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PUMPKIN SEED OIL AS A FUNCTIONAL DIETARY COMPONENT: EFFECTS ON HEPATIC AND RENAL FUNCTIONS OF DYSLIPIDEMIC RATS

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ABSTRACT
Dyslipidemia is a metabolic disorder associated with impaired hepatic and renal functions and an increased risk of cardiovascular complications. This study investigated the preventive effects of *Cucurbita pepo Linn* (pumpkin) seed oil on biochemical markers of liver and kidney function in male Wistar rats fed high-cholesterol diets. Thirty-six rats were randomly assigned into six dietary groups. Groups 1, 2 and 3 were fed diets based on palm oil (Control group), high-cholesterol diet containing palm oil and diet based on pumpkin seed oil respectively, while groups 4, 5 and 6 were respectively fed diets based on palm and pumpkin seed oils, high-cholesterol diet containing pumpkin seed oil and high-cholesterol diet containing palm and pumpkin seed oils. At the end of the ninth week, the rats were sacrificed and blood collected into plain bottles. The serum activities of GGT and LDH, and serum concentrations of free fatty acids, direct and indirect bilirubin were significantly ($p<0.05$) higher in the groups fed high-cholesterol diet containing pumpkin seed oil, and high-cholesterol diet containing palm and pumpkin seed oils compared with the control, but significantly ($p<0.05$) lower in comparison with the dyslipidemic group, while ALT and AST activities were not affected significantly ($p>0.05$). All the groups had significantly ($p<0.05$) lower concentrations of urea, creatinine and electrolytes in comparison with the dyslipidemic group. These findings suggest that pumpkin seed oil may exert hepatoprotective and renoprotective effects and could serve as a functional dietary component in the nutritional management of dyslipidemia and its associated complications.

Keywords: Dyslipidemia; Cucurbita pepo; High cholesterol diet; Hepatoprotective; Renoprotective.

INTRODUCTION
Dyslipidemia, is a condition characterized by abnormal levels of serum lipids such as cholesterol, triglycerides, and lipoproteins and is a major risk factor for cardiovascular diseases and metabolic disorders worldwide (Santos *et al.*, 2020). The prevalence of dyslipidemia particularly in developing regions undergoing rapid urbanization and dietary transitions is rising. Increased consumption of processed foods that are high in saturated fats has been associated with the growing burden of metabolic syndrome and organ dysfunction in Nigeria (Eze *et al.*, 2022).
The liver and kidneys are among the primary organs adversely affected by dyslipidemia. The liver plays a central role in lipid metabolism and detoxification, and hence hepatocellular damage due to dyslipidemia is often reflected in elevated serum levels of liver enzymes such as AST, ALT, ALP,

and GGT (Adedayo *et al.*, 2021). Similarly, the kidneys regulate electrolyte balance and waste elimination and as a result, dyslipidemia-induced renal injury is evidenced by increased levels of serum urea and creatinine, as well as alterations in serum electrolytes like sodium, potassium, and chloride homeostasis (Al-Malki & Sayed, 2023).

Cucurbita pepo Linn (Pumpkin) seed oil or PSO has garnered attention as a functional food because of its high content of polyunsaturated fatty acids (PUFAs), phytosterols, tocopherols, and antioxidant compounds (Hassan *et al.*, 2020). These bioactive constituents have been shown to modulate lipid metabolism, reduce oxidative stress, and support organ health (Mohamed *et al.*, 2021). Previous research has indicated that *Cucurbita pepo Linn* (Pumpkin) seed oil may ameliorate dyslipidemia and oxidative liver damage, but studies exploring its effects on both hepatic and renal markers under conditions of high-cholesterol diet remain limited.

This study therefore aimed to evaluate the hepatoprotective and renoprotective potentials of pumpkin seed oil in male Wistar rats fed high-cholesterol diets. It is hypothesized that PSO supplementation will mitigate biochemical derangements in the liver and kidneys associated with dyslipidemia, thereby supporting its use as a dietary strategy for preventing organ dysfunction.

