



Effects of extracts of *anchomanes difformis* on haematological parameters of albino wistar rats

Egwurugwu J.N.^{1*}, Ohamaeme M.C.², Chinko B.C.³, Ebuenyi M.C.⁴, Akunneh-Wariso C.C.⁵, NGWU E.E.¹, Ugwuezumba P.C.¹ and Ezekwe S.R.⁶

¹Department of Human Physiology, College of Medicine, Imo State University, Owerri, Nigeria

²Department of Community Medicine, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria

³Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Nigeria

⁴Health Promotion Unit, Adolescent Rights and Care Foundation, Owerri, Nigeria

⁵Department of Human Physiology, College of Medicine & Health Sciences, Abia State University, Uturu, Nigeria

⁶Department of Medical Biochemistry, College of Medicine, Imo State University, Owerri, Nigeria

judenuel@gmail.com

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Abstract

This study evaluated the effects of hydro-methanol extracts of *Anchomanes difformis* on haematological parameters of male albino wistar rats. Twenty adult rats, weighing 150 g to 200 g, divided into 4 groups of 5 rats each were used for the study that lasted for 6 weeks. Group 1 which served as the control received normal rat feed and normal saline ad libitum while the test groups 2, 3, and 4 received 100, 200, and 300 mg/kg body weight of the extracts respectively, in addition to the normal rat chow and water ad libitum. Ether was used to anaesthetize the rats at the end of the second, fourth and sixth weeks respectively, and via cardiac puncture, blood samples were collected from all the rats in each group and analyzed for white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB) concentration, haematocrit (HCT) count, lymphocyte (LYM) count, platelet (PLT) count and the calculated blood indices (MCV, MCH, MCHC). The results showed statistically significant dose dependent increase in WBC, LYM and PLT at the end of the second and fourth weeks compared to control ($p < 0.05$). After the second week, MCHC also increased significantly while MCH decreased ($p < 0.05$) compared to control. At the end of the sixth week, there were statistically significant reductions in HGB and HCT while MCHC and PLT increased marginally ($p < 0.05$) compared to control. In conclusion, hydro-methanol extracts of *Anchomanes difformis* increased the levels of most of the haematological indices of male rats in first 2-4 weeks of administrations, followed by a steady decline of the blood indices assayed for at the end of the sixth week. *Anchomanes difformis* should therefore be used with caution especially on long term basis as it may depress haemopoiesis.

Keywords: *Anchomanes difformis*, extracts, haematological parameters, albino wistar rats, chronic administration.

Introduction

Around the globe, virtually all cultures have used medicinal plants as sources of medicine¹⁻². There is renewed and increased interest in natural vegetal medicines³ for reasons such as high cost of antibiotics and antibiotic resistance⁴, high cost of producing patentable chemicals and drugs⁵, increasing debt burden and high cost of modern healthcare in developing countries⁶, unavailability of modern equipment⁷⁻⁹ and the increasing occurrence of anaemia from varied causes with natural ways to ameliorate it. In most medicinal plants, the active principles are more concentrated in their storage organs such as roots, leaves, seeds, barks and in most cases, the flowers and woody parts of herbaceous stem are usually relatively inert¹⁰.

Anchomanes difformis commonly known as forest anchomanes in English, belongs to the family, *Araceae*. It is a native plant of the African continent, particularly the following countries: Nigeria, Cameroon, Ghana, Cote d'ivoire, Sierra Leone, Senegal

and Togo¹¹⁻¹². It is a tropical herb that grows in shady terrestrial areas and can grow up to 2 m high. It has a stem and spathe that arises from a horizontal tuber¹³⁻¹⁴.

A. difformis is known by different names in Nigeria including "olumahi" by the Igbos (Umuahia), "ebaenan" by the Efik, "chakara" by the Hausas, "boubekedou" by the Ijaws¹⁴, as "abrisoko" by the Yourbas in South West of Nigeria¹⁵; and "Olikhoror" by the Bini tribe of Edo state¹¹.

Quantitative and qualitative analyses of *A. difformis* have shown the significant presence of the following phytochemical constituents: carbohydrates, crude proteins, fats, fibres, calcium, magnesium, manganese, copper, iron, zinc, alkaloids, saponins, flavonoids and steroids¹⁶⁻¹⁸. These phytochemical constituents may be responsible for the many functions ascribed to *A. difformis*.

