

## PHARMACOLOGICAL ASSESSMENT, PHYTOCHEMICAL ESTIMATION AND HYPERLIPIDEMIC POTENTIAL OF MEDICINAL PLANT *OXALIS STRICTA* EXTRACT ON EXPERIMENTAL RATS

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### ABSTRACT

**Background:** Hyperlipidemia, accompanied by oxidative stress, is a major risk factor for cardiovascular and metabolic disorders. *Oxalis stricta*, traditionally used in folk medicine, has not been extensively validated for its therapeutic efficacy. This study aimed to investigate the antioxidant, antihyperlipidemic, and hepatoprotective potential of the methanolic extract of *O. stricta* leaves in high-fat diet (HFD)-induced hyperlipidemic rats. **Methods:** Fresh leaves of *O. stricta* were collected, authenticated, shade-dried, and successively extracted using petroleum ether and methanol. The extracts were evaluated for phytochemical constituents, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity by the DPPH assay. Acute oral toxicity was studied following OECD guideline 423. Hyperlipidemia was induced by feeding Wistar rats an HFD for 21 days. Animals were divided into five groups (n = 6): normal control, HFD control, lovastatin (10 mg/kg), and two test groups receiving methanolic extract (100 and 200 mg/kg, p.o.). Body weight, lipid profile, serum glucose, and liver enzymes were analyzed. Institutional Animal Ethics Committee (IAEC approval no. 1649/PO/Re/S/11/CCSEA). **Results:** Soxhlet extraction yielded 2.11% methanolic extract, rich in phenolics (64.6 mg GAE/g) and flavonoids (17.66 mg RE/g). The extract demonstrated dose-dependent antioxidant activity with IC<sub>50</sub> of 51.27 µg/mL versus 20.09 µg/mL for ascorbic acid. In vivo, the 200 mg/kg dose significantly reduced body weight gain (286.97 ± 3.45 g vs. 319.86 ± 1.84 g in HFD control), lowered total cholesterol (1.27 ± 0.16 g/L) and LDL-C (0.33 ± 0.16 g/L), while elevating HDL-C (0.50 ± 0.06 g/L). It also reduced liver enzymes, including ALP (120.30 ± 53.23 U/L vs. 179.90 ± 34.38 U/L in HFD). Results were dose-dependent and comparable to lovastatin. **Conclusion:** Methanolic extract of *O. stricta* exhibited potent antioxidant, lipid-lowering, and hepatoprotective effects, validating its ethnomedicinal use and supporting its potential as a natural therapeutic candidate for metabolic disorders.

**KEYWORDS:** *Oxalis stricta*; Antihyperlipidemic activity; Antioxidant potential; Phenolic and flavonoid content; High-fat diet-induced hyperlipidemia; Hepatoprotective effect.

### INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, with a steadily rising prevalence projected in the coming years. Among the major contributors to CVDs, hyperlipidemia, characterized by elevated levels of cholesterol, triglycerides, and low-density lipoproteins (LDL), is considered a primary risk factor for atherosclerosis and subsequent complications such as myocardial infarction and ischemic stroke. Conventional lipid-lowering therapies, including statins, fibrates, and bile acid sequestrants, are effective but often associated with adverse effects such as hepatotoxicity, myopathy, gastrointestinal disturbances, and poor patient compliance. These limitations have intensified the search for alternative, plant-based therapeutic agents that are

safe, effective, and economical. Herbal medicines have historically played a pivotal role in the prevention and management of metabolic disorders. A large proportion of the global population still relies on plant-based formulations for primary healthcare. Plants are naturally rich in bioactive secondary metabolites such as alkaloids, flavonoids, tannins, phenolics, and terpenoids, many of which exhibit antioxidant, anti-inflammatory, and lipid-lowering activities. Several medicinal plants have already been reported to possess significant antihyperlipidemic effects, validating their traditional use and supporting further scientific investigation. *Oxalis stricta* L. (family: Oxalidaceae), commonly known as yellow woodsorrel or upright yellow sorrel, is widely distributed across North America, Europe, and Asia. Traditionally, it has been used for the treatment of

gastrointestinal disorders, wounds, fever, and infections. Preliminary investigations of related species such as *Oxalis corniculata* have demonstrated the presence of diverse bioactive constituents with antioxidant, anti-inflammatory, antidiabetic, and wound-healing properties. However, despite its broad ethnomedicinal applications, the antihyperlipidemic potential of *Oxalis stricta* has not been scientifically validated to date. Given the global burden of hyperlipidemia and the limitations of current pharmacotherapy, there is a compelling need to explore novel plant-based alternatives. The present study was therefore designed to investigate the phytochemical composition, antioxidant capacity, and antihyperlipidemic activity of the methanolic extract of *Oxalis stricta* in high-fat diet (HFD)-induced hyperlipidemic Wistar rats. The study also aimed to assess its safety through acute toxicity studies, thereby providing scientific evidence to support its traditional use and laying the groundwork for future drug development.

