

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

31 INTRODUCTION

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

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

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

54 Ariwo (Yoruba) [9], Erhe (Urhobo), Ehinawosin (Ikale), Uyengben (Edo) [11]. *M. myristica*
 55 seed is oblongoid in shape and pale brown in colour with a thin seed coat and hard kernel.
 56 Phytochemically, *M. myristica* seeds have been reported to contain secondary metabolites like
 57 saponins, tannins, flavonoids, glycosides, alkaloids and steroids [13] [14] [15]. They also contain
 58 minerals like potassium, sodium, magnesium, phosphorus and iron [13]. They have also been
 59 reported to contain amino acids like phenylalanine, tyrosine, arginine, glutamic acid and
 60 asparagines and vitamin C and E and sugars [16] [17]. *M. myristica* has been used as herbal plant
 61 and spices since ancient times. It is used in the treatment of hemorrhoids stomach ache, fiber
 62 pain, and constipation. [18] [9], control passive uterine hemorrhage after childbirth [19] [20] [9]
 63 and has also been associated with antisickling effectiveness [17]. As spices, the seeds are grinded
 64 and used in cooking pepper soup and stew [13] [21]. The seeds are also used as an aromatic and
 65 stimulating addition to medicines and snuff [7]. In Central African Republic, the seeds are used
 66 as condiment and drug in the treatment of headache and hypertension [22]. The direct action of
 67 these plant extracts is on the liver, which is central to drug metabolism [23].
 68 The liver is the largest internal and very vital organ in the body, constituting about 2.5% of an
 69 adult's body weight. The liver plays an important role in maintaining blood glucose levels. It
 70 also regulates the circulating blood lipids by the amount of very low density lipoproteins
 71 (VLDLs) it secretes. Liver takes up numerous toxic compounds and drugs which may include
 72 medicinal plant formulations from the portal circulation [24]. Many drugs and metabolites are
 73 hydrophobic, and the liver converts them into hydrophilic compounds and in the process some
 74 may adversely affect the liver. Such drugs or metabolites are said to be hepatotoxic, and their
 75 effects on the liver are determined by measuring the plasma concentration of some biochemical
 76 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°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 LD₅₀ 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.

119 Three rats from each group were sacrificed after every seven days, that is, on day 8, day 15, day
120 22 and day 29 day. After each sacrifices, blood was collected by cardiac puncture into lithium
121 heparin bottles for biochemical analysis, while the liver of each rat was collected via abdomino-
122 thoracic dissection into plain bottle containing buffered formalin for histological study.

123 **Biochemical Parameters**

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

132 **Histopathological Examination**

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

135 **Statistical Analysis**

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

140

141

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 <i>M. myristica</i>	197.33±42.08*	198.00±4.73*	212.67±7.27*	205.00±15.18*
200mg/kg <i>M. myristica</i>	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 <i>M. myristica</i>	501.67±204.6	388.33±54.23	480.67±26.59	632.33±88.89
200mg/kg <i>M. myristica</i>	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 <i>M. myristica</i>	326.33±41.86	312.00±14.93	353.67±34.37	356.00±89.50
200mg/kg <i>M. myristica</i>	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 <i>M. myristica</i>	62.67±2.60	65.00±2.08	65.67±3.84	63.33±2.73
200mg/kg <i>M. myristica</i>	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 <i>M. myristica</i>	34.00±1.53	30.00±0.58	27.67±0.67	33.00±1.73
200mg/kg <i>M. myristica</i>	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

