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Physiological Effects of Aqueous Myristica fragrans Extract on Lipid Profiles, Oxidative Stress, and Organ Function in Hyperlipidemic Male Albino Rats

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Abstract. Hyperlipidemia is a leading risk factor for cardiovascular diseases and metabolic disorders, often exacerbated by high-fat diets. This study evaluates the physiological effects of aqueous Myristica fragrans (nutmeg) extract on hyperlipidemia-induced male albino rats. A total of 30 rats were divided into four groups: a control group, a high-fat diet group, a high-fat diet supplemented with 50 mg/kg nutmeg extract, and a nutmeg-only group. Over a month, lipid profiles, glucose levels, oxidative stress markers, and liver and kidney functions were assessed, nutmeg extract significantly reduced blood glucose levels from 188.5 mg/dL in the high-fat diet group to 124.25 mg/dL in the treated group ($p < 0.05$). Oxidative stress markers showed notable improvement, with malondialdehyde (MDA) levels decreasing from 2.94 µmol/L in the high-fat diet group to 1.43 µmol/L in the treated group. However, glutathione (GSH) levels decreased to 1.74 µmol/L in the treated group compared to 2.34 µmol/L in controls, indicating a selective antioxidant response. Lipid profile analysis revealed mixed results; triglycerides increased to 134.53 mg/dL in the treated group, suggesting limited lipid-lowering effects. Notably, liver function tests indicated hepatotoxic effects in the nutmeg-only group, with ALT levels rising to 142.17 mg/dL (control: 101.2 mg/dL), warranting caution for standalone use. Kidney function remained relatively stable, with creatinine levels unaffected, but urea levels increased to 68.87 mg/dL in the high-fat diet group with nutmeg supplementation. The findings suggest that nutmeg extract has promising hypoglycemic and antioxidant properties but limited efficacy in improving lipid profiles and potential risks to liver health. Further research is recommended to optimize dosage and evaluate long-term safety for clinical applications in managing hyperlipidemia.

Highlights:

1. Hypoglycemic Effects: Nutmeg extract reduced blood glucose levels significantly (188.5 → 124.25 mg/dL).
2. Antioxidant Properties: Improved oxidative stress markers but decreased glutathione levels selectively.
3. Risks: Limited lipid-lowering effects; potential hepatotoxicity observed in nutmeg-only group.

Keywords: Myristica fragrans, hyperlipidemia, Lipid Profiles, Oxidative Stress, and Organ , Albino Rats

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Introduction

Hyperlipidemia is a critical risk factor for chronic diseases such as cardiovascular disorders, atherosclerosis, and renal dysfunction [1,2], and given the strong association between elevated plasma cholesterol levels and atherosclerosis, which can lead to ischemic heart disease, myocardial infarction, and stroke [3], the focus has shifted toward preventive strategies with an emphasis on early detection and management of cardiovascular risk factors [4]. By 2030, more than 24 million people are expected to suffer from cardiovascular events annually [5]. Increases in cholesterol and triglyceride levels contribute to oxidative stress, inflammation, and metabolic disturbances, causing organ damage, especially in the liver and kidneys, and may lead to chronic kidney disease [6,7]. Cardiovascular diseases account for 30% of global deaths and 10% of the global health burden, making them the leading causes of death and healthcare expenditure worldwide , they contribute to approximately 12 million deaths annually. High-cholesterol diets contribute significantly to hyperlipidemia, atherosclerosis, and ischemic heart disease, increasing the risk of cardiovascular complications [8,9,10]. Lifestyle factors such as diets high in saturated fats, age, family history, and hypertension significantly influence the risk of cardiovascular disease. However, high low-density lipoprotein (LDL-C) remains the primary factor in the onset of the disease [11]. Studies have also reported intracellular lipid accumulation and structural and functional changes in myocardial tissue due to high-cholesterol diets [12,13]. Previous findings suggest that hyperlipidemia induced by such diets reduces the cardioprotective effects of ischemic conditioning through nonvascular mechanisms [14,15], over the past three decades, studies have emphasized the use of medicinal plants in traditional medicine as alternative therapies, highlighting the pharmacological potential of plant-derived products [16]. Research shows that extracts from Averrhoa carambola are rich in bioactive compounds, including flavonoids, saponins, alkaloids, tannins, proanthocyanins, vitamin C, and gallic acid, all of which exhibit antioxidant and therapeutic properties [17,18]. Previous studies have shown that the methanolic extract of A. carambola leaves (MEACL) has anti-hyperlipidemia and antioxidant effects in a mouse model of Poloxamer 407-induced hyperlipidemia, which is comparable to atorvastatin [19].

