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Research Article

Chronic toxicity evaluation of ethanolic stem bark extract of *Randia (Xeromphis) nilotica* Stapf. (Rubiaceae) in Wistar rats

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Background: *Randia nilotica* is known in northern Nigeria as Gial-goti, but has also been reported to be widespread in Sudan and India. The plant is used traditionally for its ethno-medicinal claims in managing mental-illnesses, convulsion or epilepsy, jaundice, infertility, snake bites and other ailments. The leaf, root and stem bark of the plant had been scientifically validated for CNS depressant activity and the stembark is particularly used for CNS-related disorders. However, information related to the toxicity potential of the plant is not available.

Objective: To investigate the effect of 90-days administration of ethanol stem-bark extract of the plant on some physiological-biomarkers and vital-organs' histology.

Methods: Oral median-lethal dose (LD_{50}) estimated from acute-toxicity test and extract doses of 250, 500, 1000mg/kg for 3-groups of 20-rats each and normal-saline control group were used. The rats were euthanized on the 90th-day following daily oral treatments per-body-weight. Blood-samples in plain and anticoagulated (EDTA) sample-bottles for biochemical and haematological analyses were collected from each group and vital-organs isolated, weighed and kept in fixatives for histo-analyses.

Result: The oral acute extract-administration up to 5000mg/kg caused no observable toxic-sign or mortality. PCV, Hb and RBC counts decreased significantly at 500 and 1000mg/kg, but only at 1000mg/kg for MCV, with no significant changes in other haematological-indices. Significant increase in blood-urea-nitrogen at all test-doses and in high-density lipoprotein at 250mg/kg occurred. Brain-weight was significantly decreased and all organs histologically showed blood-vessels congestion and inflammatory-cells' infiltration, in addition to dose-dependent neuronal-degeneration and cerebral-oedema in brain, lymphocytes' depletion in spleen, necrositic-hepatocytes, myocardial-haemorrhage with oedematous-fragmentations and glomerular-atrophy, haemorrhage, tubular-necrosis, glomerular hypercellular-vacuolation and Bowman's-capsule adhesion to parietal surface.

Conclusion: Haematological, biochemical and histological analysis revealed evidence of chronic toxicity to various major organ systems. In addition to dose, duration of use also contributes to the toxic effects of the plant.

Key-words: *Randia-nilotica,* stem-bark, ethanol extract, chronic-toxicity, rats

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1. Introduction

The dose-related toxicity evaluation of extracts of natural products often provides valid information related to their biochemical, physiological and pharmacological effects on vital organs and their processes. It also provides an estimate of safety margin for initial safe starting dose of agents for clinical trials

(Robbinson et al, 2009). The routine toxicity evaluations which are often used in screening new chemical agents include the acute, sub-acute and chronic toxicity studies (Gupta et al, 2012). The practice of using medicinal plants to maintain and promote health, prevent and/or cure a wide variety of diseases is being carried out over ages (Siddharthan, 2007; Madhulika, 2010). Quite a number of medicinal plants used traditionally or locally have proven to be important sources of potential therapeutic agents, but most often little or no attention is given in identifying the toxic effects associated with their use for relatively prolonged periods at both low and high doses.

Randia nilotica (Stapf.) of the family, Rubiaceae and genus, catunaregam has common names as *Xeromphis nilotica* or *Catunaregam nilotica* and is known in the northern part of Nigeria as barbaji, tsibra, kwanarya or Gial-goti ((Dalziel, 1937; Burkill, 1985). It is used traditionally in many countries including Sudan, India and Northern Nigeria for mental illness, convulsion, epilepsy, jaundice, anti-infertility and for snake bites amongst other uses (Chabra et al, 1991; Ismaila et al, 2012). The leaf, root and stem bark of the plant had been scientifically validated for CNS depressant activity (Lemmich et al, 1995; Danjuma, et al, 2009). However, aside the acute toxicity studies, variously reported by the different researchers, no intensive toxicity study (sub-chronic or chronic) on the medicinal use of any part of *Randia nilotica* had been reported. This study thus, evaluated the toxicity profile of the stem bark of this plant in rats.

