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Effect of prolonged administration of ethanolic seed extract of *Monodora myristica* on some liver function enzymes and antioxidant status in rats

[1]Okafor, Azubuike Ikechukwu and [2]Igwe, Christopher Odey

[1,2]**Department of Medical Biochemistry, University of Cross River State, Calabar, Nigeria**
Email: okafor.azubuike@gmail.com; phone number: +2348035723454

ABSTRACT

This study investigated the effect of prolonged consumption of ethanolic seed extract of *Monodora myristica* (ESEM) for 21 day on some liver function enzymes and antioxidant status in rats. Eighteen (18) male Wistar rats were randomly divided into three groups (n=6) and treated orally as follows: Group I: (Control) received 0.4 mL of 0.1 % vehicle (tween 80) for 21 consecutive days; Group II: (Low dose) and Group III (High dose) received 50 mg/kg and 100 mg/kg of ethanolic seed extract of *Monodora myristica* (ESEM) for 21 consecutive days respectively. The animals were sacrificed under anaesthesia (via ketamine; 90 mg/kg) 24 h after the last treatment. Blood and liver tissue samples were collected and processed for analysis of some liver function parameters and antioxidant status. The oral LD_{50} value of ESEM in rats exceeded 5000 mg/kg body weight, indicating a relatively low toxicity. ESEM administration for 21 days at doses of 50 mg/kg and 100 mg/kg significantly ($p<0.05$) improved the serum levels of ALT (13.60±0.40 U/L; 12.42±0.42 U/L), AST (24.24±0.88 U/L; 21.19±0.90 U/L) and GGT (1.86±0.07 U/L; 1.15±0.08 U/L) compared to the normal control group (16.40±0.46 U/L; 28.46±0.94 U/L; 2.44±0.10 U/L) respectively. Normal level of SOD (9.24±0.12 U/mg protein) and CAT (1.74±0.04 µmol/min/mg protein) were significantly increased to 10.44±0.10 U/mg protein; 2.03±0.02 µmol/min/mg protein and 11.98±0.14 U/mg protein; 2.42±0.05 µmol/min/mg protein, in the animals that received 50 and 100 mg/kg of ESEM respectively. Also, the normal concentration of MDA (1.05±0.03 nmol/mg protein) was significantly ($p<0.05$) attenuated to 0.82±0.04 and 0.65±0.02 nmol/mg protein, in the animals that received oral administration of ESEM at 50 mg/kg and 100 mg/kg respectively. The findings suggest that long-term use of ethanolic seed extract of *Monodora myristica* at low dose is unlikely to induce hepatotoxicity, but rather exerts hepatoprotective effects, enhancing the overall liver health.

Keywords: Prolong consumption, *Monodora myristica* seed, oral administration, hepatoprotective, liver enzymes, antioxidant system

INTRODUCTION

Monodora myristica (also know as African nutmeg), belonging to the family Annonaceae, is a perennial edible plant food predominantly in the evergreen forests of West Africa including Nigeria [1]; [2]. Studies have shown that almost every part of the *M. myristica* plant is of importance, particularly the seed [3]. The seed of *Monodora myristica* posses various health benefits, including antimicrobial and anti-inflammatory properties, making them effective against infections, inflammation, cancer and ulcer [4]; [5]. They also exhibit anti-oxidant activity protecting against cell damage [6]. In Eastern Nigeria, the seeds of *M. myristica* serve multiple purposes, including as condiments and species for flavoring pepper soup and stew, as well as in traditional medicine to prevent and treat postpartum hemorrhage [7]; [8]. Nevertheless, long term consumption of *M. myristica* seeds may have adverse effects on liver health, which is a significant concern due to the liver's essential roles in metabolizing nutrients, eliminating toxins, and storing energy [9]. Despite the traditional use of *M. myristica* seeds, there is limited scientific data on their long term effects on liver health.Therefore, this study aims to investigate the effect of prolonged administration of *M. myristica* seed ethanolic extract on some liver function enzymes and antioxidant status in rats

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MATERIALS AND METHODS

Chemicals and reagents

Sodium chloride, sodium hydroxide, trichloro acetic acid, thiobarbituric acid, glacial acetic acid, ethanol and potassium heptaoxodichromate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); Disodium hydrogen orthophosphate, sodium hydroxide, hydrochloric acid, and sodium dihydrogen phosphate were products of BDH Company (UK, England); Ketamine hydrochloride (Gujarat, India); Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Gamma glutamyltransferase (GGT) were procured from Randox Laboratories limited, Antrim United Kingdom.

