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# Evaluation of acute and subacute oral toxicity of ethanol seed extract of *Myristica fragrans* (Houtt.) in female rodents

**Adaeze P. UCHENDU[1*], Ifeoma R. NWAFOR[2], Stellamaris O. OTOGHILE[1], Efe F. ENABULELE[1], Favour F. OLUSHEGUN[1], Fabian C. AMAECHINA[1]**

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.*
[2]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.*

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**Abstract**
*Myristica fragrans* seed (Nutmeg) is widely used as a culinary spice and traditional medicinal agent, but concerns remain regarding its safety at high doses. This study evaluated the acute and subacute oral toxicity of the ethanol seed extract of *M. fragrans* in female rats. Acute toxicity was assessed in female Swiss albino mice using Lorke's method, while subacute toxicity was evaluated in 20 non-pregnant female Wistar rats randomly divided into four groups. The control group received distilled water, while the treatment groups received the ethanol seed extract at various oral doses for 28 consecutive days. Parameters assessed included weekly body weight, haematological and biochemical indices, relative organ weight, and histopathology of the liver, kidneys, heart, and lungs. The acute study showed no mortality or observable signs of toxicity. Subacute administration did not affect body weight or relative organ weights. While most haematological parameters remained normal, significant reductions in platelet count, plateletcrit, and platelet distribution width were observed at 100 mg/kg. Liver and kidney function indices and lipid parameters showed no significant differences compared with the control. Histopathological evaluation revealed normal tissue architecture. These findings indicate that the ethanol seed extract of *M. fragrans* exhibits a low toxicity profile.

*Keywords*: Acute toxicity; Histopathology; *Myristica fragrans*; Nutmeg; Subacute toxicity

## INTRODUCTION

*Myristica fragrans* (Houtt.) is an evergreen tree belonging to the family Myristicaceae [1]. It is the source of two popular spices: nutmeg and mace. Nutmeg is the most valuable part of the plant and is contained in the fruit, while mace is the red covering on the kernel [2,3]. *Myristica fragrans*, although indigenous to Indonesia, is primarily used in West Africa as a flavouring for pastries, meats, sauces, soups, and stews, owing to its aromatic flavour and slightly sweet taste [4-6].

In addition to its culinary use, nutmeg has a long history of use in traditional medicine for the treatment of conditions such as inflammations, gastrointestinal disturbances, respiratory disorders, and female reproductive ailments including dysmenorrhea and amenorrhea [7]. These therapeutic effects are supported by its diverse pharmacological activities, including anti-inflammatory, analgesic, antimicrobial, antioxidant, hepatoprotective, anticonvulsant, and antispasmodic actions [8-10]. Such properties are largely attributed to its complex

*Correspondence. *E-mail*: adaeze.uchendu@uniben.edu *Tel*: +234-8032065268. ISSN 0189-8442

phytochemical composition, which comprises terpenes, alkaloids, phenolics, and bioactive volatile constituents such as myristicin, safrole, and elemicin [11,12].

Existing toxicological studies on *M. fragrans* have predominantly focused on acute exposure, essential oil preparations, or male animal models. However, sex-related differences in hormonal milieu, metabolic enzyme activity, and physiological responses can significantly influence the disposition and toxicity of xenobiotics. As a result, data derived from male models may not accurately reflect toxicological outcomes in females. This limitation is particularly relevant for *M. fragrans*, given its traditional use in managing female-specific conditions, which may increase exposure among female populations. Despite these considerations, there is limited information on the subacute toxicity of ethanol seed extracts of *M. fragrans* in female models. Therefore, this study evaluated the haematological, biochemical, and histopathological parameters following a 28-day oral administration of the ethanol seed extract in female rats, providing insights into its potential systemic toxicity and establishing a basis for safe therapeutic use. This study provides insights into the safety profile and therapeutic implications associated with *M. fragrans* consumption in female rodents. In addition, we believe that our study findings would pave the way for further research into its potential therapeutic applications and optimal dosage.

## EXPERIMENTAL METHODS

**Plant materials.** *M. fragrans* seeds (nutmeg) were sourced from Oba Market, situated at Ring Road, Oredo Local Government Area, Benin City, Edo State, Nigeria, in July 2024. The seeds were identified and botanically authenticated by Professor Henry Akinnibosun Adewale of the Department of Plant Biology and Biotechnology (PBB), University of Benin. The voucher specimen was assigned a number UBH-M537 and was deposited there for future reference.

**Preparation of plant extract.** The seeds were thoroughly cleaned to remove any foreign particles and were ground into a fine powder using an electric blender. A total of 920 g of the powdered seeds underwent extraction with 5 L of absolute ethanol, using a Soxhlet extraction apparatus at 78ºC for 48 hours. The filtrate was then concentrated via solvent evaporation using a rotary evaporator (Bibby Scientific Ltd, Staffordshire, UK), set at a controlled temperature of 60°C with a rotation speed of 90 rpm. The dried extract, was stored in an airtight container and refrigerated at 4°C (to maintain stability) until required.

