290 g), casein (265 g), maize oil (10 g), cholesterol (10 g), vitamins and minerals mix (60 g), DL-methionine (3 g), yeast powder (1.0 g), and sodium chloride (1.0 g). [9]. The diets were administered to individual rats on days one, two, and three, respectively, and then repeated in the same order for 84 days.[10].

Experimental Design

The seven groups of six adult Wistar rats were separated.

Group I: Normal control - Rats given regular rat food.

Group II: Obese control - Rats fed with fat filled diet for 84 days.

Group III: Rats fed with fat filled diet + treated with the reference drug orlistat at a dose level of 30 mg/kg body weight, once a day orally for 84days.

Group IV: Rats fed with fat filled diet+treated with aqueous leaves extract of *Bryophyllum pinnatum* at a dose level of 200 mg/kg body weight, once a day orally for 84 days.

Group V: Rats given a diet high in fat were given an oral dose of 400 mg/kg body weight of *Bryophyllum pinnatum* leaf extract once daily for 84 days.

Group VI: *Bryophyllum pinnatum* ethanolic leaf extract was administered once daily for 84 days at a dose of 200 mg/kg body weight to rats fed a high-fat diet.

Group VII: Rats were fed a high-fat diet and administered 400 mg/kg body weight of *Bryophyllum pinnatum* ethanolic leaf extract once daily for 84 days.

At the end of the 84-day research, the animals were killed by cervical decapitation following a 12-hour fast. For several biochemical tests, blood was drawn. After being removed and weighed, the testis, liver, kidney, and fat pads were prepared for biochemical examination. Testicular, liver, and kidney samples were subjected to histopathological examinations. Liver tissues homogenized in 0.1 M phosphate buffer (pH 7.4) were used for the analysis of numerous biochemical parameters.

In vitro antioxidant activity assay for GSH and DPPH of *Bryophyllum pinnatum*.

All of the drugs were administered orally via gavage each day. Each cage's daily food intake was monitored at regular intervals for 12 weeks, and the average consumption was computed. At the conclusion of the trial (day 85), blood was drawn into a tube using the tail vein procedure, and the serum was separated by centrifugation. The liver, mesenteric (MES), epididymal (EPI), and retroperitoneal (RET) adipose tissue were immediately removed after blood was drawn, cleaned with phosphate-buffered saline, and

weighed. Prior to analysis, the liver, fat tissue, and serum samples were stored at -70°C [15,16].

Morphological parameters to measure obesity

Body weight and feed intake

To determine weight gain, each rat's initial and final body weights were noted. Additionally, feed intake was noted.

Collection of blood and samples

After an overnight fast, three tail venous blood samples were taken from each rat before they were all killed at the end of the study. The initial blood sample was taken in di-potassium ethylene diamine tetra acetate (EDTA) salt in order to measure hematological parameters. The second blood sample was taken using a basic tube. The sera were separated and stored in aliquots at -20°C until they were required for the computation of the lipid profile, which included total cholesterol (TC), triglycerides, LDL cholesterol (LDL-C), VLDL cholesterol (VLDL-C), and HDL cholesterol (HDL-C), using commercial kits and enzymatic colorimetric techniques. Separate sera for biochemical analysis, adiponectin, and leptin. The third blood sample were put in a sodium fluoride tube in order to estimate glucose. Kidneys, testicles, and liver were removed and stored in 10% formalin for histological examination. When the abdomen was opened, the liver and adipose tissues (mesenteric, epididymal, and retroperitoneal) were removed [14].

Morphological parameters to measure obesity

The body weights were determined once a week. Body mass index (BMI), WHR, Adiposity index, Obesity index was calculated from the formula [15,16].

- $BMI = \frac{Body\ Weight(g)}{Length^2\ (cm^2)}$
- *Waist – hip ratio*
- $Adipose\ Index = \frac{Perennial\ WAT + Retero\ WAT + Epididy\ WAT}{WAT + Epididy\ WAT} \times 100$
- $Obesity\ Index = \frac{Body\ weight}{Body\ weight\ of\ Rat} \times 10^4$

Biochemical assessment

Calculation of Total Cholesterol

To assess total serum cholesterol, a Bayer Diagnostic kit (Bayer Diagnostic India Ltd) was used.

Calculating High-density lipoprotein Cholesterol levels

A Bayer diagnostic kit (Bayer Diagnostic India Ltd.) was used to assess the amount of high-density lipoprotein cholesterol.

Calculating Triglyceride levels

A kit from Erba Diagnostics in Mannheim, Germany, was used to estimate the triglyceride level.

Calculating Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) Levels

The Fried Ewald equation was used to determine the concentrations of VLDL and LDL.

VLDL Level

$$Serum\ VLDL\ mg/dl\ levels = \frac{Triglyceride\ level}{5}$$

LDL Level

Serum LDL levels(mg/dl) = Total cholesterol – (HDL level+ VLDL level) [16].

Estimation of Serum glucose

Total serum glucose was estimated by the glucose-peroxidase method.

Methods for Assessment of Oxidative Stress

Calculating the amount of Reduced Glutathione (GSH)

A common chemical technique was used to determine the levels of reduced glutathione (GSH). The GSH content was directly correlated with the generated chromogen's absorbance, which was evaluated at 405 nm. A common chemical technique was used to determine the levels of reduced glutathione (GSH). In short, the tissue homogenate was made by homogenizing tissue samples in an appropriate cold buffer. After treating an aliquot of the homogenate with 10% trichloroacetic acid (TCA) to precipitate proteins, it was centrifuged for ten to fifteen minutes at a speed of roughly 3000 to 5000 rpm. After gathering the clear supernatant, it was treated with the proper chromogenic reagent to produce colour. A UV-visible spectrophotometer was used to evaluate the generated chromogen's absorbance at 405 nm against an appropriate reagent blank. A standard calibration curve made with known reduced glutathione concentrations was used to calculate the GSH concentration, and the results were reported in relation to the protein content or tissue weight [17].

Histopathological analysis

Adipose tissue was extracted and preserved in 10% neutral buffered formalin prior to being embedded in paraffin for histological analysis. A light microscope was used to view the standard 5 mm thick slices after they had been sliced and stained with hematoxylin and eosin [18].

