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Biochemical Changes in Haematological and Liver Function Parameters in Intoxicated Male Albino Rats Treated with *Hymenocardia acida* Leaves Ethanolic Extract

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Abstract

In the current study, the biochemical changes in haematological and liver function parameters in Intoxicated Male Albino Rats treated with *Hymenocardia acida* leaves ethanolic extract on $AlCl_3$ -toxicity was investigated. Twenty albino rats assigned to four groups of five each were used. Exactly 100 mg/kg bw of the plant extract was administered to groups 3 and 4 for the experimental period of seven days. At the end of the experimental period, animals were sacrificed and blood collected by cardiac puncture. Biochemical, haematological parameters and Thiobarbituric acid reactive substances (TBARS) were determined. The levels of all serum biochemical parameters measured (alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), Glucose, Potassium and total Bilirubin) in the aluminium chloride ($AlCl_3$)-induced (negative control) increased significantly ($p < 0.05$) when compared with the normal control, while the ethanolic leaves extract non-significantly ($p > 0.05$) decreased some of these parameters. Furthermore, the leaves extract showed a slight moderation effect on selected haematological parameters. However, comparative effect of the leaves extract on the normal control and on the negative control show that the leaf could possibly be toxic. This result show that the ethanolic extract of *Hymenocardia acida* leaves may possess mild ameliorative effect against aluminium chloride-induced toxicity and may also be toxic in male albino rats.

Keywords: *Hymenocardia acida*; Kidney; Lipid peroxidation; Liver; Toxicity

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Introduction

The use of herbal medicines in human and animal health care systems is well documented in ancient literature. In many parts of the World, ethno-therapies are no longer seen as myth, superstition, witchcraft or ungodly practices and indeed is gaining popularity with the belief that "natural is better". It is believed that nearly 80% of world population relies primarily on herbal remedies for the treatment of human and animal diseases. It is in this light that World Health Organization encourages the use of herbal preparations for the treatment of some local health problems particularly in developing countries where they are readily available, easily affordable and already integrated into the people's cultures. Several medicinal plants have been proved beneficial through extensive laboratory tests [1].

In many African countries, herbal remedies play important role in the health of millions of people, particularly the poor living in rural and peri-urban areas where medicinal plants are mostly available and affordable [2]. Generally, people begin treating themselves using medicinal plants before going to an herbalist or modern doctor. It is a common practice in Nigeria that herbal products are administered over prolonged period and by persons that have little or no knowledge of science [3,4].

Hymenocardia acida (Tul.) is a small brown tree or shrub with palatable foliage, widely distributed within the savanna region of Nigeria. Several researchers have reported the antimicrobial activities [5], anti-ulcer [6], anti-diarrhoeal [7], anti-HIV and anti-inflammatory [8] activities of *H. acida*.

Despite the acclaimed popular and numerous therapeutic benefits of *H. acida*, little is known about its effects on Aluminium chloride-

toxicity. Since the past decade, traditional medical practices have become a topic of global relevance. In many developing nations, a significant number of indigenous populations rely on medicinal plants to meet their health care needs [9]. *Hymenocardia acida* is very popular in African Traditional medicine. It has been used by different culture to treat diseases. This research seeks to investigate the effect of its ethanolic leaves extract on aluminium chloride-induced toxicity in male albino rats.

Materials and Methods

Sample collection and preparation

The leaves of the plant (*Hymenocardia acida*) were collected from bushes within the Federal University Wukari campus, Wukari Local Government Area of Taraba State, Nigeria. The leaves were examined to be free from diseases. Only healthy plant parts were used. The leaves were thoroughly rinsed with clean water and dried under shade. The dry leaves were pulverized into powdered form using a laboratory blender.

Sample extraction

Exactly 100 g of the pulverized leaf was soaked in 500 mL ethanol in the ratio 1: 5 for 48 hrs. The extracts were filtered out first using a clean white sieving mesh and then using the Whatman No. 1 filter paper. The filtrates were concentrated using a thermostat water cabinet at 40°C. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator at 4°C until required.

