Journal of King Saud University – Science 34 (2022) 101694

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Hepato-renal toxicity of *Myristica fragrans* Houtt. (Myristicaceae) seed extracts in rats

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ARTICLE INFO

Article history:
Received 19 January 2021
Revised 19 September 2021
Accepted 3 November 2021
Available online 9 November 2021

Keywords:
Spices
Myristica fragrans
Toxicity
Renotoxic
Hepatotoxic
Histology

ABSTRACT

Objectives: *Myristica fragrans* is used in many parts of the world as a common spice and herbal medicine for managing a wide variety of ailments. The rich nutritional factors in M. fragrans seeds show potential application in food biofortification; however, the long-term safety of using a high dose of the seeds needs to be validated. This study seeks to investigate how oral administration of high doses of methanol (ME) and n-hexane (NHE) extracts of *M. fragrans* seed for one or two weeks affects the histology and serum markers of kidney and liver of rats.

Methods: Adult male Swiss mice (6–7 weeks, 20–30 g) were used for acute toxicity study by standard methods, while male Wistar rats (6–8 weeks, 100–120 g) were used for sub-acute toxicity test. The sixty rats were distributed into five groups of 12 rats each: Group 1 received normal saline, groups 2 and 3 were orally treated with 500 and 1000 mg/kg b.w/day ME while groups 4 and 5 were fed 500 and 1000 mg/kg b.w/day NHE, respectively for 14 days. Six rats from each group were sacrificed on days 8 and 15 after 24 h of fasting. Markers of kidney and liver status of test and control animals were compared using one-way analysis of variance.

Results: The presence of terpenoids, flavonoids, alkaloids, phenols, steroids, and tannins were detected in both extracts, although at varying levels. There was no obvious sign of toxicity nor mortality in acute toxicity test after 24 h of administration of extracts up to 5000 mg/kg b.w. However, there were significant ($p < 0.5$) elevations in urea, total bilirubin and creatinine concentrations, alkaline phosphatase, aspartate and alanine aminotransferases, and lactate dehydrogenase activities in rats fed extracts for seven or fourteen days relative to control. Furthermore, the n-hexane extract at 1000 mg/kg elicited some histological changes consistent with hepatotoxicity.

Conclusions: Although the extracts were rich in some essential phytochemicals, this study demonstrated that long-term administration of high doses of the extracts elicits hepato-renal toxicities. Hence, consuming a large amount of the seed over a long duration is discouraged.

1. Introduction

Foods derived from plants have been widely acclaimed as sources of nutrients needed to promote good health and wellbeing (Hirschi, 2009). Although many plants are rich in nutritional factors and are medicinal, which are beneficial in combating nutritional deficiency and managing common health challenges, their abuse could be harmful. Considering that natural source of nutritional factors for nutrient-fortification is perceived to be safer and maybe more acceptable by the consumers, using common spices for nutritional fortification of food and beverages need to be investigated. However, there is a need to investigate the safety of using a high dose of these spices over a long time to avoid creating problems while trying to solve another. The need to confirm the safety of this plant is because a handful of plants are toxic, majorly when consumed at higher doses (Mounanga et al., 2015), including the common spice, *Myristica fragrans* Houtt. (Myristicaceae) (Benzeid et al., 2018).

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101694
1018-3647/

Myristica fragrans is traditionally used in managing paralysis because it increases blood circulation, scavenge radical species, and kill cancer cells (Ginting et al., 2021). Other properties include aphrodisiac, hypolipidaemic, anti-thrombotic, anti-fungal, nervous stimulant, aromatic, anti-dysenteric and anti-inflammatory, fungicidal (Fernando and Senevirathne, 2020; Zhao et al., 2020), anti-diarrheal, and memory-enhancing (Nikolic et al., 2021) potentials have been documented. On the other hand, it has been shown to induce convulsions, palpitations, and hallucinations, indicating that it elicits psychoactive effects (Gupta, 2020). Essential oils from the seed formulated as potential drugs in combination with magnesium aluminometasilicate as an excipient are currently being examined for approval for broad-spectrum therapeutic purposes (Matulyte et al., 2020). Recently, the seeds were demonstrated to contain substantial amounts of essential amino and fatty acids and moderate levels of essential minerals (Anaduaka et al., 2020). Considering the nutritive and pharmacological properties of M. fragrans, the seeds have potential application in the biofortification of food to mitigate malnutrition. However, this can only be possible if the plant is demonstrated to be safe for consumption in large quantities over a long time. The involvement of the liver and kidney in detoxification processes exposes them to damage by cytotoxic chemicals. Hence, monitoring of liver and kidney status is crucial in establishing food safety. This study, therefore, seeks to evaluate the phytochemical constituents and effects of n-hexane and methanol extracts of *M. fragrans* on the liver and kidney status of rats.

