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Hepato-Curative and Antioxidative Potentials of Ethanol and Hexane Fractions of Methanol Leaf-Extract of *Annona muricata* against Paracetamol-induced Hepatotoxicity in Albino Rats

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ABSTRACT

This was designed to evaluate the hepato-curative and antioxidant effects of ethanol and hexane fractions of methanol leaf-extract of *Annona muricata* on paracetamol-induced hepatotoxicity in albino rats. A total of 50 albino rats were used for the study. Hepatotoxicity was induced by administration of 800 mg/Kg body weight of paracetamol for seven consecutive days. Hepatotoxicity-induced albino rats were distributed into 8 groups (C-J) of 5 rats each. Groups A and B served as normal controls and were given normal saline and olive oil, respectively; Group C was left without treatment (negative control); Group D was treated with 25 mg/Kg of silymarin (standard drug) (positive control); Groups E-G, were treated with 200, 400 and 600 mg/Kg of ethanol fraction of *Annona muricata* leaves, Groups H-I were treated with 200, 400 and 600 mg/Kg of hexane fraction of *Annona muricata* leaves. The treatment was done by oral intubation, once daily for 14 days. The result showed dose-dependent, significant ($p < 0.05$) decrease in liver enzymes (ALT, AST, ALP and LDH) in *A. muricata* treated groups compared to the negative control (untreated group). Activities of ALT and ALP in groups treated with ethanol fraction of *A. muricata* leaves were significantly ($p < 0.05$) lower compared with groups treated with hexane fraction. Activities of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were significantly increased ($p < 0.05$) in dose-dependent manner in all treated groups in relation to the values found in negative control (untreated) group while the level of MDA decreased significantly. Activities of catalase and superoxide dismutase and levels of GSH and MDA of groups treated with ethanol fraction of *A. muricata* leaves were not significantly ($p > 0.05$) different from groups treated with hexane fraction. The effects on 600 mg/Kg body weight of both fractions on these markers were comparable to the group treated with standard drug and normal control groups. The study demonstrates that ethanol and hexane fractions of methanol leaf-extract of *Annona muricata* could be beneficial in management of paracetamol-induced hepatic damages.

Keywords: *Annona muricata*, Hepatotoxicity, Paracetamol and Antioxidative effects

INTRODUCTION

The functions of the liver are well known. It includes: metabolism of protein, carbohydrate and lipid, production of clotting factors, storage of vitamins (vitamin A, D and B₁₂),

secretion of bile, detoxification, neutralization and elimination of toxins, drugs, environmental pollutants and xenobiotics [1]. Because the liver is a critical organ of the body for

maintaining overall health, disorders of this organ constitute a serious health problem [2]. Liver function is often compromised by numerous toxicants from food and environment. Drug metabolisms also produce harmful intermediates that cause liver injury and dysfunction [3].

Drug-induced hepatotoxicity is a frequent cause of liver injury and dysfunction [1]. More than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for a significant proportion of all instances of fulminant hepatic failure [4]. In the United States, approximately 2000 cases of acute liver failure occur annually and drugs account for over 50 % of them (39 % are due to acetaminophen, 13 % are idiosyncratic reactions due to other medications [1].

Paracetamol is a widely used analgesic and antipyretic drug [5]. However, paracetamol is a common cause of drug-induced hepatotoxicity and acute liver failure in humans and experimental animals [5]. At toxic doses, the normal metabolic detoxification pathways of paracetamol are saturated, causing

formation of N-acetyl-p-benzoquinone imine (NAPQI) and hepatic glutathione depletion [6]. It has been reported that reactive oxygen and nitrogen species (ROS and RNS) production, lipid peroxidation and mitochondrial dysfunction are involved in paracetamol-induced hepatotoxicity [1],[7].

All parts of *Annona muricata* tree (root, bark, fruit, seed and leaf) are extensively used as traditional medicines against an array of human ailments and diseases, namely, tumours, diabetes, malaria, arthritis, cough, asthma, dysentery, hypertension and ulcers [8]. *A. muricata* contain many bioactive compounds that possess pharmacological properties [9]. Hepatoprotective effects of *Annona muricata* ethanol leaf extract have been documented [10]. The present study investigates the effects of ethanol and hexane fractions of methanol extract of *Annona muricata* leaf on liver enzymes and oxidative indices in paracetamol-induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh leaves of *Annona muricata* were plucked from a tree in Usumutong, Abi LGA of Cross River State, Nigeria.

