

HPTLC Standardization, Phytochemical Profiling and Pharmacological Evaluation of *Barleria prionitis* Leaf Extract in High-Fat-Diet-Induced Obese Rats

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ABSTRACT

Obesity has emerged as a global health crisis, necessitating the exploration of safe and effective plant-based therapeutic alternatives. *Barleria prionitis* Linn. (Acanthaceae), a medicinal plant widely used in Ayurvedic medicine, has been traditionally employed for various ailments, though its anti-obesity potential remains underexplored. This study aimed to standardize *B. prionitis* leaf extract using High-Performance Thin-Layer Chromatography (HPTLC), conduct comprehensive phytochemical profiling, and evaluate its pharmacological efficacy in high-fat-diet (HFD)-induced obese rats. The hydroethanolic leaf extract was subjected to preliminary phytochemical screening, total phenolic and flavonoid content estimation, and HPTLC fingerprinting using a validated method with quercetin, gallic acid, and tannic acid as marker compounds. For pharmacological evaluation, male Wistar rats were divided into six groups: normal control, HFD control, HFD + standard drug (orlistat 30 mg/kg), and HFD + extract at three doses (100, 200, and 400 mg/kg) for 12 weeks. Body weight, food intake, adiposity indices, lipid profile, glycemic parameters, hepatic and renal function markers, oxidative stress parameters, and histopathological changes were assessed. The extract exhibited significant phenolic (86.42 ± 2.14 mg GAE/g) and flavonoid (42.18 ± 1.86 mg QE/g) content. HPTLC analysis confirmed the presence of quercetin (R_f 0.52), gallic acid (R_f 0.64), and tannic acid (R_f 0.36) with good resolution and validation parameters (linearity $r^2 > 0.998$, LOD 12.4-28.6 ng/band, recovery 97.8-99.6%). The extract at 400 mg/kg significantly ($p < 0.001$) reduced body weight gain (42.3%), adiposity index (48.6%), serum total cholesterol (38.2%), triglycerides (41.5%), LDL-C (44.8%), and glucose (36.4%), while increasing HDL-C (52.1%). It also restored hepatic and renal function markers and enhanced antioxidant enzyme activities (SOD, CAT, GSH) with concomitant reduction in MDA levels. Histopathological examination revealed attenuated hepatic steatosis and adipocyte hypertrophy. These findings demonstrate that *B. prionitis* leaf extract possesses significant anti-obesity, hypolipidemic, hypoglycemic, and antioxidant properties, likely mediated through its rich phytochemical constituents, particularly iridoid glycosides and flavonoids. The validated HPTLC method provides a reliable approach for quality standardization of *B. prionitis*-based formulations.

Keywords: *Barleria prionitis*, HPTLC standardization, phytochemical profiling, anti-obesity, high-fat diet, oxidative stress, histopathology

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INTRODUCTION

Obesity has reached pandemic proportions, affecting over 650 million adults worldwide according to the World Health Organization, with prevalence rates continuing to rise in both developed and developing nations. This

complex metabolic disorder is characterized by excessive accumulation of adipose tissue resulting from an imbalance between energy intake and expenditure, and represents a significant risk factor for type 2 diabetes mellitus, cardiovascular diseases, non-alcoholic fatty liver

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disease, hypertension, and certain cancers. The pathophysiology of obesity involves intricate interactions between genetic predisposition, environmental factors, dietary patterns, and neuroendocrine regulation of energy homeostasis. Conventional pharmacological interventions for obesity, including orlistat, sibutramine, and liraglutide, are associated with considerable adverse effects such as gastrointestinal disturbances, cardiovascular complications, and psychiatric manifestations, thereby limiting their long-term clinical utility [1, 2].

Medicinal plants have served as invaluable sources of therapeutic agents since antiquity, and approximately 60-80% of the world's population continues to rely on traditional herbal medicines for primary healthcare needs. The genus *Barleria* (family Acanthaceae) comprises over 300 species distributed across tropical and subtropical regions of Africa, Asia, and the Americas, with *Barleria prionitis* Linn. being one of the most extensively studied species. Commonly known as "Porcupine Flower" in English and "Kate Koranti" or "Saireyaka" in Ayurvedic literature, *B. prionitis* is a small, spiny shrub widely distributed throughout India, Sri Lanka, and tropical Asia. In traditional Indian medicine, various parts of this plant have been employed for the treatment of diverse ailments including toothache, fever, inflammation, gastrointestinal disorders, ulcers, bronchial asthma, and urinary infections. Notably, the flowers of *B. prionitis* are used internally for the treatment of migraine, internal abscesses, edema, urethral discharges, seminal disorders, and to reduce obesity [1,2].

Phytochemical investigations of *B. prionitis* have revealed the presence of a wide array of bioactive constituents including iridoid glycosides (shanzhiside methyl ester, barlerin, acetylbarlerin), flavonoids (6-hydroxyflavone, quercetin, scutellarein), phenolic acids (gallic acid, caffeic acid, vanillic acid), tannins, saponins, and terpenoids. The iridoid glycosides, particularly shanzhiside methyl ester and barlerin, have been identified as the major active principles, with reported content ranging from 4.91% to 17.32% w/w in different extracts. These phytoconstituents exhibit a broad spectrum of pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, antimicrobial, and anticancer effects [1,4,7,9].

Despite the traditional use of *B. prionitis* for obesity reduction and its documented antidiabetic and hypolipidemic potential, systematic pharmacological evaluation in high-fat-diet-induced obesity models remains limited. Furthermore, the quality standardization of plant extracts using validated analytical methods is essential for ensuring batch-to-batch consistency and therapeutic efficacy. High-Performance Thin-Layer Chromatography (HPTLC) has emerged as a versatile and reliable technique for the phytochemical fingerprinting and quantification of marker compounds in herbal medicines, offering advantages of simplicity, cost-effectiveness, and high sample throughput [3,9,22].

The present study was designed to address these knowledge gaps by: (1) standardizing *B. prionitis* leaf extract using a validated HPTLC method with quercetin, gallic acid, and tannic acid as marker compounds; (2) conducting comprehensive phytochemical profiling including total phenolic and flavonoid content estimation; and (3) evaluating the anti-obesity, hypolipidemic, hypoglycemic, and antioxidant effects of the extract in a high-fat-diet-induced obese rat model with correlation to histopathological changes. We hypothesized that the hydroethanolic leaf extract of *B. prionitis*, rich in iridoid glycosides and phenolic compounds, would attenuate HFD-induced obesity and associated metabolic complications through modulation of lipid metabolism and oxidative stress pathways [1,4].

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of *Barleria prionitis* Linn. were collected from the jungles of Melghat region. The plant material was authenticated by a taxonomist and a voucher specimen (voucher number: BP/2025-26/001) was deposited in the institutional herbarium for future reference. The collected leaves were washed thoroughly with distilled water to remove dust and debris, shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10-15 days, and then powdered using a mechanical grinder. The powdered material was passed through a 40-mesh sieve to obtain uniform particle size and stored in airtight containers at room temperature until further use.

Pharmacognostic Standardization

Pharmacognostic evaluation of the leaf powder was performed according to standard methods. Organoleptic characteristics including color, odor, taste, and texture were recorded. Physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water-soluble extractive, and moisture content were determined following the protocols of the Indian Pharmacopoeia. For microscopic examination, transverse sections of fresh leaves were prepared and stained with phloroglucinol-HCl and iodine solution for detection of lignified elements and starch grains, respectively. Powder microscopy was performed to identify characteristic cellular structures including trichomes, stomata, xylem vessels, and calcium oxalate crystals.