2. Materials and methods

2.1. Plant material

Myristica fragrans seeds used for this study were sourced from Ogige Market in Nsukka and were authenticated by Mr. Alfred Ozioko, a taxonomist with Bioresources Diversity and Conservation Programme, Nsukka (voucher number- InterCEDD/16308). The plant seeds were pulverized into a fine powder from which 500 g was macerated in 5 L of a solvent composed of methanol and n-hexane (2:1 v/v) for 48 h. The suspension was filtered, the filtrate was separated, and each partition was dried to a semi-solid mass to obtain methanol and n-hexane extracts (Enechi et al., 2019).

2.2. Experimental animals

Inbreed adult healthy male rats of Wistar strain (100–120 g) and adult healthy male Swiss albino mice (20–30 g) were used in the study. The animals were acclimatized to an animal laboratory (24 ± 20C and 12 h light/dark cycle) and were allowed to eat and drink freely. The Faculty of Biological Science, University of Nigeria, Nsukka Committee on Research Ethics approved the protocols for this study before its commencement (UNN/FBS/EC/1032). The study animals were ethically handled based on local and international guidelines.

2.3. Analyses of the phytochemical compositions of methanol and n-hexane extracts

The phytochemical analyses on the extracts were carried out using standard procedures as detailed in our previous study (Nkwocha et al., 2018).

2.4. Acute toxicity test on the methanol and n-hexane extracts

The acute toxicity test on the extracts was done following Lorke (1983) method as described earlier (Enechi et al., 2019).

2.5. Study design for hepato-renal toxicity study on the methanol and n-hexane extracts

The sixty rats used were distributed into five groups of 12 rats each. The extracts were administered orally by gavage to the rats for 14 consecutive days: Group 1, the control received the vehicle, normal saline, groups 2 and 3 were treated with 500 and 1000 mg/kg body weight/day *M. fragrans* methanol extract, respectively while groups 4 and 5 were fed with 500 and 1000 mg/kg body weight/day *M. fragrans* n-hexane extract, respectively. All treatments were via oral administration by gavage. Six rats from each group were sacrificed on days 8 and 15 after 24 h of fasting. Serum harvested from blood samples of the experimental rats were subjected to biochemical analyses following the procedures in commercial assay kits of Randox Diagnostics Limited (United Kingdom) according to standard methods. The activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were determined based on King (1965) and Reitman and Frankel (1957) methods, respectively. The activity of CYP 450-2E1 in the microsomal fraction of liver homogenate was assayed based on the conversion of p-nitrophenol to 4-nitrocatechol as described by Chrastil and Wilson (1975). The total bilirubin level was determined using a photometric method of Malloy and Evelyn (1937), while the renal status was evaluated by measuring the concentration of urea and serum in the serum based on Fawcett and Scott (1960) and Henry (1974) methods, respectively. On the 15th day, tissue sections of the liver and kidney of a rat in each group were fixed in 10% formalin for histological examination using standard hematoxylin and eosin solutions as stains (Disbrey and Rack, 1974).

2.6. Statistical analysis

The data obtained were analyzed statistically using one-way analysis of variance (ANOVA), and *post hoc* Duncan's test was used to compare means of the experimental groups in IBM statistical product and service solution. Student T-test was used to compare the means of the phytochemical contents of the two extracts. Means with $p < 0.05$ were considered statistically significant, and the results were presented as mean ± standard deviation.