MATERIALS AND METHODS

Plant Material Collection and Preparation

The pumpkin plant (*Cucurbita pepo*) was collected from the Wednesday market in Dutsin – ma, Katsina state, Nigeria and identified at the herbarium unit of the department of plant science and biotechnology, Federal University Dutsin – ma. The voucher number FUDMA/PSB/00114 was assigned to it. The seeds were scooped out, washed and air dried at room temperature. They were hulled and then ground to fine powder using a grinder. The powdered seeds were cold-pressed to extract oil, which was then stored in airtight containers at room temperature, before further analysis.

Cold pressing of the oil was done by following the methods of Górnaś *et al.* (2016). The seeds were fed into a mechanical screw press (Komet) where the oil was extracted through high mechanical pressure without external heating. The pressing was conducted slowly, so as to prevent friction, which could ultimately lead to the generation of heat.

The crude oil obtained was allowed to settle for 24–48 hours at room temperature to separate suspended solids. It was then filtered using a fine mesh filter to remove residual particulates and subsequently passed through filter paper as suggested by Mohdaly *et al.* (2021).

Formulation of Experimental Diets

Rat diets were formulated by appropriately mixing the feed ingredients outlined in table 1

Table 1: Components of the formulated diet

S/N	Feed ingredient	PO diet (g/100g)	PSO diet (g/100g)	PSO/PO diet (g/100g)
1	Corn starch	55.45	55.45	55.45
2	Soybean meal	32	32	32
3	Cellulose	45	45	45
4	Palm oil	6	-	3
5	Pumpkin seed oil	-	6	3
6	Salt mix	0.3	0.3	0.3

S/N	Feed ingredient	PO diet (g/100g)	PSO diet (g/100g)	PSO/PO diet (g/100g)
7	Vitamin/Mineral mix	0.25	0.25	0.25
8	Methionine	0.25	0.25	0.25
9	Bone meal	1.25	1.25	1.25

PO diet = diet formulated with palm oil; PSO diet = diet formulated with pumpkin seed oil and PSO/PO diet = diet formulated with equal quantities of palm oil and pumpkin seed oil.

Experimental Animals

Male Wister strain albino rats weighing between 100 – 120g were obtained from the department of pharmacology, faculty of pharmaceutical sciences, ABU Zaria. The animals were kept and maintained in well ventilated cages under standard laboratory conditions. Rats were maintained on grower's mash feed and provided with water *ad libitum.* They were allowed to acclimate to the animal house conditions for a period of 7 days, before the study commenced.

Animal groupings

A total of 36 rats divided into 6 groups of 6 rats each were used in this study. The groups were classified as follows:
Group 1 = fed normal diet
Group 2 = fed high cholesterol diet
Group 3 = fed pumpkin seed oil supplemented diet
Group 4 = fed diet with equal quantities of pumpkin seed oil and palm oil
Group 5 = fed high cholesterol diet supplemented with pumpkin seed oil
Group 6 = fed high cholesterol diet supplemented with equal quantities of pumpkin seed oil and palm oil

Supplementation

The diets were fed to the rats in the groups concerned daily, for a period of nine weeks. The animals were sacrificed after eight weeks and blood was collected to analyze for lipid profile and lipid metabolites.

Blood Collection and Serum Preparation

At the end of the feeding period, the rats were euthanized under mild anaesthesia. Blood was collected via cardiac puncture into plain sample bottles and centrifuged at 3000 rpm for 15 minutes. Serum was harvested and stored at 4°C until biochemical analyses were performed.
Activities of Liver Enzymes in Rats Fed High Cholesterol Diet and Palm oil/Pumpkin Seed Oil Supplemented Diet

Aspartate Aminotransferase (AST)

AST activity in serum was measured using the method outlined by Reitman and Frankel (1957). A (0.1mL) serum sample was incubated with a substrate solution containing aspartate and alpha-ketoglutarate. The reaction produces oxaloacetate, which reacts with malate dehydrogenase to

form NADH, which is quantified spectrophotometrically at 340nm. The AST activity is expressed in IU/L.

