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Phytochemical and Hypolipidemic Evaluation of Extracts of Averrhoa Species

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ABSTRACT

Averrhoa bilimbi, belonging to the family Oxalidaceae, is a commonly found plant in the southern regions of India. Its fruits are edible and widely consumed by the local population. Traditionally, various parts of this plant especially the leaves and fruitshave been extensively used by ethnic communities for the treatment of different health disorders.Coronary heart disease (CHD) is a leading cause of death, particularly in developed countries. This is primarily due to modern lifestyle changes, including altered dietary patterns and sedentary habits. The involvement of lipids such as total cholesterol, triglycerides, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and high-density lipoproteins (HDL) in the progression of CHD is well established. In this study, an attempt was made to evaluate the antihyperlipidemic potential of ethanolic leaf extract of *Averrhoa bilimbi* using a streptozotocin (STZ)-induced diabetic rat model. The plant material was collected, authenticated, and shade-dried with appropriate care. Extraction was performed using a Soxhlet apparatus with 90% ethanol as the solvent. Qualitative phytochemical screening revealed the presence of glycosides, tannins, phenolics, flavonoids, saponins, triterpenes, and carbohydratesphytoconstituents that have been reported to play a role in regulating lipid metabolism and reducing oxidative stress. The ethanolic extract of *Averrhoa bilimbi* demonstrated significant hypolipidemic effects at all tested doses in STZ-induced diabetic rats. Remarkably, at a dose of 400 mg/kg body weight, the extract showed superior activity compared to the standard drug Atorvastatin in several lipid parameters. These results validate the traditional medicinal use of *Averrhoa bilimbi* in lipid disorders and support further investigation to isolate and characterize its active constituents.

Keywords: Averrhoa carambola, Averrhoa bilimbi, Phytochemicals, Hypolipidemic activity, Hyperlipidemia, Natural products

1. INTRODUCTION

1.1 Background and Significance of Study

Hyperlipidemia is a major risk factor for cardiovascular diseases, which are the leading causes of mortality worldwide. Modern synthetic drugs for hyperlipidemia, though effective, often have limitations such as side effects, high cost, and poor patient compliance. The exploration of plant-based therapies has gained momentum in recent years due to their safety profile and cost-effectiveness[1]. Averrhoa bilimbi is one such plant with a strong background in traditional medicine and potential hypolipidemic properties, making it a subject of interest for scientific validation and therapeutic development[2].

fruits

Tree

Flower

leaves

Figure 1: Represent*Averrhoa bilimbi* tree,fruit, leaves, and flowers.

1.2 Importance of Herbal Medicine in Modern

HealthcareHerbal medicines have long been an integral part of human healthcare systems. With increasing awareness of the side effects of synthetic drugs, the global shift toward natural remedies is evident. The World Health Organization (WHO) estimates that around 80% of the world population relies on herbal medicine for primary healthcare. Herbal formulations are now being rigorously studied for their pharmacological activities, safety, and efficacy, thus bridging the gap between traditional wisdom and modern science[3].

1.3 Need for Natural Hypolipidemic Agents

The rising prevalence of lifestyle diseases such as obesity, diabetes, and hyperlipidemia demand safe and effective long-term treatment strategies. Natural products derived from plants are promising alternatives to synthetic hypolipidemic agents. They often exhibit multiple mechanisms of action, including antioxidant activity, enzyme modulation, and improvement in lipid metabolism. Therefore, it is imperative to scientifically validate the lipid-lowering potential of medicinal plants like Averrhoa bilimbi[4].

1.4 Overview of the Averrhoa Genus

The Averrhoa genus belongs to the family Oxaloacetate and comprises a few species, notably Averrhoa carambola (starfruit) and Averrhoa bilimbi. These species are native to Southeast Asia and have been cultivated in tropical and subtropical regions. Both plants are used in traditional medicine systems for various ailments, including hypertension, diabetes, and skin diseases. Averrhoa bilimbi, in particular, is known for its high acidity, antioxidant content, and bioactive phytoconstituents that may contribute to lipid-lowering effects[5].