The various medical uses of *A. difformis* include antibacterial¹⁵, anti-inflammatory^{12,19}, analgesic and hypothermic effects^{12,20},

diuresis and purgative^{12,15}, anti-hemolytic and anti-oxidant effects²⁰⁻²¹, anti-diabetic, anti-filariasis, insecticidal and anti-diarrheal properties²³⁻²⁵. It has also been found to reduce serum concentrations of some sex hormones implicated in the pathogenesis of uterine fibroids, suggesting its possible role in the management of uterine myomata¹⁶.

Despite the above mentioned and other uses of *A. difformis*, both as food and medicine, there is paucity of data on the possible effects of *A. difformis* extracts on haematological parameters. This study was therefore designed to explore the possible effects of the hydro-methanol extracts *A. difformis* on haematological parameters of albino wistar rats especially on its acute and long term usage.

Materials and methods

Experimental animals: Twenty adult male albino wistar rats weighing 150 g to 200 g were used for this study. They were purchased from the University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were kept in metal rat cages of wired mesh by the sides, in a controlled environment and allowed to acclimatize in a room temperature of $24 \pm 1^\circ\text{C}$ for 2 weeks. The rats were then grouped into four of five rats each. Group 1, the control group, received 0.3 ml of normal saline, normal rat chow and water *ad libitum*. The test groups 2, 3, and 4 received 100 mg, 200 mg and 300 mg per kilogram body weight of the hydro-methanol extract of *A. difformis* daily between the hours of 8 and 10 am in addition to normal rat feed (Pfizer Plc) and water *ad libitum*. The extracts were administered orally using 2 mL syringes without needles.

Ethical consideration: All rats were handled according to the care and use of laboratory animals as provided by international and institutional guidelines for handling of laboratory animals in biomedical research, as stated by the Canadian Council of Animal Care²⁶ and Imo State University Ethical Committee.

Collection and processing of plant sample: Rhizomes of the mature plants were harvested from Owerre-Ebeiri, in Orlu Local Government Area of Imo State, Nigeria during the month of October, 2010. The rhizomes of the plants were washed, sliced, cooked for 1-2 hours and air-dried at room temperature for three weeks. They were further pulverized using electrical blender (Binatone, China) and stored in air-tight bottles.

Extraction with solvents: A proportional methanol-water mixture of 80%v.v of methanol and 20% of water was constituted to form hydro-methanol. Thereafter, 300g of the dried powder was extracted with 400ml of methanol and 100ml of distilled water using soxhlet extractor. The hydro-methanol extract was then concentrated using a rotary evaporator into a semi-solid form. After being concentrated, 1g of aqueous methanol extract of *A. difformis* was mixed with 10ml of distilled water, thus 0.1ml of the extract being equivalent to 100mg for easy administration to the experimental animals.

Phytochemical screening: After the extraction of the solvents, the hydromethanol extract derived from *A. difformis* was analysed for the presence phytochemicals such as flavonoids, alkaloids, saponins, tannins, phlobatannins, anthraquinones, and cardiac glycosides. Harbone methods²⁷ were used to assess for alkaloids, flavonoids, cardiac glycosides and phlobatannins. For the presence of saponins, the method used by Odebiyi O.O. and Sofowora A.E.²⁸ was employed while tannins and anthraquinones were also screened as done by Trease G.E. and Evans W.C.²⁹.

Test for alkaloids: About 0.2 g of the extract was warmed with 2% of H_2SO_4 for two minutes, it was then filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicated the presence of alkaloids²⁷.

Test for anthroquinones: One milliliter of the extract was shaken with 10 ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test²⁹.

Test for cardiac glycosides: The Legal test (the Killer-Killiani) were adopted as follows: 0.5 g of the extract were added to 2 ml of acetic anhydrous plus H_2SO_4 ²⁷.

Test for Flavonoids: One milliliter of the plant extract was mixed with 2 ml of 10% lead acetate, a brownish precipitate indicated a positive test for the phenolic flavonoids. For the other flavonoids, 1 ml of the plant extract were mixed with 2 ml of dilute NaOH, a golden yellow colour indicated the presence of flavonoids²⁷.

Test for Saponins: One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture were vigorously shaken, and left to stand for 10 minute during which time the development of foam on the surface of the mixture lasting for more than 10 minutes, indicates the presence of saponins²⁸.

Test for Tannins: One milliliter of the extracts were mixed with 2 ml of FeCl_3 , a dark green colour indicated positive test for tannins²⁹.