**Animals.** Non-pregnant female Wistar albino rats (body weight 160 - 185 g; aged 9 - 10 weeks) and Swiss albino mice (body weight 22 - 26 g; aged 8 - 10 weeks) were used in this study. These animals were purchased and housed in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. They were kept in well-ventilated plastic cages of appropriate sizes and were maintained under standard laboratory conditions (12-hour cycle of light and darkness). The cages were lined with clean wood shavings as bedding material, which were changed regularly to maintain hygiene. The animals were provided with Chikun Grower Pellets Feeds (Crown Flour Mill Limited, Lagos, Nigeria) as their standard diet, with a continuous supply of drinking water. Ethical approval (EC/FP/024/13) for this study was obtained from the Faculty of Pharmacy Ethics Committee, University of Benin. All animal handling and experimental procedures followed the guidelines outlined in the Guide for the Care and Use of Laboratory Animals [13]. After a two-week acclimatization period, the animals were carefully assessed, and pregnant rats or mice were excluded from the study.

**Acute toxicity assessment.** An acute toxicity study was carried out on the *M. fragrans* extract using Lorke's model [14]. Lorke's model is a two-phase (phases 1 and 2) approach, and 12 non-pregnant female Swiss mice were used. In phase 1, nine mice were randomly divided into three groups of three mice each. The animals were orally administered different doses (10, 100, and 1000 mg/kg, respectively) of *M. fragrans* extract using an oro-gastric tube. The mice were monitored for toxic symptoms and mortality for the first 8 hours and then 24 hours after administration. In phase 2, three mice were distributed into three groups of one mouse each. Each mouse was orally administered higher doses (1600, 2900, and 5000 mg/kg, respectively) of *M. fragrans* extract and then observed for the first 8 hours, and subsequently 24 hours after administration for toxic symptoms and mortality. Animals were monitored for an additional 2 weeks to detect any potential delayed toxic effects.

**Subacute toxicity assessment.** Sub-acute toxicity of the ethanol seed extract of *M. fragrans* was assessed according to OECD 407 [15] guidelines for oral toxicity in rodents. A total of 20 non-pregnant female Wistar albino rats were randomly divided into four groups of five rats each. The control group (group 1) received 10 ml/kg distilled water, while the other three groups (group 2, 3, and 4) received *M. fragrans* ethanol extract at doses of 100, 500, and 1000 mg/kg, respectively. Treatments were administered orally for 28 consecutive days. The body weight of each rat was recorded before treatment and subsequently monitored weekly throughout the 28-day treatment period using an electronic weighing balance. On Day 29, the overnight fasted animals were anaesthetized under mild chloroform, and a blood sample of each rat was collected via the abdominal aorta into ethylenediamine tetraacetic acid (EDTA) coated tubes and plain tubes (containing no anticoagulant) to analyse haematological and biochemical indices, respectively.

**Haematological analysis.** Blood sample (1 mL) collected in EDTA-coated tubes was analysed for various haematological parameters including, red blood cell (RBC) count, haemoglobin (Hb), haematocrit (Hct), Mean corpuscular volume (MCV) Mean corpuscular Haemoglobin (MCH), Mean corpuscular Haemoglobin concentration (MCHC), White Blood cell count (WBC), platelet count (PLT), Mean corpuscular thickness (MCT), Platelet large ratio (PLR), plateletcrit (PCT), Platelet Distribution Width (PDW), Mean Platelet Volume, Red Cell Distribution Width-Coefficient of Variation (RDW-CV), and Red Cell Distribution Width - Standard Deviation (RDW-SD).

**Biochemical analysis.** Blood (5 mL) placed in the plain tubes was allowed to clot, and then centrifuged at 3000 rpm for 10 min**utes**. The serum obtained was used to analyse the biochemical parameters of the liver, kidney, and lipid profile. Lipid profile parameters analysed include total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Liver function indices included total protein (TP), total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, while renal function was measured through creatinine levels, urea levels, and electrolytes.

**Relative organ weight measurement.** Following blood collection, vital organs (liver, kidneys, lungs, and heart) were carefully excised, cleaned of connective tissues, and weighed using an analytical digital weighing balance.

**Histopathological assessment.** The harvested organs (liver, kidney, lungs, and heart) were fixed in 10% buffered formalin for histopathological examination according to the methods [16-18]. Briefly, the fixed tissues were placed in cassettes and processed, which

involved dehydration through a series of alcohol baths (70%, 75%, 90%, and 95%), followed by clearing in xylene to remove alcohol. Tissues were wax-impregnated, sectioned (5 µm), and H&E stained. Photomicrographs captured via VWR VisiScope digital microscope were evaluated by a pathologist, who was blinded to haematological and biochemical changes.