Analysis of statistics

One-way analysis of variance (ANOVA) followed by an appropriate post-hoc multiple comparison test was used to statistically evaluate the analytical data using GraphPad Prism software. Data is expressed as Mean $\pm$ standard error (SE). The biochemical data for random blood glucose, lipid profile, and fat pad weights along with the impact of ethanolic extract of *Bryophyllum pinnatum* leaves on feed intake, body weight, BMI, and obesity index were statistically analyzed using one-way analysis of variance (ANOVA) and the multiple comparison test $p < 0.05$. A p -value of less than 0.05 was considered statistically significant.

RESULTS

Anti-obesity activity of *Bryophyllum pinnatum*

The anti-obesity action of *Bryophyllum pinnatum* was examined, and the outcomes are Shown in Fig. 1.

Morphological Characteristics

Effect of aqueous and ethanolic extracts of *Bryophyllum pinnatum* leaves and orlistat on rats' body weight, body mass index (BMI), and waist-hip ratio

To induce obesity, normal rats were fed a high-fat diet for 12 weeks. The mean body weights of the seven experimental groups were similar at the start of the trial. Rats in the HFD control group had a significant increase in body weight, body mass index (BMI), and waist-hip ratio after 12 weeks, along with a decrease in feed consumption, as compared to a normal control group. However, the traditional drug Orlistat (30 mg/kg, oral) given once daily for 12 weeks significantly ($p < 0.05$) decreased body weight, BMI, waist-hip ratio, and feed intake when compared to the HFD control group. On the other hand, compared to the HFD control group, *Bryophyllum pinnatum* leaf extract (200 & 400 mg/kg; oral) given once daily for seven weeks and continued for 84 days significantly decreased body weight, BMI, waist hip ratio [19, 20].

Table 2: Effect of Orlistat and *Bryophyllum pinnatum* extracts on Body weight

Group	% Body Weight		
	Initial Body Weight (g)	Final Body Weight (g)	% Body Weight Gain
Normal control	169.33 $\pm$ 2.01 1	275.71 $\pm$ 1.36 2	62.57
HFD control	176.17 $\pm$ 3.85 1 ^a	380.84 $\pm$ 4.39 7 ^a	116.17 ^a
Orlistat (30mg/kg)	159.35 $\pm$ 1.94 5 ^b	240.06 $\pm$ 2.00 2 ^b	50.64 ^b
Aqueous Extract (200 mg/kg)	171.83 $\pm$ 1.83 5 ^b	314.89 $\pm$ 1.75 9 ^b	83.12 ^b
Aqueous Extract (400 mg/kg)	170.39 $\pm$ 2.03 6 ^b	288.86 $\pm$ 2.36 8 ^b	69.52 ^b
Ethanol Extract (200 mg/kg)	172.83 $\pm$ 1.73 9 ^b	280.66 $\pm$ 1.75 8 ^b	62.41 ^b
Ethanol Extract (400 mg/kg)	169.39 $\pm$ 2.04 4 ^b	258.46 $\pm$ 2.38 1 ^b	52.58 ^b

All values are represented as mean $\pm$ S.E.M of $n=6$ /group. Where, a = $p < 0.05$ vs Normal Control; b = $p < 0.05$ vs HFD control

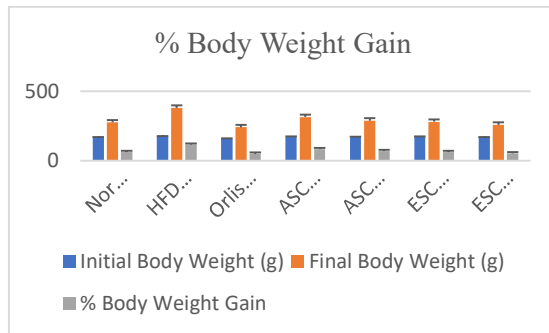


Fig.No. 1 Effect of leaves extracts of *Bryophyllum pinnatum* on % body weight gain in experimental rats

Effect of Orlistat and *Bryophyllum pinnatum* on Body mass index, Waist-hip ratio, obesity index and adiposity index of rats.

To make normal rats obese, they were fed a high-fat diet for 12 weeks. The average body weight of each of the seven experimental groups was determined at the start of the study. Rats in the HFD control group showed a significant increase in body weight, body mass index (BMI), and waist hip ratio after 12 weeks, as well as a decrease in feed consumption when compared to a normal control group. In contrast to the HFD control group, the conventional medication Orlistat (30 mg/kg, oral) administered once daily for 12 weeks significantly ($p < 0.05$) reduced body weight, BMI, waist-hip ratio, and feed consumption. In contrast to the HFD control group, both *Bryophyllum pinnatum* leaves extract at specific doses—200 and 400 mg/kg orally once daily for 84 days (12 weeks)—significantly reduced body weight (Table 1, Fig. 1), BMI, and waist-to-hip ratio (Table 3, Fig. 2) [21, 22].

Table 3: Effect of Orlistat and *Bryophyllum pinnatum* extracts on WH Ratio and BMI

Groups	WH Ratio			BMI (gm/cm ²)		
	Week 4	Week 8	Week 12	Week 4	Week 8	Week 12
Normal control	0.87 ±0.1 3	0.89 ±0.2 2	0.90 ±0.2 6	0.52 ±0.0 3	0.54 ±0.0 2	0.55 ±0.0 3
HFD control	0.93 ±0.1 9 ^a	0.98 ±0.2 7 ^a	1.05 ±0.3 1 ^a	0.55 ±0.0 3 ^a	0.64 ±0.0 4 ^a	0.65 ±0.0 3 ^a
Std Orlistat 30 mg/kg	0.90 ±0.0 21 ^b	0.95 ±0.2 4 ^b	0.91 ±0.2 9 ^b	0.53 ±0.0 19 ^b	0.58 ±0.0 08 ^b	0.56 ±0.0 3 ^b
Aqueous extract 200	0.92 ±0.1 9 ^b	0.99 ±0.2 4 ^b	0.97 ±0.2 7 ^b	0.53 ±0.0 13 ^b	0.63 ±0.0 12 ^b	0.62 ±0.0 1 ^b

mg/kg						
Aqueous extract 400 mg/kg	0.91 ±0.2 5 ^b	0.97 ±0.1 1 ^b	0.95 ±0.1 5 ^b	0.54 ±0.0 09 ^b	0.62 ±0.0 15 ^b	0.61 ±0.0 2 ^b
ethanol extract 200 mg/kg	0.92 ±0.1 9 ^b	0.98 ±0.2 5 ^b	0.94 ±0.2 7 ^b	0.54 ±0.0 13 ^b	0.61 ±0.0 12 ^b	0.60 ±0.0 2 ^b
ethanol extract 400 mg/kg	0.91 ±0.2 5 ^b	0.99 ±0.1 1 ^b	0.92 ±0.1 5 ^b	0.54 ±0.0 09 ^b	0.60 ±0.0 15 ^b	0.59 ±0.0 2 ^b

