

Evaluation of Anti-Hyperlipidemic Activity of Ethanolic Root Extract of *Psidium guajava* (Myrtaceae) in Wistar Rat

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ABSTRACT

Objective: Hyperlipidaemia is a major risk factor for cardiovascular diseases and requires effective therapeutic strategies. The present study evaluated the antihyperlipidaemic activity of the ethanolic root extract of *Psidium guajava* in a high-fat diet (HFD)-induced dyslipidaemia model in Wistar rats.

Materials and Methods: Hyperlipidaemia was induced by feeding rats an HFD for 28 days. Animals were divided into five groups (n = 6): normal control, HFD control, standard treatment with Atorvastatin (10 mg/kg, i.p.), and two extract-treated groups receiving *P. guajava* extract at 200 mg/kg and 400 mg/kg (p.o.), respectively. Body weight was monitored, and serum lipid parameters including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) were estimated using standard enzymatic methods. Liver tissues were subjected to histopathological examination.

Results: HFD-fed rats showed significant body weight gain and dyslipidaemia, evidenced by elevated TC, TG, and VLDL levels and reduced HDL compared to the normal control group. Treatment with atorvastatin significantly improved these parameters. The ethanolic extract of *P. guajava* produced dose-dependent reductions in TC, TG, and VLDL levels, along with increased HDL levels. The higher dose (400 mg/kg) demonstrated effects comparable to the standard drug. Histopathological analysis indicated that extract treatment attenuated HFD-induced hepatic alterations and preserved normal liver architecture.

Conclusion: The findings demonstrate that *Psidium guajava* ethanolic root extract exhibits significant antihyperlipidaemic activity, particularly at higher doses, and offers protective effects against diet-induced biochemical and hepatic changes.

Keywords: *Psidium guajava*, Cholesterol, Atorvastatin, Hyperlipidaemia, Atherosclerosis

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INTRODUCTION

Hyperlipidaemia is distinguished by raised amount of lipids and lipoproteins in the Blood plasma, importantly increasing the risk of cardiovascular diseases such as heart attacks, atherosclerosis, and Strokes. It requires an increase in levels of triglycerides, low density lipoprotein cholesterol (LDL-C), and total cholesterol (TC), beside a decrease in high density lipoproteins (HDL). Cholesterol deposits harden arteries, narrowing them and leading to serious health complications leads to metabolic disorders called atherosclerosis. Synthetic hypolipidemic drugs like statins frequently come with adverse effects, medicinal plants like guava (*Psidium*

guajava) engaging for their efficacy and lower side effects. The study of plant-based agents for leading hyperlipidaemia is crucial. The historical use of traditional medicine has been significant in improving long term tolerance and health outcomes [1].

Lipoproteins are large molecular complexes of lipids and proteins, made up of phospholipids, triglycerides, and cholesterol. They are made in the liver and have a hydrophobic core surrounded by a monolayer of polar lipids. Lipoproteins are of five primary classes: chylomicron, very low-density lipoprotein cholesterol (VLDL-C), intermediate density lipoprotein cholesterol (IDL-C), and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein

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cholesterol (HDL-C). High density lipoproteins reduce cardiovascular disease risk, while low density lipoprotein increases risk of coronary artery disease, heart attacks, and stroke [2].

Statins, which are Medicinal Plants, can lower LDL levels by 25-60% by suppressing the HMG-CoA reductase. Vitamin B3 reduces VLDL formation, and fibrates increase lipoprotein lipase, lowering cholesterol and LDL by 15% and 35% respectively. 2-Azetidiones inhibit sterol transporter at the brush boundary and bile acid sequestrants lower cholesterol and LDL by brush-bound bile duct sequestration. Liver cholesterol production is inhibited, thereby lowering LDL-C with ACL inhibitors. The treatment of hyperlipidaemia using herbs has limited adverse effects, is cheap, and is available locally. Medicinal plants are the “backbone” of traditional medicine, with numerous medicinal capabilities and a significant impact on the global economy [3].

