JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Study of Toxic Effect of Extracts of Myristica fragrans (Houtt.) on some Biochemical Aspects and Histopathology of Kidney of Swiss Albino Mice

Rupali Vaity
Assistant Professor, Department of Zoology, SIES College of Arts, Science & Commerce(Autonomous), Near Jain society, Sion West, Mumbai 400 022. Maharashtra

Abstract
Nutmeg is a commonly used spice not only in flavoring of food items but also in traditional medicines as a sleep inducer, an anti-diarrheal, and an aphrodisiac agent. Reports have also claimed that it is used to create euphoric feelings and hallucinations. However nutmeg when consumed in the form of traditional medicine or in the form of cheap source of euphoric and hallucinating agent, its dose is not standardized and hence can be proved to be toxic. Therefore present investigation is aimed at studying the toxic effect of aqueous and methanolic extracts of nutmeg on some biochemical aspects as well as histopathology of kidney of Swiss Albino mice.
Mice of both the sexes (n=40) were divided into four groups. Experimental groups were treated with 1000mg/kg body weight of Aqueous Nutmeg Extract (ANE) and 200 mg /kg body weight of Methanolic Nutmeg Extract (MNE) for 28 days. Animals in control groups were feed with distilled water as aqueous control and olive oil as methanolic extract control. Blood samples from all the animals were withdrawn to estimate the levels of creatinine, urea and uric acid. The animals were sacrificed and kidneys were harvested, weighed and sectioned for histopathological investigations. The results obtained were compared using paired T-test.
There was significant increase in the weight of kidney and levels of creatinine, urea and uric acid of both the treated group of animals. Histopathological sections of kidney of treated animals revealed less identified renal corpuscles, increase in the capsular space, massive lymphocytic infiltration, atrophied and sparse glomeruli with moderate vacuolation. Hence it can be concluded that consumption of nutmeg in high doses over longer time period induce toxic effect.

Keywords: Nutmeg, Histopathology, Biochemical, Aqueous extract, Methanolic extract.,

Introduction

Nutmeg, *Myristica fragrans (Houtt).* is amongst various spices used in Indian traditional dishes. It is one of the important constituents of spice mix that are used in every day cooking.The sour ripe fruits are used for preparing pickles, jams, sweets and jellies, while the seed and mace are used as flavoring agent. A Myristica genus belonging to Myristicaceae family is spread from India to South-east Asia and North Australia Islands of Pacific. Nutmeg is indigenous to Banda Islands from East Indonesia, earlier called as 'Spice Islands'. Within India nutmeg is chiefly cultivated in Southern parts of India that is Chennai, Karnataka, as well as Kerala [1].

The phytochemicals present in nutmeg seeds are responsible for its various pharmacological properties. Extracts and essential oil of *Myristica fragrans*, possess numerous pharmacological activities and since nutmeg is widely used as a spice in South Africa, India and other tropical countries it plays important role in drug development in these regions. In traditional medicines, *M. fragrans* has been used since ages, as a carminative (for relieving flatulence), narcotic, emmenagogue (substance that stimulates menstrual flow) and abortifacient [2], [3]. Nutmeg is also prescribed for the treatment of diseases, such as rheumatism, and it also possesses anti-inflammatory and analgesic properties [4], [5]. M. fragrans has been shown to possess antimicrobial, antioxidant and cytotoxic activity [6], antifungal activity [7], anti- diarrheal activity [8], [9], aphrodisiac activity [10], [11]. Thus, because of its excellent flavoring and therapeutic properties, nutmeg has become a popular ingredient in most of the spice mix used in Indian kitchen as well as medicine in Grandma's pouch.

There is a common belief amongst people that anything that is natural is safe to consume. However that is not the case always. Literature review indicates that study of nutmeg extracts and nutmeg powder on experimental animals showed hepatotoxicity [12], renal toxicity [13], cardiac toxicity [14], brain toxicity [15],[16],[17], pulmonary toxicity [18], Salivary gland toxicity [19]. Nutmeg is considered to be a cheap source of hallucinogenic and euphoric agent. Reports have indicated toxic effect of nutmeg on human beings [20]. Hence present study aimsat investigating the effect of aqueous extract and methanolic extract of nutmeg on biochemical and histopathological aspects of the kidneys of Swiss Albino mice.

Materials and Methods

Preparation of plant extracts: Nutmeg seeds were procured from local market in Mumbai. Both Aqueous and Methanolic extracts of nutmeg were prepared by maceration technique [21].

Animal study: The animals used in the study were Swiss Albino mice (*Mus musculus albinus)* Animals of both the sexes, having body weight of 20-30g, were purchased from Haffkins Institute, and maintained in the animal house of Ramnaraian Ruia College, Matunga, Mumbai. The animals were housed in groups of 6 in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (Lipton), and water was given ad libitum.

