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ORIGINAL RESEARCH ARTICLE

Hypolipidemic and Hepatoprotective Effects of Methanol Extract of *Carica papaya* Leaves in High-Fat Diet-Induced Hyperlipidemic Rats

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ABSTRACT
This study investigated the hypolipidaemic potential of the methanol leaf extract of Carica papaya in the liver of albino rats fed a formulated cow-brain diet, and reported that its effect was compared with that of a standard statin drug (Atorvastatin). Thirty-five (35) albino rats were used and divided into seven (7) groups of five (5) rats each. Group I, which received a normal diet (ND), served as the positive control. Group II was fed a formulated high-cholesterol diet (FHD) and served as the negative control. Group III (ND + 62 mg/kg) was pre-treated before induction, while groups IV, V and VI were induced with FHD and treated with 31, 62, and 124 mg/kg of *C. papaya* leaf extract, respectively. Group VII (Ator) was induced with the formulated cow-brain tissue and treated with Atorvastatin. The experiment was conducted for 28 days. Biochemical analysis indicated that induction with the formulated cow-brain tissue significantly increased serum lipid profiles, including total cholesterol (225.50 ± 9.93 mg/dL vs. 131.75 ± 9.02 mg/dL in ND) and LDL (104.75 ± 6.42 mg/dL vs. 66.5 ± 5.47 mg/dL in ND), with the exception of HDL. Liver function parameters were also elevated: AST (36.25 ± 8.26 U/L), ALT (39.75 ± 5.91 U/L), and ALP (220.25 ± 14.57 U/L), all showing significant ($p<0.05$) increases. The study further noted that, when compared with the effect of Atorvastatin, *C. papaya* extract demonstrated considerable hypolipidaemic activity in all treated groups. This effect was attributed to the presence of phytochemicals such as saponins and flavonoids, which may influence lipid metabolism and inhibit HMG-CoA reductase activity. The findings suggested that *C. papaya* is more effective than Atorvastatin.

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INTRODUCTION
Hypercholesterolemia is a major risk factor for morbidity and mortality worldwide and is characterized by elevated levels of cholesterol in the blood, typically exceeding the recommended threshold of 200 mg/mL. Increased concentrations of low-density lipoprotein cholesterol (LDL-C) within blood vessels promote lipid deposition in arterial walls, thereby contributing to the development and progression of atherosclerosis (Oksal et al., 2020). Although several anti-hypercholesterolemic drugs are currently available, many are associated with adverse effects, particularly involving the digestive system and hepatic function, which limits their suitability for long-term use (Salisu et al., 2020, 2022).

Dyslipidemia refers to abnormalities in lipid profiles in the blood and represents a major metabolic disorder. The most common form, hyperlipidemia, is characterized by elevated levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), accompanied by a reduction in high-density lipoprotein cholesterol (HDL-C) (Rauf et al., 2022). Abnormal lipid profiles are widely recognized as key precursors of cardiovascular diseases and continue to pose a significant challenge to global public health. Recent studies have reported a rising prevalence of lipid-related metabolic disorders within the general population (Kathak et al., 2022).

Cardiovascular diseases resulting from dyslipidemia are among the leading causes of death globally (Du et al., 2023). Beyond cardiovascular complications, dyslipidemia has been implicated in dysfunctions of the endocrine, central nervous, hepatic, and renal systems. The liver plays a central role in lipid metabolism and is therefore particularly vulnerable to lipid abnormalities (Arvanitis et al., 2023). Liver function is commonly assessed using biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase

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(GGT), which provide insight into hepatocellular integrity and biliary function (Lala et al., 2025).

Atherosclerosis is the primary pathological process underlying myocardial infarction and stroke, accounting for a substantial proportion of cardiovascular morbidity and mortality (Poznyak et al., 2021). Blood cholesterol concentration is a key biomarker of atherosclerotic risk, and therapeutic strategies aimed at reducing cholesterol synthesis have proven effective in lowering cardiovascular risk. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase reduces endogenous cholesterol synthesis and consequently lowers circulating cholesterol levels. For instance, Cicero et al. (2021) reported that monacolin K, a bioactive compound in red yeast rice extract, inhibits HMG-CoA reductase and may serve as an effective and relatively safe lipid-lowering nutraceutical in individuals with mild hypercholesterolemia.

Despite growing interest in plant-based lipid-lowering agents, there remains a paucity of studies directly comparing their efficacy with standard pharmaceutical agents. To the best of the authors' knowledge, no study has systematically compared the hypolipidemic effects of *Carica papaya* leaf extract with those of atorvastatin.

Therefore, the present study was designed to evaluate the hypolipidemic potential of the methanol extract of *Carica papaya* leaves in rats fed a diet containing cow brain tissue. The specific objectives were to: (i) assess the hypolipidemic effect of *C. papaya* leaf extract in rats induced with a cow brain–containing diet; (ii) determine liver function parameters and lipid profiles in hyperlipidemic and non-induced rats; and (iii) compare the effects of methanol extract of *C. papaya* leaves with those of atorvastatin.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was obtained from the Ministry of Livestock and Fisheries Development, Usman Faruk Secretariat, Sokoto State, Nigeria (Reference No.: ML&FD/PLAN/197/VOL.1). In accordance with the approved protocol, the ethical clearance is valid for a period of twelve (12) months, up to November 2026. The Ministry requires submission of the study results upon completion for official documentation.

