Comparative Histological Effects of Myristica fragrans (Nutmeg) on Heart of Male and Female Rats

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Abstract: Nutmeg (Myristica fragrans) seed is one of the important tree spices of the world. Widely used as a spice and used to flavour many kinds of baked foods. The present investigation was carried out to study the effect of nutmeg extract on heart tissue of male and female rats. Experiment conducted on the rats of both sexes (n = 40) were randomly divided into four groups (n=10 each). The rats in the treated groups (G.III and G.IV) given orally 1000 mg/kg/day b.w daily single dose of aqueous extract nutmeg for 6 weeks. The results revealed no significant differences in histological findings between both sexes of rats. Male cardiac fibers showed vacuolations, necrosis, deformed nuclei, congestion of endomysial capillaries and perimysial capillaries in male rats. Female treated rats recorded distortion, necrotic muscle bundles, loss of nuclear and cellular components and fibrosis perivascular. TEM observed swollen and deformed nuclei, segregation of nucleolar components and sarcoplasmic degeneration with dilated cisternae of sarcoplasmic reticulum, mitochondrial, desmosomal and gap junctions damaged. Female treated hearts tissues appeared deformed and distorted mitochondria with dilatation of sarcoplasmic reticulum cisternae and myofibrils loss. Thus, based on the results of this study, it can be concluded that the chronic consumption of Nutmeg in dishes and soft drinks induce histopathological changes in heart tissue of both sexes.

Key words: Myristica fragrans • Rats • Myocardium • Histopathology

INTRODUCTION

Nutmeg (Dried seed kernel of Myristica fragrans) belongs to the Myristicaceae family and is utilized by most people, either for spice and flavor many kinds of baked goods and vegetables or medicinal purposes. It has been reported that nutmeg has anti-diarrheal agent for patients with medullar carcinoma of the thyroid, antifungal, hepatoprotective and antioxidant effects [1]. Moreover, nutmeg is applicable for treatment of tuberculosis, against colds [2], fever and in general for respiratory ailments [3]. It is also used as anthelminthic and against skin diseases like eczema and scabies [4]. It also plays an important role as tonic for the heart, brain, sexual and general debility [5]. Nutmeg is a native of Banda Islands of Eastern Indonesia and is cultivated in Malaysia and in Southern India [6]. Recent findings pointed hepatocytes deleterious on the liver of both male and female rats administered Myristicafragrans seeds [7], due to nutmeg interference with metabolic activities [8]. While, nutmeg caused histopathological alterations in the urinary tissue of female and male rats[9]. However, few specific studies have been performed concerning the relationship between nutmeg and histological alterations induced tissues damage. Therefore this study was designed to characterize the possible effects of aqueous extract of nutmeg on heart tissue in male and female normal rats.

MATERIALS AND METHODS

Nutmeg Plant: The seeds of Myristica fragrans were purchased from a local super- market in Saudi Arabia (Jeddah) and identified as Myristicafragrans in the King Fahd Center for Medical Research, King Abdulaziz University according to method Olaleye et al. [5].

Preparation of Myristica fragrans (Nutmeg) Extract: The required amount of grated nutmeg seeds powdered (760g) was weighed and then soaked in 3 L of hot distilled water and left to stand for 72 hour, the extract was filtered and freeze-dried and kept frozen until used [9].
Animals and Experimental Design: Forty wistar rats (Rattus norvegicus) of both sexes weighting between 180 - 200 g were used for this work. They were purchased from Animal House of King Fahd of Medical Researchers in Jeddah, Saudi Arabia. Animals were maintained under standard laboratory conditions of temperature of 25±2°C, qualified humidity of 60 ± 5% and at 12-h light 12 h dark cycles. They were fed daily with standard pellet diet and water ad libitum. Rats were divided into four groups, ten rats in each. Groups I and II were served as control. Groups III and IV were served as treatment groups. Rats were orally received 1000 mg kg body weight of aqueous nutmeg extract for forty two days (6 weeks).

Changes in body weight, behavior of animals in all groups were noted during the experimental at standard intervals. At the end of the experiment, hearts (Left ventricle) were quickly removed from sacrificed rats and cleansed of blood using 0.09M saline solution. They were then fixed separately in 10% formalin solution overnight. Fixed tissues were dehydrated through ascending grades of ethanol. They were cleared in xylene and embedded in paraffin wax. Sections were cut at 5µm using a rotatory microtome. The sections were dewaxed in xylene and dehydrated through descending grades of ethanol to water. They were stained with Harris haematoxylin and eosin (H&E), then dehydrated, cleared in xylene and mounted in Canada balsam and viewed under the light microscope [8, 9]. For transmission electron microscope (TEM) small pieces of hearts tissues of both control and treated groups were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (PH. 7.4) for 2 hours at 4°C, washed with the buffer 4 times, post fixed in 1% cacodylate buffered osmium tetroxide (pH 7.4) at 4c for 2 hour. Dehydrated specimens were embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate. For detailed cytological study, sections were examined and photographed by electron microscope Philips-CM100 at King Fahd Medical Research/Jeddah, Saudi Arabia [8, 9].

