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**Research Article**

# A Pharmacological Mechanistic Appraisal Of Antihyperlipidaemic Activity Of *Balbisia Pedunculata* Methanol Extract In High Fat Diet Induced Hypelipidemia Model

Rajendra Madhukar Kawade[1], Krishna Chandra Panda[2], Touseef Begum[3*], Pranav Balvant Dhekale[4], Rose Saira Ekka[5], Nabin Mahar[6], Sandala Anuradha Bai[7], Dinesh Vishwakarma[8],

[1]Department of Pharmacology, Nandkumar Shinde College of Pharmacy Vaijapur Dist Aurangabad, State Maharashtra Pin- 423701
[2]Department of Pharmaceutical Chemistry, Roland Institute of Pharmaceutical Sciences, Berhampur, Ganjam, Odisha, Pin- 760010
[3*]Department of Pharmaceutical Sciences, Ibn Sina National College for Medical Studies, P.O. Box 31906, Jeddah 21418, Kingdom of Saudi Arabia.
[4]Department of Pharmacology, Rajgad Dnyanpeeths college of Pharmacy, Bhor, Pune, India
Pin- 412206.
[5]Department of Pharmacology, Dr. Ambedkar Institute of Pharmaceutical Science, Jabaghat, Rourkela, Sundergarh, Odisha,Pin-769042
[6]Department of Pharmacology, One Beat College of Medical Science, Santgarh Nagar, Bhira ,Kheri Pin- 262901
[7]Department of Pharmaceutical Quality Assurance, Sarojini Naidu Vanita Pharmacy Mahavidyalaya ,TarnKa Hyderabad, Telangana, India Pin- 500007.
[8]Department of Pharmacology, Madhyanchal Professional University Bhopal pin code -462044.

***Corresponding Author:** Touseef Begum
Department of Pharmaceutical Sciences, Ibn Sina National College for Medical Studies, P.O. Box 31906, Jeddah 21418, Kingdom of Saudi Arabia.

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**ARTICLE INFO**

**ABSTRACT**

The current study focuses on the ability of *Balbisia pedunculata* methanolic whole plant extract to decrease cholesterol with hyperlipidemia brought on by a high-fat diet. For six weeks, rats were given an oral high-fat diet to produce hyperlipidemia. After that, the extract was given to hyperlipidaemic rats for the next six weeks at two distinct doses: 250 mg/kg and 500 mg/kg body weight. As a standard, atorvastatin was utilised. The extract demonstrated dose-dependent anti-hyperlipidaemic action. Total cholesterol, LDL-C, VLDL-C, HDL-C, and the atherogenic index were among the evaluation parameters that revealed the dose of 500 mg/kg to have the highest antihyperlipidemic efficacy. These data were comparable to those of the standard medicine, atorvastatin, in each case. These results validate the extract's use in conjunction with current therapies to treat hyperlipidemia.

**Keywords**: Hyperlipidaemia, High fat diet, Atorvastatin, *Urtica dioica*, Nettle

## INTRODUCTION

Increased concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and triglycerides are specific symptoms of hyperlipidaemia, a metabolic disorder marked by a concurrent decrease in the blood circulation's concentration of high-density lipoproteins (HDL-C). Current research has demonstrated a correlation between mid- and late-life depression and blood cholesterol, including total and lipoprotein fractions (1, 2). As a primary cause of atherosclerosis and its concomitant conditions, including coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease (PVD), hyperlipidemia is one of the leading causes of death in developed countries and is more common in developing countries like India (3). The World Health Organisation (WHO) estimates that high blood cholesterol causes approximately 56% of cardiovascular disease cases worldwide and over 4.4 million deaths yearly. Because of this, medical professionals believe that managing hyperlipidemia is essential to halting the atherogenic process and reducing the risk of ischemic heart disease and other cardiovascular or cerebrovascular diseases (4).

Balbisia pedunculata (Asteraceae) has long been used as a traditional drink to treat liver diseases, diarrhoea, bronchial catarrh, and dysentery in numerous African, South Asian, and Southeast Asian countries. This is in spite of claims that the plant might infiltrate a variety of crops (5). This plant has a number of bioactive compounds that have been successfully isolated, such as 6,8,3′-trihydroxy-3,7,4′-trimethoxyflavone, 8,3′-dihydroxy-3,7,4′-trimethoxy-6-O-β-d-glucopyranosyl flavone, and puerarin, centaurein, and centaureidin (6). Lupeol, fucosterol, 30-methyl-28-oxodotriacont-29-en-l-oic acid, methyl 14-oxooctadecanoate, methyl 14-oxononacosanoate, and β-amyrone are among the lipid components of this plant that have been identified (6). Furthermore, it has been demonstrated that this plant contains phenolic acids, including benzoic, vanilic, benzeneacetic, and guiacol (7). The biological activity of Balbisia pedunculata is diverse. This plant's ethyl acetate extract demonstrated potent larvicidal and allelopathic properties. Pharmacologically, methanol and ethanol extracts showed anti-hyperglycemic, anti-fungal, anti-leshmanial, and hepatoprotective qualities, whereas ethyl acetate extract shown anti-inflammatory, anti-cyclooxygenase, and antioxidant qualities (7). The acetone extract of this herb demonstrated antibacterial, anticoagulant, and antihepertic effects (8).
The hypolipidaemic medications that are now on the market have a variety of negative effects. Synthetic drug use causes flushing, dry skin, myositis, hyperuricemia, diarrhoea, nausea, and altered liver function. Thus, it is imperative to look for alternative natural materials that, in addition to being less costly and harmful, offer superior long-term safety and efficacy. Herbal remedies are more compatible than synthetic medications and cause less harm, which increases patient compliance with long-term use. It has been stated that the use of polyherbal capsules made of different anti-lipidemic plant parts is essential for the management and treatment of dyslipidemia (4, 9). Thus, the goal of the current study is to investigate the potential pharmacological assessment of *Balbisia pedunculata*, one of the historically used medicinal herbs, in the management and treatment of hyperlipidemia (10).

