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Effect of *Lactuca sativa* supplemented diet on Poloxamer 407 induced hyperlipidemic albino rats (*Rattus norvegicus*)

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Abstract. *Ayo VI, Adondua MA, Morayo AE, Ekele JU, Amilo D, Ochuele DA, Ayantse LM, Barrah C, Abdulsalam IO, Eya SB, Iheanacho CC, Tibile ST, Mohammed RI, Barde CE. 2023. Effect of* Lactuca sativa *supplemented diet on Poloxamer 407 induced hyperlipidemic albino rats (*Rattus norvegicus*). Asian J Nat Prod Biochem 21: 67-78.* Cardiovascular diseases (CVDs) have primarily contributed to the global disease burden. They represent the leading cause of mortality and healthcare expenditures in developed and third-world nations, responsible for approximately 30% of global deaths and 10% of global diseases annually. This study investigated the effects of a *Lactuca sativa* L. (lettuce) supplemented diet on Poloxamer 407-induced hyperlipidemic albino rats. Twenty-four (24) rats were grouped into six groups of treatments, i.e., four rats in each treatment. Treatments applied in this study were: control treatment (feed and water only), a P-407 induced without Atorvastatin, a P-407 induced treated with Atorvastatin, and P-407 induced with 10%, 30%, and 50% *L. sativa* supplemented diet. P407 was administered intraperitoneally at 1000 mg/kg body weight. Body weight was measured every three days for 14 days. Blood sample collection was carried out for the analysis of lipid profiles (High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), Very Low-Density Lipoprotein (VLDL), Triacylglycerides (TAG), and Total Cholesterol (TC)) and liver function parameters (ALP, ALT, AST, GGT, TP, ALB and GLB). The liver and brain tissues were analyzed for lipid peroxidation levels. Results showed that induction of P407 resulted in a higher body weight gain ($p<0.05$) compared to other treatments. The treatment groups other than the P-407 treatment showed a significant decrease in Total Cholesterol (TC), triacylglycerides (TAG), and LDL cholesterol levels and a significant increase in HDL cholesterol. The TC, TAG, LDL-C, and HDL-C levels in treating P-407 with a 50% *L. sativa*-supplemented diet did not differ ($P>0.05$) compared to the control treatment. The atherogenic risk prediction indices indicated a decreased risk in the treated groups with Atorvastatin or *L. sativa*-supplemented diet. Furthermore, liver function parameters were better in the treatment groups with Atorvastatin or *L. sativa*-supplemented diet, including decreased liver function parameters and increased total protein, albumin, and globulin levels. The *L. sativa*-supplemented diet also exhibited anti-lipid peroxidation activity, as indicated by reduced malondialdehyde (MDA) levels. In conclusion, the *L. sativa*-supplemented diet had hypolipidemic effects, anti-lipid peroxidation activity, and hepatoprotective effects, suggesting its potential as an antihyperlipidemic agent.

Keywords: Albino rats, hyperlipidemia, *Lactuca sativa*, Poloxamer 407, supplemented diet

INTRODUCTION

Cardiovascular diseases (CVDs) have emerged as the primary contributor to the global disease burden (Shukr et al. 2019). CVDs are the leading cause of mortality and increasing healthcare expenditures in developed and third-world nations, responsible for approximately 30% of global deaths and 10% of global diseases annually (Bhatnagar et al. 2008). It is projected over 24 million people will be affected by cardiovascular ailments by 2030 (Wang et al. 2015). Hyperlipidemia, characterized by elevated levels of plasma lipids such as Total Cholesterol (TC) and triglycerides (TAG), is a well-known determinant of cardiovascular disease (CVD) (Zhang et al. 2014; Ayo et al. 2023a). Hyperlipidemia is typically defined as increased cholesterol or triglyceride-carrying lipoproteins in the blood above a defined standard limit (Avogaro and Cazzolato 1975). Hyperlipidemia, or dyslipidemia, can lead

to severe complications associated with atherosclerosis, including cardiovascular disease, cerebrovascular disease, peripheral vascular disease, and strokes (Shattat 2015). The accumulation of lipids, particularly cholesterol, in the arterial walls causes narrowing and reduced blood flow, leading to atherosclerosis. The risk of morbidity and mortality increases if hyperlipidemia occurs with other prevalent conditions such as hypertension, diabetes mellitus, and renal disorders (Amanda et al. 2013). Various factors contribute to the development of hyperlipidemia, including genetic abnormalities, type II diabetes mellitus, obesity, thyroid dysfunction, alcoholism, hormonal therapy, renal diseases, jaundice, lipoprotein lipase mutations, and certain medications (Santamarina-Fojo et al. 2004).

Lactuca sativa L., commonly known as lettuce, is the *Asteraceae* plant family and native to the Mediterranean region. It is known globally as a vegetable crop, consumed as fresh in salads or occasionally cooked (Lebeda et al. 2007). It has a low-calorie, low-fat, and low-sodium, making it a popular choice for salads. It is also a good source of dietary fiber, folate, vitamin C, and essential minerals, such as iron (Kim et al. 2016). The nutritional composition of *L. sativa* includes moisture (93.4g), protein (2.1g), fat (0.3g), minerals (1.2g), fiber (0.5g), carbohydrates (2.5g), calcium (310mg), phosphorus (80mg), iron (66mg), vitamin A (1650 IU), thiamine (0.09mg), riboflavin (0.13mg), and vitamin C (10mg) (Gopalan and Balaraman 1966). The bioactive compounds in *L. sativa*, such as phenolic compounds, are attributed to their antioxidative activity and various pharmacological effects, such as cardio-protective, antihyperlipidemic, anti-cancer, and antidiabetic activities (Hedges and Lister 2005). Several epidemiological studies have provided evidence that the consumption of vegetables, particularly green leafy vegetables like *L. sativa* (lettuce), is linked to the treatment of cardiovascular diseases (CVDs) (Nicolle et al. 2004a). A study on rats by Nicolle et al. (2004b) showed that a diet containing 20% lettuce had a cardio-protective effect by improving cholesterol metabolism and enhancing plasma antioxidant capacity. The cholesterol-lowering effect of *L. sativa* can be attributed to its fiber content. Soluble fibers such as pectin in lettuce affect lipid metabolism and reduce dietary cholesterol absorption in animals and humans (Nishimura et al. 2000; Abu et al. 2023). The mechanisms underlying the inhibition of cholesterol absorption are the disruption of micelle formation and the slowing down of cholesterol transfer through the unstirred layer (Stedronsky 1994). Furthermore, polyphenols present in lettuce have been proven to effectively inhibit the oxidation of Low-Density Lipoprotein (LDL), a significant contributor to atherosclerosis and hyperlipidemia (Aviram et al. 2000; Yakubu et al. 2019; Ekele 2023).

