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Antihyperlipidemic efficiency of extracts from different parts of the Sidr plant *(Ziziphus spina-christi)* and compare to rosuvastatin on induced hyperlipidemic rat models

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Abstract: The current work aims to study the anti-hyperlipidemic effectiveness of the ethanolic extracts from various parts of *Ziziphus spina-christi* (Sidr) plant; seed (SSF), pulp (SPF) and leaf (SLF) against rosuvastatin (ROF), 10 mg/kg) in rats induced with hyperlipidemia via a (10-week) high-fat diet. Several biochemical, hematological and physiological parameters were determined. The SSF verified greater effectiveness, mainly enhancing liver and kidney functions in particular; it reduced alanine aminotransferase (ALT) levels to 29.16 U/L (vs. 35.89 in SLF and 29.14 in control) and considerably improved kidney functions by reducing urea and creatinine levels to 1.84 mmol/L and 119.83 µmol/L, respectively. Lipid profile with the SSF group was comparable to the rosuvastatin group. Additionally, SSF group ameliorated the hematological alterations and decreased hyperlipidemia-induced organ damage more than the other extracts. In conclusion, the seed extract was the most efficient part, as it established the most positive function biomarkers, such as ALT activity, urea and creatinine, sustaining its potential for more preclinical and clinical developments.

Keywords: Anti-hyperlipidemic; Health; Immunity; Liver functions; Lipid profile; *Ziziphus spina-christi*

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INTRODUCTION

The pathophysiology of cardiovascular diseases is intimately associated with the consumption of excessive amounts of dietary fats (Heo *et al.,* 2021). The abnormally high quantity of lipids in the body, known as hyperlipidemia, might have hereditary or familial causes. Some investigations have linked a high intake of fats to several diseases in both human and animal species (Atteya *et al.,* 2021). High dietary fat consumption generally significantly reduces hemorrhageological characteristics, such as lower blood fluidity and elevated low-density lipoprotein (LDL) that causes increased erythrocyte aggregation, reduced blood flow and elevated blood viscosity (Lee *et al.,* 2022). Numerous circulatory conditions, including hypertension, thrombosis, myocardial infarction and cerebrovascular diseases, can develop or worsen as a result of the cumulative effects of hemorrhageological abnormalities manifesting as an altered blood flow profile (Indrianti *et al.,* 2023; Levin *et al.,* 2023). An inflammatory process in the vessel wall's endothelial cells initiates atherosclerosis (Al-Eisa *et al.,* 2023a). It is mainly carried on via the formation of white blood cells and macrophages, which are elevated by LDL, the plasma proteins that carry triglycerides and cholesterol, while functional high-density lipoprotein (HDL) is unable to adequately remove lipids and cholesterol from the macrophage (Rani *et al.,* 2021). Additionally, flavonoids and alkaloids are two examples of natural products that can be utilized to protect against cardiovascular illnesses in both primary and secondary ways (Al-Eisa *et al.,* 2023b; Baazeem *et al.,* 2024). Dyslipidemia is predisposed by many etiological variables, such as a high-fat diet, obesity and a lack of physical activity (Moorthy *et al.,* 2021). Unhealthy

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lifestyle choices significantly contribute to the genesis of dyslipidemia and heighten its associated problems.

Statins, niacin, fibrates and bile acid sequestrants are among the medications used to lower lipid levels. Statins are the most widely used medications for the treatment of dyslipidemia and to lower the risk of atherosclerotic cardiovascular diseases (Rauf *et al.,* 2022). Statins work by blocking the mevalonate pathway's rate-limiting enzymes, such as β-hydroxy β-methylglutaryl-coenzyme and by boosting the expression of LDL receptors, primarily in hepatocytes, which increases the clearance of LDL. Atorvastatin, Rosuvastatin, Simvastatin, Fluvastatin and Lovastatin have several dose-limiting effects while being very effective lipid-lowering medications. Hemorrhagic stroke, diabetes mellitus, cognitive decline, tendon rupture and interstitial lung disease are among the toxicities that have been reported. In addition to their negative character, statins are linked to a variety of medication interactions (Mollazadeh *et al.,* 2021).

Even if there are several pharmaceuticals available to prevent dyslipidemia, herbal remedies are still favored in some regions of the world. In order to increase the effectiveness of conventional pharmaceuticals and reduce potential side effects, herbal remedies can be utilized as adjuvants (Gyawali *et al.,* 2021). According to (Ameri *et al*., 2024), the *Ziziphus* plant had reduced lipid peroxidation, enhanced liver and kidney function and alleviated hyperlipidemia in rats with elevated cholesterol. Furthermore, the extract from the *Ziziphus* plant effectively lowers lipid peroxidation, hyperlipidemia and liver enzyme activity. The rosuvastatin-induced hyperlipidemic rat models are recommended to be treated with *Ziziphus* leaves, pulp, and seeds as a traditional medicine, this study is noteworthy. Animal groups were fed a fatty diet for ten weeks and a number of parameters were assessed, including body weight changes, hematological analysis, lipid profiles and the activities of liver and kidney biomarkers. The study aimed to evaluate and compare the efficacy of Rosuvastatin and ethanolic extracts of different parts of the Sidr plant in reducing lipid profile and monitoring organ functions in a high-fat diet-induced hyperlipidemic rat model.

MATERIALS AND METHODS

Experimental

Preparation of Ziziphus Extracts

Taif City, Saudi Arabia, is where the *Ziziphus* plants and leaves were grown and purchased from different areas from October 2024 to January 2025. Taif University, Department of Biotechnology, identified and authenticated the plants. Running tap water was used to thoroughly clean the fresh fruits and leaves and distilled water was used to rinse them. It is hand-separated into pulps and seeds using a sterilized stainless-steel knife. In a thermostatically controlled oven (DHG-9053A, Shanghai, China), leaves, pulps and seeds were dried at 45 ºC until reaching a constant weight. The dried materials were ground into powder using a milling machine. The resulting particles were then packed in an airtight, sterilized glass jar and stored at 4 ºC until use. The powder of each component (50 g), Sidr seeds (SS), Sidr pulps (SP) and Sidr leaves (SL) were mixed with 250 ml of 50 % ethanol. It had strongly shaken at intervals and was allowed to sit at the ambient temperature for 2 days. After filtering, the mixtures were evaporated at 45 °C in a water bath. Following desiccation, the crude extracts were desiccated, weighed and sealed in dark glass containers before being kept at 4 °C until further use. The resultant slurry was reconstituted with distilled water at the needed dosage for the animal model therapy (Mohammed *et al.,* 2023).

