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Hepatic Toxicity Profile of Ethanolic Fraction of the Seed of *Myristica Fragrans* Houston in Adult Wistar Rats

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ABSTRACT

We studied hepatic toxicity profile of the ethanolic fraction of the seed of *Myristica fragrans* (*M. fragrans*, nutmeg) in rats. Adult Wistar rats were randomized to receive 20 mg/kg/d or 80 mg/kg/d of oral ethanolic fraction of nutmeg for 7 days (7d; acute treatment) or 28 days (28d; chronic treatment). Hepatic histology (haematoxylin and eosin-stained paraffin sections of the liver) and plasma levels of liver enzymes were analyzed and compared with controls. Our findings showed that chronic treatment with higher dose of ethanolic fraction of nutmeg (80 mg/kg/d) but not lower dose (20 mg/kg/d), induces significant elevations ($P<0.05$) in plasma levels of alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase. These biochemical findings and the presence of hepatic sinusoidal dilatation in these treated rats suggest that long-term treatment with higher but not lower dose of the nutmeg is associated with hepatic injury. However, the specific phytochemical principles in nutmeg that is responsible for this hepatic toxicity profile of the spice is unknown and requires further studies.

Key words: Hepatic Toxicity, *Myristica fragrans,* Wistar Rats

INTRODUCTION

The seed and mace of the fruit of *Myristica fragrans* Houtt.are valued for their culinary property and thus have a role as spices for domestic and industrial usages. In addition to this property, the seed and mace of *M. fragrans* also possess several other uses. Nutmeg (seed of *M. fragrans*) has psychotropic property and is therefore used as a recreational substance [1]. There are reports of nutmeg ingestion arising from the need to achieve an euphoric, elated and hallucinogenic state at relatively low costs [2].

The psychotropic property of nutmeg is partly attributable to the conversion of some of its phytochemicals to amphetamine-like metabolites [3]. The abuse of nutmeg for recreational purpose is reportedly rife among college students, prisoners, and drug addicts [2]. In acute nutmeg toxicity, symptoms that suggest effects of the substance on the central nervous system (CNS) and the cardiovascular system (CVS) have been reported but hepatic involvement is rare [4-6].

Recent animal studies however show significant elevations of plasma levels of liver enzymes (markers of hepatic injury) in male rats on chronic treatment with oral dose of aqueous extract of nutmeg [7]. The findings of Al-Jumaily *et al*[8] in mice also show significant elevations in plasma levels of liver enzymes from exposure to 500 mg/kg and 1000 mg/kg of the methanolic extract of nutmeg. These and related findings [9] suggest a degree of hepatotoxicity of the aqueous and methanolic fractions of nutmeg in rodents. In the present work, we report hepatic sinusoidal dilation and elevated plasma concentrations of liver enzymes in adult rats treated with chronic oral dose of ethanolic fraction of nutmeg. Long-term (28 days) and higher dose (80 mg/kg/d) but not short-term (7 days) and lower dose (20 mg/kg/d) of ethanolic fraction of nutmeg is associated with hepatic injury.

MATERIALS AND METHODS

Preparation of ethanolic extract of nutmeg

Dry seeds of *M. fragrans* (152 g) were soaked in 600 ml of 75% ethanol for 72 hours to extract the ethanolic fraction of nutmeg, as described by Olaleye *et al*[10]. The filtrate was concentrated by evaporation at 40 ^{oC} in a water bath. The resulting solid mass (extract) was used in the present study.

Animals

Female Wistar rats (160-201 g) were randomized to receive oral doses of ethanolic fraction of nutmeg (20 mg/kg/d or 80 mg/kg/d) as an acute regimen for 7 days (7d) or a chronic regimen for 28 days (28d); with normal saline as vehicle. Control rats received oral normal saline. Animal handling was in line with the guidelines recommended by the Institutional Animal Care and Use Committee (IACUC) and the National Academy of Sciences Guide for the Care and Use of Laboratory Animals [11].

Collection of blood and liver samples for analysis.

At euthanasia (7d and 28d), control and treated rats were anaesthetized with diethyl ether. Blood was collected into heparinized tubes by cardiac puncture. The blood was centrifuged at 10,000 rpm for 20 minutes. The plasma was analyzed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) according to the method of Reitman and Frankel[12]; as well as for gamma-glutamyl transferase (GGT), according to the method of Smith *et al*[13].

In addition, animals were perfused with normal saline and 4% paraformaldehyde. The liver was removed and fixed in paraformaldehyde for paraffin embedding. Hepatic paraffin sections were then stained by the haematoxylin and eosin method, as described by Bancroft and Stevens [14].