Experimental animals

Twenty (20) healthy male albino rats weighing 100-150 g were obtained from the animal house of the Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria. They were kept in clean cages (plastic bottom and wire mesh top), maintained under standard laboratory conditions (Temperature 25 ± 5°C, Relative humidity 50% to 60%, and a 12/12 h light/dark cycle) and were allowed free access to standard diet and water *ad libitum*. Animals were acclimatized for 7 days in the animal house of the Department of Biochemistry, Federal University Wukari, Nigeria before the experiments. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Faculty of Pure and Applied Sciences, Federal University Wukari, Nigeria.

Extract dissolution

Two hundred milligram (200 mg) ethanol extract of *Hymenocardia acida* leaves was dissolved in 2 mL of distilled water. Exactly 100 mg/kg body weight (bw) of the extract was administered to the albino rats orally with the aid of gavage tube.

Experimental design

The rats were randomly distributed into four groups (n=5): (i) Group 1 (normal control): received only feed and water daily. (ii) Group 2 (negative control): received 100 mg/kg bw Aluminium chloride daily. (iii) Group 3: Received 100 mg/kg bw ethanol

extract of *Hymenocardia acida* leaves an hour after administering 100 mg/kg bw of Aluminium chloride. (iv) Group 4: Received 100 mg/kg bw ethanolic extract of *Hymenocardia acida* leaves only.

After the experimental period, the animals were sacrificed, blood was collected by cardiac puncture and their Liver harvested. Blood samples were collected into (1) EDTA tubes for examination of haematological parameters and (2) plain sample tubes containing no anticoagulant for other biochemical parameters. The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min.

Tissue homogenization

Liver tissues were weighed and homogenization was carried out using phosphate buffer at pH 7.4 at 10 parts per 1 (10 mL to 1 g of tissue) in a standard laboratory mortar and pestle. The homogenates were centrifuged and the supernatant was examined for Thio-barbituric acid reactive substance (TBARS).

Determination of biochemical parameters

Hepatic lipid peroxidation was determined as Thiobarbituric Acid Reactive Substances (TBARS) as described by Torres et al. [10]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined as described by Reitman and Frankel [11] using assay kits (Randox Laboratories Ltd, UK). Serum alkaline phosphatase was determined as described by Klein et al. [12]. Total Bilirubin was determined calorimetrically according to the method described by Jendrassic and Grof [13], while the amount of potassium was determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension [14]. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/L.

For the haematological analysis, a full blood count analysis was carried out to determine the volume of blood cells present in the whole blood sample. Automated method of full blood count anti-coagulated blood which is suckled through a narrow tube by equipment. The equipment then counts the type of cells via two types of sensors; Light detectors and electrical impedance.

Ethics approval

All animals received humane care according to the criteria outlined in the Institution's Guide for the care and use of laboratory animals.

Statistical analysis

Results obtained were analysed by one-way (Analysis of variance) ANOVA and Least Significant Differences (LSD), using SPSS version 20. All data were expressed as Mean ± SD (n=5) and difference between groups were considered statistically significant at p<0.05.

Results

Biochemical parameters

Administration of ethanolic extract of the leaf of *Hymenocardia acida* (100 mg/kg bw) daily for 7 days resulted in a non-

significant ($p > 0.05$) decrease in serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose and total bilirubin in rats intoxicated with $AlCl_3$ (**Table 1**). However, serum activities of alkaline phosphatase (ALP) was significantly decreased.

