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Haematopoietic Activity of *Ficus exasperata* Vahl. (Moraceae) Leaf Methanol Extract in Albino Rats

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Abstract

Twenty (20) albino rats of both sexes were divided into five groups of four animals each and tested for body weight as well as haematological parameters such as: PCV, WBC, HB, RBC, MCV, MCHC and MCH methanol leaf extract of *F. exasperata*. The test groups II, III, IV and V were fed with extract doses of 100, 200, 300 and 400mg/kg body weight of mice respectively by oral means for two weeks. Initial weights of the rats and weights after feeding were taken, and then the animals were sacrificed after two weeks. Blood samples collected from the sacrificed animals were analysed for haematological parameters. Results showed that weights of the animals increase as the doses increase in all the groups except for the control group (I) where the animals were given ordinary water. General decreases in haematological parameters PCV, HB, WBC, MCHC and MCV were witnessed whilst the RBC showed significant increases ($P \leq 0.05$) compared with the control value $5.05 \pm 0.31 \times 10^6$ mm^3. The study indicated that long term use of the leaves of *F. exasperata* as an oral medication in traditional medicine was not safe at the doses investigated in this study, as the weight increase in the animals may be due to hypertrophy (swollen organs) except in the control group without the extract.

Keywords

Haematopoietic, *Ficus exasperata*, Albino Rats, Haematological

1. Introduction

Ficus exasperata is commonly known as ‘’sand paper’’ tree because of the abrasive nature of the leaf surfaces. It is known by various local names in Nigeria such as ‘’Baure’’ in Hausa, “Ewe Ipin” in Yoruba and “Asisa” in Ibo. The plant is confined to West Africa; occurring in all kinds of vegetation particularly in secondary forest re-growth [1]. It is one of the largest genus in the angiosperms with over 800 species of woody trees, shrubs, vines, epiphytes and hemi-epiphytes belonging to the family Moraceae.

The viscid non-milky sap is used for treating sores, eye problems and stomach ache in traditional Ivory Coast medicinal systems [2]. The sap is equally used to stop bleeding during birth while the infusion of the bark is used to hasten birth in cattle in Ghana [3]. Other uses of the plant includes pain relief, stimulant, leprosy cure, birth control, treating typhoid fever, anti-ulcer agent, anti-hypertensive agent, anti-fungal agent , among other uses [4].

Chemically, the leaves contained glycosides of various types including saponins, cardiac glycosides, cyanogenic glycosides, tannins and alkaloids [5]. Steroids and flavonoids and terpenes have also been reported to be present in stem bark [6]. The presence of cyanogenetic glycosides in the leaf ethanol extracts had been reported, resulting in high levels of renaltoxicity in albino mice [5].

Therefore, this present study was undertaken to determine the effects of methanol leaf extract of *F. exasperata* on the weights and haematological parameters of albino mice to provide a scientific basis justifying the use of the plant in traditional medicine for the acclaimed treatment of multiple ailments and to assess its safety.

2. Materials and Methods

2.1. Collection and Identification of Plant

Fresh leaves of *F. exasperata* were collected from a forest

in Southern Zaria, Kaduna State, Nigeria, and identified at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria where a voucher number was deposited for the plant.

2.2. Preparation of Plant Extract

The leaves collected were air-dried and ground into powder using milling machine, and then extracted with re-distilled methanol using Soxhlet apparatus for 72 hours. The extract was then concentrated by using rotary evaporator at 64.7^0C. The residue was weighed and stored for further use. Doses of 100, 200, 300 and 400mg/kg body weight (b.w) for feeding the albino rats was prepared.

2.3. Experimental Design and Procedure

Albino rats weighing between 160 and 258g were bought from the animal house of the Department of Pharmacology and clinical therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University Zaria, Nigeria. The animals were kept in a well ventilated cage at optimum conditions, and were exposed to 12 hours of light and dark cycles, fed with water and allowed seven days acclimatization.

2.4. Grouping of Animals and Extract Administration (Oral Route)

Twenty (20) albino rats were randomized into five (5) groups of four animals each after weighing. Group I which served as control received an equivalent volume of grower's mash feed and water for fourteen (14) days. Group II, III, IV, and V received 100, 200, 300 and 400mg/kg body weight of methanol extract of *F. exasperata* and grower's mash respectively. The treatment continued for fourteen days following oral route.