Myristica fragrans a spice commonly used in traditional medicine, is recognized for its diverse pharmacological properties. Native to Indonesia and found in regions such as

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India, Sri Lanka, and Malaysia, nutmeg has been extensively cultivated and valued for its medicinal and culinary applications [20]. Numerous phytochemical and pharmacological studies report that extracts from different parts of nutmeg are rich in flavonoids, saponins, alkaloids, tannins, proanthocyanins, and essential oils, which possess antioxidant, anti-inflammatory, and therapeutic properties. Nutmeg contains bioactive compounds such as myristicin, elemicin, and essential oils that have shown protective effects against oxidative stress and lipid peroxidation [21].Nutmeg consumption has been reported to enhance immunity, detoxify the body, and exhibit anti-inflammatory, analgesic, and neuroprotective effects [22]. Additionally, nutmeg extracts have shown hepatoprotective, antihyperlipidemic, and antidiabetic properties, making them potentially beneficial in managing conditions like nonalcoholic fatty liver disease and hyperlipidemia. In a previous study, aqueous nutmeg extract demonstrated significant antihyperlipidemic and antioxidant effects in a hyperlipidemic rat model induced by a high-fat diet, comparable to those achieved with atorvastatin. This discovery has spurred further investigations into the potential benefits of aqueous nutmeg extract in managing hyperlipidemia and oxidative stress [23,24].

This study aims to explore the physiological effects of an aqueous extract of nutmeg on lipid profiles and related physiological parameters in a controlled animal model, focusing on key biomarkers of kidney health such as creatinine and urea.

Methods

Preparation and Extraction of Nutmeg

M. fragrans (Nutmeg) plants used in this study were procured from local markets in Baghdad. The plants were ground using an electric grinder to obtain a powdered form. The aqueous extraction of nutmeg was prepared following the method described by [25]. Ten grams of nutmeg powder were soaked in 500 mL of distilled water in a glass container with an additional 200 mL of distilled water. The solution was mixed using a magnetic stirrer for 10 minutes and then filtered through a gauze. The aqueous portion was further filtered using filter paper to remove impurities, concentrated again using a rotary evaporator at 45°C to dry the remaining liquid, and stored in glass bottles labeled for future use at 4°C [26].

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Experimental Design

A total of 30 animals were included in the study, with obesity induced by adding 8% animal fat, 2% cholesterol, and 3% nutmeg extract to their diet for two months [27]. At the end of the period, lipid profiles were assessed in serum (5 animals were tested to confirm obesity compared to samples from the first group). The animals were then divided into the following three subgroups:

- Control Group (C): This group consisted of 10 animals fed a standard diet and given regular water for one month.
- Treatment Group (T1): This group included 10 animals fed a high-fat diet.
- Treatment Group (T2): This group included 10 animals fed a high-fat diet and given 50 mg/kg of aqueous nutmeg extract for one month (Tuzcu et al., 2017).
- Treatment Group (T3): This group included 10 animals given 50 mg/kg of aqueous nutmeg extract only.

Study Parameters

Blood Glucose Levels: Blood glucose levels were measured following the procedure provided by Randox Laboratories (France) using three reagents. Reagent 1 contained phosphate buffer (pH 7.5) at 150 mmol/L and phenol at 7.5 mmol/L. Reagent 2 contained glucose oxidase (GOD) at 12,000 U/L, peroxidase at 660 U/L, and 4-aminoantipyrine at 0.40 mmol/L. Reagent 3 contained glucose at 5.55 mmol/L (100 mg/dL) [28].

Liver Enzyme Activity: The activities of transaminase enzymes (AST and ALT) were measured using kits from Giesse Company (Italy), following the colorimetric method described by [29].

Serum Urea Concentration: Serum urea concentration was determined enzymatically using reagents provided by BioMerieux (France). Serum creatinine levels were measured using the colorimetric method with protein removal. Reagents for this process were supplied by Randox (UK).