## MATERIALS AND METHODS

### Plant Collection and Authentication

Fresh leaves of *Oxalis stricta* were collected from the local region of Madhya Pradesh, India, in September 2023. The plant was authenticated by a taxonomist, and a voucher specimen (No. 205/Saif./Sci./Clg/Bpl) was deposited in the institutional herbarium for future reference. The collected material was washed, shade-dried for three days, followed by oven drying at 45 °C until constant weight, and stored in airtight containers.

### Preparation of Extract

The dried leaves were coarsely powdered and subjected to successive Soxhlet extraction using petroleum ether (60–80 °C) followed by methanol. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator at 40 °C and stored in airtight containers at 4 °C until use. The percentage yield was calculated based on the initial plant weight.

### Phytochemical Screening

Preliminary phytochemical tests were carried out on petroleum ether and methanolic extracts using standard procedures (Harborne, 1998) to detect alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and carbohydrates.

### Quantitative Estimation of Phytochemicals

Total Phenolic Content (TPC): Determined by the Folin–Ciocalteu method, expressed as mg of gallic acid equivalents (GAE) per gram of extract. Total Flavonoid Content (TFC): Estimated by the aluminum chloride colorimetric method and expressed as mg of rutin equivalents (RE) per gram of extract.

### Antioxidant Assay

The antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations of extract (20–100 µg/mL) were incubated with 0.1 mM DPPH solution for 30 min

in the dark. Absorbance was measured at 517 nm, and the percentage inhibition was calculated. Ascorbic acid served as the reference standard.

### Acute Oral Toxicity Study

Acute toxicity was evaluated according to OECD guideline 423 (Acute Toxic Class Method). Female Wistar rats (n=3 per step) were administered *Oxalis stricta* methanolic extract orally at doses of 5, 50, 300, and 2000 mg/kg body weight. The animals were observed for 14 days for mortality, behavioral changes, and signs of toxicity.

### Experimental Animals

Healthy Wistar albino rats (2–3 months old, 250–310 g) of either sex were procured from the institutional animal house. Animals were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to standard chow and water. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC approval no. 1649/PO/Re/S/11/CCSEA) and conducted in compliance with CPCSEA guidelines.

### Experimental Design

Hyperlipidemia was induced by feeding animals a high-fat diet (HFD) comprising commercial rat chow (79.8%), sunflower oil (15%), cholesterol (5%), bile salts (0.5%), and thiouracil (0.2%) for 21 days. Animals were randomly divided into five groups (n = 6 each). Treatment was given once daily for 21 days. Body weight was recorded weekly.

- **Group I (Normal Control):** Standard diet + vehicle.
- **Group II (Negative Control):** HFD only.
- **Group III (Standard):** HFD + Lovastatin (10 mg/kg, p.o.).
- **Group IV (Test I):** HFD + *Oxalis stricta* extract (100 mg/kg, p.o.).
- **Group V (Test II):** HFD + *Oxalis stricta* extract (200 mg/kg, p.o.).

### Biochemical Analysis

At the end of the treatment, animals were fasted overnight, anesthetized, and blood samples were collected by retro-orbital puncture. Serum was separated by centrifugation at 3000 rpm for 15 min and analyzed for:

**Lipid profile:** total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C).

**Liver function markers:** alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Biochemical parameters were estimated using commercially available diagnostic kits (Span Diagnostics, India).

## RESULTS AND DISCUSSION

### Extractive Yield of Plant Material

Successive Soxhlet extraction of *Oxalis stricta* leaves produced a **0.41% petroleum ether extract** and a **2.11% methanolic extract** (Table 1). The higher yield in methanol suggests that polar phytoconstituents dominate, particularly phenolics and flavonoids, which are known to exert antioxidant and antihyperlipidemic effects.