3. Results

3.1. Phytochemical compositions of hexane and methanol extracts of Myristica fragrans seeds

Methanol extract of *M. fragrans* had higher terpenoids, flavonoids, alkaloids, phenols, and tannins contents than the n-hexane extract. In contrast, steroids content was higher in n-hexane extract when compared to the methanol extract (Table 1).

3.2. Acute toxicity profile of methanol and n-hexane extracts of Myristica fragrans seed in mice

Methanol and n-hexane extracts of *M. fragrans* seeds did not elicit any morphological and behavioral signs of toxicity. This implies that there was no sign of toxicity such as decreased locomotor activity, change of hair color, decreased feed intake, or prostration, nor mortality after 24 h of administering the two extracts up to 5000 mg/kg body weight.

Table 1
Phytochemical, vitamin and anti-nutritional compositions of hexane and methanol extracts of *Myristica fragans* seeds.

Chemical	Composition in n-hexane extract	Composition in methanol extract
Flavonoids (mg/100g)	150.62 ± 0.06[a]	166.67 ± 0.08[b]
Terpenoids (mg/100g)	667.53 ± 3.46[a]	743.50 ± 0.58[b]
Alkaloids (mg/100g)	540.55 ± 0.03[a]	638.34 ± 0.06[b]
Phenols (mg/100g)	563.45 ± 0.06[a]	626.56 ± 5.77[b]
Steroids (mg/g)	2.58 ± 0.02[b]	1.28 ± 0.03[a]
Tannins (mg/100g)	427.61 ± 0.06[a]	486.57 ± 0.06[b]
Vitamin A (mg/100g)	0.62 ± 0.03[b]	0.20 ± 0.00[a]
Vitamin C (mg/100g)	18.80 ± 3.44[a]	68.80 ± 0.00[a]
Vitamin E (mg/100g)	30.75 ± 1.14[b]	13.93 ± 1.19[a]
Vitamin B1 (mg/100g)	1.12 ± 0.45[a]	22.72 ± 3.39[b]
Vitamin B2 (mg/100g)	1.08 ± 0.33[a]	2.96 ± 0.47[a]
Vitamin B12 (mg/100g)	22.69 ± 2.30[a]	60.19 ± 1.31[b]
HCN (mg/100g)	0.03 ± 0.06[a]	0.03 ± 0.15[a]
Phytate (mg/100g)	3.90 ± 0.03[a]	2.44 ± 0.23[a]
Trypsin inhibitor (TIU/mg)	0.43 ± 0.02[a]	0.45 ± 0.04[a]
Haemagglutinin (HU/mg)	0.76 ± 0.03[a]	0.78 ± 0.15[a]

Values represent the mean ± SD of triplicate determination. The data was analysed using student T-test and values with different letters of the alphabets as superscripts are significantly different at $p < 0.05$.

3.3. Effects of methanol and n-hexane extracts of Myristica fragrans seeds on serum renal markers of rats

Unlike the placebo, rats that received 500 and 1000 mg/kg b.w. of methanol and n-hexane extracts of *M. fragrans* for 7 and 14 days had higher levels of urea, while treatment with 1000 mg/kg b.w. of both extracts for two weeks increased creatinine concentration compared to control (Table 2).

3.4. Effects of methanol and n-hexane extracts of Myristica fragrans seeds on serum liver markers of rats

After 7- and 14-day treatment of rats with 1000 mg/kg b.w. of both extracts, ALP and LDH activities were elevated compared with control. Similarly, treatment of rats with 500 and 1000 mg/kg b.w. of both extracts elevated ALT and AST activities compared to control. Meanwhile, treatment of rats with the extracts for 14 days did not affect serum lactate dehydrogenase (LDH) activity (Table 3). In addition, methanol and n-hexane extracts of *M. fragrans* seeds at 500 and 1000 mg/kg b.w. for 14 days caused elevation (though not statistically significant) in liver CYP 450-2E1 compared with control. Similarly, treating rats with 500 and 1000 mg/kg b.w. of both extracts for seven or 14 days increased total bilirubin concentrations compared to control (Table 4).

