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AMELIORATIVE EFFECTS OF *CUCURBITA PEPO* L. SEED OIL ON INFLAMMATION AND OXIDATIVE STRESS INDUCED BY HIGH-CHOLESTEROL DIET IN MALE WISTAR RATS

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ABSTRACT
Inflammation and oxidative stress are core contributors to the pathogenesis of metabolic diseases associated with dyslipidemia. The need to modulate these processes through dietary interventions remains a promising strategy. This study investigated the antioxidant and anti-inflammatory effects of *Cucurbita pepo Linn* (pumpkin) seed oil in male Wistar rats fed on 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. Serum concentrations of C-reactive protein (CRP), TNF-α, IL-1β, IL-6, as well as oxidative stress markers including SOD, CAT, GPx, GR, melatonin, CoQ10, and MDA, were measured and analysed. Rats fed high-cholesterol diets exhibited significantly elevated inflammatory markers (CRP, TNF-α, IL-1β) and compromised antioxidant defences, with reduced enzymatic activity and increased MDA levels. However, supplementation with pumpkin seed oil—alone or combined with palm oil—attenuated these effects, improving antioxidant enzyme activity, restoring melatonin and CoQ10 levels, and reducing MDA and inflammatory cytokines. Pumpkin seed oil demonstrates potent anti-inflammatory and antioxidant properties that counteract cholesterol-induced metabolic disturbances. Its inclusion in cholesterol-rich diets may mitigate systemic inflammation and oxidative damage.

Keywords: Dyslipidemia; Inflammation; Oxidative stress; Pumpkin seed oil; High cholesterol diet.

INTRODUCTION
Dyslipidemia, a condition characterized by abnormally elevated levels of plasma cholesterol and triglycerides, is a major contributor to the development of metabolic syndrome and cardiovascular diseases globally (Wong *et al.*, 2023). A high-cholesterol diet has been shown to provoke systemic inflammation and oxidative stress, both of which are critical drivers of

endothelial dysfunction, atherogenesis, as well as organ damage (Zhang *et al*., 2022). Dietary habits like high intake of saturated fats and low consumption of fiber, especially when combined with sedentary behaviour, have been implicated in the rising prevalence of dyslipidemia (Gómez *et al*., 2021).

These pathological processes are mediated through increased production of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), along with a decline in the activity of antioxidant defence mechanisms like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Suh *et al*., 2021).

Dietary lipids influence inflammation and redox balance depending on their fatty acid composition. While palm oil is a common cooking fat in many parts of Africa and Asia, its high saturated fat content raises concern in conditions of dyslipidemia (Otunola & Afolayan, 2020). In contrast, pumpkin (*Cucurbita pepo*) seed oil (PSO) is rich in mono- and polyunsaturated fatty acids, tocopherols, phytosterols, and other bioactive compounds with reported antioxidant and anti-inflammatory effects (Hassan *et al*., 2020; Palacios *et al*., 2022). These bioactive compounds may improve lipid metabolism and modulate cytokine production and oxidative stress, offering therapeutic potential in conditions of metabolic dysfunction (Djuricic & Calder, 2021).

Pumpkin seed oil has been utilized in traditional medicine for long to treat and/or manage a variety of ailments. Widely cultivated, the plant species belongs to the *Cucurbitaceae* family and stands out among the many plants used in traditional African medicine due to its versatile uses in both culinary and medicinal practices (Bunaciu *et al*., 2021). Recent research has further highlighted the potential of pumpkin seed oil as a functional food, offering promising health benefits, especially in the management of various diseases (Yang *et al*., 2020).

Despite these promising attributes, the combined effects of PSO on inflammatory and oxidative markers under cholesterol-induced stress have not been comprehensively studied. Biomarkers such as malondialdehyde (MDA), melatonin, and coenzyme Q10 (CoQ10), along with inflammatory cytokines, provide critical insight into the pathophysiological state and the efficacy of interventions. This study therefore investigates the potential of pumpkin seed oil to attenuate inflammation and oxidative stress induced by high-cholesterol diets in male Wistar rats.

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
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.

SERUM ANTIOXIDANT ENZYMES

Malondialdehyde (MDA)

MDA levels, an indicator of lipid peroxidation, were determined using the thiobarbituric acid Reactive Substances (TBARS) assay (Yagi, 1987). A serum sample (0.1 mL) was reacted with thiobarbituric acid at 95°C for 1 hour. The resulting pink-coloured complex was measured spectrophotometrically at 532 nm. MDA concentration was determined from a standard curve of 1,1,3,3-tetramethoxypropane.