**Statistical analysis.** All values were expressed as the mean ± standard error of mean (SEM), and the results were analysed statistically by one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc tests using statistical software, GraphPad Prism (GraphPad Software, San Diego, CA, USA) version 8.0. $p < 0.05$ compared to the control was considered statistically significant.

## RESULTS

**Acute oral toxicity study.** The ethanol seed extract of *M. fragrans* caused no deaths or changes in behaviour of the mice within 24 hours of administration and throughout the 14-day observation period. Hence, the estimated $LD_{50}$ for the extract was greater than 5000 mg/kg.

### Subacute oral toxicity study

**Mortality outcome.** During the 28-day subacute toxicity study, there was no mortality recorded in the animals treated with 100, 500, or 1000 mg/kg of ethanol seed extract of *M. fragrans*.

**Changes in body weight.** The effect of 28-day oral administration of *M. fragrans* on body weight is presented in Figure 1. Administration of *M. fragrans* at doses of 100, 500, or 1000 mg/kg did not result in significant body weight changes compared with the control group.

**Changes in organ/body weight ratio.** Following the 28 days of daily oral administration of *M. fragrans*, there were no statistically significant differences ($p$>0.05) in the relative weight of liver, kidney, heart, and lung between the extract-treated rats and control (Figure 2).

**Effect on haematological parameters.** The haematological parameters are summarized in Table 1. Oral administration of *M. fragrans* at the doses of 100, 500, and 1000 mg/kg did not significantly ($p$>0.05) affect most haematological parameters (WBC, RBC, HGB, HCT, MCH, MCHC, RDW-SD, and RDW-CV) compared to the control group. However, the 100 mg/kg-treated group showed decreased PLT, PDW, and PCT.

**Effect on biochemical parameters.** The results of the effect of the extract on biochemical parameters of liver and renal functions, and lipid profile are presented in Tables 2 and 3, and Figure 3, respectively. No significant differences ($p$>0.05) were observed in the measured biochemical parameters (ALT, ALP, AST, TP, bilirubin, creatinine, urea, electrolytes, and cholesterols) between the control and the extract-treated groups.

**Histopathological examination.** Histopathological examinations of vital organs (liver, kidney, heart, and lungs) confirmed normal tissue architecture across all treatment groups (Figure 4). The sections of the liver in control and extract-treated groups showed similar features of hepatocytes (arrows) with eosinophilic cytoplasm surrounding a centrally placed normochromic nucleus with indistinct nucleoli, which are features of normal hepatocytes. The kidney sections of the control and extract-treated groups showed normal glomeruli (thick arrow) containing normal mesangium, blood vessels, and epithelium. The tubules (thin arrow) are oval-shaped and lined by cuboidal epithelium, with some tubules containing pale eosinophilic material. The heart sections of all the groups showed myocytes (arrow) with peripherally placed nuclei surrounded by eosinophilic cytoplasm. The sections of the lung across all the groups showed a normal airway (thick arrow) and alveolar sacs (thin arrow) that are separated by

the interstitium. These features are in keeping with normal hepatocytes, kidneys, myocytes, and lungs.

**Table 1**: Haematological profile of female rats treated with *M. fragrans* for 28 days

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Distilled water (10 mL/kg) | *M. fragrans* (100 mg/kg) | *M. fragrans* (500 mg/kg) | *M. fragrans* (1000 mg/kg) |
| WBC ($10^3$/µL) | 8.18 ± 0.72 | 5.72 ± 0.37 | 5.58 ± 0.40 | 8.04 ± 1.34 |
| LYM (%) | 88.25 ± 1.50 | 89.88 ± 1.56 | 88.66 ± 0.98 | 87.00 ± 1.30 |
| GRAN (%) | 2.32 ± 0.34 | 2.08 ± 0.27 | 2.36 ± 0.31 | 2.92 ± 0.53 |
| RBC ($10^6$/µL) | 6.01 ± 0.08 | 5.85 ± 0.10 | 5.99 ± 0.16 | 6.30 ± 0.17 |
| HB (g/dL) | 13.93 ± 0.23 | 13.10 ± 0.26 | 13.36 ± 0.29 | 13.92 ± 0.30 |
| HCT (%) | 39.43 ± 0.19 | 36.50 ± 0.79 | 38.92 ± 1.00 | 39.64 ± 0.80 |
| MCH (pg) | 23.15 ± 0.32 | 22.34 ± 0.28 | 22.30 ± 0.20 | 22.06 ± 0.41 |
| MCHC (pg/dL) | 35.30 ± 0.51 | 35.84 ± 0.31 | 34.30 ± 0.29 | 35.06 ± 0.48 |
| RDW-SD (fL) | 35.80 ± 1.06 | 32.90 ± 0.85 | 35.46 ± 2.46 | 33.42 ± 1.09 |
| RDW-CV | 15.83 ± 0.42 | 15.30 ± 0.16 | 15.76 ± 0.40 | 15.30 ± 0.16 |
| PLT ($10^3$/µL) | 590.30 ± 40.19 | 442.20 ± 16.60* | 523.60 ± 49.79 | 541.00 ± 36.94 |
| MPV (fl) | 8.38 ± 0.30 | 7.74 ± 0.35 | 8.18 ± 0.18 | 7.96 ± 0.09 |
| PDW (%) | 11.63 ± 0.45 | 9.60 ± 0.45* | 10.76 ± 0.47 | 10.44 ± 0.49 |
| PCT (%) | 0.48 ± 0.02 | 0.34 ± 0.24* | 0.42 ± 0.05 | 0.43 ± 0.03 |