All values are represented as mean $\pm$ S.E.M of $n=6$ /group. Where, a = $p < 0.05$ vs Normal Control; b = $p < 0.001$ vs HFD control

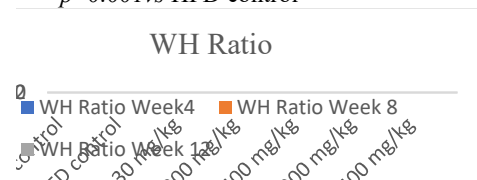


Fig. 2: Effect of leaves extracts of *Bryophyllum pinnatum* on WH Ratio in experimental rats



Fig. 3: Effect of leaves extracts of *Bryophyllum pinnatum* on BMI in experimental rats

Impact on the fat pad weights, total fat, obesity index, and adiposity index of rats

The weights of the fat pads (epididymal, mesenteric, retroperitoneal, and total fat) significantly increased when HFD control rats were compared to normal control rats. In contrast to the HFD control group (Table 4), animals treated with the conventional medications Orlistat, Aqueous and Ethanolic extracts of *Bryophyllum pinnatum* (200 and 400 mg/kg, oral) showed significant ($p < 0.05$) reductions in fat pad weights and total fat (Fig. 4), obesity index (Fig. 5), and adiposity index (Fig. 6) over a 12-week period. Total fat mass and organ weights were higher in obese

control animals fed a high-fat diet, but total fat pad mass was lower in animals given plant extracts [24].

Table 4: Effect of Orlistat and *Bryophyllum pinnatum* leaves extracts treatment on fat pad weights, total fat, obesity index and adiposity index

Groups	Parameters					
	Epididymal Fat (g)	Retro-peritoneal Fat (g)	Mesenteric Fat (g)	Total Fat (g)	Adiposity Index (%)	Obesity Index
Normal control	2.9 6±0 .51	1.5 6±0 .45	2.9 7± 0.35	7.4 9±0 .49	2.6 5±0 .09	420.07±8.90
HFD control	7.6 ±0.73 ^a	4.31 ±0.73 ^a	8.06 ±0.73 ^a	19.97 ±2.11 ^a	5.54 ±0.45 ^a	511.17±7.02 ^a
Orlistat (30mg/kg)	2.9 5±0 .67 ^b	1.98 ±0.65 ^b	3.07 ±0.65 ^b	8.69 ±0.54 ^b	4.90 ±0.25 ^b	311.25±3.37 ^b
Aq.Extr act(200 mg/kg)	7.4 1±0 .89 ^b	5.22 ±0.44 ^b	6.05 ±0.91 ^b	19.21 ±0.96 ^b	5.42 ±0.31 ^b	358.86±9.53 ^b
Aq.Extr act(400 mg/kg)	4.3 1±0 .71 ^b	2.99 ±0.56 ^b	4.05 ±0.66 ^b	11.86 ±0.63 ^b	5.13 ±0.15 ^b	337.90±7.65 ^b
Ethanol ic extract	4.1 2±0 .94 ^b	2.78 ±0.78	4.05 ±0.78	11.43 ±1.83	5.23 ±0.18	353.86±8.68 ^b

(200mg/kg)		.6 5 ^b	± 0.6 1 ^b	8 ^b	.1 4 ^b	
Ethanol ic extract(400mg/kg)	3.3 7±0 .62 ^b	2.11 ±0.28 ^b	3.08 ±0.29 ^b	9.36 ±0.92 ^b	5.08 ±0.38 ^b	318.41±11.42 ^b

All values are represented as mean ± S.E.M of n=6/group. Where, a = p<0.05 vs Normal Control; b = p<0.05 vs HFD control.

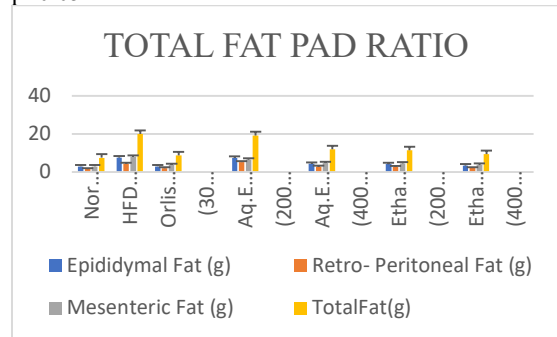


Fig. 4: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on fat pad weights in experimental rats

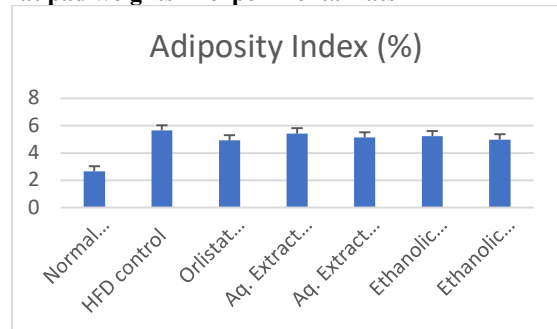


Fig. 5: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on Adiposity Index



Fig. 6: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on Obesity Index

Biochemical Characteristics
Effect on Glucose, leptin and Adiponectin levels

Table No. 5 displays the changes in blood glucose, leptin, and adiponectin levels in obese rats treated with an extract from *Bryophyllum pinnatum* leaves. The elevated blood glucose, leptin, and adiponectin levels in obese rats fed a high-fat diet nearly reverted to normal after receiving plant extracts [25, 26].