Serum Cholesterol Levels: The total serum cholesterol was determined using a kit, with the final reaction product being quinoneimine, which absorbs at 546 nm. The process involved converting cholesterol esters into fatty acids and cholesterol via cholesterol esterase, followed by the oxidation of cholesterol into cholesterol-4-en-3-one

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and hydrogen peroxide by cholesterol oxidase. In the presence of peroxidase, hydrogen peroxide reacted with aminoantipyrine and phenol to produce quinoneimine, whose color intensity was proportional to serum cholesterol levels, Low-density lipoprotein cholesterol (LDL-C) was calculated using the formula [30]:

Triglyceride levels were measured using the Biomerieux 69280 IE kit (France) via an enzymatic method involving lipase to hydrolyze triglycerides into glycerol and fatty acids. The glycerol underwent enzymatic reactions involving glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase to produce quinoneimine, whose color intensity corresponded to triglyceride concentration in serum [31].

Oxidative Stress Enzymes: Malondialdehyde (MDA) levels in serum were measured using a colorimetric method to determine oxidative stress. MDA, a primary lipid peroxidation product, reacts with thiobarbituric acid (TBA) under acidic conditions to form a colored complex with a peak absorption at 532 nm, Glutathione (GSH) levels in serum were assessed using a modified method [32], employing Ellman's reagent (DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid)). DTNB reacts with GSH to form a yellow compound measurable at 412 nm, with the resulting absorbance directly proportional to GSH concentration in the serum.

Result and Discussion

Blood Glucose Levels:

The current study's findings, as illustrated in Figure 1, revealed a significant decrease ($P < 0.05$) in blood serum glucose concentration in male white rats of the T2 group compared to the negative control group (C), Additionally, a significant decrease ($P < 0.05$) in glucose concentration was observed in group T1 compared to group T2.

The results also demonstrated a significant increase ($P < 0.05$) in glucose concentration in the T3 group (control group) compared to the negative control (C). In contrast, group T4 did not show any significant difference, rats maintained on a normal diet exhibited a mean glucose level of 140.8 mg/dL, which served as a baseline for comparison. In the high-fat diet group (T1), the glucose levels significantly increased to 188.5 mg/dL, indicating that the high-fat diet induced hyperlipidemia, commonly associated with elevated glucose levels—a hallmark of metabolic syndrome. For the high-fat diet supplemented with nutmeg extract (T2), the glucose levels significantly

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decreased to 124.25 mg/dL. This suggests that the nutmeg extract exerted a potent glucose-lowering effect, mitigating the hyperglycemic impact of the high-fat diet. Importantly, rats treated with nutmeg extract alone (T3, without a high-fat diet) exhibited glucose levels similar to the control group, averaging 137 mg/dL. This indicates that nutmeg extract does not significantly alter glucose levels in healthy rats, reinforcing its specific efficacy in counteracting diet-induced hyperglycemia.

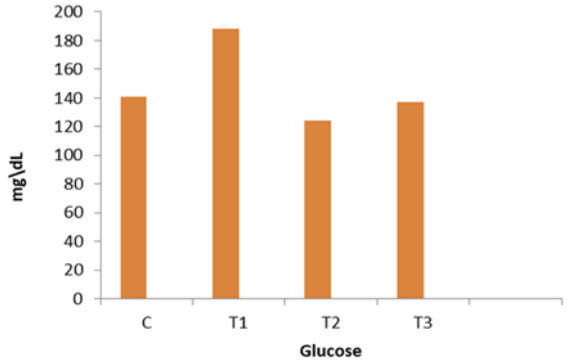

Figure 1: Effect of nutmeg extract on glucose levels in male rats in the experiment.

The LSD (least significant difference) value at $p < 0.05$ is 5.79, indicating that differences between groups exceeding this value are statistically significant. Based on these results, nutmeg extract effectively lowers blood glucose levels in hyperlipidemic rats, suggesting potential benefits in the management of conditions such as hyperlipidemia and hyperglycemia.

Blood lipid concentrations

Table 2 presents blood lipid values in male albino rats under different conditions, to evaluate the effect of nutmeg extract M. fragrans on elevated lipid levels. The measured lipid parameters include total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and triglycerides (TG).

