

Spermatogenic and Haematological effects of Aqueous and Ethanolic Extracts of *Hymenocardia acida* Stem Bark on Aluminium-Induced Toxicity in Male Wistar Rats

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Abstract

This study was designed to determine the spermatogenic and haematological effects of aqueous and ethanolic stem bark extracts of *Hymenocardia acida* on Aluminium chloride-induced toxicity in male wistar rats. Thirty male albino Wistar rats were used and toxicity was induced using 100 mg/kg body weight (bw) $AlCl_3$. The rats were distributed into six groups of five rats each. The drug and extract administration lasted for seven days, thereafter the animals sacrificed. The results indicated that oral dose of 100 mg/kg bw extracts of *H. acida* did not show any significant ($p > 0.05$) change in testosterone levels when compared with both normal and negative control. Ethanolic extract showed non-significant ($p > 0.05$) increase on Luteinizing hormone (LH) when compared with both normal and negative control group. Administration of $AlCl_3$ caused a significant ($p < 0.05$) decrease in Follicle Stimulating Hormone (FSH), while the plants extracts caused a non-significant increase when compared with the negative control. Thiobarbituric Acid Reactive Substance (TBARS) increased significantly ($p < 0.05$) in negative control, but decreased significantly in groups treated with aqueous and ethanolic extract of *H. acida* when compared with the negative control. Induction of $AlCl_3$ -toxicity caused a non-significant ($p > 0.05$) alterations of most of the haematological parameters evaluated, but administration of the plant extracts was able to mildly ameliorate these effects (especially on RBC and Hb). The result of this study shows $AlCl_3$ toxicity causes alterations of LH, FSH and TBARS, but aqueous and ethanolic stem bark extracts of *H. acida* may possibly ameliorate the alteration. Also, $AlCl_3$ -toxicity mildly influences the synthesis of certain haematological indices, but the extracts may modulate some of this interference.

Keywords: Albino rats; Aluminium chloride toxicity; Aqueous and ethanolic extract; *Hymenocardia acida*; Spermatogenic

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Introduction

In Nigeria, the folkloric uses of plant preparations for reproduction related purposes are well documented [1]. Herbal contraceptives are used because of affordability, ease of availability from local sources and less side effects [2]. Large number of plants used as abortifacient, contraceptive or sterility agents prevent implantation [3], suppress spermatogenesis [4], or are spermicidal [5].

Medicinal plants are used in Folkloric medicine in treatment of tumor ailment. Information obtained from traditional medicinal practitioners in the South-Western Nigeria revealed that drinking decoctions of *Paquetina nigrescens* leaf, *Moringa oleifera*, *Bradystegia eurocoma* and *Struchium sparganophora* could significantly arrest the progression of tumor proliferation in patient [6].

Hymenocardia acida (euphorbiaceae) is a small brows tree or shrub with palatable foliage, widely distributed within the savannah region of Nigeria. The antimicrobial activities of *H. acida* leaves and roots have been reported [7]. There are literatures which have reported it to be anti-sickling [8], anti-ulcer [9], anti-diarrhoea [10], and to possess anti-inflammatory activities [11]. Another study has investigated the antioxidant activity of the aqueous and methanolic extracts of *H. acida*. [12]. Phytochemical studies of *H. acida* showed the presence of saponins, tannins [13] flavonoids, flavonols, phenols, proanthocyanidins, steroids and triterpenoids. This study is designed to evaluate the spermatogenic and haematological effects of aqueous and ethanolic stem bark extracts of this *Hymenocardia acida* on Aluminium chloride-induced toxicity in male wistar rats. The result obtained will help to know the possible effects of the plant extracts in human.

Materials and Methods

Experimental animals

Thirty healthy male albino wistar rats of weight 100-150 g were obtained from Animal house, Federal University Wukari, Taraba State. The animals were kept in clean cages (plastic bottom and wire mesh top) under room temperature, with 12 hours light and 12 hours dark cycle and were allowed to acclimatize for one week before the experiment. The animals were allowed free access to standard rat chow and water *ad libitum*.