Table 5: Effect of *Bryophyllum pinnatum* on blood glucose, leptin and adiponectin levels in Experimental rats

Groups	Parameters		
	Serum Leptin (ng/ml)	Random Blood Glucose (mg/dl)	Adiponectin in (µg/ml)
Normalcontrol	2.58±0.39	84.65 ± 5.53	9.47±2.34
HFD control	8.76±0.58 ^a	131.72 ± 1.62 ^a	6.89±1.25 ^a
Orlistat(30mg/kg)	3.65±0.27 ^b	93.27 ± 3.38 ^b	8.39±3.15 ^b
Aq. Extract(200mg/kg)	6.76±0.42 ^b	125.42± 5.32 ^b	7.13±2.13 ^b
Aq. Extract(400mg/kg)	4.82±0.36 ^b	116.82±2.97 ^b	7.29±1.36 ^b
Ethanollic extract(200mg/kg)	5.89±0.39 ^b	110.76±5.49 ^b	7.56±4.24 ^b
Ethanollic extract(400mg/kg)	3.94±0.31 ^b	97.72±3.09 ^b	7.96±3.21 ^b

The mean ± S.E.M. of n=6/group is used to represent all values. where b = p<0.05 vs. HFD control and a = p<0.05 vs. normal control.

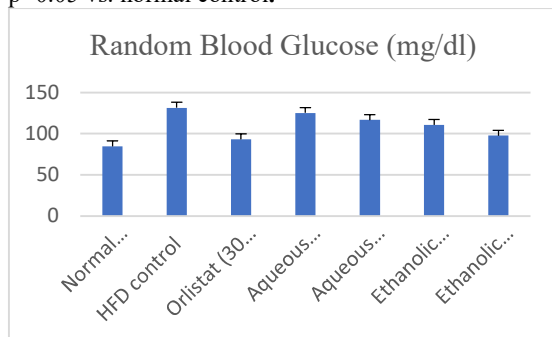


Fig. 7: Effect of leaves extracts of *Bryophyllum pinnatum* on High Fat Diet induced alterations in Random Blood Glucose Level

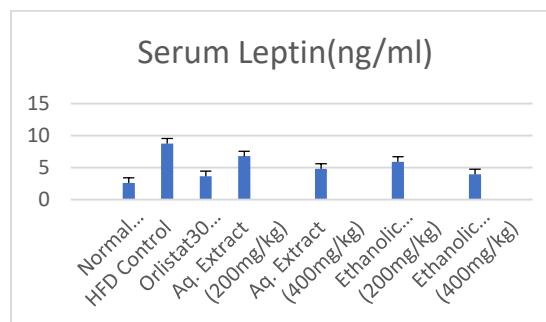


Fig. 8: Effect of leaves extracts of *Bryophyllum pinnatum* on High Fat Diet induced alterations in Leptin

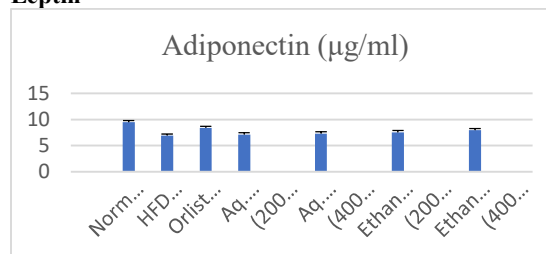


Fig. 9: Effect of leaves extracts of *Bryophyllum pinnatum* on High Fat Diet induced alterations in Adiponectin Impact on the lipid profile of the liver and serum

Table No.6 shows the effect of *Bryophyllum pinnatum* on the serum lipid profile. Blood triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) all rose when the experimental animals were fed a high-fat diet (HFD), whereas HDL decreased. Hepatic TG and TC levels were likewise elevated in rats given a high-fat diet. Oral administration of aqueous extracts of a selected plant medication at different dose levels successfully restored the aberrant lipid profiles of the obese rats to normal. The effect was statistically significant (p<0.05) and dependent on dosage. [27, 28].

Table 6: Effect of Orlistat and *Bryophyllum pinnatum* leaves extracts on lipid profile of rats

Groups	Parameters				
	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normalc control	98.64± 3.02	86.69± 5.67	46.27 ±4.83	35.03± 1.26	17.34 ±3.65
HFD control	224.32 ±2.83 ^a	189.68 ±2.7 ^a	29.69 ±3.7 ^a	156.69 ±3.29 ^a	37.94 ±2.25 ^a
Orlistat (30mg/kg)	126.72 ±4.29 ^b	113.35 ±1.32 ^b	42.68 ±3.85 ^b	61.37± 2.67 ^b	22.67 ±3.43 ^b
Aq.Extra ct (200mg/k	178.57 ±5.04 ^b	153.42 ±4.89 ^b	35.44 ±1.64 ^b	112.45 ±2.94 ^b	30.68 ±4.92 ^b

g)					
Aq.Extract (400mg/kg)	146.45 ±3.78 ^b	122.35 ±3.85 ^b	41.25 ±2.65 ^b	80.73± 2.82 ^b	24.47 ±2.91 ^b
Ethanolice extract (200mg/kg)	165.84 ±1.62 ^b	136.40 ±2.66 ^b	39.69 ±5.62 ^b	98.87± 5.72 ^b	27.28 ±4.53 ^b
Ethanolice extract (400mg/kg)	134.74 ±4.85 ^b	115.92 ±1.62 ^b	43.71 ±3.89 ^b	67.85± 2.56 ^b	23.18 ±3.48 ^b

The mean ± S.E.M. of n=6/group is used to represent all values. Where b = p<0.05 vs. HFD control and p<0.05 vs. normal control

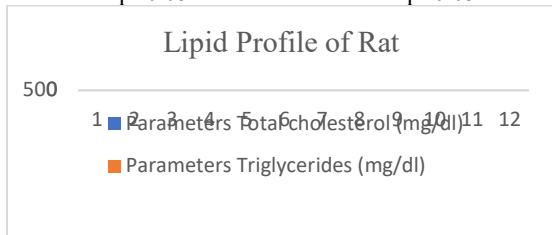


Fig. 10: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on Lipid Profile

Table.7: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on SGPT and SGOT level