## MATERIAL AND METHODS

### Chemicals, Drugs, Kits and Equipment

Only trustworthy vendors provided the chemicals, reagents, testing kits, and other supplies, all of which were of analytical grade.

### Collection of Plant Material & Its Evaluation

The botanical identification of *Balbisia pedunculata* was completed by Dr. A K Gupta of the Department of Botany. The plant was taken from the Dehradun District in Uttarakhand, India. The plant specimen was sent in with accession number MKD27078/2023/028..

### Extraction by Soxhlet extraction method

The active ingredients that are found in natural herbs and plants give them their therapeutic value. The technique of extraction involves treating plant tissues with particular solvents in order to dissolve out these medicinally active ingredients. As a result, it is an extremely important step in creating an appropriate extract for biological and phytochemical analyses. Using methanol as the solvent solution, the air-dried *Balbisia pedunculata* coarse powder was extracted using the Soxhlet method. A Soxhlet device was used to extract 500g of the dried powders of *Balbisia pedunculata* using methanol. The extract was then filtered, and the filtrate was evaporated at a temperature of 45° C using a vacuum evaporator operating at low pressure (11, 12). The physical characteristics and percentage yield of each extract type were also noted. The formula was used to assess the extract's percentage yields**:**

Percentage Yield= Weight of extract (g)/ Weight of dry powder (g) ×100

### Qualitative Preliminary Phytochemical Analysis

The plant serves as a biosynthetic laboratory for a wide range of substances known as secondary metabolites, such as alkaloids, glycosides, alkaloids flavanoids, polyphenols, phytosterols, and others that have physiological and medicinal effects, in addition to chemical compounds like carbohydrates, proteins, and lipids that humans use as food. From here on, the extracted material must undergo many chemical analyses to identify the chemical components that are present in them (13). As mentioned above, Soxhlet extraction was used to obtain the extract. A preliminary phytochemical screening was performed on the extract to check for the presence of proteins, carbohydrates, phytosterols, alkaloids, flavanoids, glycosides, saponins, and phenols.

### Determination of Total phenolic content

With certain adjustments, the Folin-Ciocalteu technique was used to calculate the total phenolic content. A methanolic extract stock solution was made in order to achieve a final concentration of 1 mg/ml. This process involved weighing 0.1 g of extract, dissolving it in 5 ml of 95% ethanol, and then adding distilled water to make 100 ml. Two millilitres of the 50% Folin-Ciocalteau reagent were added to 0.4 millilitres of the stock solution. For five minutes, the solution mixture was left to react. Four millilitres of 5% Na2CO3 were added to the mixture, and it was left in the dark for an hour. Using a Shimadzu 1800 UV spectrophotometer, the absorbance was measured at 725 nm. The absorbance readings in the 10-100 µg/ml range were compared with the gallic acid standard. Every experiment was conducted in triplicate, and the outcomes were reported in milligrammes of GAE per 100 milligrammes of extract (14). The mg/g of extract was used to express the total phenol content.

Using an equation derived from the equation of the regression line of the standard Gallic acid graph, the concentration of total phenolic components in the extract was expressed as grammes of Gallic acid equivalent (GA).

## Pharmacological evaluation: Hyperlipidaemia induced by high fat diet (HFD)

### *Animals*

For the study, Wistar rats (120–150 g) of both sexes that were roughly 6–8 weeks old were obtained from the animal facility of Bilwal Medchem and Research Laboratory Pvt. Ltd. in India. Large, spotless polypropylene cages housing the animals were housed in a temperature-controlled environment with a relative humidity of 44–55% and 12-hour light and dark cycles. Before beginning any research, all of the animals were given seven days to acclimatise to the laboratory setting. The animals were fed a normal rat diet consisting of pellets and were allowed unlimited access to water. The Institutional Animal Ethics Committee closely adhered to the criteria established by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), an Indian organisation, for the care and use of laboratory animals.

### *Composition of high fat diet (HFD)*

For the experimental diet, the following ingredients were combined: wheat flour (67.5g), barley powder (37.5g), corn powder (62.5g), milk powder (37.5g), calcium chloride (2.5-2.g), animal fat (25g), salt (2.5-2.g), vanaspati (10ml), coconut oil (10 ml), cholesterol (2g), cholic acid (2g), vitamin B12 (1 tablet) and sugar (20g). The wet dough was shaped into little balls and let to dry at room temperature before being fed to the animals (15).

### *Induction of Atherosclerosis in Rats*

Each animal received the freshly produced high-fat food with the above composition on a daily ad libitum basis for six weeks. Normal pellet chow (Amrut feeds, Pranav Agro Industries Ltd., New Delhi, India) was fed to the control animals. Lipton, India) as well as unlimited water. Following the development of hyperlipidemia, the rats were administered the plant extract suspended in 1% gum acacia at a dose of 250 mg/kg and 500 mg/kg b.w. once daily in the morning by stomach intubation. Every group was likewise given the same dosage of the atherogenic diet during these days. The vehicle and the hyperlipidaemic diet were given to the control animals. Following the treatment period, the animals were utilised to investigate a range of metabolic markers.