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Ramnaraian Ruia College, Matunga, India (CPCSEA Ref No.: CPCSEA/315)

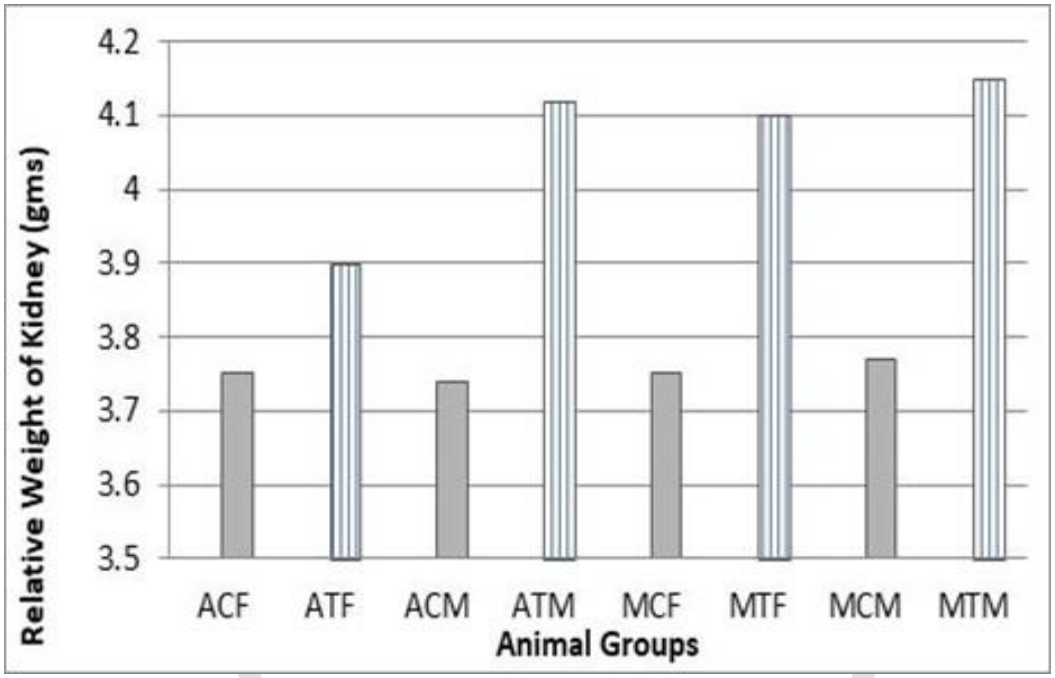
Experimental design: The study was conducted on 48 animals as per the guideline No. 407 of OECD. The animals were feed by oral gavage. The animals were divided into 8groups. The aqueous control groups- Aqueous Control Males and Females (ACM &ACF) were feed with 1 mL of distilled water, whereas aqueous treated groups- Aqueous Treated Males and Females (ATM & ATF) were feed with 1000 mg/kg body weight of Aqueous extract of nutmeg. The methanol control groups- Methanol Control Males and Females (MCM & MCF) were feed with1 mL of olive oil whereas the Methanol Treated groups- Methanol Treated Males and Females (MTM & MTF) were feed with 200 mg/kg body weight of Methanolic extract of nutmeg. The animals were feed with the respective extracts for 28 days. Body weight, food intake and water intake of all the animals were recorded every day. On 29th day of the study, blood samples were retrieved from retro-orbital plexus; plasma was separated and used for the estimation of creatinine, urea and uric acid. The animals were sacrificed by using high dose of ether anesthesia, they were dissected open and the kidneys were harvested, weighed and processed for histopathology study.

Statistical analysis

Results of relative organ weight and biochemical assays were statistically analyzed using Student's T test. Values were significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results and Discussion

Relative Kidney Weight:



There was significant increase in the weight of kidney of animals treated with ANE and MNE. Increase in the weight of kidney following cisplastin administration was reported [22]. The increase in the weight of kidney might be due to renal cell injury caused by inflammation. According to the authors, blood flow to the organ wherein inflammation has occurred increases and there is a transient stasis of blood in the area of inflammation. Inflammatory cells like neutrophils and monocytes along with the fluid enter into area of inflammation thereby increasing the weight of the organ. In rats treated with Rinbacin there was increase in the weight of kidney along with serum urea, potassium and bicarbonate ions [23]. Increased kidney weight either absolute or relative, coupled with a significant change in at least one serum parameter might be due to nephrotoxicity [24] ***[****Figure 1]*.

Figure 1. Toxic effect of nutmeg extracts on relative kidney weights of Swiss Albino mice

Biochemical Assays:

Creatinine and Urea levels

Creatinine, a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the individual's muscle mass. Creatinine is also formed due to excess metabolism of proteins. In absence of excess protein intake, increase level of creatinine in blood may be an indicator of renal damage. Because the body does not metabolise it, creatinine is filtered by the kidneys and excreted in the urine. The rise in serum level of this chemical indicates either decline or failure of renal function to filter waste products from the blood. Renal function can be assessed by measuring the levels of plasma creatinine, urea and uric acid concentrations. Creatinine and urea are known to be important markers of renal dysfunction. Urea is also a product of protein catabolism. What is true of increased creatinine level is also applicable to urea.