Groups	SGPT units/lit	SGOT units/lit
Normal	36.18±0.37	21.31±0.54
HFD Control	68.31±2.37 ^a	74.23±1.13 ^a
Orlistat	51.28±0.82 ^b	55.46±1.36 ^b
Aq.Extract(200mg/kg)	62.28±0.88 ^b	67.43±1.19 ^b
Aq.Extract(400mg/kg)	58.13±0.73 ^b	64.21±1.20 ^b
Ethanolice extract(200mg/kg)	56.21±1.52 ^b	60.54±0.17 ^b
Ethanolice extract(400mg/kg)	52.41±0.83 ^b	56.24±1.22 ^b

The mean ± S.E.M. of n=6/group is used to represent all values. Where b = p<0.05 vs. HFD control and a = p<0.05 vs. normal control

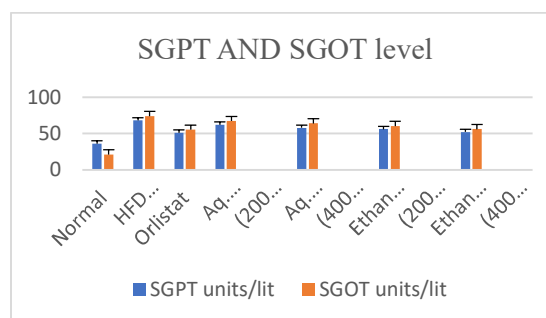


Fig. 11: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on SGPT and SGOT levels

Table No.8:Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on Organ Tissue Weight

Groups	Liver	Kidney	Testis
Normal control	10.9 ± 1.7	2.65 ± 0.7	2.45 ± 0.4
HFD Control	22.13 ± 1.3 ^a	5.87 ± 0.6 ^a	4.54 ± 0.5 ^a
Orlistat 30 mg/kg	12.26 ± 1.2 ^b	2.92 ± 0.5 ^b	2.86 ± 0.7 ^b
Aq. Extract 200 mg/kg	21.68±0.7 ^b	4.89 ±0.5 ^b	4.23 ±0.2 ^b
Aq. Extract 400mg/kg	19.72 ±0.4 ^b	4.43 ±0.3 ^b	3.94±0.4 ^b
Ethanolice Extract 200 mg/kg	16.21 ±0.6 ^b	4.25 ±0.6 ^b	3.76±0.8 ^b
Ethanolice Extract 400 mg/kg	14.13 ±0.8 ^b	3.32 ±0.5 ^b	3.23±0.7 ^b

The mean ± S.E.M. of n=6/group is used to represent all values. Where b = p<0.05 vs. HFD control and a = p<0.05 vs. normal control.

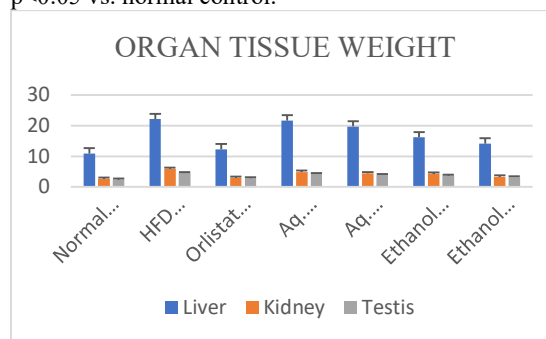


Fig.12: Effect of aqueous and ethanolic leaves extracts of *Bryophyllum pinnatum* on Organ Tissue Weight

Table.9: Effect of Aqueous and ethanolic extract of *Bryophyllum pinnatum* leaves on High Fat Diet induced alterations in tissue parameters (GSH)

GSH	
Groups	Absorbance
Normal Control	0.769±0.02

HFD Control	0.385±0.01 ^a
Orlistat30 mg/kg	0.612±0.21 ^b
Aq.Extract(200mg/kg)	0.483±0.02 ^b
Aq.Extract(400mg/kg)	0.527±0.01 ^b
Ethanoliceextract(200mg/kg)	0.539±0.037 ^b
Ethanoliceextract(400mg/kg)	0.583±0.048 ^b

All values are represented as mean±S.E.M. of n=6/group. Where, a= $p<0.05$ vs Normal Control; b = $p<0.05$ vs HFD control

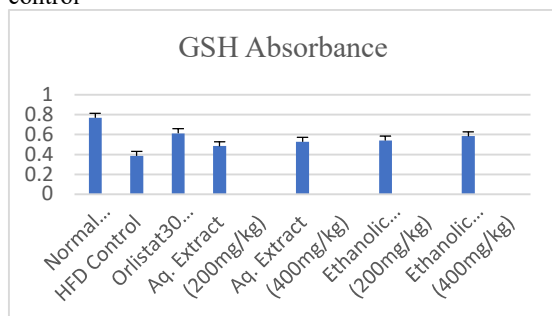


Fig. 13: Effect of aqueous and ethanolic leaves extract of *Bryophyllum pinnatum* on High Fat Diet induced alterations in tissue parameters (GSH)

In-vitro antioxidant activity

Table No. 10 DPPH radical scavenging activity

S. No	Conc (µg/ml)	(%) Scavenging effect of Aqueous Extract	(%) Scavenging effect of ethanolic Extract	(%) Scavenging effect of Ascorbic acid
1	12.5	22.41	39.38	48.8
2	25	28.69	48.73	66.25
3	50	39.88	55.37	75.00
4	75	58.69	67.68	82.40
5	100	69.66	73.48	87.35
6	125	73.56	82.74	88.49
7	150	80.68	88.90	95.72

Sample	IC ₅₀ (µg/mL)
Aqueous Extract	63.45
Ethanolice Extract	29.78

Ascorbic Acid	13.35
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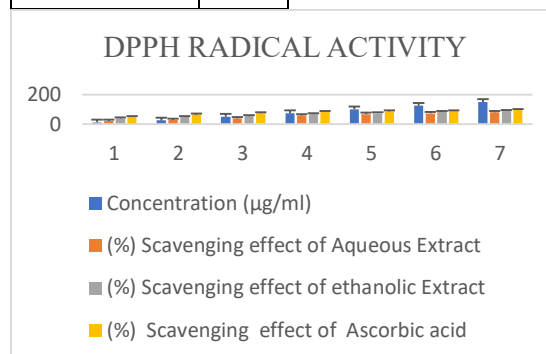


Fig.14: DPPH radical scavenging activity Identified Bioactive Compounds in LC-MS/MS

A total of 26 compounds were identified in the aqueous and ethanolic extracts of BP using the LC-MS/MS spectra. A few important classes of bioactive compounds, such as flavonoids, phenolics, steroids, and alkaloids, were detected in the extract of BP. Compounds were detected as major contributors to the anti-obesity activity. The anti-obesity activity was found to be significantly influenced by some compounds. *Bryophyllum pinnatum* leaves extracts have LOD values of 5 ng/ml and LOQ values of 10 ng/ml, respectively.

