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PHYTOCHEMISTRY AND SUBCHRONIC TOXICITY EVALUATION OF ETHANOLIC STEM BARK EXTRACT OF RANDIA (XEROMPHIS) NILOTICA STAPF (RUBIACEAE) IN WISTAR RATS

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ABSTRACT

Aim: To evaluate the sub-chronic toxicity of the ethanolic stem-bark extract of *Randia nilotica* in rats

Study Design: Observational/investigational studies

Place and Duration of Study: Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Kaduna. April to December, 2013

Methodology: Phytochemicals were assayed. Acute oral lethal-dose (LD_{50}) was estimated following Lorke's method. Four groups of 10 Wistar rats/group matched for weight and sex were orally dosed for 28 days with normal saline, 250, 500 and 1000 mg/kg extract respectively. On euthanizing, blood samples collected from each group into plain and anticoagulated (EDTA) sample-bottles were used for biochemical and haematological analyses respectively. The brain, heart, kidney, liver and spleen were isolated and each weighed and fixed in 10% buffered-formalin or Bouin's fluid (brain only) for histopathology. The mean ± standard error of mean and statistical significance at (P=.05) of obtained data were evaluated.

Results: All, except alkaloids, steroids, cardenolides and anthraquinones phytoconstituents were present. No observable toxicity or mortality at 5000 mg/kg/bw following acute oral administration. Significantly reduced PCV level occurred only at 1000 mg/kg. No significant changes in Hb, RBC, MCHC, MCH, MCV, WBC differentials and ESR; as also in the levels of BUN, creatinine or calcium; liver-enzymes, total-protein, albumin, total-cholesterol, triglycerides and high-density-lipoproteins. Changes in organ-weights relative to control group were not significant. Histologically, dose-dependent alterations occurred as neuronal degeneration and congested blood vessels in the brain; myocardial congestion, haemorrhage and infiltration of inflammatory cells; the kidney showed hyper-cellular atrophy of glomeruli, haemorrhage, tubular necrosis, cellular infiltration, and Bowman's capsule adhesion with parietal cell surface; the spleen showed congestion of blood vessels and lymphocytes' depletion, while the liver had congestion of blood vessels, perivascular infiltration of inflammatory-cells and necrotic hepatocytes.

Conclusion: This relatively short-term use changes in vital organs' histology of this plant part may be deleterious and thus, calls for caution.

Keywords: *Randia nilotica*, sub-chronic toxicity, Rats, Phytochemicals, Hazard

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1.0 INTRODUCTION

Randia nilotica (staph) 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 [1,2]. 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 [3,4]. The leaf, root and stem bark of the plant had been scientifically validated for CNS depressant activity [5,6]. However, aside the median lethal dose (LD_{50}) variously measured in an acute toxicity research studies for dose selections, no intensive toxicity study (sub-chronic or chronic) on the medicinal use of any part of *Randia nilotica* had been reported. The measure of acute lethality such as LD_{50} may not accurately reflect the full spectrum of toxicity or hazard associated with drugs or chemicals. The full spectrum of the toxic effects associated with the use of any drug or chemical is usually established from the sub-chronic and chronic toxicity studies [7]. Such overall knowledge of the toxic effects of any drug often helps in the expansion of medical care whereby an identified drug can be used with the awareness of the risk/benefit effect associated with it [8]. The use of medicinal plants like *Randia nilotica* in health care without reference to long term adverse effects may constitute further health challenge; and it had been noted that reasonably large population of people in northern Nigeria use the plant for one ailment or the other without due consideration to toxic consequences. The need to determine the dose and duration related toxic effects associated with the traditional use of herbal preparations is imperative considering the widespread use of natural products especially herbal preparations [9].

2.0 MATERIALS AND METHODS

2.1 Collection, Identification and Extraction of the Plant Material

The plant *Randia nilotica* was collected at Galadimawa village in Giwa Local Government of Kaduna State, Nigeria in the month of April, 2013. It was identified and authenticated with a voucher specimen number of 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 about 72 hours and filtered. The filtrate was concentrated at 40^{o}C over an evaporating dish and digital thermostatic water bath and it yielded 56.28g (5.628%) brownish sticky solid residue. The working concentrations of 250, 500 and 1000 mg/kg extract doses diluted with distilled water were prepared fresh for each day experiment.