Animals and housing

Forty-eight male albino rats from the Animal Holding Unit of the Biological Science, Jeddah, KSA, were used in this investigation. Each rat was 9 weeks old and weighed between 225 g and 250 g. They were acclimatized in stainless steel cages with free access to diet and tap water in a well-ventilated room, at ambient temperature with typical laboratory humidity levels and a regular light-dark cycle for two weeks to adjust to the environment before experimentation. Approximately 40 % yellow corn, 39% wheat flour, 5% wheat bran, 10% powder milk, 5% corn oil, % 8.5% berseem clover hay, 1% vitamins and minerals and 0.5% sodium chloride were the components of the typical diet, which was freshly prepared every 3 days. Frequent cleaning, removal of feces and spilled food from cages and proper handling and care were all observed. Crestor is a brand name for rosuvastatin (RO) that was bought from a pharmacy in Taif City. A freshly prepared solution of RO after being dissolved in normal saline was administered daily as a single dosage of 10 mg/kg B.W./day to the rats via oral gavage.

High-fat diet preparation

Following the guidelines outlined by (Bencheikh *et al.,* 2021) with slight adjustments, the high-fat diet was prepared. The standard rat diet was supplemented with coconut oil and ghee at a dose of 10 mL/kg body weight/day via oral administration with 0.5% cholic acid due to its ability to enhance micelle formation and aid in the intestinal absorption of cholesterol. The supplemented diet is coded as a fatty diet.

Experimental design

The control group (N=8) was considered the negative control, which was given a regular diet and distilled water at 10 mL/kg B.W./day twice daily for 10 weeks. Hyperlipidemic groups (N=40) were given a hyperlipidemic diet for 10 weeks. The hyperlipidemic groups were randomly split up into 5 subgroups (N=8) matching by weight, with 8 rats in each group, as follows (Al-Awar *et al.,* 2019):

Fatty diet (-) (F group): was considered the positive control were given a hyperlipidemic diet with distilled water via gastric gavage (10 mL/kg B.W./day) for 10 weeks.

SL + F (SSF group): which was given a hyperlipidemic diet and 150 mg/kg B.W./day of seed extract orally.
SP + F (SPF group): which was given a hyperlipidemic diet and 150 mg/kg B.W./day of pulp extract orally.
SL + F (SLF group): which was given a hyperlipidemic diet and 150 mg/kg B.W./day of leaf extract orally.
Rosuvastatin + F (+) (ROF group): which was given a hyperlipidemic diet and 10 mg/kg B.W./day of rosuvastatin orally.

Fig. 1 presents the summary of the experimental work.

Body weight changes
The rats' body weight (B.W.) is among the major sensitive indicators of hyperlipidemia, which was gravimetrically recorded regularly for the duration of the experiment at (0, 2, 4, 6, 8 and 10) weeks with a laboratory scale in grams (XPR, Shanghai, China) (Sokoła *et al.,* 2024).

Animal sacrifice
At the end of the experimental period and after overnight fasting for 8 hours, all rats were humanely sacrificed and euthanized under chloral hydrate (250 mg/kg B.W. pentobarbital) (Tai *et al.,* 2010). Kits for biomarkers were obtained from Diamond, Mid-Egypt, Egypt. To produce the liver and kidney homogenate, tissues (10% w/v) were homogenized in an ice bath with 0.1 M sodium phosphate-buffered saline at a pH of approximately 7.4. For the biochemical parameters, the samples were then centrifuged for 40 minutes at 4°C and 8,000 rpm.

Blood collection
Blood samples were drawn from the treated rats via cardiac puncture in heparinized tubes for the hematological analysis. EDTA with a concentration of 1.5 mg/mL as an anticoagulant was applied. Serum was collected in plain bottles, isolated through centrifugation at 3000 g for 10 minutes at a temperature of 4°C and kept at -20 °C in aliquot tubes until they were used to estimate the lipid profile (Shawky, 2015).

Hematological analysis
Subsequently, fresh blood samples were taken from the tubes with EDTA for the hematological tests. White and red blood (WBC) and (RBC) cells with hematocrit (Hct) were measured using a hematology analyzer (Huma, German).

Biochemical analysis
Liver function biomarkers
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum activities were evaluated colorimetrically at a wavelength of 546 nm and expressed as (U/L). Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were determined and expressed as (U/mL) at a wavelength of 340 nm and 405 nm, respectively (Baazeem *et al.,* 2024). Liver proteins such as total protein, albumin and globulin were evaluated and expressed as (g/dL). Total protein was evaluated by the biuret reagent, albumin was evaluated by the bromocresol reagents, while globulin was calculated as follows: total protein - albumin. Bilirubin and conjugated bilirubin were determined by using Randox reagents and expressed as (µmol/L) (Nuru *et al.,* 2024).

Kidney function biomarkers
Urea concentration was evaluated colorimetrically based on the transformation of the urease enzyme's hydrolysis into ammonia and carbon dioxide, detected at a wavelength of 546 nm and expressed as (mmol/L). Creatinine concentration was evaluated colorimetrically based on the reaction of creatinine and picric acid with an alkaline solution, detected at a wavelength of 492 nm and expressed as (µmol/L) (Nuru *et al.,* 2024).

Lipid profile biomarkers
Analysis was done on the serum samples by using the enzymatic-colorimetric techniques of the commercial kits with the help of a Hitachi 7450 analyzer. Plasma samples were examined for lipid profile biomarkers, including total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides. Results were expressed as (mg/dL).

Statistical analysis
The mean ± standard deviation (SD) was used to illustrate each outcome of the data. One-way analysis of variance (ANOVA) and Tukey post hoc were employed to identify significant pairs. P-values were considered statistically significant if they were less than 0.05. For statistics and graphical depiction, GraphPad Prism (GraphPad Software, Version 5, San Diego, California, USA) was used.

RESULTS

Body weight
According to the measurements, the weight increases throughout the experiment were determined for all groups. The experimental work evaluated body weight changes during 10 months at (0, 2, 4, 6, 8 and 10) in all groups, fig. 2. All groups gained a rise in body weight after the experiment's duration. The rats' weight increased, while the control group's rise was more maintained than the other treatment groups. The body weight increased to reach (279.04 g) consistently in the control group, whereas, on the 8th week, rats in the negative control (F group) died compared with the remaining treatments before reaching (293.77 g) on the 6th week. During the 10 weeks of treatment, the SLF group was administered daily with the ethanolic leaf extract, had a reduction in the increase in body weight and reached (295.43 g) compared

to the ROF group (291.01 g). The SSF group and SPF group reached the highest values of weight gain (306.55 g and 303.25 g), respectively. The hyperlipidemic diet raised body weight in the F group while lowering it in other groups that received rosuvastatin and other ethanolic extracts of *Ziziphus* plant parts, such as seeds, pulp and leaves.