The effects of the extracts on blood components are presented in **Table 2**. Non-significant decreases ($p < 0.05$) were observed in the haemoglobin level and Red Blood Cells (RBC) of the negative control group when compared to the normal control. Whereas non-significant increase in Packed Cell Volume (PCV) and White Blood Cells (WBC) were noticeable only in the group 2 (negative control), while platelet was significantly decreased in the same group. The White Blood Cells (WBC) and Red Blood Cells (RBC) increased in the group that received only the ethanol extract of *H. acida*, while RBC and Hb increased in the $AlCl_3$ + *H. acida* treated group when compared with the negative control. Packed Cell Volume (PCV) reduced non-significantly in $AlCl_3$ -intoxicated (negative control) groups while decreasing significantly in other groups when compared to the normal Control.

TBARS level was significantly increased in the group intoxicated with $AlCl_3$ whereas a significant decrease occurred in the negative control after treatment with the plant extract.

Discussion

Health is the subject of priority as far as life is concerned, but despite effort to maintain good health, man and animals alike still confront disease conditions which are due to exposure to chemical agents [14], such as aluminium chloride in the environment. Though the body system is made in such a way that it tackles invading foreign substances in most cases, the body system needs to be protected, enhanced and activated [15,16]. This ability to activate the body defence mechanism or to protect the body system has been found to be present in some

natural vegetation/herbal sources. So, it has become expedient to examine scientifically the protective effects of these herbal plants.

From **Table 1**, it is observed that the ALT, AST, Bilirubin, Glucose, ALP and K^+ levels of the negative control group are (47.00 ± 2.15), (283.75 ± 56.48), (22.88 ± 6.64), (7.68 ± 6.19), (277.00 ± 51.56), and (12.00 ± 0.00) respectively. Apart from potassium, these levels are significantly increased as compared to the normal control group. This significant increase is an evidence of hepatotoxicity which may be as a result of leakage from the cells through peroxidative damage of membranes [17]. Hepatocellular necrosis or membrane damage leads to very high levels of serum ALT and AST released from liver to circulation which was in consonance with the increased level of these enzymes in the $AlCl_3$ intoxicated rats (group 2). The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver [18]. In the present study, administration of ethanolic leaves extract of *H. acida* to groups induced with $AlCl_3$ -toxicity mildly suppressed the elevated serum levels of these parameters towards the respective normal values. This clearly indicates that on continued treatment, the plant extract may possibly stabilise the plasma membrane as well as help in healing of the hepatic tissue damage clearly: suggesting that the plant extract has the ability to heal hepatic tissue damage [19]. However, the result of the effects of the plant (leaves) extract on the normal control group also show possible toxic effect of *Hymenocardia acida*.

Haematological parameters provide valuable information on the health status of an animal. The administration ethanolic extract of *H. acida* leaves on the negative control did not modulate the haematological parameters slightly altered by the induction of $AlCl_3$ -toxicity, an indication that there was no much interference on red blood cell and Hb production. Red blood cells (RBC) and

Table 1 Results of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin, Glucose (GLU), Alkaline Phosphatase (ALP) and Potassium ion (K^+).

Group	Normal Control	$AlCl_3$ Control	$AlCl_3$ + HA Eth.	N. + HA Eth.
ALT (U/L)	18.43 ± 01.49^a	47.00 ± 02.15^d	45.35 ± 10.78^d	37.30 ± 11.73^d
AST (U/L)	052.00 ± 10.25^a	283.75 ± 56.48^d	$255.00 \pm 64.03^{c,d}$	$249.00 \pm 82.05^{b,c}$
Bilirubin (mg/dl)	19.20 ± 05.58^a	22.88 ± 06.64^b	21.25 ± 34.14^b	22.03 ± 4.76^b
Glucose (mmol/L)	04.90 ± 01.22^a	07.68 ± 02.19^b	07.17 ± 03.66^b	07.99 ± 02.49^b
ALP (U/L)	78.58 ± 10.10^a	277.00 ± 51.56^c	243.00 ± 57.57^b	228.75 ± 47.17^b
K^+ (mg/dl)	09.50 ± 02.00^a	12.00 ± 00.00^a	12.00 ± 00.82^a	12.00 ± 00.00^a

Each value represent mean of five rats \pm SD.

