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RESEARCH PAPER

EFFECTS OF NUTMEG (*MYRISTICA FRAGRANS)* POWDER ON THE MICROSCOPIC ARCHITECTURE OF THE LIVER OF ADULT MALE WISTAR RATS

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ABSTRACT

The present study was designed to investigate some effect of nutmeg (*Myristica fragans*) powder on the microscopic architecture of the liver of adult Wistar rats. Twenty (20) adult male Wistar rats of an average weight of 140g were used in the study. The rats were randomly distributed into four (4) groups (n= 5). Group A served as the control group while group B, group C and group D served as the treatment group receiving low (250mg/kg), medium (500mg/kg) and high (750mg/kg) doses of nutmeg powder for 4 weeks respectively. After 4 weeks of administration, animals were sacrificed with overdose of anesthetic and the liver harvested, weighed and immediately fixed in 10% formol saline. Afterwards, the tissues were processed for histological assessment. The result revealed that the administration of nutmeg powder had no noxious effect on the liver but only that there was dilation of sinusoids in the liver of animals in group D with a little of hepatocytes concentration towards the central vein. Also animals were seen to be gaining weight significantly every week during the period of administration. There was also no effect on the relative organ weight of animals. The study concludes that nutmeg showed no toxic or noxious effect on the liver.

Keywords: Nutmeg, Liver, Wistar rats, Microscopic architecture.

INTRODUCTION

The Nutmeg plant, *Myristica fragrans* (Houtt), is a member of the small primitive family called Myristicaceae, taxonomically placed between the Annonaceae and Lauraceae (Stein *et al*, 2001). At present, Myristicaceae is considered as a member of Magnotiales or its taxonomical equivalents. The name nutmeg comes from the Latin word *nux muscat*, meaning musky nut (Cronquist, 1983).

Nutmeg is a warm, rich, somewhat sweet, brownish-red spice that comes from the evergreen nutmeg tree, which produces both nutmeg and mace (Cronquist, 1983). This spice comes from the seed of the fruit of the nutmeg tree, which looks similar to a walnut. Although the outside of the seed is very hard, it's surprisingly easy to grate. The lacy reddish membrane of the seed can be dried and used as the spice called mace. Nutmeg is widely used in variety of ways and for various purposes. Dating back to the 16th century, nutmeg has been known for its psychoactive properties, which includes antidepressant, anxiogenic and hallucination. Nutmeg, now a common household spice, comes from the tree *Myristica fragrans*, which originates from the Indonesian Banda Islands (also known as the Spice Islands) (Cronquist, 1983).

Research has it that when *M. fragrans* set seed, the musky smell of the nutmeg is so overpowering that it causes birds of paradise fall to the ground. This may have more to do with the narcotic properties of nutmeg that with its characteristic scent, but it is the musky quality that has made nutmeg a popular flavoring for both sweet and savory dishes (Josiah and Ezekiel, 2006). Nutmeg has been described to have the following health benefits; promoting digestion, supporting oral health, detoxifying the body, supporting kidney health, relieving pain and also treating cancer (Natural Food Series, 2011). The active ingredient in nutmeg is called Myristicine. Cytotoxic and apotoxic effects of Myristicine have been reported such that cell viability was reduced by exposure to Myristicine in a dose dependent manner (Eweka and Eweka, 2010). It has been reported that the phytochemical constituents of nutmeg includes alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins, while tannins were absent in the aqueous extract (Olaleye *et al*, 2006). Nutmeg spice has been used to prevent flatulence, aid digestion, improve appetite, help control asthma, relax muscles and is also used in some cultures as aphrodisiac (Truitt *et al*, 1983). Aphrodisiac/Psychoactive dose is 500mg/kg (Olaleye *et al*, 2006).

Liver is an abdominal organ (which belongs to the gastrointestinal tract) which plays a vital role in detoxification and excretion of many endogenous and exogenous substances. The liver is a natural chemical factory which aids metabolism and detoxification of complex molecules. It neutralizes toxins, and manufactures bile which aids fat digestion and removes toxins through the bowels (Maton *et al*, 1993; Ajilore and Ayannuga, 2012). The live plays an important role in breaking down fats, converting glucose to glycogen and also maintaining proper blood glucose level. Nutmeg which is commonly used in homes for baking, flavouring agents and also used medicinally has been said to have some histological effects on the medial geniculate body (Josiah and Ezekiel, 2006) also on the kidney distorting the cyto-architecture of the renal corpuscle (Eweka and Eweka, 2010) at higher dose (500mg/kg) as aphrodisiac dose and psychoactive agent in male wistar rats. Allergies to nutmeg are very rare and it is a very safe spice.

