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Toxicity Studies of the Hydroethanolic Leaf Extract of *Clerodendrum polycephalum* (*Lamiaceae*) in Rats

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Abstract

Background: *Clerodendrum polycephalum* has been traditionally used as a pain killer, management of paralysis, epilepsy and convulsion. Irrespective of its use in Traditionally African Medicine, no data on sub acute toxicity has been described.

Objectives: The present study was designed to assess the acute and sub-acute toxicity of Hydroethanolic leaf extract of *Clerodendrum polycephalum* (HeCP) in rats for 24 h and 28 days respectively.

Materials and Methods: Fasting mice (n=5) were subjected to acute toxicity test at doses of HeCP 1000 mg/kg p.o; 2000 mg/kg p.o; 5000 mg/kg p.o. After 24 h, the animals were then observed for behavioral changes, signs of toxicity and mortality. In the sub acute toxicity test (n=10; HeCP 100 mg/kg p.o. 200 mg/kg p.o. 400 mg/kg p.o. daily). Control group received distilled water (0.2 ml) daily. Blood samples were collected for biochemical and hematological analyses as well as serum antioxidant. Tissues were harvested for histopathological analysis.

Results: It showed that treatment of mice with 1000 mg/kg, 2000 mg/kg, and 5000 mg/kg of the extract produced no lethality in the mice following acute toxicity testing. Results also showed that the HeCP did not produce any significant ($P<0.05$) difference in all the hematological parameters when compared to the control. HeCP (200 mg/kg) produced significant decrease ($P<0.05$) in the level of catalase when compared to the control. At 200 mg/kg of HeCP, there is a significant increase in the level of AST (Aspartate aminotransferase) in comparison to the control. No significant difference was observed in the rest of biochemical parameters.

Conclusion: We conclude from the result obtained in this study that there is a wide margin of safety for the use of this plant for long time administration.

Keywords: *Clerodendrum polycephalum*; Toxicity; Hematological and biochemical assays; AST

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Introduction

Today in Nigeria, there is an increase in the acceptance and utilization of traditional phytotherapies partly because of validation of some of their medicinal values [1]. The basic goal of drug development hinges on developing new synthetic drugs with enhanced therapeutic efficacy and low toxicity profile [2]. Despite the achievement recorded in drug development from plant source, there is an arsenal of local medicinal plants used for disease treatment which have not been evaluated for their toxicity profile [3], one of which is *Clerodendrum Polycephalum* (CP). This plant under study belongs to the family Lamiaceae. It is present in tropical Africa, South Asia, Cameroun, Ghana, Sierra Leone and Guinea [4,5]. In Nigeria, the Yorubas commonly refer to it as Aporo which means it kills pain and as an antidote to venomous stings and bites. It has anti-nociceptive and anti-inflammatory effects [6]. It is also used as pain killer and medicines for the treatment of paralysis, epilepsy and convulsion [5].

The interest in the current study was motivated by increasing acceptance and use of CP despite lack of its human and animal toxicity assessment. In this study, the toxic effects of the hydroethanolic leaf extract of CP on blood biochemical and hematological parameters as well as its histopathological lesions on selected body organs were investigated in adult mice and young male rats.

Materials and Methods

Plant material

Fresh leaves of CP were obtained from a farm land at Okeletu, Ijede in Ikorodu, Lagos State,

Nigeria. The botanical identification and authentication of the plant was done by Mr O.O. Oyebanji, a forestry expert of the Department of Botany Herbarium in the Faculty of Science, University of Lagos, Lagos, Nigeria where the herbarium voucher specimen (LUH 7080) was deposited for reference.

Experimental animals

A total of 25 male adult mice (15 g to 20 g) and 25 white albino rats (male) weighing between 140 g to 150 g obtained from and kept at the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Lagos. Nigeria. The animals were kept in well ventilated and hygienic compartments, maintained under standard environmental conditions and fed with standard feed (Livestock Feed Plc, Lagos Nigeria) and water ad libitum. The experimental procedure adopted in this study was in accordance with the United States National Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [7].