**Figure 1.** Diet heavy in fat intended to cause hyperlipidemia

### *Experimental design*

Five groups of six rats each were created from the total of thirty rats.

**Table 1.** Experimental Protocol for Hyperlipidemia Induced by a High-Fat Diet (HFD)

| S. No. | Groups | Treatment given |
|---|---|---|
| 1. | Normal Control | Normal diet + vehicle (1 ml/kg/day of 1% gum acacia; p.o) |
| 2. | Hyperlipidaemic Control | HFD p.o for 6 weeks |
| 3. | HFD + BP-ME (250 mg/kg) body weight | After 6 weeks, HFD + BP-ME 250 mg/kg p.o. for next six weeks. |
| 4. | HFD + BP-ME (500 mg/kg) body weight | After 6 weeks, HFD + BP-ME 500 mg/kg p.o. for next six weeks. |
| 5 | HFD + Atorvastatin (10 mg/kg) body weight | After 6 weeks, HFD + Atorvastatin (10 mg/kg) body weight p.o. for next six weeks. |

BP-ME : Methanolic extract of *Balbisia pedunculata*

The animals were put to death by euthanasia at the conclusion of the treatment, and blood was drawn to estimate the lipid profiles. Organs such as the liver were taken out for histological analysis and biochemical assessment.

### *Monitoring of body weight*
On the first day of hyperlipidemia induction and thereafter once a week both before and after therapy, body weight was recorded. The results are appropriately expressed in the results section.

### *Blood Collection and Serum Preparation*
At the conclusion of the research, blood was drawn using a retroorbital sinus puncture while under a light ether anaesthesia. The collected blood was allowed to coagulate, and the serum was separated for 20 minutes at 4000 rpm in order to do additional biochemical analyses. After that, animals were killed and their livers were harvested (16). Twenty four hours after the final treatment, all rats were killed by cervical dislocation. They took blood samples right before the sacrifice. Diethyl ether was used to anaesthetize the animal, and the retro-orbital plexus was used to draw blood. After allowing the drawn blood to clot, serum was extracted for 20 minutes at 4000 rpm in order to conduct additional biochemical analyses.

### *Estimations of Biochemical markers*
### *Lipid profile Determination*
Blood was drawn from the rats 24 hours after the previous medication treatment and 12 hours after they had fasted in order to determine their lipid profile. Under ether anaesthesia, blood was drawn from the retro orbital plexus and placed in non-heparinized Eppendorf tubes. The serum was then made according to the instructions above. The NEC route triglyceride testing kit (God/Pod technique) was used to analyse triglycerides in accordance with the manufacturer's instructions. The NEC route Total cholesterol test kit (Chod-PAP, endpoint technique) was used to measure serum total cholesterol, and the Enzo Pak HDL- Cholesterol test kit was used for the PTA method to estimate HDL-cholesterol.

### *Measurement of Liver function and Kidney funtion parameters*
Standard and customary laboratory procedures were used to measure the activity of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (17). Standard and regular laboratory procedures were also used to perform the assay for serum creatinine and uric acid.

### *Estimation of oxidant-antioxidant/peroxidation markers in liver homogenate*
Protein, reduced glutathione, superoxide dismutase (SOD), and malondialdehyde (MDA) assays were performed as previously mentioned. Furthermore, Catalase assaying was done using previously described standard procedures (17-19).

### *Estimation of lipid profile parameters in liver homogenate*
The removed liver homogenate was also used to evaluate lipid profile parameters such total cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C, and atherogenic index using previously developed protocols (17, 20).

### *Histopathology*
After being removed, the liver tissue was preserved in a 10% formalin solution. After that, it was cleaned in xylene, dried in ethanol (50–100%), and embedded in paraffin wax. then, 5 micron thick sections were prepared, and they were then stained with eosin and hemophthalein dye for examination under a microscope. Under a microscope, the liver sections were scored on a scale from 1-4, as shown below (21, 22).
0 = typical histology of the liver.
1 = Little and thin connective tissue septa that have no effect on the lobules' formation in the liver.
2 = Large connective tissue septa that penetrate the parenchyma and flow together. propensity to grow nodules.
3 = The architecture of the liver is nodularly transformed, and the hepatic lobules lose their structure.
4 = Excessive connective tissue production and deposition accompanied by scarring and the division of the regenerated lobules.

### Statistical analysis
Graph Pad PRISM software version 8.0 (Graphpad software, San Diego, CA) was used to do statistical analysis. Each and every result was given as mean ± standard deviation (SD) of the mean. Analysis of variance (ANOVA) was used to compare treatment groups, and Dunnett's multiple comparison tests were then performed as *post hoc*. P values less than 0.05 were deemed statistically significant.

## RESULTS AND DISCUSSION

### Qualitative Phytochemical Analysis
Table 2 reports and displays the findings of the qualitative preliminary phytochemical screening.