Experimental animals

Albino male Wistar rats weighing 180 - 200 g were obtained from Faculty Animal Farm at Okuku Campus, University of Cross River State (UNICROSS), Nigeria. The experimental animals were housed in rodent facility at ambient condition with 12-hour day/night cycle and given feed and drinking water *ad libitum*. They were acclimatised for two weeks prior to experiment.

Test sample

Dried seeds of *Monodora myristica* was purchased from local market in Calabar South Local Government Area of Cross River State, Nigeria. The seed was authenticated in the Department of Botany, University of Calabar, Nigeria. The dried seed was then pulverised and stored in plastic bags until used. The extraction was performed by refluxing 50 g of pulverized seed in 400 mL of ethanol at b.pt. 78 ^{0}C. The reflux extraction was carried out for 3 hours. The resulting filtrate was then concentrated in water bath at 45 ^{0}C. The obtained concentrate designated as ethanolic seed extract of *Monodora myristica* (ESEM) was used for the experiment. Prior to use, the extract was freshly dissolved in 0.1 % tween 80 solution.

Determination of median lethal dose (LD_{50})

The LD_{50} of ethanolic seed extract of *Monodora myristica* (ESEM) was determined in rats using the method of [10]. The LD_{50} study consists of two phases: Phases I and Phase II. In Phase I, nine rats randomly divided into three groups (n=3) were orally (via a cannula) given 10, 100 and 1000 mg ESEM/kg body weight, respectively. The animals were observed for signs of toxicity and death under 24 h. In Phase II, the procedure was repeated using three rats randomly divided into three groups (n=1), given 1600, 2900, and 5000 mg ESEM/kg body weight, respectively. The animals were also observed for signs of toxicity and mortality.

Experimental protocol

The selected doses of 50 mg/kg and 100 mg/kg correspond to 5% and 10% of the lethal dose (LD_{50}) of ESEM, suggesting a possible maximum tolerated daily dose. Eighteen (18) male Wistar rats were randomly divided into three groups (n=6) and treated orally as follows: Group A: (Control) received 0.4 mL of 0.1% tween 80 for 21 consecutive days; Group B: (Low dose) and Group C (High dose) received 50 mg/kg and 100 mg/kg of ethanolic seed extract of *Monodora myristica* (ESEM) for 21 consecutive days respectively. Twenty-four hours after the last treatment, the experimental animals were weighed, and sacrificed under anaesthesia (90 mg/kg of ketamine). Blood samples was collected via cardiac puncture into plain tubes, allowed to clot at room temperature for an hour and then centrifuged at 1500 x g for 10 min. The resulting serum was carefully harvested and subsequently used for liver function tests. Concurrently, liver tissues were excised, rinsed with saline, and homogenized in a chilled saline at 5% w/v, using a homogenizer. The homogenate was centrifuged at 3500 rpm for 20 min and the supernatant obtained were used for antioxidant analysis.

Assessment of liver function enzymes

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) were assayed in serum using commercial reagent kits from Randox Laboratories limited, Antrim United Kingdom.

Assessment of antioxidant status

The antioxidant status in liver tissue was assessed by evaluating the activities of antioxidant enzymes and biomarkers in liver homogenate. Malondialdehyde (MDA), a measure of lipid peroxidation was determined using the method described by [11]. Catalase (CAT) and superoxide dismutase (SOD) activities were also assayed by methods described by [12] and [13].

Statistical analysis

The results are presented as mean ± standard deviation (SD). The data were analyzed using SPSS V17.0 for one-way ANOVA (Analysis of variance) and the significant differences among the groups was compared by Duncan's multiple range tests at $P<0.05$ significance.

RESULTS

The lethal dose of ethanolic seed extract of *Monodora myristica* (ESEM) was assessed orally in rat (Table 1). According to the protocol, the toxicity study consists of two phases: Phases I and Phase II. Phase I involves sub-acute toxicity testing, where three increasing lower doses (10, 100, 1000 mg ESEM/kg) are administered to three distinct groups

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Table 1. Phases (I and II) of ESEM toxicity test (LD_{50}) in rats

Phase(s)	Dose (mg/kg)	Number of rat(s)	Mortality
Phase I	10	3	0/3
	100	3	0/3
	1000	3	0/3
Phase II	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

***LD_{50} >5000 mg/kg body weight. 0/3 and 0/1 signifies no death records**

of animals (n=3). The mortality rate observed over 24 hours interval showed no death. Phase II was conducted subsequent to Phase I, wherein three separate groups (n=1) received doses of 1600, 2900 and 5000 mg ESEM/kg body weight of ESEM respectively. The design and implementation of this phase were informed by the results and findings obtained during Phase I. Similarly, no mortality was observed 24 hours post-administration. However, mild behavioral changes were noted, including decreased in sensitivity to noise, touch, and movement as well as altered breathing patterns. These signs were transient and diminished within a few hours, returning to normal without any further intervention. The median lethal dose (LD_{50}) of ethanolic seed extract of *Monodora myristica* (ESEM) should be greater than 5000 mg ESEM/kg body weight.

