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# Antihyperlipidemic and Antioxidant Activities of Ethanolic Extract of Paederia foetida Leaves (EEPFL) in Albino Rats

Bikram Dutta Tassa, Navajit Sahu, Nabajit Barman, Gautam Sahu

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**Keywords:**
Antihyperlipidaemic, Antioxidant, Paederia foetida, EEPFL.

**Abstract:** The present study was designed to evaluate the antihyperlipidemic and antioxidant activities of leaves of Paederia foetida (EEPFL). The ethanolic extract was obtained by infusion method, and acute oral toxicity tests were performed according to Organization for Economic Cooperation and Development, 2006 (OECD) guidelines. Hyperlipidemia was induced by feeding the rats with a high-fat diet consisting of coconut oil and vanaspati ghee in a ratio of 2:3 v/v at a dose of 10 ml/kg body weight. The extract was given at a dose of 500mg/kg body weight. Total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol were measured for antihyperlipidemic activity. For antioxidant activity, Malondialdehyde (MDA), Catalase (CAT), and Superoxide Dismutase (SOD) were measured using standard methods. The extract showed a significant decrease in total cholesterol, triglycerides, LDL, and MDA in the blood. On the other hand, HDL, CAT, and SOD increased significantly. The study demonstrated that the ethanolic extract of leaves of Paederia foetida decreased blood lipid levels and lipid peroxidation. These findings suggest that the EEPFL may have potential therapeutic applications in the treatment of hyperlipidemia and oxidative stress-related diseases.

## Introduction

Hyperlipidemia is a major risk factor for coronary artery disease and is the leading cause of death (1). It is a highly predictive risk factor for atherosclerosis, coronary artery disease, and cerebral vascular disease (2). Clinical trials showed conclusively that lowering serum cholesterol reduces morbidity and mortality from coronary artery disease in patients with established coronary artery disease and reduces new coronary artery disease events and mortality in patients without established coronary artery disease (3). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease, cardiovascular disease, or cerebrovascular disease (4). Many patients with hypercholesterolemia do not achieve adequate cholesterol reduction with statins and other lipid-lowering drugs (5, 6). Currently, available drugs have been associated with several side effects. Synthetic drugs lead to hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin, and abnormal liver function (7).

Paederia foetida Linn. (Assamese-Vedailata, Hindi-Ghandhli) belongs to the family Rubiaceae (see Figure 1). It is a distinct part of Assamese cuisine. It is a climbing twining shrub with a bitter taste and foul smell, indigenous to the Indian subcontinent. The plant contains alkaloids (α and β-paederidine), sitosterol, vitamin C, essential oils, and flavonoid (by the Shinoda test) (8). Traditionally the whole plant is used in rheumatic disease. Leaf juice is used for diarrhea and dysentery. It is also used in vesicle calculi, piles, and ascites (9). The tea garden people of Assam have used a decoction of the leaves to treat joint pain, abdominal colic, diarrhea, and dysentery. It possesses antioxidant (10), anti-inflammatory (11), and anti-diarrhoeal activity (12). For our study, the ethanolic extract of Paederia foetida leaves was prepared by the percolation method, as described in section 2.2.



**Figure 1.** Photograph of the Paederia foetida plant, a member of the Rubiaceae family, commonly known as "skunk vine" or "stinkvine" due to its strong odor, which is traditionally used for medicinal purposes in many cultures.

There has been a revival of interest in plant-derived drugs, and there is a good scope for using herbal medicines possessing antihyperlipidemic and antioxidant properties with adequate safety and efficacy as an alternative to synthetic drugs. The antihyperlipidemic and antioxidant activities of this plant have not been scientifically evaluated. Hence, the present study was undertaken to evaluate this plant's antihyperlipidemic and antioxidant activities on albino rats fed with a high-fat diet.

# Material and Methods

## Plant Material

Paederia foetida leaves were collected from Assam Medical College and Hospital campus, Dibrugarh, from April to August 2012. The Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, authenticated plant materials.

## Extract Preparation

Paederia foetida leaves were collected, washed thoroughly, and dried separately at room temperature. The dried leaves were ground into powder. Enough powdered leaves were moistened with 95% ethyl alcohol and allowed to remain for 6 hours in a percolator. The orifice was closed when the liquid began to drop from the percolator, and the content was allowed to macerate for 24 hours. After 24 hours, it was allowed to percolate slowly, at a rate not exceeding 1 ml/min, and the solution was collected in Petri dishes. Alcohol was allowed to evaporate at room temperature (20°-25°C). When the extract was completely dried, it was scrapped out, weighed, and stored (8).