2. Methods

2.1 Collection, identification and extraction of the plant material

The plant *Randia nilotica* was collected from Galadimawa village in Giwa Local Government of Kaduna State, Nigeria in the month of April, 2013. It was identified and authenticated with Voucher Specimen Number **2867** and deposited for reference purposes in the herbarium section of the department of Biological sciences, Ahmadu Bello University, Zaria. The stem bark of *Randia nilotica* was cleaned and air-dried under a shade to a constant weight and then pounded into coarse powder using pestle and mortar. The obtained 1000 g powder was cold macerated into 2.5 litres of 70 % ethanol for 72 hours and filtered. The filtrate was concentrated at 40^{o}C over an evaporating dish and digital thermostatic water bath and it yielded 56.28 g (5.6%) brownish sticky solid residue. Working concentrations of 250, 500 and 1000 mg/kg extract doses were prepared fresh for each day experiment with distilled water.

2.2 Experimental animals

Male Wistar rats weighing between 120 – 280 g obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used. The animals were maintained on standard rodents' feed and liberal drinking water. The animals were handled in accordance with the ethical standards of the Department's experimental animal committee which approved the study and also in accordance with the regulation for the Care and Use of Laboratory animals as accepted internationally (NIH, rev 1996).

2.3 Acute toxicity study for LD_{50} determination

The LD_{50} of the extract was determined using Lorke's method (1983) of acute toxicity test. A total of 13 rats were used to assess the extract for acute toxic effect of phases I and II of this model and of which 9 rats in 3 study groups of 3 per group were given graded doses of 10, 100 and 1000 mg/kg respectively in the 1st phase test and observed for death in 24 hours. Increased doses of 800, 1600, 2,900 and 5,000 mg/kg in absence of mortality in first phase were administered to the remaining rats in study groups of 1 rat per group. From the observed outcome, the LD_{50} was then calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

2.4 Chronic toxicity study

The repeated dose of 90 days oral toxicity study of the organization of Economic Cooperation and Development Guidelines (OECD GLs) 408 of 1998, in male Wister rats was used for chronic effect investigations. Four groups of 20 rats of both sexes, matched for weight and sex per group were orally pre-treated daily for 90 days with normal saline (group 1) and extract doses of 250, 500 and 1000 mg/kg for groups 2-4. Blood samples collected at the end of treatment periods of 90 days from each rat-group into plain and anticoagulated (EDTA) sample-bottles were used for biochemical and haematological analyses respectively. The brain, heart, kidney, liver and spleen isolated from the euthanized rats were weighed and fixed in 10 % buffered formalin or Bouin's fluid (brain only) for histopathological examinations. The parameters evaluated for toxic effects of the plant extract include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels, blood urea nitrogen (BUN), creatinine, calcium, total protein (TP), albumin, total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL); packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV), white blood cell count (WBC) and differentials – neutrophils, lymphocytes, monocytes, basophils, eosinophils; erythrocytes sedimentation rate (ESR); organ-weight variation and histological analysis of the brain, heart, kidney, liver and spleen.

2.5 Statistical analysis

Data were expressed as mean ± standard error of mean (M±SEM) and results presented as tables and figures. Data were analyzed using one way Analysis of variance (ANOVA) followed by Post-Hoc Dunnett's test for multiple comparisons at the significance level of $p<0.05$.

3. Results

The oral median lethal dose (LD_{50}) estimation

There were no observable toxic signs or mortality at up to 5000 mg/kg body-weight oral administration of the

extract. Oral median lethal dose (LD_{50}) was thus estimated to be greater than 5000 mg/kg.

Effect of 90 days daily oral administration of ethanol extract of *Randia nilotica* on haematological and biochemical parameters in rats

The result of the haematological analysis in **Table 1** below showed significant ($p<0.05$) decrease in PCV, Hb and RBC counts at both 500 and 1000 mg/kg extract doses. The mean cell volume (MCV) was also significantly ($p<0.05$) increased, but only at 1000 mg/kg.