Extraction

The dried leaf powder (500 g) was subjected to Soxhlet extraction using petroleum ether ($60-80^\circ\text{C}$) for defatting, followed by sequential extraction with chloroform and methanol. The methanolic extract was concentrated under reduced pressure using a rotary evaporator at 40°C . Additionally, a hydroethanolic extract (70% ethanol) was prepared by maceration of the defatted powder (100 g) in 70% ethanol (500 mL) for 72 hours with occasional shaking, followed by filtration and concentration. Both extracts were stored in amber-colored containers at 4°C until further analysis. The percentage yield of each extract was calculated on a dry weight basis.

Phytochemical Screening

Preliminary phytochemical screening of the extracts was performed using standard qualitative tests to detect the presence of various phytoconstituents including alkaloids (Mayer's test, Dragendorff's test), flavonoids (ferric chloride test, Shinoda test), tannins (ferric chloride test, lead acetate test), saponins (foam test), steroids and terpenoids (Liebermann-Burchard test, Salkowski test), glycosides (Keller-Killiani test, Borntrager's test), and phenolic compounds (ferric chloride test) [1,4,8].

Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method with gallic acid as the standard. Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (10% v/v) and 2 mL of sodium carbonate solution (7.5% w/v). The mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 765 nm. TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content (TFC) was estimated using the aluminum chloride colorimetric method with quercetin as the standard. Extract (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of aluminum chloride (10% w/v), 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water. After incubation for 30 minutes, absorbance was recorded at 415 nm, and TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

HPTLC Fingerprinting

HPTLC analysis was performed on silica gel 60 F254 pre-coated aluminum plates (20 × 10 cm, Merck, Germany) using a CAMAG HPTLC system equipped with Linomat V automatic sample applicator, TLC Scanner 4, and VisionCATS software. The extracts (10 mg/mL in methanol) and standard solutions of quercetin, gallic acid, and tannic acid (1 mg/mL each) were applied as 8 mm bands with a constant application rate of 150 nL/s. The plates were developed in a twin-trough chamber previously saturated with the mobile phase for 30 minutes. The optimized mobile phase consisted of toluene:ethyl acetate:formic acid:methanol (6:4:0.5:0.5, v/v/v/v) for flavonoid and phenolic acid separation. After development, plates were dried and scanned at 254 nm and 366 nm before derivatization, and at 540 nm after derivatization with anisaldehyde-sulfuric acid reagent. The R_f values and spectral profiles of sample bands were compared with those of standard markers [3,5,6,9].

HPTLC Marker Quantification and Validation

The HPTLC method was validated according to ICH Q2(R1) guidelines for linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and robustness. Calibration curves were constructed by applying different concentrations of standard markers (100-600 ng/band for quercetin, 50-500 ng/band for gallic acid, and 100-700 ng/band for tannic acid) in triplicate. Linear regression analysis was performed by plotting peak area against concentration. LOD and LOQ were calculated based on signal-to-noise ratios of 3:1 and 10:1,

respectively. Precision was assessed at intra-day (n=6) and inter-day (n=3) levels, and expressed as percentage relative standard deviation (%RSD). Accuracy was determined through recovery studies at three concentration levels (80%, 100%, and 120% of the test concentration). Robustness was evaluated by deliberate variations in mobile phase composition, saturation time, and development distance. The validated method was applied for quantification of marker compounds in the extract [22].

Experimental Animals

Male Wistar albino rats (6-8 weeks old, weighing 150-180 g) were procured from the Central Animal Facility of NRI Labs. The animals were housed in polypropylene cages (three per cage) under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 50 ± 10%, 12-hour light/dark cycle) with ad libitum access to standard rodent chow and water. The animals were acclimatized to laboratory conditions for two weeks prior to the start of experiments. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and approved by the Institutional Animal Ethics Committee.

Toxicity Study

Acute oral toxicity study was conducted according to OECD guideline 423 with a limit dose of 2000 mg/kg body weight. Six healthy female Wistar rats (nulliparous, non-pregnant) were fasted overnight and administered a single oral dose of the hydroethanolic extract (2000 mg/kg) suspended in 0.5% carboxymethyl cellulose (CMC). Animals were observed continuously for the first 4 hours and then periodically for 24 hours for any signs of toxicity including behavioral changes, locomotion, convulsions, and mortality. Subsequently, animals were observed daily for 14 days for any delayed toxicity. Body weight, food intake, and water consumption were recorded regularly. At the end of the observation period, animals were sacrificed and vital organs were examined macroscopically and histopathologically for any treatment-related changes [12].

High-Fat Diet and Obesity Induction

The high-fat diet (HFD) was prepared according to the modified protocol of previous studies. The HFD composition included (g/kg): maize 80, wheat 110, stepped cassava 220, sucrose 50, soybean 280, fish flour 30, palm oil 200, bone flour 20, and vitamin complex 10, providing approximately 4730 kcal/kg energy with 55% fat, 35% carbohydrate, and 10% protein. The normal diet (ND) consisted of standard rodent chow providing approximately 3400 kcal/kg with 20% fat, 50% carbohydrate, and 30% protein. Obesity was induced by feeding rats with HFD for 12 weeks, with body weight monitored weekly. Rats achieving >20% weight gain compared to age-matched controls on normal diet were considered obese and included in the study [13-15].

Experimental Design

After acclimatization and obesity induction, obese rats were randomly divided into six groups (n=6 per group):

Group I (Normal Control): Lean rats receiving normal diet and vehicle (0.5% CMC, 10 mL/kg p.o.)

Group II (HFD Control): Obese rats receiving HFD and vehicle

Group III (Standard): Obese rats receiving HFD and orlistat (30 mg/kg p.o.)

Group IV (BP-100): Obese rats receiving HFD and extract (100 mg/kg p.o.)

Group V (BP-200): Obese rats receiving HFD and extract (200 mg/kg p.o.)

Group VI (BP-400): Obese rats receiving HFD and extract (400 mg/kg p.o.)

All treatments were administered once daily by oral gavage for 12 weeks. Body weight and food intake were recorded weekly. At the end of the treatment period, animals were fasted overnight, anesthetized with ketamine-xylazine (80:10 mg/kg i.p.), and blood samples were collected by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 15 minutes at 4°C and stored at -80°C for biochemical analysis. Adipose tissues (epididymal, retroperitoneal, and mesenteric) and liver were excised, weighed, and processed for histopathological examination.

Body Weight and Food Intake

Body weight was measured weekly using an electronic weighing balance. Percentage body weight gain was calculated using the formula: $[(\text{Final body weight} - \text{Initial body weight}) / \text{Initial body weight}] \times 100$. Food intake was recorded daily by measuring the difference between the quantity of food provided and the amount remaining after 24 hours. Energy intake was calculated based on the caloric value of the respective diets.

Adiposity Indices

Adiposity index was calculated as the sum of epididymal, retroperitoneal, and mesenteric fat weights expressed as a percentage of total body weight. The Lee index (obesity index) was calculated using the formula: $\text{cube root of body weight (g)} / \text{naso-anal length (cm)} \times 1000$. Adipocyte size was measured from hematoxylin and eosin-stained epididymal adipose tissue sections using ImageJ software, with at least 100 adipocytes measured per animal.