3.5. Histology of the kidney and liver of rats fed Myristica fragrans seed extracts

Histopathological examination of kidney sections collected from a rat in each group indicated no significant abnormality in kidney histo-architecture. The tissue sections showed normal glomeruli (G) in Bowman's capsule surrounded by normal renal tubules (proximal convoluted tubule, distal convoluted tubule, Pars recta, and collecting duct) in the cortex, outer medulla, and inner medulla. The renal interstitium was also normal, showing normal vascular channels (Plate 1a-e). Photomicrograph of the liver from rats in groups 1–4 showed normal hepatic histo-architecture (Plate 2a-d). The hepatocytes were as expected in the hepatic lobules, arranged in chords around the central vein, diverging towards the portal triad; hepatic artery, hepatic vein, and bile duct. However, liver sections from group 5 (treated with 1000 mg/kg b.w. of n-hexane extract) showed histopathological changes peculiar to hepatotoxicity (Plate 2e).

4. Discussion

The increased understanding of herbs and spices as sources of natural antioxidants in addition to nutrients has encouraged more researches on them with a view of identifying those with little or no toxicity (Chatterjee et al., 2007). *The seeds of M. fragrans* are widely used as spices and drugs by locals, some in large amounts without knowing the potential safety concerns associated with the consumption of large doses of the plant. This study investigated the impact of short-term administration of large doses of extracts of *M. fragrans* on two crucial organs, the liver and kidney. The presence of flavonoids, terpenoids, alkaloids, phenols, steroids, and tannins in both extracts of *M. fragrans* (though in varying quantities) agrees with earlier studies. Nikolic et al. (2021) reported the presence of alkaloids, flavonoids, cardiac glycosides, and saponins in *M. fragrans* seed essential oil. Similar to our findings, the presence of terpenoids and phenolics was also reported by El-Alfy et al. (2009) and Naikodi et al. (2011) in their separate studies. The existence of these secondary metabolites in the plant extracts suggests that the extracts may elicit some health-promoting potentials (Huang et al. 2010). It was shown that the extracts did not cause mortality or conspicuous sign of toxicity. The observed tolerance of mice to the extracts in this study supports an earlier report by George and Ioana (2007) that myristic acid, a constituent of *M. fragrans* (Anaduaka et al., 2020), elicits a low order acute oral toxicity in rodents. This finding implies that the extracts have a low acute toxicity profile, making the seed relatively safe for consumption at low doses over a short time.

Urea is produced as a waste product of protein digestion by the liver and is typically removed from the body through ultrafiltration in the glomeruli of the kidneys (Kasper et al., 2005). Similarly, creatinine is a metabolite of the muscle's creatine phosphate whose concentration is relatively constant due to the kidney's role in extracting it (Miller et al., 2005). Hence, urea and creatinine levels are essential indicators of renal health because higher than expected serum urea, and creatinine levels suggest impaired kidney function. The significantly higher urea and creatinine levels after 14 days daily administration of the extracts, as seen in groups 3 and 5 compared to the control, suggest that the extracts at 1000 mg/kg b.w. for 14 days elicits nephrotoxicity.

One of the liver function enzyme markers, ALP, participates in membrane transport, protein and glycogen metabolisms in addition to secretory properties (Akpanabiatu et al., 2005). Hence, elevated circulating ALP levels may arise from leakage of the enzymes

Table 2
Effects of methanol and n-hexane extracts of *Myristica fragrans* on serum renal markers of albino rats.

Parameter/Group	Urea (mg/dl)		Creatinine (mg/dl)	
	Day 7	Day 14	Day 7	Day 14
Control	17.67 ± 0.58^{a}	27.00 ± 2.65^{a}	0.76 ± 0.19^{a}	0.98 ± 0.10^{a}
500 mg/kg M.E	23.00 ± 6.08^{b}	36.00 ± 4.58^{b}	0.83 ± 0.22^{a}	1.01 ± 0.17^{a}
1000 mg/kg M.E	28.33 ± 4.73^{c}	47.00 ± 13.08^{c}	0.81 ± 0.17	2.43 ± 0.48^{c}
500 mg/kg H.E	20.33 ± 0.58^{b}	37.00 ± 3.61^{b}	0.87 ± 0.41^{a}	1.34 ± 0.13^{b}
1000 mg/kg H.E	30.33 ± 5.03^{c}	46.67 ± 11.59^{c}	0.81 ± 0.19^{a}	1.69 ± 0.42^{bc}

Each value represents the mean ± SD (n = 6). Values with different alphabets down the column are statistically significant ($p < 0.05$) compared with the test groups.

