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# Hepatoprotective Effects of *Myristica fragrans* with Concurrent Caffeine Intake: A Histopathological and Biochemical Analysis

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ABSTRACT

The growing prevalence of caffeine consumption has sparked concerns due to limited research on its potential health-related controversies. This investigation aimed to assess the hepatoprotective effect of *Myristica fragrans* following the concomitant administration of caffeine. Twenty-five male Wistar rats were randomly assigned to five groups (A-E), each comprising five rats. The control group (Group A) received distilled water (2 mL/kg), while Group B was administered caffeine (40 mg/kg). Group C received *Myristica fragrans* exclusively at 200 mg/kg. Groups D and E were administered caffeine (40 mg/kg) + *Myristica fragrans* (100 mg/kg) and caffeine (40 mg/kg) + *Myristica fragrans* (200 mg/kg), respectively. All treatments were administered orally for 21 days. Following the 21-day treatment period, the rats body weight changes were determined. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed. Histological examination of the liver was also carried out. Results indicated that the caffeine-only group experienced a statistically significant ($p < 0.05$) reduction in body weight compared to the control group. Conversely, Group C, which received only *Myristica fragrans*, significantly ($p < 0.05$) increased body weight. Serum levels of ALT and AST were significantly ($p < 0.05$) lower in the groups treated with *Myristica fragrans*. Histological analyses demonstrated normal liver architecture in the control and *Myristica fragrans*-only groups, while the caffeine-only group exhibited necrotic liver cell changes. Groups D and E maintained normal liver histology. In conclusion, the concurrent administration of *Myristica fragrans* alongside caffeine appears to mitigate the detrimental histological and biochemical effects of caffeine consumption

***Keywords:*** Histopathological, Caffeine, *Myristica fragrans*, Liver, Antihepatotoxicity.

## Introduction

Caffeine, with the chemical name 1,3,7-trimethylxanthine, is characterized by its distinct bitter flavour.[1] It consists of two interlinked rings belonging to the purine family of compounds.[2,3] This naturally occurring compound can be found in various plants, including tea leaves, cocoa seeds, and certain nuts such as bitter kola and kola nut, as well as in fruits from over sixty different species.[4] Caffeine is extensively used across various industries, particularly in coffee, tea, soft drinks, and some processed food industries. It has a variety of medical uses, such as a muscle relaxant, analgesic, and diuretic. Additionally, caffeine is noted for its neuroprotective properties, which may be attributed to its capacity to modulate and inhibit adenosine receptors.[5-7] Caffeine is among the most widely consumed active compounds globally.[4] The liver, situated in the abdominal cavity, is one of the most crucial organs of the body,[8] performing numerous essential functions.[9]

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As the primary organ responsible for detoxifying xenobiotics, the liver is prone to potential adverse effects following caffeine consumption.[10] This organ processes a significant portion of ingested substances and neutralizes harmful compounds.[11] Studies investigating how caffeine affects the liver have produced conflicting findings. While some studies indicate that caffeine may negatively impact liver health, increased intake, particularly from coffee has been correlated with a slight reduction in mortality rates.[12] Regular caffeine consumption has also been shown to significantly lower liver enzyme levels in males without pre-existing liver conditions.[13] Interestingly, higher caffeine intake correlates with reduced hepatic fibrosis,[14] but has also been associated with increased hepatocellular enzymes.[15] Several factors, including dosage, method of administration, study duration, and individual metabolic differences[16, 17] may influence the variability in these findings.

The Myristicaceae family includes *Myristica fragrans* (nutmeg), which is taxonomically located between the Lauraceae and Annonaceae groups.[10] Indigenous to the Spice Islands of Indonesia, it is also cultivated in regions such as the Caribbean, South Africa, Sri Lanka, and Malaysia.[18] Notable constituents of *Myristica fragrans* include triterpenoids, carbohydrates, alkaloids, glycosides, tannins, phenolic compounds, flavonoids, proteins, amino acids, and phytosterols.[18] Previous studies have highlighted the protective role of *Myristica fragrans* on liver health.[19,20] This plant has demonstrated a protective effect against liver damage induced by thioacetamide and may aid in preventing alcoholic liver disease.[10] Moreover, *Myristica fragrans* has exhibited hepatoprotective properties in instances of hepatocellular injury elicited by lipopolysaccharide (LPS) and D-galactosamine (D-GalN), potentially linked to the presence of myristicin in its oil extract.[21] In light of this, the growing use and industrial utilization of caffeine have revealed why minimal research is being done to resolve the debates surrounding its impacts on humans. This study, therefore, seeks

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to establish the effect of the concomitant administration of caffeine and nutmeg on the liver of Wistar rats.