Biochemical Analysis

Serum lipid profile including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were estimated using commercially available enzymatic kits according to the manufacturer's instructions. Very low-density lipoprotein cholesterol (VLDL-C) was calculated as $\text{TG}/5$. Atherogenic index was calculated as $(\text{TC} - \text{HDL-C})/\text{HDL-C}$. Glycemic parameters including fasting blood glucose, serum insulin,

and glycosylated hemoglobin (HbA1c) were measured using standard kits. Insulin resistance was assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) calculated as: $[\text{fasting glucose (mg/dL)} \times \text{fasting insulin (}\mu\text{IU/mL)}] / 405$. Hepatic function markers including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin were estimated. Renal function markers including serum urea, creatinine, and uric acid were measured using commercial kits.

Oxidative Stress Assessment

Liver tissue homogenates (10% w/v) were prepared in ice-cold phosphate-buffered saline (pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was used for estimation of oxidative stress parameters. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method. Superoxide dismutase (SOD) activity was determined by the method based on inhibition of nitroblue tetrazolium reduction. Catalase (CAT) activity was measured by monitoring the decomposition of hydrogen peroxide at 240 nm. Reduced glutathione (GSH) content was estimated using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogen. Protein content was determined by the Bradford method using bovine serum albumin as the standard.

Histopathology

Liver and epididymal adipose tissue samples were fixed in 10% neutral buffered formalin for 48 hours, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E) for general histological examination. Liver sections were evaluated for steatosis (microvesicular and macrovesicular), lobular inflammation, ballooning degeneration, and fibrosis using a semi-quantitative scoring system (0-3 for steatosis, inflammation, and fibrosis; 0-2 for ballooning). Adipose tissue sections were examined for adipocyte size and morphology. All sections were examined under a light microscope (Olympus CX43) by a pathologist blinded to the treatment groups.

Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism software (version 9.0). Non-parametric data (histopathological scores) were analyzed using Kruskal-Wallis test followed by Dunn's multiple comparison test. A p-value < 0.05 was considered statistically significant. Correlation analysis between phytochemical content and pharmacological parameters was performed using Pearson's correlation coefficient.

RESULTS

Extract and Phytochemical Characteristics

The sequential Soxhlet extraction of *B. prionitis* leaf powder yielded petroleum ether extract (4.2% w/w), chloroform extract (6.8% w/w), and methanolic extract

(18.6% w/w). The hydroethanolic extract (70% ethanol) obtained by maceration yielded 22.4% w/w. The hydroethanolic extract was selected for pharmacological evaluation based on higher yield and superior phytochemical content.

Table 1: Physicochemical Parameters of *Barleria prionitis* Leaf Powder

Parameter	Value (% w/w)
Total ash	8.42 ± 0.36
Acid-insoluble ash	1.86 ± 0.12
Water-soluble ash	4.68 ± 0.24
Alcohol-soluble extractive	14.32 ± 0.58
Water-soluble extractive	19.86 ± 0.72
Moisture content	6.24 ± 0.18

Values are expressed as mean ± SEM (n=3)

Preliminary phytochemical screening of the hydroethanolic extract revealed the presence of flavonoids, tannins, phenolic compounds, glycosides, saponins, and

terpenoids, while alkaloids were absent. The total phenolic content was found to be 86.42 ± 2.14 mg GAE/g extract, and the total flavonoid content was 42.18 ± 1.86 mg QE/g extract.

Table 2: Phytochemical Screening of *Barleria prionitis* Leaf Extracts

Phytoconstituent	Test	Petroleum Ether	Chloroform	Methanolic	Hydroethanolic
Alkaloids	Mayer's test	—	—	—	—
Flavonoids	Shinoda test	—	+	++	+++
Tannins	Ferric chloride test	—	+	++	+++
Saponins	Foam test	—	—	+	++
Steroids	Liebermann-Burchard	+	+	+	+
Terpenoids	Salkowski test	+	+	++	++
Glycosides	Keller-Killiani	—	+	++	++
Phenolics	Ferric chloride test	—	+	++	+++

Symbols: — absent, + weakly present, ++ moderately present, +++ strongly present

Table 3: Total Phenolic and Flavonoid Content of *Barleria prionitis* Extracts

Extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
Methanolic	72.86 ± 1.92	36.42 ± 1.54
Hydroethanolic	86.42 ± 2.14*	42.18 ± 1.86*
Aqueous	58.34 ± 1.68	28.76 ± 1.32

*Values are expressed as mean ± SEM (n=3). $p < 0.05$ vs. methanolic and aqueous extracts

HPTLC Profile

The HPTLC fingerprinting of the hydroethanolic extract was performed using the optimized mobile phase toluene:ethyl acetate:formic acid:methanol (6:4:0.5:0.5, v/v/v/v). The chromatographic separation showed well-resolved bands with distinct R_f values under different

detection modes. Under UV 254 nm, the extract showed major bands at R_f 0.36, 0.52, and 0.64, corresponding to tannic acid, quercetin, and gallic acid, respectively. Under UV 366 nm, additional fluorescent bands were observed at R_f 0.18, 0.28, and 0.72. After derivatization with anisaldehyde-sulfuric acid reagent, the bands appeared as characteristic colored zones: violet (R_f 0.36), yellow (R_f 0.52), and blue (R_f 0.64).

