

Histological and Cytological Effects Of Aqueous Extract of *Myristica fragrans* (Nutmeg) on the Renal Cortical of Male and Female Rats

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Abstract: Aqueous extract of *Myristica fragrans* was evaluated for its renal cortical structures of changes of male and female Wistar rats. Rats of both sexes (n = 40) were randomly assigned into two treatment and two Control groups (n=10 each). Groups (G1&G3) served as control, while the rats in the test groups (G2&G4) received oral daily single dose of 1000 mg/kg b.w of aqueous extract nutmeg for 6 weeks. Serum biomarker urea nitrogen, serum creatinine and alkaline phosphatase of kidney injury pointed at levels increased as well as body weights and the mean relative kidney weights were affected by the treatment. Examination histological showed congested and fragmented glomeruli of treated male rats. While, glomerular in the female rats appeared atrophy and tissue lysis. However proximal convoluted tubules appeared atrophy, necrosis, cytoplasmic degeneration with brush border damage and cellular infiltration in male and female treated groups. In addition, destruction of cytoplasmic organelles, irregular of basement membrane with dilation of basal in folding were observed in distal convoluted tubules both treated sexes. The results of the present study suggest that use of nutmeg in higher doses (1000 mg/kg) could be toxic to the renal cortical tissues and metabolic functions in both rats sexes.

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Key words: *Myristica fragrans* (nutmeg), kidney tubules, histopathology, ultrastructure, creatinine, urea.

1. Introduction

There is a tendency to use herbal medicines in all parts of the world, hence it is possible to misuse *M. fragrans* in order its medicinal properties. The nutmeg plant, *Myristica fragrans* Houtt, is a member of the small primitive family called Myristicaceae, taxonomically placed between the Annonaceae and Lauraceae (Joseph,1980). Medicinally, nutmeg is known for its anti-inflammatory (Olajide *et al.*, 1999), as well as anti-rheumatic, carminative properties (Prabuseenivasan *et al.*, 2006). Utilization nutmeg as a medicinal or spice suggest that it contains some constituents which are responsible for the reported biological activities. Some of these activity may at the same time possess some adverse effects. One of the active ingredients in nutmeg is called myristicine, which has neurotoxic effects (Madsen and Bertelsen,1996). It has also been found possesses anti-inflammatory (Olumayokun *et al.*, 1999) antifungal, hepatoprotective (Tatsuya *et al.*, 2003), anti-diarrheal agent, and tonic for the heart and brain (Olaleye *et al.*,2006). Nutmeg has long been known for its psychoactive properties when ingested in large quantities (1-3 nutmegs) causing convulsions, hallucinations, and possibly death (Kelly *et al.*, 2003; Forrester, 2005). It has been reported to reduce cell viability in a dose dependent manner (Lee *et al.*, 2005). Previous studies by Hummdi, (2011) reported that administration of

nutmeg may have some deleterious effects on the hepatocytes of adults rats at higher doses. Indiscriminate use is well reported previously in the medical literature (Sonavane *et al.*, 2002; McKenna *et al.*, 2004). However, subcellular pathological perspective are studied. Therefore, study possible subcellular effects of chronic consuming of nutmeg on the glomerular and renal tubules of adult male and female Wistar rats could be worthwhile. With a view to advising the consumers on the inherent dangers of excessive consumption of the *M. fragrans*.

2. Materials and Methods

Experimental animals

The study was conducted in accordance with the National Institutes of Health guidelines for the use of experimental animals. Wistar rats, a total of 20 males and 20 females were divided randomly into four groups (10 rats per group), weighing 248.60±9.553g and 234.40± 8.264 of male and female respectively. Obtained from the Animal House of the King Fahd Center for Medical Research, King Abdul Aziz University in Jeddah of Saudi Arabia. Rats were maintained on a 12 h light/dark cycle at 21 ± 1°C and 50 ± 10% humidity, and left several days of adaptation.

Preparation of extract

Seeds of *Myristica fragrans* found in Spices market in Jeddah of Saudi Arabia, they have been

brought and authentication in the King Fahd Center for Medical Research, King Abdulaziz University according to **Olaleye et al., (2006)** method grated nutmeg seeds (760g) were soaked in 3 L of hot distilled water and left to stand for 72 hrs, then filtered the extract. The extract was freeze-dried and kept frozen until used.

Nutmeg administration

The animals in treatment groups (G2 & G4) were daily given 1000 mg/kg/ b.w. of *Myristica fragrans* (Nutmeg) aqueous extract orally for forty-two days (6weeks). Control groups (G1 & G3) the rats were used as control and given orally distilled water throughout the experiment. Thereafter, during the entire experiment, all animals were checked daily for state of health and behavior. Individual body weights were recorded weekly. After 6 weeks of treatment all animals of group were sacrificed by cervical dislocation and blood biochemical assay were done and quantitative morphometrics analysis. Histopathological evaluations and electron microscopic studies were performed on kidney tissues.