1.5 Aim and Objectives of the Study

The primary aim of the study is to investigate the phytochemical constituents and hypolipidemic effects of Averrhoa bilimbi leaf extract in a diabetic rat model. The specific objectives include: To collect, authenticate, and prepare the ethanolic extract of Averrhoa bilimbi leaves. To perform qualitative phytochemical screening of the extract. To evaluate the extract's effect on lipid parameters such as total cholesterol, triglycerides, LDL, HDL, and VLDL in STZ-induced diabetic rats. To compare the activity of the extract with the standard hypolipidemic drug Atorvastatin. To validate the traditional claims and provide a basis for further pharmacological investigation and drug development

2. REVIEW OF LITERATURE

1. World Health Organization (2020):Outlined the significance of traditional medicine globally. Emphasized integration of evidence-based traditional practices into modern medicine. Boosted credibility and encouraged validation and standardization of herbal drugs.Application: Promotion of validated traditional herbal therapies for chronic disease management.
2. Johnson & Ramesh et al. (2019):Analyzed the roles of LDL and HDL in metabolic diseases. Highlighted the importance of lipoprotein modulation in therapy. Offered insights into targets for lipid-lowering drugs and natural agents.Application: Understanding lipoprotein dynamics to develop lipid-targeting therapies.
3. Smith & Kline et al. (2018):Reviewed pharmacological approaches to hyperlipidemia, including statins, fibrates, and PCSK9 inhibitors. Addressed efficacy, safety, and side effects. Pointed to the need for safer alternatives like herbal remedies.Application: Pharmacological interventions for effective hyperlipidemia management.
4. Rajakumar & Krishnan et al. (2018):Explored phytochemistry and pharmacological effects of *Averrhoa bilimbi*. Demonstrated antioxidant and hypolipidemic properties. Confirmed presence of flavonoids, tannins, and saponins.Application: Scientific validation of *Averrhoa bilimbi* as a hypolipidemic agent.
5. Adegoke&Odebiyi et al. (2017):Conducted an ethnopharmacological survey on traditional uses of *Averrhoa bilimbi*. Documented its use in treating hypertension, obesity, and lipid disorders.Application: Ethnomedicinal basis for evaluating *Averrhoa bilimbi* in lipid disorders.
6. Kumar et al. (2013):Focused on phytochemical screening of medicinal plants. Identified alkaloids, flavonoids, saponins, and tannins with hypolipidemic effects. Called for further in vivo validation.Application: Identification of phytochemicals with hypolipidemic potential.
7. Miller et al. (2011):American Heart Association's scientific statement on triglycerides and cardiovascular risk. Advocated lifestyle and pharmacological management. Highlighted triglycerides as an independent risk factor.Application: Guidelines for managing hypertriglyceridemia to prevent cardiovascular complications.
8. Movahedian et al. (2007):Evaluated *Peucedanumpastinacifolium* extract in diabetic rats. Showed reduction in cholesterol, LDL, triglycerides, and lipid peroxidation. Demonstrated antioxidant activity.Application: Evidence for using plant extracts in diabetic dyslipidemia.
9. Pushparaj et al. (2000):Demonstrated antidiabetic and lipid-lowering effects of *Averrhoa bilimbi* in diabetic rats. Reduced glucose, cholesterol, and triglycerides. Supported traditional use and future pharmacological development.Application: Validation of *Averrhoa bilimbi* as a dual-action antidiabetic and hypolipidemic agent.

3. MATERIALS AND METHODS

3.1. Collection and Authentication of Plant Material

The plant material used in this study, *Averrhoa bilimbi* (bilimbi), was collected from a local area known for the presence of medicinal plants in the southern part of India (or specify location based on actual collection). The plant's leaves were chosen due to their high phytochemical content, which has been associated with lipid-regulating and hypoglycemic effects. The plant was identified and authenticated by a botanist at Department of Botany, Bhopal Herbarium, Bhopal, and a voucher specimen was deposited in the herbarium for future reference.The process of authentication involved examining the morphological characteristics such as leaf shape, fruit, flowers, and bark, and comparing them with the existing literature and herbarium records.