The effect of prolonged administration of ESEM on liver function enzymes were assayed by measuring the activities of ALT, AST and GGT (Figure 1-3). The results showed that ESEM administration at doses of 50 mg/kg and 100 mg/kg significantly ($p<0.05$) improved the serum levels of ALT (13.60±0.40 U/L; 12.42±0.42 U/L), AST (24.24±0.88 U/L; 21.19±0.90 U/L) and GGT (1.86±0.07 U/L; 1.15±0.08 U/L) compared to the normal control group (16.40±0.46 U/L; 28.46±0.44 U/L; 2.44±0.10 U/L) respectively.

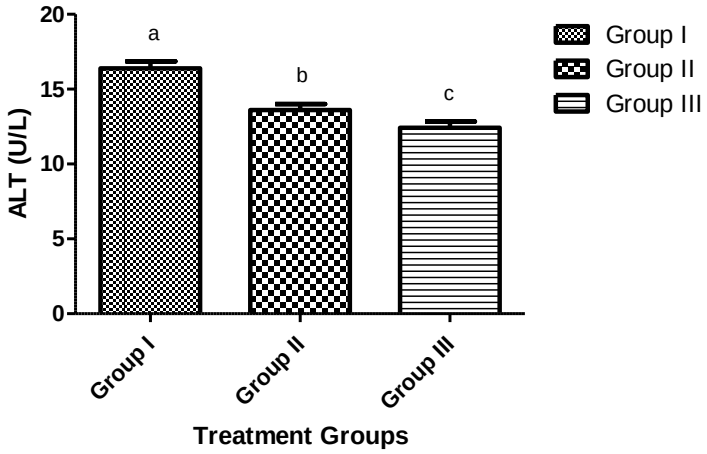

Figure 1. Effect of prolonged administration of ethanolic seed extract of *Monodora myristica* (ESEM) on rat serum alanine aminotransferase (ALT). The results are mean ± SD (n=6) and chart with different letters (a, b, c) are significantly different at $p<0.05$. Group I (Control); Group II (50 mg ESEM/kg); Group III (100 mg ESEM/kg)

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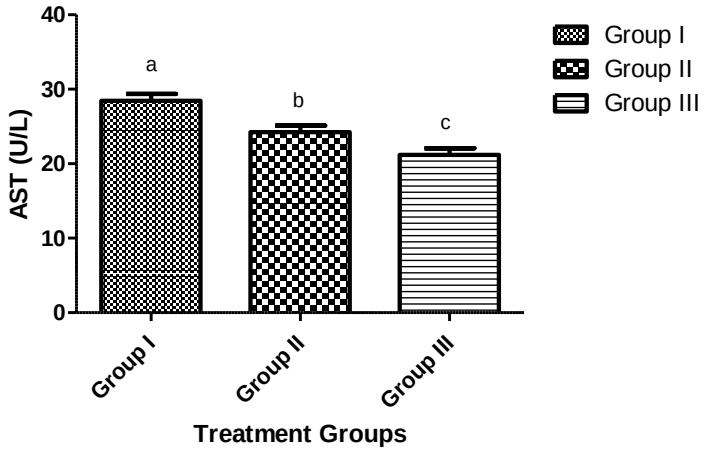

Figure 2. Effect of prolonged administration of ethanolic seed extract of *Monodora myristica* (ESEM) on rat serum aspartate aminotransferase (AST). The results are mean ± SD (n=6) and chart with different letters (a, b, c) are significantly different at $p<0.05$. Group I (Control); Group II (50 mg ESEM/kg); Group III (100 mg ESEM/kg)

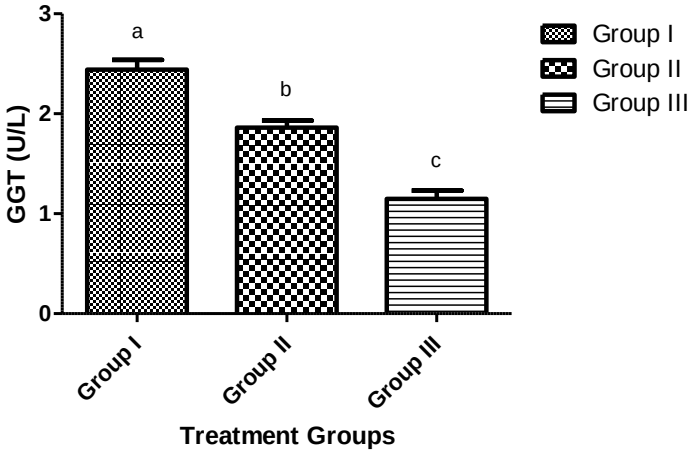

Figure 3. Effect of prolonged administration of ethanolic seed extract of *Monodora myristica* (ESEM) on rat serum gamma glutamyltransferase. The results are mean ± SD (n=6) and chart with different letters (a, b, c) are significantly different at $p<0.05$. Group I (Control); Group II (50 mg ESEM/kg); Group III (100 mg ESEM/kg)

