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Research Article

Toxicological studies of *Anchomanes difformis* Blume (Araceae) using rats and mice

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ABSTRACT

Background: The toxicity; biochemical, hematological and histopathological study of *Anchomanes difformis* aqueous extract was carried out.
Methods: Six groups; 0, 100, 1000, 5000, 7500, and 10,000 mg/kg p.o of mice consisting of four animals; two females, two male a group were used in the acute toxicology experimentation. For the sub-acute test, rats of either sex; three males and three females were shared into four groups; 0, 500, 1000, and 2500 mg/kg p.o for 21 days. Toxicity studies were done using automated techniques and prepared histology slides viewed under a light microscope.
Results: Results showed no mortality - undeterminable lethal dose, 50%. Biochemical parameters tested were within permissible limits and no pathologies in the selected organs studied.
Conclusion: It can be inferred that *A. difformis* aqueous extract is safe even at a dose as high as 10 g/kg body weight.

Keywords: Acute, Sub-acute, *Anchomanes difformis*, Rats, Mice

INTRODUCTION

Herbal medicines are thought to be safer than modern medicines and free of side effects.[1] However, natural does not make them safe.[2] Some plants are toxic and as medicines, are expected to have side effects.[3] According to the same WHO document, the toxicity of herbal medicines may result from, adulteration of herbal medicines with other potent pharmaceutical ingredients (such as corticosteroids and non-steroidal anti-inflammatory drugs), use of the wrong species of the herb, incorrect dosing, and contamination with toxic metals, micro-organisms and agrochemical residues. Toxicity of herbal medicines may also result from the simultaneous use of herbal and conventional medicines, improper storage, self-medication, and failure to report same to the physician. Mis-labeling, misidentification, and substitution are also important factors.[4,5]

In stark contrast to the claims that herbal medicines are safe and devoid of side effects, there have been several reports of toxicity from herbal remedies. A prospective study showed that 25% of the corneal ulcer in Tanzania and 26% of the childhood blindness in Nigeria and Malawi were associated with traditional eye medicine.[6]

Anchomanes difformis (Blume); a medicinal plant indigenous to Nigeria and Cameroun grows in the tropical zones in various areas of Africa.[7] It is sometimes called forest anchomanes in English,[8] Ogiriisako or Oje (Igbo language), Langbodo or Isu-igo in Yoruba language, Chakara in Hausa language, and ebe-olikhoro in bini language.[9] An *in vitro* study of the antimicrobial activities of *A. difformis* leaves and rhizomes against selected pathogens of public health importance showed that most pathogens of public health importance were susceptible to both extracts showing that *A. difformis* has got antimicrobial activity. In parts of Africa such as Tanzania, the juice from the root tuber is used in the treating river blindness.[10] Ethnomedicinal information from herbalists in Zaria city showed that a decoction of the root is used to treat diabetes, cough, and throat related problems.[11] It was reported to treat kidney-ailments, edema and as diuretic for treating urethral discharge, jaundice and as poison antidote.[12-14] But no report has furnished us with the biosafety of the plant. Thus, this study is aimed at assessing the acute toxicity and sub-acute profile of the aqueous leaf extract in mice and rats.

METHODS

Plant material and preparation of extract

Fresh leaves of *A. difformis* were collected from a bush in Ekosodin village in Ovia North East of Edo state. The plant was authenticated by Professor MacDonald Idu of the Department of Plant Biology and Biotechnology. The fresh leaves were washed in distilled water and blended using a kitchen blender. The filtrate was dried using an FD-10M freeze dryer on a temperature of −4°C at the National Centre for Energy and Environment, Benin City.