Alanine Aminotransferase (ALT)

ALT activity was determined using the same method as AST, with alanine replacing aspartate as the substrate (Reitman & Frankel, 1957). The change in absorbance due to NADH formation was measured at 340 nm, and the enzyme activity was calculated in IU/L.

Alkaline Phosphatase (ALP)

ALP activity was assessed using the method described by Bessey *et al*. (1946). A serum sample (0.1 mL) was incubated with a substrate containing p-nitrophenyl phosphate at an alkaline pH. The hydrolysis of the substrate releases p-nitrophenol, which absorbs light at 405 nm. The ALP activity was determined by measuring the increase in absorbance and is expressed in IU/L.

Total Protein and Albumin

Albumin and total protein concentrations were determined by the Biuret method (Gornall *et al*., 1949). Serum samples were reacted with a Biuret reagent, and the resulting complex was measured at 540 nm. Albumin concentration was determined using a specific albumin reagent (Bromocresol green method), while total protein was measured using the general Biuret method. The albumin-to-total protein ratio was also calculated.

Globulins

Globulins were calculated as the difference between total protein and albumin:
Globulins = Total Protein−Albumin

Gamma Glutamyl Transferase (GGT)

GGT activity was determined using a spectrophotometric method based on the Szasz (1974) procedure. Serum samples were incubated with a substrate, gamma-glutamyl-p-nitroanilide. The hydrolysis of the substrate produces p-nitroaniline, which was measured at 405 nm. GGT activity was expressed in IU/L.

Bilirubin (Total, Direct, Indirect)

Total and direct bilirubin levels were measured using the Malloy and Evelyn (1937) method. Serum (0.1 mL) was treated with diazo reagent, and the resulting colour intensity was measured spectrophotometrically at 540 nm. Indirect bilirubin was calculated as the difference between total and direct bilirubin.

Markers of Renal Function

Urea

Urea concentration was determined using the urease-berthlot method (Fawcett & Scott, 1960). A serum sample (0.1 mL) was treated with urease, which hydrolyses urea to ammonia. The ammonia was then reacted with phenol and hypochlorite to produce a colorimetric compound, which was measured at 540 nm. The urea concentration was determined using a standard curve.

Creatinine

Creatinine levels were measured using the Slot (1965) method. A serum sample (0.1 mL) was mixed with an alkaline picrate solution, which reacts with creatinine to form a coloured complex. The absorbance was measured at 510nm, and the creatinine concentration was calculated using a standard curve.

Electrolytes

Sodium (Na^+)

Sodium concentration was determined using flame photometry (Van Slyke & Palmer, 1927). A serum sample was aspirated into a flame, and the intensity of the emitted light at 589 nm was used to quantify sodium concentration, compared to a standard curve of known sodium concentrations.

Potassium (K^+)

Potassium concentration was measured in the same manner as sodium, using flame photometry, with emission detected at 766.5 nm.

Chloride (Cl^-)

Chloride concentration was determined using the Schales and Schales (1941) method, where a serum sample (0.1 mL) was mixed with a reagent containing mercuric nitrate. The resulting complex was measured at 350 nm. Chloride levels were quantified based on a standard curve.

Statistical Analysis

Data are hereby expressed as mean $\pm$ standard deviation. Analysis of variance (ANOVA) was used to compare group means, followed by Duncan's Multiple Range Test to identify statistically significant differences ($p < 0.05$).