The use of plants, including vegetables such as *L. sativa* (lettuce), to treat hyperlipidemia is crucial due to their abundance of bioactive and nutritional substances (Ejeh et al. 2022; Edogbanya et al. 2023). Research reports indicate that *L. sativa* used in this study demonstrated minimal or no adverse effects compared to synthetic drugs commonly used to treat hyperlipidemia. The *L. sativa* is readily available in Nigeria, which benefits cost and accessibility. Therefore, this study aimed to assess the antihyperlipidemic effect of an *L. sativa*-supplemented diet in Albino rats with hyperlipidemia induced by Poloxamer 407.

MATERIALS AND METHODS

Study area

The study was conducted at the Central Research Laboratory, Federal University Wukari, Taraba state, Nigeria, from October 2022 to March 2023.

Sample collection and preparation

The study utilized mature, fresh, healthy *L. sativa* from Jos, Plateau State, Nigeria. The leaves were carefully rinsed with tap water to ensure cleanliness from soil and dust particles. Subsequently, the leaves were air-dried under the sun for 6 days to reach a brittle state, followed by crushing the leaves into fine fragments using a mortar and pestle. The powdered leaves were labeled correctly and stored in dry containers until they were required for further use.

Experimental animals

The rats used in the study were procured from the Animal House located in the Department of the Central Research Laboratory, Federal University Wukari, Taraba State, Nigeria. Their weight ranged from 110g to 145g, over 3 weeks old. They were mixed-sexed (males and females). They were kept in cages with a 12-hour light-dark cycle, following animal standard laboratory protocols approved by the University's Faculty and Ethics Committee (Approval number: 1765BG). The rats were given free access to water and feed throughout the experiment. Feed was obtained from Wukari Central Market, Taraba State, and constituted wheat maize, sugar, and calcium carbonate. Feed was given ad-lib.

Treatment of animal

Twenty-four (24) Albino rats were grouped into six groups. Each group consisted of four animals. The rats were subjected to the following treatment: (i) Treatment 1: Water and feed only. (ii) Treatment 2: Induced by an intraperitoneal injection of P-407 without treatment. (iii) Treatment 3: Induced and treated with Atorvastatin. (iv) Treatment 4: Induced and treated with 90% feed with 10% *L. sativa*. (v) Treatment 5: Induced and treated with 30% feed with 70% *L. sativa*, (vi) Treatment 6: Induced and treated with 50% feed with 50% *L. sativa.*

Diet preparation

The powdered *L. sativa*, which had been air-dried and pulverized, was mixed with the Albino Rats' feed following the experimental treatments. Treatment group IV received a diet of 90% Albino Rat feed and 10% *L. sativa*. Treatment group V received a diet with 70% Albino Rats feed and 30% *L. sativa*, while treatment group VI received a diet comprising 50% Albino Rats feed and 50% *L. sativa*.

Induction of hyperlipidemia

Poloxamer 407 was utilized as the inducing agent to trigger the hyperlipidemic effect. The method outlined by Megalli et al. (2005) was employed to induce hyperlipidemia. Poloxamer 407 was administered intraperitoneally at 1,000 mg/kg BW.

Record of body weight

The body weight of the experimental animal was recorded before the experiment began. The rats' body weights were measured individually every three days throughout the experiment.

Collection of blood samples

After fourteen (14) days of treatment, the rats were subjected to a fasting period of twelve (12) hours following their last day. They were then anesthetized using chloroform. Sterilized syringes and needles were utilized to obtain whole blood samples from the heart via cardiac puncture. The blood samples were collected into lab bottles and immediately sealed with corks. The collected blood samples were centrifuged at 3,000 revolutions per minute for 10 minutes to obtain serum. The serum was used to analyze lipid profiles and liver function tests.

Lipid profile analysis

The serum samples were used for the analysis of several lipid parameters, including Very Low-Density Lipoprotein (VLDL), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein-Cholesterol (HDL-C), triacylglycerol (TAG), and Total Cholesterol (TC). These parameters were determined to assess the rats' lipid profile and evaluate the effects of treatment on their cholesterol and lipid levels.

Determination of total cholesterol

Total cholesterol in the serum was determined using the method described by Allain et al. (1974) using an Agappe reagent kit. It contains specific reagents and chemicals necessary for accurately measuring total cholesterol levels.

Determination of triglycerides

Triglycerides were analyzed using the enzymatic colorimetric method by Allain et al. (1974) and an Agappe reagent kit.

Determination of HDL-cholesterol

HDL-cholesterol was determined using the method described by Assmann (1979), which involves an Agappe reagent kit.

Estimation of LDL-cholesterol

LDL-cholesterol was determined by the Friedewald equation, as described by Friedewald et al. (1972). The equation is presented as follows:

LDL-C = TC – HDL-C – TG/5

Where: LDL-C: Low-Density Lipoprotein Cholesterol, TC: Total Cholesterol, HDL-C: High-Density Lipoprotein-Cholesterol, TAG: Triacylglycerol

Liver function test

The serum samples were also analyzed for various liver function parameters, including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT), Total protein (TP), and Albumin (ALB) using a UV/visible spectrophotometer.

Assessment of Aspartate Aminotransferase (AST) activity

AST activity in the serum was measured using the procedures described by Reitman and Frankel (1957). A Randox reagent kit was used for this analysis.

Assessment of Alanine Aminotransferase (ALT) activity

ALT activity in the serum was investigated using the method described by Reitman and Frankel (1957) using a Randox reagent kit.

Assessment of Alkaline Phosphatase (ALP) activity

The Alkaline Phosphatase (ALP) serum activity was assessed using the Agappe reagent kit, following the procedure by Schlebusch et al. (1974).

Assessment of Gamma-Glutamyl Transferase (GGT)

The method by Szasz (1976), utilizing the Agappe reagent kit, was employed to determine the serum activity of Gamma-Glutamyl Transferase (GGT).

Determination of Total Protein (TP)

The Randox reagent kit was utilized to determine the total protein concentration, following the method outlined by Weichselbaum (1946).

Determination of Albumin (ABL)

The Agappe kit was employed to determine the serum albumin concentration, following the method described by Doumas et al. (1971).

Estimation of Globulin

The estimation of globulin was performed using the following formula:

Globulin = Total protein – Albumin

Preparation of liver and brain homogenates

0.3 grams (0.3g) of liver and brain tissues were homogenized in 1.5mL of Tris HCL, resulting in 1.5% (w/v) homogenates to assess the extent of lipid peroxidation in the experimental animals. The homogenates were centrifuged for 10 minutes, and the supernatants were utilized to determine the activity of Thiobarbituric Acid Reactive Substance (TBARS).