Herbal treatments for hyperlipidaemia include onions, garlic, *Comiphora mukul*, *Asparagus racemosus*, *Withania somnifera*, and *Psidium guajava* (guava) is a traditional medicine plant used as a source of copper, Vitamin C, iron, Vitamin B, B6, Vitamin A, tannin, saponin, flavonoid, alkaloid, cardiac glycosides, flavonolmorin-3-O-lyxoside, moprin-3-oarabinoside, quercetin-3-O-arabinoside phenol and carbohydrates and dietary fibre in the Myrtaceae family. It is widely distributed throughout India and is a small tree of about 10m high with spreading branches that thrives on various soils. Guava trees are best grown in tropical, sub-tropical Asia, and parts of the United States, with dry summers and short winters. They can survive in humid conditions, drought conditions and on the other hand young crops are vulnerable to cold conditions and drought. Optimal growth and propagation temperature is 23- 26degC and well drained, fertile soil pH of 4.5-9.4 is the best. These antioxidants play a crucial role in combating oxidative stress, potentially lowering the risk of chronic diseases, including heart disease and certain cancer [4].

In our current paper we have examined the influence of the *P. guajava* ethanolic root extract on the HFD induced dyslipidaemia, in rats. Therefore, growth parameters, organ and adipose tissue weights, serum biochemical parameters and histopathology were determined in rats fed on normal diet (ND) and HFD in the current study.

MATERIALS AND METHODS

Chemicals and reagents

The experiment utilized the following chemicals and reagents: Atorvastatin (Zydus Medica) as the reference

drug; ethanol (Analytical CSS Reagent) for extraction and analytical procedures; normal saline (0.9%) (Hippocrat Biotech Private Limited) for physiological use; Ketamine (Troikaa Pharmaceuticals Ltd.) as the anesthetic agent; distilled water (CARO Care Products LLP) for laboratory applications; Heparin (Samarth Life Sciences Pvt. Ltd.) as the anticoagulant; and hematoxylin and eosin stains (Benz Microscope) for histopathological examination.

Plant material

The roots of *P. guajava* plant were collected from Lalbagh Botanical Garden in Bangalore, Karnataka and a specimen was submitted to the herbarium for reference. The identification and authentication of the plant material was done by the Revitalization of local Health Traditions in Bangalore. After collection, the roots were dried under shade and ground into a coarse powder for further purpose.

Preparation of extract

The *P. guajava* root extract was involved several careful steps to ensure effective extraction of the desired compounds. The roots were completely washed under running water to remove debris and dirt, then dried in a well-ventilated area to eliminate moisture. The dried roots were grounded into a coarse powder using an electric grinder. The powdered material (2kg) undergoes three successive extractions over 72 hrs with 4 liters of ethanol at room temperature. After extraction, the ethanol was evaporated to get a concentrated residue, which was then suspended in water and lean with petroleum ether to remove fatty substances. Finally, the defeated aqueous extract was evaporated to dryness, yielding 80 grams of concentrated extract, rich in active compounds and free from fats [5].

Yield percentage

The percentage yield of an extract is a Performance Metric that quantifies the efficiency of an extraction process, indicate the amount of desired product obtained relative to the maximum possible yield [6]. It is calculated by using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of Starting Material}}$$

In this formula, the weight of extract refers to the quantity of extract that was successfully obtained after the extraction, while the weight of starting material represents the maximum possible amount of crude drug that could be derived from the starting material, based on its composition and the extraction method used.

Qualitative photochemical analysis of *P. guajava*

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Preliminary phytochemical screening of the extract was performed to qualitatively identify the presence of major secondary metabolites, including alkaloids, carbohydrates, saponins, flavonoids, steroids, triterpenoids, phenols, tannins, and glycosides, using standard procedures.

Test for Alkaloids (Mayer's Test)

One milliliter (1 mL) of the extract was mixed with 1 mL of Mayer's reagent (potassium mercuric iodide solution). The formation of a whitish-yellow or cream-colored precipitate indicated the presence of alkaloids [7].

Test for Carbohydrates (Molisch's Test)

Two milliliters (2 mL) of the extract were treated with 1 mL of α -naphthol solution. Subsequently, concentrated sulfuric acid was carefully added along the sides of the test tube to form a separate layer. The appearance of a purple or reddish-violet ring at the interface of the two layers confirmed the presence of carbohydrates [7].

Test for Saponins (Foam Test)

A small quantity of the extract was transferred into a graduated cylinder, and 20 mL of distilled water was added. The mixture was shaken vigorously in a longitudinal manner for 15 minutes. The formation of a stable foam layer of approximately 1 cm height indicated the presence of saponins [8].