The effect of prolonged administration of ESEM for 21 days on hepatic antioxidant system, was monitored by the level of CAT, SOD and MDA in liver homogenates (Table 2). In normal control group, which did not receive ESEM, SOD (9.24±0.12 U/mg protein) and CAT (1.74±0.04 µmol/min/mg protein) levels were within normal ranges. In contrast, treatment with ESEM at 50 and 100 mg/kg resulted in a significant increase on SOD (10.44±0.10 U/mg protein; 11.98±0.14 U/mg protein) and CAT (2.03±0.02 µmol/min/mg protein; 2.42±0.05 µmol/min/mg protein) activities respectively. Also, MDA concentration was found to be 1.05±0.03 nmol/mg protein in the normal control group and was statistically significant at $p<0.05$. However, the observed MDA concentration was significantly ($p<0.05$) reduced to 0.82±0.04 and 0.65±0.02 nmol/mg protein, in the animals that received oral administration of ESEM at 50 mg/kg and 100 mg/kg respectively.

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Table 2. Effect of prolonged administration of ethanolic seed extract of *Monodora myristica* (ESEM) on hepatic antioxidant indices in Rats

	PARAMETERS		
GROUPS	**CAT**	**SOD**	**MDA**
Normal Control (Group I)	1.74 ± 0.07^{c}	9.24 ± 0.45^{c}	1.05 ± 0.04^{a}
ESEM 100 mg/kg (Group II)	2.03 ± 0.05^{b}	10.44 ± 0.34^{b}	0.82 ± 0.05^{b}
ESEM 200 mg/kg (Group III)	2.42 ± 0.08^{a}	11.98 ± 0.52^{a}	0.65 ± 0.02^{c}

Values are mean ± SD (n=5). Values with different superscript (a, b, c) down the column are significantly different ($P<0.05$). CAT = Catalase (µmol/min/mg protein); SOD = Superoxide dismutase (U/mg protein); MDA = Malondialdehyde (nmol/mg protein).

DISCUSSION

The effect of prolonged consumption of ethanolic seed extract of *Monodora myristica* (ESEM) for 21 day on some liver function enzymes and antioxidant status in rats were monitored. The oral LD_{50} value of ESEM in rats exceeded 5000 mg/kg body weight, indicating a relatively low toxicity [10]. The selected doses of 50 mg/kg and 100 mg/kg correspond to 5% and 10% of the lethal dose (LD_{50}) of ESEM, considered as the possible maximum tolerated daily doses. The biochemical indices investigated in this study, namely ALT, AST and GGT are sensitive and reliable markers of hepatic integrity [14]; [15]. Their measurement provided valuable insights into the effects of ESEM extract administration for 21 days on liver function in rats, serving as indicative parameters of hepatic health and function. The administration of 50 mg/kg and 100 mg/kg of ESEM resulted in a significant improvement in the activity of liver enzymes, including ALT, AST, and GGT. Notably, the high dose (100 mg/kg of ESEM) demonstrated a more pronounced effect, leading to a greater improvement in enzyme activity compared to the low dose. This dose-dependent response suggests that the extract's hepatoprotective properties are optimized at higher concentrations. This finding is consistent with previous study that have demonstrated the hepatoprotective effects of this plant [16]; [17]. Consistent with its hepatoprotective effects, the ethanolic seed extract of *Monodora myristica* (ESEM) also enhanced the antioxidant status in the treated rats. The extracts antioxidant properties were evident in the significant increase in the activities of SOD and CAT with concomitant decrease in MDA. By enhancing the antioxidant status, the extract may have mitigated the harmful effects of free radicals, thereby protecting the liver from oxidative injury [17]. The antioxidant effects of ESEM are likely attributed to its phytochemical constituents, such as flavonoids and phenolic compounds, which have been reported to possess potent antioxidant properties [16]; [18]. The dose-dependent increase in antioxidant parameters suggests that ESEM antioxidant effects may be optimized at higher doses.

CONCLUSION

The findings suggest that long-term use of ethanolic seed extract of *Monodora myristica* at low dose is unlikely to induce hepatotoxicity, but rather exerts hepatoprotective effects, enhancing the overall liver health.

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