Statistical Analysis: All data were presented as Mean ± SE for ten rats in each group. Differences between groups were assessed by student’s t test using the SPSS version 16 software package for Windows, USA. The P <0.05 was considered statistically significant.

RESULTS

Body and Heart Weight: There was a significant difference observed in the body and relative heart weights of both rats sexes (Table 1).

Histological Results: The light microscope examination of control male and female hearts (Left ventricles) revealed normal appearance of cardiac muscle fibers which branching and anastomosing with acidophilic sarcoplasm and central elongated vesicular nuclei (Fig.1a, b; 3a,b). Histological investigation of male treated group observed muscle fibers vacuolations, interstitial edema, focal muscle fibers necrosis and mast cells infiltration (Fig.1c, d). While, other sections pointed diffused muscle fibers degeneration as necrotic fibers with vacuolated cytoplasm, cardiomyocytes lysis, deformed nuclei, congestion of endomysial capillaries and perimysial capillaries, marked spread of macrophages and mast cells (Fig.1e, f). On the other hand, female treated group indicated distortion, fragmented, necrotic muscle bundles and damage endomysial capillaries (Fig.3c, d). Sever vacuolations, loss of nuclear and cellular components connective tissue separation or enlargement, damaged epimysial blood vessel and fibrosis perivascular areas were also seen in the other sections of myocardium of female treated rats (Fig. 3e, f).

Table 1: Body weight and absolute and relative heart weights of experimental and control rats after 6 weeks of Nutmeg administration

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Weights (g)</th>
<th>Male Control (G1)</th>
<th>Male Treated (G2)</th>
<th>Female Control (G3)</th>
<th>Female Treated (G4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight</td>
<td>M</td>
<td>296.6 ± 11.32961</td>
<td>272.8 ± 9.09615</td>
<td>254.4 ± 2.71293</td>
<td>242.2 ± 11.20893</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>± 11.32961</td>
<td>± 9.09615</td>
<td>± 2.71293</td>
<td>± 11.20893</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>-</td>
<td>0.0401*</td>
<td>-</td>
<td>0.0343*</td>
</tr>
<tr>
<td>Absolute heart weight</td>
<td>M</td>
<td>2.44 ± 0.02449</td>
<td>2.76 ± 0.29086</td>
<td>0.04899 ± 0.04899</td>
<td>± 0.03742</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>± 0.02449</td>
<td>± 0.29086</td>
<td>± 0.04899</td>
<td>± 0.03742</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>-</td>
<td>0.045*</td>
<td>-</td>
<td>0.179</td>
</tr>
<tr>
<td>Relative heart weight</td>
<td>M</td>
<td>0.828 ± 0.02672</td>
<td>1.004 ± 0.09331</td>
<td>0.390 ± 0.01643</td>
<td>± 0.0204</td>
</tr>
<tr>
<td></td>
<td>± SEM</td>
<td>± 0.02672</td>
<td>± 0.09331</td>
<td>± 0.01643</td>
<td>± 0.0204</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>-</td>
<td>0.0164**</td>
<td>-</td>
<td>0.0229*</td>
</tr>
</tbody>
</table>

* P <0.05, ** P <0.01
Plate 1a-f: Longitudinal section (a) and transverse section (b) of male rats left ventricles tissue of control group showing branching and anastomosing cardiac muscle fibers with acidophilic sarcoplasm (Thick arrows) and central elongated vesicular nuclei (Thin arrows), (c-f) sections of the heart tissues of male rats treated with 1000 ml/kg; (c, d) showing muscle fibers vacuolations (Arrows), edematous deposit (head arrows), slight muscle fibers necrosis (Nc) and mast cells (Mc) diffused, (e, f) diffused muscle fibers degeneration; areas of necrotic fibers with vacuolated cytoplasm (Arrows), cardiomyocytes lysis (Head arrows), deformed nuclei (N), congestion of endomysial capillaries (Ec) and perimysial capillary (*), marked diffused of macrophages (Ma) and mast cells (Mc), (H&E, X1000).

Electron microscope examination of left ventricles tissue of control male and female rats showed the normal nuclei and a well arrangement myofibrils within a fiber is aligned repeating series of dark bands(Myosin filaments) in the middle and light(Actin filaments) bands at the periphery (Fig. 2a, 4a). When comparing treated male hearts micrographs with the control rats observed hypertrophy and deformed nuclei, with segregation of nucleoli components, sarcoplasmic degeneration with moderate dilated cisternae of sarcoplasmic reticulum and mitochondrial lysis (Fig. 2b, c). Deformed desmosomes and gap junctions, sever sarcoplasmic degeneration with dilated cisternae of sarcoplasmic reticulum were also seen (Fig. 2d, e). Micrographs of treated female myocardium appeared deformed and distorted mitochondria with lost cristae leaving empty space, sever dilatation of sarcoplasmic reticulum cisternae and myofibrils loss (Fig. 4b - d).