There was significant increase in the creatinine levels and urea levels of ANE and MNE treated groups of animals. Our results are similar to the findings of Eweka *et al*, who reported increase in serum creatinine levels and urea levels of rats on administration of 0.1gm and 0.2 gm of nutmeg [13]. The increase in the creatinine and urea levels might be due to the phytochemicals that cause kidney damage thereby disrupting the renal functions. High dose of nutmeg consumption over a longer period of time might also be responsible for the renal damage thereby increasing the creatinine and urea levels.[Figure 2], [Figure 3].

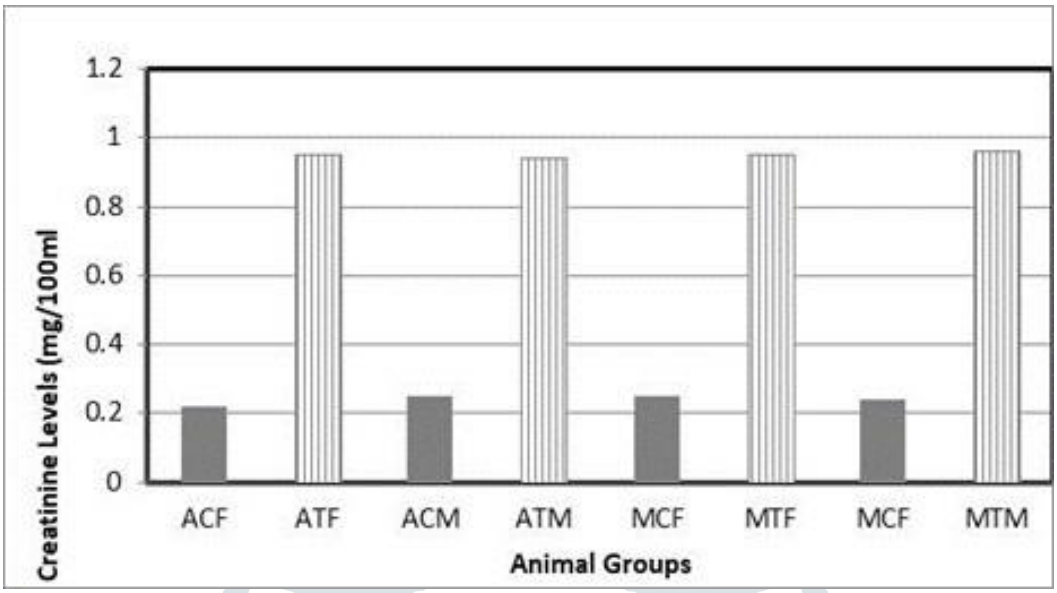


Figure 2. Toxic effect of nutmeg extracts on Creatinine levels of Swiss Albino mice

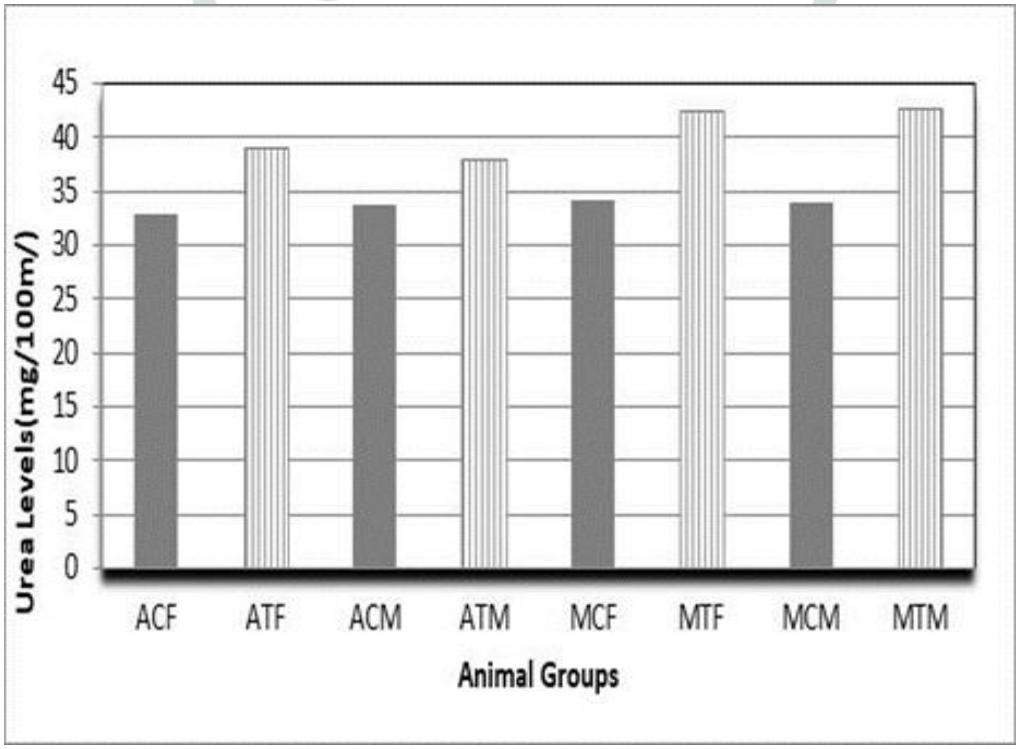


Figure 3.Toxic effect of nutmeg extracts on Urea levels of Swiss Albino mice

Administration of 50 and 100 mg/kg body weight of aqueous extract of *Hippobromus pauciflorus*, increases serum urea level, suggesting impairment in the normal kidney function of the animals as the mechanism of removing it from the blood.