Determination of Thiobarbituric Acid Reactive Substance (TBARS)

Estimating Thiobarbituric Acid Reactive Substances (TBARS) in the tissues was conducted using the method outlined by Fraga et al. (1988). Rat liver homogenates, adjusted to 10 mg protein/mL in 120 mM KCl, 50 mM

phosphate buffer, pH 7.4, were incubated with 1000, 100, and 10 µg dry weight/mL of plant extract at 37°C for 15 min. Following incubation, Sodium dodecyl sulfate (0.2 mL of 3% (w/v)) and 0.05 mL of BHT 4% in ethanol were added. After mixing, the mixture was added with 2 mL of 0.1N HCl, 0.3 mL of 10% (w/v) phosphotungstic acid, and 1 mL of 0.7% (w/v) 2-thiobarbituric acid and then heated for 60 min in boiling water, and TBARS were extracted using 5 mL n-butanol. After centrifuging at 10,000 rpm for 10 min, the fluorescence of the butanol layer was measured at 515 nm excitation and 555 nm emission using a Hitachi F-3010 fluorescence spectrophotometer. The values are expressed as the ratio of TBARS formed in the presence of plant extracts compared to control.

Statistical analysis

The obtained biochemical results were analyzed statistically using One-Way Analysis of Variance (ANOVA), followed by Duncan multiple comparisons using Statistical Package for Social Science (SPSS) version 21. Significance between means was determined at a p-value of less than 0.05 ($p<0.05$). The results for each treatment were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Impact of *Lactuca sativa* supplemented diet on weights of P407-induced hyperlipidemic rats

Table 1 and Figure 1 illustrate the results of the statistical analysis. The control treatment showed that the body weight from day 0 to 7 was not significantly different ($p>0.05$). However, the body weight in the control group from days 10 to 14 increased significantly ($p<0.05$) compared to days 0-7. The P407 treatment showed that the body weight was not significantly different ($p>0.05$) from day 0-1, but the body weight was increased constantly and significantly ($p<0.05$) from day 4. The body weight on days 10 and 12 did not differ; however, it differed significantly ($p<0.05$) compared to that of days 4, 7, and 14. Body weight on day 14 showed a significant difference ($p<0.05$) compared to days 0, 1, 4, 7, 10, and 12.

In the P407+STD treatment, the body weight on day 1 differed significantly ($p<0.05$) compared to days 0, 4, 7, 10, 12, and 14. In the treatment group of P407 with a 10% *L. sativa* supplemented diet, the body weight on days 1, 4, 7, 10, 12, and 14 differed significantly ($p<0.05$) compared to day 0. Day 1 showed a significant difference ($p<0.05$) compared to days 0, 4, 7, 10, 12, and 14. Body weight on day 4 differed significantly ($p<0.05$) compared to days 0, 1, 7, 10, 12, and 14. The body weight on days 12 and 14 was significantly higher ($p<0.05$) than on days 0, 1, 4, 7, and 10.

The treatment group of P407 with a 30% *L. sativa*-supplemented diet showed that the body weight from day 0 to 14 was not different ($p>0.05$). The treatment group of P407 with a 50% *L. sativa*-supplemented diet showed that the body weight increased significantly from day 1 ($p<0.05$). The body weight was stable until day 12 and increased significantly on day 14 ($p<0.05$).

Impact of *Lactuca sativa* supplemented diet on lipid profile of P407 induced hyperlipidemic rats

The administration of P407 increased total cholesterol levels compared to the control treatment. The P407 treatment had a significant increase ($p<0.05$) in cholesterol levels compared to the P407 with STD, P407 with 10% *L. sativa* supplemented diet, P407 with 30% *L. sativa* set, and P407 with 50% *L. sativa* treatments. However, the cholesterol levels of P407 with STD, P407 with 10% *L. sativa* set, and P407 with 30% *L. sativa* treatments did not differ ($p>0.05$). Still, it is significantly lower ($p<0.05$) than the P407 treatment and higher than the control treatment. On the other hand, the cholesterol level of P407 with a 50% *L. sativa*-supplemented diet showed no significant difference ($p>0.05$) compared to the control treatment. However, it is significantly lower ($p<0.05$) than the P407, P407 with STD, P407 with 10% *L. sativa* supplemented diet, and P407 with 30% *L. sativa* supplemented diet (Table 2 and Figure 2).

The administration of P407 increased triglyceride levels significantly ($p<0.05$) compared to the control treatment. The triglyceride level in the P407 treatment was significantly higher ($p<0.05$) than the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa* and P407 with 50% *L. sativa*. The P407+STD treatment showed a significant difference ($p<0.05$) in triglyceride levels compared to the control treatment, P407, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*

The triglyceride levels in the P407 with 10% *L. sativa* and P407 with 30% *L. sativa* did not differ. Still, they significantly differed ($p<0.05$) from the control treatment, P407 with STD and P407 with 50% *L. sativa*. The triglyceride level in P407 with a 50% *L. sativa*-supplemented diet did not differ ($p>0.05$) from the control. However, it differed significantly ($p <0.05$) compared to the P407, P407 with STD, P407 with 10% *L. sativa*, and P407 with feed with 30% *L. sativa*. Additionally, the induction of P407 resulted in a significant difference ($p<0.05$) in HDL-C levels compared to the control.

The HDL-C level in the P407 treatment was significantly reduced ($p<0.05$) compared to the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The HDL-C level in the P407 with 10% *L. sativa* significantly differed ($p<0.05$) compared to the P407 treatment. It significantly differed ($p<0.05$) compared to the P407 with STD, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The HDL-C levels of P407 with STD treatment, P407 with 30% *L. sativa*, P407 with 50% *L. sativa*, and control treatment did not differ ($p>0.05$). However, the HDL-C of the P407 treatment significantly differed from that of P407 with a 10% *L. sativa*-supplemented diet.

The induction of P407 increased significantly ($p<0.05$) in LDL-C level compared to the control treatment, P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The LDL-C levels in the treatments of P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa* were higher ($p<0.05$) compared to the control, P407 with 50% *L. sativa*. P 407

with 50% *L. sativa* showed no significant difference (P>0.05) in HDL-C level compared to the control. Still, it had a significant difference (p<0.05) in HDL-C level compared to P 407, P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa*.

The induction of P407 increased significantly (p<0.05) in the VLDL level compared to the control treatment. The VLDL level in the P407 treatment was significantly higher (p<0.05) than the P407 with STD, P407 with 10% *L. sativa*, P407 with Feed with 30% *L. sativa*, and P407 with 50% *L. sativa*, P407 with STD, P407 with 10% *L. sativa*, P407 with Feed with 30% *L. sativa* and P407 with 50% *L. sativa* showed no significant difference (P>0.05) in VLDL level compared to the control.