**DISCUSSION**

In the present study, the mean body weights of male rats were significantly depressed as well as significant increase in the mean absolute and relative heart weights
Plate 2a-e: Electron micrographs of left ventricles tissue from control and treated male rats of: (a) control group showing the nuclei (N) and arrangement of myofibrils within a fiber is aligned repeating series of dark A bands in the middle (Arrows) and light I bands at the periphery (Head arrows), also notice sarcoplasmic reticulum (*) and mitochondria (M) (X18000), (b, c) heart of treated rat shows enlargement and deformed nucleus (Ne), segregation of nucleolus (Ne) components, sarcoplasmic reticulum degeneration (Sd), dilated cisternae of sarcoplasmic reticulum (*) and mitochondrial lysis (M); (X 14000 and X18000 respectively), (d, e) marked desmosomes and gap junctions deformed (Arrows) sever sarcoplasmic degeneration (Sd), marked dilated cisternae of sarcoplasmic reticulum (Head arrows) and mitochondrial cristae lysis (M) (X7100 and X14000, respectively)

were recorded. However, no changes in body, absolute and related heart weights were indicated for female rats as compared to control rats. This came in agreement with Hummadi [8] and Alalwani [9] studied. Likely, significant increase in relative heart weights in male rats in the present study may attribute to the interstitial oedema and congestion of endomysial and perimysial capillaries which observed in this study. These findings matched with Olalaye et al. [5], Hummadi [8] and Alalwani [9] results which noticed congestion of blood vessels of liver and kidney tissues in nutmeg treated rats. On the contrary [10], stated that no significant difference in body weight of male mice treated with the aqueous extract of nutmeg seeds. Likewise, it is noted weight gain in the rats taken nutmeg extract [5].

Chronic treatment of nutmeg extract with long duration of and high dose (1000/ mg kg b.w.) induced cyto-architectural distortion of male and female rats myocardium architecture, including muscle fibers vacuolations, necrosis, or degeneration, deformed nuclei, interstitial edema, macrophages and mast cells infiltration and perivascular fibrosis. In corresponding to our results, it has been reported that chronic administration of nutmeg extract resulted in the diffuse cellular degenerative changes, vacuolations, necrosis, nuclear damaged and fibrosis on rats myocardium [5], rats brain [11], rats kidney [9,12], rat liver [8], parotid salivary glands [13]. Whereas, Alalwani [9] and Al-Jumaily et al. [14] reported that liver treated with nutmeg extract 1000 mg/kg showed focal cellular infiltration. Moubarak [15] attributed that the
Plate 3a-f: longitudinal section (a) and transverse section (b) of female rats left ventricles tissue of control group show a normal appearance of cardiac muscle fibers (Thick arrows) and normal centrally arranged nuclei (Thin arrows); (c-f) sections of the cardiac tissues of female rats treated with 1000 ml/kg; (c, d) show distortion, fragmented (Thin arrows), necrotic muscle bundles (Thick arrows) and damaged endomysial capillary (Ec); (e, f) showing vacuolations and loss of nuclear and cellular components (Thick arrows) connective tissue separation or enlargement (Head arrows), damage endomysial capillary (Ec), epimysial blood vessel (*) and fibrosis perivascular area (Thin arrows) (H&E, X1000).

Congestion of the blood vessels might be a part of inflammatory response to bring more blood to the areas of fibrosis or degeneration. In agreement with the recent work [8,9], observed nuclear alteration such as hypertrophy or pyknosis.

Ultrastructure studies of the cardiac myofibrils of male and female rats revealed sarcoplasmic degeneration, hypertrophy and deformed nucleus with segregation of nucleolus components, dilated cisternae of sarcoplasmic reticulum, mitochondria distorted and lysis. Deformed desmosomal and gap junctions [10] pointed that the some nuclear changes were noticed in the form of redistribution of chromatin granules and its adherence to the nuclear membrane. According to the present study [8] found alterations and dilatation of endoplasmic reticulum in the liver of both male and female nutmeg treated rats. It was stated that the endoplasmic reticulum from the variable cell organelles, which swells or atrophy reflects a state of altered functional activity [9]. In harmonized with this study, Hummdi [8] found swollen mitochondria with cristae disruption in nutmeg treated rats. While, Alalwani [9] adds the atrophy of mitochondria and lysis were observed in glomerular cells of kidney in male and female rats. The Ghadially [16] studied were supported by Olaleye et al. [5] observations that stated the hepatocytes of nutmeg administrated rats showed deformed desmosomal junctions.

Suggested that the histopathological may be due to nutmeg interference, since it has been reported that myristicine obtained from the nutmeg may have a cytotoxic and apototic effects on the body [11]. In addition to, Eweka and Eweca [12] pointed out that the
possible deduction from above results is that secondary metabolites of nutmeg, which are largely responsible for therapeutic or pharmacological activities of medicinal plants [17], may also account for their toxicity when the dosage is abused.

REFERENCES