Total cholesterol (TC): The control group (C) showed normal total cholesterol levels at 50.41±1.90 mg/dL. The high-fat diet group (T1) showed a slight increase to 52.19±2.43 mg/dL, reflecting an early onset of hyperlipidemia. The high-fat diet with nutmeg extract (T2) resulted in a moderate increase in total cholesterol to 56.78±2.45

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mg/dL, indicating partial efficacy in cholesterol control. However, nutmeg extract alone (T3) caused a significant increase in TC to 81.28±6.16 mg/dL, suggesting that the extract itself raises cholesterol levels when taken without a high-fat diet. This may reflect a specific effect of the extract or its components on lipid metabolism.

High-density lipoprotein (HDL): The control group (C) showed healthy levels of HDL at 28.32±2.43 mg/dL, in the high-fat diet group (T1), HDL decreased to 26.60±0.32 mg/dL, which is common in hyperlipidemia. The high-fat diet plus nutmeg extract (T2) also decreased HDL levels to 23.95±2.84 mg/dL, indicating an aggravating effect. Nutmeg extract alone (T3) significantly decreased HDL to 21.01±0.55 mg/dL, confirming a significant decrease in HDL cholesterol levels.

LDL levels in the control group (C) were normal at 7.56±0.35 mg/dL. The high-fat diet group (T1) showed an increase to 11.13±0.09 mg/dL, as expected. The high-fat diet with nutmeg extract (T2) caused a further increase to 12.77±0.21 mg/dL, indicating that the extract did not reverse the LDL increase. Nutmeg extract alone (T3) significantly increased LDL to 14.72±2.10 mg/dL, raising concerns about its independent effect on LDL levels. The control group (C) showed normal LDL levels at 12.71±0.20 mg/dL. The high-fat diet group (T1) experienced an increase to 14.29±2.05 mg/dL, consistent with hyperlipidemia. With a high-fat diet and nutmeg extract (T2), LDL increased to 16.75±0.67 mg/dL, indicating that the extract did not prevent the increase. Nutmeg extract alone (T3) significantly increased LDL to 19.09±5.43 mg/dL, indicating that the extract promoted the increase in LDL levels. Triglycerides (TG): The control group (C) showed normal triglyceride levels at 70.91±2.99 mg/dL. The high-fat diet group (T1) experienced an increase to 111.41±14.45 mg/dL, which is consistent with hyperlipidemia. With the high-fat diet and nutmeg extract (T2), triglyceride levels increased to 134.53±18.23 mg/dL, indicating an insufficient antagonistic response by the extract. Nutmeg extract alone (T3) caused a significant increase to 166.78±27.06 mg/dL, indicating that the extract independently induces a significant increase in triglyceride levels. LSD values indicate that differences beyond the specified limits (e.g., 1.29 for TC) are statistically significant at $p < 0.05$, confirming significant changes in lipid levels between groups.

Table 1: Effect of nutmeg extract on some blood lipid parameters in male rats in the experiment

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Groups	TC mg/DI	HDL mg/DI	LDL mg/DI	VLDL mg/DI	TG mg/DI
C	50.41±1.90[d]	28.32±2.43[d]	7.56 ±-0.35d	12.71±0.20[d]	70.91±2.99[d]
T1.	52.19±2.43[c]	26.60±0.32[c]	11.13 ±0.09[c]	14.29±2.05[c]	111.41±14.45[c]
T2.	56.78±2.45[b]	23.95±2.84[b]	12.77 ±-0.21[b]	16.75±0.67[b]	134.53±18.23[b]
T3.	81.28±6.16[a]	21.01±0.55[a]	14.72 ±2.10[a]	19.09±5.43[A]	166.78±27.06[a]
LSD 5%	2.87	0.15	0.07	0.78	20.07

Live Enzymes

The results presented in Table 2 demonstrate the effects of the aqueous extract of nutmeg M, fragrans on male albino rats with hyperlipidemia, focusing on alanine aminotransferase (ALT) and aspartate aminotransferase (AST), These enzymes are key markers of liver function, with elevated levels typically indicating liver damage or stress, in the control group (C), ALT levels were 101.2 ± 6.12 mg/dL, representing normal liver function. In the high-fat diet group (T1), ALT levels increased slightly to 102.37 ± 1.3 mg/dL, indicating mild liver stress associated with the high-fat diet, which may be linked to fat accumulation in the liver (hepatic steatosis). In the high-fat diet group treated with nutmeg extract (T2), ALT levels showed a slight increase to 102.71 ± 6.37 mg/dL. Nutmeg extract did not significantly reduce ALT levels in hyperlipidemic rats, suggesting minimal protection against liver stress under these conditions.