Table 1. The effect of diet supplemented with *Lactuca sativa* on the weights of hyperlipidemic rats induced by P407

Treatment	Day 0 (g)	Day 1 (g)	Day 4 (g)	Day 7 (g)	Day 10 (g)	Day 12 (g)	Day 14 (g)
Control	$119.7^{a}\pm2.51$	$124.7^{a}\pm4.51$	$130.3^{a}\pm7.02$	$134.0^{a}\pm2.64$	$140.7^{b}\pm2.89$	$143.0^{b}\pm3.00$	$149.0^{b}\pm1.00$
P 407	$124.3^{a}\pm5.69$	$127.7^{a}\pm3.06$	$150.0^{b}\pm5.57$	$167.0^{bc}\pm7.94$	$172.7^{c}\pm8.50$	$179.0^{c}\pm8.89$	$214.0^{d}\pm9.64$
P407 with STD	$136.7^{a}\pm5.51$	$148.3^{ab}\pm9.29$	$159.7^{b}\pm8.02$	$158.7^{b}\pm13.20$	$160.7^{b}\pm2.31$	$158.7^{b}\pm1.53$	$158.0^{b}\pm2.65$
P407 with 10% *Lactuca sativa*	$119.7^{a}\pm8.96$	$129.3^{ab}\pm8.08$	$138.0^{b}\pm7.55$	$149.7^{bc}\pm4.73$	$146.0^{bc}\pm1.73$	$156.7^{c}\pm4.62$	$156.3^{c}\pm7.51$
P407 with 30% *Lactuca sativa*	$115.3^{a}\pm8.50$	$124.3^{a}\pm3.06$	$126.3^{a}\pm3.21$	$131.7^{a}\pm7.23$	$129.7^{a}\pm9.02$	$128.7^{a}\pm12.50$	$127.3^{a}\pm11.37$
P407 with 50% *Lactuca sativa*	$107.7^{a}\pm7.09$	$121.0^{ab}\pm1.0$	$123.3^{bc}\pm4.16$	$131.0^{bc}\pm7.55$	$134.7^{bc}\pm9.66$	$136.3^{bc}\pm17.04$	$139.0^{c}\pm23.58$

Note: Each value represents mean ± SD, n=3; mean values with different superscripts are significantly different (P<0.05) across the row

Table 2. The effect of *Lactuca sativa* supplemented diet on the lipid profile of P407-induced hyperlipidemic rats

Treatment	TC (mg/dL)	TAG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Control	$122.17^{a}\pm1.96$	$113.23^{a}\pm2.60$	$45.00^{a}\pm0.90$	$54.62^{a}\pm0.97$	$22.65^{a}\pm0.52$
P 407	$231.57^{b}\pm6.64$	$210.73^{b}\pm2.02$	$13.78^{b}\pm2.78$	$157.64^{b}\pm3.98$	$42.15^{b}\pm0.40$
P407 with STD	$151.77^{c}\pm0.83$	$127.43^{ac}\pm4.50$	$43.34^{a}\pm2.61$	$82.70^{c}\pm3.89$	$25.49^{a}\pm0.90$
P 407 with 10% *Lactuca sativa*	$154.97^{c}\pm1.33$	$144.00^{c}\pm14.43$	$43.23^{ac}\pm1.91$	$82.77^{c}\pm2.01$	$28.77^{a}\pm2.92$
P 407 with 30% *Lactuca sativa*	$152.83^{c}\pm0.21$	$133.13^{c}\pm0.40$	$41.95^{a}\pm3.21$	$82.59^{c}\pm0.56$	$26.63^{a}\pm0.08$
P 407 with 50% *Lactuca sativa*	$132.97^{a}\pm0.96$	$110.03^{a}\pm2.57$	$44.74^{a}\pm0.3$	$66.22^{a}\pm0.90$	$22.01^{a}\pm0.51$

Note: Each value represents mean ± SD, n=3; mean values with different superscripts are significantly different (P<0.05) in the same column. LDL-C: Low-Density Lipoprotein Cholesterol; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein-Cholesterol; TAG: Triacylglycerol; VLDL-C: Very Low-Density Lipoprotein- Cholesterol

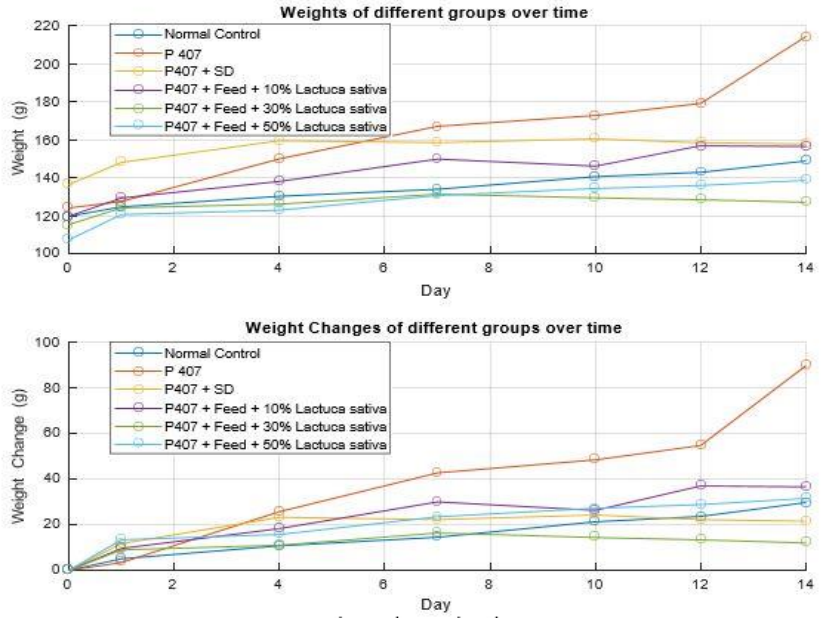

Figure 1. The effect of treatment on the body weight of rats over time

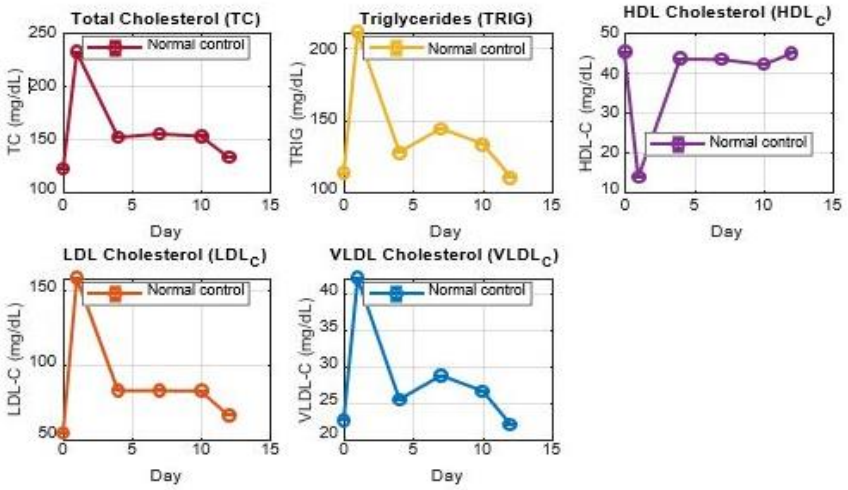

Figure 2. Lipid profile of P407-induced hyperlipidemic rats with *Lactuca sativa*-supplemented diet