Hematological analysis

The fatty diet decreased WBC counts from 9.45 $\times 10^3$ mm^3 (F group) to 6.22 $\times 10^3$ mm^3 (SLF group) when compared with the control group 8.06 $X10^3$ mm^3. However, the ROF group (7.01 $X10^3$ mm^3) had higher values than all parts of the *Ziziphus* plant. Conversely, the rosuvastatin drug did not cause any positive changes in the mean WBC counts. On the same trend, a fatty diet given to the rats for ten weeks changed RBC counts from 7.54 $X10^6$ mm^3 in the F group to 8.22 $X10^6$ mm^3 in the SLF group when compared with the control group 8.88 $X10^6$ mm^3. However, the ROF group (8.61 $X10^6$ mm^3) had higher values than all parts of the *Ziziphus* plant. Conversely, the rosuvastatin drug did not cause any positive changes in the mean RBC counts. Hyperlipidemia in treated rats with *Ziziphus* parts changed Hct values from 47.54 % in the SSF group to 48.51 %o in the SPF group Hct when compared with the control group of 50.90 %. However, the ROF group produced a slight decrease of 49.45 % in comparison with the F group of 50.22 %, Table 1

Activities of liver biomarkers

Liver enzymes

According to the results in figs. 3-6, the serum ALT levels of the hyperlipidemic rats ranged from 29.16 U/L in the SSF group to 35.89 U/L in the SLF group when compared with the control group of 29.14 U/L. However, as compared to rats with hyperlipidemia (F group, 59.56 U/L), the group treated with rosuvastatin drug (31.15 U/L) showed a slight drop in ALT levels. SPF group and SSF group showed the most efficiency in reducing ALT levels (29.47 U/L and 29.16 U/L), respectively. The current findings demonstrated a substantial difference in serum AST levels between the rats in the hyperlipidemic group, ranging from 115.25 U/L in the SLF group to 140.56 U/L in the SSF group, when compared with the control group, 109.36 U/L. However, as compared to rats with hyperlipidemia (F group, 181.60 U/L), the group treated with rosuvastatin drug (119.79 U/L) showed a slight drop in AST levels. SPF group and SLF group showed the most efficiency in reducing AST levels (117.22 U/L and 115.25 U/L), respectively. Differences in serum LDH levels between the rats in the hyperlipidemic group ranged from 1199.49 U/L in the SLF group to 1275.88 U/L in the SSF group when compared with the control group, 894.32 U/L. However, as compared to rats with hyperlipidemia (F group, 1382.47 U/L), the group treated with the rosuvastatin drug (1235 U/L) showed a small drop in LDH levels. However, compared to the hyperlipidemic rats in SPF group (1212.56 U/L) and ROF group (1235.00 U/L), there was an efficient decrease in LDH levels. The findings indicated that the hyperlipidemic rats' serum ALP levels, which ranged from 24.17 U/L in the SLF group to 28.25 U/L in the SSF group, were substantially higher than values of the control group, 22.14 U/L. However, as compared to rats with hyperlipidemia (F group, 33.56 U/L), the group treated with rosuvastatin drug (24.54 U/L) showed a slight drop in ALP levels. However, a comparison to the hyperlipidemic rats in the SLF group (24.17 U/L) and rosuvastatin drug (24.54 U/L) showed an efficient decrease in ALP levels.

Liver proteins

Hyperlipidemic rats have far higher levels of total protein, 9.55 g/dL in the liver, than the control group, 7.12 g/dL, fig. 7. The SSF group reported the lowest total protein, 7.55 g/dL, followed by the SLF group, 7.81 g/dL, compared with the ROF group, 7.88 g/dL. The increase in total protein in rats may result from reactive oxygen species (ROS) produced in hyperlipidemia, damaging cellular proteins.

According to fig. 7, all of the treated groups were generally lower than those in the control group. The albumin values were decreased to reach 3.95 g/dL in the F group when compared with values in the control group of 5.17 g/dL. The SSF group reported the highest albumin content, 5.02 g/dL, followed by the SPF group at 4.87 g/dL, compared with ROF group at 5.11 g/dL.

All of the treated groups were generally higher than values in the control group. The globulin values were increased for hyperlipidemic rats to reach 5.60 g/dL in the F group when compared with values in the control group of 1.95 g/dL, fig. 7. The SLF group reported the lowest globulin content of 2.15 g/dL followed by SSF group 2.35 g/dL compared with ROF group 2.77 g/dL. Bilirubin and conjugated bilirubin were increased in hyperlipidemic rats to reach 13.11 g/dL and 5.55 g/dL, respectively. SPF group gave similar values to the treated rats with rosuvastatin drug for bilirubin and conjugated bilirubin as 10.87 g/dL and 3.99 g/dL, respectively.

Activities of kidney biomarkers

After 10 weeks of observation, the urea and creatinine levels of experimental hyperlipidemic rats given ethanol extracts of *Ziziphus* plant parts and rosuvastatin drug were measured. Both biomarkers have increased compared with the control group, even though the urea and creatinine levels were within the typical range. Figs. 8 and 9 show the urea and creatinine levels for each group. Fatty diet raised urea and creatinine levels to reach 8.22 mmol/L and 232.55 µmol/L in the F group, respectively.

Activities of lipid profile

Table 2 presents the date of the lipid profile. The fatty diet caused a great rise in total cholesterol, LDL cholesterol,

phospholipids and triglycerides with a reduction in HDL cholesterol. The ROF group's serum total cholesterol was lower (63.48 mg/dL) than that of the other three *Ziziphus* groups (94.25 mg/dL, 90.01 mg/dL and 110.65 mg/dL). On the same trend, HDL and LDL cholesterol in all groups were altered when compared to the control and ROF groups. The effect on hyperlipidemic rats for HDL cholesterol was similar in the SLF group, 55.26 mg/dL, and the ROF group, 55.18 mg/dL. The SPF group (8.06 mg/dL) detected better values than the rosuvastatin drug group (9.41 mg/dL). The F group reported the highest value for triglycerides (429.58 mg/dL) compared with the control group (220.96 mg/dL). This implies that the *Ziziphus* extract's hypocholesterolemic action might result from LDL cholesterol redistribution to the liver and extra-hepatic tissues through these hepatocyte-level receptors, where it will be eliminated as bile acids.

DISCUSSION

Body weight

Rats-fed fatty diets were a helpful model of the potential consequences of dietary fat in humans; therefore, they can be used to induce obesity because they can easily gain weight when given meals rich in fat (Diemen *et al.,* 2006). Death can be due to an extreme fatty diet, which can cause several negative responses, such as pancreatitis or even hepatic failure (Noeman *et al.,* 2011). Hyperlipidemia and gluconeogenesis resulting in muscle atrophy and tissue protein loss may be linked to death (Mohammed *et al.,* 2023). Animals' reactions to fatty diets are gradual yet cumulative because they occur over ten weeks. Age, the genetics of the various strains and the composition of various formulations could all be contributing factors to the variations in weight gain (Noeman *et al.,* 2011). These results of the body weight are consistent with several earlier research investigations that evaluated the potential of *Ziziphus* extracts to prevent hyperlipidemia (Mohammed *et al.,* 2023). Many studies have clearly shown that rats that regularly have a diet high in fats become obese or even overweight (Bouhlali *et al.,* 2020; Bouhrim *et al.,* 2020). These findings concurred with those of (Al-Sieni*et al*., 2020), which might be because *Ziziphus* extracts contain antioxidants that lower oxidative stress and act as antihyperlipidemics.