**Table 2.** Outcomes of Soxhlet extract-based preliminary phytochemical screening.

| Phytochemical constituent | BP-ME (Soxhlet extract) |
|---|---|
| **Flavonoids** | + |
| **Alkaloids** | + |
| **Phenols** | + |
| **Phytosterols** | + |
| **Saponins** | ++ |
| **Borntrager test** | + |

**+:** Presence of moderate active constituents, **++:** Presence of maximum active constituents

## Extraction Yields

The extraction yield results were displayed as a percentage yield. Yield percentage using the Soxhlet extraction method: gramme of extract for every 100 grammes of crude medication.

## Total Phenolic Content

The mg/g of extract was used to express the total phenol content. Using an equation derived from the equation of the regression line of the standard Gallic acid graph, the concentration of total phenolic components in the extract was expressed as grammes of Gallic acid equivalent (GA):

$Y = 0.0089x + 0.0217$, $r^2 = 0.9979$ (BP-ME)

where x was the concentration and y was the absorbance.

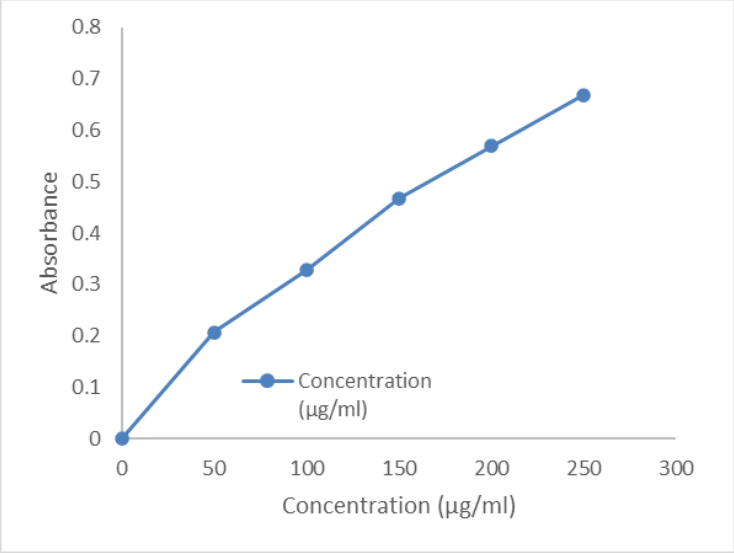


**Figure 2.** Graph using a straight-line equation to predict the extract's total phenolic content concentration. The methanolic extract's total phenolic content was determined to be 11.91 g ± 0.72 phenol/100g extract (BP-ME).

## Assessment of antihyperlipidaemic efficacy of BP-ME in HFD induced hyperlipidemia

### Impact on body weight

Compared to the normal control group, the animals fed a high-fat diet for six weeks showed a significant ($P<0.05$) increase in mean body weight. Compared to the normal control group's weight rise of 7.79%, the weight gain by the end of the sixth week was over 12.91%. For the next six weeks, a high-fat diet and treatment with methanolic extract BP-ME at doses of 250 and 500 mg/kg stopped the body weight from rising any further. For BP-ME, the dose of 250 mg/kg permitted only 3.71% body weight growth, while the dose of 500 mg/kg permitted only 3.34% body weight gain, which was equivalent to the standard medication atorvastatin. The outcomes are shown in Table 3.

### Impact on liver weight

Liver was removed and weighed following the sacrifice of all the healthy and treated animals. Rats fed a high-fat diet for six weeks showed a significant ($P<0.05$) increase in mean liver weight (11.71±0.64 g) compared to the normal control group's value of 6.76±0.19 g. However, treatment with atorvastatin and methanolic extract BP-ME at doses of 250 mg/kg, 500 mg/kg, and 8.18±0.33g, 8.03±0.28g, and 8.01±0.26g showed a significant reduction in the weight of liver tissue. Figure 3 presents the results in an expression.