The effect of *Lactuca sativa* supplemented diet on total cholesterol/HDL and LDL/HDL ratios in P407-induced hyperlipidemic albino Rats

Table 3 and Figure 3 show that the induction of P407 increases significantly ($p<0.05$) in the total cholesterol/HDL ratio compared to the control. The total cholesterol/HDL ratio in P407 treatment was also higher ($p<0.05$) than the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The total cholesterol/HDL ratio in the P407 with STD, P407 with10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa* set were significantly lower ($p<0.05$) than P407 treatment, and no significant difference ($P>0.05$) compared to control. The induction of P407 increased ($p<0.05$) the LDL/HDL ratio in the P407 treatment compared to the control. The LDL/HDL ratio in the P407 treatment also significantly differs ($p<0.05$) compared to the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa* set, and Feed with 50% *L. sativa* set.

P407 with STD set, P407 with Feed with 10% *L. sativa* set, P407 with Feed with 30% *L. sativa* set showed a significant difference ($p<0.05$) in LDL/HDL ratio compared to the control treatment, and P407 with 50% *L. sativa* supplemented diet. The treatment of P407 with a 50% *L. sativa* supplemented diet set showed no significant difference ($P>0.05$) in LDL/HDL ratio in comparison to the control treatment but showed a significant difference ($p<0.05$) in LDL/HDL ratio compared to P407 set, P407 with STD set, P407 with Feed with 10% *L. sativa* set, and P407 with Feed with 30% *L. sativa* set.

The effect of *Lactuca sativa* supplemented diet on parameters of liver function in hyperlipidemic rats induced by P407

Table 4 and Figure 4 show the analysis results of parameters for liver function: AST, ALP, ALT, GGT, TP, ALB, and GLB after treatment with a *L. sativa*-supplemented diet. The induction of P407 causes a significant increase in AST level in the P407 treatment compared to the control. The treatment of P407 caused an increase significantly ($p<0.05$) in ASP level compared to the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*, P407 with STD and P407 with 10% *L. sativa* had significantly higher ($p<0.05$) ASP levels compared to the control treatment, P407 set, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The AST level of the treatments of P407 with a 30% *L. sativa* supplemented diet and 50% *L. sativa* supplemented diet did not differ compared to the control but showed a significant difference ($p<0.05$) compared to the P407 P407 with STD and P407 with 10% *L. sativa*.

The ALP level of the P407 treatment was significantly higher ($p<0.05$) than the other treatments. The treatment of P407 had a higher ALT level ($p<0.05$) than the control. The ALT level of the P407 treatment significantly differed ($p<0.05$) from the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa* set. The ALT level of the treatments P407 with STD and P407 with 10% *L. sativa* diet showed a significant difference ($p<0.05$) compared to the control treatment, P407, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The ALT level in the treatment of P407 with a 30% *L. sativa* supplemented diet was significantly different ($p<0.05$) compared to the control and a significant difference ($p<0.05$) compared to P407, P407 with STD, P407 with 10% *L. sativa* and P407 with 50% *L. sativa*.

The ALT level in the treatment of P407 with a 50% *L. sativa*-supplemented diet shows no significant difference ($P>0.05$) compared to the control treatment. It showed a significant difference ($P>0.05$) compared to the P407, P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa*. The GGT level in the P407 treatment was significantly different ($p<0.05$) compared to the control treatment, the P407with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The GGT level in the P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa* did not differ

(P>0.05); however, their GGT levels significantly differ (p<0.05) compared to the control, P407 and P407 with 50% *L. sativa*. The treatment P407 with a 50% *L. sativa* supplemented diet showed no significant difference (p<0.05) in GGT level compared to the control. It showed a significant difference (p<0.05) in the GGT level compared to the P407, P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa* set.

The P407 treatment resulted in a lowered and significantly different (p<0.05) TP level compared to the control and P407 compared to the P407 with STD P407 with 10% *L. sativa*. P407 with 30% *L. sativa*, and P407 with Feed with 50% *L. sativa*. The TP level in the P407 with STD treatment was slightly lower (p<0.05) compared to the control. It also differed significantly (p<0.05) compared to the P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. Furthermore, the treatment of P407 with a 10% *L. sativa* supplemented diet differed significantly (p<0.05) on TP level compared to the control, P407, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The TP levels in the treatments P407 with 30% *L. sativa* and P407 with 50% *L. sativa* and control treatments showed no significant difference (P>0.05); however, their TP levels differed significantly (p<0.05) compared to P407, P407 with STD and P407 with 10% *L. sativa*.

The ALB level in the P407 treatment was lower (p<0.05) than the control. Also, it differed significantly (p<0.05) compared to P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa* set. The ALB levels in the treatment of the P407 with STD and P407 with a 30% *L. sativa*-supplemented diet did not differ (P>0.05). They differed significantly (p<0.05) compared to the control, P407, P407 with 10% *L. sativa*, and P407 with 50% *L. sativa*. The ALB level in the treatment of P407 with 10% *L. sativa* supplemented diet differed significantly (p<0.05) compared to the control, P407, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. P407 with feed with 50% *L. sativa* set showed no significant difference (P>0.05) on the ALP level compared to the control. It differed significantly (p<0.05) on the ALP level compared to the control, P407, P407 with 10% *L. sativa* and P407 with 30% *L. sativa*.

The GLB level in the P407 treatment differed significantly (p<0.05) compared to the control and P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The GLB levels in the treatments of the P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa* did not differ (P>0.05). However, they differed significantly (p<0.05) compared to the control, P407, and P407 with 50% *L. sativa*. The GLB levels in the treatments of P407 with 50% *L. sativa* and the control did not differ (P>0.05). However, they differed significantly (p<0.05) compared to the P407, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa*.