Biochemical study

At the end of the trail-24 hours after the experiment, the blood samples were collected and analyzed for serum creatinine (Cre), urea nitrogen (UN), alkaline phosphatase (ALP) were determined according to the procedures recommended by the manufacturer of the kits employed according to **Chromy et al., (2008)** procedures.

Histological study

After sacrifice rats the kidneys transported quickly dissected out and fixed in 10% formal saline for routine histological techniques. The tissues were

dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 2 microns thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with hematoxylin and eosin (**Bancroft and Gamble, 2002**). Photomicrographs of the desired sections were made by light microscope for further observations.

Electron Microscopy

Samples of kidney were fixed in 2.5% glutaraldehyde and 0.25 M sodium cacodylate, post-fixed in 1% osmium tetroxide and embedded in Spurr's epoxy. Ultrathin sections were picked up on nickel grids, stained with uranyl acetate/lead citrate (**Woods and Stirling, 2002**) and examined in a Philips TEM 100 microscope in KFCMR.

Statistical analysis

All data were presented as Means \pm SE. Multiple comparisons were performed using student's t-test. A value $p < 0.05$ were taken into consideration for determining significance. All Statistical procedures were computed using SPSS 18.0 software.

3. Results

Body and kidney weights

The *M. fragrans* extract caused a significant increased in the mean value of body weight of male rats, While there was no visible increased in body weights in females **Table 1**. Significant increased in relative kidney weights of male animals and highly significant decreased of female animals given nutmeg compared to their respective controls, it also no mortality or treatment macroscopic findings observed in both rats sexes were recorded.

Table 1: Body weight and relative kidney weight of experimental and control rats after 6 weeks of Nutmeg administration.

Weights		Experimental Groups			
		Male		Female	
		Control (G1)	Treated (G2)	Control (G3)	Treated (G4)
Body Weight	Mean	248.60	301.60	234.40	242.00
	\pm SE	± 9.553	± 10.375	± 8.712	± 8.264
	P	-	.002**	-	.586
Relative kidney weight	Mean	0.680	1.040	0.620	0.410
	\pm SE	± 0.048	± 0.132	± 0.020	± 0.001
	P	-	.057*	-	.003**

* $P < 0.05$, ** $P < 0.001$

Effects of nutmeg on renal function

Increased levels of serum creatinine (Cre), urea nitrogen (UN) and alkaline phosphatase (ALP) were

observed as biochemical parameters in the treated rats (**Table 2**).

Table 2: serum creatinine and serum urea of experimental animals kidney and control rats after 6 weeks of Nutmeg administration.

Kidney bioassay		Experimental Groups			
		Male		Female	
		Control (G1)	Treated (G2)	Control (G3)	Treated (G4)
S. creatinine mg/dl	Mean	.650	.720	.680	.780
	±SE	±.022	±.040	±.124	±.046
	P	-	.245	-	.277
Blood urea mg/dl	Mean	4.120	4.880	4.680	5.480
	±SE	±.332	±.190	±.177	±.664
	P	-	.170	-	.008**
alkaline phosphatase (U/L)	Mean	237.00	258.60	116.20	131.80
	±SE	±15.911	±21.139	±14.392	±24.374
	P	-	.053*	-	.032*
Na ⁺ (mmol/L)	Mean	14.023	10.322	8.7722	7.2856
	±SE	±.2330	±.2371	±.3200	±.5041
	P	-	.000**	-	.000**
Cl ⁻ (mmol/L)	Mean	13.0430	12.1009	8.7391	7.5911
	±SE	.392±	±.2899	±.372	±.5008
	P	-	.001**	-	.001**

* $P < 0.05$,** $P < 0.001$

Histological results

No histological changes were observed in the control groups (G1&G3) of male and female rats kidneys. Cortical zone of rats kidneys displayed urinary glomeruli, proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs) (Figs. 1a,b). Glomeruli of renal corpuscles were made up of parietal cells and podocytes with foot processes, the glomerular basement membrane (GBM), foot processes and filtration slits were observed in normal structure (Fig.2a), mesangial cells, mesangial matrix (Fig.2b) and endothelial cells in capillary (Fig.2c). The results of light microscopy of the kidney of male animals in group (G2) revealed some congested, fragmented glomeruli and obliterated urinary space (Fig. 1c). However, electron microscopic examination of the group G2 confirm the endothelial cells necrotic and capillaries filled with deformed red blood cells. Some endothelial cells appeared marked deformed nuclei, mitochondrial hypertrophied and separation of its basement membrane in some areas (Fig.2e). As appear mitochondria necrotic inside the remains of mesangial cell and decreased mesangial matrix (Fig.2f). The podocytes has appeared nuclei atrophy, different electronic density and severe podocytes pedicles fusion association with swollen pedicles and indistinct filtration slits (Figs.2d-f).