The biochemical analysis showed that the 90 days daily oral administration of ethanolic stem bark extract of Randia nilotica caused significant ($p<0.05$) increase in blood urea nitrogen at all tested doses and also in high density lipoprotein (HDL), but only at 250 mg/kg of extract dose (**Table 2**).

Table 1: Haematological parameters in 90 days treated rats with ethanol stem bark extract of *Randia nilotica*

Haematological indices	Distilled water (1 ml/kg)	Extract doses (mg/kg)		
		RNE 250	RNE 500	RNE 1000
PCV (%)	40.75 ± 1.23	41.39 ± 1.85	26.20 ± 3.60*	26.50 ± 3.50*
Hb (g/dl)	13.32 ± 0.39	13.61 ± 0.60	8.50 ± 1.20*	8.65 ± 1.35*
RBC(10^{12}/l)	4.77 ± 0.17	4.97 ± 0.23	3.08 ± 0.51*	2.90 ± 0.40*
MCHC(g/dl)	32.40 ± 0.29	40.69 ± 5.27	32.20 ± 0.37	32.50 ± 0.50
MCH(pg)	28.15 ± 0.23	27.62 ± 0.21	28.60 ± 0.75	30.00 ± 1.00
MCV (fl)	85.35 ± 0.55	83.08 ± 0.54	86.20 ± 2.94	91.50 ± 0.50*
Neutrophils (%)	25.65 ± 1.25	28.46 ± 0.91	27.40 ± 1.72	26.50 ± 3.50
Lymphocytes (%)	72.20 ± 1.26	68.62 ± 1.15	72.00 ± 1.64	73.00 ± 3.00
Monocytes (%)	2.00 ± 0.27	2.33 ± 0.33	1.00 ± 0.00	0.00 ± 0.00
Eosinophils (%)	3.00 ± 0.47	3.44 ± 0.29	2.00 ± 0.00	1.00 ± 0.00
WBC (10^9/l)	14.53 ± 0.87	14.94 ± 1.20	11.02 ± 2.14	8.95 ± 0.35
Platelets (10^9/l)	229.37 ± 8.94	256.31 ± 19.57	175.20 ± 10.37	170.50 ± 2.50

Data are presented as Mean±SEM; * = One-way ANOVA and Dunnett's post hoc test at $p<0.05$ n=20; RNE= *Randia nilotica* extract.

Table 2: Biochemical indices following 90 days daily oral administration of ethanolic stem bark extract of *Randia Nilotica* in rats

Liver Indices	Treatments(mg/kg)			
	Distilled Water (1ml/kg)	RNE 250	RNE 500	RNE 1000
AST (u/l)	64.16±3.63	63.31±4.55	73.60±4.89	66.00±20.00
ALT (u/l)	70.32±3.84	70.85±5.21	87.00±5.65	72.50±21.50
ALP (u/l)	85.00±4.38	103.46±8.77	113.00±11.81	90.50±31.50
ALBUMIN (g/l)	37.00±0.64	37.33±1.18	39.80±0.66	42.00±0.00
TP (g/l)	64.94±0.87	66.67±1.28	65.50±1.85	67.00±0.00
Triglyceride(mg/dl)	0.85±0.05	0.91±0.06	0.88±0.08	1.15±0.15
HDL (mg/dl)	0.78±0.04	0.99±0.05*	0.76±0.07	0.85±0.05
TC (mg/dl)	2.38±0.05	2.39±0.07	2.60±0.12	2.75±0.55
LDL (mg/dl)	1.42±0.06	1.22±0.10	1.66±0.08	1.67±0.57

Data are presented as mean±SEM; * = One-way ANOVA and Dunnett's post hoc test at $p<0.05$ n=20; RNE = *Randia nilotica* extract

Effect of ethanolic stem bark extract of *Randia nilotica* on organ weights and histology

Changes in organ weights relative to control group following 90 days daily oral administration were not significant. However, there was statistically significant decrease in brain weight at the various doses (**Table 3**). Histological assessment of organs following 90 days oral administration of *Randia nilotica* stem bark extract revealed neuronal degeneration, blood vessel and cerebral congestions in the brain. The kidney showed glomerular atrophy, haemorrhage, tubular-necrosis, Bowman's capsule adhesion to parietal cells, hypercellular-vacuolation, inflammatory cells' infiltration and glomerular-congestion (**Figure 1**).