## Animal Model

The study was approved by Institutional Animal Ethics Committee (IAEC), Assam Medical College, Dibrugarh, Assam. It was conducted following the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines with approval number IAEC/AMC/03 dated 07/08/11. The study was carried out on healthy adult albino rats of Wistar strain (Rattus norvegicus) of either sex (male or female), weighing 150-200 g and aged between 6 to 8 months. Animals were procured from the Central Animal House, Assam Medical College, and Hospital, Dibrugarh. They were given a standard animal diet consisting of Bengal gram, wheat, maize, and carrot in sufficient quantity, and water was given ad libitum during the entire experiment period. The animals were housed in standard conditions with natural light and dark cycles and adequate ventilation.

## Sample Collection

Under all aseptic and antiseptic measures and ether inhalation anesthesia, blood samples were collected from the retro-orbital sinus with the help of a capillary tube. The capillary tube was inserted at the medial canthus into the retro-orbital plexus with gentle rotation so that blood flowed into it by capillary action (13).

## Acute Toxicity Tests

The acute toxicity of Ethanolic Extract of leaves of Paederia foetida (EEPFL) was determined on female albino rats of Wistar Strain weighing 150-200g. After administration with different doses of the extract, the mortality with each dose was noted as per OECD (Organization for Economic Cooperation and Development, 2006) guidelines 425 (14). As the extract was found safe at 2000mg, one-fourth of the extract dose was decided to be considered for the study.

## Induction of Hyperlipidemia

A high-fat diet, consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/kg body weight, was fed to the animals orally, daily, in addition to the regular diet (15) for 8 weeks (16).

## Experimental Design

A total of 20 animals of either sex weighing 150-200 g were divided into four groups of five animals each and were treated as follows:

1. Group I (Normal Control): Received a normal diet and normal saline at a 10 ml/kg/day dose.
2. Group II (Hyperlipidemic Control): Received high-fat diet at a dose of 10 ml/kg/day.
3. Group III (Hyperlipidemic Test): Received high-fat diet at a dose of 10 ml/kg/day and EEPFL at a dose of 500 mg/kg/day.
4. Group IV (Hyperlipidemic Standard): Received

high-fat diet at a dose of 10 ml/kg/day and simvastatin at a dose of 1.8 mg/kg/day.

The drugs were administered once daily, orally, for 8 weeks by intragastric feeding tube. At the end of 8 weeks, all the animals were fasting for 18 hours. Blood samples were collected from each rat to assess lipid profile parameters and antioxidant status.

### Estimation of Biochemical Parameters

Serum was separated from the blood after clotting and centrifuged for 5 min at 3000 rpm. The serum thus obtained was used for biochemical estimations. The total serum cholesterol estimation was done by the method described by Allain CC et al. (1974) using Qualigens-Diagnostics Cholesterol Kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda (17). Triglycerides were measured by enzyme colorimetric method as described by Fossati P et al. (1982) using Qualigens-Diagnostics Triglyceride Reagent GPO manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda (18). HDL–cholesterol was assayed by the method of Izzo C et al. (1981) using a Qualigens-Diagnostics HDL-cholesterol kit manufactured by Sigma Diagnostics (India) Pvt. Ltd., Baroda (19). LDL–Cholesterol was measured by using the formula (Eq. 1) of Friedewald WT et al. (1972) (20). The Atherogenic Index (AI) and Percent Protection were calculated using Equations 2 and 3 (21).

$$LDL = Total\ Cholesterol - HDL\ \frac{Triglyceride}{5} \quad \textbf{Equation 1}$$

$$Atherogenic\ Index\ (AI) = \frac{Total\ serum\ cholesterol}{HDL} \quad \textbf{Equation 2}$$

$$Percent\ protection = \frac{AI_{hyperlipidemic\ control} - AI_{treated\ group}}{AI_{hyperlipidemic\ control}} \times 100 \quad \textbf{Equation 3}$$

### Estimation of Antioxidant Status

Malondialdehyde (MDA) was measured in plasma while Catalase (CAT) and Superoxide Dismutase (SOD) were measured in the erythrocytes. MDA level was estimated by the method described by Satoh K (22). The CAT level was estimated by the method described by Beers and Sizer (23), and the SOD level was estimated by the method described by Kakkar et al. (24).