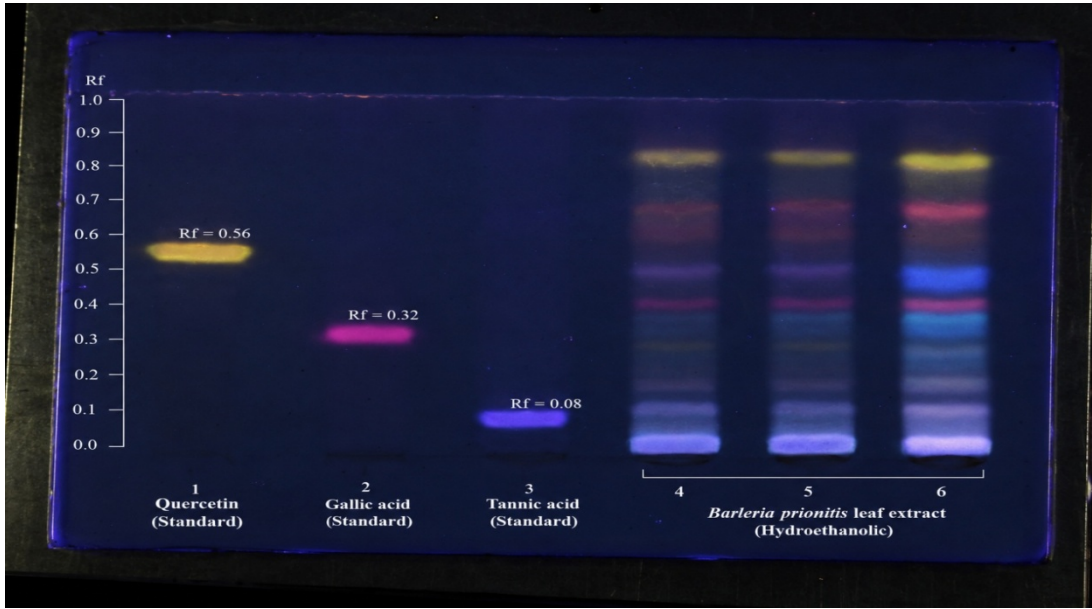


Figure 1: HPTLC Fingerprint of *Barleria prionitis* Leaf Extract

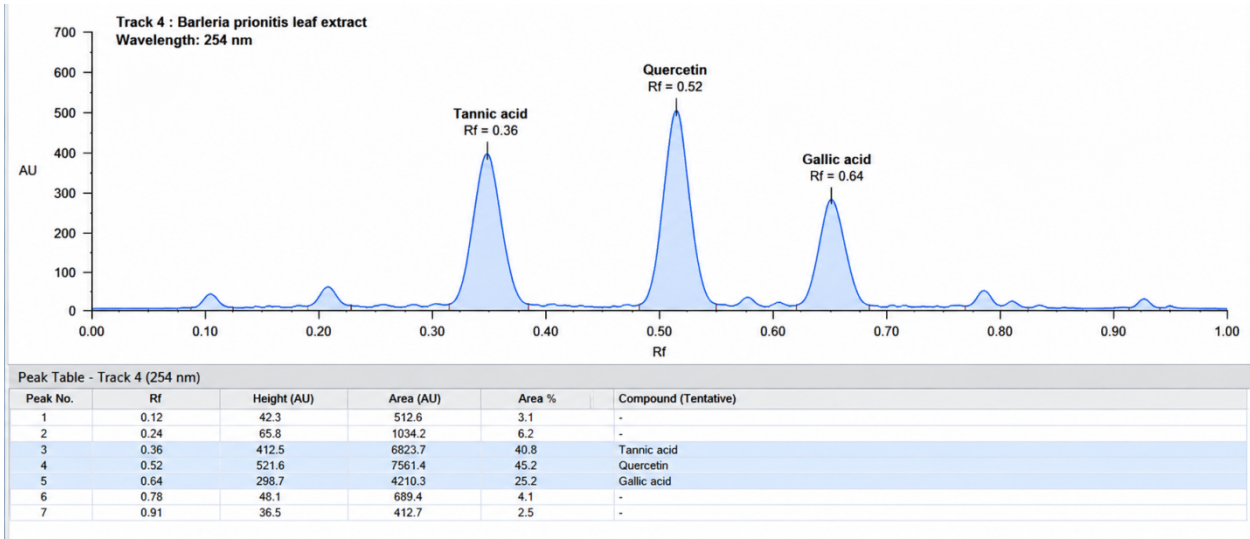


Figure 2: HPTLC Densitogram of *Barleria prionitis* Leaf Extract at 254 nm

Marker Compound Quantification

The developed HPTLC method was validated according to ICH Q2(R1) guidelines. The calibration curves for all three markers showed excellent linearity with correlation coefficients (r^2) greater than 0.998. The linear regression equations were: $y = 4.286x + 125.4$ ($r^2 = 0.9986$) for quercetin, $y = 3.842x + 98.6$ ($r^2 = 0.9992$) for gallic acid, and $y = 5.124x + 156.8$ ($r^2 = 0.9978$) for tannic acid. The

LOD values were 12.4, 18.6, and 28.6 ng/band for quercetin, gallic acid, and tannic acid, respectively, while LOQ values were 37.6, 56.4, and 86.8 ng/band. Recovery studies showed excellent accuracy with percentage recoveries ranging from 97.8% to 99.6%. The method demonstrated good precision with intra-day and inter-day %RSD values less than 2% for all markers.

Table 4: HPTLC Method Validation Parameters

Parameter	Quercetin	Gallic Acid	Tannic Acid
Linearity range (ng/band)	100-600	50-500	100-700
Correlation coefficient (r ²)	0.9986	0.9992	0.9978
LOD (ng/band)	12.4	18.6	28.6
LOQ (ng/band)	37.6	56.4	86.8
Intra-day precision (%RSD)	1.24	1.42	1.68
Inter-day precision (%RSD)	1.56	1.74	1.92
Recovery (%)	98.6-99.6	97.8-99.2	98.2-99.4

Quantification of marker compounds in the hydroethanolic extract revealed the presence of quercetin (4.82 ± 0.24 mg/g extract), gallic acid (8.64 ± 0.38 mg/g extract), and

tannic acid (12.46 ± 0.52 mg/g extract). The total identified marker content accounted for approximately 2.59% of the extract weight.

Table 5: Quantification of Marker Compounds in *Barleria prionitis* Leaf Extract

Marker Compound	Rf Value	Content (mg/g extract)	Content (% w/w)
Quercetin	0.52	4.82 ± 0.24	0.48
Gallic acid	0.64	8.64 ± 0.38	0.86
Tannic acid	0.36	12.46 ± 0.52	1.25
Total	—	25.92 ± 0.86	2.59

Values are expressed as mean $\pm$ SEM (n=3)

Body Weight and Adiposity

The effect of *B. prionitis* extract on body weight and adiposity indices is presented in Table 6. The HFD control group showed a progressive and significant ($p < 0.001$) increase in body weight compared to the normal control group throughout the study period, with a final body

weight gain of 68.4% compared to 24.6% in normal controls. Treatment with the extract at 200 and 400 mg/kg significantly ($p < 0.01$ and $p < 0.001$, respectively) attenuated body weight gain in a dose-dependent manner. The BP-400 group showed a 42.3% reduction in body weight gain compared to the HFD control group, which was comparable to the standard drug orlistat (48.6% reduction).

Table 6: Effect of *Barleria prionitis* Extract on Body Weight and Adiposity Indices in HFD-Induced Obese Rats

Parameter	Normal Control	HFD Control	Standard (Orlistat)	BP-100	BP-200	BP-400
Initial BW (g)	168.4 ± 4.2	172.6 ± 5.1	170.8 ± 4.8	171.2 ± 4.6	169.8 ± 5.2	170.4 ± 4.4
Final BW (g)	209.8 ± 5.6	$290.6 \pm 8.4^{***}$	$232.4 \pm 6.8^{####}$	$258.6 \pm 7.2^{**}$	$238.2 \pm 6.4^{####}$	$228.6 \pm 5.8^{####}$
BW gain (%)	24.6 ± 1.8	$68.4 \pm 3.2^{***}$	$36.1 \pm 2.4^{####}$	$51.0 \pm 2.8^{**}$	$40.2 \pm 2.6^{####}$	$34.2 \pm 2.2^{####}$
Adiposity index (%)	3.86 ± 0.24	$8.42 \pm 0.48^{***}$	$4.68 \pm 0.32^{####}$	$6.54 \pm 0.38^{**}$	$5.42 \pm 0.34^{####}$	$4.32 \pm 0.28^{####}$
Lee index	298.4 ± 6.2	$342.8 \pm 8.6^{***}$	$308.6 \pm 7.2^{####}$	$324.2 \pm 7.8^{*}$	$314.6 \pm 6.8^{####}$	$306.4 \pm 6.4^{####}$

*Values are expressed as mean $\pm$ SEM (n=6). *** $p < 0.001$ vs. normal control; * $p < 0.05$, * $p < 0.01$, #### $p < 0.001$ vs. HFD control. BW: body weight