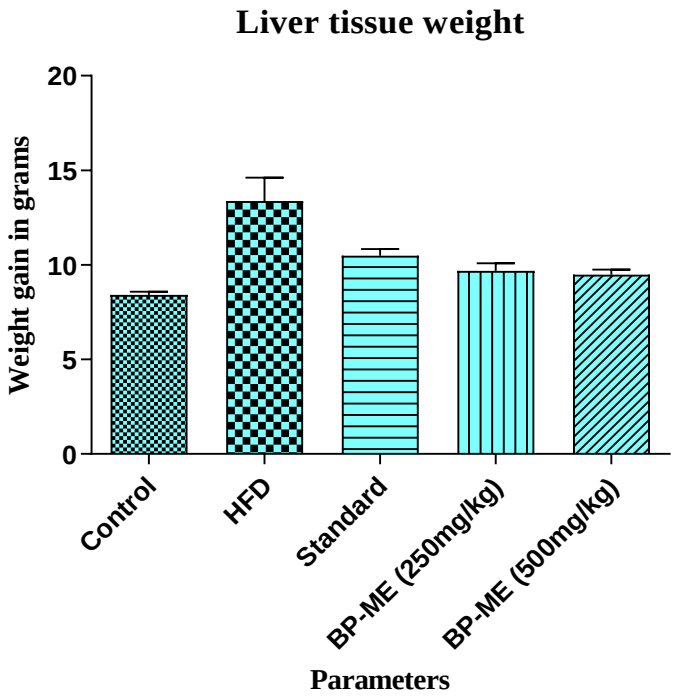


**Figure 3.** Weight gain in the liver tissue in different groups

## Impact on lipid profile

Compared to the normal control group, the high-fat diet treated group had a significant (P<.001) rise in total cholesterol. At 250 mg/kg and 500 mg/kg body weight, the methanolic extract BP-ME significantly (P<.001) reduced the blood total cholesterol levels, which were 158.31±1.11 mg/dl and 148.71±1.00 mg/dl, respectively. Table 4 presents the results. Rats on a high-fat diet had mean serum LDL-C and VLDL-C levels that were significantly (P<.001) higher than those of rats fed a normal diet. At a dose of 250 mg/kg body weight, the methanolic extracts BP-ME demonstrated a significant (P<.001) decrease in the levels of serum LDL-C and VLDL-C, with values of 100.06±1.11 (BP-ME) mg/dl and 32.45±1.01 mg/dl (BP-ME). Out of all the treated groups, the plant extracts at 500 mg/kg had the greatest impact, with values of 90.72 ± 1.43 mg/dl for LDL-C and 29.41 ± 1.03 mg/dl for VLDL-C (BP-ME). This was even better than the group treated with atorvastatin, which had values of 110.87±2.01 mg/dl and 31.56±1.06 mg/dl for LDL-C. Table 4 presents the results. When compared to the normal control group, it was shown that the mean blood HDL-C levels were considerably (P<0.001) lower in the rats treated with a high-fat diet. However, after administering 250 mg/kg and 500 mg/kg bodyweight of the methanolic extract to hyperlipidaemic rats for six weeks, the animals' blood HDL-C levels increased, measuring 29.54±0.99 mg/dl (BP-ME) and 32.34±1.17 mg/dl (BP-ME). At a dose of 10 mg/kg body weight, the results were equivalent to those of the usual medication atorvastatin, with a value of 32.00±1.18 mg/dl. Table 4 presents the findings. The mean serum triglyceride levels were significantly (P<.001) higher in the high-fat diet treated group than in the normal control group. Nonetheless, triglyceride levels were significantly reduced by atorvastatin 10 mg/kg body weight and methanolic extracts at a dose of 250 mg/kg, with values of 159.87±2.32 mg/dl and 145.87±1.49 mg/dl, respectively. It was also observed that the highest reduction in serum triglyceride levels was exhibited by both plant extracts at 500 mg/kg. The outcomes are shown in Table 5.

## Effect on glucose levels (mg/dl)

The high-fat diet treated group had considerably (P<0.001) higher mean blood glucose levels than the normal control group. Nevertheless, after six weeks of treatment with the methanolic extracts BP-ME at a dose of 250 mg/kg body weight, rats' blood glucose levels significantly decreased, as shown in table 5. The plant extracts, however, showed the greatest reduction in glucose levels at a dose of 500 mg/kg, with values of 123.76±1.12 mg/dl, lower than those of the usual medication, atorvastatin (10 mg/kg). The outcomes are shown in Table 5.

**Table 4.** Effects of methanolic extracts BP-ME on serum levels of total cholesterol, HDL-C, LDL-C, and VLDL-C in hyperlipidemia caused by HFD

| Group | Treatment Schedule | Total Cholesterol (mg/dl) | HDL-C (mg/dl) | LDL-C (mg/dl) | VLDL-C (mg/dl) |
|---|---|---|---|---|---|
| I | Normal Control | 79.80 ± 1.56 | 30.21 ± 1.03 | 35.28 ± 1.67 | 18.03 ± 0.99 |
| | HFD (Hyperlipidemic | 249.42 ± | 17.80 | 194.37±1.78 [a] | 42.00±1.07 [a] |

| | | | | | |
|---|---|---|---|---|---|
| II | control) for six weeks | 1.46[a] | ±1.45 [a] | | |
| III | HFD + BP-ME 250 mg/kg p.o for six weeks | 158.31±1.11 [b] | 29.54±0.99 [b] | 100.06±1.11 [b] | 32.45±1.01 [b] |
| IV | HFD + BP-ME 500 mg/kg p.o for six weeks | 148.71±1.00 [b,c] | 32.34±1.17 [b] | 90.72 ± 1.43[b,c] | 29.41 ±1.03 [b,c] |
| V | HFD + Atorvastatin 10 mg/kg p.o for six weeks | 168.68±2.02 [b] | 32.00±1.18 [b] | 110.87±2.01 [b] | 31.56±1.06[b] |

For n = 6, values are mean ± SEM. Using one-way ANOVA and Dunnett's test, aP<0.05 vs. Group I, bP<0.05 vs. Group II, and cP<0.05 vs. Group III

**Table 5.** Impact of BP-ME on blood glucose, atherogenic index, and triglycerides in HFD induced hyperlipidemia

| Groups | Treatment | Glucose (mg/dl) | Atherogenic index | Triglycerides (mg/dl) |
|---|---|---|---|---|
| I | Normal Control | 109.86±1.15 | 4.75±0.10 | 87.54± 1.09 |
| II | HFD (Hyperlipidemic control) for six weeks | 155.71±1.91 [a] | 18.91±0.98 [a] | 207.73±1.95 [a] |
| III | HFD + BP-ME 25 0mg/kg p.o for six weeks | 123.91±1.44 [b] | 7.92±0.83 [b] | 159.86±2.63[b] |
| IV | HFD + BP-ME 500 mg/kg p.o for six weeks | 119.92±1.61 [b] | 6.87±0.78[b] | 144.51±1.83 [b,c] |
| V | HFD + Atorvastatin 10 mg/kg p.o for six weeks | 124.87±1.22 [b] | 7.76±0.83 [b] | 145.81±1.14 [b] |