In contrast, in the nutmeg extract-only group (T3), ALT levels increased significantly to 142.17 ± 5.57 mg/dL. For AST, the control group showed levels of 34.46 ± 2.19 mg/dL, indicating normal liver enzyme activity. In the high-fat diet group (T1), AST levels increased slightly to 37.62 ± 3.5 mg/dL, reflecting some liver stress caused by the high-fat diet. In the high-fat diet group with nutmeg extract (T2), AST levels were 35.25 ± 3.41 mg/dL. Although this value was higher than the control group, it was comparable to the control group, suggesting that nutmeg extract might have a slight protective effect in preventing further AST elevation in hyperlipidemic rats, in the nutmeg extract-only group (T3), AST levels significantly increased to 41 ± 4.03 mg/dL, indicating significant liver stress when nutmeg extract was administered alone. These findings are consistent with the elevated ALT levels observed in this group. The least significant

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difference (LSD) value for ALT at $p < 0.05$ was 4.38, indicating that differences between groups exceeding this value are statistically significant.

The results suggest that while nutmeg extract does not significantly affect ALT and AST levels in rats on a high-fat diet (T2), it has a pronounced negative effect on liver enzyme levels when administered alone (T3). The significant increases in ALT and AST in the T3 group indicate that nutmeg extract may have hepatotoxic effects under certain conditions, particularly when used independently. The high-fat diet alone caused mild liver stress (T1), but combining the high-fat diet with nutmeg extract (T2) did not lead to additional increases in liver enzymes. This could imply a partially protective or neutral effect on liver enzyme regulation under hyperlipidemic conditions, overall, while nutmeg extract may not exacerbate liver enzyme levels in hyperlipidemic rats, its standalone use raises concerns about potential hepatotoxicity, as evidenced by the significant elevation of both ALT and AST in the T3 group. Further research is needed to clarify the conditions under which nutmeg extract is safe for liver health.

Table 2: Effects of nutmeg extract on liver enzymes in male albino rats.

Treatments	AST mg/Dl	ALT mg/Dl
C	34.46±2.19a	101.2±6.12a
T1	37.62±3.5b	102.37±1.3b
T2	35.25±3.41ac	102.71±6.37c
T3	41±4.03d	142.17±5.57d
LSD0.05	12.65	4.38

Kidney enzymes

Table figure 2 the levels of urea and creatinine, two key indicators of kidney function. Elevated levels of these indicators can indicate kidney stress or dysfunction, as the kidneys are responsible for filtering waste products such as urea and creatinine from the blood. The urea levels in the control group (C) were 36.75 mg/dL, reflecting normal kidney function. In the high-fat diet group (T1), urea levels increased slightly to 39.95 ± 3.46 mg/dL, indicating mild, though not severe, kidney stress from the high-fat diet.

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In the high-fat diet group supplemented with nutmeg extract (T2), urea levels increased significantly to 68.87 mg/dL, representing a significant increase compared to both the control and T1 groups, This suggests that while nutmeg extract may regulate other parameters (as previously observed with glucose), it appears to cause kidney stress or impair kidney function when combined with a high-fat diet.

In contrast, the group that consumed only nutmeg extract (T3) had urea levels of 36.023 mg/dL, which is close to the levels of the control group, suggesting that nutmeg extract alone does not significantly affect urea levels and reflects normal kidney function. In this case, regarding creatinine levels, the control group (C) showed values of 0.023 mg/dL, representing normal kidney function. In the group that consumed a high-fat diet (T1), creatinine levels rose significantly to 4.46 mg/dL, suggesting some kidney stress or possible impairment caused by the high-fat diet. This increase suggests that the kidneys may be struggling to filter waste products from the blood effectively. In the group that consumed a high-fat diet with nutmeg extract (T2), creatinine levels were 3.41 mg/dL, slightly lower than in the T1 group. Despite the significant increase in urea within the same group, creatinine levels remained closer to the normal range. This may suggest that nutmeg extract has a complex effect on kidney function, varying depending on the waste product being measured. In the group that consumed nutmeg extract alone (T3), creatinine levels were 0.353 mg/dL, similar to control values, suggesting that nutmeg extract alone does not cause significant stress to the kidneys, as creatinine filtration remained within the normal range.