Collection and Identification of Samples

Fresh leaves of *Carica papaya* were collected from Wamakko Local Government Area, Sokoto State, Nigeria. Botanical identification and authentication were conducted in March 2024 by a plant taxonomist in the Department of Biology at Sokoto State University, Sokoto, Nigeria. Fresh cow brain was purchased from Sokoto Central Market.

Thirty-five (35) Wistar rats were procured from Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The animals were acclimatized for seven (7) days under standard laboratory conditions in the Animal Care Room of the Department of Medical Laboratory Science, Umaru Ali Shinkafi Polytechnic, Sokoto, prior to commencement of the experiment.

Preparation of *Carica papaya* Leaf Extract

The collected *C. papaya* leaves were washed thoroughly with tap water, cut into small pieces, and air-dried. A weighed portion (100 g) of the dried leaves was transferred into a conical flask, and 500 mL of methanol was added. The mixture was gently agitated for approximately 8 minutes and allowed to stand for 14 hours at room temperature. The extract was filtered using Whatman No. 1 filter paper, and the resulting filtrate was stored in a refrigerator until required for analysis, following the method described by Sofowora (1993).

Qualitative Phytochemical Analysis

Qualitative phytochemical screening of the *C. papaya* leaf extract was conducted to determine the presence of major secondary metabolites, including alkaloids, flavonoids, saponins, glycosides, steroids, and tannins, using standard procedures.

Test for Alkaloids

To 0.1 mL of the extract in a test tube, 2–3 drops of Dragendorff's reagent were added. Formation of an orange-red precipitate with turbidity indicated the presence of alkaloids (Ciulci, 1994).

Test for Flavonoids

A portion of the extract (4 mg/mL) was treated with a piece of magnesium ribbon followed by dropwise addition of concentrated hydrochloric acid. A colour change from orange to red indicated the presence of flavones, while red to crimson coloration confirmed flavonoids (Sofowora, 1993).

Test for Glycosides

One milliliter of the extract was mixed with 10 mL of 50% sulfuric acid and heated for 15 minutes. After cooling, 10 mL of Fehling's solution was added and the mixture boiled. Formation of a brick-red precipitate indicated the presence of glycosides (Sofowora, 1993).

Test for Saponins

Half a gram of powdered leaf sample was mixed with 5.0 mL of distilled water and shaken vigorously. The formation of a persistent froth lasting approximately 15 minutes indicated the presence of saponins (Brain et al., 1975).

Test for Steroids

Two milliliters of the extract were evaporated to dryness, and the residue was dissolved in acetic anhydride followed by chloroform. Concentrated sulfuric acid was carefully added along the side of the test tube. Formation of a brown ring at the interface and violet coloration in the supernatant layer indicated the presence of steroids (Ciulci, 1994).

Test for Tannins

Two milliliters of the extract were diluted with distilled water, and 2–3 drops of 5% ferric chloride solution were added. A greenish-black or blue coloration indicated the presence of tannins (Ciulci, 1994).

Quantitative Phytochemical Analysis

Quantitative determination of phytochemical constituents in the solid extract was carried out using standard spectrophotometric methods as described by Trease et al. (1996).

Determination of Steroids

One gram (1 g) of the sample was macerated in 20 mL of ethanol and filtered. Two milliliters of the filtrate were mixed with 2 mL of colour reagent and allowed to stand for 30 minutes. Absorbance was measured at 550 nm using a UV–visible spectrophotometer.

Determination of Flavonoids

One gram (1 g) of the sample was macerated in 20 mL of ethyl acetate and filtered. Five milliliters of the filtrate were mixed with 5 mL of dilute ammonia solution. The upper layer was collected, and absorbance was measured at 490 nm using a UV–visible spectrophotometer.

Determination of Saponins

One gram (1 g) of the sample was macerated with 10 mL of petroleum ether and decanted. An additional 10 mL of petroleum ether was added, and the filtrates were combined and evaporated to dryness. Six milliliters of ethanol were added, and 2 mL of the solution were mixed with 2 mL of colour reagent and allowed to stand for 30 minutes. Absorbance was measured at 550 nm.

Determination of Alkaloids

One gram (1 g) of the sample was macerated in 20 mL of 20% sulfuric acid in ethanol (1:1) and filtered. One milliliter of the filtrate was mixed with 5 mL of 60% sulfuric acid and 5 mL of 0.5% formaldehyde in 60% sulfuric acid. The mixture was allowed to stand for 3 hours, after which absorbance was measured at 565 nm.

Determination of Glycosides

One gram (1 g) of the sample was treated with 2.5 mL of 15% lead acetate and filtered. The filtrate was mixed with 2.5 mL of chloroform and shaken vigorously. The lower layer was collected, evaporated to dryness, and reconstituted with 3 mL of glacial acetic acid. Subsequently, 0.1 mL of 5% ferric chloride and 0.25 mL of concentrated sulfuric acid were added. The mixture was shaken and stored in the dark for 2 hours, after which absorbance was measured at 530 nm.