**Table 1: Percentage yield of extracts of *Oxalis stricta*.**

Extract	% Yield (w/w)
Petroleum ether extract	0.41%
Methanolic extract	2.11%

### Phytochemical Screening

Phytochemical tests revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenolics, and tannins in the methanolic extract, whereas the petroleum ether extract primarily contained steroids and saponins (Table 2). Since flavonoids and phenolics are strongly associated with lipid-lowering and antioxidant properties, the methanolic extract was selected for pharmacological evaluation.

**Table 2: Phytochemical screening of petroleum ether and methanolic extracts of *Oxalis stricta*.**

Phytoconstituents	Petroleum ether extract	Methanolic extract
Alkaloids	–	+
Carbohydrates	–	+
Glycosides	–	+
Flavonoids	–	+
Phenolics	–	+
Tannins	–	+
Saponins	+	–
Steroids	+	–

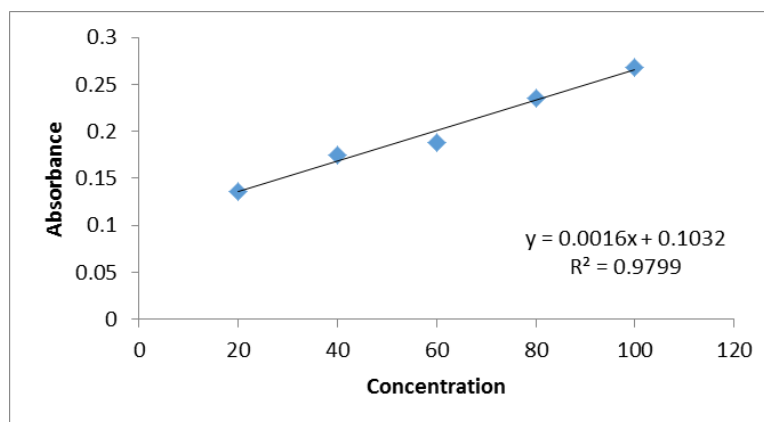
(+ = *Present*, – = *Absent*)

### Total Phenolic and Flavonoid Content

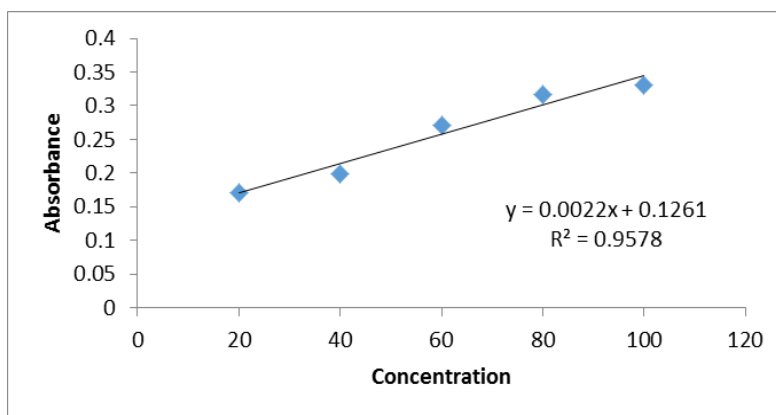
The methanolic extract exhibited a Total Phenolic Content (TPC) of 64.6 mg GAE/g (Table 3; Figure 1) and a Total Flavonoid Content (TFC) of 17.66 mg RE/g (Table 3; Figure 2). The high content of phenolic compounds and flavonoids suggests strong free radical scavenging and lipid-modulating potential, consistent with prior studies on other Oxalidaceae members.

**Table 3: Total Phenolic and Flavonoid Content of methanolic extract of *Oxalis stricta*.**

Sample	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RE/g)
Methanolic extract	64.6	17.66