RESULTS

Oral nutmeg treatment induces hepatic sinusoidal dilatation:

Oral treatment with ethanolic fraction of nutmeg is associated with hepatic sinusoidal dilatation in all the treatment regimens (Fig. 2). However, no necrotic changes in the liver parenchyma were observed.

Long-term treatment with higher oral dose of nutmeg raises plasma levels of liver enzymes.

To test the toxicity profile of oral nutmeg on the liver, we assayed plasma levels of ALT, AST and GGT in the treated and control rats. Significant elevations ($P<0.05$) in the plasma levels of these markers of hepatic injury occurred in rats on long-term (28d) treatment with higher dose of nutmeg (80 mg/kg/d) (Fig. 1). Plasma levels of ALT and AST were not significantly different from the controls in rats that received 20 mg/kg/d and 80 mg/kg/d of the ethanolic fraction of nutmeg for 7d (Fig. 1A and 1B).

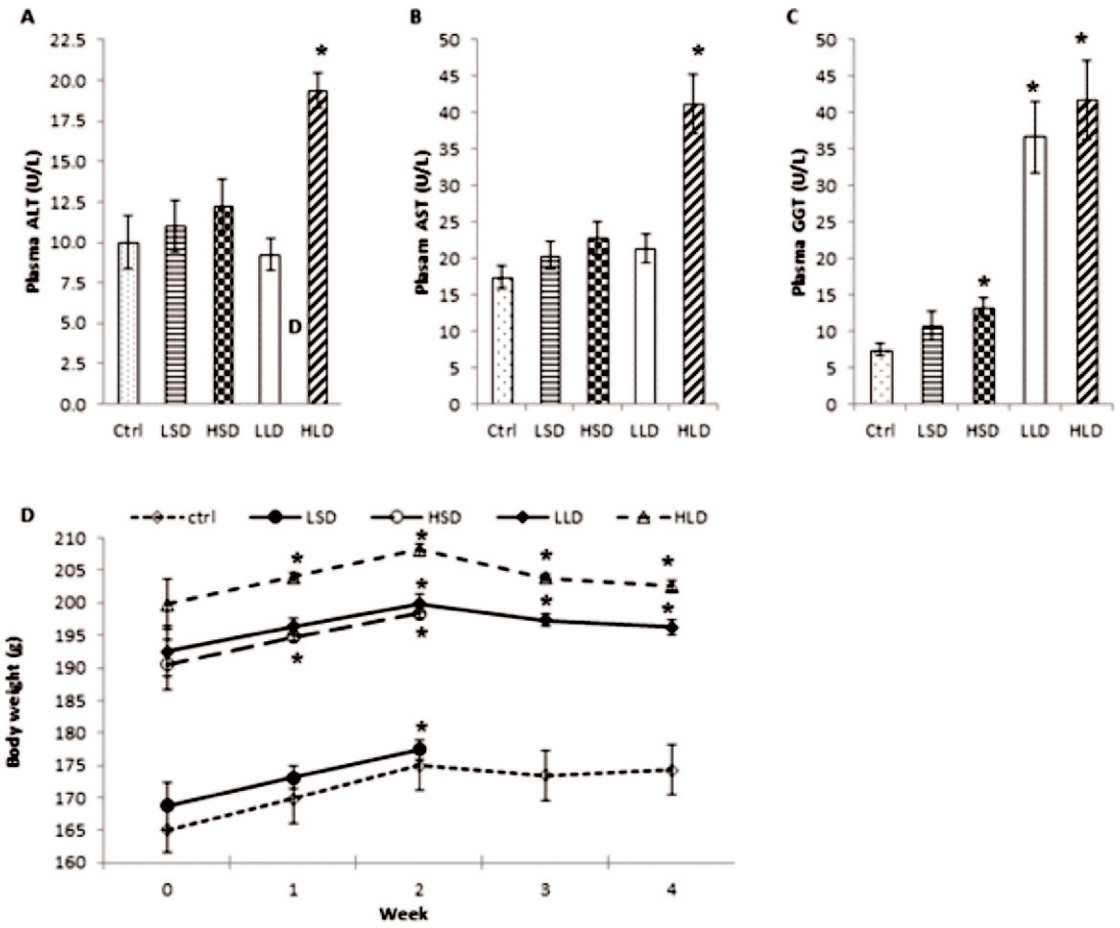

Figure 1.Body weights and plasma levels of liver enzymes in the treated and control rats. (A-C), Significant increases ($P<0.05$) in alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase have occurred in rats on chronic regimen of higher dose (80 mg/kg/d) of the ethanolic fraction of nutmeg. Values are mean ± SEM of 5 animals; * indicates $P<0.05$ compared with controls. (D), Body weight of the control and treated animals. Significant increases had occurred in the body weights of the treated animals at euthanasia, when compared with the basal weights at week 0. Values are mean ± SEM; * indicates $P<0.05$ compared with the basal weights of rats in each group.