Hematological analysis

Hematological parameters are thought to be sensitive indicators of physiological changes in response to any external impacts (Alzomor *et al.,* 2021). The fatty diet decreased WBC counts (SLF group) when compared with the control group. However, the ROF group had higher values than all parts of the Ziziphus plant. Conversely, the rosuvastatin drug did not cause any positive changes in the mean WBC counts. On the same trend, a fatty diet ten weeks increased RBC counts in the SLF group when compared with the control group. However, the ROF group had higher values than all parts of the Ziziphusplant. The current finding reported the positive effects of hematological analysis for all parts of the *Ziziphus* plant against the rosuvastatin drug.

Activities of liver biomarkers

Liver enzymes

Hyperlipidemic rats had higher serum levels of ALT, AST, LDH and ALP than control groups, which suggests that a fatty diet has a detrimental effect on the liver. A rise in serum marker enzyme levels is generally considered to be one of the most sensitive indications of toxicity-induced liver damage due to leaking into the bloodstream from the liver cytosol. Localized endoplasmic reticulum injury or the risky energy effect that is liberated through fatty diet metabolism can be considered as other reasons for liver damage (Rhiouani *et al.,* 2008; Sookoian *et al.,* 2015).

The *Ziziphus* plant ethanolic extracts were linked to a decrease in enzymes and an improvement in disease severity. Daily administration of rosuvastatin (10 mg/kg) raised the serum enzymes of hyperlipidemic rats. This result was in line with a study conducted by (Dodiya *et al*., 2013) that discovered that giving rats oral rosuvastatin in doses of 40 mg and 80 mg for 21 days markedly raised their levels. Rosuvastatin's direct irritating impact on hepatic cells may be the cause of the elevated serum ALP (Mojarrad *et al.,* 2020). The study's findings showed that rosuvastatin drug reduced the severity of steatohepatitis in the hyperlipidemic group. This was made abundantly clear by the liver pathology, which indicated a decrease in the increased enzyme levels and an improvement in the disease's severity level close to remission. The current results are consistent with earlier research that demonstrated rosuvastatin's advantageous effects (Nascimbeni *et al.,* 2016). In the current investigation, we found that the results of the *Ziziphus* plant administered both independently and in combination with extracts were nearly identical to the control group. The current findings support those of (Al-Sieni*et al*., 2020), who found that administering *Ziziphus* plant extracts returned the liver enzymes to normal levels. (El Rabey *et al*., 2014) found that rats fed a hyperlipidemic diet had an increase in serum levels, which was efficiently decreased by *Ziziphus* leaf extract, which could be due to the presence of saponins that have hepatoprotective properties.

Liver proteins

Evaluating total protein may be a more accurate marker for assessing the condition of the liver, while damaged proteins are essential markers due to their longer lifespans, higher stability, dependability and comparatively early synthesis (Noeman *et al.,* 2011). Total protein values were nearly identical to those in the control group following the administration of ethanolic extracts of *Ziziphus* parts in combination with rosuvastatin drug.

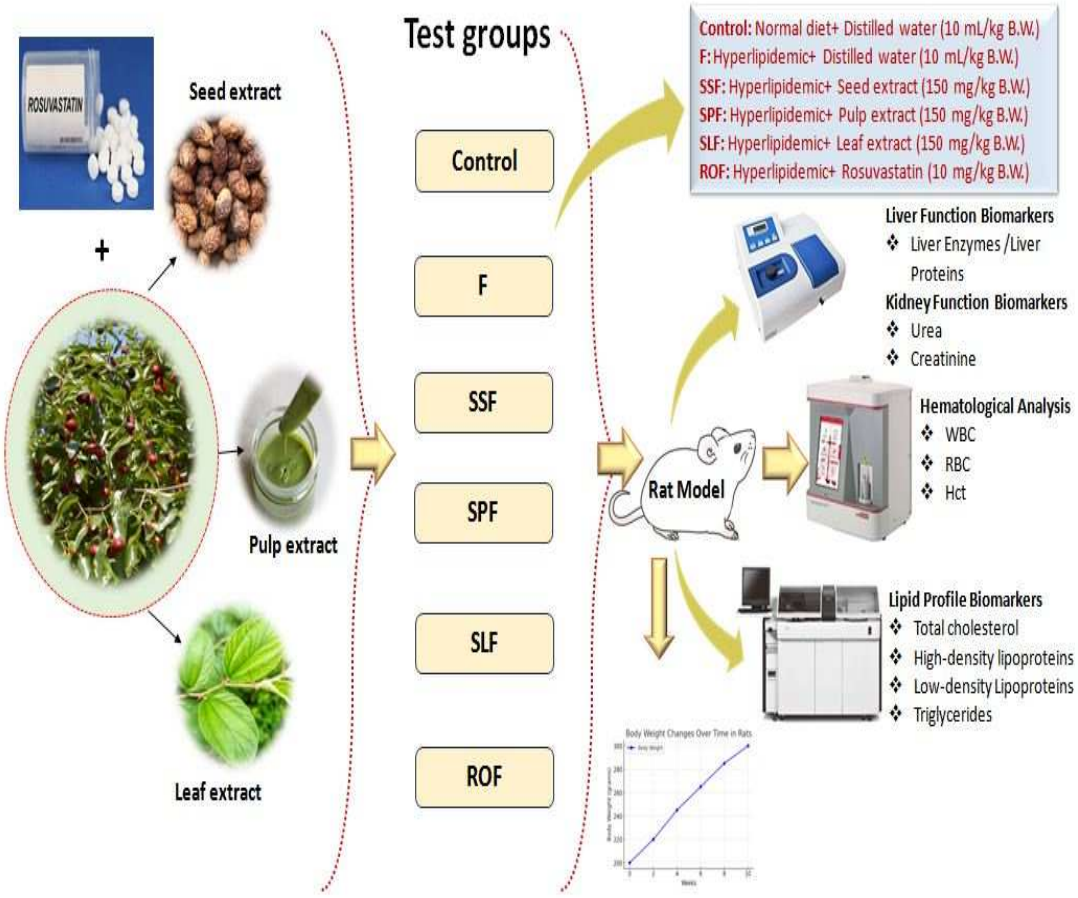

Fig. 1: The summary of the experimental hyperlipidemic rats' procedure

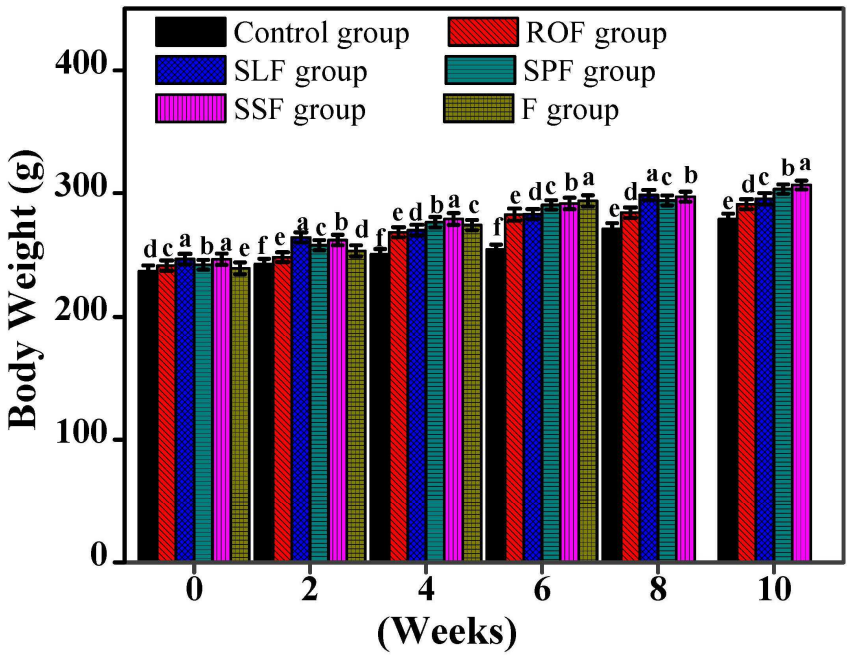

Fig. 2: The effect of high-fatty diet on the body weight at (0, 2, 4, 6, 8, and 10 weeks); Different letters mean significant differences among rat groups (6 groups with 8 rats each). (n=48 rats)