RESULTS

Table 2: Serum Markers of Liver Function in Rats Fed High-Cholesterol Diet Supplemented with Pumpkin Seed Oil

	1	2	3	4	5	6
AST (U/L)	4.67 ± 0.67^a	5.00 ± 0.58^a	4.67 ± 0.33^a	4.67 ± 0.33^a	5.00 ± 0.58^a	4.33 ± 0.33^a
ALT (U/L)	2.00 ± 0.58^a	2.33 ± 0.33^a	1.67 ± 0.33^a	1.67 ± 0.33^a	1.67 ± 0.33^a	2.00 ± 0.58^a
ALP (U/L)	85.77 ± 1.69^b	147.20 ± 3.88^d	60.00 ± 1.70^a	63.77 ± 1.08^a	136.20 ± 2.11^c	138.9 ± 4.85^c
GGT (U/L)	24.73 ± 0.65^a	99.67 ± 1.20^d	23.40 ± 0.40^a	25.07 ± 0.71^a	58.93 ± 0.58^b	72.50 ± 2.26^c
LDH (U/L)	114.45 ± 0.80^a	225.83 ± 5.73^c	112.48 ± 2.61^a	116.33 ± 1.20^a	130.83 ± 0.74^b	151.20 ± 1.91^c
T. Prot (g/dl)	4.22 ± 0.87^d	7.62 ± 1.04^f	4.42 ± 1.36^d	4.42 ± 0.19^d	4.94 ± 0.26^d	5.45 ± 0.87^e
Alb (g/dl)	2.48 ± 0.92^b	4.37 ± 0.24^d	2.68 ± 0.21^b	2.91 ± 0.15^b	2.73 ± 0.18^b	3.79 ± 1.42^c
Glob (g/dl)	1.70 ± 0.15^a	3.21 ± 0.99^c	1.70 ± 0.19^a	1.49 ± 0.76^a	2.17 ± 0.38^b	1.65 ± 1.51^a
Total B (mg/dl)	2.99 ± 0.04^a	11.46 ± 0.13^d	2.95 ± 0.06^a	3.92 ± 0.05^b	7.54 ± 0.26^c	7.49 ± 0.12^c
DB (mg/dl)	2.12 ± 0.02^a	8.02 ± 0.04^d	2.09 ± 0.53^a	2.93 ± 0.07^b	4.87 ± 0.07^c	4.84 ± 0.04^c
IB (mg/dl)	0.87 ± 0.03^a	3.44 ± 0.14^c	0.86 ± 0.03^a	0.99 ± 0.02^a	2.67 ± 0.24^b	2.65 ± 0.10^b

Values represent the means of 3 determinations. Same superscripts across the same row means they are not significantly ($p>0.05$) different, but differ significantly ($p<0.05$) if the superscripts are different

AST = aspartate transferase; ALT = alanine amino transferase; ALP = alkaline phosphatase; Alb = albumin, T. Prot = total protein; Glob = globulins; GGT = gamma glutamyl transferase; DB = direct bilirubin; IB = indirect bilirubin, LDH = lactate dehydrogenase and Total B = total bilirubin.

1- Group fed diet based on palm oil (Control group)
2- Group fed high-cholesterol diet containing palm oil
3- Group fed diet based on pumpkin seed oil
4- Group fed diet based on palm and pumpkin seed oils
5- Group fed high-cholesterol diet containing pumpkin seed oil
6- Group fed high-cholesterol diet containing palm and pumpkin seed oils

Table 3: Serum Markers of Renal Function in Rats Fed with High-Cholesterol Diet Supplemented with Pumpkin Seed Oil

GROUPS	UREA (mmol/L)	CRT (µmol/L)	Na+ (mg/dL)	K+(mg/dL)	Cl-(mg/dL)
1	2.50 ± 0.17b	5.41 ± 0.15a	122.20 ± 3.68a	4.07 ± 0.33a	73.33 ± .67a
2	4.77 ± 0.15e	15.35 ± 0.11b	214.37 ± 9.00c	7.40 ±0.42c	306.67 ± 6.67b
3	1.23 ± 0.13a	5.15 ± 0.00a	119.60 ± 1.29a	4.47 ± 0.13a	83.33 ± 3.33a
4	2.63 ± 0.17b	6.87 ± 1.72a	124.77 ± 2.39a	5.57 ± 0.49b	110.00 ± 5.78a
5	3.07 ± 0.18c	15.49 ± 0.33b	126.30 ± 1.50a	5.83 ± 0.18b	126.67 ± 7.63a
6	3.93 ± 0.33d	6.87 ± 1.72a	191.73 ± 2.49b	4.83 ± .37ab	123.33 ± 3.33a