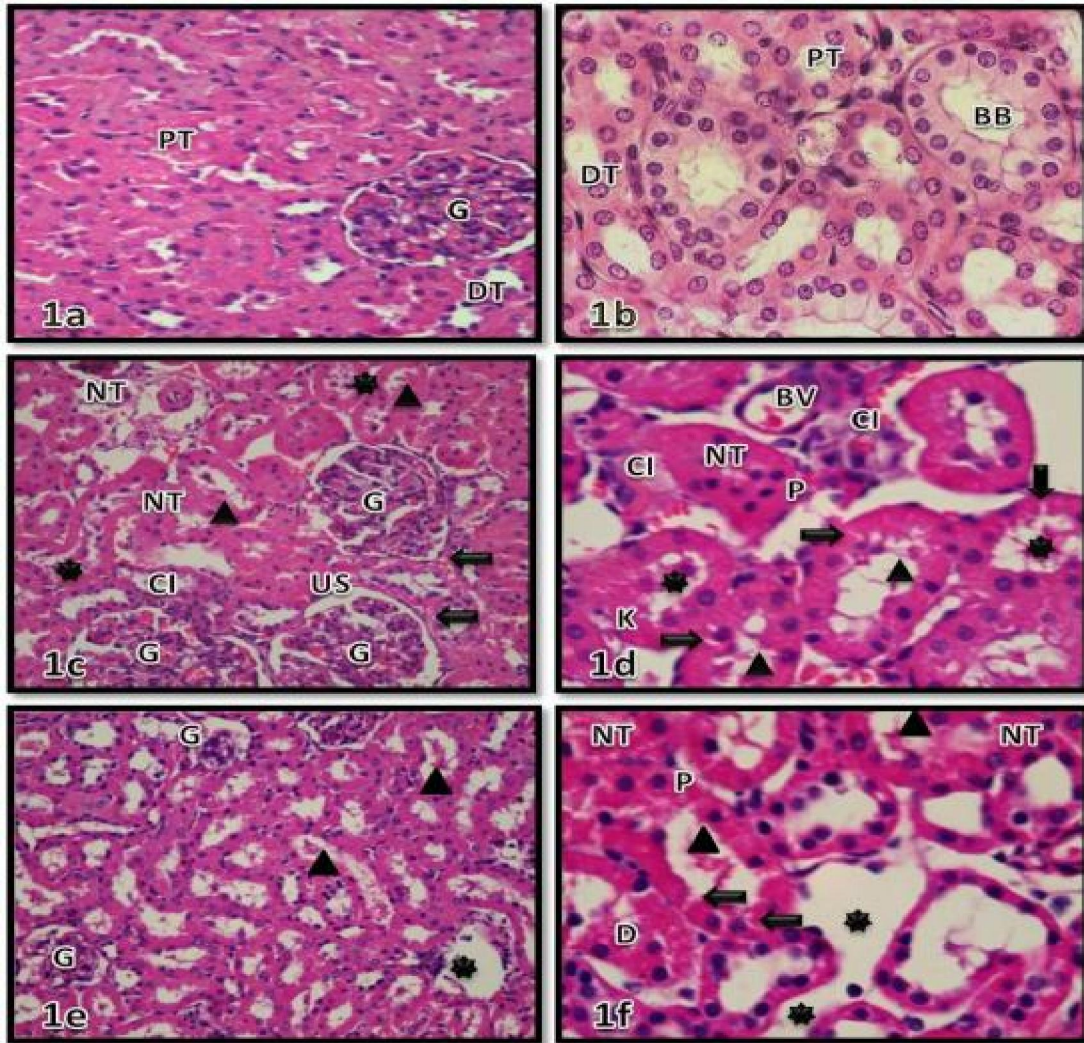
On the other hand, nutmeg caused marked histopathological alterations in the kidney of female animal groups (G4) as distortion of the renal cortical structures, and reduction in the number of renal corpuscle, some glomerular were atrophied and lysis (Fig.1e). However, electron microscopic indicated capillaries lesions associated with analyzed of cells

and GBM thickening in several areas (Fig.3a), vesiculation endoplasmic reticulum of endothelial cells and nuclei hypertrophy, internal chromatin condensation and conversion to active chromatin (Fig.3b). Hypertrophy mesangial cells, necrosis nuclei, atrophy mitochondrial and lysis (Fig.3c). Nuclei of the podocytes appeared atrophic, chromatin condensation and manifested deterioration fused pedicles and ruptured lead to cytoplasmic organelles lysis than those observed in (G2) (Figs.3a-d), in addition to, affected and deformed nuclei were indicated in the treated group (G4).