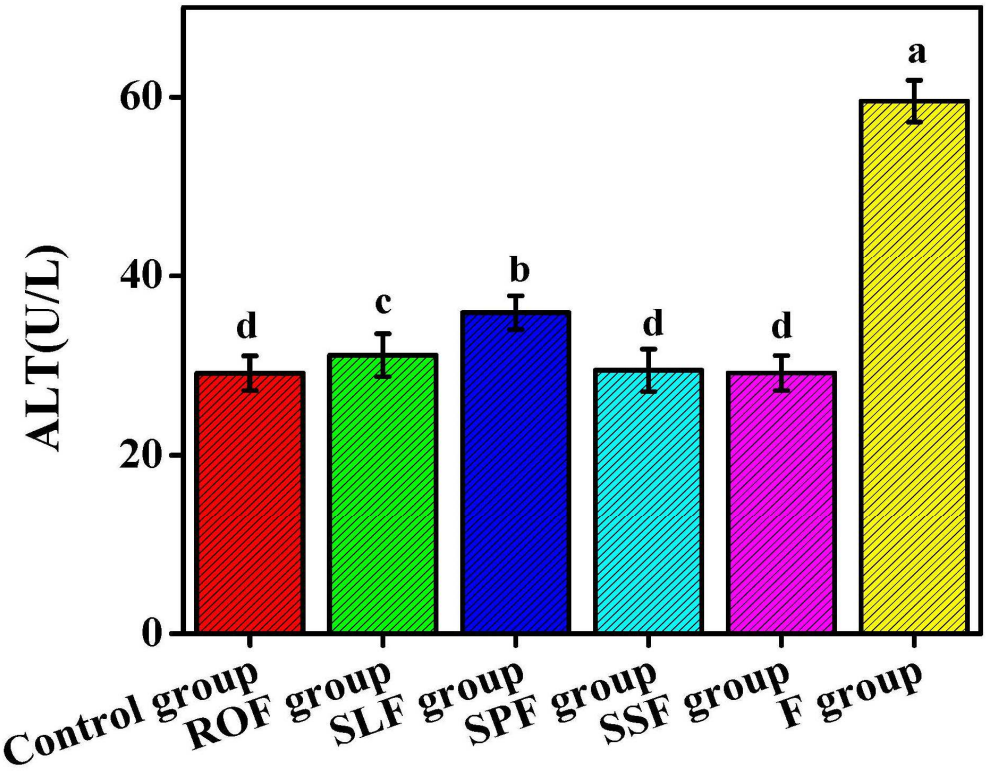

Fig. 3: The effect of high-fatty diet on alanine aminotransferase (ALT) liver enzyme; Different letters mean significant differences among rat groups.

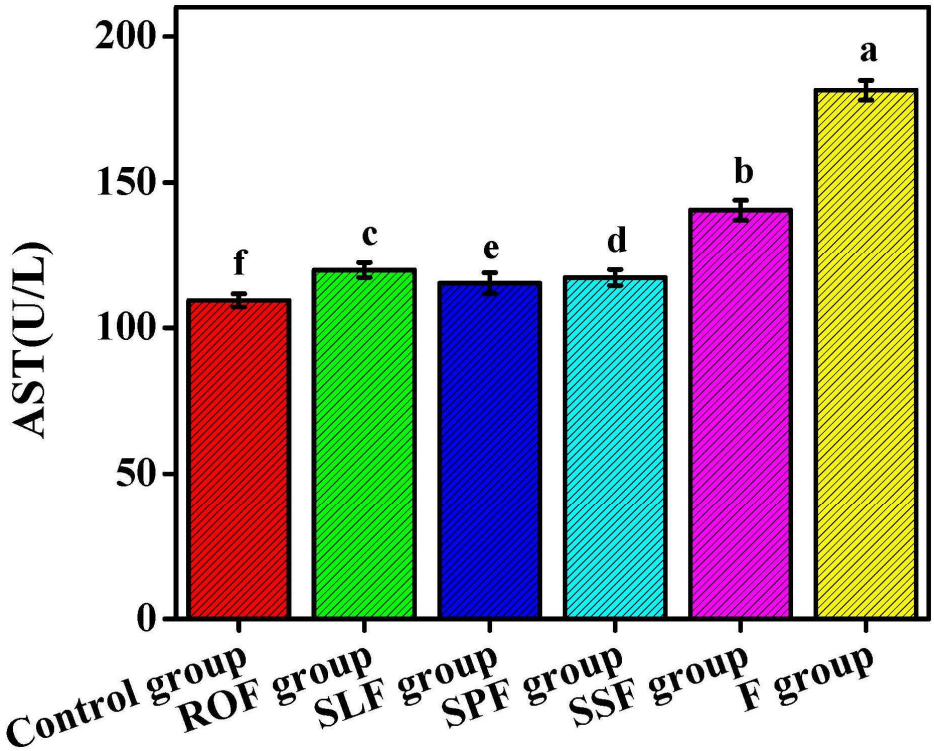

Fig. 4: The effect of high-fatty diet on aspartate aminotransferase (AST) liver enzyme; Different letters mean significant differences among rat groups.