Animals

Rats and mice of either sex weighing 21.64±4.01 (mean±standard error mean [SEM]) and 165±2.51 (mean±SEM) were bought from the livestock market, Aduwawa, Benin City. They were kept in the Animal House of the Department of Animal and Environmental Biology, University of Benin. The animals had access to grower's marsh (got from Bendel Feeds and Flour Mill, Ewu, Edo State) and tap water *ad libitum*. The animals were kept at 27±1.5°C temperature, 60%±5% relative humidity, and 12 hrs day and night cycle and were handled according to standard procedures for laboratory animals as adopted and cited by Ozolua et al.[15]

Acute toxicological experiments

The oral median lethal dose of *A. difformis* aqueous leaf extract was determined following methods described by Lorke.[16] Six groups of mice consisting of four animals; two females, two male a group were used. Group 1 served as control and was administered distilled water while Groups 2-6 were administered 100, 1000, 5000, 7500, and 10,000 mg/kg of the extract using an oral gastric tube. Four groups of rats comprising four animals; two females and two males a group were used. Group 1 (control) was administered distilled water while Groups 2-4 were administered 5000, 7500, and 10,000 mg/kg using an oral gastric tube. Mortality and other physical signs of acute toxicity were monitored for 2 weeks.

Sub-acute toxicological experiments

Rats of either sex; three males and three females were shared into four groups. Group 1 was administered distilled water; Groups 2-4 were administered 500, 1000 and 2500 mg/kg for 21 days. On the 21st day, the animals were anesthetized with 2% chloroform in an airtight container. Blood for hematological and biochemical assays was withdrawn from the abdominal aorta. The heart, left kidney, spleen, lungs, and liver were isolated, fixed in 10% formal saline and processed using an automated tissue processor,[17] using Leica Tp 1020, Leica rotary microtome Leica 1160 Medite tissue embedding unit, Leica RM 2125 RT the final Rotary microtome to produce the final sample on glass slides for the various histochemical staining techniques,[18] including: hematoxylin and eosin technique for normal histological features and cellular response.[19] The processed tissues/glass slides produced were subjected to microscopic examination at the anatomy department of the University of Benin, using Olympus Cx 21 model optical microscope.[20] Photomicrographs of the observed changes were got using Samsung digital camera SV 20, 12 megapixels attached to the optical lens of a light microscope connected to a Compaq Hp laptop computer system by a fiber-optic USB cord producing a triocular complex.[21]

Biochemical and hematological assays

The blood sample was taken from each animal in all four groups (control and treatment groups) in both ethylenediaminetetraacetic acid and lithium heparin bottles and taken to the University of Benin Teaching Hospital Hematology laboratory where the samples were analyzed using an automated machine from ERMA Inc. Tokyo Japan after the blood samples have been centrifuged at 3000 revolutions per mins to separate serum from plasma using a pasteur pipette. Lipid profile parameters and liver function analysis (aspartate aminotransferase [AST], alanine aminotransaminase [ALT], alkaline phosphatase, total protein, total bilirubin, albumin, and conjugated bilirubin) were carried out using Elitech clinical systems. Electrolytes (Sodium [Na^+], potassium [K^+], bicarbonate [HCO_3^-], and chlorine [Cl^-]) were determined using ISE 4000 analyzer by SFRI-Lieudit Berganton 33127 St. Jean dilac-France. Urea and creatinine contents were also quantified.

Data presentation and statistical analysis

Results are presented as mean±SEM and n represents the number of animals a group. Data were analyzed using

ordinary one-way ANOVA and multiple comparisons using Graph pad computer software (UK) version 6.0. $p<0.05$ indicates statistically significant difference.

RESULTS

Oral lethal dose, 50% (LD_{50}) of the extract was indeterminable as there was no mortality observed and no obvious toxicological signs at 10,000 mg/kg body weight as depicted in Table 1. Effect of repeated doses of *A. difformis* aqueous leaf extract on the relative abundance of fundamental electrolytes in rats after 21 days administration is reported in Table 2. Effects of sub-acute oral administration of *A. difformis* on hematological limits are shown in Table 3. The effect of *A. difformis* aqueous leaf extract on some biochemical and the histology of rats after 21 days oral administration are reported in Tables 4 and 5. Photomicrographs of the histopathological diagnosis of some vital organs in rats used for subacute toxicological experiment after 21 days treatment with aqueous leaf extract of *A. difformis* are reported in Figures 1-5.

DISCUSSION

Using high doses in toxicological tests indicates the safety margin of an extract.[22] Despite several criticisms, LD_{50} determination has remained a useful tool in the safety assessment of substances.[23] Absence of death and gross toxicological manifestations after an oral dose of 5 g/kg body weight suggests the extract may be safer by this route.[24,25]

Table 1: Acute toxicological analysis of *A. difformis* aqueous leaf extract in mice and rats.