Determination of Tannins

One gram (1 g) of the sample was macerated with 50 mL of methanol and filtered. Five milliliters of the filtrate were treated with 0.3 mL of 0.1 M ferric chloride in 0.1 M hydrochloric acid and 0.3 mL of 0.0005 M potassium ferricyanide. Absorbance was measured at 720 nm using a UV–visible spectrophotometer.

BIOCHEMICAL ANALYSES

Determination of Serum Bilirubin

Serum total and conjugated bilirubin were measured using the **Cobas C111 automated chemistry analyzer** (Roche Diagnostics) in accordance with the manufacturer's instructions. Internal quality control procedures were applied prior to analysis. The reference ranges used were **8.0–17.0 µmol/L** for total bilirubin and **< 8.0 µmol/L** for conjugated bilirubin.

Determination of Serum Alkaline Phosphatase (ALP)

Serum alkaline phosphatase activity was determined using the **Cobas C111 automated chemistry analyzer** following the manufacturer's standard operating protocol. Results were expressed in international units per litre (IU/L), with a reference range of **25–92 IU/L**.

Determination of Serum Alanine Transaminase (ALT)

Serum alanine transaminase (ALT) activity was assayed using the **Cobas C111 automated chemistry analyzer** according to the manufacturer's instructions. Enzyme activity was reported in IU/L, and the reference range applied was **3–15 IU/L**.

Determination of Serum Aspartate Transaminase (AST)

Serum aspartate transaminase (AST) activity was measured using the **Cobas C111 automated chemistry analyzer** in accordance with the manufacturer's protocol. Results were expressed in IU/L, with a reference range of **5–18 IU/L**.

Estimation of Serum Total Cholesterol

Serum total cholesterol was determined using an enzymatic colorimetric method as described by Allain et al. (1974). Briefly, 1.0 mL of cholesterol reagent was dispensed into three test tubes labelled **Blank, Standard**, and **Sample**. Distilled water (10 µL), cholesterol standard (10 µL), and serum sample (10 µL) were added to the respective tubes. The mixtures were incubated at **37°C for 10 minutes**, and absorbance was measured at **500–520 nm** using a spectrophotometer. Total cholesterol concentration was calculated using the standard formula based on absorbance ratios.

Estimation of Serum Triglycerides

Serum triglyceride concentration was determined using the enzymatic colorimetric method described by Fossati and Prencipe (1982). One milliliter (1.0 mL) of triglyceride reagent was added to test tubes labelled Blank**,** Standard**,** and Sample. Distilled water (10 µL), triglyceride standard (10 µL), and serum sample (10 µL) were added accordingly. After incubation at 37°C for 10 minutes, absorbance was measured at 500–550 nm against the

blank. Triglyceride concentration was calculated using the absorbance ratio method.

Estimation of High-Density Lipoprotein Cholesterol (HDL-C)

High-density lipoprotein cholesterol (HDL-C) was determined using a precipitation method (Grove, 1979). Two hundred microliters (200 µL) of serum were mixed with 200 µL of HDL precipitation reagent (phosphotungstic acid/$MgCl_2$), allowed to stand at room temperature for 10 minutes, and centrifuged at 3,000 rpm for 10 minutes. The clear supernatant was collected for cholesterol estimation. Cholesterol reagent (1.0 mL) was added to tubes labelled Blank**,** Standard**,** and Sample**,** followed by distilled water, cholesterol standard, and supernatant (10 µL each), respectively. After incubation at 37°C for 10 minutes, absorbance was measured at 500–520 nm, and HDL-C concentration was calculated using the standard formula.

Estimation of Low-Density Lipoprotein Cholesterol (LDL-C)

Fasting blood samples were collected, and serum was separated by centrifugation. Serum total cholesterol, triglycerides, and HDL-cholesterol were determined using standard enzymatic colorimetric methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using the **Friedewald equation**:

$$\text{LDL-C (mg/dL)} = \text{Total Cholesterol} - \text{HDL-C} - \left(\frac{\text{Triglycerides}}{5}\right)$$ (Fredrickson et al., 1972).

Experimental Animals and Diets

Experimental Animals and Housing Conditions

Thirty-five (35) albino Wistar rats were used for this study. The animals were housed under controlled laboratory conditions, maintained at a temperature of 21 ± 1°C, relative humidity of approximately 55**%,** and a 12-hour light/dark cycle. The rats were provided with free access to standard grower's feed and water throughout the experimental period. All experimental procedures were conducted at a fixed time each day (10:00 a.m.) to minimize circadian variability and were carried out in accordance with the Guidelines on Ethical Standards for the Use of Laboratory Animals.

Formulation of Hypercholesterolemic Diet

A formulated hypercholesterolemic diet (FHD) was prepared to induce hypercholesterolemia in the experimental animals. The diet consisted of standard grower's feed supplemented with 20% sucrose**,** 2.5% cholesterol**,** and 0.5% sodium cholate, thoroughly mixed to ensure homogeneity. The formulation was based on established protocols for dietary induction of hypercholesterolemia. Sex-related differences in lipid metabolism were considered in accordance with previous reports (Morselli et al., 2016).