**Figure 1: Represent standard curve of Gallic acid.**



**Figure 2: Represent standard curve of Rutin.**

### Antioxidant Activity (DPPH Assay)

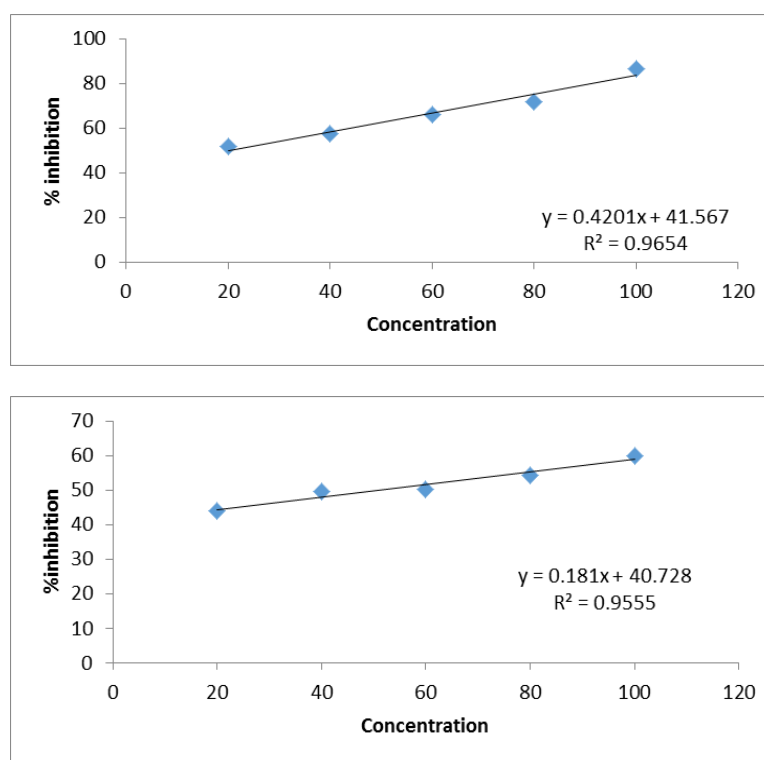
The antioxidant potential of the methanolic extract of *Oxalis stricta* was assessed using the DPPH radical scavenging assay. The extract demonstrated concentration-dependent activity, with a maximum inhibition of 74.21% at 100 µg/mL. The calculated IC<sub>50</sub> value was 51.27 µg/mL, whereas the standard

antioxidant ascorbic acid exhibited an IC<sub>50</sub> of 20.09 µg/mL. Although less potent than the standard, the extract showed substantial free radical scavenging activity, suggesting its potential role in reducing oxidative stress associated with hyperlipidemia (Table 4; Figure 3).

**Table 4: DPPH radical scavenging activity of methanolic extract of *Oxalis stricta* compared with ascorbic acid.**

Concentration (µg/mL)	% Inhibition (Methanolic extract)	% Inhibition (Ascorbic acid)
20	28.36 ± 1.42	46.21 ± 1.17
40	42.18 ± 1.25	61.54 ± 1.09
60	55.72 ± 1.36	73.86 ± 1.32
80	66.47 ± 1.14	81.42 ± 1.21
100	74.21 ± 1.28	89.33 ± 1.08
IC <sub>50</sub> (µg/mL)	51.27	20.09

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



**Figure 3: DPPH radical scavenging activity of methanolic extract of *Oxalis stricta* compared with Ascorbic Acid.**

### Effect on Body Weight

High-fat diet (HFD) feeding induced a significant increase in body weight compared to the normal control group over the 21-day study period. Treatment with the methanolic extract of *Oxalis stricta* (100 and 200 mg/kg)

significantly attenuated body weight gain in a dose-dependent manner. Interestingly, the higher dose (200 mg/kg) produced effects comparable to lovastatin (10 mg/kg), indicating potent anti-obesity activity of the extract (Table 5; Figure 4).

**Table 5: Effect of methanolic extract of *Oxalis stricta* on body weight of HFD-induced rats.**

Treatment groups	Day 0	Day 7	Day 14	Day 21
Normal control	294.31 ± 2.12	298.60 ± 1.87	299.21 ± 1.68	300.88 ± 1.10
High-fat diet (HFD) control	300.52 ± 1.45	313.29 ± 2.34	314.65 ± 1.98	319.86 ± 1.84
Lovastatin (10 mg/kg)	296.18 ± 1.02	291.24 ± 2.10	283.97 ± 1.91	284.86 ± 1.65
<i>Oxalis stricta</i> (100 mg/kg)	299.87 ± 0.89	297.13 ± 0.57	290.64 ± 0.99	289.78 ± 2.66
<i>Oxalis stricta</i> (200 mg/kg)	297.57 ± 1.13	292.46 ± 2.65	286.55 ± 1.56	286.97 ± 3.45

Values are mean ± SEM (n = 6).