It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney [25]. The mean concentrations of urea and creatinine increased significantly in rabbits treated with 200 mg/kg body weight of *Fumaria officinalis* hydro-alcoholic extract [26]. There was increase in serum urea and creatinine levels in group of animals treated with gentamicin. The increase in concentration of gentamicin causes impairment of renal microcirculation and glomerular hemodynamics thereby decreasing glomerular filtration rate [27], [28].

Uric acid

Uric acid is synthesized by liver; it is the end product of dietary purine metabolism. Majority of the uric acid formed in the body is mainly excreted by the kidneys and some amount by intestine. Serum uric acid levels can increase due to many factors. Consumption of purine rich food, obesity, renal disease are some of the factors responsible for increased uric acid levels.[Figure 4].

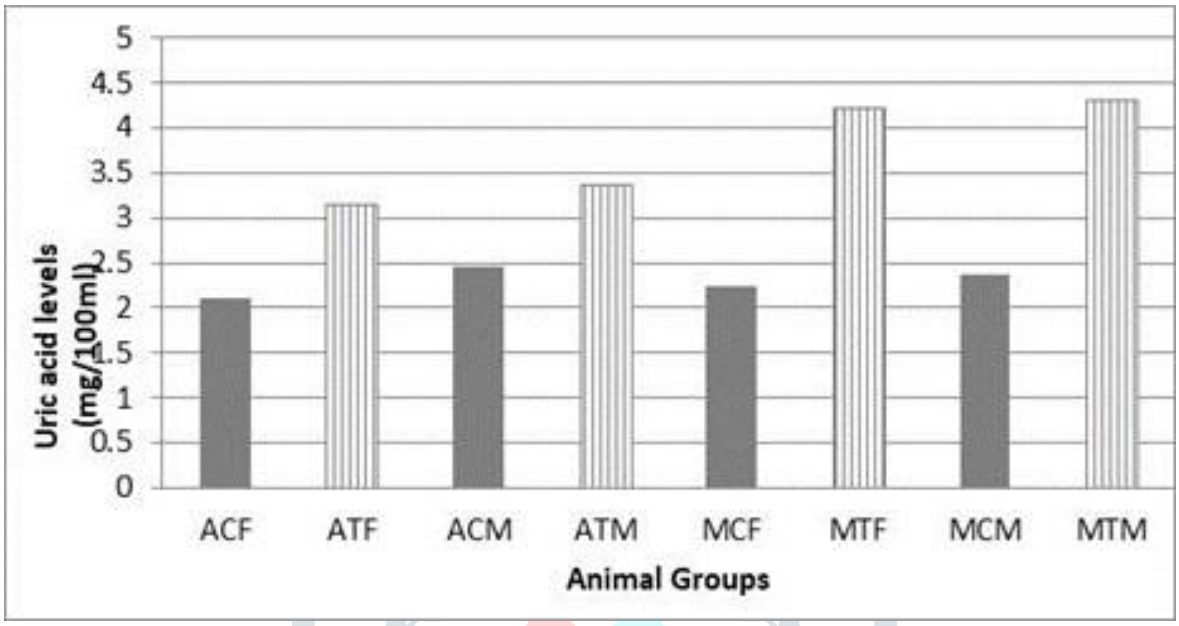


Figure 4. Toxic effect of nutmeg extracts on Uric acid levels of Swiss Albino mice

Sub-chronic exposure of fipronil on biochemical parameters of male albino rats indicated significant increase in serum uric acid levels in treated animals. Increased uric acid levels might be due to degradation of purines and pyrimidines [29]. Overproduction of uric acid or inability of the kidney to excrete it out of the body might be the reason for increased serum uric acid [30]. Study of nephron-protective effect of *Berberis vulgaris* (BV) on lead acetate induced toxicity in mice showed increased serum uric acid levels in mice treated with lead acetate [31].

The Lead acetate induced hyperuricemia might result from over-production and/or reduced renal excretion of uric acid. Lead acetate induced nephrotoxicity was inhibited by co- treatment with BV extract as indicated by significant restoration of serum creatinine, urea and uric acid [32].

Histopathology of Kidney:

The histopahtological examination is considered to be an important standard for assessing treatment related to pathological changes in tissues and organs [33]. In the present study, histopathological evaluation of oral administration of ANE and MNE indicated that both the extracts cause toxicity of kidney. The histopathological changes were in agreement with the results of biochemical analysis.