## Materials and Methods

*Preparation of Myristica fragrans extract*

A sample of *Myristica fragrans* powder, weighing approximately 490 g, was sourced from Ado-Ekiti in Ekiti State. The powder was macerated in 500 mL of absolute ethanol for 72 hours, with occasional stirring with the aid of a mechanical shaker. The extract was filtered using a Whatman No. 1 filter paper. The resulting filtrate was concentrated by gradual heating and constant stirring in a water bath maintained at 40°C until it evaporated to dryness. The dark brown residue obtained was collected for further use, and the percentage yield of the extract was calculated using the formula below (equation 1).

$$\frac{W2}{W1} X\ 100 \quad \text{..... (Eq. 1)}$$

W2 is the final weight of the extract after the extraction process
W1 is the initial weight of the powder sample before extraction
Stock solutions of the extract at concentrations of 100 mg/mL and 200 mg/mL were prepared by dissolving 2 g and 4 g of the extract in 20 mL of distilled water, respectively.

*Preparation of caffeine solution*

High-purity caffeine used in this study was a product of Sigma, Missouri, USA. Stock solution of caffeine (40 mg/mL) was prepared by dissolving 0.8 g of the pure caffeine powder in 20 mL of distilled water, and the resulting solution w was refrigerated until needed.

*Animals*

Twenty-five (25) healthy male Wistar rats, weighing 180 to 220 g, were obtained from the Animal Research Center at Afe Babalola University, Ado-Ekiti (ABUAD), Ekiti State, Nigeria. The animals were housed under standard laboratory conditions. They were fed a diet of conventional laboratory rat pellets with free access to clean water *ad libitum*, and they were acclimatized for two weeks.

*Ethical consideration*

The Ethics and Grant Committees at Afe Babalola University, Ado Ekiti (ABUAD) approved this study under the reference number: ABUADHREC/08/03/2024/2025. All experimental procedures conducted in this research complied with the institutional regulations established by the committee for animal care and use.

*Animal grouping and treatment*

Twenty-five (25) male Wistar rats were randomly assigned to five groups (A-E), each comprising five rats. The control group (Group A) received distilled water at a dose of 2 mL/kg, Group B was administered caffeine at 40 mg/kg, Group C received *Myristica fragrans* exclusively at 200 mg/kg, Group D was administered caffeine (40 mg/kg) and *Myristica fragrans* at 100 mg/kg, and Group E was administered caffeine (40 mg/kg) and *Myristica fragrans* at 200 mg/kg. All treatments were administered orally once daily for 21 days. The treatment schedule is presented in Table 1.

**Table 1:** Administration schedule and dose regimen

| S/N | Group | Treatment | Doses | Route | Duration |
|---|---|---|---|---|---|
| 1 | A | Distilled water | 2 mL/kg | orally | 21 days |
| 2 | B | Caffeine only | 40 mg/kg | orally | 21 days |
| 3 | C | *Myristica fragrans* (nutmeg) only | 200 mg/kg | orally | 21 days |
| 4 | D | Caffeine + *Myristica fragrans* (nutmeg) | 40 mg/kg + 100 mg/kg | orally | 21 days |
| 5 | E | Caffeine + *Myristica fragrans* (nutmeg) | 40 mg/kg + 200 mg/kg | orally | 21 days |

*Blood sample collection and animal sacrifice*

On the 22nd day following the treatment, blood samples were obtained from the rats via cardiac puncture, and used for biochemical analysis. Subsequently, the animals were euthanized through cervical dislocation. The liver was excised and preserved in 10% formal saline before being processed for paraffin wax embedding. Tissue sections were subsequently cut to a thickness of 5 microns.

*Body weight assessment*

Body weights of the rats were measured weekly using a Mettler Toledo P163 weighing scale. The percentage change in weight was calculated using the following formula (Equation 2):

$$\frac{W2-W1}{W1} \text{ x } 100 \quad \text{..... (Eq. 2)}$$

Where;
W1 represents the initial weight of the rats.
W2 denotes the weight measured at the time of sacrifice, referred to as the final weight.