The effect of *Lactuca sativa* supplemented diet on lipid peroxidation in the liver and brain of P407-induced hyperlipidemic rats

The induction of P407 results in a significant increase (p<0.05) in lipid peroxidation in the liver compared to control. The lipid peroxidation in P407 treatment also differed significantly (p<0.05) compared to the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*. The lipid peroxidation in the treatments of the P407 with STD, P407 with 10% *L. sativa*, and P407 with 30% *L. sativa* did not differ (p<0.05), they differed significantly (p<0.05) compared to the control, P407 and P407 with 50% *L. sativa* as shown in Figure 5.

The induction of the P407 caused a significant increase (p<0.05) in lipid peroxidation in the brain compared to the control. Also, it differed significantly (p<0.05) compared to the P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa* (Figure 6). P407 with STD, P407 with 10% *L. sativa*, P407 with 30% *L. sativa*, and P407 with 50% *L. sativa*, and the control treatment had no significant difference (P>0.05) in lipid peroxidation. However, the lipid peroxidation of these treatment groups differed significantly (p<0.05) compared to the P407 treatment.

Table 3. The effect of *Lactuca sativa* supplemented diet on total cholesterol/HDL and LDL/HDL ratios in P407-induced hyperlipidemic rats

Treatment	TC/HDL-C ratio	LDL-C/HDL-C ratio
Normal control	2.71^{a}±1.96	1.21^{a}±0.97
P 407	16.80^{b}±6.64	11.44^{b}±3.98
P407 with STD	3.48^{a}±0.83	1.91^{c}±3.89
P 407with 10% *L. sativa*	3.58^{a}±1.33	1.91^{c}±2.01
P 407 with 30% *L. sativa*	3.64^{a}±0.21	1.97^{c}±0.56
P 407 with 50% *L. sativa*	2.97^{a}±0.96	1.48^{a}±0.90

Note: Each value represents mean ± SD, n=3; mean values with different superscripts are significantly different (P<0.05) in the same column. Atorvastatin is denoted as STD.

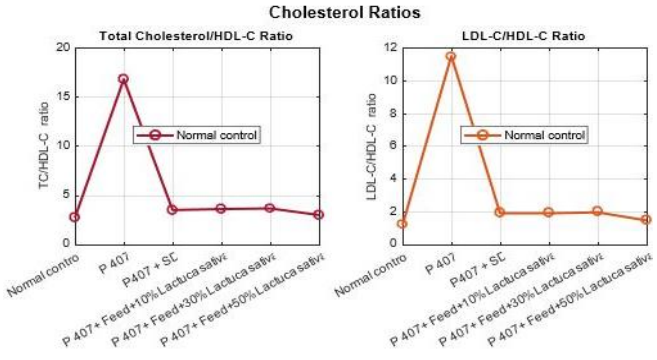

Figure 3. The effect of *Lactuca sativa* supplemented diet on total cholesterol/HDL and LDL/HDL ratios in P407-induced hyperlipidemic rats

Table 4. The effect of *Lactuca sativa* supplemented diet on the liver parameters of P407-induced hyperlipidemic rats

SETS	AST (U/L)	ALP(U/L)	ALT(U/L)	GGT (U/L)	TP(g/dL)	ALB(g/dL)	GLB (g/dL)
Normal control	21.85^{a}±2.07	41.37^{a}±1.56	9.33^{a}±4.62	22.30^{a}±3.77	5.62^{a}±0.02	2.04^{a}±0.02	2.58^{a}±0.04
P 407	52.70^{b}±2.88	79.83^{b}±5.15	19.67^{b}±2.31	38.49^{b}±1.77	2.72^{b}±0.01	0.68^{b}±0.41	1.06^{b}±0.06
P 407 with STD	30.77^{c}±3.37	38.78^{a}±1.34	13.33^{c}±2.31	30.94^{c}±0.48	4.79^{ac}±0.06	1.64^{ac}±0.03	2.13^{c}±0.08
P 407 with 10% *L. sativa*	30.37^{c}±3.00	32.23^{a}±1.02	13.67^{c}±2.3	29.56^{c}±0.94	4.66^{c}±0.05	1.43^{c}±0.21	2.23^{c}±0.20
P 407 with 30% *L. sativa*	23.93^{a}±3.81	32.56^{a}±0.80	11.00^{ac}±5.20	31.47^{c}±2.23	5.74^{a}±0.06	1.67^{ac}±0.08	2.05 ^{c}±0.11
P 407 with 50% *L. sativa*	22.06^{a}±4.22	32.90^{a}±1.84	6.67^{a}±2.89	26.34^{a}±1.06	5.67^{a}±0.11	1.75^{a}±0.02	2.93 ^{a}±0.13

Note: Each value represents mean ± SD, n=3; mean values with different superscripts are significantly different (P<0.05) in the same column. AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; GGT: Gamma-Glutamyl Transferase; TP: Total Protein; ALB: Albumin; GLB: Globulin

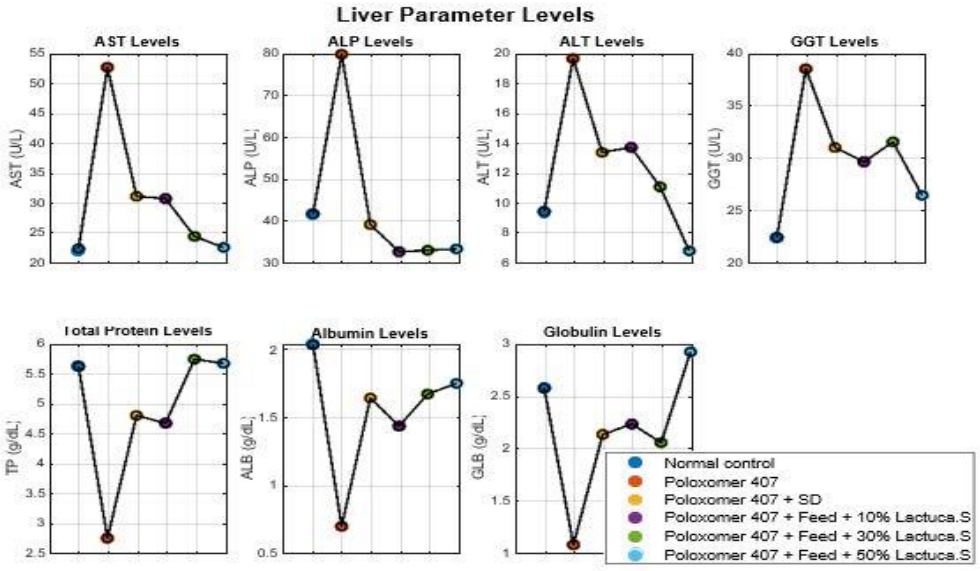

Figure 4. The effect of *Lactuca sativa* supplemented diet on the liver parameters of P407-induced hyperlipidemic rats**.** Atorvastatin is denoted as STD.