The aim of this study is access the possible effect of nutmeg commonly used as spice in various dishes, as components of teas and soft drinks or mixed in milk and alcohol on the microscopic architecture of the liver because of its wide range of functions, including detoxification, protein synthesis and production of biochemical necessary for digestion.

MATERIALS AND METHODS

Animals: Twenty (20) adult Male Wistar rats of an average weight of 140 were purchased and housed in the animal holding of Ladoke Akintola University of Technology (LAUTECH), Ogbomosho. They were allowed acclimatization period of 2 weeks and fed with standard laboratory mouse chow (Ladokun feeds, Ibadan) and were given distilled water. Animals confirmed to be free of any pathological condition and the rule guiding good laboratory practice was adhered to.

Plant: Nutmeg was bought at Oja Igbo, a popular market in the town of Ogbomoso, Oyo state. It was authenticated by an Angiosperm Taxonomist in Department of Pure and Applied Biology, LAUTECH (Voucher Number: LHO 235). Authenticated nutmeg was grinded into a powdered form using a manual grater.

Experimental Protocol: All experimental investigations were done in compliance with "humane animal" as stated in the "Guide to the care and use of Laboratory Animals Resources" (NRC, 2011). After 2 weeks of acclimatization, animals were randomly distributed in 4 groups (n= 5). Group A is the control group and group B, C and D are the control group.
Group A (control group): Receiving feeds and distilled water only for 4 weeks
Group B: Received nutmeg powder 250mg/kg daily for 4 weeks
Group C: Received nutmeg powder 500mg/kg daily for 4 weeks
Group D: Received nutmeg powder 750mg/kg daily for 4 weeks

***Animal Sacrifice and Organ Harvesting*:** At the end of the experiment (i.e. 24hours after last administration), animals were sacrificed with overdose of anesthetic (diethyl ether). Each animal was placed on the dissecting board,

pinned to the board and dissecting set (sharp scalpel on scalpel holder for making incision; scissors for cutting and dissecting forceps for harvesting) were used to harvest the liver which was immediately weighed before transferring into 10% formol saline for proper fixing for histological sectioning and analysis.

Statistical Analysis: Data were expressed as Mean ± Standard Deviation (SD) using tables. The differences between the groups were compared for statistical significance by using ANOVA and student 't' test. Data were statistically significant when $P<0.05$.

RESULT

Physical Observation: Daily oral administration of nutmeg powder; 250mg/kg, 500mg/kg and 700mg/kg to animals in Group B, C, and D respectively for 4weeks showed no physical change in animals as animals were seen to be active every day. There was no sign of sluggishness in their responses; rather animals tend to grow more appetite for the nutmeg powder.

Statistical Analysis: Statistical analysis of the weight of animals revealed that animals in all groups were gaining weight throughout the period of administration (Table 1).

However, there was an increase in the relative organ weight of animals treated with nutmeg when compared with the control group (Table 2).

Table 1: Showing mean and standard deviation (Mean ± S.D) of weight of animals in each during the period of administration.

	WEEKS				
Groups	Week 1	Week 2	Week 3	Week 4	P-Value
A (Control Group)	140g±4.000	143g±6.892*	147g±3.873*	150g±7.969*	0025
B(250mg/kg Nutmeg)	140g±3.162	140.5g±3.742*	142g±5.000*	146g±9.214*	0.001
C(500mg/kg Nutmeg)	141.5g±3.165	144±1.225*	146.3g±5.7888*	149g±7.969*	0.000
D(700mg/kg Nutmeg)	140g±3.000	145g±4.743*	147.8g±9.354*	152g±9.656*	0.000

*Significant difference ($p<0.05$)

Analysis of variance (ANOVA) showed significant increase ($p<0.05$) in weight of animals throughout the period of administration.