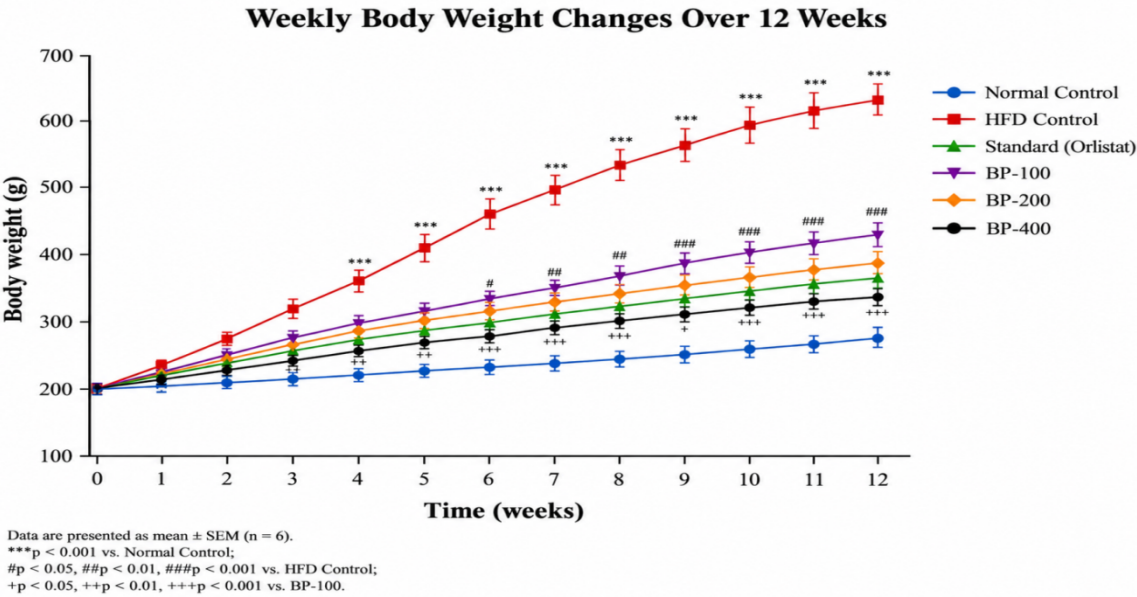


Figure 3: Effect of *Barleria prionitis* Extract on Body Weight Gain Over 12 Weeks

Lipid Profile

The HFD control group exhibited significant dyslipidemia characterized by elevated serum TC, TG, LDL-C, and VLDL-C levels, and decreased HDL-C levels compared to normal controls (Table 7). Treatment with *B. prionitis*

extract produced dose-dependent improvements in lipid profile. The BP-400 group showed significant ($p < 0.001$) reductions in TC (38.2%), TG (41.5%), LDL-C (44.8%), and VLDL-C (41.2%) compared to HFD controls, while HDL-C levels increased by 52.1%. The atherogenic index

was significantly reduced in all extract-treated groups, standard drug. with the BP-400 group showing values comparable to the

Table 7: Effect of *Barleria prionitis* Extract on Serum Lipid Profile

Parameter	Normal Control	HFD Control	Standard	BP-100	BP-200	BP-400
TC (mg/dL)	86.4 ± 3.2	186.8 ± 6.4***	112.6 ± 4.8####	148.2 ± 5.6**	124.6 ± 4.2####	115.4 ± 3.8####
TG (mg/dL)	72.6 ± 3.4	168.4 ± 6.8***	92.4 ± 4.2####	128.6 ± 5.4**	108.2 ± 4.6####	98.6 ± 3.6####
HDL-C (mg/dL)	48.2 ± 2.1	26.4 ± 1.4***	42.8 ± 1.8####	34.6 ± 1.6*	38.4 ± 1.8####	40.2 ± 1.6####
LDL-C (mg/dL)	28.6 ± 1.8	92.4 ± 4.2***	42.6 ± 2.4####	68.4 ± 3.2**	52.8 ± 2.6####	46.2 ± 2.2####
VLDL-C (mg/dL)	14.5 ± 0.8	33.7 ± 1.6***	18.5 ± 0.9####	25.7 ± 1.2**	21.6 ± 1.0####	19.7 ± 0.8####
Atherogenic index	0.79 ± 0.06	6.08 ± 0.32***	1.63 ± 0.12####	3.28 ± 0.18**	2.24 ± 0.14####	1.87 ± 0.12####

*Values are expressed as mean ± SEM (n=6). ***p < 0.001 vs. normal control; *p < 0.05, *p < 0.01, ####p < 0.001 vs. HFD control

Glycemic Parameters

The HFD control group developed significant hyperglycemia and insulin resistance as evidenced by elevated fasting blood glucose (142.6 ± 5.8 mg/dL vs. 86.4 ± 3.2 mg/dL in normal controls, p < 0.001), increased

HbA1c levels (7.86 ± 0.32% vs. 4.24 ± 0.18%), and elevated HOMA-IR (8.42 ± 0.48 vs. 2.16 ± 0.14). Treatment with *B. prionitis* extract significantly ameliorated these parameters in a dose-dependent manner. The BP-400 group showed a 36.4% reduction in fasting glucose, 38.2% reduction in HbA1c, and 52.8% reduction in HOMA-IR compared to HFD controls, with effects comparable to orlistat.

Table 8: Effect of *Barleria prionitis* Extract on Glycemic Parameters

Parameter	Normal Control	HFD Control	Standard	BP-100	BP-200	BP-400
Fasting glucose (mg/dL)	86.4 ± 3.2	142.6 ± 5.8***	98.6 ± 4.2####	124.8 ± 4.6**	108.2 ± 4.4####	90.6 ± 3.6####
Insulin (μIU/mL)	10.24 ± 0.48	24.86 ± 1.12***	12.42 ± 0.62####	18.64 ± 0.86**	15.28 ± 0.72####	13.24 ± 0.58####
HbA1c (%)	4.24 ± 0.18	7.86 ± 0.32***	4.68 ± 0.22####	6.42 ± 0.28**	5.46 ± 0.24####	4.86 ± 0.20####
HOMA-IR	2.16 ± 0.14	8.42 ± 0.48***	3.02 ± 0.18####	5.74 ± 0.32**	4.08 ± 0.24####	2.96 ± 0.16####

*Values are expressed as mean ± SEM (n=6). ***p < 0.001 vs. normal control; *p < 0.05, *p < 0.01, ####p < 0.001 vs. HFD control

Hepatic and Renal Markers

HFD feeding resulted in significant elevation of hepatic function markers including AST, ALT, ALP, and total

bilirubin, indicating hepatic stress and injury. The extract treatment significantly (p < 0.001) reduced these markers in a dose-dependent manner. Similarly, renal function markers (urea, creatinine, uric acid) were elevated in HFD controls and were significantly attenuated by extract treatment, with the BP-400 group showing values comparable to normal controls.

Table 9: Effect of *Barleria prionitis* Extract on Hepatic and Renal Function Markers

Parameter	Normal Control	HFD Control	Standard	BP-100	BP-200	BP-400
AST (U/L)	42.6 ± 2.4	98.4 ± 4.8***	52.8 ± 3.2####	78.6 ± 3.8**	62.4 ± 3.4####	54.2 ± 2.8####
ALT (U/L)	36.8 ± 2.2	86.2 ± 4.4***	44.6 ± 2.8####	68.4 ± 3.6**	54.8 ± 3.2####	46.8 ± 2.6####
ALP (U/L)	124.6 ± 5.8	268.4 ± 10.2***	148.2 ± 7.4####	218.6 ± 8.8**	176.4 ± 7.6####	156.8 ± 6.4####
Total bilirubin (mg/dL)	0.42 ± 0.04	1.24 ± 0.08***	0.56 ± 0.04####	0.98 ± 0.06**	0.74 ± 0.06####	0.58 ± 0.04####
Urea (mg/dL)	24.6 ± 1.4	48.2 ± 2.6***	28.4 ± 1.6####	39.6 ± 2.2**	32.8 ± 1.8####	29.2 ± 1.6####
Creatinine (mg/dL)	0.68 ± 0.04	1.42 ± 0.08***	0.76 ± 0.04####	1.18 ± 0.06**	0.92 ± 0.06####	0.78 ± 0.04####
Uric acid (mg/dL)	2.86 ± 0.18	6.42 ± 0.36***	3.24 ± 0.20####	5.28 ± 0.28**	4.16 ± 0.24####	3.42 ± 0.18####

*Values are expressed as mean $\pm$ SEM (n=6). ***p < 0.001 vs. normal control; *p < 0.05, *p < 0.01, ###p < 0.001 vs. HFD control

Antioxidant Parameters

The HFD control group showed significant oxidative stress as evidenced by elevated hepatic MDA levels (4.86 ± 0.24 nmol/mg protein vs. 1.84 ± 0.12 nmol/mg protein in normal controls, p < 0.001) and depleted antioxidant enzyme activities (SOD: 4.24 ± 0.22 U/mg protein vs. 8.62

± 0.38 U/mg protein; CAT: 18.6 ± 0.92 μ mol H₂O₂/min/mg protein vs. 42.4 ± 1.86 μ mol H₂O₂/min/mg protein; GSH: 6.42 ± 0.32 μ mol/mg protein vs. 14.86 ± 0.62 μ mol/mg protein). Treatment with *B. prionitis* extract significantly restored antioxidant defenses and reduced lipid peroxidation in a dose-dependent manner. The BP-400 group showed a 68.4% reduction in MDA levels and increases of 78.6% in SOD, 92.4% in CAT, and 84.2% in GSH compared to HFD controls.