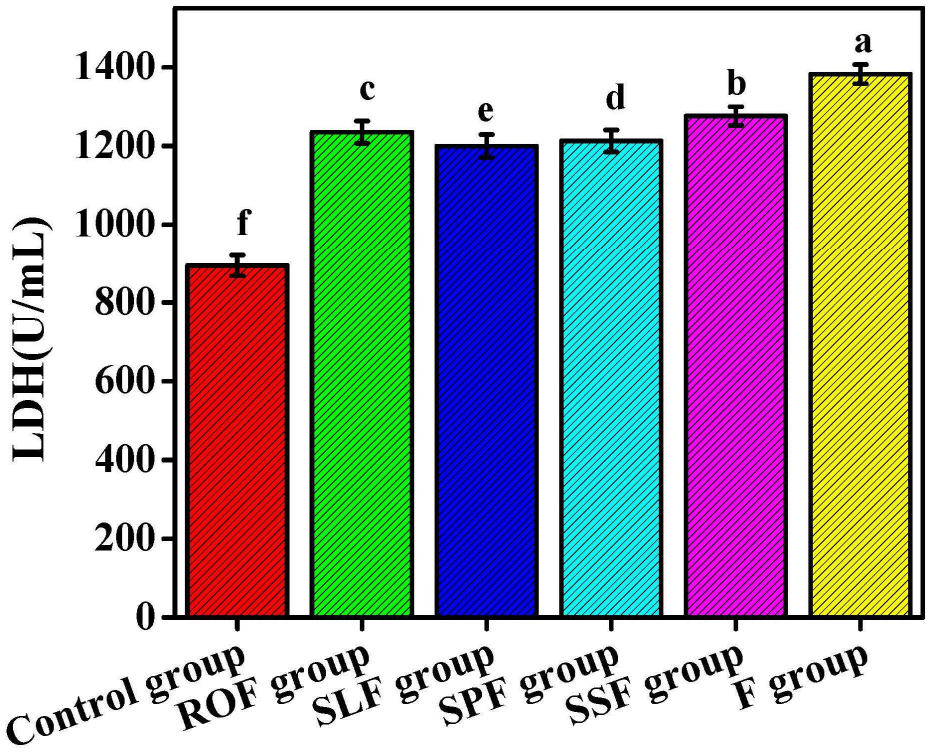

Fig. 5: The effect of high-fatty diet on lactate dehydrogenase (LDH) liver enzyme; Different letters mean significant differences among rat groups.

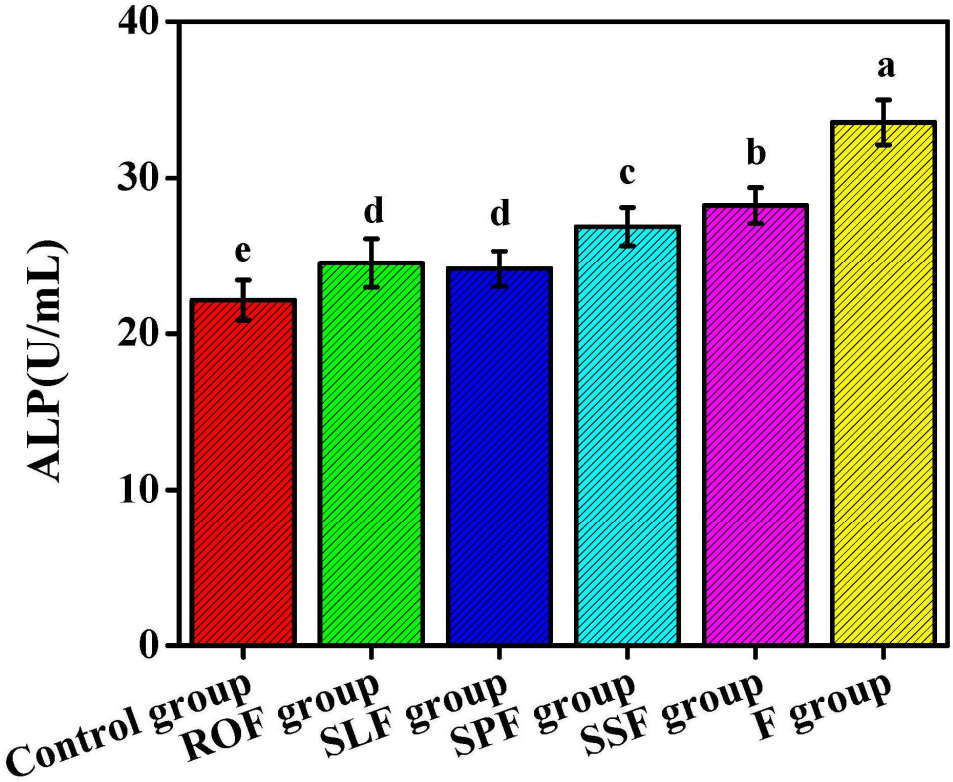

Fig. 6: The effect of high-fatty diet on alkaline phosphatase (ALP) liver enzyme; Different letters mean significant differences among rat groups.

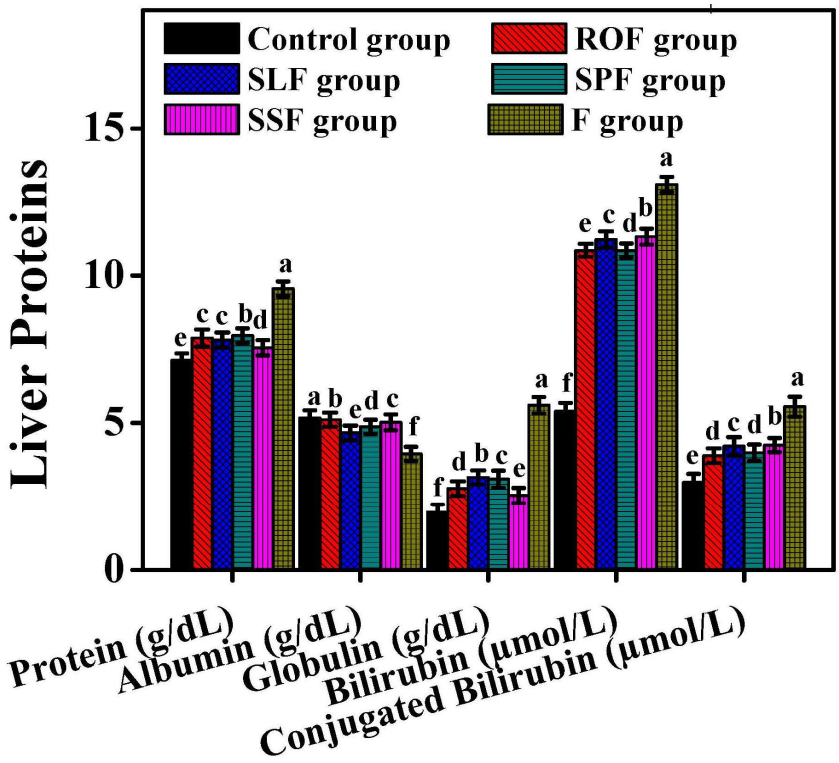

Fig. 7: The effect of high-fatty diet on liver proteins; Different letters mean significant differences among rat groups.