2.5. Collection of Blood Samples

After fourteen days of extract administration, the animals were re-weighed. Blood samples were collected from the animals through cardiac puncture into Ethylene - Diamine - Tetra Acetic acid (EDTA) bottles after anaesthetizing the animals with chloroform. The bottle contains EDTA to avoid coagulation of blood samples. Blood samples were then taken to Haematology Unit of Ahmadu Bello University Teaching Hospitals (ABUTH), Shika, Zaria, for haematological analysis.

2.6. Analysis of Haematological Parameters on Blood Sample Collected from the Animals

Haematological parameters were analysed using the following standard procedures: PCV [7], WBC (Counting chambers), HB (HCl Acid Haematin Test), RBC [8], MCV, MCH and MCHC [9].

Packed Cell Volume (PCV)

Materials: Blood sample, centrifuge machine, heparinized capillary tube, haematocrite reader, blood mixer and sealant.

Procedure: Blood were mixed thoroughly with a blood mixer and the samples were filled in heparinized capillary tube to at least 2/3 of the tube. The capillary tube was sealed with sealant and was carefully inserted into a centrifuge machine and centrifuged for 5 minutes. The results were determined using haematocrite reader.

White Blood Cell Total Count (WBC)

Materials: Blood sample, counting chamber and pasture pipette.

Procedure: 0.38mL of diluting distilled water was measured and dispensed into a small test tube while 2 mL of well mixed EDTA anticoagulant blood were added. The grids chamber was filled with the blood sample carefully using pasture pipette held at an angle of about $45^o$and the chamber was left undisturbed for 2 minutes to allow the cells to settle. The chamber was then cleaned beneath and placed on the microscope stage and observed using X10 objective lens. The cell was counted in the four large corner squares of the chamber marked W_1, W_2, W_3, and W_4.Results were recorded using the following method: Divide the number of cells counted by 2; divide the result obtained by 10 and the final result obtained is multiplied by 10^9/L

Haemoglobin Test (HB)

Haemoglobin test was carried out using Sialic Acid Haematin Method as described below:

Materials: HCl, heparinised blood and graduated dilution tubes.

Procedure: 0.02mL blood was mixed in a tube containing 0.1ml of HCl. After a period of 10minutes, 0.1mL of HCl was added in drops and stirred until the colour of the solution matched the colour of the glass standard, positioned alongside the dilution tube. The concentration of haemoglobin was recorded from the graduated scale of *AccubaseA1C test kit Model 881001* (made in USA) in g/l; from where other haemoglobin parameters such as MCH (mean corpuscular haemoglobin) and MCHC (mean corpuscular haemoglobin concentration) were calculated.

Red Blood Cell Count (RBC)

Modification: Whole blood is diluted using isotonic diluents to avoid lyses of red cells. The number of red cells in a known volume and of known dilution is counted using counting chamber.

Procedure: 0.02ml of whole blood was added to 3.98mL of diluting fluid. Improved Neuberger chamber was carefully charged with the mixed blood and the cells were allowed to settle in a moist chamber for 5 minutes. The ruled area of the counting chamber was located under X10 objective of the microscopeand ensured that the cells were evenly distributed. Using X40 objective, the total number of red cells were counted in five groups of 16 small squares in the central ruled area (A, B, C, D and E) which is $0.2mm^2$ [8].

2.7. Statistical Analysis

The results obtained were expressed as mean ± SE of four animals. Significance of differences compared to their control group was determined using T-test ($P \leq 0.05$).

3. Results

The administration of the methanol extract from the leaves of *Ficus exasperata* at 100, 200, 300 and 400 mg/kg body weight caused an increase in the body weight of the treated animals relative to control [Tables 1 and 2]. The extract showed significant effect on RBC, HB, PCV, MCV (mean corpuscular volume) and MCHC and WBC

Table 1. *Percentage (%) body weight gained by animals on Ficus exasperata extracts following oral administration.*

Group	Mean initial weight(g)	Mean final weight(g)	% weight gained
Control(I)	211 ± 0.20	236±0.23	11.8±0.10
Group II100mg/kg	210±0.14	233±0.27	5.0±0.11
Group III200mg/kg	233±0.22	[a]273±0.36	17.2±0.13
Group IV 300mg/kg	212±0.22	[*]275±0.38	11.8±0.10
Group V 400mg/kg	236±0.25	[a]265±0.30	15.5±0.12

Results are means of four animals per group ± SE, [a] increase in weight of animals (probably due to organ swollen),[*] Statistical significant difference at $P \leq 0.05$.