*Biochemical analysis for liver enzymes*

Blood samples intended for liver enzyme analysis were collected via cardiac puncture at the apex of the heart. The blood samples were placed in plain specimen containers, allowed to clot, and then centrifuged at 4000 rpm for 15 minutes. The resulting sera were transferred into vials capped with plastic screw tops. Liver enzyme activities, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were quantified using standard assay kits, adhering strictly to the provided protocols.

*Histological examination*

The liver tissue morphology was assessed using histological and histochemical staining methods, including Hematoxylin and Eosin for general structural evaluation, Masson trichrome for collagen visualization, and Gordon and Sweet stains to highlight connective tissue and reticular fibers.[22]

*Photomicrography*

Stained liver tissues were examined with an Olympus binocular microscope paired with a 5.1-megapixel MV550 camera, integrated with a computer for precise image capture and analysis. The imaging system was calibrated to enhance clarity and resolution. Digital images were acquired at a magnification of 400x for subsequent examination.

*Statistical analysis*

Data were analyzed using GraphPad Prism 5 (Version 5.03; GraphPad Software Inc., San Diego, California). Differences between means were analyzed using one-way analysis of variance (ANOVA), followed by the student-Newman-Keuls post-hoc test for mean comparisons, with statistical significance set at $p < 0.05$.

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## Results and Discussion

*Effect of Myristica fragrans and caffeine on body weight*

A significant reduction in body weight was observed in caffeine-only treated group (Group B) ($F = 3.256$; $p = 0.0373$) compared to the control group. Post-hoc analysis indicated that the body weight of rats in the caffeine-only group was markedly lower than that of the vehicle-treated group. However, the administration of *Myristica fragrans* (nutmeg) extract significantly mitigated the weight-reducing effects of caffeine in a dose-dependent manner (Figure 1).

The mechanisms underlying caffeine-induced weight loss are multifaceted, including increased basal metabolic rates and thermogenesis, with potential inhibition of adipocyte proliferation through suppression of adipogene-related factors.[22,23] Prior studies have linked high doses of caffeine to reduced offspring birth weights, suggesting a possible dose-dependent relationship in the weight-reducing effects of caffeine.[24]

*Myristica fragrans* has been recognized for its pharmacological benefits.[10] The plant has been documented to exhibit antioxidant, analgesic, and anti-inflammatory properties, among others.[25] The weight-stabilizing potential of antioxidant-rich plants such as *Myristica fragrans*, observed in groups treated concomitantly with caffeine, reinforces their role in counteracting caffeine-induced weight loss.[26-27]

*Effect of Myristica fragrans and caffeine on liver enzymes*

Treatment with *Myristica fragrans* extract resulted in no significant alteration in alanine aminotransferase ($F = 0.3527$, $p = 0.8365$) (Figure 2A) and aspartate aminotransferase ($F = 0.2343$; $p = 0.9127$) in comparison to the control group (Figure 2B). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are enzymes primarily located in the liver, heart, and skeletal muscle, among other tissues.[28,29] ALT is a key marker of hepatic health, with elevated levels indicating potential liver damage.[30] This study revealed increased serum activities of both ALT and AST, suggesting hepatocellular disturbances following the administration of caffeine (40 mg/kg). Although conflicting reports exist regarding the impact of caffeine on the liver, some studies documented its hepatoprotective effects, while others highlight its potential hepatotoxicity.[15]

*Liver histopathological findings*

This investigation aimed to elucidate the effects of concurrent caffeine administration and *Myristica fragrans* extract on the hepatic histomorphology of adult Wistar rats. Hematoxylin and eosin staining indicated a normal histomorphological appearance of the liver in both the control and *Myristica fragrans*-only groups, with sinusoids radially oriented towards the central vein. No damage, necrosis, or fatty acid accumulation were observed, hence the organized histoarchitecture of the liver was preserved (Figure 3A and C). In contrast, the caffeine-only group exhibited notable deviations in liver histology, with apparent abnormalities in hepatocytes and portal structures.