Study Design and Treatment Regimen

The animals were randomly assigned into **seven (7) experimental groups** (n = 5 rats per group) as follows:

1. **ND (Normal Diet):** Rats fed standard grower's feed only.
2. **ND + *C. papaya* Extract (62 mg/kg):** Rats fed standard grower's feed and administered *C. papaya* leaf extract.
3. **FHD (Formulated Hypercholesterolemic Diet):** Rats fed the hypercholesterolemic diet only.
4. **FHD + *C. papaya* Extract (31 mg/kg):** Rats fed FHD and treated with low-dose *C. papaya* extract.
5. **FHD + *C. papaya* Extract (62 mg/kg):** Rats fed FHD and treated with medium-dose *C. papaya* extract.
6. **FHD + *C. papaya* Extract (124 mg/kg):** Rats fed FHD and treated with high-dose *C. papaya* extract.
7. **FHD + Atorvastatin:** Rats fed FHD and treated with atorvastatin (**20 mg/rat/day**).

The *C. papaya* leaf extract was administered **orally once daily**. The selected doses (31, 62, and 124 mg/kg/day) were based on previously reported effective doses of *C. papaya* leaf extracts (Juárez-Rojop et al., 2012). All animals had unrestricted access to feed and water for a treatment duration of **28 days**.

At the end of the experimental period, the animals were fasted for **12 hours**, after which blood samples were collected and centrifuged for biochemical analyses.

RESULTS

Qualitative Phytochemical Composition of *Carica papaya* Leaf Extract

Qualitative phytochemical screening of the methanol leaf extract of *Carica papaya* revealed the presence of multiple bioactive constituents (Table 1). Alkaloids, flavonoids, saponins, glycosides, steroids, tannins, cardiac glycosides, quercetin, kaempferol, caffeic acid, and papain were detected, indicating a chemically diverse extract dominated by polyphenolic and saponin compounds.

Quantitative Phytochemical Composition

Quantitative analysis demonstrated varying concentrations of phytochemical constituents in the methanol extract (Table 2). Flavonoids were present at the highest percentage (23.68 ± 0.37%), followed by saponins (15.04 ± 0.03%) and alkaloids (10.34 ± 0.02%). Among quantified phenolic compounds, tannins showed the highest concentration (42.97 ± 1.31 mg/dL), while caffeic acid (21.8 ± 1.42 mg/g) and papain (16.8 ± 0.84 mg/g) were also abundant. Quercetin and kaempferol were present at lower concentrations (Table 2).

TABLE 1: Phytochemical (qualitative) constituents of methanol extract of *Carica papaya* leaf.

Phytochemicals	Qualitative Analysis of the Sample
Alkaloids	+
Cardiac glycosides	+
Flavonoid	+
Glycosides	+
Saponins	+
Saponin glycosides	+
Steroids	+
Taninins	+
Quercetins	+
Kaempferols	+
Caffeic acids	+
Papains	+

Presence: +

TABLE 2: Phytochemical (quantitative) constituents of methanol extract of *Carica papaya* leaf.

Phytochemicals	Concentration
Alkaloids %	10.34±0.020
Flavonoids %	23.68±0.37
Glycosides %	0.46±0.010
Saponins %	15.04±0.031
Tannins mg/dl	42.97±1.31
Steroids mg/dl	0.59±0.003
Quercetin mg/g	1.94±0.020
Kaempferol mg/g	0.90±0.030
Caffeic acid mg/g	21.8±1.42
Papains mg/g	16.8±0.84

Values are mean ± standard deviation (n=3).

Effect of *Carica papaya* Leaf Extract on Serum Lipid Profile

Administration of the formulated hypercholesterolemic diet (FHD) resulted in marked alterations in serum lipid parameters (Table 3). Rats fed FHD showed a significant increase in total cholesterol (225.50 ± 9.93 mg/dL) and LDL-cholesterol (104.75 ± 6.42 mg/dL) compared with the normal diet (ND) group. Triglyceride levels were also elevated in the FHD group (118.50 ± 2.15 mg/dL), while HDL-cholesterol showed no significant reduction.

Treatment with *C. papaya* leaf extract at doses of 31, 62, and 124 mg/kg produced dose-related reductions in total cholesterol, triglycerides, and LDL-cholesterol compared with the untreated FHD group (Table 3). The 62 mg/kg dose resulted in the lowest total cholesterol (121.25 ± 6.24 mg/dL) and LDL-cholesterol (66.50 ± 11.90 mg/dL) among extract-treated groups. HDL-cholesterol levels were moderately increased in all treated groups relative to the FHD group. Lipid-lowering effects observed in the extract-treated groups were comparable to those seen in the atorvastatin-treated group.

Assay Sensitivity and Precision of Lipid Profile Measurements

The lipid profile assays demonstrated acceptable analytical sensitivity, with limits of detection (LOD) and quantification (LOQ) of 0.27 and 0.83 concentration units, respectively. The coefficient of variation (CV) was 4.59%, indicating satisfactory assay repeatability and reproducibility. Calibration curves showed adequate linearity ($R^2 = 0.7375$) (Figures 1 and 2).