Superoxide Dismutase (SOD)

SOD activity was measured using the method described by Marklund and Marklund (1974). Serum samples (0.1 mL) were added to a reaction mixture containing pyrogallol. The change in absorbance at 420 nm due to inhibition of pyrogallol oxidation by SOD was used to calculate the enzyme activity.

Catalase

Catalase activity was determined by measuring the decrease in absorbance of hydrogen peroxide at 240 nm using the method by Aebi (1984). A 0.1 mL serum sample was added to a reaction mixture containing hydrogen peroxide, and the rate of decomposition was monitored spectrophotometrically.

Glutathione Peroxidase (GPx)

GPx activity was measured using the method described by Paglia and Valentine (1967). Serum samples were incubated with hydrogen peroxide and glutathione. The reduction of NADPH was monitored at 340 nm, which correlates with GPx activity.

Glutathione Reductase (GR)

GR activity was determined by monitoring the reduction of glutathione disulfide in the presence of NADPH, following the method of Carlberg and Mannervik (1985). The decrease in absorbance at 340 nm was used to determine the activity.

Melatonin

Melatonin levels were measured using ELISA, as described by Hassan *et al.* (2015). Serum samples were reacted with anti-melatonin antibodies, and the bound complexes were quantified by the colorimetric reaction at 450 nm.

Coenzyme Q10 (CoQ10)

CoQ10 levels were determined using high-performance liquid chromatography (HPLC), following the method of Ganguly *et al.* (2009). A serum sample was extracted and analysed on a reverse-

phase HPLC column. The CoQ10 concentration was determined by comparing the sample peak to a CoQ10 standard.

SERUM MARKERS OF INFLAMMATION

C-reactive Protein (CRP)

CRP levels were measured using an enzyme-linked immunosorbent assay (ELISA) according to the method described by Westwood *et al.* (2000). A 100 µL serum sample was added to a pre-coated plate with anti-CRP antibodies. After incubation and washing, a substrate solution was added, and the absorbance at 450 nm was measured. CRP concentrations were calculated from a standard curve.

Tumour Necrosis Factor-alpha (TNF-α)

TNF-α levels were determined by ELISA using a commercial TNF-α kit (e.g., R&D Systems). A serum sample (100 µL) was added to a plate coated with specific anti-TNF-α antibodies. The reaction was developed using a colorimetric substrate, and absorbance was measured at 450 nm.

Interleukin-6 (IL-6)

IL-6 levels were quantified using ELISA following the protocol by Feldmann *et al.* (1996). A serum sample was incubated with a specific anti-IL-6 antibody, and the bound complexes were detected using a secondary enzyme-linked antibody. The colour intensity was measured at 450 nm.

Interleukin-1β (IL-1β)

IL-1β was also quantified by ELISA, following the protocol by Dinarello (2009). The assay involved coating a plate with anti-IL-1β antibodies and detecting bound IL-1β using a colorimetric reaction, measured at 450 nm.

RESULTS

Serum Markers of Inflammation in Rats Fed With High-Cholesterol Diet Supplemented with Pumpkin Seed Oil

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

GROUPS	C-RP (mg/ml)	TNFα (ng/L)	IL-6 (ng/L)	IL-1B (ng/L)
1	117.07 ± 1.57^{a}	99.47 ± 1.35^{b}	13.17 ± 0.09^{a}	22.80 ± 0.20^{a}
2	229.33 ± 2.03^{c}	154.97 ± 2.29^{e}	13.89 ± 0.11^{a}	51.57 ± 1.22^{d}
3	112.43 ± 4.34^{a}	79.67 ± 1.20^{a}	13.34 ± 0.28^{a}	23.20 ± 0.20^{a}
4	111.00 ± 1.16^{a}	99.44 ± 0.97^{b}	13.48 ± 0.13^{a}	22.52 ± 0.27^{a}
5	183.67 ± 11.67^{b}	127.00 ± 0.58^{c}	13.57 ± 0.20^{a}	39.03 ± 1.18^{b}
6	180.67 ± 1.20^{b}	143.76 ± 0.88^{d}	13.37 ± 0.36^{a}	47.50 ± 2.26^{c}

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

CRP = C – reactive protein; TNFα = tumour necrosis factor alpha; IL6 = interleukin 6; IL – 1B = interleukin 1B
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