Values represent the means of 3 determinations. Same superscripts along the same column means they are not significantly ($p>0.05$) different, but differ significantly ($p<0.05$) if the superscripts are different

CRT = creatinine; Na+ = sodium; K+ = phosphorus and Cl+ = chlorine 1- Group fed diet based on palm oil (Control group)
2- Group fed high-cholesterol diet containing palm oil
3- Group fed diet based on pumpkin seed oil
4- Group fed diet based on palm and pumpkin seed oils
5- Group fed high-cholesterol diet containing pumpkin seed oil
6- Group fed high-cholesterol diet containing palm and pumpkin seed oils

Discussion

Serum activities of enzymes such ALT, AST, ALP, GGT, LDH, and some other metabolites such as concentrations of serum proteins, total and conjugate bilirubin are reliably used to assess liver functions and health as their activities/concentrations change when the liver dysfunctions (Bari *et al.*, 2023).

In this study, there were no variations in the serum activities of AST and ALT, an indication that the diets including the high-cholesterol diet may not have caused any damage to the membranes of the hepatocytes. As severally reported, AST and ALT are markers of hepatocellular injury as they leak into the blood when the hepatocytes are damaged thereby elevating the activities of the enzymes (Sangouni *et al.*, 2022; Idoko *et al.*, 2022).

The non-significant difference in the AST and ALT activities notwithstanding, the high-cholesterol diet may portend great danger to the liver as seen in the higher serum activities of GGT and LDH in the groups concerned. GGT is in the bile duct epithelium and microsomal membranes of the hepatocytes and plays roles in glutathione metabolism and detoxification (Ikeda & Fujii, 2023; Koenig & Seneff, 2015). Its activity is affected more by biliary dysfunction and oxidative stress, and not necessarily by hepatocellular damage (Allameh *et al.*, 2023; Xing *et al.*, 2022). A non-liver specific enzyme, LDH is also found in the kidney, muscle, lung and heart (McGill *et al.*, 2023; Klein *et al.*, 2020; Tapper & Lok, 2017). Like the GGT, its activity is affected by tissue stress and mild membrane injury (Farhana & Lappin, 2025; Forkasiewicz *et al.*, 2020), and not necessarily liver necrosis. High-cholesterol diet has been reported to cause oxidative damage to the biliary duct and metabolic stress of the tissues (Zhang *et al.*, 2024; Pereira *et al.*, 2023; Nuño-Lámbarri *et al.*, 2016) which may have resulted in the elevation of GGT and LDH activities in the serum of the group fed the high-cholesterol diet as shown in the result. The serum activities of the enzymes were not affected in the groups fed diets containing pumpkin seed oil either alone or in combination with palm oil, an indication that the cholesterol and not the palm oil was responsible for the elevation of the GGT and LDH in the group fed high-cholesterol diet containing palm oil. The activities of these enzymes were lowered in the groups fed high-cholesterol diets containing pumpkin seed oil or a combination of it and palm oil which may be due to the anti-oxidative properties of the pumpkin seed oil. As presented earlier, pumpkin seed oil is rich in both MUFA and PUFA. PUFA are precursors of eicosanoids, a class of lipids that modulates inflammation and enhances antioxidant capacity (Palacios *et al.*, 2022; Djuricic & Calder, 2021). Intake of diets rich in MUFA has also been reported to be helpful in managing inflammation (Kim *et al.*, 2025; Grosso *et al.*, 2022) through activation of the peroxisome proliferator-activated receptors (PPARs) by which it reduces pro-inflammatory cytokines (Ali *et al.*, 2023).