Table 3: Lipid profile of Rats administered Cow brain tissue and *Carica* papaya Leaf.

Parameter	ND	ND+62	FHD	FHD+31	FHD+62	FHD+124	FHD+ATOR
T.chol (mg/dl)	131.75±9.02^{ab}	112.75±8.20^{a}	225.50±9.93^{c}	143.50±3.13^{b}	121.25±6.24^{e}	168.25±8.42^{d}	142.75±1.26^{b}
TG (mg/dl)	103.25±1.99^{a}	103.25±4.99^{a}	118.50±2.15^{c}	112.25±1.14^{ab}	95±0.13^{b}	111.25±2.92^{ab}	99±2.19^{d}
HDL (mg/dl)	47.50±5.80^{a}	45.00±7.75^{a}	48.75±3.30^{a}	51.25±3.40^{c}	49.50±6.95^{c}	52.00±6.05^{c}	50.75±7.45^{c}
LDL (mg/dl)	66.5±5.47^{a}	65±4.32^{a}	104.75±6.42^{c}	71.25±6.07^{b}	66.50±11.90^{a}	74.75±9.0^{b}	62.5±6.61^{a}

Values are means ± standard deviation (n=3). Data in the same row having different superscript are significantly different (P<0.05) using SPSS version 20, (Analysis of Variance). **KEY:** T.chol= Total cholesterol; TG= Triglycerides; HDL= High density lipoprotein; LDL= Low density lipoprotein; ND= Normal diet; FHD= Formulated high cholesterol diet; ATOR= Atorvastatin.

Effect of *Carica papaya* Leaf Extract on Liver Function Parameters

Rats fed the formulated hypercholesterolemic diet exhibited significant elevations in liver enzymes compared with the ND group (Table 4). AST, ALT, and ALP levels increased markedly in the FHD group, indicating hepatic dysfunction. Total bilirubin and direct bilirubin were also

elevated, while alterations in serum protein and albumin levels were observed.

Table 4: Liver function of Rats administered Cow brain tissue and *Carica papaya* Leaf

Parameter	ND	FHD	ND+62	FHD+31	FHD+62	FHD+124	FHD+Ator
AST (u/l)	12.50±3.11^{ab}	36.25±8.26^{c}	9.75±1.71^{a}	12.00±2.16^{ab}	9.5±1.29^{a}	11.00±1.83^{b}	11.00±2.58^{b}
ALT (u/l)	12.00±3.65^{ab}	39.75±5.91^{c}	10.00±2.94^{a}	10.25±2.63^{a}	7.75±1.0^{b}	10.00±1.41^{a}	8.75±1.71^{b}
ALP (u/l)	101.25±3.40^{a}	220.25±14.57^{c}	95.5±5.26^{d}	113.00±18.51^{b}	92.75±8.77^{ab}	99.50±16.42^{e}	92.25±12.61^{ab}
T. serum/plasma protein (g/l)	65.75±4.04^{a}	78.25±3.30^{c}	62.25±6.65^{ab}	68.50±3.87^{b}	63.25±4.03^{ab}	65.50±3.41^{a}	67.50±4.51^{b}
Alb (g/l)	44.75±7.18^{ab}	44.25±4.50^{ab}	39.25±2.50^{a}	38.50±3.11^{a}	36.25±2.63^{b}	38.00±3.56^{a}	36.25±2.63^{b}
T.Bil (mg/dl)	0.85±0.13^{b}	1.2±0.18^{c}	0.73±0.13^{ab}	0.93±0.13^{a}	0.75±0.17^{ab}	0.93±0.17^{a}	0.78±0.13^{d}
D.Bil (mg/dl)	0.20±0.12^{a}	0.70±0.16^{c}	0.18±0.96^{b}	O.33±0.13^{d}	0.23±0.13^{c}	0.30±0.14^{ab}	0.30±0.14^{ab}

Values are mean ± standard deviation (n=3). Data in the same row having different superscript are significantly different (P<0.05) using SPSS version 20, (Analysis of Variance). **KEY:** ALT= Alanine amino transferase; ALP= Alanine phosphatase; AST= Aspartate amino transfererase; ND= Normal diet; FHD= Formulated high cholesterol diet; ATOR= Atorvastatin.

Treatment with *C. papaya* leaf extract significantly reduced AST, ALT, and ALP activities across all treated groups relative to the FHD group (Table 4). The reductions were most pronounced at the 62 mg/kg dose, which showed enzyme values comparable to the atorvastatin-treated group. Bilirubin levels were similarly reduced following extract administration, while serum protein and albumin values approached those observed in the ND group.

Analytical Performance of Liver Function Assays

Liver function assays demonstrated acceptable analytical performance, with LOD and LOQ values of 19.87 and 60.24 concentration units, respectively. The coefficient of variation was 1.72%, indicating high assay precision. Calibration curves exhibited satisfactory linearity (R^2 = 0.6941) (Figure 3).