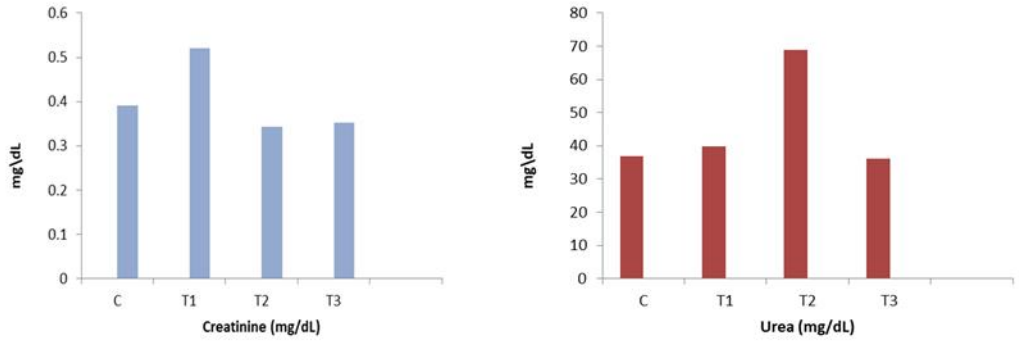

Figure 2: Effect of nutmeg extract on kidney enzymes in male rats.

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The least significant difference (LSD) values for statistical significance were 8.54 for urea and 5.054 for creatinine at $p < 0.05$. Changes in these parameters that exceed these values are statistically significant, and while nutmeg extract alone does not appear to negatively affect kidney function, its combination with a high-fat diet raises concerns about potential stress on the kidneys, especially with regard to urea levels.

Oxidative stress enzymes

Figure 3 shows data on glutathione (GSH) and malondialdehyde (MDA) levels, which are key indicators of oxidative stress. Glutathione is an antioxidant enzyme that protects cells from oxidative damage, while malondialdehyde is a by-product of lipid peroxidation and serves as an indicator of oxidative stress. In the control group (C), glutathione levels were 2.34 µmol/L, reflecting normal antioxidant capacity in the absence of oxidative stress. In the high-fat diet group (T1), glutathione levels increased to 2.95 µmol/L, indicating a compensatory increase in antioxidant activity in response to the oxidative stress induced by the high-fat diet. This suggests that the body is trying to upregulate glutathione to combat oxidative stress caused by fat accumulation. In the group that consumed a high-fat diet with nutmeg extract (T2), GSH levels decreased significantly to 1.74 µmol/L, indicating a decrease in antioxidant defense when nutmeg extract was added to the high-fat diet. This suggests that nutmeg extract may not support, or may even weaken, antioxidant defenses in the context of a high-fat diet. In the group that consumed nutmeg extract alone (T3), GSH levels were 1.93 µmol/L, lower than the control group but higher than the T2 group. This suggests that nutmeg extract alone may slightly reduce antioxidant capacity, but not as severely as when combined with a high-fat diet.

The control group demonstrated a stable antioxidant defense mechanism with optimal GSH levels. A high-fat diet alone induces oxidative stress, leading to a compensatory increase in GSH levels to combat lipid peroxidation and protect cells. When nutmeg extract was added to a high-fat diet, the antioxidant response was significantly impaired, as reflected by decreased GSH levels. This suggests that nutmeg extract may interfere with the body's ability to manage oxidative stress under high-fat conditions. Nutmeg extract itself moderately reduced GSH levels, suggesting a slight decrease in antioxidant efficacy, but not to a worrying extent under normal dietary conditions.