Serum Markers of Oxidative Stress in Rats Fed With High-Cholesterol Diet Supplemented With Pumpkin Seed Oil

Table 3: Serum Markers of Oxidative Stress in Rats Fed with High-Cholesterol Diet Supplemented with Pumpkin Seed Oil (mg/dL)

	1	2	3	4	5	6
SOD	3.62 ± 0.17^{a}	2.57 ± 0.29^{b}	3.59 ± 0.26^{a}	3.60 ± 0.90^{a}	3.59 ± 0.01^{a}	3.60 ± 0.23^{a}
CAT	$0.77 \pm .02^{b}$	0.48 ± 0.15^{d}	0.87 ± 0.22^{a}	0.80 ± 0.07^{a}	0.67 ± 0.2^{c}	0.60 ± 0.12^{c}
GPx	14.13 ± 0.13^{c}	6.67 ± 0.33^{a}	20.43 ± 0.27^{d}	19.83 ± 0.60^{d}	11.40 ± 0.00^{b}	11.22 ± 0.14^{b}
GR	2.57 ± 0.18^{b}	0.96 ± 0.04^{a}	2.43 ± 0.27^{b}	3.16 ± 0.12^{c}	1.07 ± 0.08^{a}	1.07 ± 0.08^{a}
MLTN	35.33 ± 0.88^{e}	14.34 ± 0.08^{a}	37.63 ± 0.86^{f}	30.00 ± 0.32^{d}	25.27 ± 0.27^{c}	22.23 ± 0.12^{b}
CoQ10	0.90 ± 0.06^{c}	0.35 ± 0.01^{a}	0.87 ± 0.04^{c}	0.87 ± 0.02^{c}	0.61 ± 0.01^{b}	0.35 ± 0.03^{a}
MDA	0.11 ± 0.00^{d}	0.30 ± 0.01^{a}	0.12 ± 0.01^{d}	0.13 ± 0.0^{c}	0.15 ± 0.02^{c}	0.19 ± 0.01^{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

MDA = malondialdehyde; SOD = superoxide dismutase; CAT = catalase and MLTN = melatonin
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

The enzymes; catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are essential components of the body's enzymatic antioxidant defence. They help in neutralizing reactive oxygen species (ROS) and limit oxidative stress (Asatiani *et al*., 2025; Ighodaro & Akinloye, 2017). Other markers of oxidative stress assessed include melatonin, coenzyme Q10 (CoQ10) and MDA (malondialdehyde). Melatonin and coenzyme Q10 (CoQ10) are also important in determining the antioxidant status, while MDA is more particular about cellular damage (Zarezadeh *et al*., 2022; Hacişevki & Baba, 2018). The activities of all the studied antioxidant enzymes were decreased in the group of high-cholesterol diet containing palm oil. This is indicative of an overwhelmed antioxidant defence. SOD scavenges free radicals by converting highly reactive and damaging radical into a less reactive form (H_2O_2),

which is finally converted to harmless compounds (H_2O and O_2) by catalase or GPx (Ighodaro & Akinloye, 2018). In addition to this, GPx is a major detoxifier of peroxides in lipid membranes, preventing oxidative damage to lipids, proteins and DNA (Iglesias-Matesanz *et al*., 2024). Like dyslipidemia, its low activity is positively correlated with cardiovascular diseases (Alexander *et al*., 2024). The low activity of GR in this group is expected since GPx was also low, and since its main function is to support continuous GPx activity by supplying glutathione (Negm *et al*., 2025; Dawi *et al*., 2024).

Also lowered in the group fed high-cholesterol diet containing palm oil are melatonin and coenzyme Q10 (CoQ10). Melatonin is a hormone, but also a powerful endogenous antioxidant, scavenging not only reactive oxygen species (ROS) but also reactive nitrogen species (RNS) like hydroxyl radicals, hydrogen peroxide and peroxynitrite (Monteiro *et al*., 2024; Hardeland, 2018). Furthermore, melatonin upregulates antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Zhang *et al*., 2024). This has made it one of the most studied biomarkers in nutraceutical researches for conditions that are oxidative stress related. The lower CoQ10 in this group means that production of ATP is less efficient as CoQ10 is helpful in shuttling in the mitochondrial respiratory chain, a mechanism by which it reduces free radical generations (Borges, 2024; Fišar & Hroudová, 2024). Being a lipid-soluble antioxidant, it protects the cell membranes and lipoproteins from lipid peroxidation, and also helps in regenerating vitamin E, another lipid-soluble antioxidant (Marcheggiani *et al*., 2023). MDA was raised in the group fed high-cholesterol diet, another important pointer that this group suffered oxidative stress. MDA is formed when polyunsaturated fatty acids in cell membranes are attacked by free radicals (Saeed *et al*., 2024; Mohideen *et al*., 2023). With the reduced CoQ10 which protects the cell membranes and lipoproteins from lipid peroxidation, and decrease in other defence oxidative systems, the increased attack on the membrane is not surprising. It is however not clear why the activities of AST and ALT were not affected by the high-cholesterol diets. These enzymes do leak into the blood when the cell membranes are attacked and damaged as the results of inflammation and oxidative stress suggest.