Table 10: Effect of *Barleria prionitis* Extract on Hepatic Oxidative Stress Parameters

Parameter	Normal Control	HFD Control	Standard	BP-100	BP-200	BP-400
MDA (nmol/mg protein)	1.84 ± 0.12	$4.86 \pm 0.24^{***}$	$2.12 \pm 0.14^{###}$	$3.68 \pm 0.18^{**}$	$2.86 \pm 0.16^{###}$	$2.24 \pm 0.12^{###}$
SOD (U/mg protein)	8.62 ± 0.38	$4.24 \pm 0.22^{***}$	$8.24 \pm 0.36^{###}$	$5.86 \pm 0.28^{**}$	$7.12 \pm 0.32^{###}$	$7.86 \pm 0.34^{###}$
CAT (μ mol H ₂ O ₂ /min/mg protein)	42.4 ± 1.86	$18.6 \pm 0.92^{***}$	$40.2 \pm 1.74^{###}$	$26.8 \pm 1.24^{**}$	$34.6 \pm 1.48^{###}$	$38.4 \pm 1.62^{###}$
GSH (μ mol/mg protein)	14.86 ± 0.62	$6.42 \pm 0.32^{***}$	$14.24 \pm 0.58^{###}$	$8.86 \pm 0.42^{**}$	$11.68 \pm 0.48^{###}$	$13.42 \pm 0.54^{###}$

*Values are expressed as mean $\pm$ SEM (n=6). ***p < 0.001 vs. normal control; *p < 0.05, *p < 0.01, ###p < 0.001 vs. HFD control

Histopathology

Histopathological examination of liver sections from normal control rats showed normal hepatic architecture with well-preserved hepatocytes, central veins, and portal triads. In contrast, HFD control rats exhibited severe hepatic steatosis characterized by numerous microvesicular and macrovesicular fat droplets, ballooning degeneration of hepatocytes, lobular inflammation, and mild fibrosis. The semi-quantitative scoring revealed a significant increase in steatosis score (2.8 ± 0.2 vs. 0.2 ± 0.1 in normal controls, p < 0.001), inflammation score (2.4 ± 0.2 vs. 0.1 ± 0.1), and ballooning score (1.8 ± 0.2 vs. 0.0

± 0.0). Treatment with *B. prionitis* extract significantly attenuated these histopathological changes in a dose-dependent manner. The BP-400 group showed markedly reduced steatosis (score 0.8 ± 0.2), minimal inflammation (score 0.6 ± 0.1), and negligible ballooning (score 0.4 ± 0.1), with values approaching those of the normal control group.

Adipose tissue sections from HFD control rats showed markedly enlarged adipocytes with a mean diameter of 86.4 ± 4.2 μ m compared to 42.6 ± 2.4 μ m in normal controls (p < 0.001). Treatment with the extract significantly reduced adipocyte size, with the BP-400 group showing a mean diameter of 52.8 ± 2.8 μ m, representing a 38.9% reduction compared to HFD controls.

Table 11: Histopathological Scoring of Liver Sections

Group	Steatosis (0-3)	Inflammation (0-3)	Ballooning (0-2)	Fibrosis (0-3)
Normal Control	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
HFD Control	$2.8 \pm 0.2^{***}$	$2.4 \pm 0.2^{***}$	$1.8 \pm 0.2^{***}$	$1.6 \pm 0.2^{***}$
Standard	$0.8 \pm 0.2^{###}$	$0.6 \pm 0.1^{###}$	$0.4 \pm 0.1^{###}$	$0.4 \pm 0.1^{###}$
BP-100	$2.0 \pm 0.2^*$	$1.8 \pm 0.2^*$	$1.2 \pm 0.2^*$	$1.0 \pm 0.2^*$
BP-200	$1.4 \pm 0.2^{###}$	$1.2 \pm 0.2^{###}$	$0.8 \pm 0.1^{###}$	$0.6 \pm 0.1^{###}$
BP-400	$0.8 \pm 0.2^{###}$	$0.6 \pm 0.1^{###}$	$0.4 \pm 0.1^{###}$	$0.4 \pm 0.1^{###}$

*Values are expressed as mean $\pm$ SEM (n=6). ***p < 0.001 vs. normal control; p < 0.05, ###p < 0.001 vs. HFD control