Table 3: Organ weight changes following 90 days daily oral administration of stem bark extract of *Randia nilotica* in rats

Organs (g)	Distilled water (1 ml/kg)	Doses (mg/kg)		
		RNE 250	RNE 500	RNE 1000
Heart	0.54 ± 0.54	0.54 ± 0.03	0.55 ± 0.07	0.58 ± 0.03
Spleen	0.57 ± 0.04	0.61 ± 0.06	0.77 ± 0.06	0.60 ± 0.00
Liver	4.17 ± 0.11	4.37 ± 0.24	4.72 ± 0.71	4.70 ± 0.20
Kidney	0.81 ± 0.03	0.81 ± 0.06	0.98 ± 0.11	0.90 ± 0.01
Brain	1.72 ± 0.04	1.50 ± 0.07*	1.42 ± 0.11*	1.30 ± 0.10*

Data are presented as mean±SEM; * = One-way ANOVA and Dunnett's post hoc test at $p<0.05$ n=20; RNE = *Randia nilotica* extract

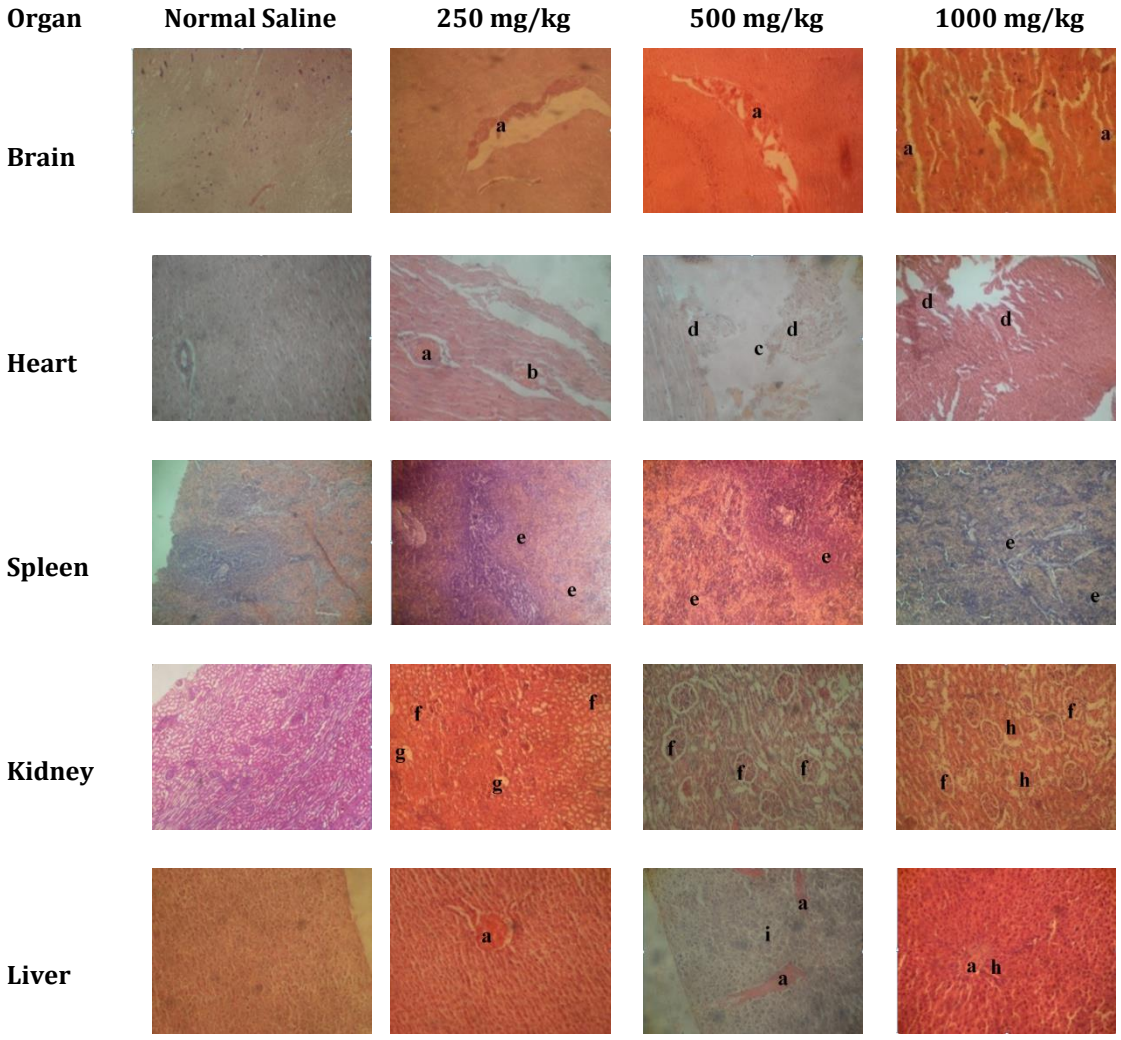

Figure 1: Photomicrographs of organ sections (H & E at × 200 – 400) of rats following 90 days daily oral administration of ethanol stem bark extract of *Randia nilotica* showing: Congestion (**a**); Haemorrhage (**b**); Oedema (**c**); Fragmentation of muscle fibres (**d**); Depletion of lymphocytes (**e**); Hypertrophy of glomeruli (**f**); Vacuolation of glomeruli (**g**); inflammatory cells infiltration (**h**); Necrosis of hepatocytes (**i**).

4.0 Discussion

This study revealed that *Randia nilotica* stem bark extract used traditionally for various diseases is practically non-toxic at up to and above 5000 mg/kg dose from acute oral administration. This was in line with the documentation of Lorke (1983) that compounds with an oral LD_{50} above 5000 mg/kg body weight are practically non toxic

In an earlier sub-chronic toxicity study of 28 days daily oral administration, only the packed cell volume (PCV) was significantly (*p<0.05*) decreased and at 1000 mg/kg extract dose. However, in this study of 90 days daily oral administration for chronic toxicity evaluation, the PCV was significantly decreased even at 500 mg/kg dose and together with other related haematologic indices such as Hb and RBC counts, but the MCV was increased significantly at 1000 mg/kg.