Quantitative Analysis Sample Report of Aqueous Extract

Table 11. Compounds identified in BP aqueous extract by positive mode of analysis

Compound	Concentration	Unit
Quercetin	126.6656	ng/ml
Kaempferol	207.2681	ng/ml
Rutin	54.9181	ng/ml
Isoquercetin	126.5249	ng/ml
Cyanidin	78.2632	ng/ml
Delphinidin	144.7157	ng/ml
Malvidin	67.4616	ng/ml
Bufadienolides	187.9368	ng/ml
Brevillin A	14.8112	ng/ml
Bryotoxin	45.1075	ng/ml
Isoflavones	22.7336	ng/ml
Luteolin	156.5692	ng/ml
Thymol	24.3419	ng/ml
Terpineol	180.9838	ng/ml
Fenchone	34.2041	ng/ml
Coumarins	97.3562	ng/ml
Limonene	48.9888	ng/ml
β-Caryophyllene	35.5274	ng/ml
α-Pinene	17.2995	ng/ml
β-Pinene	13.9839	ng/ml
α-Thujene	243.5528	ng/ml
Sabinene	43.3848	ng/ml
Myrcene	209.7641	ng/ml

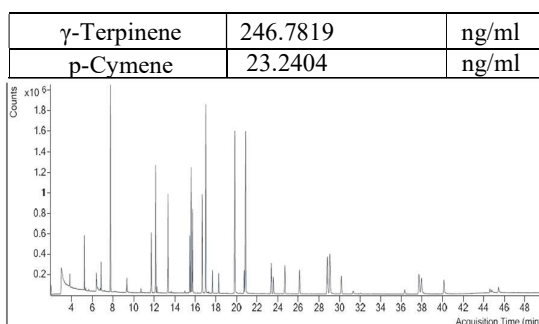


Fig 15. LC-MS/MS Report of Aqueous Extract (Sample Chromatogram)

Quantitative Analysis Sample Report of Ethanolic Extract

Table.12: Compounds identified in BP Ethanolic extract by negative mode of analysis

Compound	Concentration	Unit
Quercetin	102.8940	ng/ml
Kaempferol	168.3697	ng/ml
Rutin	66.0568	ng/ml
Isoquercetin	98.6860	ng/ml
Cyanidin	86.8321	ng/ml
Delphinidin	174.0675	ng/ml
Malvidin	78.3207	ng/ml
Bufadienolides	228.8045	ng/ml
Brevillin A	16.8054	ng/ml
Bryotoxin	56.9963	ng/ml
Isoflavones	17.0809	ng/ml
Luteolin	188.3251	ng/ml
Thymol	31.1509	ng/ml
Terpineol	217.6916	ng/ml
Fenchone	41.1415	ng/ml
Coumarins	121.4822	ng/ml
Limonene	59.0669	ng/ml
β -Caryophyllene	42.7332	ng/ml
α -Pinene	20.8083	ng/ml
β -Pinene	16.8202	ng/ml
α -Thujene	197.8448	ng/ml
Sabinene	52.1843	ng/ml
Myrcene	252.3092	ng/ml
γ -Terpinene	200.4679	ng/ml
p-Cymene	27.9541	ng/ml

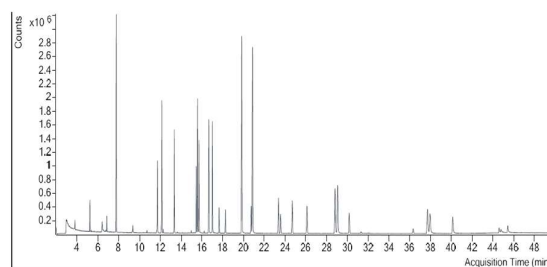


Fig.16: LC-MS/MS Report of Ethanolic Extract (Sample Chromatogram)

Quantitative Analysis Sample Report of blank sample

Table 13. Compounds identified by the Blank mode of analysis

Compound	R T	Re sponse	Co nc	Unit
Quercetin	2.824	49	0.0000	ng/ml
Kaempferol	6.175	40B	0.0000	ng/ml
Luteolin	6.316	41	0.0000	ng/ml
Anthraquinones	7.505	1626	0.0000	ng/ml
Pterocarpan	11.340	354	0.0000	ng/ml
Homopterocarpan	11.751	70B	0.0000	ng/ml
Lanceolatin	12.912	704	0.0000	ng/ml
Cambcdianol	14.949	70	0.0000	ng/ml
Isofiavonone	15.077	5B7	0.0000	ng/ml
Kaempferol	16.118	959	0.0000	ng/ml
Dihydrochalcone	17.069	177	0.0000	ng/ml
Quindone	19.181	1345	0.0000	ng/ml
Trifasciatine	27.884	149	0.0000	ng/ml
Delphinidin	28	20	0.00	ng/