Dose	Number of deaths	Mortality
0	0/4	0
100	0/4	0
1000	0/4	0
5000	0/4	0
7500	0/4	0
10,000	0/4	0

Animals were observed for 2 weeks after drug administration. *A. difformis: Anchomanes difformis*

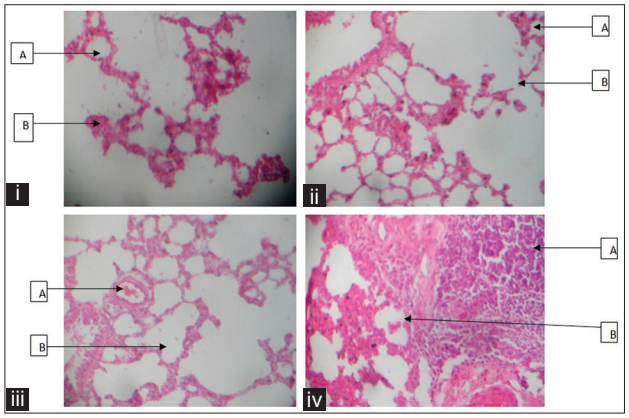

Figure 1: Lungs (i) Control; rat lungs composed of A - Alveolar sacs separated by B - interstitial space, (ii) rat lungs treated with 500 mg/kg *Anchomanes difformis*; A - Mild interstitial congestion and B - Normal alveoli, (iii) rat lungs treated with 1000 mg/kg *A. difformis*; A - Mild vascular congestion and B - Normal alveoli, (iv) rat lungs treated with 2500 mg/kg *A. difformis*; A - Moderate activation of bronchioloalveolar lymphoid aggregates and B - Normal alveoli (H and E, ×100).

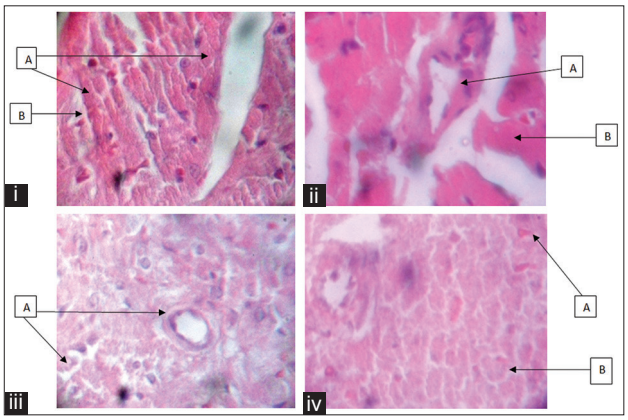

Figure 2: Heart (i) Control; rat heart composed of bundles of A - Myocardiac fibers and B - Interstitial space, (ii) rat heart treated with 500 mg/kg of *Anchomanes difformis*; A - Mild vascular congestion and B - Normal myocardiac fibers, (iii) rat heart treated with 1000 mg/kg of *A. difformis*; A - Normal myocardiac fibers pierced by a coronary vessel, (iv) rat heart treated with 2500 mg/kg *A. difformis*; A - Mild interstitial congestion and B - Normal myocardiac fibers (H and E, ×400).

Table 2: Effects of 21 days (sub-acute) oral administered of *A. difformis* leaf extract on some electrolytes.

Groups	Urea	Creatinine	Sodium Na^+	Potassium K^+	Bicarbonate HCO_3^-	Chlorine Cl^-
Control	28.60±2.04	0.72±0.05	134.2±1.83	5.44±0.68	19.80±0.37	98.4±2.62
500 mg/kg	30.00±2.21	0.90±0.07	136.6±1.03	3.90±0.68	19.00±0.84	100.2±0.20
1000 mg/kg	37.00±4.83	0.78±0.12	134.6±1.78	5.02±0.40	20.00±0.55	100.0±0.71
2500 mg/kg	36.60±4.38	0.82±0.18	128.0±4.51	5.30±0.48	19.00±0.45	99.80±2.91

Each of the treatment groups including the control group consists of five animals each. Values are not significantly different from control and every other dose. *A. difformis: Anchomanes difformis*

Table 3: Effects of 21 days (sub-acute) oral administered of *A. differmis* leaf extract on hematological parameters.

