Original Research Article

EFFECTS OF ETHANOLIC EXTRACT OF *MONODORA MYRISTICA* SEED (AFRICAN NUTMEG) ON SOME LIVER FUNCTION PARAMETERS USING ALBINO WISTAR RATS

ABSTRACT

Aim: This study was carried out to Investigate the effects of *Monodora myristica* on the physiological status of the liver of consumers who consume it for medicinal and nutritive purposes.

Study design: This research was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria; between May and October, 2014.

Methodology: Thirty six (36) albino Wistar rats weighing between 180 - 220g were used in the study. They were grouped into 3 groups of 12 rats each (2 test groups and a control group). The animals were fed with standard feed and clean water, in addition, those of test groups 2 and 3 also received 400mg/kg and 200mg/kg of ethanolic extract of *M. myristica* seed respectively on daily basis for twenty eight days (four weeks). After each week of administration, three rats from each group were sacrificed and blood samples collected by cardiac puncture for biochemical analysis of some liver function parameters (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), serum total protein and serum albumin). The liver of the sacrificed animals were also harvested for histological study.

Results: The results from the biochemical assay were subjected to statistical analysis and it showed a significant ($P<0.05$) increase in ALT values, non significant increase in AST and ALP values, and a non significant effect on total serum protein and serum albumin. The histological study of the harvested liver showed hepatocytes degeneration and periportal inflammations, which are indications of alteration on the normal physiological status of the liver.

Conclusion: Findings from this study showed that the extract produced an adverse alteration in the normal functioning of the liver. Therefore, the plant should be used with caution, especially when used for medicinal purposes.

Keywords: Ethanolic extract, *Monodora myristica,* liver enzymes, hepatocytes and physiological status

INTRODUCTION

The use of medicinal plants as therapy for health conditions is an age long practice [1]. This usage has gained prominence worldwide over the last three decades and has been estimated that at present over two third of the developing countries' population relies on plant preparation as medicines to take care of their health needs [2]. This surge in the use of medicinal plants as therapy is believed to be due to their accessibility and affordability and the perceived failure of synthetic drugs in the treatment of some chronic diseases like hypertension, diabetes, arthrosclerosis etc. [2]. According to Akerele (1993) [3], about 65-80% of the world's population in developing countries, due to poverty and lack of access to modern medicine, depend essentially on plants and their formulations for their primary health care.

Medicinal plants and their formulations have continued to attract attention as a result of the strong speculations and belief that they are safe and very efficacious [4] [5]. This strong speculation and assumption has led to the indiscriminate use of medicinal plants and their formulations especially in developing countries, like Nigeria [1].

M. myristica is a species of plant which belong to the family of annonaceae [6]. *M. myristica* forms a large branching tree with a gray-barked trunk and reach 35m high in nature. It has large leaves (35cm long and 18cm wide) at the end of its branches. The leaves are purple at first but turn a smooth deep green on the upper side with paler green underneath. They are prominently veined and the petiole is purplish [7] [8]. This is widely distributed from Africa to Asia, Central and South America and Australia [9] [10]. It is native to Central, East and West Africa [11]. *M. myristica* grows very well in the ever green forest of West Africa and in Nigeria are most prominent in the Southern part [12] [13]. Its common names include; Calabash nutmeg, African nutmeg, False nutmeg, Jamaican nutmeg, while its local names include Ehuru or Ehiri (Igbo),

Ariwo (Yoruba) [9], Erhe (Urhobo), Ehinawosin (Ikale), Uyengben (Edo) [11]. *M. myristica* seed is oblongoid inshape and pale brown in colour with a thin seed coat and hard kernel. Phytochemically, *M. myristica* seeds have been reported to contain secondary metabolites like saponins, tannins, flavonoids, glycosides, alkaloids and steroids [13] [14] [15]. They also contain minerals like potassium, sodium, magnesium, phosphorus and iron [13]. They have also been reported to contain amino acids like phenylalanine, tyrosine, arginine, glutamic acid and asparagines and vitamin C and E and sugars [16] [17]. *M myristica* has been used as herbal plant and spices since ancient times. It is used in the treatment of hemorrhoids stomach ache, fiber pain, and constipation. [18] [9], control passive uterine hemorrhage after childbirth [19] [20] [9] and has also been associated with antisickling effectiveness [17]. As spices, the seeds are grinded and used in cooking pepper soup and stew [13] [21]. The seeds are also used as an aromatic and stimulating addition to medicines and snuff [7]. In Central African Republic, the seeds are used as condiment and drug in the treatment of headache and hypertension [22].The direct action of these plant extracts is on the liver, which is central to drug metabolism [23].