Preparation of the plant extract

The fresh leaves of CP washed and were dried. It was then grounded into powdered form by mechanical grinder and (3350 g) coarse powder was loaded into a percolator. Extraction was done with 4 liter of absolute alcohol for 72 h.

After filtration, the residue was discarded and the final filtrate was concentrated in a rotary evaporator (40°C under vacuum). The yield was 7.65 w/w. The greenish solid extract obtained was always reconstituted in distilled water to appropriate concentrations before administration to experimental animals.

Quantitative phytochemical analysis

Preliminary phytochemical analysis of CP was carried out to identify the constituents, using methods described [8,9].

Test for alkaloids

One gram of extract was stirred in 10 ml distilled water and then filtered. The filtrate was divided into 3 portions and the following reagents were used to test for the presence of alkaloids.

Mayer's test: The filtrate was treated with few drops of Mayer's regent (potassium mercuric iodide). The formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's test: The filtrate was treated with few drops of Wagner's reagent. The formation of a brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's test: The filtrate was treated with few of Dragendroff's reagent (solution of potassium bismuth iodide). The formation of reddish precipitate indicates the presence of alkaloids.

Test for flavonoids

Shinoda's test: The extract was dissolved in 2 ml of distilled water and warmed in a test tube. Four drops of concentrated hydrochloric acid were then added to 1 ml of the solution followed by addition of same magnesium ribbons, immediate appearance of orange color denotes the presence of flavones, red crimson indicates presence of flavonoids and pink magenta color denotes the presence of flavones.

Sodium hydroxide test: The other 1 ml from above was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Test for tannins

Lead acetate test: To 1 ml of extract (dissolved in distilled water), 2 drops of lead subacetate solution was added. A colored precipitate indicates the presence of tannins.

Bromine water test: 1 ml of extract solution was treated with 3 drops of bromine water. Non-formation of colored precipitate indicates the presence of hydrolysable tannins.

Test for saponins

Frothing test: 0.5 g of extract dissolved in 5 ml distilled water and shaken in a test tube for 5 min. The occurrence of a honey comb froth, which lasts for about 45 minutes, indicates the presence of saponins.

Hemolysis test: 0.5 g of extract was dissolved in 5 ml distilled water, 2 drops of fresh whole blood was added to the solution. Immediate hemolysis of indicates the presence of saponins.

Test for phytosterols/ triterpenoids

One g of extract dissolved in distilled water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into two portions and used for:

Salkowsk's test: A few drops of concentrated sulphuric acid were added to the filtrate by the wall of the text tube. The appearance of golden yellow color indicates the presence of triterpenes.

Test for anthraquinones

Borntrager's test: One gram of extract was dissolved in distilled water and fractionated with petroleum ether. The petroleum ether fraction was concentrated and shaken with 5 ml of 25 % ammonia solution. A cherry-red color of the alkaline solution indicates the presence of antraquinones.

DPPH radical scavenging activity assay

The free radical scavenging capacity of HeCP was determined using the Stanley free radical DPPH [10]. Extract was mixed with 95 % ethanol to prepare a stock solution (5 mg/ml). DPPH solution (0.004% w/v) was placed in test tubes followed by serial dilution of the extract (20 to 100) was added in every test tubes so that the final volume was 3 ml, after 10 min, the absorbance test read at 515 nm using a spectrophotometer (HACH 400 DR visible spectrophotometer, USA). Ascorbic acid was used as a reference standard and dissolved in distilled water to prepare a stock solution with the same concentration (5 mg/ml). A control sample of the same volume was prepared without any extract and reference ascorbic acid. The % scavenging of the DPPH free radical was measured using the following equation.

% scavenging activity = absorbance of control × Absorbance of the test sample/Absorbance of the control × 100

The IC50 value is the concentration of the sample required to inhibit 50% of the radical served as blank. Antioxidant activity was expressed as the number of gram equivalent of ascorbic acid [11].