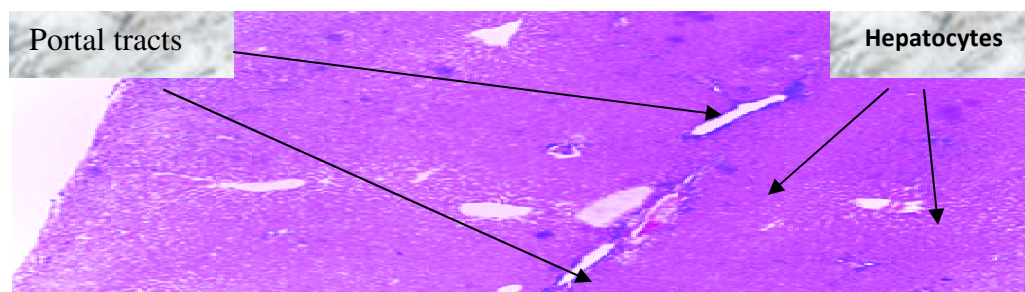


Fig. 2: Histology of normal liver

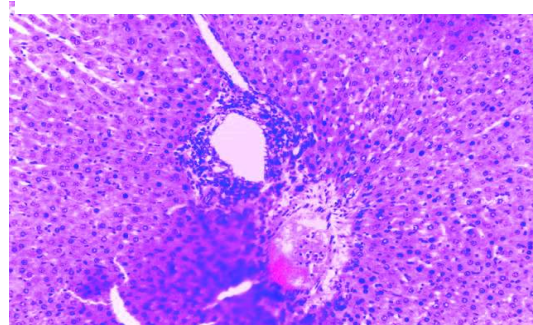


Fig. 3: Effects of one week administration of 200mg/kg *M. myristica* on histology of liver: Periportal inflammation

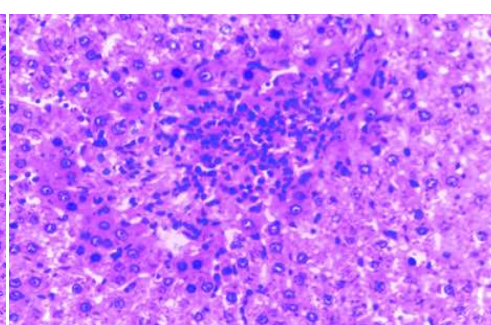


Fig. 4: Effects of one week administration of 400mg/kg *M. myristica* on histology of liver: Mild inflammatory cells infiltrate in the liver parenchyma

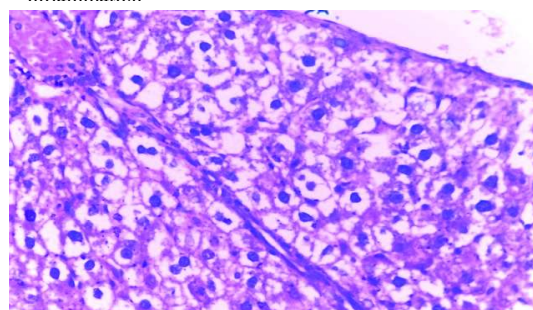


Fig. 5: Effects of four weeks administration of 200mg/kg *M. myristica* on histology of liver: Severe cytoplasmic swelling and degeneration.

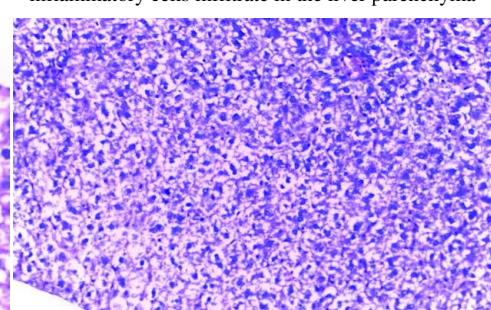


Fig. 6: Effects of four weeks administration of 400mg/kg *M. myristica* on histology of liver: Severe hepatocyte degeneration

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

221 serum protein, albumin, bilirubin, triglycerides, total cholesterol, high and low density
 222 lipoproteins, etc. Normally ALT and AST are mainly present and in high concentrations within
 223 the liver cells (hepatocytes). Damage or destruction of the hepatocytes leads to the release of
 224 these enzymes into circulation thereby increasing their plasma levels. Thus, increase in the
 225 plasma or serum level of any of these enzymes is an indication of hepatocytes damage. ALP is an
 226 enzyme in the cell lining of the biliary ducts of the liver. Its plasma level increases when liver
 227 bile duct obstruction is present or there is intrahepatic cholestasis or infiltrative diseases
 228 (Angelico *et al.*, 2010). The result from the biochemical analysis of this study showed that the
 229 400mg/kg and 200mg/kg dose of the extract significantly ($P<0.05$) increased the serum ALT,
 230 and in a dose dependent manner (table 1). This increase is an indication of liver (hepatocytes)
 231 damage and is confirmed by the histological study of the harvested liver organs.

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

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

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

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

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