The packed (or red blood) cell volume (PCV) also known as haematocrit is the volume percentage (%) of red blood cells in blood that serves as a reference point of capability of RBCs to deliver oxygen. It is usually an integral part of complete blood cell counts, along with haemoglobin concentration, white blood cell count, and platelet count. For a condition such as anaemia that goes unnoticed, one way it can be diagnosed is by measuring the haematocrit levels of the blood which shows the number of red blood cells in circulation (Adewuyi, 2007). Low levels of it suggest reduced transportation of oxygen (or blood flow) from the lungs to the body tissues. This usually is indicative of certain problems including iron deficiency, haemorrhage, or kidney dysfunction amongst other ailment conditions (Katzung et al*,* 2009; Camaschella, 2015). The red cells contain Hb that oxygenates blood in the lungs and a deficiency of red cells or its Hb (aplastic anaemia) occurs in conditions of iron lack because iron is the principal element in Hb for blood production. Thus, a low haematocrit as with reduced red cell sizes or low mean corpuscular volume (MCV) suggests chronic iron-deficiency anaemia with consequent abnormal haemoglobin synthesis (Camaschella, 2015). A decrease in the concentration of Hb as was observed in this study could be related to depletion in the essential body iron. Aside alkaline or high phosphate related reduction in the availability of iron, severe iron deficiency condition is often as a result of blood loss via bleeding; and haemorrhage and which were among the pathologic effects in the histological examinations of the various organs in this study. The two predominant sites of iron storage are the reticuloendothelial system and the hepatocytes, but some storage also occurs in the muscle. The main sites of reticuloendothelial cells are bone marrow, spleen, liver and lymphoid tissues and this study showed that the spleen had lymphocytes' depletion and both the spleen and liver had blood-vessels' congestion. The liver also showed necrositic-hepatocytes with perivascular infiltration of inflammatory cells. These effects of the extract may have caused depletion in the body iron store. Myocardial haemorrhage and congestion with infiltration of inflammatory cells (myocarditis) also occurred and may have decreased the heart's ability or efficiency to pump blood and this might have contributed to the reduction in these blood parameters and/or depletion in the muscle store of iron. Resistance in flow of blood from any condition often invariably increases the total vascular resistance to cause oedematous fragmentations of the muscle fibres (Guyton and Hall*,* 2006), as also observed in the heart.

Significant decrease in brain weight was seen in the 90 days daily oral administration of the stem bark extract of *Randia nilotica* in addition to histological changes that also occurred in the heart, kidney, spleen and liver. Neuronal degeneration, blood vessel and cerebral congestions occurred in the brain. The kidney showed glomerular atrophy, haemorrhage, tubular-necrosis, Bowman's capsule adhesion to parietal cells, hypercellular-vacuolation, inflammatory cells' infiltration and glomerular-congestion. The increased vulnerability to toxic attack often seen with both the kidney and liver is due to their involvement in many important physiological and pharmacological functions (Rang and Dale, 2007). The liver is directly involved in the metabolism of most toxic agents and most times many of these agents undergo metabolic activation to form reactive metabolites in the process of metabolism. Plasma albumin and about 30% serum globulins are formed in the liver and it is also the site of urea cycle and creatinine synthesis amongst other functions (Marcovitch, 2005). Blood volume and electrolyte composition are regulated by the complex homeostatic mechanisms in which the kidneys reabsorb its important glomerular filtered materials and excretes many waste substances including urea, creatinine and albumin (Marcovitch, 2005). Kidney impairment often diminishes the glomerular filtration rate (GFR) of liver-synthesized and other materials resulting in their retention (Chen et al, 2008; Ferguson et al, 2008, Treseler, 1995). Thus, the significant increase in blood urea-nitrogen (BUN) at all test doses of *Randia nilotica* extract may solely be related to kidney changes as liver damage can impair urea cycle. This finding probably suggests that the liver damage from this extract may not have been severe for such impairment. Urea is the totality of the waste product of protein metabolism representing about 90% of the total urinary excreted nitrogen. Mild necrositic injuries of the hepatocytes may only elevate liver transaminase (ALT, AST) levels (Nyblom et al*,* 2004) and slight increase in these enzymes observed in this study was not significant.

Significant increase in high density lipoprotein (HDL) level occurred at the 250 mg/kg dose in the 90 days daily oral administration of the stem bark extract of *Randia nilotica* with slight reduction in the low density lipoprotein (LDL). The HDLs secreted in the liver and small intestines decreases the clotting potentials of blood, participates in mopping of bad cholesterol from the arterial wall and also help to inhibit the oxidation of atherogenic or LDL lipoproteins (Akubue et al*,* 2006; Katzung et al*,* 2009). HDL-Cholesterol also acts as an anti-inflammatory agent.

5.0 Conclusion

Haematological, biochemical and histological analysis revealed evidence of chronic toxicity to various major organ systems. The findings of this study also indicate that the time-course of exposure or duration of therapy is a major toxicological issue as is the dose or amount of agents taken.

Conflict of Interest declaration

The authors declare no conflict of interest.

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