Parameter	Concentration (*A. differmis* treated groups and control)	Relative abundance (mean±SEM)	Statistical difference between control and treatment groups
RBC	Control	6.97±0.31	Nil
	500 mg/kg	6.96±0.34	
	1000 mg/kg	6.06±0.28	
	2500 mg/kg	6.44±0.59	
WBC	Control	10.94±0.93	Nil
	500 mg/kg	8.65±0.95	
	1000 mg/kg	9.28±1.17	
	2500 mg/kg	7.60±1.20	
PLT	Control	490.8±13.61	Nil
	500 mg/kg	458.2±32.18	
	1000 mg/kg	419.0±12.05	
	2500 mg/kg	429.0±23.63	
LY	Control	79.88±5.98	Nil
	500 mg/kg	80.58±5.71	
	1000 mg/kg	71.58±11.05	
	2500 mg/kg	62.10±10.64	
HCT	Control	37.50±1.27	Nil
	500 mg/kg	37.88±1.44	
	1000 mg/kg	33.22±1.29	
	2500 mg/kg	37.36±2.09	
MO	Control	6.62±1.60	Nil
	500 mg/kg	6.38±1.29	
	1000 mg/kg	7.32±1.64	
	2500 mg/kg	13.36±3.87	
GR	Control	13.50±4.40	Nil
	500 mg/kg	13.06±4.42	
	1000 mg/kg	21.10±10.30	
	2500 mg/kg	24.54±6.83	
HGB	Control	14.12±0.75	Nil
	500 mg/kg	13.78±0.55	
	1000 mg/kg	11.82±0.77	
	2500 mg/kg	13.42±1.10	
MCV	Control	53.86±0.80	Nil
	500 mg/kg	54.56±0.72	
	1000 mg/kg	54.86±1.21	
	2500 mg/kg	58.72±2.19	
MCHC	Control	37.52±0.80	Nil
	500 mg/kg	36.36±0.74	
	1000 mg/kg	35.38±1.20	
	2500 mg/kg	35.68±1.15	

Each of the treatment groups including the control group consists of five animals each. RBC: Red blood cell count/erythrocyte count, WBC: White blood cell indices, PLT: Platelet count, LY: Lymphocyte, HCT: Haematocrit, MO: Monocytes (a white blood cell), GR: Globules rouges, HGB: Haemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration, *A. differmis: Anchomanes differmis*

Results in this study corroborates with studies on the aqueous extract of *A. differmis* rhizome on rats, which showed no toxicological effects at 10 g/kg.[26]

Hematological changes such as anemia are often accompaniments of bone marrow toxicity among other causes.[27] The lack-of-effect on neutrophil levels (Table 3)

Table 4: Effects of *A. difformis* Blume aqueous leaf extract on biochemical parameters; liver function test.

Groups	AP (IU/L)	ALT (IU/L)	AST (IU/L)	TB1 (mg/L)	CONJ (mg/L)	TP (g/dl)	ALB (g/dl)
Control	243.80±43.18	59.00±8.96	197.8±30.13	2.46±0.16	0.10±0.00	6.94±0.14	3.48±0.07
500 mg/kg	235.8±37.22	62.80±4.77	171.6±5.63	2.00±0.00	0.10±0.00	6.94±0.60	3.38±0.02
1000 mg/kg	228.4±32.59	51.20±2.52	185.2±28.22	2.16±0.17	0.10±0.00	6.96±0.13	3.52±0.07
2500 mg/kg	241.8±40.58	62.00±4.95	152.6±19.31	2.06±0.09	0.10±0.00	7.04±0.24	3.48±0.11

AP: Alkaline phosphate, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TB: Total bilirubin, CONJ: Conjugated bilirubin, ALB: Albumin, *A. difformis: Anchomanes difformis*

Table 5: Histopathological effects of daily oral treatment with 500, 1000 and 2500 mg/kg body weight (×21 days) of *A. difformis* aqueous leaf extract on some organs in rats.