Test for Flavonoids

Two milliliters (2 mL) of the crude extract were mixed with a few drops of ferric chloride solution. The development of a green coloration suggested the presence of flavonoids [8].

Test for Steroids and Triterpenoids (Liebermann-Burchard Test)

One gram (1 g) of the test material was dissolved in a few drops of chloroform. To this solution, 3 mL of acetic anhydride and 3 mL of glacial acetic acid were added. The mixture was gently warmed and then allowed to cool. The appearance of characteristic color changes indicated the presence of steroids and triterpenoids [9].

Test for Phenols

A small quantity of the sample was dissolved in an appropriate solvent such as ethanol or distilled water. A few drops of bromine water were added to the solution and mixed thoroughly. The formation of a precipitate or decolorization of bromine water indicated the presence of phenolic compounds [9].

Test for Tannins

If the sample was solid, it was dissolved in distilled water or ethanol to prepare a solution; liquid samples were used directly. A few drops of ferric chloride

solution were added to the sample solution' in a test tube and mixed gently. The appearance of a blue-black or greenish coloration confirmed the presence of tannins [10].

Test for Glycosides

Two milliliters (2 mL) of the extract were treated with one drop of 5% ferric chloride solution, followed by the careful addition of concentrated sulfuric acid along the side of the test tube. The formation of a reddish-brown color at the interface and a blue-green colorant in the upper layer indicated the presence of glycosides [11].

In-vivo Anti hyperlipidaemic activity

Experimental Animals

The study employs in-house bred, the healthy Wistar rats from the ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India, adhering to CPCSEA protocol no. 02/1511/CCSEA. At the initiation of the demonstration, the rats were adapted for 7 days under laboratory-based conditions to adjust the new environment and to reduce stress. The rats were enclosed in polypropylene sterile cages, which help to maintain hygiene and minimize environmental contaminants. The environmental conditions in the animal room were carefully monitored, with a temperature maintained at $23 \pm 1^\circ\text{C}$ and humidity kept at $55 \pm 5\%$ to provide comfortable habitat [12, 13]. A 12-hour light/dark cycle was implemented to align with their natural circadian rhythms and support overall well-being. They were fed with normal granule diet and water will be available ad libitum.

Preparation of High-Fat-Diet (HFD)

A high-fat diet (HFD) was formulated to induce obesity in Wistar rats. The HFD provided 414.0 kcal per 100 g, with a macronutrient distribution of 43% carbohydrates, 17% protein, and 40% fat. The diet was prepared by thoroughly mixing 20% instant milk powder, 68% standard rat chow pellets, 6% corn oil, and 6% ghee. The formulation was designed to significantly increase the fat content compared to the standard diet in order to mimic human dietary patterns associated with obesity. For comparison, the base normal rat chow provided 306.2 kcal per 100 g, comprising 48.8% carbohydrates, 21% protein, and 3% fat. All components of the HFD were mixed uniformly to ensure homogeneity and subsequently baked at 65°C overnight to achieve proper blending and consistency. After cooling, the prepared diet was stored under appropriate conditions until use. Experimental animals in the obesity induction group were fed the high-fat diet ad libitum for a period of two weeks to induce obesity. Meanwhile, the control group received standard rat chow throughout the study period

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to maintain normal metabolic status. This dietary intervention protocol was adopted to establish a short-term diet-induced obesity model as described previously [14].

Experimental Design

The antihyperlipidaemic activity of the proposed plant extract was evaluated using a high-fat diet (HFD)-induced hyperlipidaemia model in Wistar rats. A total of 30 Wistar rats of either sex were used in the study. Hyperlipidaemia was induced by feeding the animals a cholesterol-enriched high-fat diet for 28 consecutive days. The high-fat diet consisted of 2% cholesterol, 1% cholic acid, 10% peanut oil, 40% sucrose, and 47% standard laboratory diet, formulated to elevate serum lipid levels and establish a stable model of diet-induced hyperlipidaemia. The animals were randomly divided into five groups (n = 6 per group). Group I served as the normal control and received a normal diet along with normal saline (0.9%). Group II served as the hyperlipidaemic control and was fed the high-fat diet for 28 days without any therapeutic intervention. Group III comprised hyperlipidaemic rats treated with Atorvastatin at a dose of 10 mg/kg/day, administered intraperitoneally, for 14 consecutive days. Group IV included hyperlipidaemic rats treated with the plant extract at a low dose of 200 mg/kg, administered orally, for 14 days. Group V consisted of hyperlipidaemic rats treated with the plant extract at a high dose of 400 mg/kg, administered orally, for 14 days. Treatment with the standard drug and plant extract was initiated after confirmation of hyperlipidaemia induction and continued for 14 days while animals remained on their respective dietary regimens [15, 16].