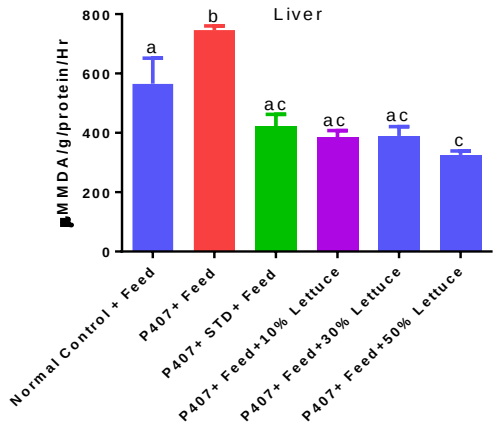

Figure 5. The effect of *Lactuca sativa* supplemented diet on lipid peroxidation in the liver of P407 induced hyperlipidemic rats. Each value represents mean ± SD, n=3, and mean values with different superscripts are significantly different (p<0.05)

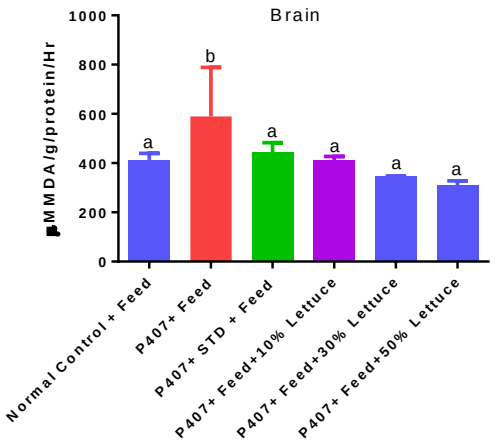

Figure 6. The effect of *Lactuca sativa* supplemented diet on lipid peroxidation in the brain of P407 induced hyperlipidemic rats. Each value represents mean ± SD, n=3, and mean values with different superscripts are significantly different (p<0.05). Atorvastatin is denoted as STD.

Discussion

Hyperlipidemia is a crucial risk factor for the emergence of Coronary Heart Diseases (CHDs), including ischemic heart disease, myocardial infarction (Ayo et al. 2023b), and stroke (Vaziri and Morris 2011). Consequently, it has become one of the greatest threats to public health (Ayo et al. 2023c). Cardiovascular risk assessment has helped reduce and manage different CVDs (Shukr et al. 2019). Plants are used to treat dyslipidemia in various ways worldwide because they have broad pharmacological effects with multiple mechanisms. The standard medicine for CVDs was Atorvastatin at 10mg/kg dose. The induction of Poloxamer 407 was carried out for the hyperlipidemia model in experimental animals. Poloxamer 407 is a non-ionic surfactant that is harmless to cellular membranes and has previously been used to cause hyperlipidemia successfully (Hyeung et al. 2006) by stimulating HMG-CoA and decreasing the activity of lipoprotein lipases. Poloxamer 407 was used in the hyperlipidemic model because of its ease of use, high repeatability, and lack of undesired underlying clinical conditions (Kim et al. 2008).

Induction of P407 increases body weight significantly ($p<0.05$), indicating the accumulation of fats in the body. However, the hyperlipidemic rats that received a *Lactuva sativa*-supplemented diet and Atorvastatin as the standard drug showed that the increased body weight was lower than the P407 treatment (Table 1). The results indicate that the *L. sativa*-supplemented diet significantly ($p<0.05$) reduced the concentrations of Total Cholesterol (TC), triglycerides (TAG), Very Low-Density Lipoprotein (VLDL), and Low-Density Lipoprotein Cholesterol (LDL-C). These reductions suggest the antihyperlipidemic effects of the *L. sativa*-supplemented diet. The *L. sativa* (lettuce) prevents weight gain by reducing fat mass accumulation and increasing energy expenditure. Consumption of lettuces enhances glucose homeostasis, increases insulin sensitivity, and improves lipid profile in albino rats. It might be attributable to the plant's abundance of bioactive molecules like esculin and chlorogenic acid (Yokozawa et al. 2006). Conversely, the P407 set significantly increased TC, TAG, LDL, and VLDL levels (Table 2).

The increase in TC levels due to the induction of P407 was likely caused by indirect activation of HMG-CoA reductase after intraperitoneal administration of P407 (Johnston 2004). The possible reduction in Total Cholesterol (TC) levels in *Lactuva sativa*-supplemented diet may be attributed to the decreased function of hepatic HMG-CoA reductase and/or the increased cholesterol-7-alpha-hydroxylase, an enzyme responsible for the conversion of cholesterol into bile acids. Furthermore, the standard drug, Atorvastatin, inhibits HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. These findings are consistent with a prior study conducted by Beckmann et al. (2009) that polyphenols had lipid-lowering properties. The increase in triglyceride (TAG) levels due to the induction of P407 primarily arises from the inhibition of TAG breakdown. P407 directly inhibits the metabolism of capillary lipoprotein lipase (LPL), the enzyme that controls plasma triglyceride breakdown (Johnston 2004). Atorvastatin decreases triglyceride (TAG) concentrations by activating lipoprotein lipase. Supplementation of *L. sativa* in the diet might lead to a reduction in TAG levels either by stimulating endothelium-bound lipoprotein lipase activity (Sikarwar and Patil 2011) or lipolysis inhibition to prevent the conversion of triglycerides into fatty acids.

The present study showed a significant ($p<0.05$) decrease in LDL cholesterol (LDL-C) and Very Low-Density Lipoprotein (VLDL) levels in all treatment groups, with the 50%:50% *L. sativa* treatment showing the most significant reduction effects. These findings are in line with the study conducted by Baum et al. (1998) and Beckmann et al. (2009), which suggested that phenolic compounds could enhance the density of LDL-C receptors in the liver, thereby facilitating the binding of apolipoprotein B and improving the liver's ability to remove LDL-C from the bloodstream efficiently.

HDL cholesterol (HDL-C) functions as a cholesterol scavenger that efficiently collects excess cholesterol and cholesterol esters from the bloodstream and peripheral tissues, transporting them to the liver for breakdown into bile acids. This process is important in lowering blood and peripheral cholesterol levels and inhibiting atherosclerotic plaque development in the aorta (Karmarkar 2008; Kim et al. 2008). The results in this study show an increase ($p<0.05$) in HDL-C after administration of the standard drug Atorvastatin and the *L. sativa*-supplemented diets. The increased HDL-C levels may be attributed to increased Lecithin-Cholesterol Acyltransferase (LCAT) activity. According to Geetha et al. (2011), LCAT is an enzyme that integrates free cholesterol into HDL-C. According to Yokozawa et al. (2006), this process increases the reverse cholesterol transport, reduces LDL-C absorption by endothelial cells, and helps prevent the production of oxidized LDL-C.