Values are expressed as mean ± SEM. Data were analysed using one-way ANOVA and Tukey's post hoc test. The mean difference is considered significant at $P<0.05$ level compared to the control, number of animals, n=5. WBC, white blood cell; LYM ($10^3$/µL), absolute lymphocyte count; MID ($10^3$/µL), absolute mixed cell count; GRAN ($10^3$/µL); absolute granulocyte count, LYM (%), lymphocyte percentage; MID (%); mixed cell percentage; Hb, haemoglobin; RBC, red blood cell; HCT, haematocrit; MCHC, mean cell haemoglobin concentration; RDW-CV, red cell distribution width coefficient of variation; RDW-SC, red cell distribution width standard deviation; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit.

**Table 2.** Liver function parameters of female rats treated with *M. fragrans* for 28 days

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Distilled water (10 mL/kg) | *M. fragrans* (100 mg/kg) | *M. fragrans* (500 mg/kg) | *M. fragrans* (1000 mg/kg) |
| AST (U/L) | 148.8 ± 10.37 | 179.80 ± 7.97 | 165.4 ± 13.23 | 143.4 ± 11.33 |
| ALT (U/L) | 69.75 ± 4.77 | 83.20 ± 5.62 | 83.00 ± 6.77 | 79.80 ± 4. 95 |
| ALP (U/L) | 258.30 ± 12.72 | 248.20 ± 21.36 | 278.40 ± 36.32 | 272.20 ± 29.54 |
| Total bilirubin (mg/dL) | 0.28 ± 0.03 | 0.24 ± 0.02 | 0.28 ± 0.04 | 0.28 ± 0.04 |
| Direct bilirubin (mg/dL) | 0.15 ± 0.03 | 0.12 ± 0.02 | 0.12 ± 0.02 | 0.12 ±0.02 |
| Total proteins (g/dL) | 6.68 ± 0.24 | 6.82 ± 0.23 | 7.16 ± 0.19 | 6.64 ± 0.16 |
| Albumin (g/L) | 3.20 ± 0.16 | 3.44 ± 0.27 | 3.78 ± 0.38 | 3.32 ± 0.12 |

Values represent mean ± SEM. Data were analysed using one-way ANOVA and Tukey's post hoc test, number of animals, n = 5. AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase

**Table 3.** Renal function parameters of female rats treated with *M. fragrans* for 28 days

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Distilled water (10 mL/kg) | *M. fragrans* (100 mg/kg) | *M. fragrans* (500 mg/kg) | *M. fragrans* (1000 mg/kg) |
| Urea (mg/dL) | 27.00 ± 2.74 | 26.00 ± 4.21 | 24.80 ± 2.89 | 25.80 ± 2.89 |
| Creatinine (mg/dL) | 0.50 ± 0.07 | 0.50 ± 0.04 | 0.32 ± 0.02 | 0.50 ± 0.05 |
| Sodium (mmol/L) | 139.3 ± 1.25 | 138.00 ± 1.67 | 137.80 ± 1.16 | 142.0 ± 1.30 |
| Potassium (mmol/L) | 3.95 ± 0.16 | 4.08 ± 0.18 | 4.18 ± 0.22 | 4.44 ± 0.24 |
| Bicarbonate (mmol/L) | 24.50 ± 0.96 | 24.40 ± 0.68 | 23.00 ± 1.30 | 22.80 ± 1.53 |
| Chloride (mmol/L) | 99.75 ± 1.03 | 98.20 ± 1.07 | 102.60 ± 1.12 | 100.2 ± 1.16 |

Values represent mean ± SEM. Data were analysed using one-way ANOVA and Tukey's post hoc test, number of animals, n = 5.