There were focal areas of hepatic necrosis characterized by leukocytic infiltration and congestion of the central vein (Figure 3B). Conversely, the 100 mg/kg and 200 mg/kg *Myristica fragrans* groups displayed histomorphological characteristics of the liver that closely resembled normal liver architecture, with minimal perivascular and interstitial inflammatory cell infiltration (Figure 3D and E). The above observation indicates that *Myristica fragrans* potentially gives protective effects against caffeine-induced liver alterations due to its polyphenolic compounds, which enhance antioxidant defenses. Studies have demonstrated the efficiency of Hematoxylin and Eosin stain in histological analysis of the liver.[31-33] Masson trichrome staining was used to evaluate collagen fiber distribution. The control group demonstrated a normative distribution of collagen fibers forming a supportive meshwork around the centrilobular region and in the spaces of Disse (Figure 4A). The caffeine-only group exhibited sparse collagen fiber distribution surrounding the portal triad and sinusoids (Figure 4B). No collagen fiber was detected around the central vein and liver sinusoids in Group C (Figure 4C). The distribution of collagen fibers in groups D and E approached that of normal liver (Figure 4D and E).

The Gordon and Sweet staining revealed a delicate meshwork of reticular fibers in the periportal and centrilobular regions of the liver in the control group (Figure 5A). In group B, collapsed reticular fibers accompanied by irregular reticulin distribution were observed (Figure 5B). Reticular fibers were well-defined in group C, while restoration of reticular fibers was noted in groups D and E. Reticular fibers serve an essential supportive role in liver connective tissue.[34]

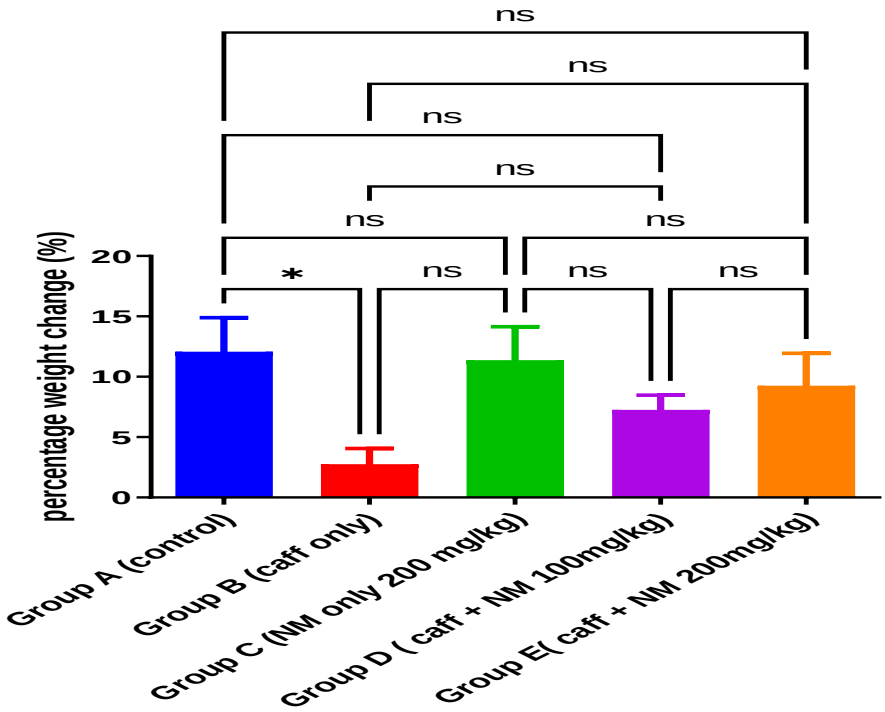


**Figure 1:** Effect of caffeine and M*yristica fragrans* on body weight.
Data represent mean percentage weight change ± SEM (n = 5). * Significant difference ($P < 0.05$); ns = non-significant