Table 2: Showing the mean and standard deviation (Mean±S.D) of the relative organ (liver) weight of animals in all groups.

Groups	Relative organ weight	P-value
A	0.028±0.2662	
B	0.030±0.3722	(A vs B) → 0.666
C	0.030±0.1516	(A vs C) → 0.268
D	0.031±0.3082	(A vs D) → 0.07
P-Value	(A vs B vs C vs D)	0.673

* Significant difference ($p<0.05$)

Analysis of variance (ANOVA) showed no significant different ($p<0.05$) in relative organ weight across the group. Post-hoc analysis also showed no significant difference ($p<0.05$) in relative organ weight when comparing Group A and other treatment groups (i.e. A vs B; A vs B; A vs D)

HISTOLOGICAL FINDINGS

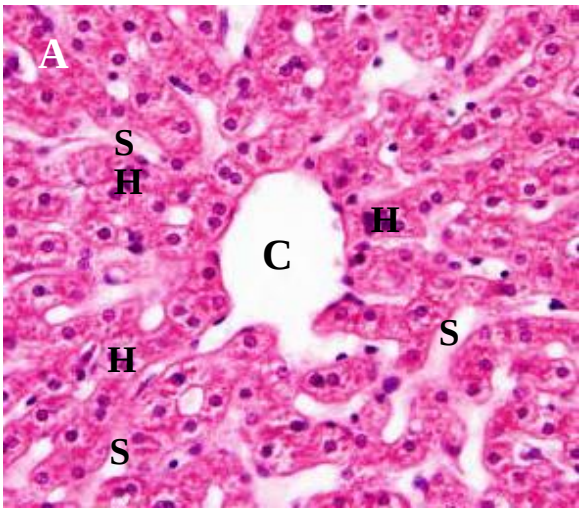

Plate A: Section of liver of Group A- 400X (*Control group*). The photomicrograph shows normal liver microscopic architecture with numerous hepatocytes (H), sinusoids (S) well fenestrated and central vein (CV) normal.

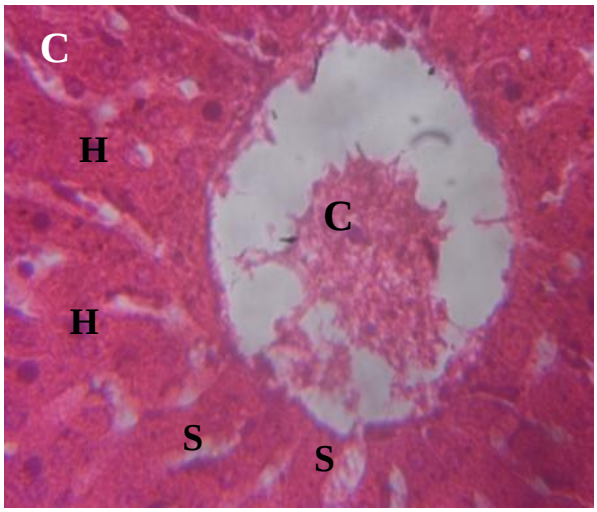

Plate C: Section of liver of Group C- 400X (*Nutmeg powder 500mg/kg*- Group). The photomicrograph shows normal liver microscopic architecture, i.e. hepatocytes (H), sinusoids (S) and central vein (CV) are normal.

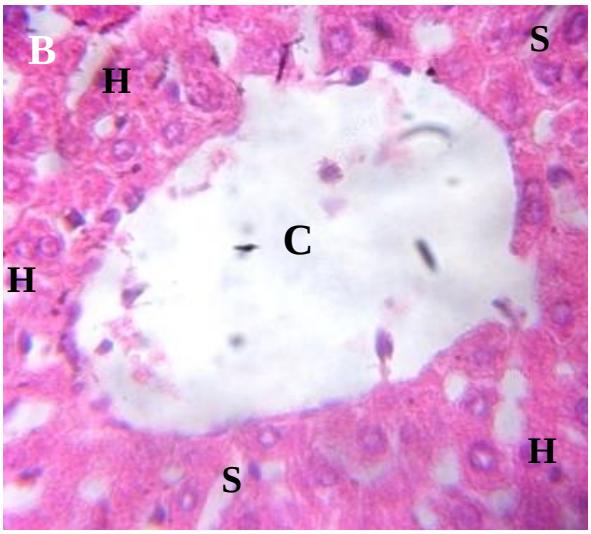

Plate B: Section of liver of Group B- 400X (*Nutmeg powder 250mg/kg*- Group). The photomicrograph shows normal liver microscopic architecture. The hepatocytes (H), sinusoids (S) and central vein (CV) are normal.