Concentrations	Histopathological appearance/diagnosis				
	Lungs	Heart	Spleen	Liver	Kidney
Control	Normal	Normal	Normal	Normal	Normal
500 mg/kg	Mild interstitial congestion	Mild vascular congestion	Moderate activation of lymphoid aggregates	Mild vascular congestion	Mild interstitial congestion
1000 mg/kg	Mild vascular congestion	Normal myocardiac fibers	Mild activation of lymphoid aggregates	Mild vascular congestion	Mild interstitial congestion
2500 mg/kg	Moderate activation of bronchioloalveolar lymphoid aggregates	Mild interstitial congestion and normal myocardiac fibers	Mild activation of lymphoid aggregates	Mild vascular congestion	Mild interstitial congestion

A. difformis: Anchomanes difformis

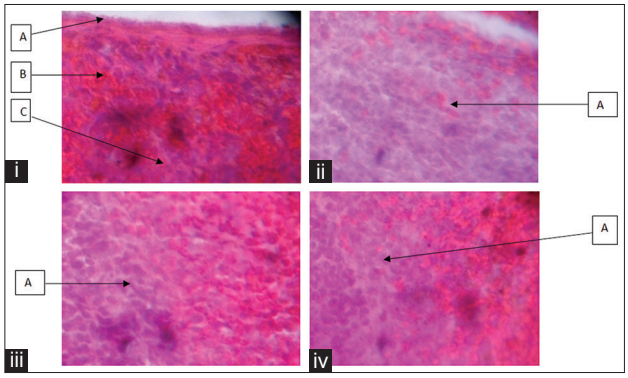

Figure 3: Rat spleen (i) Control; A - The capsule, B - Red pulp and C - White pulp, (ii) rat spleen treated with 500 mg/kg *Anchomanes difformis*; A - Moderate activation of lymphoid aggregates, (iii) rat spleen treated with 1000 mg/kg *A. difformis*; A - Mild activation of lymphoid aggregates, (iv) rat spleen treated with 2500 mg/kg *A. difformis*; mild activation of lymphoid aggregates (H and E. ×400).

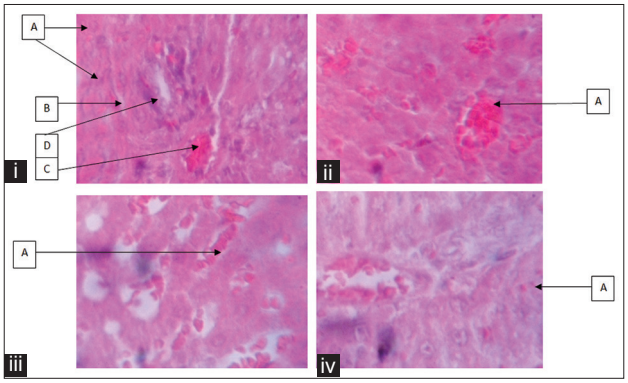

Figure 4: Liver (i) Control; A - Hepatocytes, B - Sinusoids, C - Portal vein and D - Bile ducts, (ii) rat liver treated with 500 mg/kg *Anchomanes difformis*; A - Mild vascular congestion, (iii) rat liver treated with 1000 mg/kg *A. difformis*; A - Mild vascular congestion, (iv) rat liver treated with 2500 mg/kg *A. difformis*; A - Mild vascular congestion (H and E, ×400).

shows that the extract may not have induced any inflammatory process since these cells are elevated in inflammation.[28] Many compounds are metabolized in the liver, but if too many demands are made on this organ's capacity, the continued role of its cells is no longer ensured.[29]

The liver and kidneys play significant roles in various metabolic processes. The liver plays an important role in xenobiotic function, and the kidneys are the main organs involved in drugs elimination, and so, exposed to the toxic effects of exogenous compounds.[30] It was thus important to investigate the effect of *A. difformis* on the function of these organs. Although,[9] reported that *A. difformis* used to handle different ailments is toxic to the renal tissue at a dose-dependent level, acute treatment of rats with 10,000 mg/kg in this study did not cause death or any toxicological manifestations in the rats (Table 1).