The values (n = 6) are mean ± SEM. Using one-way ANOVA and Dunnett's test, aP<0.05 vs. Group I, bP<0.05 vs. Group II, and cP<0.05 vs. Group III

## Impact on liver parameters

### Impact on serum ALP, SGOT, SGPT and Bilirubin levels

The mean levels of bilirubin, SGOT, SGPT, and ALP were significantly (P<0.001) higher in the high-fat diet treated group than in the normal control group. Serum ALP levels in the rats treated for six weeks with 250 mg/kg of BP-ME, 500 mg/kg of body weight, and 10 mg/kg of atorvastatin all significantly decreased to 191.22±2.08 U/L, 108.17±1.99 U/L, and 150.87±1.98 U/L, respectively. Treatment with plant extract BP-ME at 250 mg/kg, 500 mg/kg body weight, and atorvastatin (10 mg/kg) resulted in a significant reduction in the levels of SGPT, with values of 50.69±1.85 U/L, 38.29±1.91 U/L, and 55.97±1.65 U/L, respectively. Significant decreases in SGOT levels were also observed in all three groups, with values of 64.45±1.56 U/L, 65.91±1.63 U/L, and 72.79±1.42 U/L. The plant extract at 250 g/kg, 500 mg/kg, and atorvastatin doses shown a substantial decrease in bilirubin levels as well, with respective values of 0.65±0.01 mg/dl, 0.38±0.01 mg/dl, and 0.26±0.01 mg/dl. Table 7 presents the results.

### Effect on serum creatinine and uric acid levels

Serum creatinine and uric acid levels increased significantly (P<0.05) in the high-fat diet group as compared to the normal control group. Following treatment with 250 mg/kg of the methanolic extract BP-ME, the levels of uric acid and creatinine were lowered to 4.31±0.02 mg/dl and 1.06±0.01 mg/dl, respectively. The results of the study indicate that, of all the groups, the plant extract at a dose of 500 mg/kg had the greatest reduction in creatinine (value of 0.88±0.002 mg/dl) and uric acid (value of 4.22±0.02 mg/dl). In contrast, the group treated with atorvastatin had values of 1.08±0.03 mg/dl and 4.81±0.02 mg/dl for these two parameters. Table 8 presents all of the findings.

**Table 7.** Impact of BP-ME on blood levels of ALP, SGOT, and SGPT in HFD-induced hyperlipidemia.

| Groups | Treatment | ALP (U/L) | SGPT (U/L) | SGOT (U/L) | Bilirubin (mg/dl) |
|---|---|---|---|---|---|
| I | Normal Control | 75.94±2.09 | 41.65±1.87 | 51.88±1.09 | 0.65±0.02 |
| II | HFD (Hyperlipidemic control) for six weeks | 312.29±1.92 [a] | 121.94±1.77 [a] | 135.92±1.22 [a] | 0.76±0.02[a] |
| III | HFD + BP-ME 250 mg/kg p.o for six weeks | 191.22±2.08 [b] | 50.69±1.85 [b] | 72.79±1.42 [b] | 0.65±0.01 [b] |
| IV | HFD + BP-ME 500 mg/kg p.o for six weeks | 108.17±1.99 [b,c] | 38.29±1.91 [b,c] | 65.91±1.63 [b] | 0.38±0.01 [b,c] |
| V | HFD + Atorvastatin 10 mg/kg p.o for six weeks | 150.87±1.98 [b] | 55.97±1.65 [b] | 64.45±1.56 [b] | 0.26±0.01 [b] |

The values (n = 6) are mean ± SEM. Using one-way ANOVA and Dunnett's test, aP<0.05 vs. Group I, bP<0.05 vs. Group II, and cP<0.05 vs. Group III

**Table 8.** Impact of BP-ME on renal parameters in HFD caused hyperlipidaemia, specifically creatinine and uric acid in blood.

| Groups | Treatment | Creatinine (mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|
| I | Normal Control | 0.80±0.001 | 3.79±0.01 |
| II | HFD (Hyperlipidemic control) for six weeks | 2.01±0.02 [a] | 6.78±0.03 [a] |
| III | HFD + BP-ME 250 mg/kg p.o for six weeks | 1.06±0.01 [b] | 4.31±0.02 [b] |
| IV | HFD + BP-ME 500 mg/kg p.o for six weeks | 0.88±0.002 [b,c] | 4.22±0.02 [b,c] |
| V | HFD + Atorvastatin 10 mg/kg p.o for six weeks | 1.08±0.03 [b] | 4.81±0.02 [b] |

For n = 6, values are mean ± SEM. Using one-way ANOVA and the Dunnet test, aP<0.05 vs. Group I, bP<0.05 vs. Group II, and cP<0.05 vs. Group III.

## Impact of BP-ME on antioxidant profile in hyperlipidemia caused by a HFD

### *Effect of BP-ME on MDA*

When compared to the normal control group, the high fat diet-treated rats (i.e., positive control group) had greater MDA levels (0.688±0.005 nmol/mg pr), indicating enhanced lipid peroxidation. Rats given the usual medication atorvastatin showed a substantial decrease in MDA levels to 0.517±0.010, while the group treated with BP-ME at a dose of 250 mg/kg showed a significant decrease to 0.470±0.007 nmol/mg pr. But as indicated by a greater drop in MDA levels, the extracts at the dose of 500 mg/kg showed the greatest reduction in lipid peroxidation. The outcomes are shown in Table 9.