Sozen *et al*. (2018) reported accumulation of oxidized cholesterol derivatives in the heart following consumption of high-cholesterol diet. These derivatives activate scavenger receptors and consequently lead to inflammation and oxidative stress. Dietary cholesterol had long been reported to increase the susceptibility of low-density lipoprotein (LDL) to oxidation (Schwab *et al*., 2000) which is a leading process in the development of atherosclerosis since oxidized LDL is more readily taken up by macrophages. This leads to foam cell formation and plaque buildup in arterial walls (Sun *et al*., 2024). Oxidative stress was also reported in liver tissues of rabbits fed high-cholesterol diet as demonstrated by decreased SOD and glutathione peroxidase (GPx) (Attia *et al*., 2010).

Interestingly, these oxidative stress biomarkers were significantly increased mostly in a dose dependent manner, though most were below the values in the control. This also gives confirmation to earlier assumptions that pumpkin seed oil is high in antioxidants, and that the antioxidant properties of the seed oil are majorly responsible for the cardio-protection, hepato-protection, reno-protection and improved inflammatory response in the groups fed high-cholesterol diets containing the seed oil. The inclusion of the seed oil having up regulated the antioxidant defence system, was able to significantly reduce the consequences of oxidative stress

on the organs. The anti-oxidation also improved the lipid profile which implies reduced risks of cardiovascular diseases.

The earlier result had shown improved inflammatory response in the groups fed high-cholesterol diets containing the pumpkin seed oil, and it aligns with the improved anti oxidative defence in these groups, as inflammation and oxidative stress are bidirectional and synergistically related. Each can be initiated, amplified, and sustained by the other (Nediani *et al.*, 2024; Muro *et al.*, 2024). Recently, some studies demonstrated the potential of pumpkin seed oil as a natural antioxidant which could improve oxidative status and prevent oxidative tissue damages (Hegab *et al.*, 2025; Mukhtar *et al.*, 2025; Abdelnour *et al.*, 2023; Abou-Zeid *et al.*, 2018).

The values of these oxidative markers were mostly stable in the groups fed the diets based on the pumpkin seed oil and a combination of the seed oil and palm oil without cholesterol. This is consistent with previous results, and consistently showing that the cholesterol is to blame for the metabolic dysregulations, and not palm oil. And that the pumpkin seed oil is better than palm oil in preventing and managing high-cholesterol-induced metabolic defects. It is therefore scientifically logical to reason that patronizing pumpkin seed oil particularly in diets prone to containing high cholesterol would raise the defence system against the consequences of oxidative stress.

C-reactive protein (CRP) is well-established as a non-specific marker of systemic inflammation, and rises when there is immune disorder, infections and cardiovascular disease (Smith *et al.*, 2023). Interleukin – 6 (IL-6), tumour necrosis factor – α (TNF-α) and interleukin – 1β (IL-1β) play crucial roles in initiating and sustaining inflammatory responses, and very helpful in determining the nature of inflammation (Katkenov *et al.*, 2024; Paranga *et al.*, 2024; Chang *et al.*, 2023). While TNF-α and IL-1β are particularly elevated in autoimmune disease, auto-inflammatory diseases and infections respectively, IL-6 elevation is associated with severity of disease (Sooranahalli *et al.*, 2025; Tylutka *et al.*, 2024). Therefore, their concentrations are useful in monitoring response to inflammatory managements with their decline signalling successful treatment (Faraj & Jalal, 2023; Zhang & Jiang, 2019).