Kidney is the filtering unit of the body. Toxic effect of many components can be easily observed on the structure, via histopathology or changes in the biochemical parameters of the kidney. Sections of kidney from mice feed with 1000 mg/kg body weight of ANE showed distortion of histo-architecture of renal cortical structures.

The renal corpuscles were less identified and there was increase in the capsular space. Whereas mice feed with 200 mg/kg body weight of MNE revealed massive lymphocytic infiltration, glomeruli were sparse and atrophied. The capsular space was increased and moderatevacuolation was also observed in the sections of kidney.

Study of the antioxidant properties of *Myristica fragrans (Houtt)* and its effect on selected organs of albino rats also indicated similar results[34]. Study of effect of chronic consumption of nutmeg on histopathology of kidney

revealed varying degree of cyto-archtechtural distortion and reduction in the number of renal corpuscle in the treated groups compared to control group [13].

There were several diffuse degeneration and necrosis of the tubular epithelial cells in the kidneys of the treated animals. The degenerative and atrophic changes where observed more in the kidneys of rat that received the higher dose (0.2 g) of nutmeg.

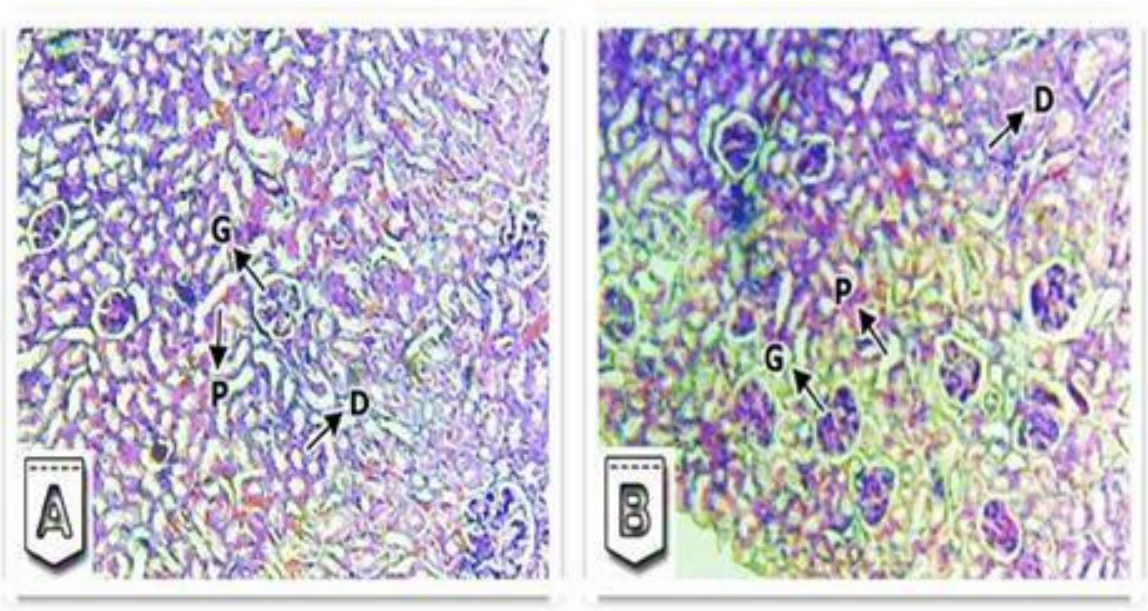


Figure A & B Illustrating histology of kidney of ANE control and MNE control groups treated with distilled water and olive oil respectively for 28 days.The section shows glomerulus (G), proximal convoluted tubule (P), distal convoluted tubule (D). H & EStain. Magnification: 100X.