The liver is the largest internal and very vital organ in the body, constituting about 2.5% of an adult's body weight. The liver plays an important role in maintaining blood glucose levels. It also regulates the circulating blood lipids by the amount of very low density lipoproteins (VLDLs) it secretes. Liver takes up numerous toxic compounds and drugs which may include medicinal plant formulations from the portal circulation [24]. Many drugs and metabolites are hydrophobic, and the liver converts them into hydrophilic compounds and in the process some may adversely affect the liver. Such drugs or metabolites are said to be hepatotoxic, and their effects on the liver are determined by measuring the plasma concentration of some biochemical compounds and enzymes produced by the liver (liver makers). Such liver makers include total

serum protein, albumin, triglycerides, total cholesterol, high and low density lipoproteins, and liver enzymes like alkaline phosphatase, aspartate transaminase and alanine transaminase, etc. Presently, some research studies have showed that some of these used medicinal plants adversely affect some vital organs in the body while exhibiting their therapeutic potentials [25]. Consequently, it has become imperative to ascertain the effects of plants used as herbs on the physiological status of vital organs. Hence this study which is aimed at investigating the effects of the ethanolic extract of *M. myristica* seed (African Nutmeg) on the physiological status of the liver using albino Wistar rats.

Figure 1: Fruit (pod) and seeds of African nutmeg.

METHODS

This research was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Nigeria; between May and July, 2014.

Experimental Animals

Thirty six (36) male albino Wistar rats weighing (180 – 220g) were purchased and kept at the animal house, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria. The animals were kept in a spacious and well ventilated cage at room temperature; under 12 hours light and dark cycle and acclimatize for 14 days. They were

allowed free access to feed diet (Top Feeds, Broiler finisher – Product of Eastern premier feed mills Ltd.) and water *ad libitum*.

All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub No. 85-23, Revised 1985).

Collection and Identification of Plant Materials

Dried seeds of *M. myristica* were purchased from Abacha Market along Udu road, Udu LGA., Delta State. The plant sample was correctly identified and authenticated in the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Port Harcourt.

Preparation of Ethanolic Extracts of *Monodora Myristica* Seed

The ethanolic extract of *M. myristica* seeds was prepared according to [9]. The seeds of *M. myristica* were dehulled (the coat removed), and rid of bad seeds and dirt. Thereafter, the seeds were milled to fine powder using manual engine grinder (Model Corene, A.5 lander YCIA S.A). The milled sample of the plant were soaked in 5L of 80% Ethanol for 48 hours, thereafter filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was concentrated under reduced pressure in a vacuum at 45^{o}C using a rotary evaporator (Searl Instruments Ltd. England) to produce a brownish gel like fluid called ethanolic extract.

Experimental Design

Thirty six (36) male albino Wistar rats weighing (160 – 220g) were randomized into three (3) groups of 12 rats each. Group I: Served as the Control, and received water and normal feed. Group II: Received 400mg/kg of the extract. Group III: Received 200mg/kg. The doses of the extract (400mg/kg and 200mg/kg) were chosen based on a previous work that determined the LD_{50} of *M. myristica* seed to be >5000mg/kg [26]. The extracts were administered orally in the early hours of each day (within 8am to 10am) throughout the period of administration.

Three rats from each group were sacrificed after every seven days, that is, on day 8, day 15, day 22 and day 29 day. After each sacrifices, blood was collected by cardiac puncture into lithium heparin bottles for biochemical analysis, while the liver of each rat was collected via abdomino-thoracic dissection into plain bottle containing buffered formalin for histological study.

Biochemical Parameters

The collected blood samples were centrifuged at 5000rpm for 10 minutes to obtain clear serum for the biochemical analysis. The serum supernatant was then carefully aspirated with needled syringe and stored in a plain sample bottle for biochemical analysis. The biochemical analysis for serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), total serum protein and serum albumin were done using Mindray Auto-analyzer machine (Model: BS – 800M) in the laboratory of the Department of Chemical Pathology at the University of Port Harcourt Teaching Hospital following standard laboratory procedures.

Histopathological Examination

Histopathological examination was done following the method outlined by Ekeanyanwu and Njoku (2014).

Statistical Analysis

The data were statistically analyzed using SPSS VERSION 20.0, and was analyzed for statistical significance by one way Analysis of Variance (ANOVA) followed by Dunnett's post-test for comparison with control group. The difference was considered to be significant at 5% level ($P<0.05$).

RESULTS

Results are presented in tables 1-5 and figure 1-5 below;

Table 1: Effects of ethanolic extracts of *M. myristica* on serum ALT (IU/L) in albino

Groups	ALT (IU/L) Week 1	ALT (IU/L) Week 2	ALT (IU/L) Week 3	ALT (IU/L) Week 4
Control	72.67±8.76	76.28±4.18	71.73±6.34	69.97±2.88
400mg/kg *M. myristica*	197.33±42.08*	198.00±4.73*	212.67±7.27*	205.00±15.18*
200mg/kg *M. myristica*	162.67±32.2	146.00±7.77	198.33±112.10*	154.67±2.33

Values are expressed as Mean±SEM; n=3; *: Significant at P<0.05

The result of serum ALT concentrations of rats administered 400mg/kg and 200mg/kg doses of the ethanolic extract of *M. myristica* is presented in table 1 above. The result showed a dose dependent significant P<0.05 increase in the test groups in comparison with the control group.