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 for this study. The animals were maintained on standard rodents' feed and liberal drinking water. All experiments were carried out in compliance with Animal Research: Reporting of In Vivo Experiments

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(ARRIVE) guidelines (2010) and in accordance with the EU Directive 2010/63/EU for animal experiments [10,11].

2.3 Equipment

Mettler scale analytical weighing balance (P162, Gallenkamp), Weighing scale (W.T. Avery, U.K.), Biochemical Analyser (Selectra XL; Vitascientifics, Netherlands), Hetteich Universal Centrifuge (Universal 32, Austria), Evaporating dish with water bath (Biotech. Laboratories, U.K.).

2.4 Acute Toxicity Study for LD_{50} Determination

The LD_{50} of the extract was determined using Lorke's method [12] of acute toxicity testing. A total of 13 rats were used to assess the extract for acute toxic effect of the 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 for the first phase test and observed for death in 24 hours. Three or four increased or reduced doses were then administered to the remaining animals respectively depending on the absence or presence of death in the first phase experiment. The LD_{50} was then calculated from the observed outcome as the geometric mean of the highest non-lethal dose and the lowest lethal dose

2.5 Preliminary Phytochemical Screening

The established standard test tube analysis procedure as described by Trease and Evans [13] was used to screen the extract for the presence of saponins, carbohydrates, cardiac glycosides, triterpenes, flavonoids, tannins, alkaloids, steroids, cardenolides and anthraquinones.

2.6 Subchronic Toxicity Studies

The repeated dose of 28-day oral toxicity studies of the organization of Economic Cooperation and Development Guidelines (OECD GLs) 407 [14] in Wistar rats was used to assess the plant for sub-chronic toxicity. Four groups of 10 rats of both sexes, matched for weight and sex per group were orally treated daily for 28 days with normal saline (group 1) and extract doses of 250, 500 and 1000 mg/kg for groups 2-4. The rats were euthanized at the end of 28 days' treatment duration to collect the blood samples from each group into plain and anticoagulated (EDTA) sample bottles for biochemical and haematological analyses respectively. Vital organs (brain, heart, kidney, liver and spleen) were isolated and weighed prior to fixing in 10% buffered formalin or Bouin's fluid (brain only) for histopathological examinations. The toxic effects of the plant extract were investigated on 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 histopathological analysis of the brain, heart, kidney, liver and spleen.

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2.7 Statistical Analysis

Data were expressed as mean ± standard error of mean and results presented as tables and figure. Data were analysed using one-way Analysis of Variance (ANOVA) followed by Dunnett's Post-Hoc test for multiple comparisons at the significance level of $p=.05$.

3.0 RESULTS

3.1 Phytochemical Constituents of Ethanol Stem Bark Extract of *Randia nilotica*

The result obtained from the preliminary phytochemical screening of the stem bark extract of *Randia nilotica* is as summarised in Table 1 below:

Table 1: The Phytochemical Constituents from the Preliminary Screening of Ethanol Stem Bark Extract of *Randia nilotica*

Constituents	Result
Saponins	+
Carbohydrates	+
Cardiac glycoside	+
Triterpenes	+
Flavonoids	+
Tannins	+
Alkaloids	-
Steroids	-
Cardenolides	-
Anthraquinone	-

Key: + = Present; - = Absent

3.2: Estimation of the Oral Median Lethal Dose (LD_{50}) of Randia nilotica Stem-bark Extract

The oral median lethal dose (LD_{50}) for the extract was estimated to be greater than 5000 mg/kg body weight as no death was recorded in both the first and second phases of the experiments of Lorke's acute toxicity test.