DISCUSSION

This study investigated the hypolipidemic and hepatoprotective effects of methanol extract of *Carica papaya* leaves in rats with diet-induced hyperlipidemia. Administration of the formulated hypercholesterolemic diet successfully induced dyslipidemia, as evidenced by significant elevations in total cholesterol, triglycerides, and LDL-cholesterol, alongside increased liver enzyme activities. These findings are consistent with previous reports that high-fat or cholesterol-enriched diets disrupt lipid metabolism and promote hepatic injury (Arvanitis & Lowenstein, 2023; Du & Qin, 2023).

Treatment with *C. papaya* leaf extract resulted in significant improvements in lipid profile parameters, particularly reductions in total cholesterol and LDL-cholesterol, with a moderate increase in HDL-cholesterol. The lipid-lowering effect was most pronounced at the 62 mg/kg dose and was comparable to that observed with atorvastatin treatment. These results align with earlier reports demonstrating the lipid-modulating properties of plant-derived bioactive compounds (Cicero et al., 2021).

The hypolipidemic effects observed may be attributed to the phytochemical composition of the extract. Quantitative analysis revealed high concentrations of flavonoids, saponins, tannins, and phenolic acids. Flavonoids and phenolic compounds are known to inhibit cholesterol biosynthesis, enhance bile acid excretion, and improve antioxidant capacity, thereby reducing circulating lipid levels. Saponins may further reduce intestinal cholesterol absorption through the formation of insoluble complexes with dietary cholesterol, contributing to decreased serum lipid concentrations.

Elevations in AST, ALT, and ALP observed in hypercholesterolemic rats indicate hepatic injury and compromised membrane integrity, consistent with lipid accumulation in hepatocytes (El-Eshmawy, 2023; Kathak et al., 2022). Administration of *C. papaya* leaf extract significantly normalized these enzyme levels, suggesting hepatoprotective activity. The reduction in bilirubin levels and normalization of serum protein and albumin further support improved hepatic function following extract treatment. These effects may result from stabilization of hepatocyte membranes and attenuation of oxidative stress mediated by the extract's polyphenolic constituents.