Experimental Animals

Animals used for the study were adult albino rats weighing between 100 - 140 g. they were obtained from the Department of Animal Science, University of Nigeria Nsukka, Enugu State. The animals were kept for 7 days to acclimatize in the Animal House of the Department of Biochemistry, Ebonyi

State University, Abakaliki. They were allowed free access to grower's mash feed and clean water.

Experimental Design

Fifty (50) albino rats of male and female sexes were grouped according to their body weights into 10 groups (A - J) containing 5 rats each. Groups A and B (normal control groups) were given normal saline and olive oil, respectively. Group C (negative control) was given only paracetamol daily at dose of 800 mg/kg to induce liver damage for 7 days. Group D (positive control) was given paracetamol at dose of 800 mg/kg for 7 days and treated with 25 mg/kg of silymarin (standard drug). Groups E - G were given paracetamol (800 mg/kg) for 7 days and were treated with ethanol fraction of *A. muricata* leaf at doses of 200, 400 and 600 mg/kg body weight, respectively. Groups H-J were given paracetamol (800 mg/kg) for 7 days and treated with hexane fraction of *A. muricata* leaf at doses of 200, 400 and 600 mg/kg, respectively. The administration was done by oral intubation with the aid of a feeding needle connected to syringe at stated doses and appropriate volume. Treatment of hepatotoxicity was done once daily for a period of 14 days. At the end of the treatment, all animals were fasted for 24 hrs and blood samples collected from all groups by vein puncture via left leg.

Crude Plant Extraction and Preparation of Ethanol and N-Hexane Fractions

The fresh leaves of *Annona muricata* were washed with clean water, dried at an ambient temperature and ground into powdered form for the extraction. A portion (1500 g) of the pulverized leaves of *Annona muricata* was thereafter soaked in 2500 ml of methanol (99.8 %) for 72 hours. The mixture was filtered with Muslim cloth. The filtrate was concentrated using a rotary evaporator, to obtain a crude extract weighing 200 g. The crude methanol extract of *Annona muricata* leaf was partitioned following the solvent-partitioning protocol designed by [11] and modified by [12]. The crude extract obtained (200 g) was dissolved in 500 ml of ethanol (99 %), and thereafter partitioned successively with same volume of hexane (99 %) in a separating funnel. The ethanol and hexane fractions were concentrated to dryness using rotatory evaporator. The resultant ethanol and hexane fractions, weighing 130g and 65g respectively, obtained were stored in airtight containers and later used for administration.

Biochemical Analysis

The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed according to the method of [13]. Alkaline phosphatase (ALP) activity was assayed by colorimetric method as described by [14]. Lactate dehydrogenase (LDH) activity was assayed by the method described by [15]. Determination of serum total protein was done according

to the method of [16]. The total bilirubin concentration was determined according to the method described by [8]. Catalase (CAT) and superoxide dismutase (SOD) activities were determined by the

methods described by [17] and [9]. Malondialdehyde (MDA) and reduced glutathione (GSH) levels were determined according to the methods described by [18] and [19] respectively.

STATISTICAL ANALYSIS

The results were expressed as mean \pm standard deviation (SD). Comparisons between groups was performed using one way analysis of variance (ANOVA)

followed by Tukey post-hoc test. Significant differences were obtained at $p < 0.05$. Data was analyzed using SPSS version 20.

RESULTS

The result of the effects of ethanol and hexane fractions of methanol extract of *A. muricata* leaf on ALT, AST, ALP and LDH in paracetamol-induced hepatotoxicity in albino rats is presented in Table 1. The result showed that the enzyme activity of ALT, AST, ALP and LDH of the negative control/untreated group (Group C) was significantly ($p < 0.05$) decreased compared with the treated (Groups D -

J) and normal controls (Groups A and B) groups. The result revealed significant ($p < 0.05$) dose-dependent decreases in ALT, AST, ALP and LDH in all the treated groups compared to the positive control (untreated) group. The result showed that the effects of 600 mg/Kg of the ethanol fraction of *A. muricata* leaf on ALT, AST, ALP and LDH were comparable to the effect of the standard drug (silymarin) (positive control).