### *Effect of BP-ME on GSH levels*

The GSH level of tissue homogenate in the high fat treatment group, or positive control group, was found to be considerably (P<0.001) lower in the current study than the GSH level in the normal control group. Following a six-week therapy with extracts at a dose of 250 mg/kg for hyperlipidaemic rats, the GSH level increased in a highly significant way (P<0.001), measuring 22.58±0.618 ug/mg pr. However, the plant extract at a concentration of 500 mg/kg nearly entirely restored glutathione to normal levels across all treatment groups, suggesting maximum in vivo antioxidant efficacy. This number is comparable to the group treated with atorvastatin, which had a value of 27.78±1.24 ug/mg pr. Table 9 displays the effect of extract on glutathione content.

### *Effect of BP-ME on SOD levels*

SOD levels dropped in the group that received the high-fat diet treatment, indicating a decrease in antioxidant activity. BP-ME treatment for six weeks at 250 mg/kg and 500 mg/kg body weight elevated SOD levels significantly (P<0.001), with values of 16.79±0.99 U/mg pr and 20.43±1.15 U/mg pr, respectively. The group that received 500 mg/kg of the plant extract BP-ME showed the highest rise in SOD levels compared to the group that received 10 mg/kg of atorvastatin, with values of 19.52±0.99 U/mg pr. The outcomes are shown in Table 9.

### *Effect of BP-ME on catalase activity*

Table 9 summarises the findings of the in vivo antioxidant experiment and shows that, in comparison to the normal control groups, the administration of a high-calorie/high-fat meal dramatically lowered catalase (CAT) levels. But when the rats were also given the extracts at a dose of 250 mg/kg, the catalase levels rose dramatically towards normal, with a value of 18.04±1.61 U/ml. Furthermore, the plant extract BP-ME at 500 mg/kg demonstrated the highest rise in catalase activity, measuring 18.36±0.60 U/ml, in comparison to the group taking the usual medication atorvastatin, which displayed a value of 17.92±1.09 U/ml.

**Table 9.** Impact of BP-ME on protein and in vivo antioxidant markers in a hyperlipidemic animal produced by HFD

| Groups | Treatment | MDA Malondialdehyde) (nmoles/mg protein) | GSH (Reduced glutathione (µg/mg protein) | SOD (Superoxide dismutase) (Unit/mg protein) | Catalase (Unit/ml) | Total protein (mg/ml) |
|---|---|---|---|---|---|---|
| I | Normal Control | 0.411±0.005 | 28.71±0.968 | 20.81±1.71 | 21.87± 1.04 | 21.71±1.08 |
| II | HFD (Hyperlipidemic control) for six weeks | 0.688±0.005 | 19.86±0.510 | 9.82±0.95 | 12.45± 0.99 | 23.87±1.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| III | HFD + BP-ME 250 mg/kg p.o for six weeks | 0.470±0.007 | 22.58±0.618 | 16.79±0.99 | 18.04± 1.61 | 24.54±1.07 |
| IV | HFD + BP-ME 500 mg/kg p.o for six weeks | 0.458±0.009 | 27.37±1.61 | 20.43±1.15 | 20.56± 1.06 | 24.77±1.05 |
| V | HFD + Atorvastatin 10 mg/kg p.o for six weeks | 0.517±0.010 | 27.78±1.24 | 19.52±0.99 | 17.92± 1.09 | 21.16±1.08 |

## Histopathological studies

It was confirmed that a high-fat diet caused histological alterations in the liver. The effects of a high-fat diet on the liver are lessened by atorvastatin, BP-ME (250 mg/kg), and BP-ME (500 mg/kg) therapies for six weeks (Figure 4).

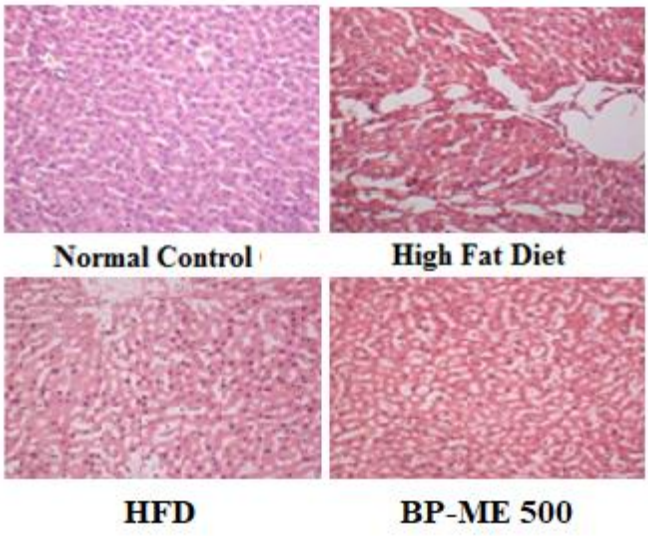


**Figure 4.** Histopathological evaluations of treated and normal groups (100X magnification)

The following were the histopathology results of rat livers::

1) The hepatocytes in the normal control group had normal architecture. The portal triads and major vein also seemed normal.

2) Spotty parenchymal necrosis, mild lobular inflammation, and ballooning degeneration of hepatocytes were observed in the HFD + Vehicle treated group. Additionally, small fat vacuoles (liposomes) were observed in the vicinity of the endoplasmic reticulum (micro vesicular fatty changes) and around the nucleus (macro vesicular fatty changes). Triglycerides accumulate intracytoplasmically as a result of fatty alterations.