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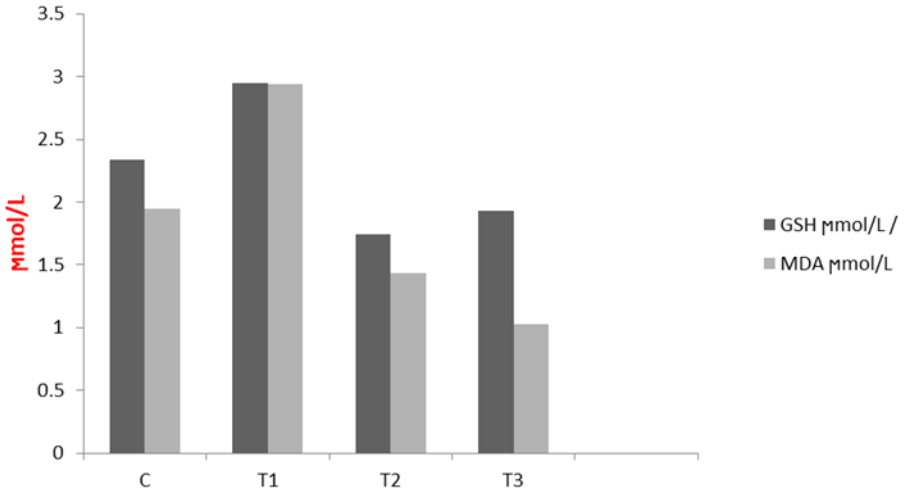

Figure 3: Effect of nutmeg extract on oxidative stress enzymes in male rats.

MDA levels (an indicator of oxidative stress) in the control group (C 1.95 µmol/L, indicating normal levels of lipid peroxidation and oxidative stress, The high-fat diet group (T1) had significantly elevated MDA levels to 2.94 µmol/L, indicating increased lipid peroxidation and oxidative stress due to the high-fat diet. This is consistent with the known oxidative damage that accompanies hyperlipidemia. The high-fat diet with nutmeg extract (T2) had decreased MDA levels to 1.43 µmol/L, indicating that nutmeg extract has a protective effect against oxidative stress when combined with a high-fat diet. Despite the decreased GSH levels, nutmeg extract appeared to reduce lipid peroxidation and oxidative damage. Nutmeg extract alone (T3) decreased MDA levels further to 1.03 µmol/L, indicating the strongest reduction in oxidative stress, indicating Nutmeg extract alone has potent antioxidant properties, significantly reducing MDA levels and indicating reduced oxidative damage. The Least Significant Difference (LSD) values for GSH and MDA are 0.43 and 0.32, respectively. Any differences in these parameters greater than these values are considered statistically significant at $p < 0.05$. In the control (C) treatment, normal GSH and MDA levels indicate a balanced oxidative stress state with adequate antioxidant defense. In the high-fat diet (T1) group, increased GSH and MDA levels reflect an increased state of oxidative stress due to the high-fat diet. The body responds to increased oxidative damage by upregulating antioxidant defenses, as in the case of elevated GSH, although lipid peroxidation remains elevated. The decrease in MDA levels at T2 suggests that nutmeg extract helps reduce oxidative stress in hyperlipidemic rats, although the decrease in GSH suggests that this protection

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may come at the expense of decreased antioxidant enzyme activity. Nutmeg extract alone shows a strong antioxidant effect [31,32], as evidenced by the significant decrease in MDA levels and the relatively modest decrease in GSH. This suggests that nutmeg extract can effectively alleviate oxidative stress, possibly by preventing lipid peroxidation and reducing oxidative damage. It also shows a dual role in managing oxidative stress. In (T2), nutmeg extract reduces oxidative stress (reduced MDA levels) but also appears to reduce antioxidant enzyme activity (reduced GSH levels), suggesting that its protective effect may involve mechanisms other than upregulation of GSH. When taken alone (T3), nutmeg extract significantly reduces oxidative stress (as reflected by decreased MDA levels) and has a mild effect on antioxidant enzyme levels, suggesting a strong antioxidant potential under normal conditions [33,34].