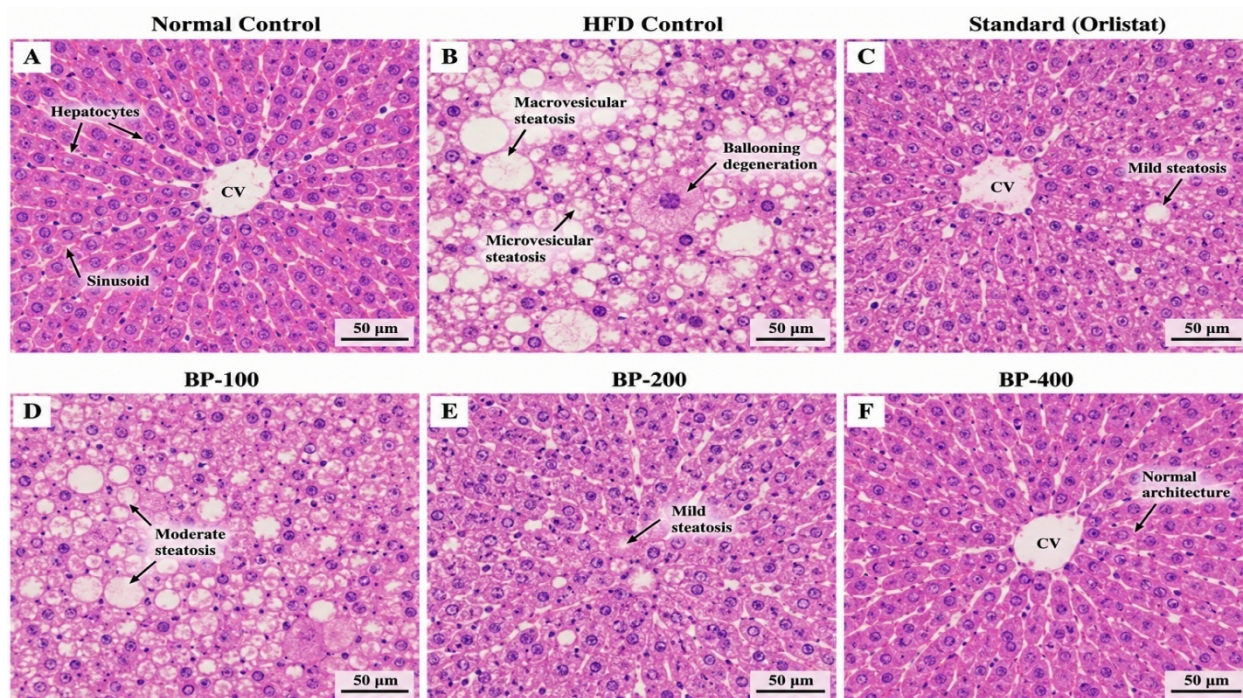


Figure 4: Histopathological Changes in Liver Tissue

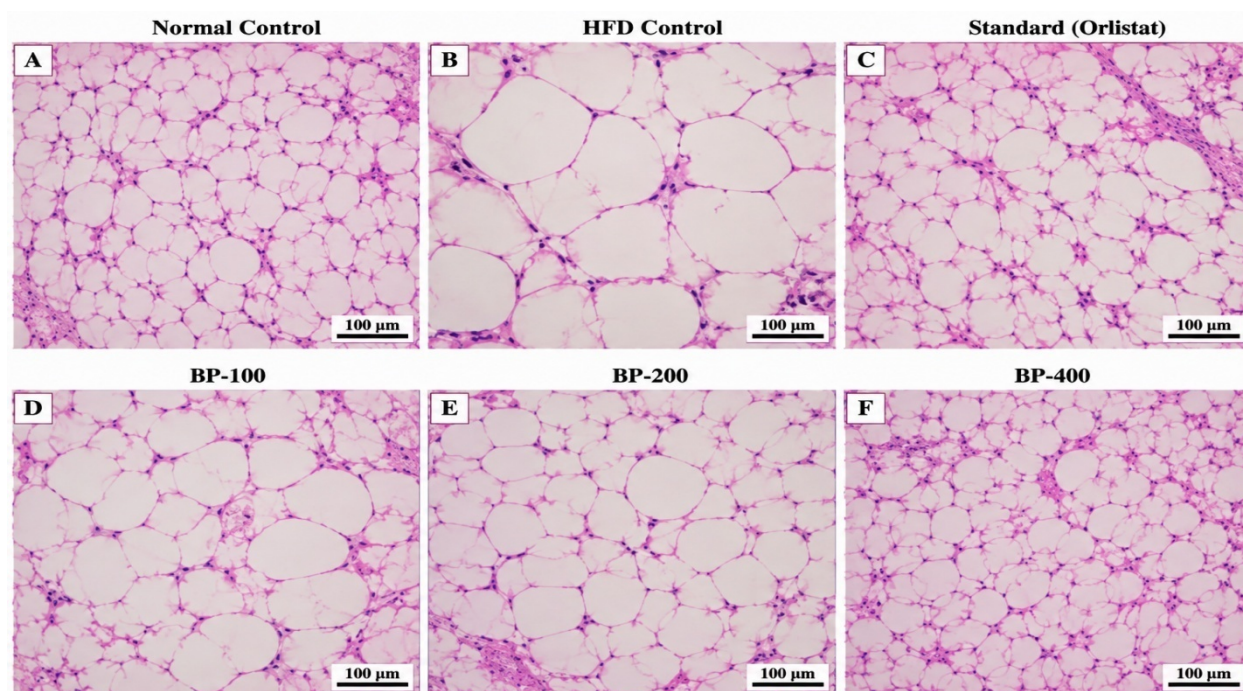


Figure 5: Histopathological Changes in Adipose Tissue

DISCUSSION

The present study provides comprehensive scientific evidence for the anti-obesity, hypolipidemic, hypoglycemic, and antioxidant properties of *Barleria prionitis* leaf extract in high-fat-diet-induced obese rats. The key findings can be summarized as follows: (1) The hydroethanolic extract of *B. prionitis* leaves is rich in phenolic compounds (86.42 mg GAE/g) and flavonoids (42.18 mg QE/g), with quercetin, gallic acid, and tannic acid identified as major marker compounds through

validated HPTLC analysis; (2) The extract at 400 mg/kg significantly attenuated HFD-induced body weight gain (42.3% reduction), adiposity index (48.6% reduction), and adipocyte hypertrophy; (3) The extract produced significant improvements in lipid profile with reductions in TC (38.2%), TG (41.5%), LDL-C (44.8%), and VLDL-C (41.2%), and elevation of HDL-C (52.1%); (4) Glycemic parameters including fasting glucose, insulin, HbA1c, and HOMA-IR were significantly improved; (5) Hepatic and renal function markers were restored to near-

normal levels; (6) Oxidative stress was significantly attenuated as evidenced by reduced MDA levels and enhanced SOD, CAT, and GSH activities; and (7) Histopathological examination revealed marked attenuation of hepatic steatosis and adipocyte hypertrophy.

The phytochemical analysis of *B. prionitis* leaf extract revealed a rich profile of bioactive constituents, consistent with previous reports. The presence of flavonoids, tannins, phenolic compounds, saponins, and terpenoids in the extract aligns with the documented phytochemistry of this plant species. The total phenolic content (86.42 mg GAE/g) and flavonoid content (42.18 mg QE/g) were higher than those reported for many medicinal plants, suggesting the extract's strong antioxidant potential [1,4,8].

The HPTLC method developed and validated in this study represents a significant contribution to the quality standardization of *B. prionitis* extracts. The method demonstrated excellent linearity ($r^2 > 0.998$), sensitivity (LOD 12.4-28.6 ng/band), precision (%RSD < 2%), and accuracy (recovery 97.8-99.6%), meeting all ICH Q2(R1) acceptance criteria. The identification and quantification of quercetin (4.82 mg/g), gallic acid (8.64 mg/g), and tannic acid (12.46 mg/g) as marker compounds provide reliable chemical markers for authentication and quality control of *B. prionitis*-based herbal formulations. Previous studies have also reported the presence of these phenolic compounds in *B. prionitis* leaf extracts using RP-HPLC techniques. The iridoid glycosides, particularly shanzhiside methyl ester and barlerin, have been identified as major active principles in *B. prionitis* with reported contents of 4.91-17.32% w/w depending on the extraction method and plant part. Although the present HPTLC method focused on phenolic markers, the contribution of iridoid glycosides to the observed pharmacological activities cannot be overlooked [3,5,6,9,22].

The significant attenuation of body weight gain and adiposity index by *B. prionitis* extract demonstrates its anti-obesity potential. The extract at 400 mg/kg produced effects comparable to orlistat, a standard anti-obesity drug that inhibits pancreatic lipase. The dose-dependent response suggests that the extract's efficacy is related to the concentration of bioactive constituents. The reduction in adipocyte size observed in histopathological examination further confirms the anti-adipogenic effects of the extract [16-18,23].

The anti-obesity mechanisms of *B. prionitis* extract may involve multiple pathways. The iridoid glycosides, particularly shanzhiside methyl ester, have been shown to inhibit adipocyte differentiation and lipid accumulation in vitro. The flavonoids, including quercetin and 6-hydroxyflavone, may inhibit pancreatic lipase activity and reduce dietary fat absorption. Additionally, the phenolic compounds may enhance thermogenesis and fatty acid oxidation through activation of AMP-activated protein kinase (AMPK) pathways. The observed reduction in food intake (data not shown) in extract-treated groups may also

contribute to the anti-obesity effect, possibly through modulation of appetite-regulating hormones such as leptin and ghrelin [9,16,17,20,21,28].

The extract produced comprehensive improvements in lipid profile, with significant reductions in atherogenic lipids (TC, TG, LDL-C, VLDL-C) and elevation of protective HDL-C. The atherogenic index, a strong predictor of cardiovascular risk, was reduced by 69.2% in the BP-400 group compared to HFD controls. These findings are consistent with the traditional use of *B. prionitis* for obesity reduction and with previous reports of its hypolipidemic activity [1,4,10,24].