Table 3 and Figure 3 show the effect of a *L. sativa* supplement diet on total cholesterol/HDL and LDL/HDL ratios in Poloxamer 407-induced hyperlipidemic albino rats. Atherogenic risk prediction indices (TC/HDL-c and LDLc/HDL-c) are numerical correlations between TC, LDL-c, and HDL-c that were effectively used as indicators of measuring atherosclerosis progression and the risk possibility of CHDs (Nicholls et al. 2007). An LDL-c/HDL-c ratio of less than 2.3 and an HDL-c/TC ratio of more than 0.3 means a lower risk of peripheral artery disease (Ojiakor and Nwanjo 2005). The *L. sativa*-supplemented diet substantially ($p<0.05$) increased the TC/HDL-c ratio and decreased the LDL-c/HDL ratio compared to the P-407 treatment without the *L. sativa*-supplemented diet. In addition, compared to rats in the induced untreated group, the standard medicines revealed a significant ($p<0.05$) increase in the TC/HDL-c ratio with a decrease in the LDL-c/HDL ratio. Increasing the TC/HDL-c ratio and decreasing the LDL-c/HDL ratio in the *L. sativa*-supplemented diet indicated that the *L. sativa*-supplemented diet has anti-atherogenic activity, lowering the possibility of coronary atherosclerosis (Dobiasova and Frohlich 2001).

Table 4 and Figure 4 show the effect of *L. sativa* supplementation on liver function parameters in P407-

induced hyperlipidemic albino rats. Hyperlipidemia is one of the diseases that harm the liver; it can cause fatty infiltration in the liver, resulting in non-alcoholic fatty liver disease (Assy et al. 2000). Fatty liver is a buildup of triglycerides and lipids in the liver cells that, if left untreated, leads to liver inflammation. Liver damage could be distinguished from steatosis to steatohepatitis, fibrosis, and necrosis (Assy et al. 2000). The increased concentrations of AST, ALT, GGT, and ALP seen in the serum of the P-407 treatment without receiving Atorvastatin may be related to liver damage caused by the buildup of triglycerides along with additional lipids in the liver cells (Hyeung et al. 2006). Supplementation of *L. sative* in the diet resulted in a substantial ($p<0.05$) recovery of liver enzyme concentrations. The reversal of these liver enzymes approaching normal concentrations may be due to polyphenols in the lysates with their membrane stabilizing effect, preventing intracellular enzyme leaking (Muthu et al. 2008). The *L. sativa*-supplemented diet reduced the serum transaminase level to normal due to the hepatic parenchymal repair and hepatocyte regeneration (Chavan et al. 2012). It indicated that the *L. sativa*-supplemented diet possesses curative effects on the liver, particularly the diet with 50% supplementation of *L. sativa*, which has the highest impact.

Total protein, albumin, and bilirubin concentrations are also decreased in hyperlipidemic rats with fatty liver lesions. Total protein, albumin, and bilirubin are all indicators of liver function; the liver releases bilirubin and hence interferes with proper liver function, affecting the process of conjugation or excretion. Total protein and bilirubin levels assess liver function and bile excretion efficiency (Usha et al. 2008). The current study showed a significant ($p<0.05$) decrease in total protein, albumin, and bilirubin levels in the P407 treatment compared to the control. These alterations might be an indication of hyperlipidemia-induced fatty liver damage. However, the *L. sativa*-supplemented diet restores total protein, albumin, and bilirubin levels to normal, with the 50%:50% *L. sativa*-supplemented diet group exhibiting the most benefit. The *L. sativa*-supplemented diet appears to improve liver function. The treatment of Atorvastatin also increased total protein, albumin, and globulin concentrations.

Figure 5 shows the effect of *L. sativa* supplementation on lipid peroxidation in the liver and the brain of P407-induced hyperlipidemic rats. Aldehydes, hydrocarbon gases, and chemical leftovers such as malondialdehyde (MDA) are the end products of the lipid peroxidation process. MDA, a prominent lipid peroxidation product, is one of the most important indicators for investigating oxidative damage on lipids (Maryam et al. 2014). MDA is a significant reactive carbon molecule often utilized as a lipid peroxidation biomarker (Karataş et al. 2006). Abnormally increased amounts of lipid peroxidation and reduced antioxidant defense systems can cause cellular organelle damage and oxidative stress (Mahboob et al. 2005). The extent of tissue damage caused by free radicals is determined by the balance between free radical formation and the endogenous antioxidant defense mechanism (Sanilkumar and Muthu 2013). The oxidative degradation of Polyunsaturated Fatty Acids (PUFA), which are abundant in cell membranes, sets off a self-perpetuating chain reaction that produces a variety of harmful products such as malondialdehyde (MDA) and 4-hydroxynonenal. Lipid peroxidation is a spontaneous radical-related process that has the potential to be damaged due to the destruction of membranes, lipids, and additional cell components when uncontrolled and self-enhancing (Mahboob et al. 2005). An increase in MDA showed that oxidative stress might result in free radical-mediated lipid peroxidation in cell membranes. MDA is an excellent marker for evaluating oxidative stress in degenerative diseases, including hyperlipidemia and diabetes mellitus (Padalkar et al. 2012).

In this present study, the induction of Poloxamer 407 significantly increased the MDA concentration. The significant ($p<0.05$) increase in lipid peroxidation in the poloxamer 407 treatment might be due to a reduction in antioxidant defense or increased free radical generation (Ceretta et al. 2012). The findings agreed with a previous study that reported increased lipid peroxidation in hyperlipidemic rats (Gopalakrishnan and Dhanapal, 2014). However, the administration of 10 mg atorvastatin, which served as the positive control, also significantly decreased the MDA concentration compared to the control treatment. Compared to the control, the MDA levels in the *L. sativa*-supplemented diet treatment groups were significantly lower. The ability of the *L. sativa*-supplemented diet to inhibit lipid peroxidation in hyperlipidemic rats may be attributed to the presence of steroids, flavonoids, phenols, and tannins, as found in the preliminary phytochemical screening. Steroids and flavonoids have antihyperlipidemic activity (Ghule et al. 2009; Patel et al. 2009).

In conclusion, this study revealed that a *L. sativa*-supplemented diet has a significant hypolipidemic effect, anti-lipid peroxidation activity, and hepatoprotective effects, with the best result obtained in the treatment of 50% *L. sativa*-supplemented diet which could be used as an antihyperlipidemic agent. The *L. sativa* could be added to the diet of those with high blood lipids. Further research must identify and isolate the active compounds with antihyperlipidemic compounds in *L. sativa*, as they have many therapeutic properties. Pharmaceutical industries should also study *L. sativa* leaves so that derivative drugs have minimal side effects.

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