Assessment of Body Weight

Body weight is an important physiological parameter reflecting the overall health status, nutritional condition, and metabolic efficiency of experimental animals. In the present study, changes in body weight were monitored as a general parameter to evaluate the effect of dietary induction and subsequent treatments. Body weight of all animals was recorded at baseline and at the end of the treatment period.

Blood Collection and Biochemical Analysis

At the end of the experimental period (Day 25), blood samples were collected from overnight fasted (8 hours) Wistar rats to ensure stable metabolic conditions for accurate lipid profiling. Blood was withdrawn via retro-orbital sinus puncture under mild ether anaesthesia to minimize animal distress. The collected blood samples were allowed to clot at room temperature for approximately 30 minutes. Thereafter, samples were centrifuged at 3000 rpm for

20 minutes to separate the serum. The clear supernatant serum was carefully collected and stored at -20°C until further biochemical analysis [18].

Serum lipid parameters, including total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein (HDL), were estimated using standard enzymatic reagent kits with the help of a semi-automatic biochemical analyser. Very low-density lipoprotein (VLDL) levels were calculated using Friedewald's formula: $VLDL \text{ (mg/dL)} = \text{Triglycerides} / 5$.

All biochemical estimations were carried out in a certified clinical laboratory under strict quality control procedures to ensure analytical accuracy and precision [19].

Histopathological Analysis of Liver

The euthanasia with ketamine was carried out and liver tissues were collected for histopathological examination to assess morphological changes induced by hyperlipidaemia and the effects of *P. guajava* extract. Through 10% formalin the liver samples were fixed in to preserve tissue structure and cellular detail, then embedded in paraffin wax to facilitate sectioning. Thin deviation (approximately 5µm thick) was cut from embedded in paraffin samples, using a microtome. To provide visualization of the cellular details, these sections were stained using Hematoxylin and Eosin (H&E), and therefore, in the staining, the cell nucleus (blue) was stained using Hematoxylin, whereas the cytoplasm and the extra cellular matrix (pink) were marked using Eosin. Histopathologist examined the stained areas beneath light microscope by looking at the pathological alterations that include steatosis, inflammation, necrosis or fibrosis [20, 21].

Statistical Analysis

The study results were reported as mean ± SEM, highlighting the precision of mean values across treatment groups. To compare means one-way ANOVA was used, followed by Newman-Keuls Multiple comparison test for post hoc analysis against the control group, controlling for type I error. A p value of smaller than 0.0001 (p<0.0001) indicated statistical significance, suggesting meaningful treatment effects of *P. guajava* extract on hyperlipidaemia. Overall, the statistical analyses validated the potential therapeutic benefits of the extract. GraphPad Prism (10.6.1) was used for the analysis.

RESULTS

Phytochemical Analysis

A preliminary phytochemical evaluation of ethanolic extract of *P. guajava* roots (EEPG) confirmed the

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existence of carbohydrates, glycosides, Alkaloids, Tannin, Flavonoids, Phenols, Steroids and triterpenoid (table 1).

Table 1: Phytochemical evaluation of ethanolic extract of *P. guajava* roots

Chemical Constituents	Ethanolic Extract
Carbohydrates	+
Glycosides	+
Alkaloids	+
Tannin	+
Flavonoids	+
Phenols	+
Steroids & triterpenoid	+

Effect of *P. guajava* treatment on rodent's body weight

In the study, body mass changes were monitored at conclusion of the treatment period. Rats medicated with Atorvastatin showed a remarkable depletion in body weight measured to the vehicle control and treatment groups. Both, EEPG doses (200 mg/kg *p.o.* and 400 mg/kg *p.o.*) significantly reduced body weight compared to the induced group. Notably, the 400 mg/kg EEPG treatment brought body weight parameters close to those of the standard control group, indicating its potential anti-hyperlipidaemic activity.