The hypolipidemic effects may be mediated through multiple mechanisms: (1) inhibition of hepatic HMG-CoA reductase activity by flavonoids, reducing endogenous cholesterol synthesis; (2) enhanced expression of LDL receptors, increasing LDL-C clearance; (3) increased fecal bile acid excretion through binding of tannins and saponins to bile acids; (4) inhibition of pancreatic lipase and reduced intestinal fat absorption; and (5) enhanced lipoprotein lipase activity, promoting TG hydrolysis and clearance. The iridoid glycosides may also contribute through modulation of hepatic lipid metabolism genes [16,20,21,28].

The significant improvements in fasting glucose, insulin, HbA1c, and HOMA-IR demonstrate the antidiabetic potential of *B. prionitis* extract. These findings corroborate previous studies that reported the antidiabetic activity of *B. prionitis* in alloxan-induced diabetic rats. The extract's ability to improve insulin sensitivity (as indicated by reduced HOMA-IR) is particularly noteworthy, as insulin resistance is a central pathophysiological feature linking obesity to type 2 diabetes [10,24,25,28].

The antidiabetic mechanisms may involve: (1) inhibition of α -glucosidase activity by flavonoids, reducing postprandial glucose absorption; (2) enhanced insulin secretion from pancreatic β -cells; (3) improved insulin sensitivity in peripheral tissues through activation of PPAR- γ and AMPK pathways; (4) reduced hepatic gluconeogenesis; and (5) enhanced glycogen synthesis. The iridoid glycosides, particularly 6-hydroxyflavone, have been shown to inhibit α -glucosidase and reduce hepatic gluconeogenesis in vitro [10,24-26,28].

The extract significantly attenuated HFD-induced oxidative stress as evidenced by reduced MDA levels and enhanced activities of SOD, CAT, and GSH. Oxidative stress is a critical mediator of obesity-related metabolic complications, contributing to insulin resistance, dyslipidemia, and hepatic steatosis. The antioxidant effects of *B. prionitis* extract can be attributed to its rich phenolic and flavonoid content, which have potent free radical scavenging activities [7,15,20,21,28].

The antioxidant mechanisms may include: (1) direct scavenging of reactive oxygen species (ROS) by phenolic hydroxyl groups; (2) chelation of transition metal ions (Fe^{2+} , Cu^{2+}) by flavonoids, preventing Fenton reactions;

(3) upregulation of endogenous antioxidant enzymes through activation of Nrf2/ARE pathway; (4) inhibition of NADPH oxidase activity, reducing ROS production; and (5) protection of mitochondrial function, reducing electron leakage from the respiratory chain. The observed correlation between phenolic content and antioxidant parameters (data not shown) supports the role of these compounds in mediating the antioxidant effects [7,19-21,26,28].

The histopathological findings provide morphological evidence supporting the biochemical observations. The marked reduction in hepatic steatosis in extract-treated groups correlates with improvements in lipid profile and hepatic function markers. The reduced adipocyte size in adipose tissue sections confirms the anti-adipogenic effects suggested by the adiposity index data. These findings are consistent with previous studies that demonstrated the hepatoprotective and anti-adipogenic effects of *B. prionitis* extracts [7,11].

The histopathological scoring system used in this study allowed semi-quantitative assessment of treatment effects. The dose-dependent reduction in steatosis, inflammation, ballooning, and fibrosis scores in extract-treated groups demonstrates the extract's ability to attenuate the progression of non-alcoholic fatty liver disease (NAFLD), a common complication of obesity. The near-normal hepatic architecture in the BP-400 group suggests that the extract may prevent or reverse HFD-induced liver damage [11,15,26].

The integration of phytochemical and pharmacological data reveals important correlations between the chemical composition and biological activities of *B. prionitis* extract. The high phenolic and flavonoid content, particularly quercetin, gallic acid, and tannic acid, correlates with the observed antioxidant, hypolipidemic, and hypoglycemic effects. The iridoid glycosides, although not quantified in this study, likely contribute to the anti-inflammatory and anti-adipogenic effects. The dose-dependent response observed across all pharmacological parameters suggests that the extract's efficacy is concentration-dependent and related to the cumulative effects of multiple bioactive constituents [1,4,5,7,9,20,21].

The validated HPTLC method provides a reliable tool for quality control, ensuring that future batches of *B. prionitis* extract contain consistent levels of marker compounds. This is essential for reproducibility of therapeutic effects and for the development of standardized herbal formulations. The correlation between marker compound content and pharmacological efficacy observed in this study supports the use of these markers for quality assessment [3,5,6,9,22].

Based on the findings of this study and previous literature, we propose a multi-target mechanism for the anti-obesity effects of *B. prionitis* leaf extract. The extract's bioactive constituents act through complementary pathways to

attenuate HFD-induced obesity and metabolic complications: [16-21,25,26,28]

At the level of energy balance, the extract reduces food intake and enhances energy expenditure through modulation of hypothalamic appetite-regulating pathways and activation of brown adipose tissue thermogenesis. The flavonoids and iridoid glycosides inhibit pancreatic lipase and α -glucosidase, reducing absorption of dietary fats and carbohydrates. In the liver, the extract inhibits HMG-CoA reductase and enhances LDL receptor expression, improving lipid profile. The iridoid glycosides suppress hepatic gluconeogenesis and enhance glycogen synthesis, improving glycemic control. In adipose tissue, the extract inhibits adipocyte differentiation and lipid accumulation through modulation of PPAR- γ and C/EBP- α pathways. The phenolic compounds activate AMPK, enhancing fatty acid oxidation and inhibiting lipogenesis. The antioxidant constituents scavenge ROS and upregulate endogenous antioxidant enzymes, reducing oxidative stress and its metabolic consequences [16,17,20,21,25,26,28].

CONCLUSION

In conclusion, this study demonstrates that the hydroethanolic leaf extract of *Barleria prionitis* possesses significant anti-obesity, hypolipidemic, hypoglycemic, and antioxidant properties in high-fat-diet-induced obese rats. The validated HPTLC method provides a reliable approach for quality standardization of the extract, with quercetin, gallic acid, and tannic acid identified as marker compounds. The extract at 400 mg/kg produced effects comparable to orlistat in reducing body weight gain, adiposity, dyslipidemia, hyperglycemia, and oxidative stress, while restoring hepatic and renal function and attenuating histopathological changes. These findings provide scientific validation for the traditional use of *B. prionitis* for obesity management and support its potential as a natural therapeutic agent. The multi-target mechanism of action, involving modulation of lipid metabolism, glucose homeostasis, and oxidative stress pathways, suggests that *B. prionitis* extract may offer advantages over single-target synthetic drugs. Further research is warranted to isolate and characterize the active constituents, elucidate the molecular mechanisms, and evaluate clinical efficacy in human subjects.

Future research directions should include: (1) isolation and characterization of individual iridoid glycosides and evaluation of their anti-obesity effects; (2) mechanistic studies using transcriptomic and proteomic approaches to identify molecular targets; (3) evaluation of the extract's effects on gut microbiota composition and function; (4) long-term toxicity studies to establish safety profile; (5) pharmacokinetic studies to determine bioavailability of active constituents; (6) formulation development for improved delivery and efficacy; and (7) clinical trials to validate the anti-obesity effects in human subjects. Additionally, the HPTLC method should be extended to include iridoid glycoside markers for more comprehensive quality control.

Competing Interests

The authors declare that they have no competing interests.

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