Table 2: Effects of ethanolic extracts of *M. myristica* on serum AST (IU/L)

Groups	AST (IU/L) Week 1	AST (IU/L) Week 2	AST (IU/L) Week 3	AST (IU/L) Week 4
Control	323.33±22.85	333.45±8.47	325.63±19.65	320.43±21.58
400mg/kg *M. myristica*	501.67±204.6	388.33±54.23	480.67±26.59	632.33±88.89
200mg/kg *M. myristica*	328.33±141.21	378.33±6.89	383.67±511.36	432.67±37.55

Values are expressed as Mean ± SEM; n=3; *; Significant at P<0.05

The result of serum AST concentrations of rats administered 400mg/kg and 200mg/kg doses of the extract is presented in table 2 above. The result showed a non significant P>0.05 increase in the test groups in comparison with the control group.

Table 3: Effects of ethanolic extracts of *M. myristica* on serum ALP (IU/L)

Groups	ALP (IU/L) Week 1	ALP (IU/L) Week 2	ALP (IU/L) Week 3	ALP (IU/L) Week 4
Control	298.33±18.32	293.72±06.24	289.94±14.12	298.33±12.98
400mg/kg *M. myristica*	326.33±41.86	312.00±14.93	353.67±34.37	356.00±89.50
200mg/kg *M. myristica*	321.00±134.97	294.00±14.57	317.00±151.24	307.67±11.20

Values are expressed as Mean±SEM; n=3; *: Significant at P<0.05

The effects of 400mg/kg and 200mg/kg doses of the extract on serum ALP of rats is presented in the table above. The result showed a non significant increase in the test groups in comparison with the control group.

Table 4: Effects of ethanolic extracts of *M. myristica* on serum total protein (g/L)

Groups	Total Protein(g/L) Week 1	Total Protein(g/L) Week 2	Total Protein(g/L) Week 3	Total Protein(g/L) Week 4
Control	64.00±0.58	65.03±1.35	62.14±0.33	65.32±0.29
400mg/kg *M. myristica*	62.67±2.60	65.00±2.08	65.67±3.84	63.33±2.73
200mg/kg *M. myristica*	61.67±1.20	66.00±1.73	64.33±4.70	59.33±4.06

Values are expressed as Mean±SEM; n=3; *: Significant at P<0.05

Table 5: Effects of ethanolic extracts of *M. myristica* on serum albumin (g/L)

Groups	Albumin(g/L) Week 1	Albumin(g/L) Week 2	Albumin(g/L) Week 3	Albumin(g/L) Week 4
Control	29.00±0.58	29.68±0.18	30.00±0.47	33.53±1.08
400mg/kg *M. myristica*	34.00±1.53	30.00±0.58	27.67±0.67	33.00±1.73
200mg/kg *M. myristica*	31.33±1.45	30.33±0.67	26.33±3.48	30.67±0.88

Values are expressed as Mean±SEM; n=3; *: Significant at P<0.05

The results of serum total protein and serum albumin of rats administered 400mg/kg and 200mg/kg doses of the extract are presented in tables 4 and 5 respectively. The results showed non significant effects.

The figures below show the histology of

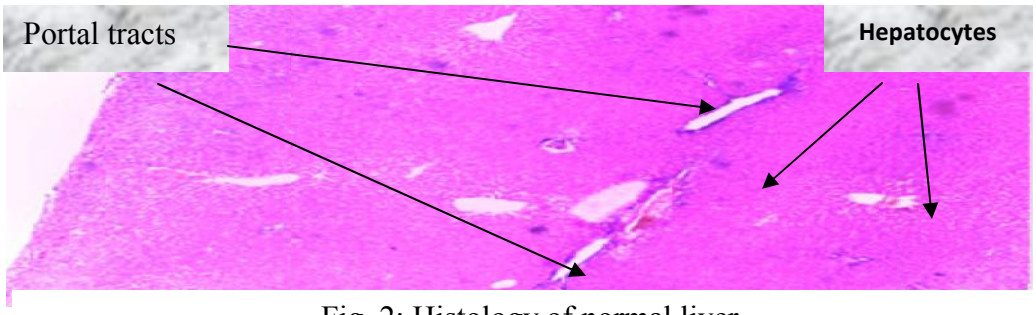

Fig. 2: Histology of normal liver

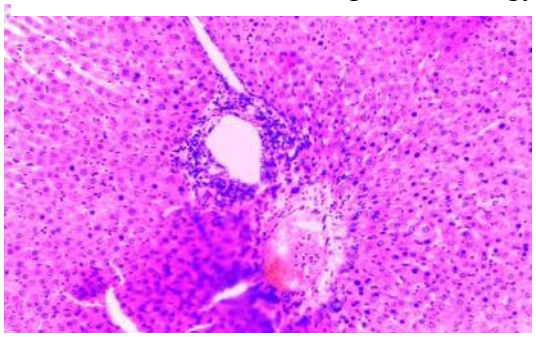

Fig. 3: Effects of one week administration of 200mg/kg *M. myristica* on histology of liver: Periportal inflammation