Serum total protein concentration is an essential index of the synthetic function of the liver, while albumin is a major protein synthesized exclusively by hepatocytes. Globulins, which comprise a group of immunoglobulins and other serum-binding proteins, also contribute to the body's defence mechanisms and are influenced by nutritional and hepatic states.

In this study, rats fed high-cholesterol diet with palm oil exhibited a significantly elevated total protein level (7.62 ± 1.04 g/dl) compared to the control group (4.22 ± 0.87 g/dl), suggesting a compensatory hepatic response or possible systemic inflammation associated with dyslipidemia. Elevated serum protein levels have been reported in dyslipidemic models and are often reflective of an acute-phase response, characterized by increased production of inflammatory globulins (Kwon *et al.*, 2020).

In contrast, the groups which received diets containing pumpkin seed oil either alone or in combination with palm oil, recorded significantly lower total protein concentrations, ranging from 4.42 to 4.94 g/dl, values comparable to or slightly higher than the control group. This suggests that pumpkin seed oil may exert a stabilizing effect on the synthesis of hepatic proteins, potentially through its anti-inflammatory and antioxidant properties (Hassan *et al.*, 2020). The group fed a combination of palm and pumpkin seed oils with cholesterol showed a moderately elevated total protein value (5.45 ± 0.87 g/dl), indicating a partial modulation of the cholesterol-induced protein response.

A similar pattern was observed with serum albumin. The dyslipidemic group presented the highest albumin concentration (4.37 $\pm$ 0.24 g/dl), which, while seemingly beneficial, could be misleading, as albumin can also be upregulated during compensatory hepatic reactions to inflammation or dehydration (Al-Ghamdi *et al.*, 2023). The groups fed diet with pumpkin seed oil either alone or in combination with palm oil recorded more physiologically consistent albumin levels, the same can be said for the group fed high cholesterol diet with pumpkin seed oil (ranging from 2.68 to 2.91 g/dl), while the group fed high cholesterol diet with a combination of pumpkin seed oil and pal oil showed a higher albumin value (3.79 $\pm$ 1.42 g/dl), reflecting a partially restored synthetic capacity under mixed oil feeding.

The pattern of globulin levels further supports these observations. The highest globulin concentration was recorded in the group fed high cholesterol diet (3.21 $\pm$ 0.99 g/dl), which may reflect heightened immune activation and systemic inflammation due to the cholesterol-rich diet (Mohamed *et al.*, 2021). The group fed diet consisting of pumpkin seed oil either alone or in combination with palm oil, as well as the group fed high cholesterol diet with a combination of pumpkin seed oil and palm oil on the other hand showed globulin levels similar to the control (ranging from 1.49 to 1.70 g/dl), suggesting that pumpkin seed oil may modulate the inflammatory milieu and protein synthesis balance. The group which received high cholesterol diet with pumpkin seed oil had a moderately elevated globulin level (2.17 $\pm$ 0.38 g/dl), possibly indicating an intermediate response.

Collectively, the modulation of serum protein fractions by pumpkin seed oil highlights its potential to maintain hepatic synthetic function and mitigate inflammatory responses under dyslipidemic stress. These findings align with previous reports that bioactive-rich oils can influence protein metabolism and immunomodulation in metabolic disorders (Saleh *et al.*, 2024).