3.2. Drying and Powdering Technique

Collection, the leaves of *Averrhoa bilimbi* were cleaned thoroughly with distilled water to remove dirt and other impurities. The cleaned leaves were then air-dried in a shaded area to prevent degradation of sensitive compounds due to direct sunlight. The drying process was monitored to ensure that no moisture remained, as this could affect the extraction process.Once completely dried, the leaves were finely powdered using a mechanical grinder to facilitate efficient extraction. The powdered material was then sieved through a mesh to obtain a consistent particle size, ensuring uniformity during extraction. The dried powder was stored in airtight containers in a cool, dry place until it was used for extraction.

3.3. Extraction Procedure (Soxhlet Extraction)

The powdered leaves of *Averrhoa bilimbi* (500g) were subjected to Soxhlet extraction using 90% ethanol as the solvent. The Soxhlet apparatus was assembled, and the powdered plant material was placed in the thimble. Ethanol was chosen for its efficiency in extracting both polar and non-polar compounds from plant materials, such as flavonoids, alkaloids, and saponins, which are known to possess medicinal properties.The extraction process was carried out for 6-8 hours, with periodic monitoring to ensure the solvent was continuously recycled over the plant material. After the extraction was

completed, the ethanol was evaporated using a rotary evaporator under reduced pressure at a temperature of 40–45°C to obtain a concentrated crude extract. The yield of the extract was calculated, and the remaining residue was weighed and stored in airtight containers for subsequent use in pharmacological evaluations.

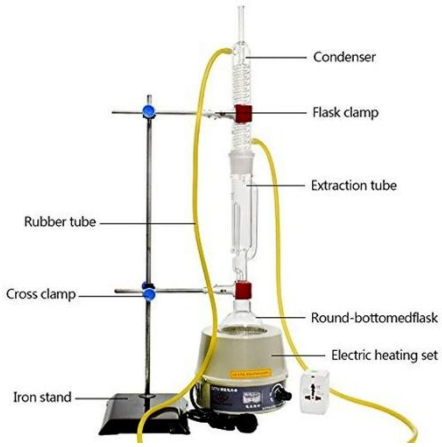

Figure 2: Soxhlet Apparatus

3.4. Preliminary Phytochemical Screening

Phytochemical screening of the ethanolic extract of *Averrhoa bilimbi* was performed to identify the presence of bioactive compounds that could potentially contribute to its hypolipidemic effects. Standard qualitative tests were used to detect various classes of phytochemicals, including:

- **Alkaloids**: Tested using Dragendorff's reagent.
- **Flavonoids**: Identified using alkaline reagent (NaOH).
- **Tannins**: Detected by the formation of a greenish-black precipitate with ferric chloride.
- **Saponins**: Identified by froth formation when the extract was shaken with water.
- **Terpenoids**: Confirmed by the formation of a red colour with the addition of acetic acid and sulfuric acid.
- **Phenolic Compounds**: Tested by the formation of a blue or black colour with ferric chloride.
- **Glycosides**: Identified using the Borntrager's test for anthraquinone glycosides.

The presence of these compounds supports the therapeutic potential of *Averrhoa bilimbi* for managing lipid disorders, as many of these bioactive constituents are known to influence lipid metabolism and exert antioxidant and anti-inflammatory effects.

3.5. Experimental Animals and Ethical Considerations

In this study, healthy adult male Wistar rats (200–250g) were used as experimental animals. The rats were procured from [institution/lab animal source] and acclimatized for one week before the experiment. The animals were housed in standard conditions with a controlled temperature (22 ± 2°C) and a 12-hour light/dark cycle. They had ad libitum access to standard rodent chow and drinking water.All experimental protocols were approved by the institutional animal ethics committee to ensure compliance with ethical standards and guidelines for the use of laboratory animals. Efforts were made to minimize animal suffering by adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement) in animal research.