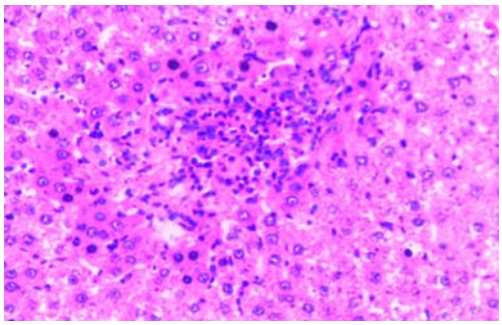

Fig. 4: Effects of one week administration of 400mg/kg *M. myristica* on histology of liver: Mild inflammatory cells infiltrate in the liver parenchyma

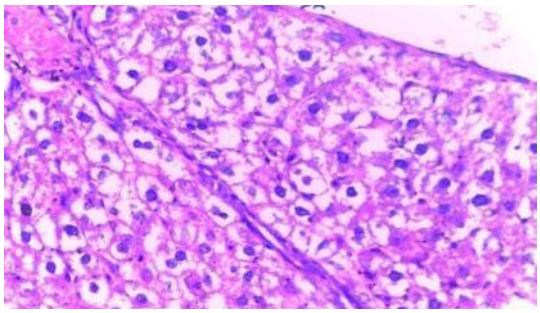

Fig. 5: Effects of four weeks administration of 200mg/kg *M. myristica* on histology of liver: Severe cytoplasmic swelling and degeneration.

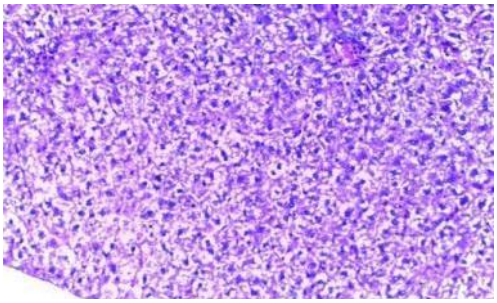

Fig. 6: Effects of four weeks administration of 400mg/kg *M. myristica* on histology of liver: Severe hepatocyte degeneration

DISCUSSION

This study is aimed at investigating the effects of ethanolic extract of *M. myristica* seed on the physiological status of the liver.

The physiological status (functionality or health state) of the liver is mainly determined by measuring the plasma levels of some enzymes called the liver enzymes which include alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (AST), etc [27]; Ekeanyanwu and Njoku 2014) and other biochemicals produced by the liver which include total

serum protein, albumin, bilirubin, triglycerides, total cholesterol, high and low density lipoproteins, etc. Normally ALT and AST are mainly present and in high concentrations within the liver cells (hepatocytes). Damage or destruction of the hepatocytes leads to the release of these enzymes into circulation thereby increasing their plasma levels. Thus, increase in the plasma or serum level of any of these enzymes is an indication of hepatocytes damage. ALP is an enzyme in the cell lining of the biliary ducts of the liver. Its plasma level increases when liver bile duct obstruction is present or there is intrahepatic cholestasis or infiltrative diseases (Angelico *et al*., 2010). The result from the biochemical analysis of this study showed that the 400mg/kg and 200mg/kg dose of the extract significantly ($P<0.05$) increased the serum ALT, and in a dose dependent manner (table 1). This increase is an indication of liver (hepatocytes) damage and is confirmed by the histological study of the harvested liver organs.

Though, both administered doses cause general increase in the serum AST and ALP, but the increase was not significant as shown in tables 2 and 3.

From the results, it was observed that the ethanolic extract of *M. myristica* do not have any effect on the total serum protein and albumin as shown in tables 4 and 5.

The histological study of the harvested liver shows that the ethanolic extract of *M. myristica* negatively alters the physiological status of the liver in a dose and time depended manner, as various degrees of hepatocytes and periportal inflammation and degeneration was observed.

In conclusion, this study reveals that *Monodora myristica* seed have negative effects on the anatomical physiology of the liver. Therefore, we advised that caution should be taken in employing their medicinal effects. Further studies should be done in order to ascertain the biochemical components of the plant responsible for this impairment of the liver and the mechanisms.

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