3) Hepatocyte deterioration is prevented by the combination of a HFD and atorvastatin (10 mg/kg), albeit periportal mono nuclear infiltration was noted. Hepatocytes showed no abnormalities; patchy parenchyma and tiny fat vacuoles were visible near the endoplasmic reticulum. No fatty alterations were noticed.

4) In experimental animals, the HFD + BP-ME (250 mg/kg) treatment resulted in micro vesicular fatty alterations with patchy parenchymal necrosis and periportal mono nuclear infiltration with minor lobular inflammation of hepatocytes.

5) The HFD + BP-ME (500 mg/kg) treatment showed no fat vacuoles (liposomes) and the reversal of both macro and micro vesicular fatty alterations. The portal triads and central vein remained intact, and the hepatocytes displayed their typical size and form.

## CONCLUSION

The current study showed the potential antihyperlipidemic and antioxidant benefits of methanol extract (BP-ME), and it also provided additional support for these benefits through the use of serum biological indicators and molecular markers. The task involved carefully planning which solvent would be best for extracting the plant material and which extraction technique would perform best. An animal model of hyperlipidemia brought on by a high-calorie, high-fat diet was used to assess the antihyperlipidemic activity. The outcomes of the biochemical measures corroborated the results, which showed a considerable antihyperlipidemic action of the plant extracts in the studied animal models. Additionally, histopathological analyses using liver tissue from both models showed that plant extracts at 250 mg/kg and 500 mg/kg body weight could reconstruct the hepatic architecture to resemble normal liver tissue.

**DECLARATION OF INTEREST**
None.

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None

## REFERENCE

1. Ross R, Harker LJS. Hyperlipidemia and atherosclerosis. 1976;193(4258):1094-100.
2. Havel RJ, Rapaport EJNEJoM. Management of primary hyperlipidemia. 1995;332(22):1491-8.
3. Kumari CS, Govindasamy S, Sukumar EJJoE. Lipid lowering activity of Eclipta prostrata in experimental hyperlipidemia. 2006;105(3):332-5.
4. Choudhary RJPJoP. Benificial effect of Allium sativum and Allium tuberosum on experimental hyperlipidemia and atherosclerosis. 2008;4(2):7-10.
5. Ali M, Ravinder E, Ramachandram R. A new flavonoid from the aerial parts of Tridax procumbens. Fitoterapia. 2001;72(3):313-5.
6. Kpodar MS, Karou SD, Katawa G, Anani K, Gbekley HE, Adjrah Y, et al. An ethnobotanical study of plants used to treat liver diseases in the Maritime region of Togo. J Ethnopharmacol. 2016;181:263-73.
7. Jachak SM, Gautam R, Selvam C, Madhan H, Srivastava A, Khan T. Anti-inflammatory, cyclooxygenase inhibitory and antioxidant activities of standardized extracts of Tridax procumbens L. Fitoterapia. 2011;82(2):173-7.
8. Andriana Y, Xuan TD, Quan NV, Quy TN. Allelopathic potential of Tridax procumbens L. on radish and identification of allelochemicals. Allelopath J. 2018;43:223-38.
9. Bhalavi V, Deshmukh P, Atram M, Mahajan BJP. Study of hypertension and hyperlipidemia in the adolescent of central India. 2014;90(99):24.4.
10. Huq A, Jamal JA, Stanslas JJE-BC, Medicine A. Ethnobotanical, phytochemical, pharmacological, and toxicological aspects of Persicaria hydropiper (L.) Delarbre. 2014;2014.
11. Sultana B, Anwar F, Ashraf MJM. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. 2009;14(6):2167-80.
12. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1-10.
13. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis: Springer; 1998.
14. Olayinka AA, Anthony IO. Phyochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum pedulanculatum*. Int J Mol Sci. 2009;10(11):46-58.
15. Satishchandra A, Sumithra M. Synergistic effect of Mimusops elangi and Moringa oleifera on high fat diet induced atheroma in rats. International Journal of Advances in Pharmaceutical research. 2011;2(6):293-300.
16. Ansarullah, Jadeja RN, Thounaojam MC, Patel V, Devkar RV, Ramachandran AV. Antihyperlipidemic potential of a polyherbal preparation on triton WR 1339 (Tyloxapol) induced hyperlipidemia: A comparison with lovastatin. Int J Green Pharm. 2009;3:119-24.
17. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics-e-book: Elsevier Health Sciences; 2012.
18. Sethi J, Sood S, Seth S, Talwar AJIJoCB. Evaluation of hypoglycemic and antioxidant effect ofOcimum sanctum. 2004;19(2):152-5.
19. Uikey A, Hazarey V, Vaidhya SJJoO, Pathology M. Estimation of serum antioxidant enzymes superoxide dismutase and glutathione peroxidase in oral submucous fibrosis: A biochemical study. 2003;7(2):44.
20. Ugochukwu N, Babady N, Cobourne M, Gasset SJJob. The effect ofGongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. 2003;28(1):1-5.
21. Hirayama C, Morotami I, Hiroshige K. Quantitative and metabolic changes of hepatic collagens in rats after carbon tetrachloride poisoning. Biochemical Journal. 1979;118:229-32.
22. Ala-Kokko L, Stenback F, Ryhanen L. Preventive effect of malotilate on carbon tetrachloride induced liver damage and collagen accumulation in the rat. Biochemical Journal. 1987;246:503-9.