3.3 Effect of 28-days Daily Oral Administration of Ethanolic Stem Bark Extract of *Randia nilotica* on Haematological Parameters in Rats

The result of the haematological analysis in table 2 below showed a significant ($p<0.05$) decrease in packed cell volume (PCV) at 1000 mg/kg following 28-day oral administration with no significant alterations in the other assessed indices.

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Table 2: Changes in Haematological Indices in Rats following 28 Days Daily Oral Administration of Ethanolic Stem Bark Extract of *Randia nilotica*

Data are presented as Mean ± SEM; One-way ANOVA, Dunnett's post hoc test; n= 10; RNE= *Randia nilotica* extract; *Statistical significance level at P<0.05

Haematological Indices	Distilled Water (1ml/kg)	Extract doses (mg/kg)		
		RNE 250	RNE 500	RNE 1000
PCV (%)	44.70±0.99	45.00±0.85	45.50±0.64	40.88±1.09*
Hb (g/dl)	14.54±0.43	14.98±0.33	15.18±0.36	13.59±0.50
RBC(x 10^{12}/L)	4.72±0.20	4.88±0.26	5.17±0.28	3.96±0.16
MCHC(g/dl)	35.20±1.60	33.56±0.24	34.00±0.37	33.25±0.56
MCH(pg)	29.50±1.46	28.78±0.36	28.00±0.33	29.25±0.75
MCV(Fl)	87.80±0.98	86.44±0.67	84.10±0.97	85.00±1.02
Neutrophil (%)	20.30±2.47	22.33±2.01	23.80±2.15	16.00±4.36
Lymphocytes (%)	79.40±2.44	77.33±2.04	75.30±2.15	84.38±3.46
Monocytes (%)	0.00±0.00	1.00±0.00	4.00±0.00	0.00±0.00
Eosinophils (%)	2.00±0.00	2.00±1.00	1.67±0.33	2.00±0.00
WBC (10^9/L)	8.44±0.38	8.76±0.43	9.37±0.29	8.70±0.53
ESR (mm/Hr)	2.30±0.26	3.22±0.40	2.20±0.33	2.63±0.46

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3.4: Effect of 28 Days Daily Oral Administration of Ethanolic Stem Bark Extract of *Randia nilotica* on Biochemical Parameters in Rats

The 28 days' oral daily administration of ethanolic stem bark extract of *Randia nilotica* caused no alteration in any of assessed biochemical parameters including the liver and renal indices (Table 3).

Table 3: Changes in Some Biochemical Indices following 28 Days Daily Oral Administration of Ethanolic Stem Bark Extract of *Randia nilotica* in Rats

Biochemical parameters	Treatments (mg/kg)			
	Distilled Water (1ml/kg)	RNE 250	RNE 500	RNE 1000
AST(u/l)	68.70±7.80	69.80±5.95	81.44±8.19	81.00±7.38
ALT(u/l)	81.40±5.54	75.90±6.33	84.63±7.49	85.44±7.46
ALP(u/l)	87.70±6.95	73.80±9.78	97.00±7.05	102.00±8.24
Albumin(g/l)	38.50±3.25	41.50±4.06	34.11±1.01	38.11±1.35
Total Protein (g/l)	64.50±0.97	64.50±0.81	62.89±0.96	66.33±0.75
Triglyceride (mg/dl)	0.98±0.83	0.95±0.10	0.92±0.07	0.83±0.07
HDL(mg/dl)	0.91±0.09	0.75±0.08	0.84±0.07	0.84±0.07
T. Cholesterol (mg/dl)	2.34±0.09	2.42±0.06	2.39±0.07	2.42±0.09
LDL(mg/dl)	1.23±0.08	1.48±0.10	1.36±0.04	1.41±0.08
BUN (mmol/L)	4.39±0.31	4.38±0.35	4.30±0.34	4.58±0.36
Creatinine (mmol/L)	55.40±3.23	55.60±3.92	58.00±3.30	64.22±4.12
Calcium (mmol/L)	2.31±0.04	2.32±0.06	2.42±0.06	2.43±0.04

Data are presented as mean ± SEM; One-way ANOVA, Dunnett's post hoc test.