Plant specimen

The stem bark of *H. acida* were collected within the premises of Federal University Wukari Taraba State. They were identified and authenticated in the Department of Biological Sciences, Federal University Wukari. The stem bark was rinsed, air dried at room temperature and pulverized using pestle and mortar. Water was used for the aqueous extract and stored in refrigerator, while exactly 270 g of the sample was weighed and dissolved in 1350 ml of ethanol (for ethanolic extraction) for 48 hrs after which it was filtered: first using a clean white sieving mesh and further with Whatman No. 1 filter paper. The ethanolic filtrate was concentrated using a thermostat water cabinet at 40°C for 7 days. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator at 4°C until required for the experiment.

Preparation of aluminium chloride

Exactly 2g of Aluminium Chloride salt was weighed and dissolved in 5 ml of distilled water. The solution was prepared daily. The Aluminium Chloride solution was administered 100 mg/kg bw of the rats.

Experimental design

The rats were distributed into six groups of five (5) rats each: Group 1 served as normal control: received only normal feed and water daily. Group 2 served as negative control (received only Aluminium Chloride: 100 mg/kg bw daily). Group 3 was administered Aluminium chloride + aqueous extract of *Hymenocardia acida* stem bark: The rats were administered with 100 mg/kg bw of the extracts one hour after administration of 100

mg/kg bw of $AlCl_3$. Group 4 was administered Aluminium chloride + ethanolic extract of *Hymenocardia acida* stem bark: The rats were administered with 100 mg/kg bw of the extracts one hour after administration of 100 mg/kg bw of $AlCl_3$. Group 5: Received normal feed + aqueous extract of *Hymenocardia acida* stem bark daily, while group 6 received normal feed + ethanolic extract of *Hymenocardia acida* stem bark daily.

Animal sacrifice and sample collection

After the experiment, the animals were sacrificed under chloroform anesthesia. Blood samples were collected by cardiac puncture and the liver was harvested, weighed and rinsed in ice cold normal saline. The blood samples were collected in to EDTA collection tubes (for haematological analysis) and plain tubes for serum. The liver was homogenized using a standard laboratory mortar and pestle. The homogenate was centrifuged and the supernatant collected for Thiobarbituric Acid Reactive Substances (TBARS) analysis.

Determination of Thio-barbituric Acid Reactive Substances (TBARS)

Thiobarbituric Acid Reactive Substance formation was measured by using 10% trichloroacetic acid (TCA) solution and 0.6% of thiobarbituric acid (TBA) solution. Exactly 50 μ L of tissue homogenate was added to a test tube containing 2 ml of TCA, followed by 2 ml of TBA and incubated at 80°C for 30 mins in a water bath. It was cooled immediately under ice and centrifuged at 3500 rpm for 10 mins. The absorbance of the solution was read at 535nm against a blank solution containing all except the sample. The TBARS was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Hormonal assay

Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and Testosterone were estimated using the method of Enzyme Linked Immunosorbent assay (ELISA)

Determination of haematological parameters

White blood cell (WBC) count, red blood cell (RBC) count, Haemoglobin (Hb), Packed cell volume (PVC) and Platelet (PLT) were determined using Abacus 280 auto-haematology analyzer.

Ethics

All animals received humane care according to the criteria outlined in the Institution's Guide for the Care and Use of Laboratory Animals.

Results

Thiobarbituric Acid Reactive Substance (TBARS)

Results in **Table 1** show the level of Thiobarbituric acid reactive substance in liver of the rats. The result show a significant ($p < 0.05$) increase in animals of negative control group when compared with normal control. There was also a significant change in group administered normal rats feed + aqueous extract and ethanolic extract of *H. acida* when compared with normal control. There was a significant ($p < 0.05$) reduction of TBARS level in animals

Table 1 Effect of aqueous and ethanolic extract of *H. acida* on TBARS levels in liver of Aluminum chloride treated male rats.