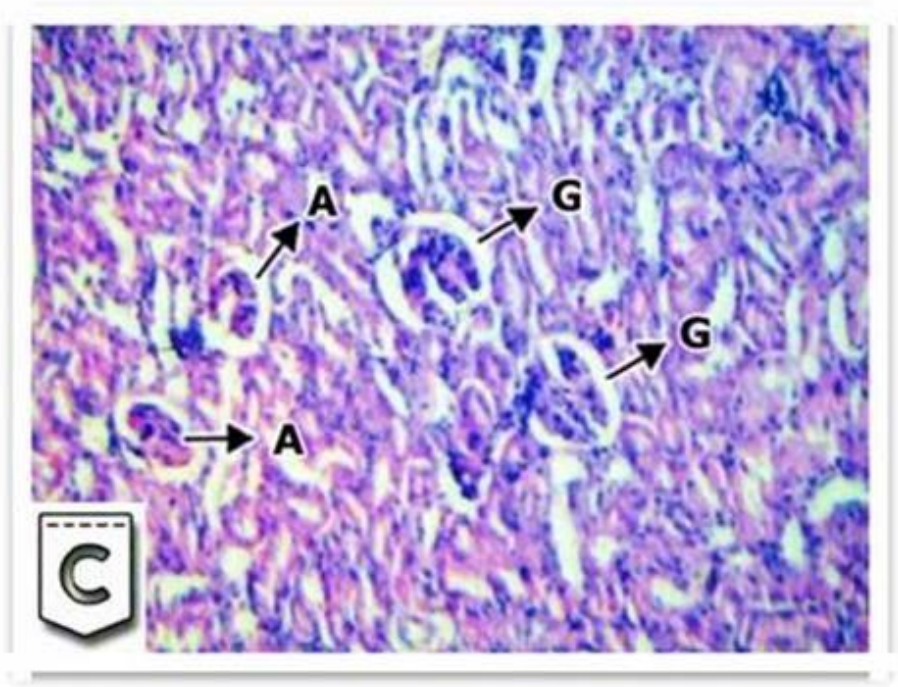


Figure C. Iillustrating histology of kidney treated with 1000mg/kg body weight of ANE for 28 days. The section shows glomerulus with increased capsular space (G), atrophied
glomerulus (A) H & E Stain. Magnification: 100X

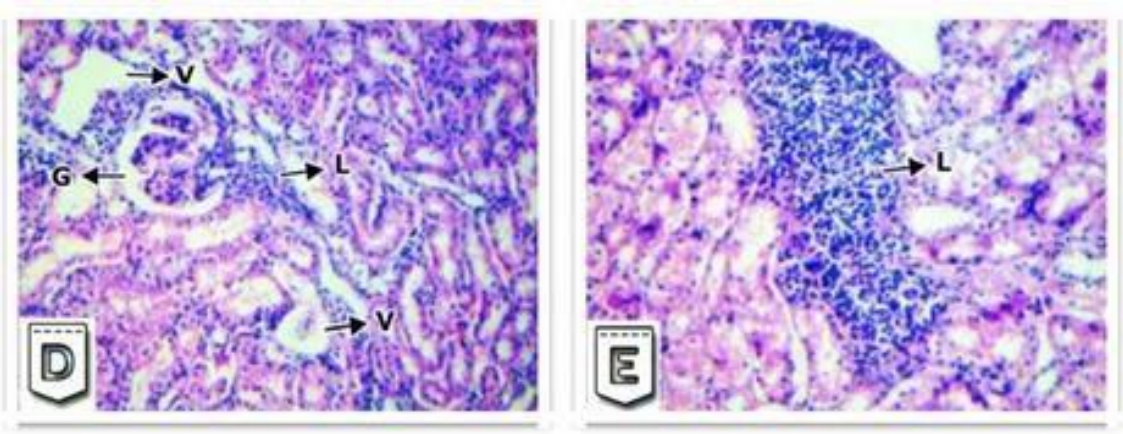


Figure D & E Figures D and E illustrating histology of kidney treated with 200mg/kgbody weight of MNE for 28 days. The section shows glomerulus with increased capsular space (G), lymphocytic infiltration (L), moderate vacuolation (V). H & E Stain.
Magnification: 100X

There were no changes in the histopathology of the kidney when the mice were treated withlow doses of ginger, however at a higher dose (40 mg/kg/48 h), observable chances of renal toxicity was significantly enhanced. There were interstitial inflammation, formation of hyaline cast, regeneration of renal tubules, glomerulonecrosis, hypertrophy of glomeruli and basement membrane thickening [35]. The effect of aqueous leaf extract of rinabacin on pre-pubertal rat kidney revealed renal pathological changes including necrosis and cellular infiltration of glomeruli and epithelia of the tubules[22].

Histopathological effects of *Annnona muricata* (AM) aqueous leaf extract on the liver and kidney of albino mice revealed atrophied glomeruli, haemmorhagic foci with tubular necrosis, degeneration of the proximal convoluted and distal tubules and hypercellularity of glomeruli. High doses AM leaf extract caused toxic effect on both liver and kidney of mice whereas at low doses it exhibited protective effect [36]. The kidney toxicity of *Annona muricata* leaf extract may be due to increasing calcium concentration, ROS production and Bax expression and Bax/ Bcl-2 ratio [37]. These observations may be due to either dose relativity or the route of administration [38].

Conclusion

Nutmeg induces sleep and gives euphoric feeling, and hence it is highly addictive. Further it also used for its pharmacological properties like antidiarrheal, anti-inflammatory and aphrodisiac properties. The study indicated toxic effect of nutmeg for both the extracts, hence long term consumption of nutmeg in higher doses is therefore not recommended. The Phytochemicals that were present in both the extracts might be responsible for the toxic effects.

References

1.Krishnamoorthy B & Rema J. Nutmeg and mace. In: Hand Book of Herbs and Spices. Peter K V (ed.), Woodhead Publishing Limited, Cambridge, England. 2001; 239-248.
2.Asgarpanah J, Kazemivash N. Phytochemistry and pharmacologic properties of *Myristica fragrans Houtt.*: A review. *African Journal of Biotechnology*. 2012 ;11:12787–12793.