The serum concentrations of bilirubin followed the same trend as in the GGT and LDH; increased in the group fed high-cholesterol diet containing palm oil and lowered in the groups fed high-cholesterol diets containing pumpkin seed oil. Bilirubin is usually elevated when its production, processing by the liver and excretion are disrupted (Vítek & Tiribelli, 2025; Kumbhar *et al.*, 2024). Just as was postulated for GGT and LDH, the elevation of bilirubin in the group fed the high-cholesterol diet containing palm oil is due to metabolic stress by the cholesterol since the groups fed diets based on pumpkin seed oil and a combination of the oil and palm oil without the cholesterol didn't vary from the group fed diet containing palm oil without the cholesterol (control group 1). Its lowering in the groups fed high-cholesterol diet containing pumpkin seed oil, and group fed high-cholesterol diet containing a combination of pumpkin seed oil and palm oil could still be due to the possible anti-oxidative and anti-inflammatory properties of the pumpkin seed oil as earlier discussed. Factors that may cause increased serum bilirubin include oxidative damage to the transport system. For instance, reduced flow of bile as in cholestasis, impaired processing of bilirubin and obstruction of the bile ducts which may result from oxidative damage to hepatocytes and inflammation does cause bilirubin to elevate in the serum (Liu *et al.*, 2023; Martins & Oliveira, 2023). Therefore, anti-oxidants as expected in the pumpkin seed oil may have countered the oxidative and inflammatory effects of the high-cholesterol diet which is reflected in the lowering of the serum bilirubin. Bilirubin, particularly the indirect one is also

raised when red blood cells are damaged (Al-Mashhadani & Bakir, 2024; Rahman & Basu, 2023; Khan & Patel, 2023).

The data on liver function suggest that high-cholesterol diets may not cause liver necrosis, but may cause excretory dysfunction of the liver through oxidative damage to bile ducts which possibly leads to cholestasis. Inclusion of MUFA and PUFA-rich pumpkin seed oil ameliorates the liver dysfunction possibly by countering the cholesterol-causing oxidative stress and inflammation.

The serum concentrations of urea and creatinine are commonly used to assess how well the kidneys are functioning in terms of eliminating waste products from the blood (NFK, 2020; Thomas, 2017). Urea is a waste product of protein catabolism while creatinine is produced during normal protein metabolism (McCance & Huether, 2019). Although eliminated by the kidney, its elevation could be caused by other factors like heart failure (Pottel *et al.*, 2019). **Creatinine is more of a specific marker for renal function and its elevation is mostly due to inefficient filtration rate by** the kidneys (Dixon *et al.*, 2017).

The group fed high-cholesterol diet containing palm oil (group 2) exhibited the highest levels of urea (4.77 ± 0.15 mmol/L) and creatinine (15.35 ± 0.11 μmol/L), indicating renal stress likely due to dyslipidemia-induced nephropathy. These findings align with previous studies that suggest hypercholesterolemia contributes to renal dysfunction via oxidative stress and endothelial dysfunction (Adeniyi *et al.*, 2021). The elevated urea levels in this group also suggest impaired renal clearance or increased protein catabolism, potentially indicative of renal dysfunction (Smith *et al.*, 2022), while the elevated creatinine levels reflect reduced glomerular filtration rates (GFR) as reported by Johnson *et al.* (2023).

Rats fed diet based on pumpkin seed oil without cholesterol showed significantly reduced urea (1.23 ± 0.13 mmol/L) and creatinine (5.15 ± 0.00 μmol/L) suggesting that cholesterol and not palm oil is to blame for the elevated urea and creatinine in the group fed high-cholesterol diet containing palm oil (group 2). The group which received a high-cholesterol diet containing pumpkin seed oil, exhibited improved renal function parameters. This suggests a nephroprotective effect of pumpkin seed oil, possibly due to its rich antioxidant and anti-inflammatory components, such as tocopherols and polyunsaturated fatty acids (Lemhadri *et al.*, 2020). This improvement may indicate that while pumpkin seed oil provides some protection, it may not entirely counteract the adverse effects of excessive cholesterol intake. This is consistent with the improved lipid profiles in the groups fed high-cholesterol diet containing pumpkin seed oil or a combination of the pumpkin seed oil and palm oil as earlier presented. **Kidney disease** often causes **abnormal lipid profiles** due to **impaired clearance** of lipoproteins as a result of reduced kidney function, just as inflammation and oxidative stress in kidney disease contribute to lipid abnormalities. This creates a vicious cycle as dyslipidemia also contributes to cardiovascular disease, and consequently kidney disease (Abidor *et al.*, 2025; Badve *et al.*, 2024; Suh and Kim, 2023).