Toxicity tests

Acute toxicity: Three groups of 5 mice each fasted overnight prior to the experiment were administered doses of the extract (1000 mg/kg p.o, 2000 mg/kg p.o and 5000 mg/kg p.o). The control animals (n=5) received distilled water 10 m/kg p.o. Mice were closely observed for 2 h post-treatment for behavioral changes and signs of toxicity. Mortality in each group within 24 h was recorded and

Table 1: Phytochemical constituents of HeCP.

Constituents Tested	Test Method	Result
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendoff's test	+
Anthraquinones	Bontrager's test	
Cardiac glycosides	Keller-Keliani test	
Flavonoids	Shinoda's test	+
	Sodium hydroxide test	+
Saponins	Frothing test	+
	Hemolysis	+
Steroids	Liebermann-Burchad's test	+
Tannins	Lead acetate test	+
	Bromine water	+

() = Absent; (+) = Present

surviving animals were observed for a further 7 days for any signs of delayed toxicity [12].

Sub-acute toxicity study: Twenty adult male rats were randomly allotted into four groups consisting of the control (n=5) three extract groups, 100 mg/kg, 200 mg/kg and 400 mg/kg. The doses represent half of the pharmacologically active dose, the pharmacologically active dose and double the pharmacologically active dose respectively [13]. The animals were daily treated p.o. with distilled water and HeCP at doses of 100, 200 and 400 mg/kg. They were closely observed for behavioral and general morphological changes. Mortality in each treatment group was recorded in the course of the experiment. After 28 days, 12 rats from the entire groups were sacrificed and blood samples were collected through retro-orbital plexus vein of the rat eye for hematological and biochemical analysis [14].

Hematological assessment

Blood samples from experimental animals were collected into EDTA (Ethylenediamine Tetraacetate) bottles and analyzed using standard procedures. Erythrocyte (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV), mean Corpuscular Volume (MVC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Red Cell Distribution Width (RDW) and total and differential leucocyte (WBC) were determined using automated hematology analyzer (QBC Autoread plus, UK).

Biochemical assessment

Whole blood samples of rat collected into plain bottles were allowed to coagulate for 30 min and serum was separated by centrifugation at 3000 rpm and then separated by use of Pasteur pipettes into clean bottles. The serum samples were stored at – 20°C until analysis using Chemwell chemistry autoanalyser (Awaress Technology, USA model 2910), Alkaline Phosphate (ALP), Alanine Amino Transaminase (ALT), Alkaline Phosphatase (ALP) were assayed by the method described [15]. Serum creatinine was quantified by kinetic method [16]. Total serum protein was assayed by Biuret method [17]. Serum albumin was quantified by the method described [18].

Measurement of *in vitro* antioxidants

From liver and kidney samples of sacrificed rats, determination of Catalase (CAT), Superoxide Dismutase (SOD), reduced Glutathione (GSH), Glutathione Peroxide (GPX) and Malondialdehyde (MDA) was done according to the protocol [19].

Histopathological assessment

After the collection of blood, the liver and kidney were immediately excised, freed from advent, blotted, weighed and fixed in 10% formol saline for histological studies. Fixed sections were dehydrated in graded alcohol, embedded in paraffin and cut into 4 µm to 5 µm thick sections. The sections were stained with hematoxylin-eosin for photomicroscope (CEL-TECH) Diagnostics, Hamburg, Germany [20,21].

Results

Phytochemical screening

Preliminary phytochemical screening of HeCP showed the

Table 2: Effects of HeCP on hematological parameters.