However, on comparing the treatment groups with the standard control groups, the EEPG at 400mg/kg *p.o* has managed to bring the general parameters to the near standard control values indicating that the extract has antihyperlipidemic activity (table 2).

Table 2: Assessment of Rodent Body Weight

Group	Day-3 (g)	Day-6 (g)	Day-9 (g)	Day-12 (g)	Day-15 (g)
Group I (Control Normal Saline)	182.4 ±2.1	185.6 ±2.3	189.8 ±2.5	193.2 ±2.7	197.5 ±2.9
Group II (HFD-Induced)	191.3 ±2.4	199.7 ±2.8	210.6 ±3.1	223.4 ±3.6	238.9 ±4.2
Group III (Atorvastatin 10 mg/kg, <i>i.p.</i>)	189.7 ±2.3	195.8 ±2.6	202.4 ±2.9	209.4 ±3.1	214.3 ±3.4
Group IV	190.5 ±2.2	197.2 ±2.7	205.8 ±3.0	214.7 ±3.3	223.6 ±3.8

(EEPG 200 mg/kg, <i>p.o.</i>)					
Group V (EEPG 400 mg/kg, <i>p.o.</i>)	188.9 ±2.1	193.6 ±2.4	198.5 ±2.6	204.2 ±2.9	210.8 ±3.2

Values are expressed as Mean ± SEM (n = 6). The HFD-induced group shows progressive weight gain, while standard drug and extract-treated groups demonstrate moderated body weight increase compared to the hyperlipidaemic control group.

Effect of EEPG on Serum Lipid Profile in HFD-Induced Hyperlipidaemic Rats

The effect of EEPG on serum lipid parameters in high-fat diet (HFD)-induced hyperlipidaemic rats is presented in Table 3. The HFD-induced group exhibited a significant elevation in total cholesterol (TC), triglycerides (TGs), and very low-density lipoprotein (VLDL) levels, along with a marked reduction in high-density lipoprotein (HDL), when compared with the normal control group. Treatment with the standard drug, Atorvastatin (10 mg/kg, *i.p.*), significantly restored the altered lipid parameters toward normal values. Similarly, administration of EEPG at doses of 200 mg/kg and 400 mg/kg (*p.o.*) resulted in dose-dependent improvement in lipid profile, characterized by a reduction in TC, TGs, and VLDL levels and an increase in HDL concentration compared to the HFD-induced group. The high-dose EEPG group demonstrated a more pronounced antihyperlipidemic effect, approaching that of the standard treatment group.

Table 3. Effect of EEPG on Serum Lipid Profile

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Group I (Control Normal Saline)	92.4±3.1	84.6±2.8	48.5±1.9	16.8±0.6
Group II (HFD-Induced)	186.7±5.4***	168.2±4.7***	28.4±1.3***	33.6±0.9***
Group III	104.8±3.6####	96.3±3.1###	44.7±1.6###	19.3±0.6###

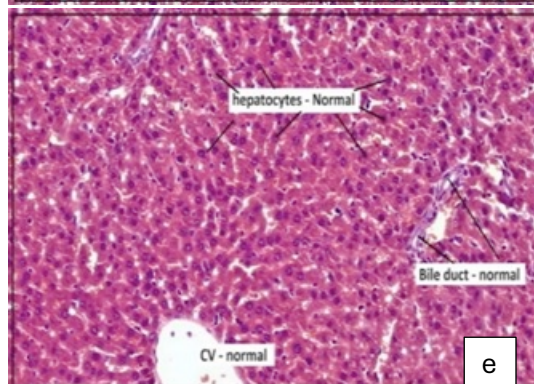
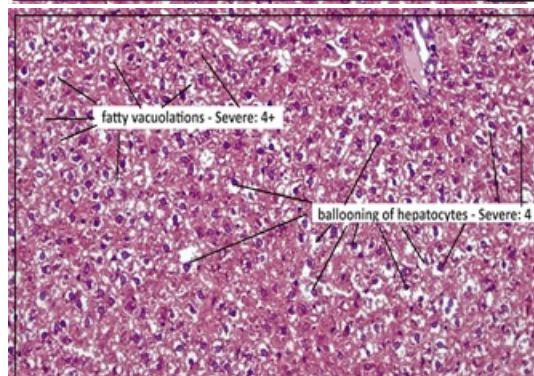
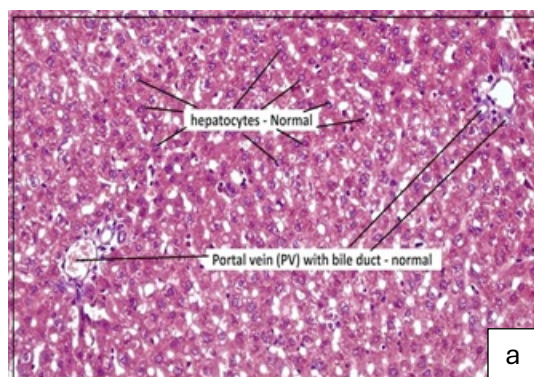
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(Atorvastatin 10 mg/kg, <i>i.p.</i>)				
Group IV (EEPG 200 mg/kg, <i>p.o.</i>)	128.5±4.2 ^{##}	118.6±3.8 ^{##}	38.2±1.4 ^{##}	23.7±0.8 ^{##}
Group V (EEPG 400 mg/kg, <i>p.o.</i>)	112.9±3.9 ^{###}	102.4±3.3 ^{###}	42.6±1.7 ^{###}	20.5±0.7 ^{###}