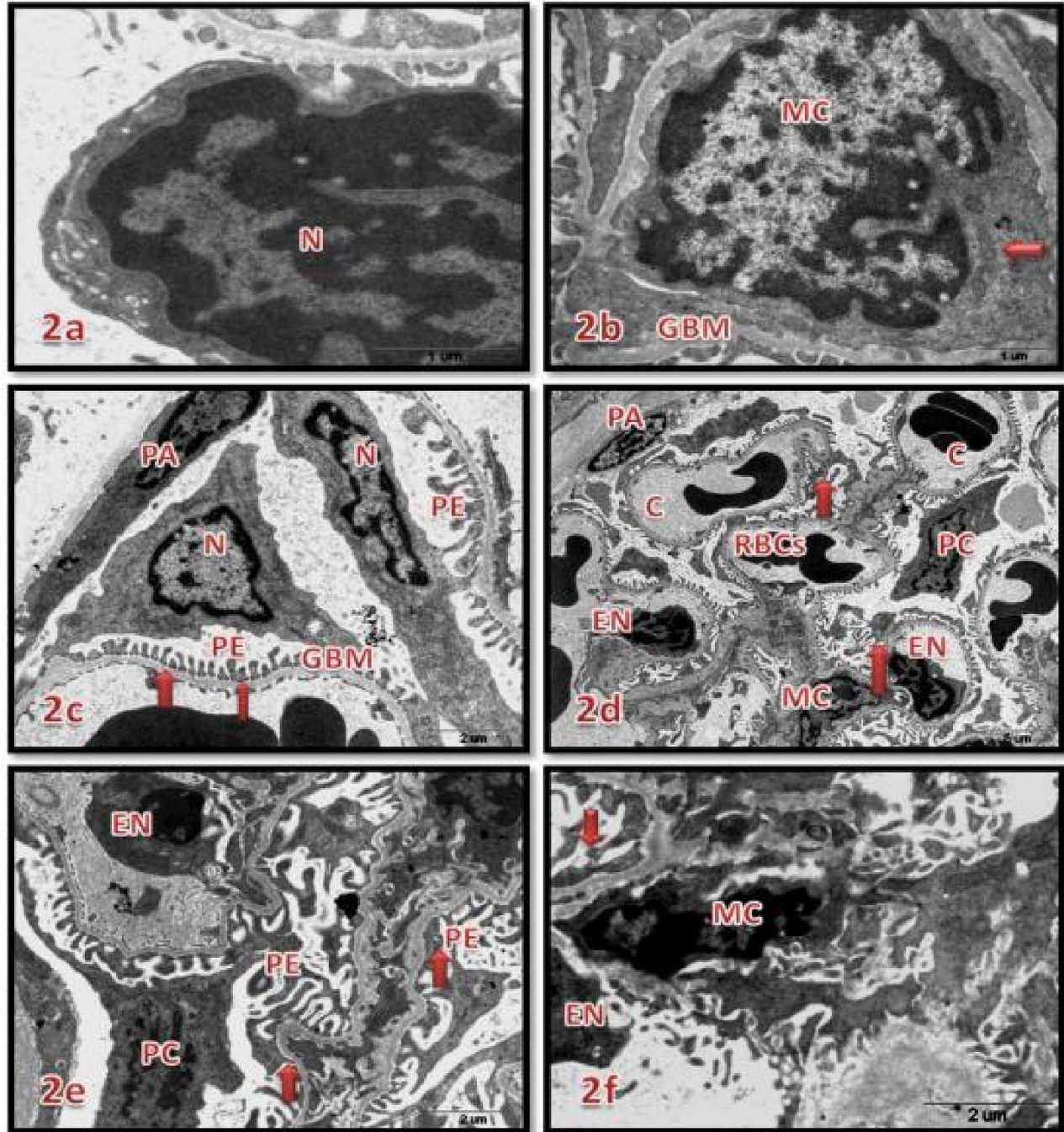
Histological examination restricted the same findings in the PCTs and DCTs in male and female treated animals G2 & G4 (Figs. 1c,f). Diffuse cytoplasmic vacuolation, widen irregular luminal surface, dilated with accumulation of eosinophilic homogenous material in tubular lumina, fragmented brush border, dilated intercellular spaces in epithelial lining cells and necrosis with nuclear pyknosis of tubular cells were seen in PCTs in treated animals. Hence, the changes showed in the DCTs displayed tubular atrophy and necrosis, cytoplasmic degeneration and separated the cells from the basement membrane. Also, deformed and nuclear margination of the tubular epithelial cells were showed (Figs. 1d,f). Through examination of the kidney of treated animal groups under transmission electron microscopy, indicated that nutmeg induced various degrees of damage on the architecture of proximal and distal tubular epithelium (Figs.4a,d), severe impairment of PCTs brush border, such as disorganized, coalescence, shrinkage, loss and vacuolated with swollen mitochondrial, fragmented and dilatation of RER in treated group (Figs.4b,c).