Table 1: Effects of ethanol and hexane fractions of *Annonamuricata* leaf on liver enzymes in paracetamol-induced hepatotoxicity in albino rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
A	41.85 ± 1.53 ^f	40.96 ± 1.46 ^g	51.12 ± 2.17 ^f	80.31 ± 1.39 ^f
B	41.78 ± 1.65 ^f	40.13 ± 1.47 ^g	51.73 ± 1.86 ^f	81.70 ± 2.27 ^f
C	129.60 ± 1.06 ^a	161.92 ± 1.82 ^a	207.33 ± 2.52 ^a	233.66 ± 2.29 ^a
D	53.32 ± 2.15 ^e	54.82 ± 1.01 ^f	53.08 ± 2.11 ^f	95.64 ± 0.91 ^e
E	83.21 ± 2.36 ^c	82.08 ± 1.72 ^c	120.26 ± 2.40 ^c	152.80 ± 1.54 ^c
F	74.12 ± 2.00 ^d	67.48 ± 1.51 ^e	91.97 ± 1.62 ^e	123.88 ± 2.52 ^d
G	57.62 ± 1.52 ^e	55.49 ± 0.91 ^f	54.95 ± 1.06 ^f	86.73 ± 1.44 ^f
H	91.99 ± 1.54 ^b	93.56 ± 0.90 ^b	154.62 ± 2.57 ^b	165.42 ± 2.21 ^b
I	82.69 ± 1.85 ^c	75.25 ± 1.07 ^d	119.29 ± 1.15 ^c	127.08 ± 1.50 ^d
J	72.62 ± 1.50 ^d	65.48 ± 0.98 ^e	82.48 ± 1.55 ^d	97.28 ± 2.62 ^f

Values are expressed as means ± SD (n = 5). Mean values of groups that do not share a letter are significantly different (p < 0.05). A and B = Normal controls, C = Negative control, D = Positive control, E

– G = 200, 400 and 600 mg/kg, respectively of ethanol fraction, H - J = 200, 400 and 600 mg/kg, respectively of hexane fraction.

The result of the effects of ethanol and hexane fractions of methanol extract of *Annona muricata* leaf on CAT and SOD activities, GSH and MDA levels in paracetamol-induced hepatotoxicity in albino rats are presented in Table 2. The result showed that the activity of CAT and SOD, and GSH level significantly increased (p<0.05) in all treated groups compared with the negative control (untreated) group. The activity of CAT and SOD at 400 mg/kg and 600 mg/kg dosage groups of both fractions of *A. muricata* were not significantly different

(p>0.05) from the normal and positive (silymarin) control groups. MDA level of all the treated groups showed significant decreases (p<0.05) compared with the negative control (untreated) group. MDA levels of groups treated with 600 mg/kg of both ethanol and hexane fractions of *A. muricata* were not significantly different (p>0.05) from the normal and negative (silymarin) control groups. The effects of the ethanol and hexane fractions of *A. muricata* leaf on oxidative stress indices were comparable.

Table 2: Effects of ethanol and hexane fractions of *Annona muricata* leaves on oxidative stress indices in paracetamol-induced hepatotoxicity albino rats

Groups	CAT (U/L)	SOD (U/L)	GSH ($\mu\text{g/ml}$)	MDA (mg/dl)
A	$0.69 \pm 0.06^{a,b}$	$1.144 \pm 0.001^{a,b}$	1.53 ± 0.09^a	10.35 ± 0.02^d
B	$0.71 \pm 0.04^{a,b}$	$1.143 \pm 0.001^{a,b}$	1.49 ± 0.07^a	10.35 ± 0.02^d
C	0.36 ± 0.03^d	1.121 ± 0.001^d	0.24 ± 0.02^c	18.54 ± 0.29^a
D	$0.75 \pm 0.05^{a,b}$	1.142 ± 0.001^b	1.46 ± 0.07^a	11.44 ± 0.23^c
E	0.55 ± 0.02^c	1.130 ± 0.001^c	0.76 ± 0.09^b	13.58 ± 0.19^b
F	$0.69 \pm 0.03^{a,b}$	1.140 ± 0.001^b	1.38 ± 0.34^a	11.59 ± 0.32^c
G	0.76 ± 0.06^a	1.146 ± 0.001^a	1.54 ± 0.08^a	10.49 ± 0.26^d
H	0.51 ± 0.02^c	1.133 ± 0.001^c	0.92 ± 0.05^b	13.62 ± 0.20^b
I	0.67 ± 0.01^b	1.140 ± 0.001^b	1.41 ± 0.02^a	11.75 ± 0.27^c
J	$0.70 \pm 0.04^{a,b}$	$1.143 \pm 0.001^{a,b}$	1.56 ± 0.09^a	10.61 ± 0.24^d