These results suggest that nutmeg extract may have antioxidant potential, particularly for reducing oxidative stress, although its effect on antioxidant enzyme systems such as GSH requires further investigation. Hyperlipidemia is a major risk factor for coronary heart disease, making it a major public health concern. The use of nutmeg as a treatment for dyslipidemia is a universal approach due to its broad pharmacological effects through different mechanisms. In this physiological study, the aqueous extract of nutmeg was investigated for its effect on hyperlipidemia in male albino rats, the process of extracting bioactive compounds from plant materials is a crucial first step in the exploitation of phytochemicals for the development of dietary supplements, pharmaceuticals, and food ingredients. Water is a plant known to effectively extract bioactive compounds, including semipolar compounds. A previous study revealed that the extract exhibited strong anti-hyperlipidemia and antioxidant activities, attributed to its phenolic and flavonoid content, especially high levels of flavonoids. Hence, the aqueous extract of Myrisinus was selected for further investigation in this study of hyperlipidemia in a high-fat diet (HFD) model. Studies have reported that apigenin, a flavonoid found in many plants, possesses biological activities such as antioxidant, tissue protection, and inhibition of hepatic cholesterol synthesis [35,36]. Apigenin has also been shown to improve cardiovascular conditions, stimulate the immune system, reduce plasma LDL-C levels, inhibit platelet aggregation, and reduce cell proliferation. Therefore, apigenin was used as a composite marker to standardize and quantify nutmeg extract, In this study. The extraction process showed minimal loss of apigenin, and its recovery

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data were satisfactory. The accuracy of the method, both intra- and inter-day, indicated reliability, repeatability [37], and reproducibility. The limit of detection (LOD) of apigenin was 0.15 g/mL, while the limit of quantification (LOQ) was 0.625 g/mL, both of which are within the acceptable ranges compared with previous studies [38], in the present study, a 15-day high-fat diet significantly increased total cholesterol (TC) and triglyceride (TG) levels in experimental rats. Oral administration of different doses of aqueous extract of nutmeg significantly reduced the levels of lipids and increased HDL-C to near-normal levels. The dose of 1000 mg/kg was the most effective in reducing TC and TG after five weeks of treatment, which is comparable to atorvastatin in its anti-hyperlipidemia activity [39], HDL-C plays a crucial role in transporting cholesterol from tissues to the liver, where it is metabolized into bile acids, thereby preventing atherosclerosis. Conversely, high triglyceride levels have been linked to the risk of coronary artery disease. Furthermore, oxidized LDL-C in arterial walls promotes cholesterol accumulation, leading to the formation of atherosclerotic plaques, a major factor in atherosclerosis.

The plant extract has dose-dependent inhibitory effects on HMG-CoA reductase and pancreatic lipase, two key enzymes involved in cholesterol and lipid metabolism. Inhibition of pancreatic lipase prevents its hydrolysis and subsequent absorption in the small intestine, a mechanism that may help control hyperlipidemia and obesity. These inhibitory effects may be one of the mechanisms behind the anti-hyperlipidemia properties of nutmeg extract, Oxidative stress has been identified as an early factor in the development of hyperlipidemia【40】. High cholesterol levels can lead to increased oxidative stress, disrupting the integrity of cell membranes and causing lipid peroxidation and protein oxidation [41,42], nutmeg extract exhibited antioxidant effects, significantly increasing the levels of endogenous antioxidant enzymes such as GSH, SOD, CAT, and GPx, while reducing the levels of lipid peroxidation and MDA in a dose-dependent manner. These results highlight the protective role of nutmeg against oxidative damage [43], Nutmeg extract was also found to contain phenolic compounds, including flavonoids such as apigenin, which protect cells from oxidative stress by enhancing antioxidant enzyme activity and reducing inflammation【44] Activation of Nrf2 pathways and inhibition of NADPH oxidase also contributed to the reduction of oxidative stress【45], These mechanisms underscore the potential of Myristica fragrans in the

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management of hyperlipidemia and its associated complications, such as coronary heart disease and atherosclerosis.

Conclusion

This study highlights the dual role of aqueous Myristica fragrans (nutmeg) extract in managing hyperlipidemia-induced physiological changes in male albino rats. The extract demonstrated significant hypoglycemic and antioxidant effects, notably reducing glucose levels to 124.25 mg/dL and malondialdehyde (MDA) levels to 1.43 µmol/L in treated groups. However, its impact on lipid profiles was mixed, with elevated triglyceride levels observed in the nutmeg-only group. Furthermore, hepatotoxic effects were noted, evidenced by ALT elevations to 142.17 mg/dL. Kidney function remained relatively stable. These findings suggest that while nutmeg extract offers therapeutic potential, its clinical application requires caution, dosage optimization, and further safety evaluations.

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