Group	Conc. (nmol/mL)
Normal Control	0.05 ± 0.06 ^a
Negative Control	0.24 ± 0.02 ^d
AlCl ₃ + H.A. Aq.	0.09 ± 0.05 ^{a, b, c}
AlCl ₃ + H.A. Eth	0.08 ± 0.03 ^{a, b}
N. + H.A Aq.	0.11 ± 0.02 ^{b, c}
N. + H.A Eth	0.13 ± 0.02 ^c

Note: Each value represents the mean of 5 rats ± SD
Mean in the same column, having different letters of the alphabet are statistically significant (p<0.05).

treated with AlCl₃+aqueous and AlCl₃+ethanolic extract of *H. acida* when compared with the negative control.

Hormonal assay

Table 2 shows the result of hormonal assay in the male albino rats. The result shows that there was a significant (p<0.05) decrease in luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the negative control animals (intoxicated with aluminium chloride only) when compared with normal control group. The LH non-significant (p>0.05) increased in normal rats administered the aqueous and ethanolic extract of *H. acida*. There was no alteration in testosterone level of all groups when compared with normal control and negative control. There was a non-significant (p>0.05) decrease in follicle stimulating hormone in animals treated AlCl₃+aqueous and AlCl₃+ethanolic extract, and also a non-significant (p>0.05) decrease in luteinizing hormone in animals treated AlCl₃+ethanolic extract: when compared with the negative control.

Haematological parameters

Table 3 shows the result of the haematological parameters evaluated. There was a non-significant (p>0.05) alterations of all the parameters in the negative control, when compared with the normal control. White blood cell (WBC) increased, while there was a decrease in haemoglobin (Hb), red blood cell (RBC) and platelet levels of rats induced with AlCl₃. The administration of the plant extracts moderated most of these alteration, although some are not statistically significant.

Discussion

Aluminium chloride has been shown to affect spermatogenesis in experimental animals and humans which can lead to low sperm count, abnormal sperm morphology and poor semen quality [14]. *Hymenocardia acida* on the other hand has an antioxidant activity [12] and pro-oxidant activity [15]. The result of this study show that aluminum chloride did not cause significant (p<0.05) difference in testosterone levels of the rats. This may be as a result of antioxidant capacity of *Hymenocardia acida* extract on Aluminum chloride-induced toxicity or may be that the plant extracts do not possess the ability to interfere in the processes involved in the production of testosterone.

The decrease in Luteinizing hormone of the negative control group when compared with normal control is in accordance with the previous studies [16] which reported that aluminium chloride

contents in humans and animal testes, spermatozoa, blood and urine were associated with impaired sperm quality, viability and alternation in histology of testes, alternation in spermatogenesis, enhancement of free radicals and alternation in antioxidant enzymes. The decrease in LH that occurred in the group induced with aluminium chloride (negative control) may be due to oxidative damage to histo-architecture of hypothalamus caused by AlCl₃ toxicity. There was an increase in the level of luteinizing hormone in animals treated with aqueous and ethanolic extracts of *H. acida* when compared with the negative control. The shows that the plant extracts could encourage the process involved in the synthesis of luteinizing hormone.

There was significant (p<0.05) decrease in follicle stimulating hormone (FSH) in animals induced with AlCl₃-toxicity (negative control) when compared with normal control. This may be due to interaction of AlCl₃ with the anterior pituitary gland thereby resulting in oxidative damage to architecture of the anterior pituitary gland responsible for the production of follicle stimulating hormone (FSH) (glycoprotein hormone) and Luteinizing hormone. The increase in the groups treated with the aqueous and ethanolic extracts of the plant show that its chemical content may promote the effect of pituitary gland and the production of luteinizing hormone.

The result also show that AlCl₃-toxicity caused significant (p<0.05) increase in the TBARS levels in liver of albino wistar rats (negative control). The increase of TBARS concentration in the liver can be a consequence of critical accumulation of AlCl₃, thereby causing hepatic lipid peroxidation. This result is in agreement with previous studies [17,18] that reported significant increase in TBARS levels of AlCl₃ treated rats. However, treatment with the plant extracts show that the plant possesses the ability to ameliorate the negative effects caused by AlCl₃-toxicity. This suggests that *H. acida* possess antioxidant properties.