Collection of blood samples and analyses: The rats were anaesthetized with chloroform and then blood samples were collected from five rats per group, via cardiac puncture every two weeks. The blood was collected in ethylenediaminetetra acetic acid (EDTA) bottles, taken to haematology laboratory of Imo State University Teaching Hospital, Orlu, where it was centrifuged at 5000 rpm, and transferred to an automated machine ERMA Inc. (Tokyo, Japan) for analysis of the following haematological parameters: white blood cell count (WBC), red blood cell count (RBC), haemoglobin (HGB) concentration, haematocrit (HCT) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocyte (LYM) count and platelet (PLT) count.

Statistical analysis: Results from five rats/group were presented as Mean±SD. ANOVA and LSD multiple comparisons were done using SPSS version 21 and p value of 0.05 or less was deemed statistically significant.

Results and discussion

Results: Table-1 shows the qualitative analysis of the phytochemical constituents of *A. difformis*. It showed that it contains alkaloids, tannins, saponins, steroids, flavonoids. Cardiac glycosides, anthraquinones and phlobatannins are absent.

Table-2 show that the hydro-methanol extracts of *A. difformis* statistically and significantly increased the concentrations of white blood cells count, mean cell haemoglobin, mean cell haemoglobin concentration, lymphocyte count and platelets counts (p <0.05) when compared with the control. These positive changes observed were dose dependent. No statistically significant differences were observed in the levels of RBC, HGB, HCT and MCV when compared with the control (p>0.05) after two weeks of administration.

Table-3 indicate that after 4 weeks administration of hydro-methanol extracts of *A. difformis*, there was statistically significant dose dependent increase in the levels of WBC, LYM, and PLT (p<0.05) when compared with the control. However, there was increase in RBC, HGB and HCT in groups

3 and 4 in a dose-dependent manner though not statistically significant when compared to control (p>0.05).

Table-4 show that after 6 weeks administration of hydro-methanol extracts of *A. difformis*, there is statistically significant dose dependent decrease in the levels of HGB and HCT, while MCHC and PLT had marginal increases (p<0.05) when compared with the control. However, WBC, RBC, MCV, MCH, and LYM were not statistically significant (p>0.05) when compared to control.

Table-1: Qualitative analysis of the phytochemical constituents of *A. difformis*.

Phytochemicals	Anchomanes difformis (Rhizome)
Flavonoids	Present
Steroids	Present
Saponins	Present
Alkaloids	Present
Tanins	Present
Phlobatannins	Absent
Cardiac glycosides	Absent
Anthraquinones	Absent

Table-2: Effects of *A. difformis* on blood indices after two weeks administration.

Group	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	LYM	PLT
Control	8.24± 1.19	7.00± 0.58	11.89± 0.62	35.67± 1.86	53.39± 4.56	17.07± 1.52	33.03± 0.18	71.24± 2.83	468.41± 19.09
100mg/kg	8.46± 1.43	7.28± 0.59	11.59± 0.93	34.78± 2.78	52.95± 5.04	16.01± 1.88	33.31± 0.23	72.24± 2.13	462.39± 14.29
200mg/kg	*10.90± 0.86	7.25± 0.51	10.51± 0.88	31.54± 2.64	49.11± 3.18	*14.56± 1.74	*33.49± 0.21	73.80± 2.67	*512.78± 17.66
300mg/kg	*11.19± 0.78	7.04± 0.69	11.30± 0.62	33.91± 1.89	51.81± 8.48	16.12± 1.02	33.44± 0.24	*74.03± 2.14	*619.92± 47.82

*Statistically significant. p<0.05, n=5.

Table-3: Effects of *Anchomanes difformis* on blood indices after four weeks administration.

Group	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	LYM	PLT
Control	8.17± 0.80	7.07± 0.36	11.60± 1.02	34.80± 3.07	49.27± 4.07	16.42± 1.35	33.35± 0.29	71.79± 2.47	453.91± 24.88
100mg/kg	9.66± 1.40	7.04± 0.62	11.64± 0.78	34.93± 2.36	49.97± 5.99	16.68± 1.97	33.20± 0.44	73.57± 3.73	*459.32± 16.36
200mg/kg	*12.03± 0.48	8.11± 0.67	12.59± 0.73	37.77± 2.19	46.88± 5.36	15.62± 1.79	33.49± 0.29	*80.22± 1.30	*542.93± 32.03
300mg/kg	*13.23± 2.16	8.20± 0.68	12.91± 1.08	38.73± 3.27	47.60± 6.38	15.86± 2.13	33.46± 0.25	*81.00± 2.00	*730.48± 39.69

* Statistically significant. p<0.05; n=5.