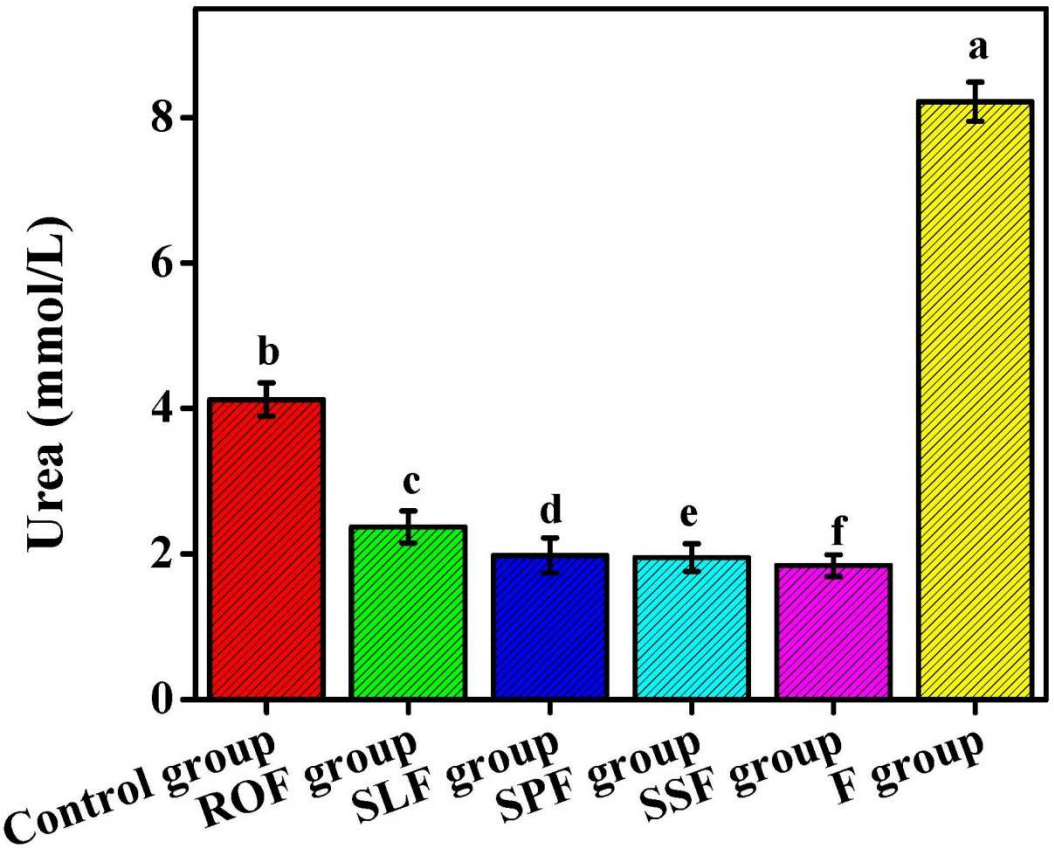

Fig. 8: The effect of high-fatty diet on urea; Different letters mean significant differences among rat groups.

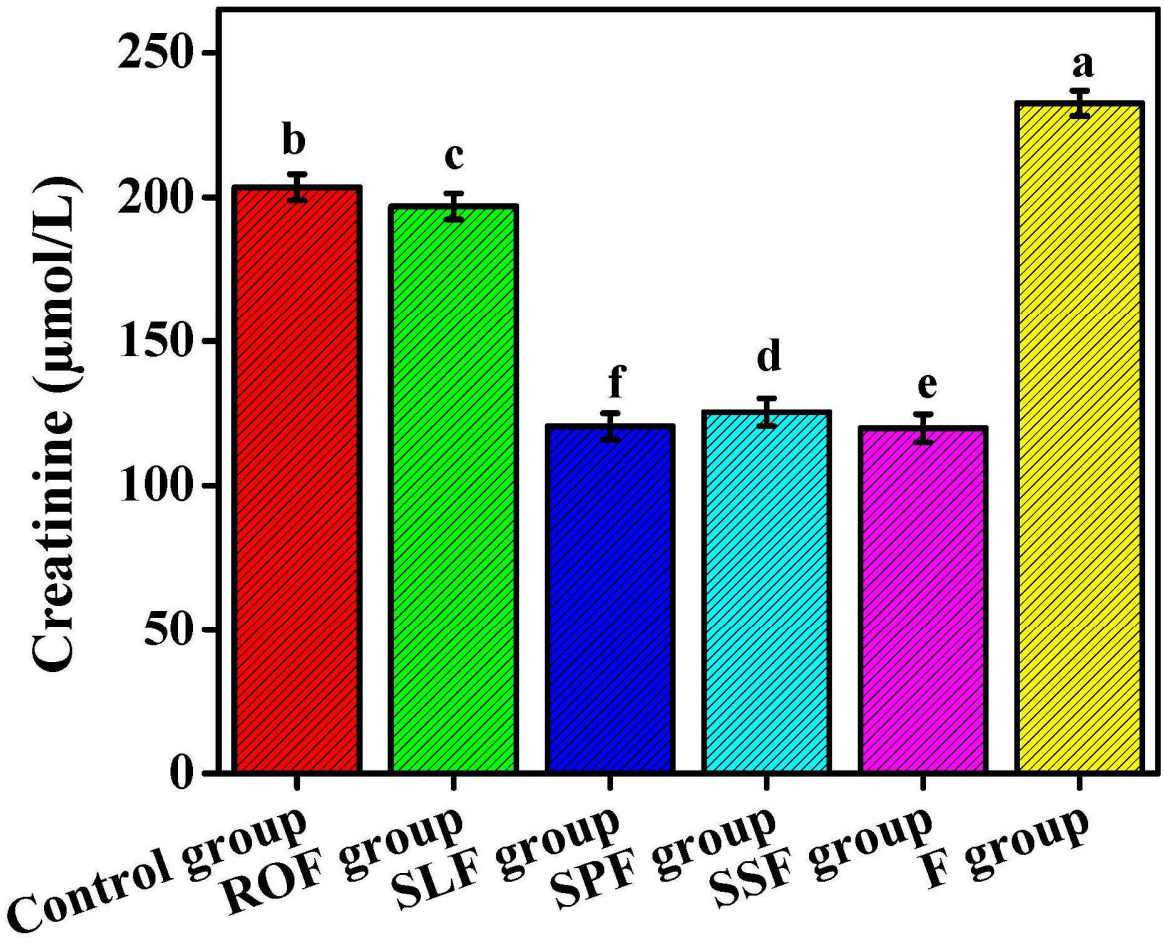

Fig. 9: The effect of high-fatty diet on creatinine; Different letters mean significant differences among rat groups.

Table 1: The effect of high-fatty diet on the hematologic analysis

Hematologic analysis	WBC ($X10^3$ mm^3)	RBC ($X10^6$ mm^3)	Hct (%)
Control group	8.06 ± 0.16^b	8.88 ± 0.24^a	50.90 ± 0.25^a
ROF group	7.01 ± 0.15^c	8.61 ± 0.26^b	49.45 ± 0.22^c
SLF group	6.24 ± 0.21^e	8.22 ± 0.21^d	48.24 ± 0.23^e
SPF group	6.30 ± 0.17^d	8.24 ± 0.25^d	48.51 ± 0.25^d
SSF group	6.22 ± 0.26^e	8.31 ± 0.17^c	47.54 ± 0.21^f
F group	9.45 ± 0.18^a	7.54 ± 0.23^e	50.22 ± 0.24^b

*The mean ± standard deviation is displayed for each value. Different letters mean significant differences among rat groups. White and red blood (WBC) and (RBC) cells with hematocrit (Hct) Significant changes among rat groups are indicated by different letters (n=3).