In addition to the appearance of cytoplasmic lysis with loss most of the cytoplasmic electron density and cellular organelles, irregularly- shaped of nuclei with loss chromatin materials. In addition to, thickened, irregular and widening the basal

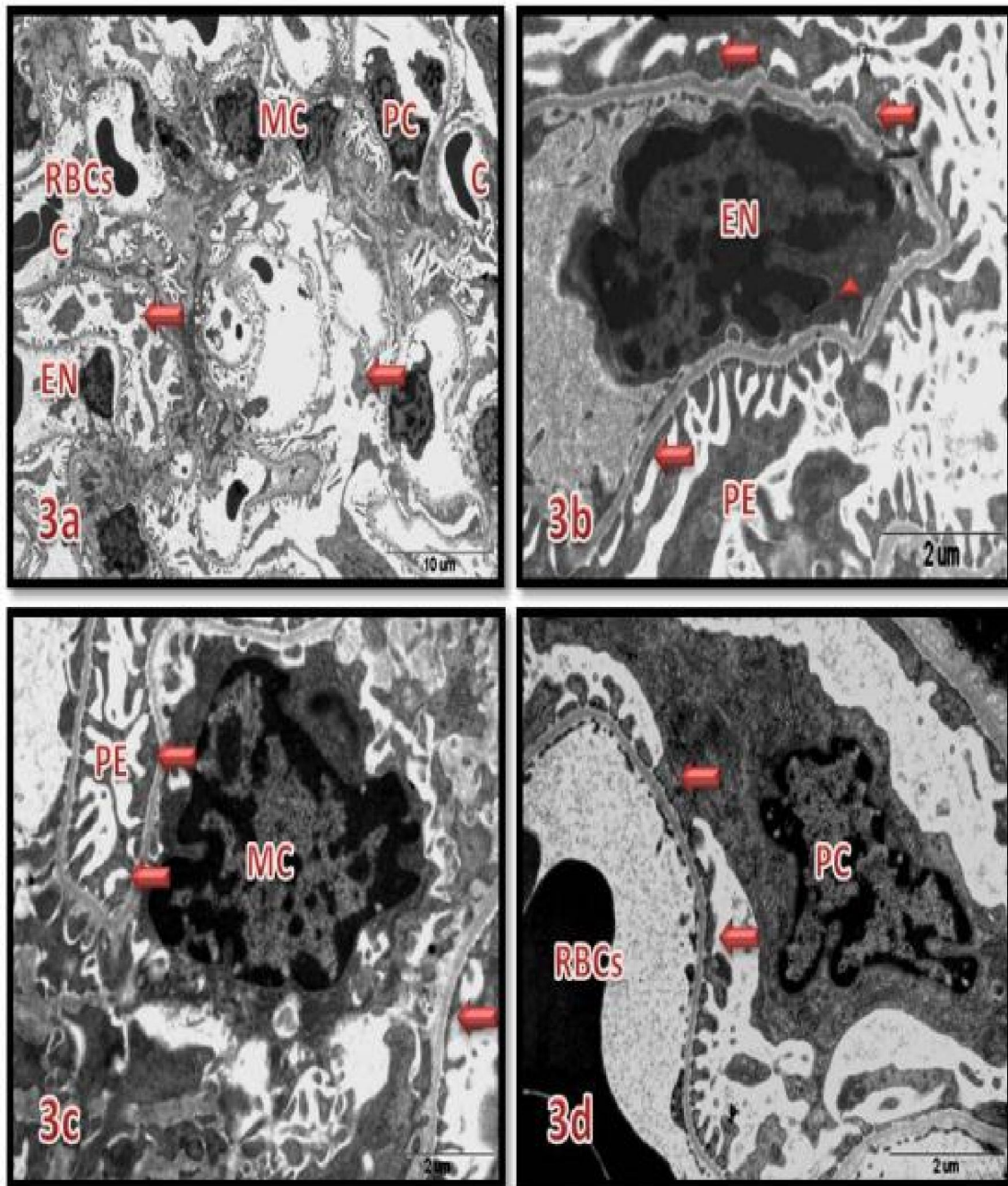
membrane infolding with scattered deformed electron dense mitochondria, tubular lumen filled with damaged mitochondria and accumulation of lipid droplets were observed in DTCs (Figs. 4e,f) in treated animal groups G2&G4.