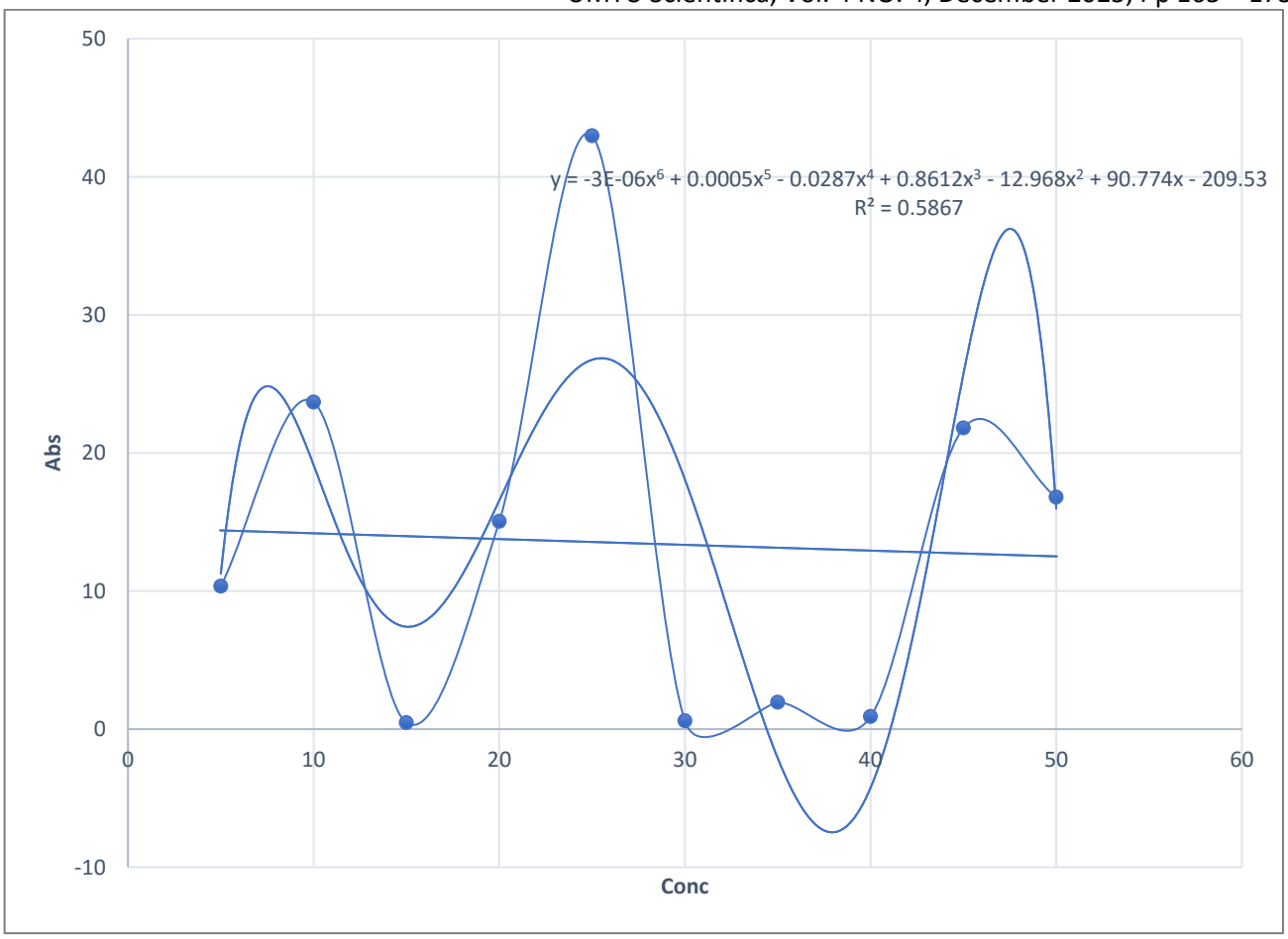

Figure 1: Calibration Curve for Quantitative Analysis

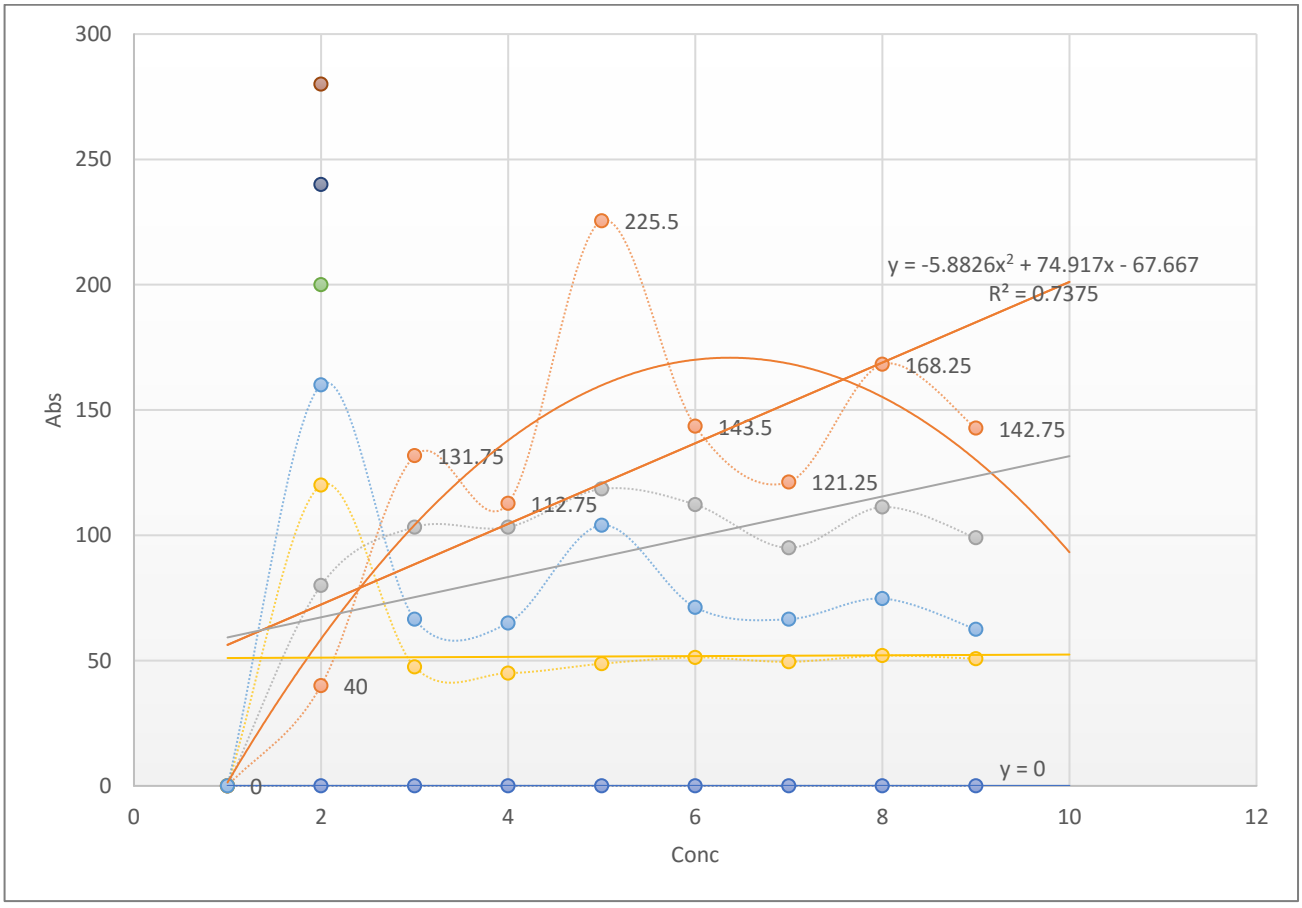

Fig 2: Calibration Curve of Lipid Profile Assay
Series 1=LDL, 2=T.Chol 3=TG and 4=HDL