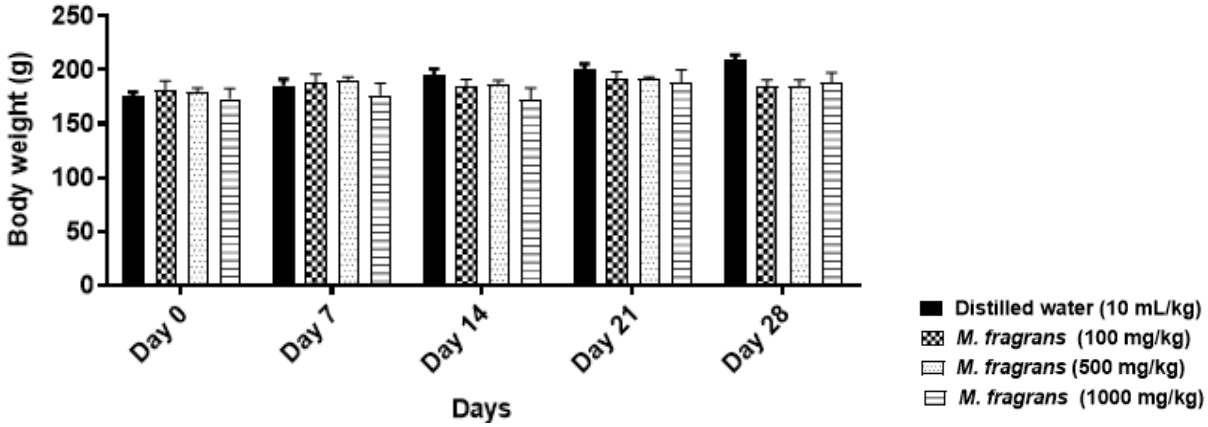


**Figure 1.** Mean body weight of female rats treated with *M. fragrans* for 28 days. Values are expressed as mean ± SEM. Data were analysed using one-way ANOVA and Tukey's post hoc test, number of animals, n = 5

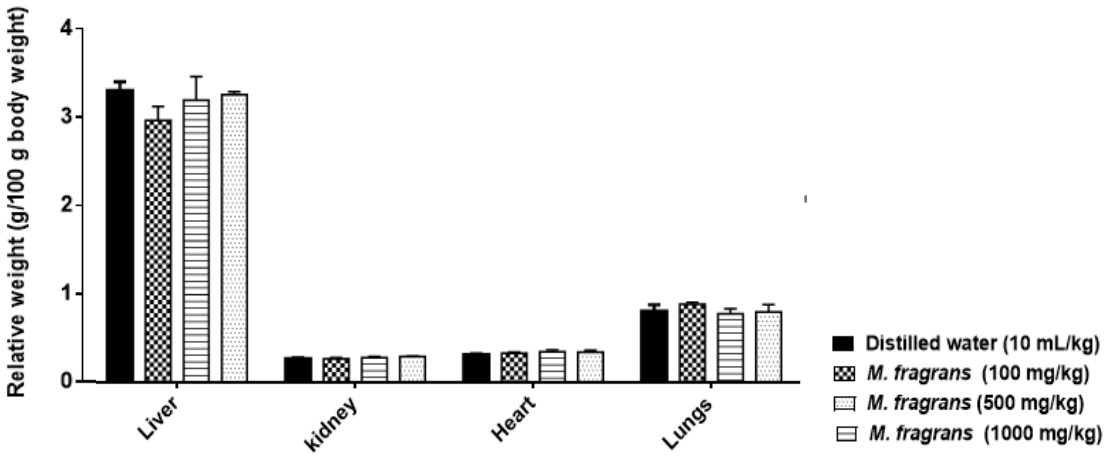


**Figure 2.** Mean relative organ weight of female rats treated with *M. fragrans* for 28 days. Values are expressed as mean ± SEM. Data were analysed using one-way ANOVA and Tukey's post hoc test, number of animals, n = 5.