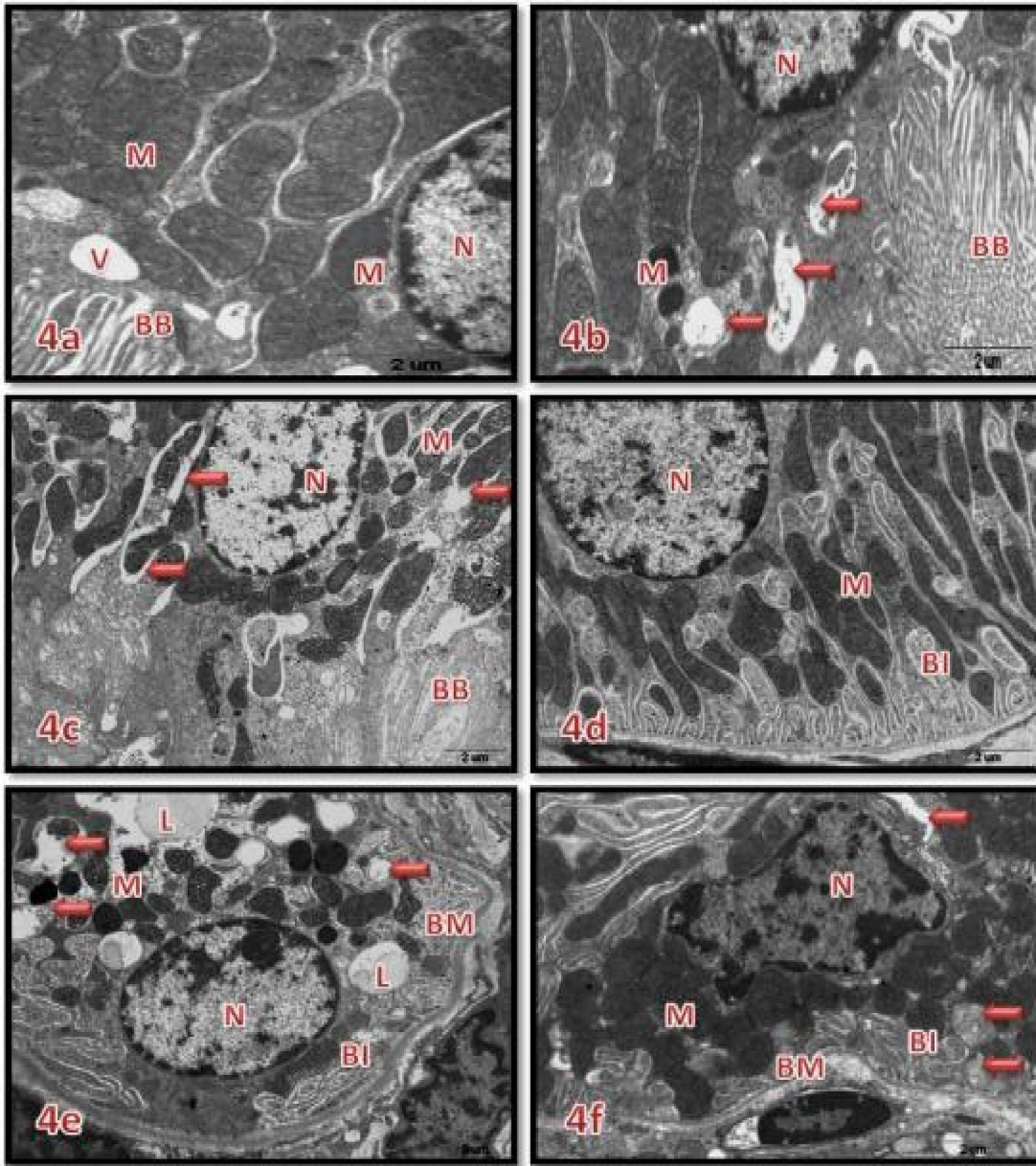
Plate(1a-f):Cross sections of kidney cortex of male and female Wister rats; H&E.(a-b): kidney sections of control rats (G1&G3):(a): showing the normal histological structure of the glomerulus(G), proximal convoluted tubules(PT) and distal convoluted tubules(DT) in the cortical portion ;x400. (b):High power from previous section showing proximal convoluted tubules(PT), brush border (BB) and distal convoluted tubules(DT) in the cortical portion ;x1000.(c-d) sections of males treated kidney(G2):(c): showing a fragmented and congested of glomeruli(G), obliterated urinary space(US), tubular atrophy and necrosis(NT), cytoplasmic degeneration (arrows), brush border damaged in some proximal tubules(head arrow), eosinophilic casts inside widen proximal tubules lumen(asterisk) and cellular infiltration(CI) ;x400.(d): showing dilation of the cortical blood vessel(BV) with red blood cells stasis,cellular infiltration(CI), tubular necrosis(NT), brush border fragmented(headarrows), pyknosis(P), karyolysis(K) of tubular cells nuclei and dilated intercellular spaces(arrows) ;x1000. (e-f) sections of females treated kidney (G4):(e):showing glomerular atrophy(G) and tissue lysis(asterisk)x400. (f): showing necrotic tubules (NT) with pyknotic nuclei (P) and tissue lysis (asterisk),blocked of some proximal tubular lumen with eosinophilic materials(headarrows), dilated intercellular spaces in epithelial lining(arrows), distal tubules with wide lumen, separated the epithelium lining from the basement membrane (D) ;x1000.