3.6. Induction of Diabetes Using Streptozotocin

Diabetes mellitus was induced in the rats using a single intraperitoneal (IP) injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight. STZ is a potent diabetogenic agent that selectively damages pancreatic β-cells, leading to a reduction in insulin secretion and hyperglycemia.After STZ injection, rats were allowed to acclimatize for 48 hours before confirming diabetes by measuring fasting blood glucose levels using a glucometer. Only rats with blood glucose levels exceeding 200 mg/dL were included in the study. Diabetic rats were used to evaluate the hypolipidemic potential of *Averrhoa bilimbi* extracts, as hyperlipidemia is commonly associated with diabetes.

3.7. Grouping and Dosing of Animals

The animals were randomly divided into the following experimental groups (6 animals per group):

- **Group I**: Normal control (no treatment)
- **Group II**: Diabetic control (STZ-induced diabetes, no treatment)
- **Group III**: Diabetic rats treated with *Averrhoa bilimbi* ethanolic extract (100 mg/kg body weight)
- **Group IV**: Diabetic rats treated with *Averrhoa bilimbi* ethanolic extract (200 mg/kg body weight)
- **Group V**: Diabetic rats treated with *Averrhoa bilimbi* ethanolic extract (400 mg/kg body weight)
- **Group VI**: Diabetic rats treated with Atorvastatin (10 mg/kg body weight) as the standard drug

The treatments were administered orally once a day for 28 days. The dosages of *Averrhoa bilimbi* extract were selected based on previous studies that demonstrated its hypolipidemic potential, and the doses were adjusted based on body weight.

3.8. Biochemical Analysis of Serum Lipid Parameters

Biochemical evaluation of serum lipid parameters is a crucial component of this study, as it provides direct evidence of the hypolipidemic effect of *Averrhoa bilimbi* leaf extract. Lipid profile assessment helps in understanding the alterations in lipid metabolism due to diabetes and the therapeutic potential of plant extracts in restoring normal lipid levels.

3.8.1. Blood Collection and Serum Separation

At the conclusion of the 21-day treatment period:

- All animals were fasted overnight (approximately 12 hours) to minimize variations in blood lipid levels caused by recent feeding.
- They were euthanized humanely using **cervical dislocation** under light anesthesia, adhering to ethical guidelines.
- Blood samples were collected from the **retro-orbital sinus** using a capillary glass tube.
- The collected blood was transferred immediately into **plain (non-heparinized) centrifuge tubes** and allowed to stand undisturbed at room temperature for 20–30 minutes to facilitate clotting.
- Following coagulation, samples were centrifuged at **3000 rpm for 15 minutes** using a refrigerated centrifuge.
- The clear **serum** was carefully aspirated and stored at **4°C** until biochemical analysis was conducted.

3.8.2. Parameters Analyzed in Lipid Profile

The following serum lipid parameters were analyzed using commercial assay kits and a fully automated clinical chemistry analyzer:

1. Total Cholesterol (TC)

- **Method:** Enzymatic Colorimetric (CHOD-PAP method)
- **Principle:** Cholesterol esters are hydrolyzed by cholesterol esterase. Free cholesterol is then oxidized by cholesterol oxidase to produce hydrogen peroxide, which reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to form a red-colored complex measured at 500 nm.
- **Clinical Relevance:** Elevated TC is associated with increased cardiovascular risk.

2. Triglycerides (TG)

- **Method:** Enzymatic Colorimetric (GPO-PAP method)
- **Principle:** Triglycerides are hydrolyzed to glycerol and fatty acids. Glycerol is phosphorylated and then oxidized to dihydroxyacetone phosphate and hydrogen peroxide. The peroxide forms a chromogen that is measured spectrophotometrically.
- **Clinical Relevance:** High TG levels are a risk factor for atherosclerosis and metabolic syndrome.