The group on high-cholesterol diet containing palm oil exhibited the highest levels of C-RP, TNFα and IL-1B suggesting a strong pro-inflammatory response induced by hypercholesterolemia. This finding aligns with previous studies indicating that excess cholesterol intake promotes systemic inflammation through oxidative stress and immune activation (Johnson *et al.*, 2021). It confirms the postulation that systemic inflammation may be the root cause of other metabolic alterations in the group fed the high-cholesterol diet containing palm oil as earlier presented.

It is worthy of note that inclusion of pumpkin seed oil alone or combined with palm oil may not eliminate the inflammatory effect of the cholesterol but could however ameliorate the effect as shown in the intermediate values of C-RP, TNFα and IL-1B between the control and the group fed high-cholesterol diet containing palm oil. This modulatory effect of the pumpkin seed oil against cholesterol-induced inflammation makes it clearer that the oil has anti-inflammatory properties which is traceable to its contents of unsaturated fats and other bioactive components that include tocopherols and phytosterol. These compounds interfere with inflammation signalling cascades and down regulate the production of pro-inflammatory cytokines such as TNFα and IL-1B through down-regulation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (Sun *et al.*, 2024; Micallef & Sathyapalan, 2023). Further, they down

regulate the expression of COX-2 and iNOS all of which lead to suppression of inflammation (Konuskan *et al.*, 2019).

When pumpkin seed oil is used alone or combined with palm oil without cholesterol, no adverse inflammatory response was observed as they compared well with the group fed diet based on palm oil without cholesterol (control). The results have consistently shown that palm oil which is one of the most common edible oils has no deleterious effect if used in normal diets. However, it cannot offer protections against high-cholesterol-induced metabolic abnormalities.

The results also show that IL-6 may not be a sensitive indicator of diet-induced inflammation since the serum concentration was not affected by different dietary treatments. Its elevation is associated with severity of disease (Sooranahalli *et al.*, 2025). In addition, IL-6 had been reported to play both anti- and pro-inflammatory roles, and to be influenced by different factors that include both acute and chronic inflammation (Hunter & Jones, 2015). This makes it special, and makes its interpretation a bit challenging.

Recently, it was demonstrated that pumpkin seed oil has nutraceutical impact on expression levels of EZH-2 and KRT-14 genes against DSS-induced inflammatory bowel disease in the rat model (Solomon *et al.*, 2025). Similarly, Abdel-Hakeem *et al.* (2024) reported that the seed oil has potential in controlling inflammation and pathogenicity during experimental trichinellosis.

This anti-inflammatory effect of the oil is very instructive since many non-communicable, and many of the metabolic diseases are strongly and positively correlated with inflammation. The nutraceutical effects of the seed oil may therefore be helpful in managing many inflammation-related diseases.

CONCLUSION

High-cholesterol intake was shown to impair antioxidant defence mechanisms, elevate lipid peroxidation, and provoke systemic inflammatory responses, as indicated by increased serum levels of malondialdehyde (MDA), C-reactive protein (CRP), TNF-α, and IL-1β. The present study demonstrates that supplementation with *Cucurbita pepo* (pumpkin) seed oil significantly ameliorates inflammation and oxidative stress induced by a high-cholesterol diet in male Wistar rats. However, dietary inclusion of pumpkin seed oil either alone or in combination with palm oil markedly improved antioxidant enzyme activities (SOD, CAT, GPx, GR), restored melatonin and CoQ10 concentrations, and attenuated inflammation. These findings reinforce the therapeutic potential of pumpkin seed oil in mitigating the biochemical and physiological consequences of dyslipidemia. Therefore, the inclusion of pumpkin seed oil in diets susceptible to cholesterol overload may serve as a nutraceutical strategy for preventing or managing metabolic dysfunctions.

RECOMMENDATIONS

1. Dietary Intervention: Regular dietary incorporation of pumpkin seed oil is recommended, especially in populations at risk of dyslipidemia or cardiovascular diseases, to help reduce inflammation and oxidative damage.
2. Further Research: Future studies should investigate the molecular mechanisms by which pumpkin seed oil exerts its antioxidant and anti-inflammatory effects, including its role in gene expression and modulation of signalling pathways such as NF-κB and MAPK.

3. Clinical Relevance: Given the promising results observed in animal models, clinical trials are warranted to validate the efficacy of pumpkin seed oil in human populations suffering from or predisposed to metabolic syndrome, dyslipidemia, or inflammatory conditions.
4. Development of Functional Food: Food scientists and nutritionists should explore the incorporation of pumpkin seed oil into functional food formulations or supplements aimed at promoting metabolic health and reducing chronic disease risk.

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