Values are expressed as means \pm SD ($n = 5$). Mean values of groups that do not share a letter are significantly different ($p < 0.05$). A and B = Normal controls, C = Negative control, D = Positive control, E – G = 200, 400 and 600 mg/kg,

respectively of ethanol fraction, H - J = 200, 400 and 600 mg/kg, respectively of

hexane fraction.

DISCUSSION

In the present study, the curative and antioxidative effects of ethanol and hexane fractions of methanol extract of *Annona muricata* against paracetamol-induced hepatotoxicity in albino rats were investigated. Hepatotoxicity in albino rats was induced by oral intubation of paracetamol at a dose of 800 mg/kg for 7 days. Assessment of hepatotoxicity and recovery were determined by measuring biochemical markers of liver toxicity, namely, serum ALT, AST, ALP and LDH [5]. Significantly ($p < 0.05$) increased activities of liver enzymes (ALT, AST, ALP and LDH) were observed after paracetamol intoxication compared to normal rats indicating considerable hepatocellular damage. The rise in serum liver enzymes has been attributed to the damaged structural integrity of the liver because these are confined in the cytoplasm and released into circulation after cellular damage. However, treatment of the albino rats which were induced hepatotoxicity with silymarin and fractions of *A. muricata* leaf extract caused a significant ($p < 0.05$) reversal of the effects, as indicated by reduced levels of liver enzymes in treated groups. The highest reductions in liver enzymes were observed in groups treated with 600 mg/kg of both fractions of *A. muricata* leaf. This suggests that the effects of the ethanol

and hexane fractions of *Annona muricata* are dose-dependent. The findings of this study are consistent with the report of [19], where extract of *A. muricata* was shown to cause significant reductions in levels of serum transaminases, ALP and LDH in albino rats induced paracetamol-hepatotoxicity.

Concurrent significant ($p < 0.05$) depletion in GSH level was observed in the paracetamol-intoxicated group. Paracetamol-induced hepatotoxicity results from the formation of a toxic metabolic intermediate, N-acetyl-p-benzoquinone imine (NAPQI), which on bioaccumulation depletes hepatic GSH levels (Hinson *et al.*, 2010, Yoon *et al.*, 2016). Besides GSH level depletion, significant ($p < 0.05$) reductions in the activity of SOD and CAT, and increased MDA level were observed in the group induced paracetamol-hepatotoxicity. Oxidative stress and lipid peroxidation are notable mechanisms implicated in paracetamol liver toxicity. This may account for the significantly ($p < 0.05$) decreased levels of GSH, SOD and CAT, and increased MDA observed in the group induced paracetamol-hepatotoxicity (Hinson *et al.*, 2010). Treatment of rats induced hepatotoxicity with ethanol and hexane fractions of *A. muricata* caused

significant ($p < 0.05$) dose-dependent increase in the levels of GSH, CAT and SOD and decrease in MDA. The effects of the treatment with 600 mg/Kg of ethanol and hexane fractions of *A. muricata* on oxidative stress indices were comparable with silymarin (standard drug) used as positive control in the study. Levels of oxidative stress indices in groups treated with 600 mg/Kg of ethanol and hexane fractions of *A. muricata* were comparable to each other and with the normal control groups.

The results of this study demonstrates that paracetamol at dose of 800 mg/kg induces oxidative stress and consequent hepatic injury. The study showed that ethanol and hexane fractions of *A. muricata* had dose-dependent effects in promoting liver recovery following paracetamol-induced hepatotoxicity. Further studies should be conducted to elucidate the precise mechanisms triggering the therapeutic effects of *A. muricata* leaf in paracetamol-induced hepatotoxicity.

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