Plate(2a-f):Electron micrographs(E.M.) of renal corpuscle. **(a-c):** kidney cortex of control rats (G1&G3): **(a):** showing endothelial cell with large irregular nucleus (N). also note glomerular basement membrane(GBM) and pedicles of podocytes (PE); x19000.**(b):** showing mesangial cell (MC) mesangial matrix and normal mitochondria (arrow), note glomerular basement membrane (GBM) ;x13500. **(c):**showing podocytes with normal nucleus (N), pedicles(PE), normal appearing glomerular basement membrane (GBM) and filtration slits with diaphragm(arrows), parietal epithelial cell (PA) ;x7900. **(d-f):**section of renal corpuscle in kidney cortex of male rats treated(G3) :**(d):** a part from glomerulus showing congested capillaries (C) filled with deformed red blood cells (RBCs) and necrotic endothelial cell (EN) damaged mesangial cell (MC), mesangial matrix and focal fused foot processes (arrows) of podocytes, parietal epithelial cell (PA) ;x2600.**(e):**showing deformation nuclei of endothelial cell (EN) and separation the (EN) from its basement membrane, note podocytes (PC) with electron dense cytoplasm, swollen pedicles (PE), indistinct filtration slits (arrows);x7900.**(f):**showing necrotic mesangial cell(MC), note also necrotic endothelial cell (EN), swollen pedicles of podocytes and indistinct filtration slits(arrow) ; x7900.



Plate(3a,d): E.M. of renal corpuscle in kidney cortex of female rats (G4): (a): apart of glomerulus showing atrophied podocytes(PC) and lysis (arrows), mesangial cell hypertrophied (MC), necrotic endothelial cell (EN), deformed (RBCs) in capillary loops (C);x1950.(b):showing vesiculation endoplasmic reticulum(head arrow) of endothelial cell(EN), nucleus hypertrophy and condensation of peripheral chromatin, focal fused of podocytes (PE) foot processes(arrows); x10500. (c): showing hypertrophied mesangial cell(MC) with nucleus necrosis, mitochondrial atrophied and lysis, also note pedicles(PE) fused on epithelial side of thickened indistinct GBM(arrows) ;x7900. (d):showing podocytes (PC) nucleus appeared atrophic, chromatin condensation, focal fused foot processes (arrows) and deformed red blood cells(RBCs) ;x7900.



Plate(4a-f): (a-c) E.M. of a parts of PCT cells of rats kidney: (a): control PCT(G1&G3):showing central located nucleus(N) with normal chromatin distribution, numerous well organized elongated mitochondria (M), pinocytotic vesicles (V) and brush border (BB); x7900. (b):a part of PTC cell of treated kidney of male rat (G2): showing irregular of nuclei (N), membrane with condense chromatin materials,atrophied mitochondria (M),cytoplasmic lysis (arrows) and brush border disorganized (BB);x2600. (c): a part of damaged PCT cell of treated kidney of female rat (G4): showing deformed nucleus(N)dilation of basal membrane infolding (arrows) and scattered deformed electron dense mitochondria (M) coalescence of brush border (BB) ; x7900. (d-f) E.M. of a parts of DCT cells of rats kidney: (d): a part of normal distal convoluted tubules cell (G1&G3): showing apical spheroid nuclei(N) of DCT, well developed basal infolding (BI) surrounding elongated mitochondria (M) ;x7900.(e): a part of a DTC cell of treated kidney of male rat (G2) showing condense chromatin nucleus (N), mitochondria (M) dilation and distortion of basement membrane infolding (BI), destruction of cytoplasmic organelles (arrows), lipid droplets(L) and irregular basement membrane (BM). Note also the tubular lumen filled with secretion and lipid droplets (L) ;x5800.(f):a part of DCT cell of female treated rat (G4) showing necrotic cells with pyknotic nucleus(N), low electron dense cytoplasm, massive electron dense mitochondria(M) loss cellular organelles(arrows),shorter and damage basal infolding (BI) with irregular of basement membrane (BM) ;x7900.

4. Discussion

Increase in body weight and relative kidney weight in male rats in present work may be due to the congestion of glomeruli which observed in this study and as previously described (**Olaleye et al., 2006; Eweka and Eweka, 2010**). Other authors have reported that no significant difference in body weight of male mice treated with the aqueous extract of nutmeg seeds at the end of the 6 weeks of injection with low dose range employed 20-80 mg/kg (**Al-Hazmi et al., 2004**). While, decreased in kidney weight of female rats in the present work may be due to the atrophic changes and necrosis of epithelial cells in glomeruli and renal tubules.

Kumar et al., (1988) display that the changes in serum UN, Cre, Na^+ and Cl^- are associated with impairment of renal function. The elevated serum levels of UN and Cre and the decreased serum levels of serum Na^+ and Cl^- indicate reduced ability of the kidney to eliminate the toxic metabolic substances and reabsorb the metal and non-metal ions. These changes in serum biochemical parameters are consistent with renal histological structure degenerations (**Prasad et al., 2011**). Damage the brush border and leakage of ALP into urine could have been a result of toxin binding to the brush border. This enzyme is associated with the brush border of the renal tubules, and the urinary concentration of ALP in particular has been used as an early marker of toxic tubular agent (**Cooper et al., 1986; Porter, 1994**).