	Control	HeCP 100 mg/kg	HeCP 200 mg/kg	HeCP 400 mg/kg
WBC (10/µl)	9.93 ± 0.93	5.30 ±1.78	8.80 ±1.60	7.06 ±1.88
LYMPH (%)	3.86 ± 0.22	3.50 ± 0.55	2.86 ± 0.21	1.93 ± 0.68
MID (%)	38.5 ± 2.63	37.6 ± 2.37	39.0 ± 0.61	1.3 ± 2.57
GRAN%	57.5 ± 3.66	58.9 ± 2.76	58.1± 0.78	66.7 ± 3.07
HGB g/dl	11.83 ± 0.51	11.70 ± 0.61	12.26 ± 0.64	12.60 ± 0.80
RBC106/µl	6.28 ± 0.22	6.35 ± 0.15	6.92 ± 0.46	6.80 ± 0.31
HCT%	37.16 ± 1.53	36.8 ± 1.66	38.60 ± 1.98	39.6 ±2.58
MCV (fl)	59.26 ± 2.71	58.03 ± 2.05	55.96 ± 1.42	58.26 ± 1.65
MCH (pg)	31.76 ± 0.20	18.33 ± 0.86	17.73 ± 0.41	18.43 ± 0.43
MCHC (g/dl)	31.76 ± 0.20	31.70 ± 0.37	31.73 ± 0.12	31.73 ± 0.23
RDW-CV %	15.53 ± 0.08	14.73 ± 0.08	15.66 ±1.03	15.50 ± 0.75
RDW-SD %	31.76 ± 0.56	29.36 ± 0.56	30.73 ± 0.23	31.46 ± 1.46
PLT (104/µl)	775.00 ± 84.05	257.0 ± 83.18	782.33 ±18.35	766.0 ± 49.41
MPV%	7.10 ± 0.15	7.10 ± 0.40	7.23 ± 0.203	6.66 ± 0.13
PDW %	15.60 ± 0.15	15.53 ± 0.13	15.73 ± 0.23	15.20 ± 0.10
PCT %	0.55 ± 0.06	0.30 ± 0.15	0.56 ± 0.05	0.50 ± 0.02

Values are expressed as mean ± SEM (n=5). *p>0.05, ****p>0.0001 *vs.* control (two way ANOVA followed by Dunnet's multiple comparison tests)

Table 3: Effects of HeCP on serum antioxidant indices in rats.

Control	100 mg/kgHeCP	200 mg/kgHeCP	400 mg/kgHeCP
GSH 3.367 ± 0.609	2.730 ± 0.272	4.837 ± 0.872	2.237 ± 0.506
SOD 2.617 ± 0.023	2.690 ± 0.120	2.473± 0.240	2.693± 0.286
CAT 19.310 ± 1.142	18.537 ± 1.517	10.797± 1.392****	19.173 ± 2.263
MDA 0.597 ± 0.055	0.693 ± 0.426	1.493 ± 0.111	0.553 ± 0.081

Values are expressed as mean ± SEM (n=3). *p<0.05, ****p<0.0001 *vs.* control (two way ANOVA followed by Dunnet's multiple comparison test)

Table 4: Effect of HeCP on blood chemistry parameters.

Control	100mg/kgHeCP	200 mg/kgHeCP	400mg/kgHeCP
AST(U/L) 418.000 ± 276.832	130.3 ± 16.9*	805.4 ± 346.34**	436.3 ± 315.44
CREA(U/L) 56.80 ± 15.80	41.2 ± 5.48	71.66 ± 13.73	53.83 ± 14.07
ALT(U/L) 56.80 ± 18.76	33.4 ± 7.92	129.96 ± 43.6	91.66 ± 34.26
UREA(mmol/L) 7.56 ± 0.88	5.63 ± 15.83	6.033± 30.55	6.60 ± 0.20
ALBUMIN(g/dl) 56.80 ± 18.76	42.9 ± 1.57	38.06 ± 2.64	42.167 ± 4.14
S-TP 56.80 ± 18.77	75.00 ± 2.32	74.56± 4.45	76.03 ± 4.70
CHO 2.00 ± 0.18	1.71 ± 0.25	1.65 ± 0.18	1.86 ± 0.10
TRIGL 0.55 ± 0.06	0.39 ± 0.02	0.59± 0.12	0.77 ± 0.06
ALP 147.2 ± 17.85	159.73 ± 31.23	129.06 ± 15.33	116.40 ± 0.60
BIL (mg/dl) 1.66 ± 0.176	2.36 ± 0.12	2.16 ± 0.18	1.43 ± 0.120
HDL1.20 ± 0.319	1.22 ± 0.266	0.65 ± 0.08	1.10 ± 0.27

Values are expressed as mean ± SEM (n=5). p>0.05 *vs.* Control (two way ANOVA)

Table 5: Effect of hydroethanolic leaf extract of *Clerodendrum polycephalum* on histology of the liver and kidney.