This study has also shown that sub-acute treatment with the extract causes none significant change in hematological or histopathological parameters examined (Tables 2-5). Added proof that organ injuries might not have occurred is seen in ALT and AST levels which were not different in the

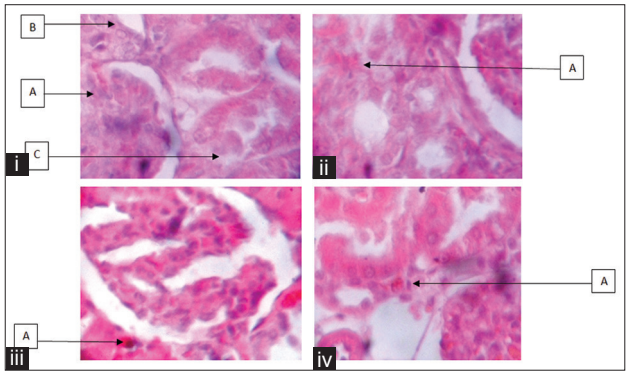

Figure 5: Kidney (i) Control; A - Glomerulus, B - Tubules and C - Interstitial spaces, (ii) rat kidney treated with 500 mg/kg *Anchomanes difformis*; A - Mild interstitial congestion, (iii) rat kidney treated with 1000 mg/kg *A. difformis*; A - Mild interstitial congestion, (iv) rat kidney treated with 2500 mg/kg *A. difformis*; A - Mild interstitial congestion.

treated and control groups. Increased levels of AST and ALT are often associated with underlying cellular injuries.[31,32] There was no effect on the levels of transaminases ALT and creatinine, which are good indicators of liver and kidney functions.[33]

All the results suggest that despite small disparities between the groups, there were no significant (t-test; $p>0.05$) differences in all the biochemical limits tested on the sub-acute administration. The aqueous leaf extract of *A. difformis* did not induce alterations in the blood biochemical parameters or damage to the liver and kidneys of the rats. Histopathologically (Figures 1-5), results show that the effect of the plant was mild vascular congestion and activation of the local lymphoid aggregates in almost all the cases including the highest tested concentration (2500 mg/kg) on the rats. These reactions pose no adverse effect on the animals rather; they ensure a better and increased activity of the white blood cells in the spleen which is a positive effect. Thus, *A. difformis* can be said to be safe as it has a broad safety margin.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