Table 2. *Animal vital organ weight, before and after oral administration of leaf methanol extracts of F. exasperata.*

Group	Organ weight (means of initial/final weights) (g)			
	Lung	Pancreas	Spleen	Liver
Control	20/21	15/16	10/12	22/24
Group II	22/26	15/19	11/14	21/22
Group III	21/28	16/22	13/15	22/26
Group IV	22/32	14/18	12/18	23/28
Group V	25/36	15/22	13/19	24/30

Note: Results are means of the initial and final organ weights using electronic weighing balance (Neman 0023 model, made in China)

Table 3. *Haematological parameters of the animals on administration of crude methanol extract of leaves of F. exasperata (oral route).*

Treatment	PCV(%)	Hb(gd/L)	WBC(106)mm3	RBC(106)mm3	MCHC (%)	MCV (F/L)
Control(I)	21.5±1.23	10.96±0.25	2.85±0.25	1.05±0.01	6.18±0.39	28.37±0.63
Group I 100mg/kg	44.5±1.76	14.50±0.64	5.75±0.19	6.30±0.17	3.50±0.47	23.83±1.70
Group III 200mg/kg	39.0±1.23	14.20±0.62	5.72±0.13	5.50±0.46	3.25±0.19	20.90±0.39
Group IV 300mg/kg	37.5±1.23	10.87±0.38	4.28±0.32	4.55±0.34	2.66±0.45	18.79±1.31
Group V 400mg/kg	34.0±1.16	10.23±0.22	3.37±0.14	2.50±0.32	1.06±0.30	14.22±0.64

Note: Results are Mean± SE in three replicates; numbers in bold are not significantly different from the control ($P \leq 0.05$), (t-test).

4. Discussion

Blood is a reflector of the overall animal health and provides important profiles for the toxicological impact on animal tissues [10]. The result of our haematological study revealed significant decreases in RBC, PCV, HB, MCHC, and MCV ($P \leq 0.05$) values of the treated mice when compared with the control [Table 3]. The non-significant effects of the extract on RBC could mean that the balance between the rate of production and destruction of red blood corpuscles (Erythropoiesis) was not affected negatively.

MCV and MCHC relate to individual red blood cells while HB, RBC, and PCV are associated with the total population on red blood cells. The absence of observable significant effect of the extract on these parameters may be an indication that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [11]. This obviously contradicts the results in table 3 above where progressive decrease in these parameters were witnessed in all the treated groups.

An adequate haemoglobin percentage is needed for the normal physiology of animals, which depends on the erythrocyte count. *F. exasperata* may have induced inhibition of RBC formation that reduces the RBC counts and leads to a decrease in Hb contents which was observed in the animals, and this supports the work of Choudhari and Deshmukh[12].

The dose dependent and reduction in number of RBC may occur due to the haemolytic activity of the extract on the animals [Table 2] or it could also be as a result of a suppressive action of the extract on erythropoiesis as well as the presence of cyanogenetic glycosides and saponins in the extract as was reported by Lawrence *et al.* [13] as saponins are class of glycosides and are known to cause haemolysis of red blood cells.

WBC counts increased significantly in mice treated with 100mg/kg dose of the methanol extract of *F. exasperata* with value 5.75± 0.19 [Table 2] ($P \leq 0.05$), and it was reported to possess the property of anti-inflammation. The administration of the plant extract appears to exhibit stimulatory effect on the effectors cell of the immune system [14], an indication that the extract could boost immune systems of animals.

The findings indicate that the administration of methanol extract of *F. exasperata* to the experimental animals resulted in significant increase in body weight relative to control.

Groups II, III, IV, and V which received 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg body weight respectively gained more weight than the control [Table 1] and this increase appeared to be dose dependent. The weight increase may be due hypertrophy or organ swollen which was in an agreement with the works by Amresh *et al*. [15] and Ashafa *et al*. [16], [Tables 1 and 2].

5. Conclusions

This study showed that the administration of methanol extract of *F exasperata* orally, support increase in weight. The mean body weight of animals increased in a dose dependent manner. Also from the result obtained, it could be seen that the extract have significant effect on PCV, HB, RBC, MCV and MCHC following the administration of *F exasperata* extract, but there is significant increase on WBC. Alterations in weight and haematological parameters observed in the present study point to selective toxicity of *F. exasperata* leaf extract on the immune system of experimental animals. Hence, the herb may not besafe as an oral medication at the doses investigated because of the presence of some glycosides such as cyanogenic glycosides and saponins, as well as alkaloids, in the leaf extract of the plant.

For ethno-medicinal prescription of the plant, it is recommended that ways to remove these poisonous glycosides from the plant during extraction should be studied to guarantee the use of the leaf extract in disease management traditionally.

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