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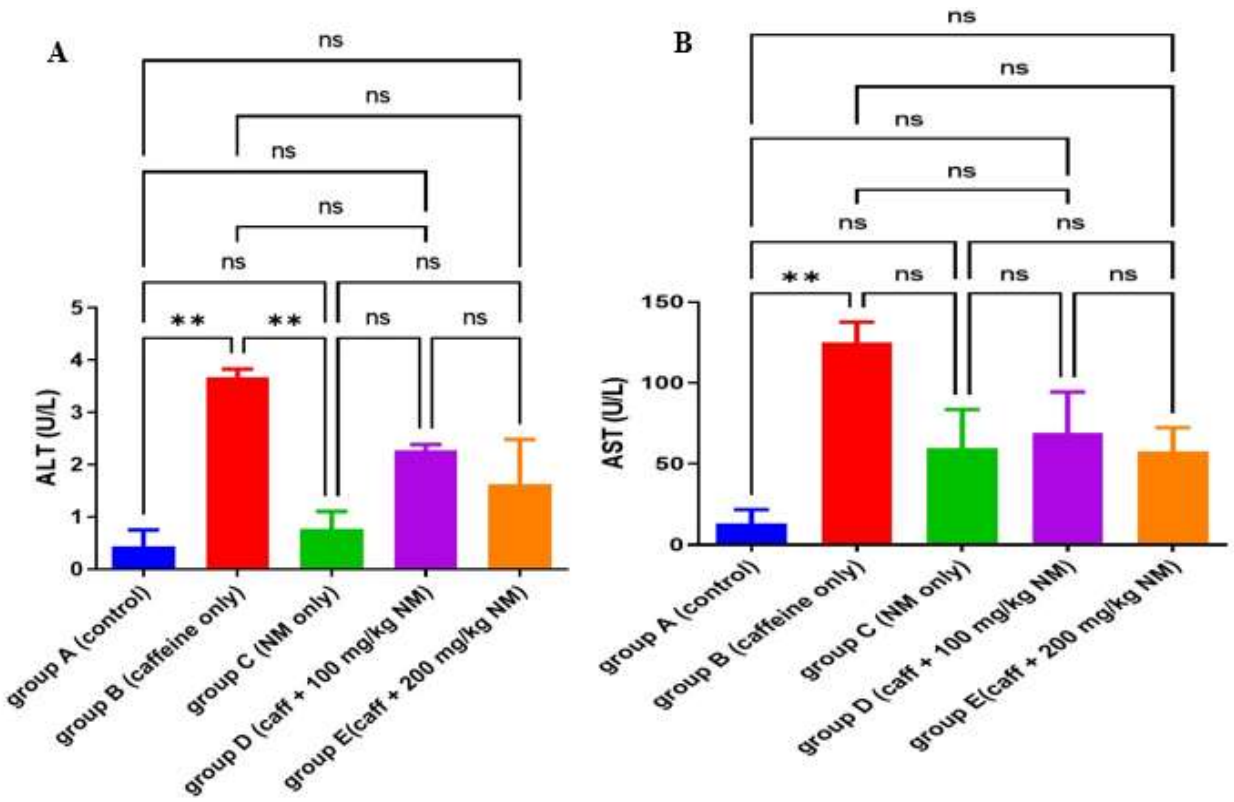


**Figure 2:** Effect of caffeine and M*yristica fragrans* on the activity of serum alanine and aspartate aminotransferases.
Data represent mean ± SEM (n = 5). ** Significant difference (P < 0.05); ns = non-significant