3.Min BS, Cuong TD, Hung TM, Min BK, Shin BS, Woo M.H. Inhibitory Effect of Lignans from *Myristica fragrans* on LPS-induced NO Production in RAW264.7 Cells. *Bulletein of Korean Chemical Society.* 2011; 32:4059–4062.

4.Santos FA, Rao VSN, Silverira ER. Antiinflammatory and analgesic activities of the essential oil of *Psidium guianense*. *Fitoterapia.* 1997; 68:65-68.

5.Olajide, O. A., Ajayi, F. F., Ekhelar, A. L., Awe, S. O., Makinde, J. M., & Alada, A. R. A. Biological effect of *Myristica fragrans* (nutmeg) extract. *Phytotherapy Research*. 1999; 13, 344e345.

6.Shazia M., Moiunuddin Saba, Fatima Jasmine, Kahn Farhan, Mustafa Huma. Antibacterial, antioxidant and cytotoxic potential of various extracts of *Myrsitica fragrans*. *International Journal of Research in Ayurveda and Pharmacy*. 2015; 6(5)

7.Pooja V, Goyal SH, Sandashwani AB, Srivastava AK. Activity of *Myristica fragrans* and its effect against filamentous and non- filamentous fungus. *International Journal of Pharmacy and Pharmaceutical Science.* 2012; 4(1):17-20.

8.Gupta S, Yadava JNS, Mehrotra R, Tandon JS. Anti-diarrhoea1 profile of an extract and some fractions from *Myristica fragrans* (Nutmeg) on Escherichia coli enterotoxin-induced secretory response. *International Journal of Pharmacognosy*.1992;30:179-83.

9.Grover JK, Khandkar S, Vats V, Dhunnoo Y, Das D. Pharmacological studies on *Myristica fragrans* antidiarrhoeal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. *Methods and Findings in Experimental and Clinical Pharmacology*.2002;24:675-80.

10. Tajuddin AS, Latif A, Qasmi IA, Amin KM. An experimental study of sexual function improving effect of *Myristica fragrans Houtt.* (nutmeg). *BMC Complementary and Alternative Medicine.* 2005; 05:16.

11. Tajuddin AS, Latif A, Qasmi IA. Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans Houtt.* (nutmeg) and *Syzygium aromaticum* (L.) Merr. & Perry. (clove) in male mice: a comparative study. *BMC Complementary and Alternative Medicine*.2003;03:06

12. Al-Jumaily Essam F. Maytham H. A. Al-Amiry Jaleel I. Assad and Jasim Mohammed Abdullah. Hepatotoxic Effect of Methanolic Extract from Nutmeg (*Myristica fragrans*) Compared to Carbon Tetrachloride- Induced Hepatic Damage in Mice. *Dav International Journal of Science*. 2012; Volume-1, Issue-1.

13. Eweka Andrew Osayame and Abieyuwa Eweka. Histological effects of oral administration of nutmeg on the kidneys of adult Wister rats. *North American Journal of Medical Science.* 2010; 2(4): 189–192.

14. Al-Mosaibih Mai A. Comparative Histological Effects of *Myristica fragrans* (Nutmeg) on Heart of Male and Female Rats. *Middle-East Journal of Scientific Research.* 2015; 23 (8): 1877-1883.

15. Adjene J. O. Histological effects of chronic consumption of Nutmeg on the superior colliculus of adult Wistar rats. *International Journal of Biomedical and Health Sciences*. 2009; (Vol. 6 No1).

16. Adjene JO, Nwose EU. Histological effects of chronic administration of *Phyllanthus amarus* on the kidney of adult Wistar rat. *North American Journal of Medical Science*. 2010; 2(4): 193-195.

17. El-Kohly Wael B and Neveen M El-Sherif. Effect of Nutmeg Administration on the Anterior Cingulate Cortex (Area 24a) of Adult Male Albino Rats and the Protective Role of Vitamin C, A Histological and Immunohistochemical Study. *Anatomy Physiology Biochemistry International Journal*.2017; 2(5).

18. Ghada Hasan El-saify; Nadia Said Badawy khair and Nermeen Mohamed Noor Eldien. Role of Tumor Necrosis Factor-alpha and Interleukin-6 in Nutmeg Induced Pulmonary Injury in Adult Albino Rats: A Light Microscopic and Molecular Study. *Journal of American Science.*2017; 13(3).

19. Moheb, M., El-Sakhawy, M. A., Sherif, H. and Shredah, M. Histological evaluation of the effect of nutmeg on parotid salivary glands of adult male albino rats. *Life Science Journal*. 2014;11(10).

20. Stein U, Greyer H, Hentschel H. Nutmeg (myristicin) poisoning--report on a fatal case and a series of cases recorded by a poison information centre. *Forensic Science International.* 2001; 118(1):87-90. doi:10.1016/s0379-0738(00)00369-8.