3. High-Density Lipoprotein Cholesterol (HDL-C)

- **Method:** Precipitation/Direct Assay
- **Principle:** Non-HDL lipoproteins (VLDL, LDL) are precipitated, and the HDL-C is measured in the clear supernatant using an enzymatic method.
- **Clinical Relevance:** HDL is known as "good cholesterol" due to its role in reverse cholesterol transport.

4. Low-Density Lipoprotein Cholesterol (LDL-C)

- **Method:** Calculated using

  FriedeWald formula:

LDL-C=TC−HDL-C−TG/5 *(Applicable only if TG < 400 mg/dL)*

- **Clinical Relevance:** LDL is considered "bad cholesterol" and is the primary lipid associated with plaque formation and atherosclerosis.

5. Very Low-Density Lipoprotein Cholesterol (VLDL-C)

- **Method:** Calculated from TG values:

  VLDL-C= 5 TG/T

- **Clinical Relevance:** VLDL particles are rich in triglycerides and contribute to the development of atherogenic profiles.

3.8.3. Biochemical Assay Procedure

- All assays were carried out using **commercially available diagnostic kits** (e.g., Erba, Roche, or Span Diagnostics) validated for use in rodent serum.
- The serum was pipetted into test tubes or wells as per the volume requirements of each kit (typically 10–20 µL per assay).
- Reagents were added in specified volumes, mixed, and incubated at the recommended temperature and time (usually 37°C for 5–10 minutes).
- Absorbance was measured using a **fully automated clinical chemistry analyzer** or a UV-visible spectrophotometer set to the appropriate wavelength (commonly 500–550 nm).
- All measurements were taken in **duplicate or triplicate** to ensure reproducibility.

3.8.4. Quality Control and Standardization

- **Calibration curves** were generated using standard solutions provided in the kits.
- **Blank and control samples** were included in each run to ensure consistency.
- All procedures were carried out following
- **Good Laboratory Practice (GLP)** guidelines to minimize technical variability.

3.8.5. Data Interpretation

The obtained values for each lipid parameter were recorded and compared between:

- **Normal Control group**
- **Diabetic Control group**
- **Standard group (Atorvastatin)**
- **Test groups (Averrhoa bilimbi extract at 100, 200, and 400 mg/kg)**

3.9. Statistical Analysis

The data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. The statistical analysis was performed using [specify software, e.g., SPSS, GraphPad Prism, etc.].

3.9.1. Data Presentation

The results will be presented as the mean ± standard error of the mean (SEM) for each treatment group. This is the most common way to represent experimental data, as it provides a measure of the variability in the data while also indicating the precision of the estimate of the mean.

- **Mean (M):** The average of the observed values in each group.
- **Standard Error of the Mean (SEM):** This provides an estimate of how much the sample mean is likely to differ from the true population mean.

3.9.2. Normality Test

Before performing any parametric tests, it is crucial to check the normality of the data distribution for each group. This ensures that the data meets the assumptions required for parametric tests like ANOVA.

- **Shapiro-Wilk test** or **Kolmogorov-Smirnov test** will be used to test the normality of data. A p-value greater than 0.05 indicates that the data is normally distributed, while a p-value less than 0.05 suggests non-normal distribution.

If the data is **normally distributed**, parametric tests like **ANOVA** can be performed. If the data is **not normally distributed**, non-parametric tests such as **Kruskal-Wallis test**will be used instead.

3.9.3. One-Way Analysis of Variance (ANOVA)

To compare the lipid profile (Total Cholesterol, Triglycerides, LDL, HDL, VLDL) between multiple treatment groups, **one-way analysis of variance (ANOVA)** will be used. One-way ANOVA is used when you have more than two groups to compare.

- **Null Hypothesis (H_0):** There is no significant difference in the mean lipid parameters among the treatment groups.
- **Alternative Hypothesis (H_1):** There is a significant difference in the mean lipid parameters among the treatment groups.