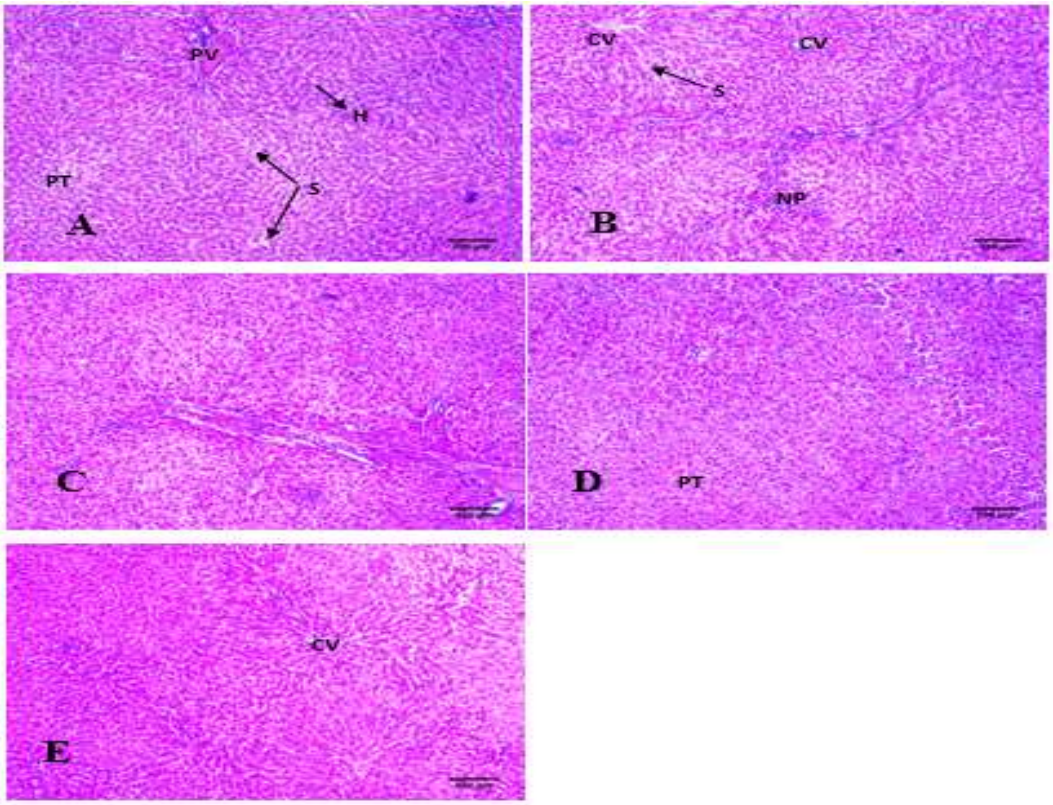


**Figure 3:** Representative light photomicrographs of H and E stained sections of the liver. (A) Normal histoarchitectural organization of the liver, the hepatocytes (H) are disposed in sheet and are separated by radially apparent sinusoids (S) and portal triad (PT) is remarkable in group A; (B) There is congestion of the central vein (CV) and hepatocytes undergoing necrotic changes in group B; (C) The central vein (CV) and portal triad (PT) are well outlined in group C; (D) Liver sections were not free from inflammatory cellular infiltration in group D. (E) The central vein (CV) is well outlined in group E. Scale Bars: 250 µm

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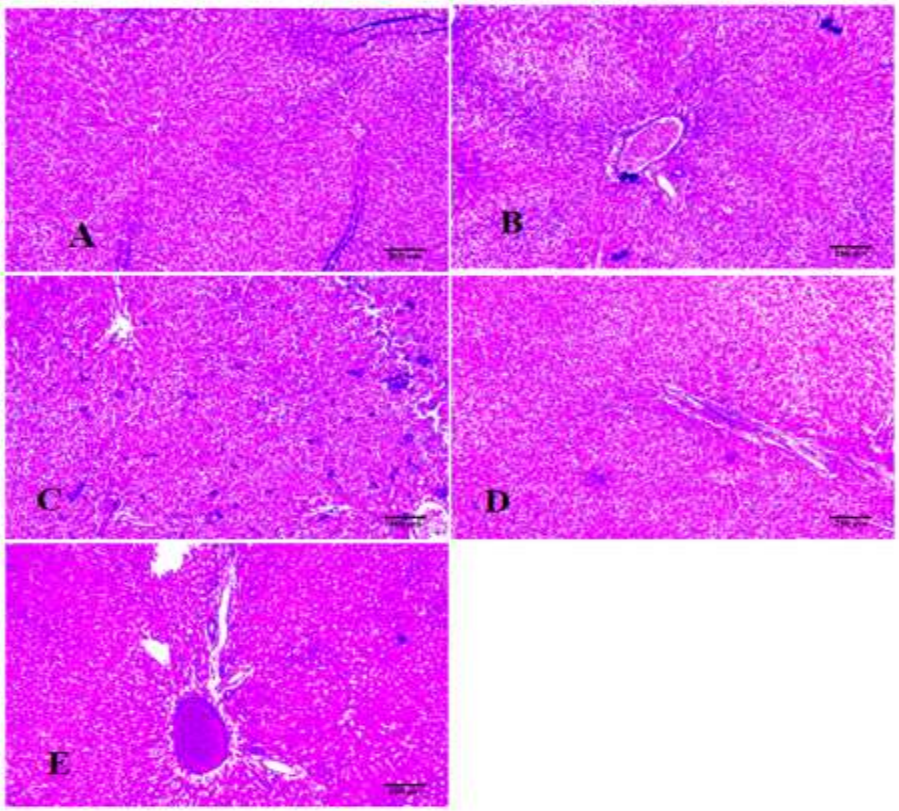