*Statistical significance level at $P<0.05$; n=10; RNE = *Randia nilotica* extract

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3.5 Effect of Ethanolic Stem Bark Extract of *Randia nilotica* on Organ Weights and Histology

Changes in organ weights relative to control group following 28 days daily oral administration were not significant (Table 4), but the histopathology of the organs showed dose dependent alterations. The brain section showed congestion of blood vessels and neuronal degeneration and heart also had congestion and infiltration of inflammatory cells (myocarditis) with myocardial haemorrhages. Slight depletion of lymphocytes as well as congestion were seen in the spleen, while the kidney showed atrophy of glomeruli, haemorrhages, renal tubular necrosis, cellular infiltration, hypercellular glomeruli with adhesion of the parietal surface on Bowman's capsule, and glomerular vacuolation. Hepatic necrosis, cellular infiltration and haemorrhages also occurred in the liver.

Table 4: Effect of Stem Bark Extract *Randia Nilotica* on Organ Weights Following 28 Days Daily Oral Administration in Rats

Organs	Distilled water (1ml/kg)	Treatment (mg/kg)		
		RNE 250	RNE 500	RNE 1000
Heart	0.57±0.04	0.56±0.04	0.51±0.07	0.56±0.04
Spleen	0.69±0.05	0.62±0.04	0.83±0.09	0.66±0.03
Liver	4.86±0.29	4.46±0.32	5.02±0.41	4.26±0.16
Kidney	0.90±0.05	0.87±0.04	1.06±0.08	0.92±0.04
Brain	1.49±0.04	1.45±0.07	1.52±0.07	1.38±0.04

Data are presented as mean ± SEM; One-way ANOVA, Dunnett's post hoc test.

*Statistical significance level at $P<0.05$; n=10; RNE = *Randia nilotica* extract

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The Histological Changes in some Vital Organs Following 28 days Daily Oral Administration of *Randia nilotica* Methanol Extract in Rats

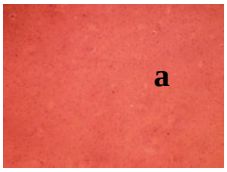
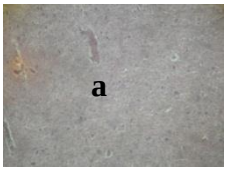
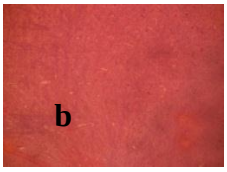
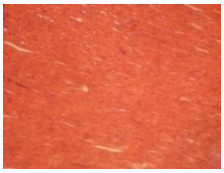
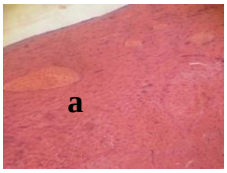
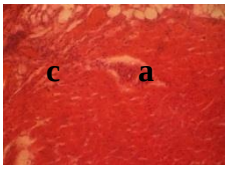
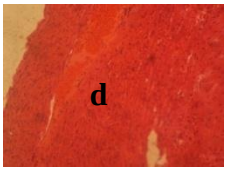
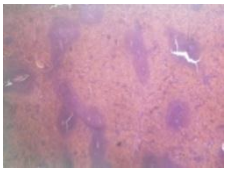
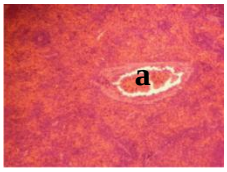
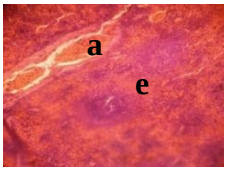
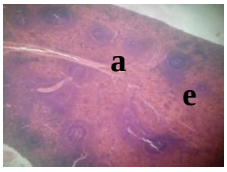
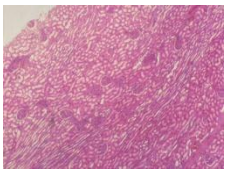
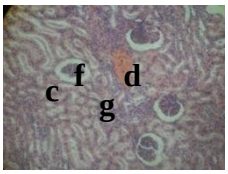
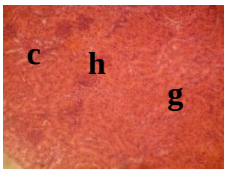
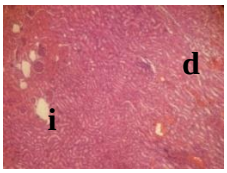
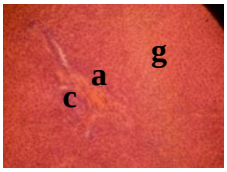
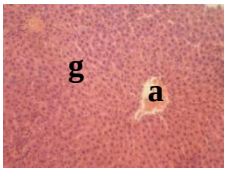
Organs of treated rats	Normal saline	250 mg/kg	500 mg/kg	1000 mg/kg
i. Brain				
ii. Heart				
iii. Spleen				
iv. Kidney				
v. Liver				