Effects of oral nutmeg treatment on body weight of Wistar rats:

At euthanasia, significant increases ($P<0.05$) in body weight occurred in the nutmeg-treated rats compared with the basal weight of these animals (weight at week 0). In the control rats, increases in body weight at euth anasia were not significantly different from the basal body weight (Fig. 1D).

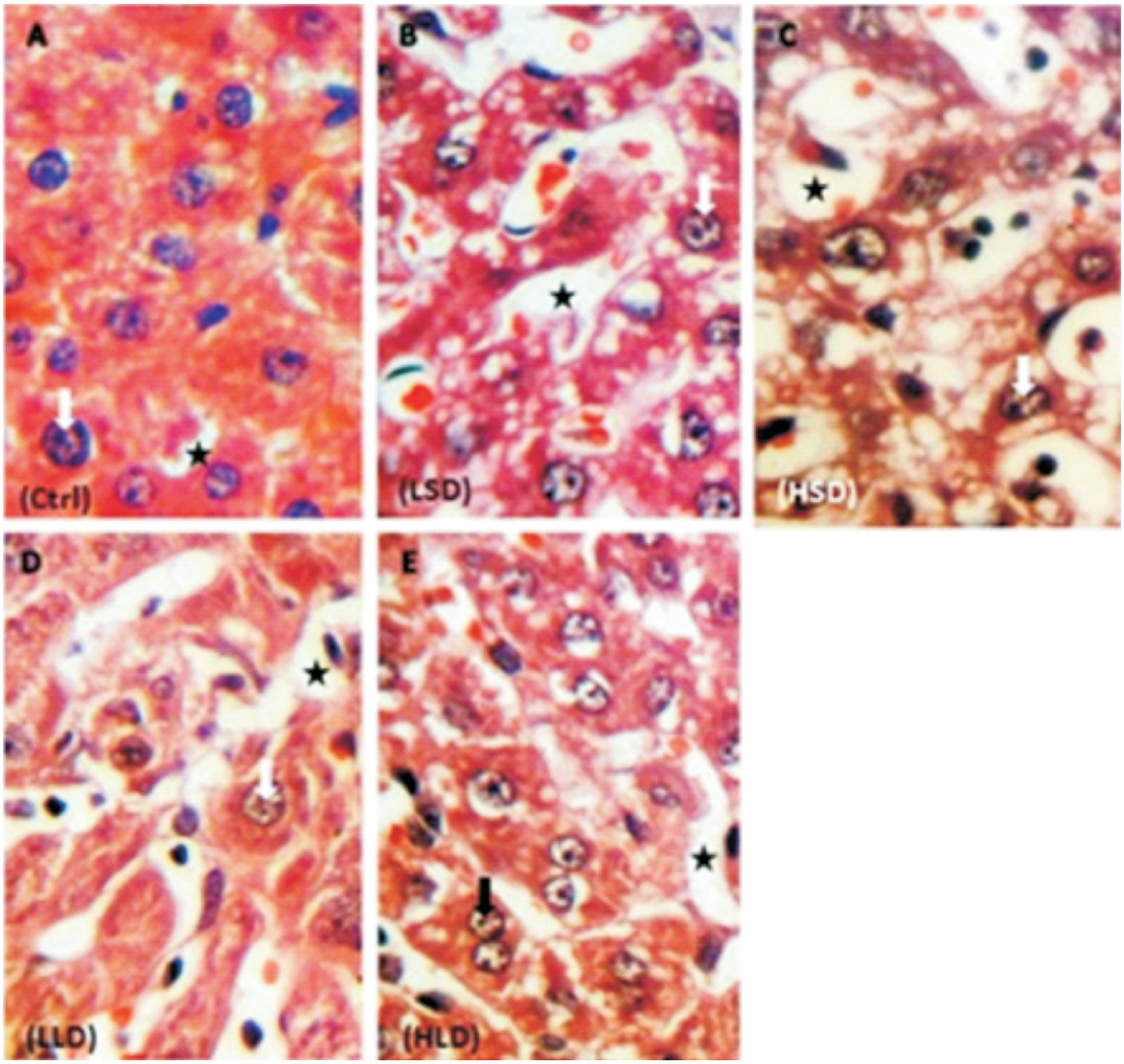

Figure 2. Representative light microscopic images of the liver of control and treated rats. Hepatic sinusoidal dilatation is obvious in all nutmeg-treated rats (Figure 2B-E) compared with the control where sinusoidal dilatation is absent. Figure 2A. Arrows indicate hepatocyte nuclei; * indicates hepatic sinusoid. H&E stain; x400.