	.1		00	ml
	15			
Herpœtine	36	4B	0.00	ng/
	.4		00	ml
	92			

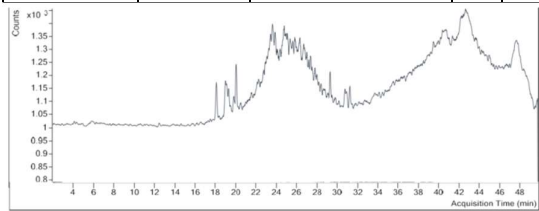
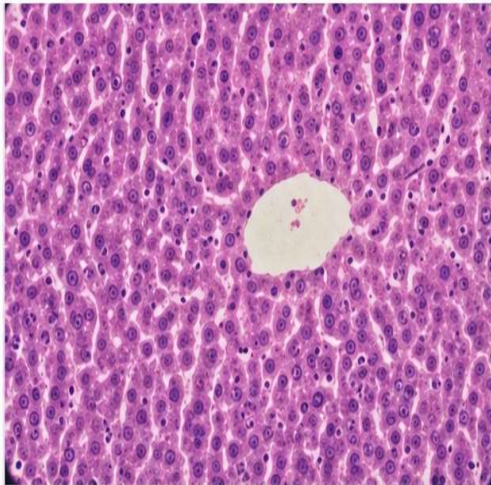
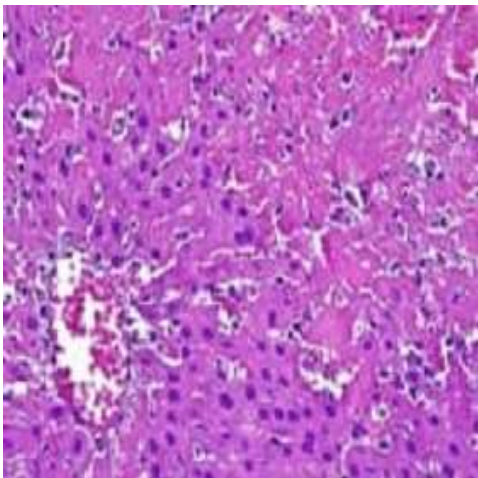
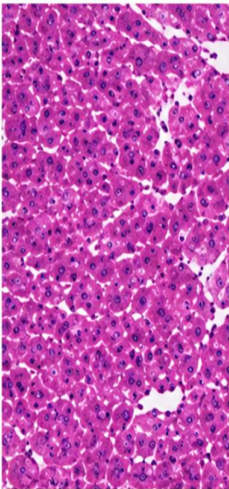
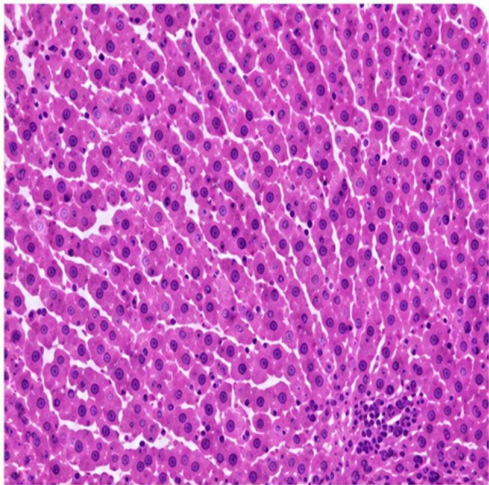


Fig.17:Fig.16 LCMS Report of Blank Sample (Sample Chromatogram)

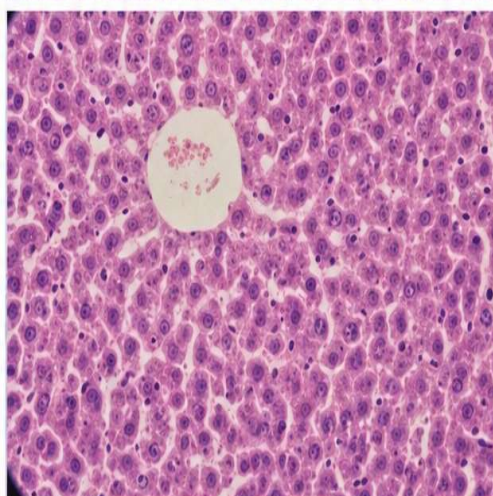
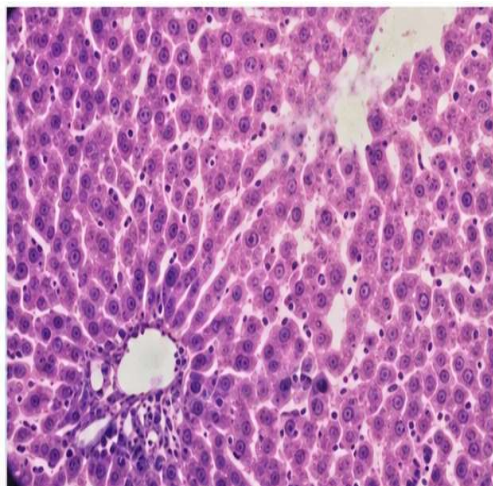


**Std. Drug Orlistat
mg BP**

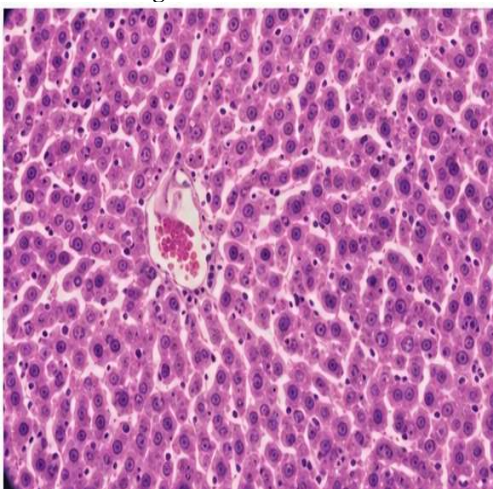
Aq. Extract 200



**Normal Control
HFD Control**



Aq. Extract 400 mg BP
Extract 200 mg BP



Et. Extract 400 mg BP

Figure 17: Effect of BPEe&BP Ae (200 and 400mg/kg, oral), on Adipose tissue of HFD fed Rats (Magnification-40x).

DISCUSSION

It is thought that obesity results from an imbalance between daily energy intake and expenditure, which causes weight growth. Eating a high-fat diet (HFD) is believed to be a risk factor for the development of obesity, despite the fact that the disorder has many different causes [29,30]. The well-known medicinal plant *Bryophyllum pinnatum* has demonstrated a variety of biological and pharmacological properties, such as lowering blood sugar and serum cholesterol [31].