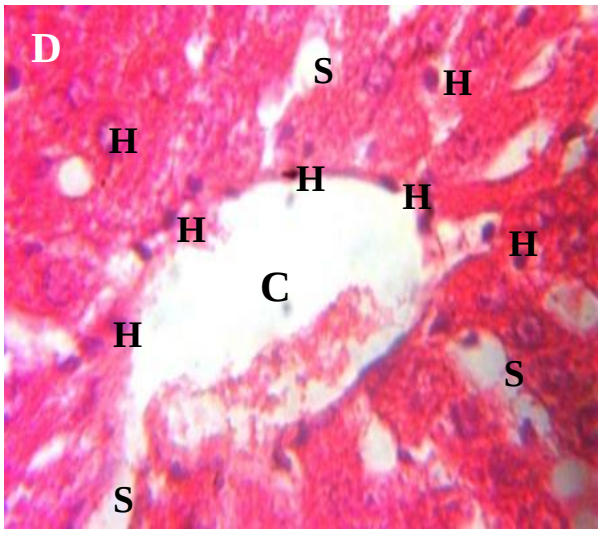

Plate D: Section of liver of Group D-400X (*Nutmeg powder 700mg/kg*- Group). The photomicrograph shows an alteration in the liver microscopic architecture. The hepatocytes (H) appeared to be a bit concentrated towards the central vein (CV) while the sinusoids appeared dilated.

DISCUSSION

Dating back to the 16th century, nutmeg has been known for its psychoactive properties, which includes antidepressant, anxiogenic and hallucination is now used as household spice for baking and flavouring agent. In relation to the consumption doses of nutmeg by experimental animals in the present study, there was significant increase in weight of animals in all groups throughout the period of administration i.e. animals did not experience any decrease in body weight. This corresponds to the findings of Al-Mosaibih, (2015) who stated that there was a significant difference observed in the body weights of rats of both sexes used in his study on Comparative Histological Effects of *Myristica fragrans* (Nutmeg) on Heart of Male and Female Rats. As regards to the relative organ (liver) weight, animals showed varying relative organ weight, however, there was no significant difference in the relative organ weight of animals in the treatment group when compared with control group.

Comparing the photomicrograph of the control group to the treatment groups, group A, B and C showed a normal micro-architecture of the liver with the hepatocytes, sinusoids and central vein being normal. However, only group D showed a slight difference in the micro-architecture of the liver of animals in this group. The sinusoids appear dilated (little widening) but the hepatocytes and central vein remains normal with little hepatocytes concentration towards the central vein. This contradicts the findings of Adjene and Nwose, (2010) who reported some cellular degenerative changes such as hypertrophy in the medial geniculate body of male Wistar rats. It is also contradicts Eweka and Eweka, (2010) that found that nutmeg produced distortion on the cyto-architecture of the kidney (renal corpuscles) of Wistar rats. This also contradicts the findings of Adjene and Igbigbi, (2010) who reported that nutmeg produced obvious signs of proliferation, hyperplasia, and atrophic changes in the treated stomach sections of animals treated with 2g of nutmeg powder when compared to other groups, indicating that nutmeg may have some deleterious effects on the microanatomy of the stomach of adult Wistar rats; however, this may be due to high dose (2g) of nutmeg administration.

CONCLUSION

In conclusion, results obtained in this study revealed that nutmeg seems not have noxious effect on the liver micro-architecture in relation to the doses and not producing any liver damage, and also having known for its uses (such as medicinal uses; controlling asthma, relax muscles, aid digestion, recovery of gout, arthritis, aches, pains, nausea, and dietary uses; complimenting the flavor of many foods, drinks and snacks) (Weil and Andrew, 1966), it is safe for consumption at a low dose and medium dose.

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AUTHORS CONTRIBUTIONS

All authors contributed in one way or the other to the success of this work.