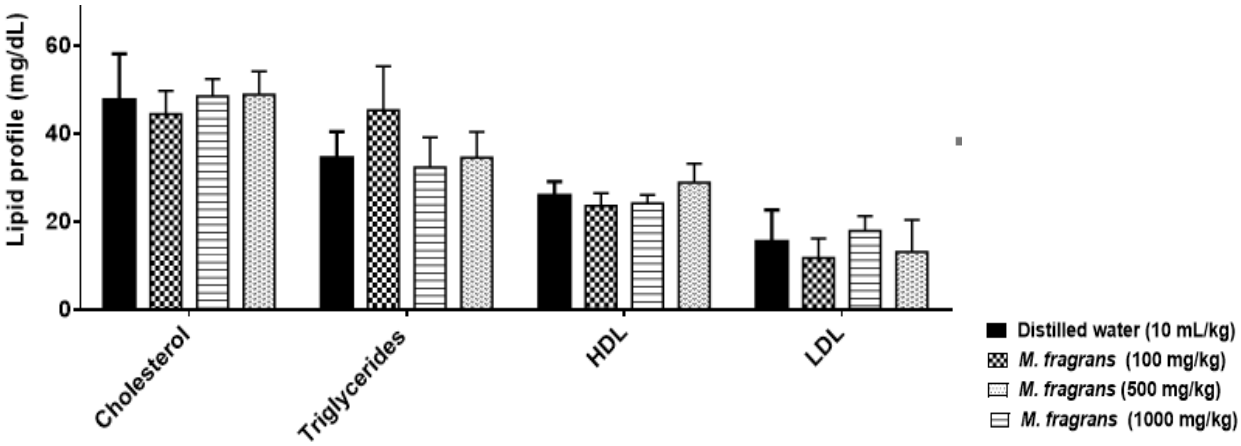


**Figure 3.** Lipid profile of female rats treated with *M. fragrans* extract for 28 days.
Values are expressed as mean ± S.E.M. Data were analysed using one-way ANOVA and Tukey's post hoc test, number of animals, n = 5. LDL, low density lipoprotein; HDL, high density lipoprotein.

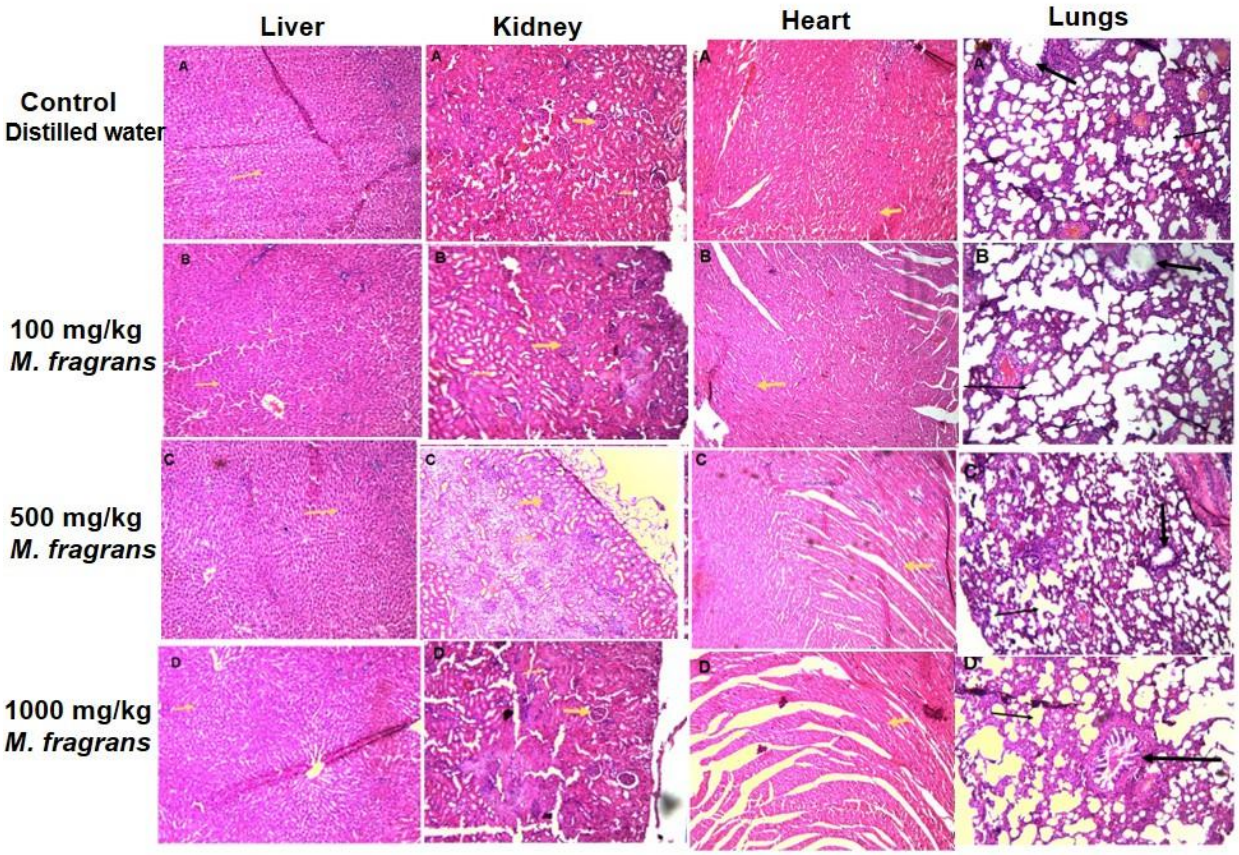


**Figure 4.** Photomicrograph of the liver, kidney, heart, and lungs of the female rats in control (A) and treatment groups (B, C, & D) exposed to 100, 500, and 1000 mg/kg *M. fragrans* respectively for 28 days. Arrows were used to show histological features. Staining was done using H & E, and the magnification used was x100

## DISCUSSION

The study provides a comprehensive evaluation of the subacute effects of ethanol seed extract of *M. fragrans* on haematological indices, biochemical parameters, lipid profile, and organ histology in female Wistar rats. Although some parameters remained unchanged, their consistency offers valuable information regarding the relative safety profile of the extract.