**Figure 4:** Representative light photomicrographs of sections of the liver subjected to Masson trichome stain. (A) Normal supporting collagen fibers forming a meshwork around the spaces of Disse in group A (Control); (B) Increased deposition of collagen fibers was noticed in group B (Caffeine only); (C) Low to no collagen fiber was observed in group C (*Myristica fragrans* only); (D and E) Collagen fibers were observed to be near normal compared to control in group D (40 mg/kg of Caffeine + 100 mg/kg of *Myristica fragrans*), and group E (40 mg/kg of Caffeine + 200 mg/kg of *Myristica fragrans*). Scale Bars: 250 µm

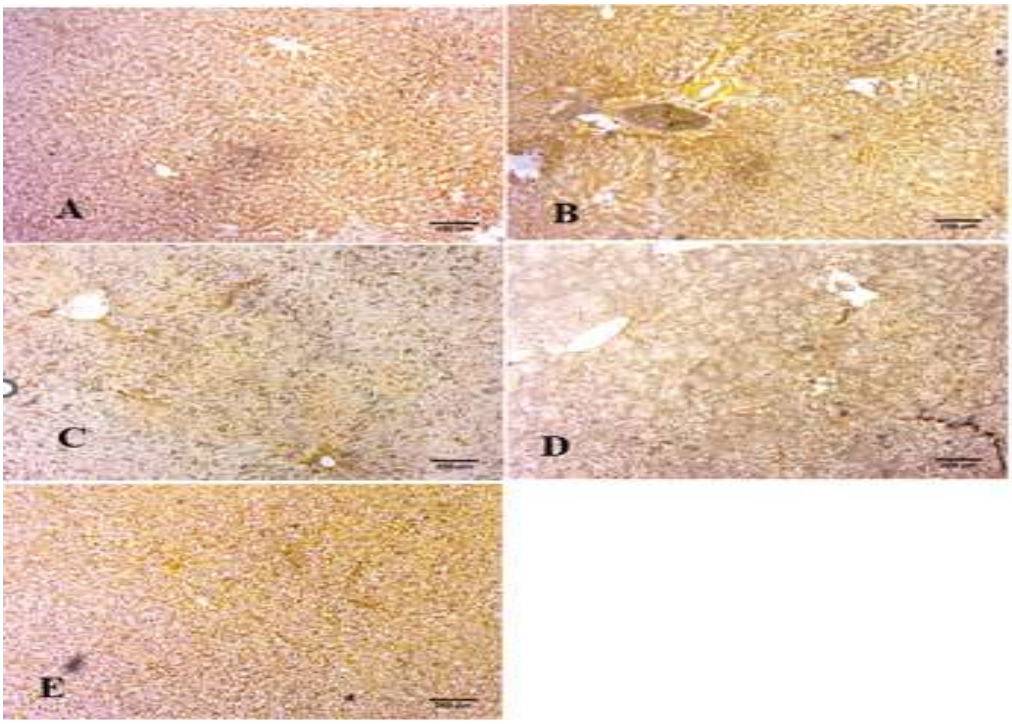


**Figure 5:** Representative light photomicrographs of liver sections impregnated in Gordon and Sweets silver stain. In group A (Control), group B (Caffeine only), group C (*Myristica fragrans* only), group D (Caffeine 40 mg/kg + *Myristica fragrans* 100 mg/kg), and group E (Caffeine 40 mg/kg + *Myristica fragrans* 200 mg/kg), there were reticular fibers forming a delicate meshwork in the periportal and centrilobular areas. In group B, there were areas of collapsed reticular fibers with lumpy reticulin distribution. Scale Bars: 250 µm

An organized reticular fiber meshwork was noted in the control and *Myristica fragrans* groups. Conversely, the caffeine group revealed areas of collapsed reticular fibers, potentially linked to altered extracellular matrix stability due to glycosaminoglycan depletion.[35] The structural integrity of reticular fibers was restored in groups treated with *Myristica fragrans*, indicating a beneficial role of polyphenolic compounds in antioxidant defense mechanisms.[36,37]

## Conclusion

The findings of this study suggest that caffeine may exert adverse effects on liver health, leading to degenerative changes. However, the concurrent oral administration of *Myristica fragrans* demonstrated protective effect on hepatic integrity.

## Conflict of interest

The authors declare no conflicts of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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