### Statistical Analysis

Statistical analysis was done using the software Graph pad Prism version 5. All the values were expressed as mean ± SEM. The results were analyzed for statistical significance using one-way ANOVA, followed by Dunnett's test. A significance level of $p<0.05$ was used to evaluate statistical significance.

## Results

### Acute Toxicity Test

There was no mortality recorded for the extracts of the leaves of Paederia foetida (EEPFL) among the rats up to the maximum dose of 2000 mg/kg when administered orally. Hence, the LD50 can be above 2000mg/kg. The ethanolic extract given to the subjects can be seen in Figure 2.

### Changes in Blood Lipid Profile

At the end of the experiment, the hyperlipidemic control group showed a significant ($p<0.05$) elevation of total cholesterol, triglycerides, and LDL cholesterol, together with a significant ($p<0.05$) decrease in HDL cholesterol when compared with the normal control group.



**Figure 2.** Ethanolic extract of Paederia foetida leaves (EEPFL).

In the hyperlipidemic test group and the hyperlipidemic standard group, there was a significant ($p<0.05$) reduction in total cholesterol, triglycerides, and LDL Cholesterol. Both groups also increased HDL cholesterol ($p<0.05$). This indicates that EEPFL effectively reduces total cholesterol, triglycerides, and LDL cholesterol and increases HDL cholesterol. Changes in serum lipid profile of each subject group can be seen in Table 1.

**Table 1.** Changes in serum lipid profile.

| Groups | Total Cholesterol(mg/dL) | Triglyceride (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | Atherogenic index | % protection |
|---|---|---|---|---|---|---|
| Normal control | 72.44±1.98 | 68.28± 1.73 | 23.91± 1.77 | 36.07± 1.57 | 3.03 | - |
| Hyperlipidemic Control | 239.38± 2.05[a] | 217.10± 1.86[a] | 11.44± 2.03[a] | 184.52± 1.75[a] | 20.92 | - |
| Hyperlipidemic test (EEPFL) | 111.76± 2.55[b] | 76.76± 1.83[b] | 42.86± 3.11[b] | 53.55± 2.14[b] | 2.61 | 87.85 |
| Hyperlipidemic Standard | 75.36± 2.30[b] | 59.82± 1.79[b] | 34.71± 1.88[b] | 28.69± 1.63[b] | 2.17 | 89.63 |

**Note:** values are expressed as MEAN ± SEM; (n=5). One Way ANOVA followed by Dunnett's multiple comparison tests is done. ([a], $p<0.05$) when compared to the normal control group. ([b], $p<0.05$) when compared to the hyperlipidemic control group.

### Changes in Lipid Peroxidation and Blood Antioxidant Levels

At the end of the experiment, there was a significant increase ($p<0.05$) in the serum MDA levels in the hyperlipidemic control group compared to the normal control group. In contrast, blood CAT and SOD levels are significantly ($p<0.05$) decreased in the hyperlipidemic control group compared to the normal control group. Table 2 shows the variations in blood antioxidant levels and lipid peroxidation among each group.

**Table 2.** Changes in lipid peroxidation and blood antioxidant levels.

| Groups | MDA (nmol/mL blood) | CAT (µ/mg protein) | SOD (µ/mg protein) |
|---|---|---|---|
| Normal Control | 1.52+0.72 | 396.2+0.86 | 7.2+0.21 |
| HyperlipidemicControl | 4.34+0.04[a] | 143.8+0.37[a] | 4.1+0.61[a] |
| HyperlipidemicTest(EEPFL) | 2.75+0.16[b] | 202.8+0.45[b] | 5.6+0.38[b] |
| Hyperlipidemic Standard | 1.82+0.95[b] | 327.8+3.86[b] | 6.9+0.17[b] |

**Note:** values are expressed as MEAN + SEM; (n= 5). One Way ANOVA followed by Dunnett's multiple comparison tests was done. (a, p<0.05) when compared to the normal control group. (b, p<0.05) when compared to the hyperlipidemic control group.

There was a significant ($p<0.05$) decrease in serum MDA levels in the hyperlipidemic test group and Hyperlipidemic standard group compared to the hyperlipidemic control group. Blood CAT and SOD levels increased significantly in the hyperlipidemic test group and hyperlipidemic standard group compared to the hyperlipidemic control group. This indicates that EEPFL decreases lipid peroxidation and increases the antioxidant enzymes in the blood.