Rats fed a high-fat, lard-based diet showed signs of oxidative stress, dyslipidemia, hyperglycemia, and visceral adiposity—all of which are frequently associated with human obesity. It is well known that rats on a high-fat diet consumed more calories on average than rats fed a regular diet [32, 33]. Thus, a high-fat diet (HFD) was administered to Wistar rats for 12 weeks in order to cause obesity in the present study. It is well established that obesity in rats and other animal models is caused by excessive fat intake. Obesity and dyslipidemia, a condition characterized by an unfavorable lipid profile, have been linked in numerous studies. [34, 35]. Elevated blood levels of fatty acids, total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides (TG) are associated with obesity, but serum HDL cholesterol is decreased [36, 37]. It has been demonstrated that eating a lot of fat raises blood sugar levels. The blood glucose levels of rats fed a high-fat diet in this study were significantly higher than those of rats fed a regular diet. Thus, as markers of hyperlipidaemia and hyperglycaemia, serum lipid levels (including total cholesterol, HDL, and triglycerides) and glucose levels were assessed in this investigation. An increase in fat tissue may lead to obesity. Studies on animals reveal a stronger correlation between body fat and fat intake than with total caloric intake [38, 39]. Reduced adipocyte size from fat storage (adipocyte hypertrophy) or decreased adipocyte creation from precursor cells (adipocyte differentiation) could be the cause of the weight loss. Rats on a high-fat diet (HFD) had a significantly larger total weight of visceral fat depots (epididymal, mesenteric, and retroperitoneal) than rats fed a normal diet (ND). Therefore, three adipose tissues (epididymal, retroperitoneal, and mesenteric fat depots) were weighed in this study as a measure of adiposity, and the size of the adipose tissue was examined histologically under a light microscope. Additionally, consuming a high-fat diet increases the generation of reactive oxygen species (ROS), which results in oxidative stress [40,41]. Reduced glutathione (GSH) levels were used to measure oxidative stress, and we found that eating a high-fat diet (HFD) decreased GSH levels in comparison to the normal control group. Therefore, we measured obesity in this study using a variety of metrics, including body weight, BMI, obesity index, food intake, adipose tissue weight, adiposity index, serum glucose levels, altered lipid profiles, and assessment of oxidative stress [42, 43].

The current work used an HFD-induced obese rat model to examine the anti-obesity effects of aqueous (BP Ae) and ethanol extracts (BP Ee) of *Bryophyllum pinnatum* leaves (Crassulaceae). Because BMI is nearly unaffected by height and has a significant correlation with body fat levels, it is frequently used as an indication of body fat in epidemiological research. The BMI of the groups treated with BP Ae and BP Ee significantly decreased, according to our findings. Obesity is caused by the expansion of adipose tissue, which includes two growth processes: hypertrophy (an increase in cell volume) and hyperplasia (an increase in cell quantity). Since treatment with BP Ae and BP Ee also significantly decreased the weight of adipose tissues (epididymal, retroperitoneal, and mesenteric fat) in comparison to HFD-fed rats, the reduction in body weight growth of HFD-fed rats was accompanied by a depletion of body fat stores. These findings demonstrated that BP Ae and BP Ee suppress adipose tissue hypertrophy and hyperplasia brought on by a high-fat diet, which reduces body weight increase. BP Ae and BP Ee treatment significantly reduced plasma TC and TG levels, raised HDL levels, and enhanced glucose tolerance in HFD rats in addition to its weight-loss impact. According to reports, dyslipidemia—a condition marked by high TG and decreased HDL concentrations—is linked to obesity, particularly abdominal obesity [44, 45]. TGs are linked to several illnesses, including metabolic syndrome, and are involved in the ectopic accumulation of lipid reserves in the liver. While high HDL helps move extra cholesterol to the liver for bile elimination, VLDL carries TG and cholesterol to the tissues. Good antihyperglycemic and antidy lipidemic properties have been demonstrated by BP Ae and BP Ee. Together, obesity and hyperlipidemia increase systemic oxidative stress and the imbalance between antioxidants and tissue free radicals [46, 47, 48].

In our investigation, GSH, a significant endogenous antioxidant, reduced obesity in rats fed a high-fat diet. The activity of these enzymatic antioxidants was reversed when BP Ae and BP Ee were administered to rats on a high-fat diet. Thus, in HFD-fed obese rats, BP Ae and BP Ee therapy enhances oxidative balance. High doses of *Bryophyllum pinnatum* have been shown to have a positive effect that is nearly identical to that of orlistat, well-reported leptin, and adiponectin in reducing body weight increase caused by a high-fat diet. Furthermore, our histological analysis showed that rats treated with BP Ae and BP Ee had much smaller adipocytes. BP Ae and BP Ee supplementation, however, significantly reduced the degree of steatosis, indicating that BP Ae and BP Ee may control adipocyte lipid storage and mobilisation [49, 50, 51].

Conclusion

According to the current study, *Bryophyllum pinnatum* extract dramatically lowers obesity in Wistar rats given a diet high in fat. Adipose tissue accumulation and body weight growth were considerably decreased upon administration of 400 mg/kg of ethanolic extract. While

high-density lipoprotein levels were raised, lipid profile measures like total cholesterol, triglycerides, and low-density lipoprotein levels were also improved. The extract has also demonstrated beneficial effects on metabolic markers and oxidative stress, suggesting that it may enhance metabolic homeostasis and fortify antioxidant defence mechanisms. Histopathological analyses supported these findings by demonstrating that the treated groups had less fat accumulation in their adipose tissues and liver than the high-fat diet control group.

Bioactive phytoconstituents like flavonoids, phenolics, and saponins, which are known to control lipid metabolism, prevent adipogenesis, and lessen oxidative stress, may be responsible for anti-obesity action.

In conclusion, *Bryophyllum pinnatum* has encouraging anti-obesity potential and may be used as a natural medicinal agent to treat obesity and its associated problems.

DECLARATION OF COMPETING INTEREST

None

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AUTHORS CONTRIBUTIONS

Abhishek Marwari conceptualized the study, designed the research, analysed the data, and drafted the manuscript. Dr. Akash Jain and Dr. Jasmine Chaudhary contributed to assisting data collection and preliminary analysis under supervision. Both authors reviewed and approved the final version of the manuscript.

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