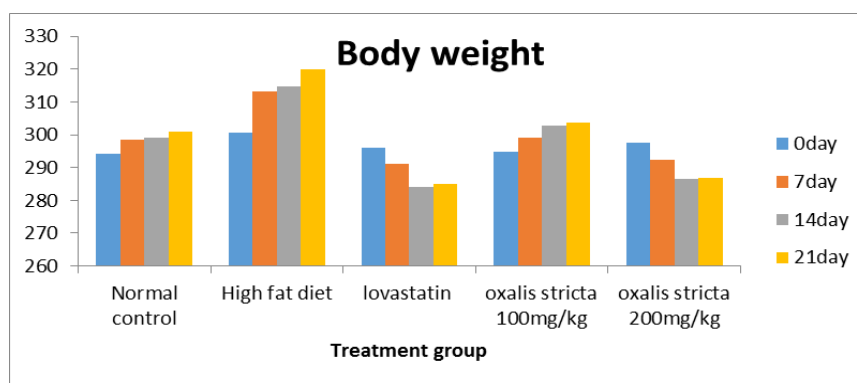


Figure 4: Effect of methanolic extract of *Oxalis stricta* on body weight in HFD-induced rats.

#### Effect on Biochemical Parameters

High-fat diet (HFD) administration significantly elevated serum glucose, triglycerides (TG), total cholesterol (TC), and LDL-C, while reducing HDL-C compared to the normal control group. Treatment with the methanolic extract of *Oxalis stricta* (200 mg/kg) markedly improved

these biochemical parameters, showing effects comparable to lovastatin (10 mg/kg). The extract at 100 mg/kg produced partial improvement, but the higher dose demonstrated more robust activity (Table 6; Figure 5).

Table 6: Effects of methanolic extract of *Oxalis stricta* on glucose levels and lipid profiles in HFD-induced rats.

Treatment Groups	Glucose (mmol/L)	TG (g/L)	TC (g/L)	HDL-C (g/L)	LDL-C (g/L)
Normal control	5.14 ± 0.21	0.76 ± 0.10	1.09 ± 0.15	0.52 ± 0.18	0.22 ± 0.02
HFD control	7.01 ± 0.21	1.10 ± 0.40	1.79 ± 0.37	0.45 ± 0.21	0.51 ± 0.09
Lovastatin (10 mg/kg)	5.22 ± 0.24	0.78 ± 0.10	1.19 ± 0.23	0.56 ± 0.25	0.28 ± 0.05
<i>O. stricta</i> (100 mg/kg)	7.01 ± 0.87	0.96 ± 0.08	1.55 ± 0.10	0.40 ± 0.06	0.45 ± 0.15
<i>O. stricta</i> (200 mg/kg)	5.39 ± 0.34	0.79 ± 0.09	1.27 ± 0.16	0.50 ± 0.06	0.33 ± 0.16

Values are mean ± SEM (n = 6).

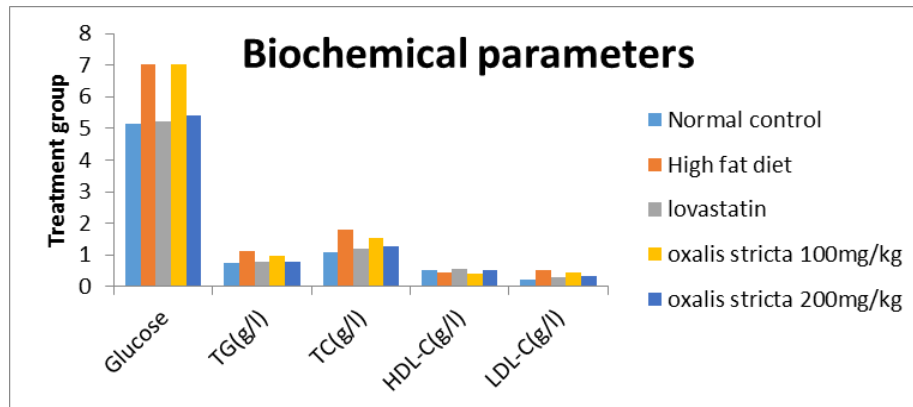


Figure 5: Effect of methanolic extract of *Oxalis stricta* on glucose levels and lipid profiles in HFD-induced rats.