Values expressed as Mean ± SEM, n=6, Statistical analysis performed using one-way ANOVA followed by Newman-Keuls Multiple Comparison Test. ***p < 0.001 vs Control group ^{##}p < 0.01 vs HFD-Induced group ^{###}p < 0.001 vs HFD-Induced group.

Histopathological Evaluation of Liver C

Histopathological examination of liver sections revealed normal hepatic architecture in all experimental groups. Microscopic analysis showed well-preserved hepatocytes arranged in normal hepatic cords radiating from the central vein, with intact cell morphology and distinct nuclei. The sinusoids appeared normal without evidence of congestion, fatty infiltration, inflammatory cell infiltration, necrosis, or fibrosis. No structural deformities or pathological alterations were observed in the treatment groups when compared to the control group. These findings indicate that administration of EEPG at both tested doses did not produce any adverse morphological changes in hepatic tissue and demonstrated hepatic safety under the experimental conditions.



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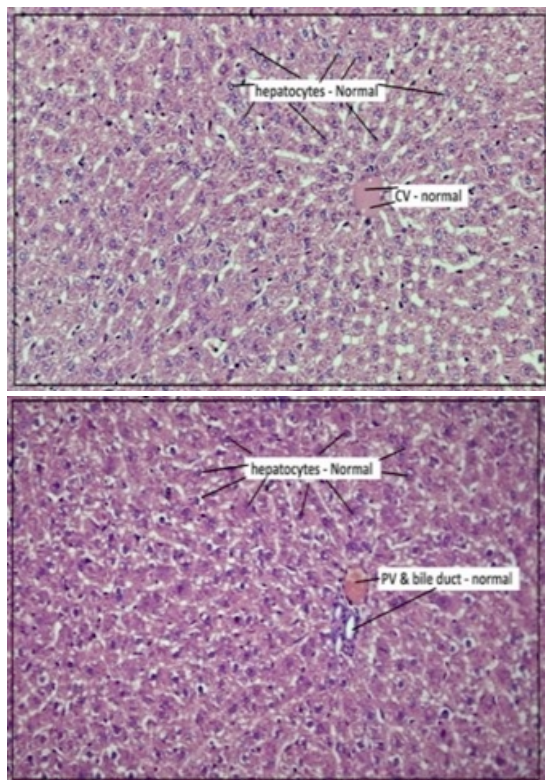


Figure 1: Photomicrograph of histopathological H&E Staining (a) Normal Control – Rat Liver: showing: Normal hepatocytes, Portal vein (PV) with bile duct – normal - NAD⁺ (X100). (b) Disease Induced (HFD) – Rat Liver: hepatic fatty vacuolations with ballooning of hepatocytes - Severe: 4+ (X100). (c) Standard treated – Rat Liver: Normal hepatocytes, around central vein (CV) – normal (X100). (d) Test Drug – Low Dose (200mg/kg EEPG) Rat Liver: showing: Normal hepatocytes - NAD⁺, Central vein (CV) – normal - (X100). (e) Test Drug – High dose (400mg/kg EEPG)-Rat Liver: showing: Normal hepatocytes, around Portal vein (PV) – normal Bile ducts - normal - NAD⁺ (X100).