Table-4: Effects of *Anchomanes difformis* on blood indices after six weeks administration.

Group	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	LYM	PLT
Control	8.24± 1.19	7.00± 0.58	11.89± 0.62	35.67± 1.86	53.39± 4.56	17.07± 1.52	33.03± 0.17	71.24± 2.89	468.41± 19.09
100mg/kg	8.46± 1.43	7.28± 0.59	10.68± 0.43	32.05± 1.28	44.28± 4.45	14.75± 1.48	33.33± 0.08	72.24± 2.13	462.39± 14.29
200mg/kg	10.38± 0.66	7.25± 0.51	*10.96± 0.62	*32.89± 1.87	45.60± 4.90	15.19± 1.63	*33.22± 0.29	70.26± 0.88	*512.78± 17.66
300mg/kg	9.24± 0.80	7.04± 0.69	*10.25± 0.87	*30.75± 2.63	43.99± 5.83	14.66± 1.94	*33.48± 0.25	69.67± 1.88	*514.67± 16.00

* Statistically significant. p<0.05.

Discussion: Different cultures and tribes have used different parts of *Anchomanes difformis* either as food or medicine^{11,30}. *A. difformis* has sub-served various functions such as anti-bacterial, anti-diabetic, anti-malarial, diuretic, analgesic, anti-inflammatory, anti-hemolytic and anti-oxidant effects amongst others^{13,21,22,31}.

The results of the second and fourth weeks of administration of the extracts of *A. difformis*, show that there was statistically significant increase (p<0.05) in the levels of WBC, MCH, LYM, and PLT when compared with the control. The observed increases were dose dependent. There were also dose dependent increases in RBC, HGB, and HCT as well as PLT in groups 3 and 4 when compared with controls though not statistically significant (p>0.05). The observed effects of the extracts may be due to the phytochemical constituents present in the plant. The extracts of *A. difformis* have been shown to contain iron, zinc, calcium, magnesium, manganese, protein, carbohydrates, fats, fibre¹⁷⁻¹⁸. The aforementioned constituents are important haemopoietic factors³²⁻³³, thus can increase the blood indices in the rats. Extracts of *A. difformis* have also been shown to contain flavonoids, saponins, alkaloids among other phytochemicals^{16,18,34}. Flavonoids have been demonstrated to possess strong anti-oxidants and anti-haemolytic properties²¹⁻²².

However, after six weeks of administration, the results showed statistically significant dose dependent decrease in the levels of HGB and HCT, while MCHC, and PLT had marginal increases (p<0.05) when compared with the control. This may suggest that the medicinal effects of *A. difformis* which has been exploited by the natives, may also have toxic effects on some haematological parameters as seen in the results of the sixth week study.

Studies have shown that extracts of *Anchomanes Difformis* can be toxic at higher doses and/or at lower doses consumed over a long duration^{12,13,35}. A study has noted that at dose of 300mg/kg, the extract of *A. difformis* was toxic to Guinea pigs with some haemorrhagic challenges observed in the animals³⁵. It has also been demonstrated that at a dose of 2.5 g/kg/day, rhizome extracts of *A. difformis* caused statistically significant reduction in haemoglobin concentration and haematocrit in rats³⁶. Extracts of *A. difformis* at higher have been reported to cause distortion of

the cyto-architecture of rat kidneys³⁶. The kidney is the main source of erythropoietin in the body. Erythropoietin is very important in the formation of erythrocytes for it increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors and later to mature red blood cells³⁷. Distortion of renal architecture if severe or prolonged, can affect some functions of the kidney including blood formation.

Conclusion

In conclusion, aqueous extracts of *A. difformis* increased the levels of most of the blood indices in Albino wistar rats in a dose dependent manner during the second and forth weeks of administration and showed a steady decline in all the indices during the sixth week of administration. Therefore, aqueous extracts of the plant may possess haemopoietic enhancing properties when used over a short period of time. It may not be safe to use over long periods of time without taking adequate precautions. Further studies are recommended to fully explore the potentials of *Anchomanes difformis* on haematological parameters especially the constituent(s) and doses that enhance/inhibit blood formation.

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