REFERENCES

1. Gesler WM. Therapeutic landscapes: medical issues in light of the new cultural geography. Soc Sci Med. 1992;34(7):735-46.
2. Erah PO, Nwazuoke JC. Identification of standards for pharmaceutical care in Benin City. Trop J Pharm Res. 2002;1(2):55-66.
3. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: WHO; 2000.
4. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian Pac J Cancer Prev. 2003;4(4):281-8.
5. Greensfelder L. Alternative medicine. Herbal product linked to cancer. Science. 2000;288(5473):1946.
6. Harries AD, Cullinan T. Herbis et orbis: the dangers of traditional eye medicines. Lancet. 1994;344(8937):1588.
7. Tchiakpe L, Balansard G, Benard P, Placidi M. Chemical and toxicological study of *Anchomanes difformis* (Engl.). Herb Hung. 1980;19:55-63.
8. Morton JF. West African lilies and orchids. In: Savory HJ, editor. The Useful Plants of West Africa. Lioydia: London: Royal Botanic Gardens; 1961: 234-46.
9. Ataman JE, Idu M, Odia EA, Omogbai EK, Amaechina F, Akhigbe AO, et al. Histopathologic effects of *Stachytarpheta jamaicensis* (L.) Vahl. on wistar rats. Pak J Biol Sci. 2006;9:477-82.
10. Okpo SO, Ching FP, Ayinde BA, Udi OO, Alonge PO, Eze GO. Gastroprotective effects of the ethyl acetate fraction of *Anchomanes difformis* (Engl). Int J Health Res. 2011;4(4):155-61.
11. Aliyu AB, Ibrahim MA, Musa AM, Musa AO, Kiplimo JJ, Oyewale AO. Free radical scavenging and total antioxidant capacity of root extracts of *Anchomanes difformis* Engl. (Araceae). Acta Pol Pharm. 2013;70(1):115-21.
12. Dalziel JM. Useful Plants of West Tropical Africa. London: Crown Agent; 1937.
13. Akah PA, Njike HA. Some pharmacological effects of rhizome aqueous extract of *Anchomanes difformis*. Fitoterapia. 1990;61:368-70.
14. Adeleke O, Adetunji T. Antimicrobial activity of *Anchomanes difformis* (Blume) Engl. (Family Araceae). J Life Physical Sci. 2010;3(2):87-90.
15. Ozolua RI, Idogun SI, Tafamel GE. Acute and sub-acute toxicological assessment of aqueous leaf extract of *Bryophyllum Pinnatum* (Lam.) in Sprague-Dawley rats. Am J Pharm Toxicol. 2010;5(3):145-51.
16. Lorke D. A new approach to practical toxicity testing. Arch Toxicol. 1983;54(4):275-87.
17. Lillie RD, Fullmer HM. Histopathologic Technic and Practical Histochemistry. 4th Edition. New York: McGraw-Hill Books Company; 1976.
18. Pearse AG. Histochemistry. 3rd Edition. London: Churchill; 1968.
19. Sheehan DC, Hrapchak BB. Theory and Practice of Histotechnology. Columbus, Ohio: Battelle Press; 1980.
20. Bradbury S. Peacock's Elementary Microtechniques. 4th Edition. London: Arnold; 1974.
21. Bradbury S, Evennet PJ, Hasselmann H, Piller H. Dictionary of Light Microscopy Royal Microscopical Society. Oxford: Oxford University Press; 1989.
22. Ozolua RI, Anaka ON, Okpo SO, Idogun SE. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (Lauraceae) in rats. Afr J Tradit Complement Altern Med. 2009;6(4):573-8.
23. Nayak BS, Sandiford S, Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. Leaf. Evid Based Complement Alternat Med. 2009;6(3):351-6.
24. Angeles-López G, Pérez-Vásquez A, Hernández-Luis F, Déciga-Campos M, Bye R, Linares E, et al. Antinociceptive effect of extracts and compounds from *Hofmeisteria schaffneri*. J Ethnopharmacol. 2010;131(2):425-32.
25. Krishnaraju AV, Rao CB, Sundararaju D, Sengupta K, Trimurtulu G. Anti-inflammatory activity of *Vitex leucoxylon* L. bark extracts against Freund's complete

adjuvant induced arthritis in Sprague Dawley rat. Am J Infect Dis. 2009;5:68-73.
26. Okpo SO, Ozolua RI, Ching FP, Eze GO. Acute and short term toxicity studies on the aqueous extract of *Anchomanes difformis*. Niger J Pharm Sci. 2013;12(2):1-10.
27. Flanagan RJ, Dunk L. Haematological toxicity of drugs used in psychiatry. Hum Psychopharmacol. 2008;23 Suppl 1:27-41.
28. Formela LJ, Galloway SW, Kingsnorth AN. Inflammatory mediators in acute pancreatitis. Br J Surg. 1995;82:6-13.
29. Mdhluli M. Toxicological and antifertility investigations of oleanolic acid in male vervet monkeys (*Chlorocebus aethiops*). Ph.D Thesis, Discipline of Physiological Sciences. Bellville: University of the Western Cape; 2003.
30. Bidhe RM, Ghosh S. Acute and subchronic (28-day) oral toxicity study in rats fed with novel surfactants. Am Assoc Pharm Sci J. 2004;6(2):7-16.
31. Karthikeyan S, Gobianand K, Pradeep K, Mohan CV, Balasubramanian MP. Biochemical changes in serum, lung, heart and spleen tissues of mice exposed to sub-acute toxic inhalation of mosquito repellent mat vapor. J Environ Biol. 2006;27 2 Suppl:355-8.
32. Wittekind C. Prognostic factors in liver tumors. Verh Dtsch Ges Pathol. 1995;79:109-15.
33. El Hilaly J, Israili ZH, Lyoussi B. Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. J Ethnopharmacol. 2004;91(1):43-50.

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