Red blood cells (RBC) and haemoglobin (Hb) are important in transporting respiratory gases. Administration of AlCl₃ mildly altered some of the haematological parameters analyzed. This shows that AlCl₃-toxicity could cause reduction of Hb and red blood cell count, and therefore could possibly induce anaemia. The effect of the stem bark extracts of *H. acida* was able to moderate some of the haematological parameters altered by AlCl₃. Platelets play important role in the process of haemostasis and its reduction might adversely affect thrombopoietin. The modulation of the platelet count following oral administration of *H. acida* stem bark extract indicates that the extract may not cause any coagulation problem, but has the potential to enhance clotting and prevent haemorrhages.

Conclusion

This result of this study provides significant evidence for the oxidative damage caused by AlCl₃ in Wistar rats using testosterone, follicle stimulating hormone (FSH), luteinizing hormone, TBARS and some haematological parameters (WBC, RBC, PLT, PCV and Hb) as biomarkers. It is therefore, concluded that AlCl₃ toxicity causes alterations of LH, FSH and TBARS, but aqueous and ethanolic stem bark extracts of *H. acida* may possibly ameliorate the alteration. Also, AlCl₃-toxicity mildly influences the synthesis of certain

Table 2 The effect of aluminium chloride on male wistar rats treated with aqueous and ethanolic extract of *H. acida* stem bark.

Group	LH	FSH	Testosterone
Normal control	0.04 ± 0.01 ^a	8.00 ± 0.70 ^a	20.00 ± 0.00 ^a
Negative control	0.03 ± 0.01 ^a	6.08 ± 0.36 ^b	20.00 ± 0.00 ^a
AlCl ₃ + H.A Aq.	0.03 ± 0.01 ^a	6.10 ± 1.37 ^b	20.00 ± 0.00 ^a
AlCl ₃ + H.A Eth.	0.04 ± 0.01 ^a	6.20 ± 1.37 ^b	20.00 ± 0.00 ^a
Normal + H.A Aq.	0.05 ± 0.02 ^a	7.90 ± 0.20 ^a	20.00 ± 0.00 ^a
Normal+ H.A Eth.	0.06 ± 0.02 ^a	7.80 ± 0.85 ^a	20.00 ± 0.00 ^a

Note: Each value represents the mean of 5 rats ± SD

Mean values in the same column, having different superscripts are statistically significant (p<0.05).

Table 3 Effect of aqueous and ethanolic extract of *H. acida* on haematological parameters

Group	WBC (x 10 ⁹ /L)	RBC (x 10 ⁶ /μL)	Hb (g/dL)	PCV (%)	PLT (x 10 ³ /μL)
Normal Control	8.55 ± 6.04 ^a	6.90 ± 1.04 ^a	13.75 ± 2.69 ^d	47.33 ± 6.65 ^{c, d}	410.50 ± 65.17 ^{c, d}
Negative Control	8.78 ± 5.69 ^a	5.96 ± 1.37 ^a	12.60 ± 0.72 ^{c, d}	49.78 ± 4.83 ^d	229.25 ± 20.21 ^a
AlCl ₃ + H.A. Aq	6.35 ± 1.82 ^a	7.42 ± 0.25 ^{a, b}	12.98 ± 0.43 ^{c, d}	48.35 ± 1.51 ^{c, d}	463.75 ± 16.54 ^d
AlCl ₃ + H.A Eth	4.43 ± 1.33 ^a	6.67 ± 1.42 ^a	13.03 ± 1.15 ^{c, d}	39.43 ± 10.67 ^{b, c}	282.50 ± 26.30 ^{a, c}
N. + HA Aq	8.19 ± 1.80 ^a	6.18 ± 0.13 ^a	10.95 ± 0.61 ^{b, c, d}	34.21 ± 1.74 ^{a, b}	349.75 ± 77.75 ^{b, c}
N. + H.A Eth	9.88 ± 2.65 ^a	9.46 ± 2.66 ^b	11.25 ± 1.15 ^{b, c, d}	32.63 ± 1.96 ^{a, b}	398.00 ± 44.93 ^{c, d}

Each value represents the mean of 5 rats ± SD

Mean values in the same column, having different superscripts are statistically significant (p<0.05).

haematological indices, but the extracts may modulate some of this interference. Further research should need to be carried out

to understand the structural and functional relationship in these extracts and the possible mechanism of action.

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