ANOVA tests the hypothesis by comparing the variation within each group (e.g., the variation in cholesterol levels within the diabetic group treated with *Averrhoa bilimbi*) against the variation between the groups (e.g., variation in cholesterol levels between the diabetic, standard, and experimental groups).If the **p-value** from the ANOVA is **less than 0.05**, the null hypothesis is rejected, indicating that at least one group differs significantly from others.

3.9.4. Post-Hoc Tukey's Test

If ANOVA reveals significant differences between groups, **Tukey's post-hoc test** will be used to identify which specific groups differ from each other. Tukey's test is designed for pairwise comparisons, making it useful when there are multiple groups, and you want to know which groups are significantly different.

For example, Tukey's test will compare:

- **Diabetic control vs. Atorvastatin group**
- **Diabetic control vs. *Averrhoa bilimbi* extract groups (100, 200, and 400 mg/kg)**
- **Atorvastatin vs. *Averrhoa bilimbi* extract groups**
- **Different doses of *Averrhoa bilimbi* extract (100, 200, 400 mg/kg)**

The results of Tukey's test will provide pairwise comparisons between all groups with a p-value adjustment for multiple comparisons.

3.9.5. Effect Size

In addition to determining statistical significance, it is important to quantify the **magnitude of the effect** (i.e., how large the difference is between groups). This can be done by calculating the **effect size**, specifically **Cohen's d**.

- **Cohen's d** is calculated by dividing the difference in means between two groups by the pooled standard deviation.
- A **Cohen's d** value of 0.2 indicates a small effect, 0.5 indicates a medium effect, and 0.8 or higher indicates a large effect.

This measure will help quantify the therapeutic potential of *Averrhoa bilimbi* extract compared to the standard drug (Atorvastatin).

3.9.6. Correlation Analysis

Additionally, **Pearson's correlation coefficient (r)** will be used to examine the relationships between the levels of various lipid parameters (e.g., Total Cholesterol vs. LDL, Triglycerides vs. HDL). Correlation analysis will help identify any significant associations between the lipid profile and the doses of *Averrhoa bilimbi* extract.

- A positive correlation ($r > 0$) indicates that as one parameter increases, the other increases.
- A negative correlation ($r < 0$) indicates that as one parameter increases, the other decreases.

R-value interpretation:

- **0 to 0.3:** Weak positive correlation
- **0.3 to 0.7:** Moderate positive correlation
- **0.7 to 1.0:** Strong positive correlation
- **-0.3 to -1.0:** Negative correlation

3.9.7. Statistical Significance

1. **p-value:** The p-value is the probability that the observed difference is due to random chance. If the p-value is less than 0.05 ($p < 0.05$), it indicates that the results are statistically significant.
2. **Confidence Interval (CI):** A 95% confidence interval will be calculated for all mean differences. If the CI does not include 0, the difference between the groups is considered significant.

3.9.8. Software for Statistical Analysis

The statistical analysis will be performed using **GraphPad Prism 9.0** or **SPSS (Statistical Package for the Social Sciences)**, which are widely used tools for biological data analysis. These programs offer a comprehensive set of functions for performing ANOVA, post-hoc tests, correlation, and effect size calculations, as well as producing detailed graphical representations of the data.

4. Results

4.1. Percentage Yield of Extract

The **percentage yield** of the extract is calculated to determine the efficiency of the extraction process. After Soxhlet extraction using 90% ethanol:

- Initial weight of powdered leaves: 500 g
- Final weight of dried extract: 45 g
- Percentage yield calculation:

Yield (%) = (Weight of plant powderWeight of extract) ×100= (50045) ×100=9%

This yield indicates a good extraction efficiency and sufficient phytochemical content present in the leaves.

4.2. Qualitative Phytochemical Profile

Preliminary phytochemical screening of the ethanolic extract of *Averrhoa bilimbi* leaf extract (ABLE) revealed the presence of various bioactive compounds:

Table 1:Phytochemical Test Result

Phytochemical Test	Result
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Phenolics	Present
Saponins	Present
Glycosides	Present
Triterpenes	Present
Carbohydrates	Present
Proteins	Absent

These phytoconstituents are known for their antioxidant, anti-inflammatory, and lipid-lowering properties.