Electrolytes balance is regulated by the kidneys and abnormalities in their serum concentrations signals possible kidney dysfunction (Martin, 2024; Sumida *et al.*, 2018). The serum concentrations of these electrolytes which include sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) are therefore vital indicators of the kidney health (Martin, 2024; Khan

et al., 2024). Elevated Na^+ (214.37 ± 9.00 mg/dL), K^+ (7.40 ± 0.42 mg/dL), and Cl^+ (306.67 ± 6.67 mg/dL) were observed in the group fed high-cholesterol diet containing palm oil, suggesting potential disruptions in renal filtration and electrolyte handling. Similar findings have been reported in studies linking dyslipidemia to altered sodium and potassium regulation, which may predispose individuals to hypertension and cardiovascular disease (Okoye *et al*., 2022). Conversely, the group fed on pumpkin seed oil supplemented diet exhibited Na^+ (119.60 ± 1.29 mg/dL), K^+ (4.47 ± 0.13 mg/dL), and Cl^+ (83.33 ± 3.33 mg/dL) levels comparable to the control group. This normalization of electrolytes suggests that pumpkin seed oil may help mitigate electrolyte imbalances associated with high cholesterol intake. The group which received a high-cholesterol diet with pumpkin seed oil, showed improved but still elevated electrolyte levels compared to the high-cholesterol-only group, reinforcing the protective role of pumpkin seed oil. The study suggests that a high-cholesterol diet negatively impacts renal function likely due to oxidative damage and lipid accumulation in the kidneys (Kumar *et al*., 2023). Pumpkin seed oil supplementation demonstrated a potential reno-protective effect, reducing renal stress markers and restoring electrolyte balance if included in high-cholesterol diet.

CONCLUSION

The present study demonstrates that pumpkin (Cucurbita pepo Linn) seed oil exhibits significant protective effects on hepatic and renal function in rats fed high-cholesterol diets. Elevated levels of liver enzymes and renal markers observed in the cholesterol-only group confirm that dyslipidemia compromises organ function. However, dietary supplementation with pumpkin seed oil, either alone or in combination with palm oil, attenuated these pathological changes and brought most parameters closer to baseline values observed in the control group. These findings underscore the potential role of pumpkin seed oil as a functional dietary component capable of modulating lipid-induced hepatic and renal impairments.

The hepatoprotective and renoprotective effects observed in this study may be attributed to the rich profile of bioactive constituents in pumpkin seed oil, particularly polyunsaturated fatty acids, phytosterols, and antioxidants. These compounds are known to suppress oxidative stress, enhance membrane stability, and regulate lipid metabolism (Hassan *et al*., 2020; Saleh *et al*., 2024). The results provide experimental evidence supporting the inclusion of pumpkin seed oil in the diet for individuals at risk of or currently managing dyslipidemia and its associated organ dysfunctions.

RECOMMENDATIONS

1. Dietary Application: Based on these findings, it is recommended that pumpkin seed oil be considered as a dietary supplement for individuals with dyslipidemia, particularly as an alternative to oils high in saturated fats.
2. Clinical Evaluation: While the current study provides strong preclinical evidence, clinical trials in human populations are necessary to validate the efficacy and safety of pumpkin seed oil in modulating hepatic and renal functions in dyslipidemic patients.
3. Mechanistic Studies: Further investigations should explore the molecular mechanisms underlying the observed protective effects, with a focus on the antioxidant and anti-inflammatory pathways modulated by pumpkin seed oil.

4. Long-term Studies: Extended-duration studies are needed to assess the long-term safety, efficacy, and potential cumulative effects of dietary pumpkin seed oil consumption on metabolic health.

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