May be inferred from the results of the present study that higher doses of nutmeg consumption may have resulted in degenerative and atrophic changes observed in the renal corpuscle. The possible deduction from these results is that secondary metabolites, which are largely responsible for therapeutic or pharmacological activities of medicinal plant (**Perry, 1980**), may also account for their toxicity when the dosage is abused.

In consistent with recent work the nutmeg has a specific potential toxicity to the kidney parenchyma was confirmed (**Eweka and Eweka, 2010**). It has been reported that the chronic administration of high dose of nutmeg resulted in cellular structures hypertrophy due to the adverse effects of nutmeg on the organ tissue (**Adjene, 2010**). As well, **Al-Hazmi et al., (2004)** found fatty degeneration and cellular vacuoles.

Correspond to present work, the previous studies have reported ultrastructural changes in rats glomeruli at treatment with various pollutants, pesticides and toxins represented in glomerular cells (**Abd El-Aal and Fares, 1993**), podocytes vacuolation with pedicles fusion and GBM indistinct layers with electron dense deposits (**El-Banhawy et al., 1994**;

Rahmy and Hemmaid, 1999; Ahmed, 2000), mitochondrial, rough endoplasmic reticulum (RER) damage with nuclei pyknosis (**Ramadan, 1996; Luty et al., 1998; Latuszynska et al., 1999; Rahmy and Hemmaid, 1999**).

Moreover, **Hummdi, (2011)** stated ballooning degeneration, mitochondrial swollen with cristae disruption, dilatation with fragmented of RER, a marked distortion of the bile canaliculi microvilli, Sinusoid congestion with some degree of endothelia necrosis, increased in the number of lysosomes and lipid droplets in rats injected by nutmeg extract.

It observed in this study, the presence of tubular injuries. There were several factors that may have played a role in tubular injuries, such as decrease in glomerular capillary permeability, back-leakage of glomerular filtrate through tubular cell walls (**Alden and Frith, 1992**). All these degenerative changes are consistent with the several studies reported renal toxicity associated with toxic substances leading to DCTs histological damage (**Ramadan, 1996; Latuszynska et al., 1999; Rahmy and Hemmaid, 1999**).

Tubular obstruction may be caused by release of cytoplasmic fragments and lipid droplets mixed with intratubular proteins. This can lead to increased intratubular pressure, which again may result in sufficient back pressure to alter the transglomerular hydrostatic pressure. These observations may be due to nutmeg interference with metabolic activities, since it has been reported that myristicine obtained from the nutmeg may have a cytotoxic and apoptotic effects on the body cells (**Lee et al., 2005**). These results supported by present biochemical study of experimental animals and other studies subjected to various noxious influences (**Ghadially, 1988**). ALP enzyme is associated with the brush border of the renal tubules, and the urinary concentration of ALP in particular has been used as an early marker of toxic tubular insult (**Cooper, et al., 1986 and Porter, 1994**).

The pathological changes in mitochondria as swollen or cavitations are an important indicator of cellular damage leading to loss of functional efficiency (**Robbins, 1995**), the morphological alterations of male rats mitochondria in recent study were pointed out earlier by **Ghadially, (1988)** that the swollen or hydropic mitochondria is due to the entry of water and solutes into the organelle and can be engendered by numerous agents, together with swelling and vesiculation of RER which produce cell damage.

Additionally, **Ericsson, (1969) and Ghadially, (1988)** reported with ongoing cellular toxicity the SER comes to occupy most of the cell both by proliferation and dilatation of its cisternae with

vesiculated. As well as **Ghadijally, (1988)** suggested that the RER from the variable cell organelles, which swells or atrophy reflects a state of altered functional activity. **Ghadijally, (1988)** and **Albano et al., (1989)**. Added that the degranulation of RER is an early, relatively mild lesion can produce more drastic alterations of membranes structure such as vesiculation, fragmentation or dissolution of the membranes of RER, accompanied by a general decrease in ribosomes, both attached to and lying free in the cytoplasm after administration of noxious agents and various drugs.

Nevertheless, actual mechanism by which nutmeg induced cellular necrosis observed in this experiment needs further investigation. The necrosis observed is probably due to the high concentration of nutmeg on the kidney. Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell as osmotic thermal, toxic and traumatic effect. The process of cellular necrosis involves disruption of membranes, as well as structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in programmed cell death (PCD), but by an abrupt environmental perturbation and departure from the normal physiological conditions (**Hayes,1994**).

5. Conclusion

These findings indicate that administration of nutmeg may have some deleterious effects of the renal cortical of adult Wistar rats in both sexes at higher doses and may affect on kidney metabolic function. *Myristica fragrans* seeds should therefore be cautiously used in both man and animals.

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