Table 3
Effects of methanol and hexane seed extracts of *Myristica fragrans* on serum liver and cardiac status markers enzymes of albino rats.

Parameter/Group	ALP (U/L)		ALT (U/L)		AST (U/L)		LDH (U/L)	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	51.33 ± 1.79 ^{a}	50.67 ± 2.08^{a}	31.33 ± 4.04^{a}	29.67 ± 3.06^{a}	101.33 ± 7.64^{a}	106.33 ± 2.42^{a}	6.38 ± 0.37 ^{a}	6.59 ± 0.00^{a}
500 mg/kg M.E	50.67 ± 4.04^{a}	55.00 ± 3.61^{a}	28.00 ± 2.00^{a}	42.00 ± 1.00^{b}	100.67 ± 9.10^{a}	129.33 ± 6.43^{b}	8.79 ± 3.81^{a}	7.05 ± 0.45^{a}
1000 mg/kg M.E	65.00 ± 2.49^{b}	69.00 ± 6.56^{b}	18.33 ± 4.16^{b}	37.00 ± 5.30^{b}	104.00 ± 9.29^{a}	143.33 ± 1.97^{c}	6.59 ± 0.00 ^{a}	6.60 ± 0.45^{a}
500 mg/kg H.E	53.33 ± 3.10^{a}	57.33 ± 3.06^{a}	23.33 ± 5.86^{a}	45.67 ± 0.58^{b}	108.00 ± 7.52^{a}	134.00 ± 8.68^{b}	12.32 ± 1.50^{b}	6.62 ± 0.69^{a}
1000 mg/kg H.E	66.00 ± 2.65^{b}	70.33 ± 1.24^{b}	30.00 ± 4.58^{a}	53.33 ± 5.03^{c}	117.67 ± 3.58^{b}	159.67 ± 1.72^{d}	34.68 ± 1.33^{c}	36.70 ± 0.03^{b}

Each value represents the mean ± SD (n = 6). Values with different alphabets down the column are statistically significant at $p < 0.05$. H.E = hexane seed extracts of *Myristica fragrans*; M.E = methanol seed extracts of *Myristica fragrans.*

Table 4
Effects of methanol and hexane seed extracts of *Myristica fragrans* on other serum liver markers of albino rats.

Parameter/Group	Total bilirubin (mg/dl)		CYP 450 2E1 (ng/ml)	
	Day 7	Day 14	Day 7	Day 14
Control	0.73 ± 0.06^{a}	0.70 ± 0.16^{a}	2.06 ± 0.01^{a}	2.38 ± 0.15^{a}
500 mg/kg M.E	1.47 ± 0.25^{b}	1.23 ± 0.06^{b}	2.09 ± 0.10^{a}	2.61 ± 0.05^{a}
1000 mg/kg M.E	1.65 ± 0.12^{c}	1.37 ± 0.12^{bc}	2.06 ± 0.02^{a}	2.69 ± 0.06^{a}
500 mg/kg H.E	1.40 ± 0.53^{b}	1.47 ± 0.23^{c}	2.09 ± 0.05^{a}	2.27 ± 0.07^{a}
1000 mg/kg H.E	1.63 ± 0.15^{c}	1.72 ± 0.12^{d}	2.10 ± 0.01^{a}	2.45 ± 0.31^{a}

Each value represents the mean ± SD (n = 6). Values with different alphabets down the column are statistically significant ($p < 0.05$) compared with other test groups.

from the hepatic membrane in hepatotoxic conditions (Malomo et al., 2006). The increase in the activity of aminotransferases is generally produced by cellular necrosis (Etim et al., 2008). The elevated levels of ALT and AST in some of the treated groups on days 7 and 14 treatment could increase the metabolism of toxic metabolites and enhance the production of free radicals, thereby causing hepatocellular injury. In all the liver marker enzymes activities assessed, there was a general elevation in their activities in serum, and the activities increased on day 14 compared with day 7, suggesting that *M. fragrans* seed extracts exhibit dose and duration of consumption-dependent hepatotoxicity, with n-hexane extract causing being more toxic.