Treatment Dose Organ Observation Interpretation (Mg/Kg)		
Dose of HeCP Administered	**Organ**	**Summary of Histologic Findings**
Control	Kidney	Histologic sections of kidney tissue show normocellular glomerular tufts disposed on a background containing renal tubules. No abnormalities are seen NORMAL KIDNEY
100 mg	Kidney	Histologic sections of kidney tissue show normocellular glomerular tufts disposed on a background containing renal tubules. No abnormalities are seen NORMAL KIDNEY.
200 mg	Kidney	Histologic sections of kidney tissue show normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels are seen VASCULAR CONGESTION
400 mg	Kidney	Histologic sections of kidney tissue show normocellular glomerular tufts disposed on a background containing viable tubules. Congested blood vessels are seen VASCULAR CONGESTION.
Control	Liver	Histologic sections of liver tissue show parallel radially arranged plates of hepatocytes No abnormalities are seen NORMAL LIVER
100 mg	Liver	Histologic sections of liver tissue show parallel radially arranged plates of hepatocytesNo abnormalities are seen NORMAL LIVER
200 mg	Liver	Histologic sections of liver tissue show parallel radially arranged plates of hepatocytes No abnormalities are seen NORMAL LIVER
400 mg	Liver	Histologic sections of liver tissue show parallel radially arranged plates of hepatocytes, Congested blood vessels are also seen VASCULAR CONGESTION

presence of alkaloids, flavonoids, saponins, steroids and tannins while cardiac glycosides triterpenoids and anthraquinones were absent (Table 1).

Acute toxicity in mice

Acute oral administration of HeCP (1000 mg/kg, 2000 mg/kg, or 5000 mg/kg) produced no mortality in mice after 24 h observation.

Sub-acute toxicity study

Effect of HeCP on hematological parameters in rats: Sub-chronic administration of HeCP (100 mg/kg, 200 mg/kg, and 400 mg/kg) produced no significant increase (P>0.05, P>0.0001) in the level of all the hematological parameters respectively when compared to the control group (Table 2).

Effect of HeCp on serum antioxidant indices: Sub-chronic administration of HeCP (100, 200, 400 mg/kg) did not produce any significant change in the serum level of GSH, SOD and MDA when compared to the control, but with CAT there is a significant reduction in the serum level compared to the control group (Table 3).

Effect of HeCP on biochemical parameters in rats: There is a reduction of Aspartate (AST) at 100 mg/kg of HeCP compared to the control group but at 200 mg/kg of HeCP there is significant increase in AST level compared to the control. However, no significant change in the level of other biochemical parameters of HeCP compared to the control group (Table 4).

Discussion

Herbal plants with medicinal values have been widely used as therapeutic agents for the treatment of various diseases affecting humanity. However, studies have proven that a lot of medicinal plants which have been presumed are actually toxic [22]. Various reasons have been adduced to be responsible for the toxicities of these medicinal plants, including presence of constituents such as alkaloids, viscotoxin, saponins, pyrrolizidine alkaloids [22]. CP is an important plant with antinociceptive and anti-inflammatory activities [6]. The leaves have been used in the treatment of paralysis, epilepsy and convulsion by Traditional Medicinal Practitioners [23] and no