Groups with similar superscripts ^a in the row are not significantly different.

Table 2 Effects of the extracts on haematological parameters.

Group	Normal Control	$AlCl_3$ Control	$AlCl_3$ + HA Eth.	N. + HA Eth.
WBC ($\times 10^9/L$)	08.55 ± 6.04^a	08.78 ± 5.69^a	04.43 ± 1.33^a	09.88 ± 2.65^a
RBC ($\times 10^6/\mu L$)	06.90 ± 1.04^b	05.96 ± 1.37^b	06.67 ± 1.42^a	09.46 ± 2.66^c
Hb (g/dL)	13.75 ± 2.69^b	$12.60 \pm 0.72^{a,b}$	$13.03 \pm 1.15^{a,b}$	11.25 ± 1.15^a
PCV (%)	47.33 ± 6.65^c	42.78 ± 4.83^c	39.43 ± 10.67^b	32.63 ± 1.96^a
Plt ($\times 10^3/\mu l$)	$410.50 \pm 65.17^{c,d}$	229.25 ± 20.21^a	$282.50 \pm 26.30^{a,c}$	$398.00 \pm 44.93^{c,d}$

Each value represents the mean of 5 Rats \pm SD.

Groups with similar superscripts ^a in the same row are not significantly different

haemoglobin (Hb) are important in transporting respiratory gases. That there were no significant treatment related effects on RBC and Hb implies that the extract did not adversely affect the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissues [19]. Some medicinal plants are known to cause destruction of red blood cells leading to anaemia [20].

The observed increase in total WBC count of group 4 administered ethanolic extract of *H. acida* indicates an enhanced phagocytic function of the leucocytes [21]. The modulation of the platelet count following oral administration of *H. acida* leaves extract indicates that the extract may not cause any coagulation problem, but has the potential to enhance clotting and prevent haemorrhages.

Administration of $AlCl_3$ increases TBARS levels in the liver homogenate of rats compared to the normal group. This means that $AlCl_3$ can increase lipid peroxidation (TBARS) in animals [22,23]. Treatment of $AlCl_3$ -intoxicated rats with ethanolic extract of *H. acida* significantly reduced the TBARS concentration (Table 3).

The ability of the extract to inhibit the process of lipid peroxidation *in vivo* may be due to the free radical scavenging activities of its phytochemical components.

Conclusion

The results of this study revealed that the ethanolic leaves extract of *H. acida* possesses mild moderation effect on $AlCl_3$ -induced toxicity. *H. acida* leaf extract slightly reduced levels of

Table 3 Results of thio-barbituric acid reactive substances (TBARS).

Group	TBARS (nmol/ml)
N. Control	0.05 ± 0.030 ^a
$AlCl_3$ Control	0.24 ± 0.010 ^d
$AlCl_3$ + HA Eth.	0.08 ± 0.007 ^b
N. + HA Eth.	0.13 ± 0.014 ^c

Each value represents the mean of 5 Rats ± SD
Groups with similar superscripts ^a in a column are not significantly different.

serum enzymes associated with liver and was also able to mildly reduce the level of serum glucose. This may contribute to the reason for the local use of *H. acida* leaves for the treatment of diabetes mellitus. The extract of *H. acida* leaves was able to slightly modulate some of the haematological parameters evaluated, indicating that there is no much interference on red blood cell production (at the dose used and duration of administration). The plant extract was also able to reduce TBARS levels in the liver homogenate, which means the extract has the ability to inhibit the process of lipid peroxidation *in vivo* due to its free radical scavenging activities possibly resulting from its phytochemical constitution. Comparative effect of the leaves extract on the normal control and on the negative control show that the leave could possibly be toxic. This result show that the ethanolic extract of *Hymenocardia acida* leaves may possess mild ameliorative effect against aluminium chloride-induced toxicity and may also be toxic in male albino rats.

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