After 14 days of oral ingestion, the extracts did not alter lactate dehydrogenase (LDH) activity in serum. This indication of no toxicity could be attributed to the presence of myristicine that has been shown to prevent lipopolysaccharides-induced liver injury (Morita et al., 2003). As a marker for liver and cardiac injuries, the presence of a large amount of LDH in circulation indicates severe damage to cardiac and hepatic tissues (King and Perry, 2001). LDH activity increased after 14 days of consuming n-hexane extract, indicating that high doses of this extract caused severe liver damage. Luster et al. (2000) reported that hepatotoxins' first point of assault is the centrilobular regions that harbor CYP 450 mixed-function oxidases. This group of enzymes catalyzes the detoxification of toxic metabolites with the release of free radicals as by-products. Among these groups of enzymes, CYP 2E1 is mainly found in the liver with a smaller amount in extra-hepatic tissues, including lungs, kidney, brain, endothelium of large blood vessels, heart, bone marrow, and nasopharyngeal tissue (Kang et al., 2008). CYP 2E1 is responsible for the breakdown of many low molecular weight toxins and carcinogens (Malhotra, 1998). Methanol and n-hexane extracts of *M. fragrans* seeds at 500 and 1000 mg/kg b. w. for 14 days caused elevation (though not statistically significant) in liver CYP 450-2E1 compared with control. Yokota et al. (1988) earlier demonstrated that 1-terpinen-4-ol and eugenol increases phase II detoxifying enzymes' activities for xenobiotics in the liver, such as UDP-glucuronyl transferase, DT-diaphorase, and glutathione-S-transferase without significantly increasing CYP 450. A higher-than-expected level of bilirubin in serum is a good marker of hepatic malfunction (Eteng et al., 2009). Hence, the ability of the extracts to elevate total bilirubin at 1000 mg/kg after 14 days of oral consumption suggests hepatotoxicity. The elevated bilirubin level in this study might have resulted from a reduction in the liver's ability to metabolize and conjugate them due to compromise, such as obstruction of bile ducts. It could also be due to a reduction in levels of reducing equivalents such as NADPH and GSH. Reduced glutathione contributes to protecting the membrane of erythrocytes from hemolysis, decreasing bilirubin levels (Noori et al., 2009). The result of the histological examination shows indications of hepatotoxicity only at 1000 mg/kg. This indication of hepatotoxicity agrees with the observed degeneration of cells of the kidney, heart, and stomach characterized by cellular necrosis involving disruption and distortion of cellular membranes structural and functional integrity by *M. fragrans* seeds and its component, myristin (Eweka and Eweka, 2010; AL-Mosaibih, 2015; Anaduaka et al., 2020). Generally, despite the numerous health benefits of the spice, *M. fragrans*, consumers are advised to consume only a small quantity over a short duration

to avert toxicological effects associated with chronic consumption of a high dose of the spice.

5. Conclusions

The presence of substantial quantities of phytochemicals including terpenoids, flavonoids, alkaloids, phenols, steroids, and tannins were detected in methanol and n-hexane of *Myristica fragrans* seeds, although at varying concentrations. This study also demonstrated that at high doses, the extracts induced hepatotoxicity and renotoxicity, with a higher impact on the liver. Compared to the methanol extract, the n-hexane extract was shown to be more toxic. Based on the findings of this study, consumption of a large quantity of *M. fragrans* seed over a long duration is discouraged. Further study is needed to ascertain the molecular mechanism behind the observed hepato-renal toxicity induced by the seed extracts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors thank Professor Paul Nnodim of Massachusetts College of Liberal Arts (MCLA), North Adams, United State of America for providing grammatical editing for this manuscript.

Funding

This study received no external funding and was hence funded by the authors.

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