Plate 1: Photomicrographs of organ sections (H & E at ×40-400) of rats following 28 days daily oral administrations of ethanol stem bark extract of *Randia nilotica* showing:

i. (a) congestion of blood vessels, (b) degeneration
ii. (c) infiltration of inflammatory cells, (d) haemorrhage, and (a),
iii. (e) depletion of lymphocytes and (a)
iv. (f) atrophy of glomeruli, (g) necrosis, (h) hypercellular glomeruli with parietal surface adhesion to Bowman's capsule (i) glomerular vacuolation, (c) and (d)
v. (c), (d) and (g)

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4.0 DISCUSSION

The time-course of exposure or duration of therapy is a major toxicological issue as with the doses or amount of agents taken. Most herbal drugs, including *Randia nilotica*, are often regarded as natural food plants and thus, used continuously and even in large quantities for prolonged periods [6]. This study has shown that, the consumption of the stem bark of *Randia nilotica* in large quantity of up to 5000 mg/kg for one or two days either, for its CNS or other effects may not cause any immediate toxic effects or mortality but may, however, not be healthy if used in such large quantity for prolonged period. The vital constituents expressed in this plant part that may have been responsible for its CNS effects include saponins, the fraction of which had been evaluated for sedative and antidepressant activities [6]. Other constituents found present in this plant part were cardiac glycosides, known to enforce myocardial contraction and to reduce conductivity of the arterio-ventricular (AV) node and thus useful in the management of supraventricular tachycardias or heart failures [15], tannins, reportedly used in the management of varicose vein, heavy menstrual flow and inflammatory conditions [13]. Triterpenes, flavonoids and carbohydrates were also present and often play vital physiological roles. The toxicities found with this extract were majorly of dose and probably duration related. None of the haematological indices was significantly altered in the 28 days' extract administration except the packed cell volume (PCV) that decreased significantly ($p = .05$) (44.70 – 40.88), and only at 1000 mg/kg extract dose. 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 oxygen delivering ability of RBCs. 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 [16]. Low levels of it suggest reduced transportation of oxygen (or blood flow) from the lungs to the body tissues. This usually is an indication of certain problems including iron deficiency, haemorrhage or kidney dysfunction amongst other ailment conditions [17,18].

The alterations found in the morphology and histology of the brain, kidney, heart, liver and spleen were also dose related. The brain showed neuronal degeneration; and also had congestion of blood-vessels which also occurred in liver, heart and spleen. There was infiltration of inflammatory cells in all the assessed organs; and haemorrhage occurred in both the heart and kidney. The kidney also had hyper-cellular atrophy of glomeruli, tubular-necrosis and Bowman's capsule adhesion to parietal cell-surface. It is possible that these toxicities may not have occurred at doses very much lower than the smallest dose of 250 mg/kg (5% LD_{50}) used in this study. Thus, it may be necessary to ascertain the lowest effective dose that could be safely taken for longer periods or continuously and at which the composite beneficial effects of its secondary metabolites are not masked by toxicity [14].