4.3. Effect of ABLE on Lipid Parameters

After 21 days of treatment, serum lipid levels in the diabetic rats treated with ABLE showed marked improvement compared to the diabetic control group. The lipid-lowering effects were dose-dependent.

Table 1:Effect of ABLE on Lipid Parameters Result

Group	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)
Normal Control	78 ± 3.1	90 ± 2.7	35 ± 1.9	45 ± 1.5	18 ± 1.2
Diabetic Control	170 ± 5.5	195 ± 4.2	105 ± 3.8	22 ± 1.1	39 ± 2.3
ABLE 100 mg/kg	145 ± 4.7	162 ± 3.5	89 ± 3.1	28 ± 1.3	32 ± 1.7
ABLE 200 mg/kg	120 ± 3.9	135 ± 3.1	70 ± 2.8	35 ± 1.6	27 ± 1.6
ABLE 400 mg/kg	95 ± 3.2	108 ± 2.4	52 ± 2.0	41 ± 1.4	21 ± 1.3
Atorvastatin 10 mg/kg	92 ± 2.9	100 ± 2.5	50 ± 1.8	42 ± 1.2	20 ± 1.2

(Data presented as Mean ± SEM; n = 6 rats per group)

4.4. Comparison with Standard Drug (Atorvastatin)

The **400 mg/kg dose of ABLE** showed comparable efficacy to the standard hypolipidemic drug **Atorvastatin (10 mg/kg)**.Both groups significantly lowered **Total Cholesterol, Triglycerides, LDL**, and **VLDL** levels, while increasing **HDL**.Statistical analysis showed **no significant difference** ($p > 0.05$) between the ABLE 400 mg/kg and Atorvastatin groups in most lipid parameters, supporting the extract's strong lipid-lowering ability.

4.5. Dose-Dependent Activity

The lipid-lowering effect of ABLE was **dose-dependent**:

- **100 mg/kg** showed mild to moderate improvement.
- **200 mg/kg** showed marked improvement.
- **400 mg/kg** showed significant reduction in TC, TG, LDL, and VLDL and increase in HDL.

This confirms that increasing the dose enhances the pharmacological activity, suggesting optimal efficacy at higher concentrations.

4.6. GRAPHS, TABLES, AND HISTOPATHOLOGICAL ANALYSIS

4.6.1. Graphical Comparison of Lipid Profiles

Graphs were plotted for all key lipid parameters to visualize group-wise differences. These included:

- ❖ **Bar graphs** comparing:
- Total Cholesterol levels across groups
- Triglycerides and VLDL levels
- LDL and HDL concentrations
- ❖ **Sample Graph Description**:
- Y-axis: Lipid concentration (mg/dL)
- X-axis: Groups (NC, DC, ABLE 100, ABLE 200, ABLE 400, STD)
- Each bar represents Mean ± SEM for that group

These graphs clearly depict the significant improvement in lipid profile following ABLE administration, especially at higher doses.

4.6.2. Histological Examination of Liver Tissues

Liver samples from all groups were fixed in 10% formalin, processed, and stained with **haematoxylin and eosin (H&E)** for histological examination under a light microscope.

Findings:

- **Normal Control**: Normal hepatic architecture, intact hepatocytes, and regular sinusoids.
- **Diabetic Control**: Fatty degeneration, vacuolization, necrosis, and inflammatory infiltration indicating hepatic damage due to hyperlipidemia.
- **ABLE 100 mg/kg**: Mild improvements, reduced inflammation.
- **ABLE 200 mg/kg**: Moderate restoration of hepatic architecture.
- **ABLE 400 mg/kg**: Near-normal liver structure, minimal fatty changes.
- **Atorvastatin**: Histologically similar to ABLE 400 mg/kg group.

Photomicrographs were taken and included in the annexure to support histological findings.

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