21. Mohammed R, Kulkarni P, Syed A, Karigar A, Koshy S,Chandur V, Pande SS, Parveen S. Hepatoprotective and antioxidant effect of whole plant extract of *Polygonum glabrum* wild.on CCl4 induced hepatic damage in rats. *Unique Journal of Pharmacologial and Biological Science.* 2013; 01: 25-32.

22. Rajakrishnan R., R. Lekshmi, P.B. Benil, J. Thomas, A.H. AlFarhan, V. Rakesh, S. Khalaf. Phytochemical evaluation of roots of *Plumbago zeylanica* L. and assessment of its potential as a nephroprotective agent. *Saudi Journal of Biological sciences.* 2017; 24,760-766.

23. Affone OJ, Orisakwe OE, Dioka CE, Obi E, Ezejiofor T, Asomugha L, Ukoha U. Effects of rinbacin extract on rat kidney. *Biological and Pharmaceutical Bulletin.* 2002; (8):1022-5.

24. Simmons Jane Ellen, Raymond S. H. Yang, and Ezra Berman. Evaluation of the Nephrotoxicity of Complex Mixtures Containing Organics and Metals: Advantages and Disadvantages of the Use of Real-world Complex Mixtures. *Environmental Health Perspectives.* 1995; Volume 103, Supplement 1.

25. Pendota S C, M T Yakubu, D S Grierson, and A J Afolayan. Effect of administration of aqueous extract of *Hippobromus pauciflorus* leaves in male Wistar rats. *African Journal of Traditional and Complementary Alternative Medicine.* 2010; 7(1): 40–46.

26. Khoshvaghti Ameneh, Masood Hashemian, Vahab Dehghan and Hamid R. Maleki Babehoveizy. Toxicological and Biochemical Studies of *Myristica fragrans* Hydroalcoholic Extracts in Albino Rats. *European Journal of Medicinal Plants.* 2013; 6(3): 154-166, Articleno.EJMP.2015.051.

27. Stojiljkovic N, Ueljkovic S, Mihailovic D, Stoiljjovic M, Radovanovic D and Randelovic P. The effect of calcium channel blocker verpamil on Gentamicin nephrotoxicity in rats. *Bosnian Journal of Basic Medical. Sciences.* 2008; 8(2): 170-176.

28. Patil CR, Jadhav RB, Singh PK, Mundada S and Patil PR. Protective effect of oleanolic acid on Gentamicin Induced Nephro toxicity in rats. Phytotherapy Research. 2010; 24 : 33- 37.

29. Mossa Abdel-Tawab H., Eman S. Swelam, Samia M.M. Mohafrasha. Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histopathological changes in the liver and kidney of male albino rats. Toxicology Reports 2. 2015; 775–784.

30. Mossa A.H., M.A. Abbassy. Adverse haematological and biochemical effects of certain formulated insecticides in male rats. Research. Journal of Environmental Toxicology.6 2012;160–168.

31. Laamech El Hilaly J., J., H. Fetoui, Y. Chtourou, A. Tahraoui and B. Lyoussi. Nephroprotective Effects of Berberis Vulgaris L. Total Extract on Lead Acetate-induced Toxicity in Mice. Indian Journal of Pharmaceutical Sciences. 2016; 78(3):326-333.

32. Ruilope LM, Garcia-Puig J. Hyperuricemia and renal function. Current Hypertension Reports. 2001; 3:197-202.

33. Jothy, S. L., Zakaria, Z., Chen, Y., Lau, Y. L., Latha, L. Y., & Sasidharan, S. Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. Molecules (Basel, Switzerland). 2011; 16(6), 5268–5282. https://doi.org/10.3390/molecules16065268

34. Olaleye M.T., Afolabi C. Akinmoladun and A.A.Akindahunsi. Antioxidant properties of Myristica fragrans (Houtt) and its effect on selected organs of albino rats. African Journal of Biotechnology. 2006; Vol. 5 (13), pp. 1274-1278.

35. Modaresi Mehrdad and Mozhgan Ghobadi Pour. Effect of Zingiber Extract on Histopathologic Changes in Mice Kidneys. Research Journal of Applied Sciences. 2011; Volume: 6, Issue: 5, Page No.: 295-298.

36. Rana Bitar, Rajaa Fakhoury, Ragaee Fahmi and Jamilah Borjac. Histopathological Effects of the Annona muricata Aqueous Leaves Extract on the Liver and Kidneys of Albino Mice. Translational Medicine Sunnyvale, 2017; 7:2.

37. Chen Y, Chen JW, Zhai JH, Wang SL, Wang Y. Antitumor activity and toxicity relationship of Annonaceous acetogenins. Food and Chemical Toxicology.2013;58: 394-400.

38. Odigie Efosa Bolaji, Peter U Achukwu. Histopathological effects of acetaminophen abuse in male Wistar rats, and prevalence in human subjects: An experimental and cross-sectional study. Journal of Experimental and Clinical Anatomy. 2014; Volume : 14, Issue : 2, Page : 81-87.