## Discussion

The present study was undertaken to evaluate the antihyperlipidemic and antioxidant activities of Paederia foetida. In the hyperlipidemic control group, the total cholesterol, triglycerides, and LDL cholesterol levels in the blood significantly ($p<0.05$) increased, together with a decrease in HDL cholesterol level. The elevated level of total cholesterol (except the HDL) is one of the major factors for occurring of coronary heart disease (CHD) (25). Hyperlipidemia, high cholesterol diet, and oxidative stress increase serum LDL levels resulting in an increased risk of development of atherosclerosis (26).

The hyperlipidemic test group fed with the high-fat diet (HFD) and EEPFL showed significant ($p<0.05$) decreases in the total Cholesterol, Triglyceride, and LDL Cholesterol which is almost comparable to the Standard group fed with HFD and simvastatin. On the other hand, HDL cholesterol level is significantly ($p<0.05$) increased in the hyperlipidemic test group and the standard group.HDL cholesterol is referred to as the 'good cholesterol' because HDL is involved in the transport of cholesterol from peripheral tissues to the liver, thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques (27).

Atherogenic index (AI), calculated as the ratio between total and HDL cholesterol, is used as a marker to assess the susceptibility of atherogenesis (28). It is an essential indicator of CHD risks at both high and low serum cholesterol levels (29). Compared with the hyperlipidemic control group, there is a significant decrease in the atherogenic index in the group fed with EEPFL (2.61), which is almost comparable to the Standard (2.17).

The persistence of a hypercholesterolemic state causes enhanced oxidative stress, leading to atherosclerosis, coronary artery disease (CAD), and other complications of obesity (30). Hypercholesterolemia increases the levels of the lipid peroxidation product Malondialdehyde. The increase in MDA levels in the liver suggest enhanced lipid

peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (31). Oxygen free radicals have been implicated in the development of hyperlipidemia atherosclerosis. SOD and catalase effectively mimic the detoxification of Oxygen free radicals and Hydrogen peroxide. Among these, SOD converts the highly toxic superoxide (O2 ) to less toxic hydrogen peroxide, and O2 is the first line of defense to protect the cells from the harmful effects of superoxide. Hydrogen peroxide is produced by superoxide dismutase, further metabolized by catalase (32). The hyperlipidemic control group fed with HFD showed a significant increase in MDA and a decrease in CAT and SOD, indicating increased lipid peroxidation and decreased antioxidant enzyme levels. The hyperlipidemic test group fed with EEPFL showed a significant decrease in MDA and an increase in CAT and SOD.

It is well known that flavonoids and polyphenols have been shown to have hypolipidemic and antioxidant activity (33-35). Flavonoids in medicinal plants protect LDL from oxidation, thereby decreasing atherosclerotic plaques. They are reported to significantly increase superoxide dismutase and catalase activity (36). Several naturally occurring active components found in Paederia foetida may be the reason behind its antihyperlipidemic and antioxidant activity.

## Conclusion

The present study suggests that Ethanolic Extracts of Paederia foetida leaves have antihyperlipidemic and antioxidant activity. Paederia foetida leaves reduce oxidative stress by free radical scavenging and protect against lipid peroxidation and are also able to manage hyperlipidemia by decreasing serum levels of cholesterol, triglycerides, and LDL Cholesterol and increasing serum levels of HDL cholesterol. This effect could be due to flavonoids in Ethanolic Extracts of Paederia foetida leaves, which could react to trap free radicals to prevent lipid peroxidation. Further pharmacological and biochemical investigations are needed to determine the precise mechanism and site of action and the active constituents involved so that these herbal drugs can be used as a safer alternative to synthetic drugs.

## Declarations

### Author Informations

**Bikram Dutta Tassa**
*Affiliation:* Assam Medical College & Hospital, Dibrugarh, Assam.
*Contribution:* Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing.

**Navajit Sahu**
*Affiliation:* Assam Medical College & Hospital, Dibrugarh, Assam.
*Contribution:* Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing.

**Nabajit Barman**
*Affiliation:* Assam Medical College & Hospital, Dibrugarh, Assam.
*Contribution:* Data Curation, Formal analysis, Investigation, Methodology, Validation.

**Gautam Sahu**
*Affiliation:* Jorhat Medical College & Hospital, Jorhat, Assam.
*Contribution:* Data Curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation.

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### Conflict of Interest

The authors declare no conflicting interests

### Data Availability

The reader could access the supplemental or any data related to the study by mailing the corresponding authors.

### Ethics Statement

The study was approved by Institutional Animal Ethics Committee (IAEC), Assam Medical College, with approval letter number of IAEC/AMC/03 dated 07/08/11.

### Funding Information

Not Applicable

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