#### Effect on Liver Enzymes

The HFD control group exhibited marked elevation in serum liver enzymes (ALP, ALT, AST), indicating hepatic stress and lipid-associated toxicity. Treatment with the methanolic extract of *Oxalis stricta* (200 mg/kg)

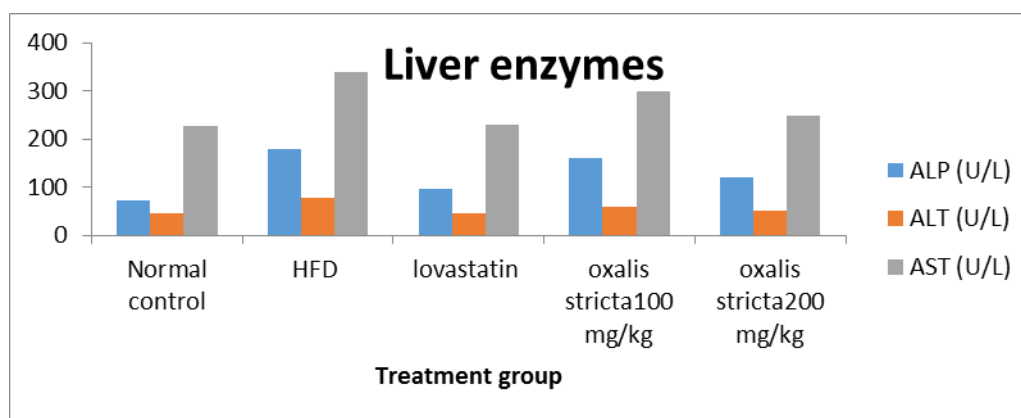
significantly reduced enzyme levels, approaching values of the lovastatin-treated group. The lower dose (100 mg/kg) showed moderate hepatoprotective effects, demonstrating a dose-dependent response (Table 7; Figure 6).

Table 7: Effect of methanolic extract of *Oxalis stricta* on liver enzymes in HFD-induced hyperlipidemic rats.

Treatment groups	ALP (U/L)	ALT (U/L)	AST (U/L)
Normal control	73.00 ± 24.85	45.90 ± 13.45	226.00 ± 95.57
HFD control	179.90 ± 34.38	78.20 ± 30.09	339.30 ± 138.06
Lovastatin (10 mg/kg)	95.80 ± 35.03	46.11 ± 9.82	230.80 ± 52.76
<i>O. stricta</i> (100 mg/kg)	159.10 ± 25.53	58.30 ± 6.90	299.90 ± 52.22
<i>O. stricta</i> (200 mg/kg)	120.30 ± 53.23	50.80 ± 8.28	249.50 ± 37.12

Values are mean ± SEM (n = 6).





**Figure 6:** Effect of methanolic extract of *Oxalis stricta* on liver enzyme levels (ALP, ALT, AST) in HFD-induced rats.

## CONCLUSION

The methanolic extract of *Oxalis stricta* demonstrated significant antioxidant, antihyperlipidemic, and hepatoprotective effects in high-fat diet-induced hyperlipidemic rats. Successive Soxhlet extraction yielded 2.11% methanolic extract rich in phenolics (64.6 mg GAE/g) and flavonoids (17.66 mg RE/g), which contributed to strong radical scavenging activity ( $IC_{50} = 51.27 \mu\text{g/mL}$ ) compared to ascorbic acid ( $20.09 \mu\text{g/mL}$ ). In vivo, treatment with *O. stricta* (200 mg/kg) significantly reduced body weight gain ( $286.97 \pm 3.45 \text{ g}$  vs.  $319.86 \pm 1.84 \text{ g}$  in HFD control), improved lipid parameters by lowering total cholesterol ( $1.27 \pm 0.16 \text{ g/L}$  vs.  $1.79 \pm 0.37 \text{ g/L}$ ) and LDL-C ( $0.33 \pm 0.16 \text{ g/L}$  vs.  $0.51 \pm 0.09 \text{ g/L}$ ), and restored HDL-C ( $0.50 \pm 0.06 \text{ g/L}$  vs.  $0.45 \pm 0.21 \text{ g/L}$ ). The extract also normalized liver enzymes, reducing ALP from  $179.90 \pm 34.38 \text{ U/L}$  (HFD) to  $120.30 \pm 53.23 \text{ U/L}$  and AST from  $339.30 \pm 138.06 \text{ U/L}$  to  $249.50 \pm 37.12 \text{ U/L}$ . These results were dose-dependent and statistically comparable to lovastatin (10 mg/kg). Collectively, the findings provide scientific validation for the traditional use of *O. stricta* in metabolic disorders and highlight its potential as a natural therapeutic candidate for hyperlipidemia. The observed lipid-lowering, antioxidant, and hepatoprotective effects suggest that its bioactivity is linked to phenolic and flavonoid constituents. While the current study establishes strong preclinical evidence, further work involving mechanistic studies, bioactive compound isolation, and well-designed clinical trials is essential to confirm translational relevance and therapeutic applicability.

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