5.0 CONCLUSION

In conclusion, this study has shown that while there appears to be no apparent derangements in the organs, there are strong indications that the plant may be deleterious when taken over prolonged period,

especially at high doses, as is seen in the histopathology and blood parameters assayed.

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Competing Interests

The Authors declare that there were no competing interests of any sort in the writing and publication of this manuscript.

Authors' Contributions

Timothy, M designed the study, performed the experiments, did the statistical analyses, wrote the first draft of the manuscript. Mallam, NM, Ejiofor, JI and Oladele, SB. Participated in the experiments and made significant inputs to the write-up. Mallam, D proof-read and made meaningful contributions. All authors read and approved the final manuscript.

REFERENCES

[1] Dalziel JM. The Useful Plants of West Tropical Africa. Crown Overseas Agents for the Colonies, London, 1937, pp. 300.

[2] Taxonomy of *Randia nilotica*. http://www.zipcodezoo.com. (accessed 2014)

[3] Chabra SC, Mahunnah RLA, Mshiu EN. Plants used in traditional medicine in Eastern Tanzania *V. Angiosperms* (Passifloraceae to Spindaceaea), J. Ethnopharmacol. 1991; 33(1/2):143-157.

[4] Ismaila MS, Adamu SA. The impact of traditional methods of managing snake bite in humans and livestock among the Hausa-Fulani communities of Sokoto State (North-western Nigeria), J. Med. Plants Res. 2012; 6 (28):4489-4493.

[5] Lemmich E, Cornett C, Furu P, Jorstian CL, Knudsen AD, Olsen CE, et al., Molluscidal Saponins from *Catunaregam nilotica*, Phytochem. 1995; 39 (1):63-68.

[6] Danjuma NM, Abdu-Aguye I, Anuka JA, Hussaini IM, Zezi AU, Yaro AH. Central nervous system depressant effect of the hydroalcoholic extracts of leaves, stem and root barks of *Randia nilotica* Stapf. (Rubiaceae), E. J. Sci. Res. 2009; (3):353-361.

[7] Cassarette I, Klaassen CD, Amdur MO, Doulls J. Toxic responses of kidney, In: C.D. Klaassen (Ed), Cassarette and Doulls Pharmacology, the Basic Science of poison, 5th edn. Mc Graw-Hill, New York, 1996, pp 403 – 414.

[8] Timbrell J, The poison paradox; Chemicals as friends and foes, Oxford University Press. 2005, pp 282 -313.

[9] Agrahari AK, Panda SK, Meher A, Padhan AR. Screening of wound healing activity of *Curculigo orchioides* Gaertn root tubers' methanolic extract, Int. J. Pharmaceut. and Appl. Sci. 2010; 1 (1):1-5.

[10] Kikenny C, Browne WJ, Cuthill IN, Emerson M, Altman DS. The ARRIVE Guidelines. Animal Research: Reporting of *In Vivo* Experiments. National Centre for the Replacement Refinement and Reduction of Animals Research, 2010.

[11] DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33, 2010.

[12] Lorke DA. A new approach to practical Acute Toxicity testing. Arch. Toxic. 1983; 54:275 – 287.

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[13] Evans WC. Trease and Evan's Pharmacognosy, 13th edn. W.B. Saunders and Co. Ltd, 1989, pp. 116-462.
[14] Organization of Economic and Cooperative Development (OECD), Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guideline for Testing of Chemicals, 407 (1995) pp. 1-8.
[15] Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology, 7th edn. Elsevier Ltd. 2012, pp. 246-264.
[16] Adewuyi OA, Companion to Practical Haematology. A Manual for the practical haematology course in the medical undergraduate programme in developing countries, Klobex Academic Publishers, Ilorin, Nigeria, 2007, pp. 21-24.
[17] Katzung GB, Masters SB, Trevor AL, Basic and Clinical Pharmacology, 11th edn. Mc Graw Hill, 2009, pp 569-989.
[18] Camaschell C. Iron-Deficiency Anaemia. N. Engl. J. Med. https://doi:10.1056/nejmra1401038