The absence of mortality or observable behavioural changes across all dose levels in the acute toxicity study indicates that *M. fragrans* seed extract has a wide safety margin. According to the OECD guidelines for acute toxicity assessments [19], substances with an $LD_{50}$ (median lethal dose) exceeding 5000 mg/kg are considered practically non-toxic, supporting the low acute toxicity of the extract, consistent with the earlier study [20]. Similarly, the subacute oral administration of the seed extracts caused no mortality and no behavioural changes in the animals throughout the 28-day study period.

The absence of significant variations in mean body weight among the treatment groups relative to the control indicates that the extract did not adversely affect the overall growth or nutritional status of the rats. Likewise, the comparable relative weights of the liver, kidney, heart, and lungs suggest the absence of extract-related organ toxicity.

Haematological assessment revealed a non-significant reduction in total white blood cells (WBC) counts, consistent with earlier findings [21]. Importantly, lymphocyte percentages remained relatively stable across all groups, indicating preservation of adaptive

immune function and unaffected lymphopoiesis during the 28-day exposure. This haematological stability may be attributed to the antioxidant and anti-inflammatory properties of flavonoids and phenolic compounds present in *M. fragrans*, which are known to protect blood cells from oxidative damage [22-24]. Collectively, these findings suggest that the extract does not exert immunotoxic effects at the tested doses. A significant reduction in mixed cell (MID) percentage was observed at 100 mg/kg, indicating a possible suppression of mild inflammatory processes. MID cells, comprising monocytes, eosinophils, and basophils, are critical mediators of inflammatory and allergic responses. Their reduction may reflect the anti-inflammatory actions of phytoconstituents such as myristicin and elemicin, which inhibit inflammatory mediator release and monocyte/macrophage activity [25-27]. Granulocyte levels remained largely unchanged, suggesting that neutrophil-mediated innate immune responses were not adversely affected. RBC indices revealed mild, dose-dependent alterations. Slight reductions in RBC count, Hb, and HCT were observed at lower doses. The increase in RBC count at 1000 mg/kg may reflect either enhanced erythropoiesis or haemoconcentration secondary to mild physiological stress. A significant reduction in MCV at 100 mg/kg suggests microcytic changes; however, stable MCH, MCHC, and RDW values indicate preserved haemoglobin content and uniformity of erythrocytes, arguing against overt anaemia or haemolysis. Platelet parameters exhibited the most pronounced changes at 100 mg/kg. The significant reduction in PLT, accompanied by alterations in MPV, PDW, and PCT, suggests a transient disruption of megakaryopoiesis or platelet maturation at this dose. Interestingly, these effects normalized at higher doses, demonstrating a non-monotonic dose-response pattern.

Biochemical analysis further supports the extract's systemic safety. The kidneys are essential in filtering out waste substances, balancing electrolytes, and regulating fluid levels. In this study, renal function markers (urea, creatinine, and electrolytes) remained largely within physiological ranges, indicating preserved kidney function. Minor variations in potassium and bicarbonate at higher doses suggest a mild metabolic acid load rather than renal dysfunction, as urea and creatinine levels remained stable. Electrolyte homeostasis, including chloride balance, was effectively maintained.

Liver function was assessed through essential biochemical markers, such as AST, ALT, alkaline phosphatase (ALP), bilirubin, total proteins, and albumin. ALT, AST, and ALP are core liver enzymes. These enzymes play a crucial role in metabolic functions, including amino acid metabolism, energy production, and bile synthesis [28,29]. Increased concentrations of these enzymes in the bloodstream frequently suggest liver problems, although they may also point to complications in other organs such as the heart, muscles, and bones [30-32]. In this study, ALT and AST levels peaked at 100 mg/kg, and ALP levels peaked at 500 mg/kg; however, these values were not statistically significant compared to the control, indicating that the extract did not cause hepatic stress, injury, or potential liver damage. Bilirubin levels (total bilirubin (0.24–0.28 mg/dL) and direct bilirubin (0.12–0.15 mg/dL) were comparatively stable, indicating no significant impairment in hepatic bilirubin metabolism. This agrees with [33], who noted minimal fluctuations in bilirubin levels following the administration of *M. fragrans* extract, highlighting preserved hepatobiliary function. The stable bilirubin confirms intact hepatic conjugation and excretory function, and supporting the lack of haemolysis observed in haematological indices. Slight fluctuation in total protein and albumin suggests metabolic

adaptation rather than overt hepatotoxicity, aligning with previous reports that moderate doses of nutmeg may enhance protein synthesis, while higher doses may induce mild metabolic stress [34].