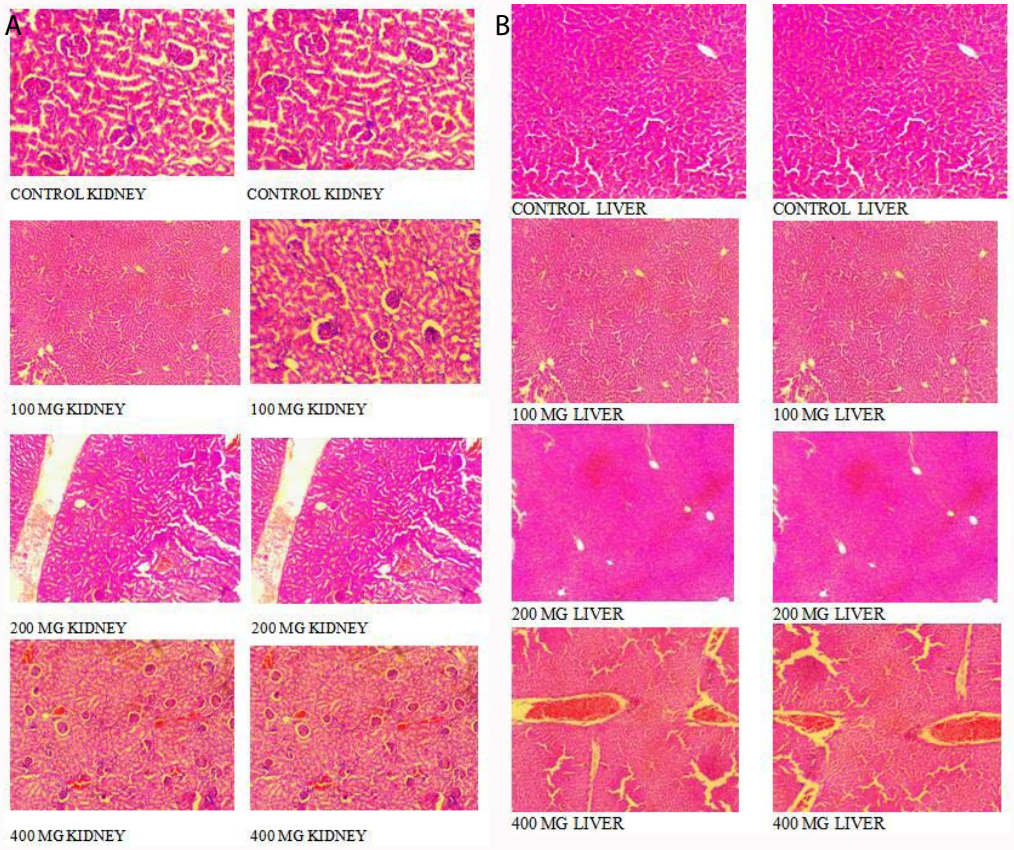

Figure 1: Dose of HeCP administered on Liver and Kidney.

information is available regarding safety following its long use. This present study investigated the safety profile of CP in rats. Decreased antioxidant defence mechanism and increased lipid peroxidation are signals for organ toxicity and damage [24]. Catalase (CAT) is one of the important antioxidant enzymes that cause oxidative stress by breaking down cellular hydrogen peroxide to produce water and oxygen radicals [25]. Sub-acute administration of HeCP (200 mg/kg) produced significant reduction in the serum level of CAT compared to the control. It could therefore be said that administration of HeCP 200 mg/kg over extended period could produce nephrotoxicity (Table 5). However, no significant change in the serum level of GSH, SOD and MDA when compared to the control group.

The liver and kidney are vital organs that play significant roles in metabolic activities in the body. The increase in the level of serum AST after sub-acute administration of HeCP 200 mg/kg is indicative of hepatotoxicity [26]. Urea and uric acid are regarded as important markers for kidney malfunction [27]. However, there were no differences in levels of urea and uric acid as compared to the control group.

Blood system is one the most important targets for toxic compounds and important reference for physiological and pathological status. A blood profile usually highlights vital details on the reaction of the body to injury and stress [27,28]. No significant change in the levels of all hematological parameters was observed when compared to the control after sub-acute administration of HeCP in the animals.

Histological examination of the kidney treated with HeCP 200 mg showed some degree of vascular compression compared to the control, central vein was intact. At HeCP 400 mg mild focal degenerative changes in the form of inflammatory infiltration were seen in the histopathological examination of kidney at high doses, mild vascular compression were seen in both liver and kidney (Figure 1).

Conclusion

In conclusion, the results suggest that *Clerodendrum polycephalumis* relatively safe.

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