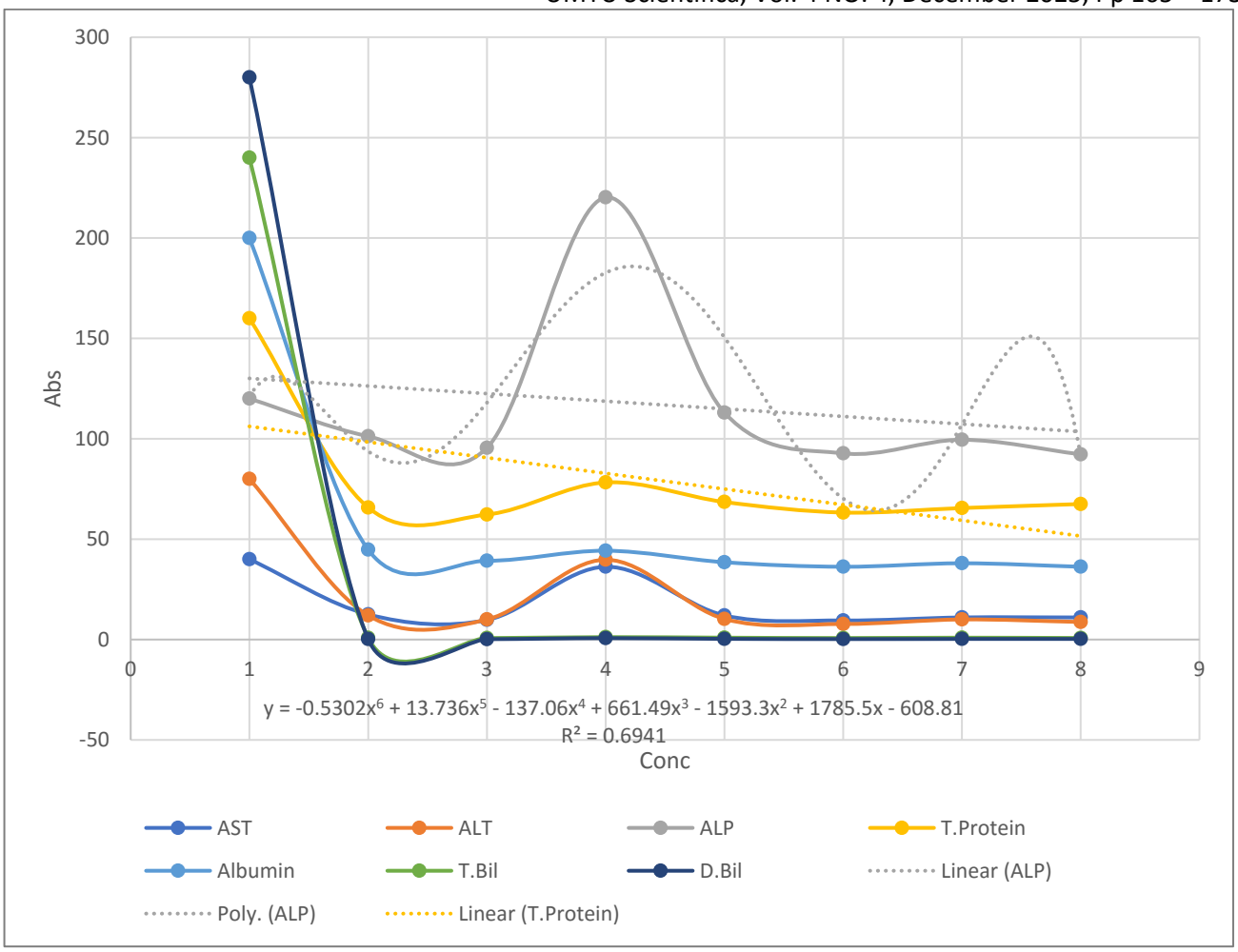

Figure 3: Calibration curve of liver function test.

Although the extract demonstrated effects comparable to atorvastatin, the mechanism of action remains indirect. Unlike statins, which directly inhibit HMG-CoA reductase, the observed effects of *C. papaya* extract are likely mediated through combined antioxidant, lipid absorption–modulating, and metabolic regulatory pathways. The presence of multiple bioactive compounds suggests a synergistic mechanism rather than a single-target effect.

Several limitations of this study should be acknowledged. The study relied on biochemical markers without histopathological confirmation of hepatic protection. Additionally, molecular assays to directly assess lipid metabolism pathways or HMG-CoA reductase activity were not performed. The sample size was relatively small, and the study duration was limited to 28 days, which may not fully capture long-term effects or toxicity.

Overall, the findings support the hypolipidemic and hepatoprotective potential of methanol extract of *Carica papaya* leaves, particularly at moderate doses. However, further mechanistic studies and controlled clinical investigations are necessary to validate these effects and establish translational relevance.

CONCLUSION

The findings of this study demonstrate that methanol extract of Carica papaya leaves possesses significant hypolipidemic and hepatoprotective effects in high-fat-diet induced hyperlipidemic rats. The extract effectively reduced serum total cholesterol, triglycerides, and low density lipoproteins levels while elevating HDL concentration. Additonally improved hepatic injury by normalizing liver enzyme activities. These effects are likely due to the actions of the phytochemicals, the extract showed dose-dependent efficay comparable to atorvastain, highlighting its potential as a natural therapeutic agent for reducing hyperlipidemia and associated liver dysfunction. Clinical studies are recommended to validate the therapeutic applicability in humans, particularly for managing hyperlipidemia and liver disorders.

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