DISCUSSION

We studied hepatic toxicity profile in rats treated with chronic and acute oral doses of the ethanolic fraction of nutmeg. Our findings include elevated plasma levels of liver enzymes in rats that received higher (80 mg/kg/d) and long-term (28d) dose of ethanolic fraction of nutmeg, but not in rats on short-term (7d) lower dose (20 mg/kg/d) of the extract (Fig. 1).

In related studies in mice [8, 9], acute (7d) oral treatment with methanolic fraction and oil of nutmeg at 500 mg/kg/d and 1000 mg/kg/d was associated with elevated plasma concentrations of liver enzymes. This contrasts with our findings in the present work; where acute (7d) treatment with ethanolic fraction of nutmeg was not associated with elevation of plasma levels of ALT and AST. This difference could arise from the relatively high doses of nutmeg extract (500 mg/kg/d and 1000 mg/kg/d) used in the work of Al-Jumaily *et al*[8, 9]. Furthermore, Hummdi [7] reported elevated plasma levels of liver enzymes in male rats on chronic (6 weeks) treatment with 1000 mg/kg/d of aqueous extract of nutmeg. Their findings indicate hepatic toxicity of nutmeg extract (at 1000 mg/kg/d) in rats on chronic regimen; and this agrees with our report in the present study. Despite the relatively low dose (80 mg/kg/d) of ethanolic fraction of nutmeg administered to rats in our work, the significant increases in plasma levels of liver enzymes in these chronically-treated rats was comparable to the findings of Hummdi [7]. This suggests that even at a low dose of 80 mg/kg/d, chronic (28d) treatment with extract of nutmeg in rats produces hepatic injury comparable to findings in rats treated with much higher doses of 500 mg/kg/d and 1000 mg/kg/d [7-9].

In addition to biochemical indices of hepatic injury, we studied haematoxylin and eosin-stained paraffin sections of the liver. As shown in Fig. 2, hepatic sinusoidal dilatation was present in all rats that received ethanolic fraction of nutmeg extract compared with the control animals. Hepatic sinusoidal dilatation could indicate sinusoidal congestion that may be associated with impaired hepatic venous drainage. This condition may stem from impairment of cardiac functions in the nutmeg-treated rats. The observed sinusoidal congestion could predispose to hepatocyte injury owing to poor hepatic circulation [15, 16]. Thus, our findings of hepatic sinusoidal dilatation in this work may suggest deleterious effects of nutmeg extract on cardiac contractile functions. In human, cardiovascular effects of nutmeg have been reported [2, 6].

In a related study by Hummdi [7], aqueous extract of nutmeg at 1000 mg/kg/d induces sinusoidal congestion with endothelial necrosis [5] at 6 weeks of treatment. These hepatic structural changes are in accordance with our histologic findings in the present study. They do suggest that chronic treatment with nutmeg extract does induces hepatic pathology characterized by sinusoidal dilatation and congestion (Fig. 2), in conjunction with elevation of plasma levels of liver ALT, AST and GGT (Fig. 1). Nevertheless, there were no adverse effects of the extract on the body weights of these rats (Fig. 1D).

Whether the biochemical and histological changes induced by chronic nutmeg treatment are attributable to phytochemicals in this spice is unclear, and require clarification. In the literature, reports of acute toxicity associated with nutmeg overdose in human do not often include hepatic lesions, but CNS and CVS effects [1, 2, 4-6]. The latter effects are reportedly produced by such phytochemicals in nutmeg that include myristicin [5], which is the most widely reported active principle in nutmeg.

However, could the hepatic toxic effects of nutmeg reported in the present and past studies [7-9] be induced by mycotoxin contamination of the seed of *M. fragrans*? Laboratory analysis of a number of common spices for the presence of mycotoxins clearly shows the high rates of aflatoxin contamination of nutmeg. Contamination of nutmeg with aflatoxins B1, B2, G1, and G2 is a common occurrence [17, 18]. It is therefore not impossible that the elevation of plasma levels of liver enzymes associated with chronic exposure to nutmeg extract was a result of deleterious aflatoxin action on the liver tissue. In the present and related studies [7-9], mycotoxin levels of the administered nutmeg were not analyzed. This is therefore strongly recommended in future animal and human studies of the hepatic effects of chronic nutmeg treatment.

In conclusion, our findings in the present work show that oral treatment with long-term (28d) and higher dose (80 mg/kg/d) but not short-term (7d) and lower dose (20 mg/kg/d) of ethanolic fraction of nutmeg is associated with hepatic injury characterized by sinusoidal dilatation and elevated plasma level of liver enzymes. Whether this hepatic toxicity of nutmeg is produced by the phytochemicals in this spice or is a result of mycotoxin contamination of nutmeg remains to be clarified; and is a subject for further investigation in our laboratory.

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