Table 2: The effect of high-fatty diet on the lipid profile

Lipid profile	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
Control group	69.45 ± 4.46^e	61.27 ± 5.06^a	7.20 ± 0.69^f	220.96 ± 5.15^b
ROF group	63.48 ± 6.38^f	55.18 ± 5.03^c	9.41 ± 0.56^c	118.25 ± 5.96^f
SLF group	94.25 ± 4.72^c	55.26 ± 5.85^c	9.11 ± 0.76^d	128.77 ± 5.45^e
SPF group	90.01 ± 6.67^d	54.10 ± 6.11^d	8.06 ± 0.73^e	198.30 ± 5.18^c
SSF group	110.65±4.46b	58.65 ± 5.47^b	9.77 ± 0.65^b	154.07 ± 5.04^d
F group	160.15 ± 5.63^a	44.12 ± 5.22^e	11.21 ± 0.75^a	429.58 ± 5.26^a

*The mean ± standard deviation is displayed for each value. Different letters mean significant differences among rat groups. High-density lipoproteins (HDL), low-density lipoproteins (LDL). Significant changes among rat groups are indicated by different letters (n=3).

Albumin is a key transporter of medications and other minerals that move through the immunological response (Nuru *et al.,* 2024). Cell function may be hampered by the accumulation of oxidized proteins. Localized damage in the endoplasmic reticulum or the hazardous effect of energy released during metabolism could be the cause of the alterations in protein levels (Shawky *et al.,* 2015). Globulins are protein molecules that are used to evaluate the liver's condition (Sunmonu *et al.,* 2013). Furthermore, our findings concurred with those of (Yossef *et al.,* 2011), who found that a daily administration of Ziziphus plant raised the levels of albumin against the carbon tetrachloride. An essential blood metabolic byproduct of physiologic and diagnostic significance is bilirubin. It is a good predictor of the liver's health because the liver eliminates it from the body (Sunmonu *et al.,* 2013). The current study's observation of a higher serum level of bilirubin in experimental rats could be due to decreased absorption brought on by liver illness (Nuru *et al.,* 2024).

Activities of kidney biomarkers

Concentrations of urea and creatinine are regarded as important indicators of kidney function (Mohammed *et al.,* 2023). Urea and creatinine levels decreased, according to another earlier study, after inducing fatty liver in animal models (Mahfouz *et al.,* 2020). Moreover, it was noticed that the *Ziziphus* plant therapy decreased kidney biomarkers, especially in the SSF group, which reached 1.84 mmol/L and 119.83 µmol/L for urea and creatinine, respectively. According to (Okasha *et al*., 2017) the addition of *Ziziphus* leaf extract may have contributed to the minor decrease in kidney damage in the experimental rats. The antioxidant potential of the *Ziziphus* leaves may be the reason for the kidney ameliorative effects (Al-Ghamdi *et al.,* 2019). By neutralizing urine, the kidneys preserve the chemical compositions of bodily fluids and active transport in the kidney tubules releases certain excreted materials into the urine (Nuru *et al.,* 2024). These materials include medications like penicillin as well as urea, creatinine and H^+ and K^+ ions. High urea and creatinine levels are important markers of kidney failure that indicate a glomerular filtration rate inactivity (Berredjem *et al.,* 2015).

Activities of lipid profile

The fatty diet caused a great rise in total cholesterol, LDL cholesterol, phospholipids and triglycerides with a reduction in HDL cholesterol. The study results for lipid profile (total cholesterol, HDL and LDL cholesterol) are in line with earlier research that showed rats given a fatty diet had higher total cholesterol and triglyceride levels (Kempaiah *et al.,* 2004). Similar outcomes were observed in a study of rats fed a fatty diet high in canola oil; HDL cholesterol levels dropped with a great increase in triglycerides when compared with values in the control group (Ellis *et al.,* 2002).It has recently been noted that triglycerides regulate lipoprotein interactions, which is essential for preserving proper lipid metabolism. This finding suggests that our extracts might help in resuming the triglyceride catabolism. This effect might result from enhanced absorption of triglycerides carried in LDL by peripheral organs and enhanced removal of triglyceride-rich lipoproteins via increased lipase lipoprotein activity (Jawed *et al.,* 2019). The findings concurred with those of Suliman (Suliman *et al.,* 2008), who claimed that a fatty diet raises cholesterol levels, as the elevated cholesterol level may be caused by the liver's overabundance of cholesterol, which inhibits the LDL receptors that convey cholesterol and causes it to be recirculated in the blood. High dietary fat consumption increases the rise in LDL, which causes erythrocyte aggregation, elevated blood viscosity and reduced blood flow.

The findings of triglycerides and LDL cholesterol concurred with those of (Vincent and Hinder, 2009) after consumption of a fatty diet. The current study demonstrates the anti-hyperlipidemic impact of rosuvastatin by modifying lipid metabolism. Saponins have been linked to an anti-hyperlipidemic action because they promote fecal lipid excretion and form an insoluble combination with cholesterol (Zhao *et al.,* 2005). Furthermore, HDL must return the excess cholesterol in peripheral tissues to the liver in order to prevent cholesterol buildup in blood arteries (Bencheikh *et al.,* 2021). Our results are consistent with previous research (Ejelonu *et al.,* 2021), which found that the phenolic components such as flavonoids, catechin and glycosides of *Ziziphus* extracts decreased the hypercholesterolemia in rats fed a fatty diet by decreasing ALT/triglycerides. *Ziziphus* extracts may reduce LDL oxidation by chelating pro-oxidizing metal ions and sustaining paraoxonase activity linked to HDL, as well as by absorbing free radicals to halt the chain reaction of lipid oxidation (Hanafi *et al.,* 2007). In contrast, these levels of lipid profile were raised and nearly equaled in the negative control group, as rosuvastatin, demonstrating that those extracts help to regulate lipid profiles and reduce cardiovascular disease.

CONCLUSION

According to the study, the ethanolic extracts of *Ziziphus* parts such as seeds, pulp and leaves showed improvement in hyperlipidemic rats by decreasing fats and positive changes in the liver and kidney functions. Seed extract was the most efficient, rivaling rosuvastatin in lipid profile regulation, as offering greater holistic benefits, considering it as a potential contestant for additional preclinical developments for treating hyperlipidemia. Our study highlights the necessity of a well-rounded approach to conventional medicine, fusing the advantages of natural cures with thorough scientific analysis to guarantee their safe and efficient application.

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Authors' contributions
Conception or design: AMA, RS, NKA, BA, SA, MM, MH, RA, AA, FA, NA; Acquisition, analysis, or interpretation of data: AAH, AA, FA, NA; Drafting the work or revising: AMA, RS, NKA, BA, SA, MM, MH, RA; Final approval of the manuscript: AAH, AA, FA, NA, AMA, RS, NKA.

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Data availability statement
The corresponding authors can provide the examined datasets during the current investigation upon reasonable request.

Ethical approval
The Gulf Countries Association of Sciences in Experimentation on Animals granted the Ethics Committee (ethical code: 8901/3177) approval.

Conflict of interest
There are no conflicts to declare.

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