DISCUSSION

The present study evaluated the antihyperlipidaemic potential of the ethanolic root extract of *Psidium guajava* (EEPG) in a high-fat diet (HFD)-induced hyperlipidaemia model in Wistar rats. The HFD model successfully induced dyslipidaemia, as evidenced by significant body weight gain, elevated total cholesterol (TC), triglycerides (TG), and VLDL levels, along with reduced HDL levels. These findings confirm the validity of the experimental model and are consistent with earlier reports demonstrating that cholesterol- and fat-enriched diets disrupt lipid metabolism and promote hepatic lipid accumulation.

Administration of EEPG resulted in dose-dependent improvement in serum lipid parameters. The higher dose (400 mg/kg) significantly reduced TC, TG, and

VLDL levels while elevating HDL levels, showing effects comparable to Atorvastatin. The lipid-lowering effect may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenols, tannins, and saponins identified in the preliminary phytochemical screening. Flavonoids and phenolic compounds are known to inhibit lipid peroxidation, enhance LDL receptor activity, and suppress hepatic cholesterol synthesis.

Similar antihyperlipidaemic effects have been reported with other medicinal plants. For example, *Allium sativum* demonstrated significant reductions in TC and TG levels in fat diet-induced hyperlipidaemic rats [22]. Likewise, *Commiphora mukul* showed improvement in lipid parameters through modulation of lipid metabolism pathways [23]. Studies on *Gymnema sylvestre* [24] and *Lagenaria siceraria* [25] also reported significant reductions in serum cholesterol and triglycerides in HFD models. These findings support the hypothesis that plant-derived phytoconstituents exert protective effects against dyslipidaemia.

Histopathological examination further corroborated the biochemical findings. The HFD group showed hepatic fatty vacuolation and ballooning degeneration, indicating steatosis. In contrast, rats treated with EEPG, particularly at 400 mg/kg, exhibited preserved hepatic architecture comparable to the standard drug-treated group. The hepatoprotective effect may be related to antioxidant activity and stabilization of hepatocellular membranes.

Collectively, the results suggest that EEPG exerts antihyperlipidaemic activity possibly through inhibition of lipid absorption, suppression of hepatic cholesterol synthesis, and antioxidant-mediated hepatoprotection.

CONCLUSION

The present investigation demonstrates that the ethanolic root extract of *Psidium guajava* possesses significant antihyperlipidaemic activity in high-fat diet (HFD)-induced hyperlipidaemic Wistar rats. Chronic administration of the extract produced dose-dependent improvements in lipid metabolism, as evidenced by marked reductions in serum total cholesterol, triglycerides, and very low-density lipoprotein levels, along with a significant elevation in high-density lipoprotein levels. The higher dose (400 mg/kg, p.o.) showed lipid-lowering efficacy comparable to the standard drug, Atorvastatin, indicating strong therapeutic potential.

In addition to improving biochemical parameters, the extract demonstrated protective effects on hepatic

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architecture, as confirmed by histopathological evaluation. The restoration of normal liver morphology suggests that the extract not only regulates lipid levels but may also prevent hepatic lipid accumulation and steatosis associated with diet-induced dyslipidaemia. The antihyperlipidaemic effect of *P. guajava* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenols, tannins, and saponins, which are known to modulate lipid metabolism, inhibit cholesterol synthesis, enhance antioxidant defense mechanisms, and improve lipoprotein balance.

Overall, the findings support the potential of *Psidium guajava* root extract as a promising natural therapeutic candidate for the management of hyperlipidaemia and prevention of associated cardiovascular complications. However, further studies involving detailed mechanistic investigations, isolation of active compounds, long-term toxicity assessment, and clinical validation are necessary to establish its safety, efficacy, and translational applicability in human subjects.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

Authors declare no conflict of interest

AUTHOR CONTRIBUTION

Arundhati Kashyap, Deeparani Urolagin, Ravindra Mishra: Conceptualization of the study, experimental design, animal handling and dosing, data acquisition, maintenance of study records, and preparation of the initial draft of the manuscript. Deeparani Urolagin, Sowmya BA, Sukrutha SK: Supervision of the experimental work, data interpretation, histopathological evaluation, critical revision of the manuscript for intellectual content, and final approval of the version to be submitted.

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