The lipid profile results indicate that administration of *M. fragrans* ethanol extract produced only mild and dose-dependent alterations in serum lipid parameters. A minor reduction in total cholesterol levels was observed at the 100 mg/kg dose, while no notable changes were observed at the higher doses of 500 mg/kg and 1000 mg/kg. This pattern suggests that the extract does not exert a strong hypocholesterolaemic effect across the tested dose range, and the slight reduction observed at the lowest dose may reflect a transient or adaptive metabolic response rather than a consistent dose-dependent effect. Triglyceride (TG) levels showed a slight increase at the 100 mg/kg dose, with no significant variations observed at 500 mg/kg and 1000 mg/kg. The absence of marked changes at higher doses indicates that the extract does not significantly disrupt triglyceride metabolism, and the minor elevation at the lowest dose is unlikely to be biologically relevant, particularly as it did not persist across increasing doses. High-density lipoprotein (HDL) cholesterol levels exhibited a slight increase at the 1000 mg/kg dose. Although this increase was modest and not statistically significant, it may suggest a potential protective effect at higher doses, given the role of HDL (commonly known as "good cholesterol") in reverse cholesterol transport, facilitating the removal of excess cholesterol from peripheral tissues and conveying it to the liver for elimination, and cardiovascular protection [35]. In contrast, the levels of low-density lipoprotein (LDL), commonly referred to as “bad cholesterol” due to its involvement in atherosclerotic plaque formation, showed a slight but non-significant decrease in the 100 and 1000 mg/kg groups compared with the control. Although not statistically significant, this downward trend is noteworthy, as reductions in LDL may indicate a mild modulatory influence of *M. fragrans* extract on lipid metabolism. Together, the minimal changes observed across lipid parameters suggest that *M. fragrans* ethanol extract does not adversely affect lipid metabolism at the administered doses. The absence of significant dyslipidaemia further supports the relative safety of the extract with respect to lipid homeostasis during subacute exposure.

Histopathological examination provided compelling evidence supporting the safety of *M. fragrans* seed extract at the tested doses. Microscopic evaluation of the liver, kidneys, heart, and lungs revealed preserved tissue architecture across all treatment groups, with no observable lesions or pathological changes suggestive of toxic effects. Liver sections showed normal hepatic architecture, with well-organized hepatocytes, intact sinusoidal spaces, and preserved portal triads. No signs of inflammation, necrosis, or steatosis were observed. These findings are notable given reports of hepatotoxicity associated with high doses of nutmeg constituents such as myristicin and safrole [36]. The absence of hepatic damage in this study suggests that, within the subacute dosing regimen employed, the bioactive compounds did not accumulate to levels capable of impairing liver function. Renal histology similarly demonstrated intact glomerular and tubular structures, with no evidence of sclerosis, tubular degeneration, proteinaceous casts, or interstitial inflammation. This indicates that the extract did not exert nephrotoxic effects and supports its renal safety, consistent with previous toxicological evaluations of plant extracts [37]. Cardiac tissues exhibited well-arranged myocardial fibres without fibrosis or inflammatory infiltration, suggesting that the extract does not compromise myocardial integrity or cardiac function. Likewise, lung sections showed

normal alveolar architecture with clear air spaces and intact bronchiolar structures, with no sign of oedema, haemorrhage, or inflammatory infiltration, indicating an absence of respiratory toxicity.

Collectively, this study demonstrates that *M. fragrans* ethanol seed extract exhibits a favourable safety profile under subacute exposure, producing only mild, dose-dependent, and reversible changes in haematological and metabolic parameters. The discrepancies between this study and earlier studies are likely to be attributed to differences in extraction techniques, dosage levels, and duration of exposure. Notably, the ethanol extract employed in this study appears to support a protective metabolic effect, possibly through the regulation of antioxidant activity and lipid metabolism.

Nevertheless, certain limitations are acknowledged. The range of doses evaluated may not adequately define the extract's toxicological threshold; studies involving higher doses and prolonged administration are necessary for a more comprehensive safety assessment. Furthermore, the relatively small sample size may reduce the sensitivity for detecting subtle changes. Future investigations using larger sample sizes and extended treatment periods are therefore recommended.

**Conclusion.** This study demonstrates that the ethanol seed extract of *M. fragrans* is relatively safe under subacute exposure in female rats. No mortality, behavioural changes, or histopathological alterations were observed, and haematological, biochemical, and lipid parameters remained largely within normal ranges. Mild, dose-dependent changes were reversible and did not cause organ damage. Further studies with higher doses and extended exposure are warranted to comprehensively assess the extract's safety and therapeutic potential.

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