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ORIGINAL ARTICLE

Toxicity of methanol seed extract of Moringa Oleifera on Haematological indices of Albino Wistar rats

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Abstract

Introduction: The effect of methanol extract of *Moringa Oleifera* seeds were determined in albino wistar rats, though several research works have been conducted on the *Moringa Oleifera* leaves extract however there is limited data available on the effect of the seeds extract on haematological parameters . This study was conducted to investigate the phytochemical, hematological and toxicological effect of extract of *Moringa oleifera* seeds in albino Wistar rats.

Materials and Methods: Thirty healthy albino wistar rats weighing between one hundred and twenty and one hundred and seventy grams randomized into five groups of six rats each and ad libitum with Moringa oleifera diets for fourteen days after two weeks of acclimatization in control. The groups were classified on varying concentrations of 25mg/kg,50mg/kg,100mg/kg ,125mg/kg and control for groups A,B,C,D,E respectively.The total white blood cells ,Red blood cells, Haemoglobin values, Haematocrit and Platelet count tests were carried out using Haematology auto-analyzer ,phytochemical and histological evaluations of any toxicities of *Moringa oleifera* seeds extract in albino Wistar rats were also investigated.

Results: Phytochemicals such as tannins, saponin, alkaloid/glucoside were found in low, moderate and high concentrations respectively in methanol extract of the *Moringa oleifera* seeds . The Red Blood Cells, Haemoglobin and Haematocrit values were significantly increased ($p<0.05$) with decreased Total white cell count for group A on day eight of the administration when compared to the control group. The extract however did not significantly ($p>0.05$) affect any parameter on day fifteen compared to the control group and day eight.

Conclusion: *Moringa oleifera* seeds extract demonstrated dose and time dependent haemopoietic properties.

Key words: Moringa, Oleifera,Haematological,Toxicities,Seeds, Rats.

Introduction

Moringa oleifera Lam (Synonym *Moringa Pteryosperma Gaertn*) is scientifically classified as the most widely cultivated specie of the genus *Moringa* which is the only genus in the family Moringaceae of the order Brassicales, of the class Magnoliopsida of the division magnoliophyta of the kingdom plantae.The Moringaceae is single genus with 14 known species, of these only *Moringa oleifera* is the most widely known species and is planted in the whole tropical belt(1). *Moringa oleifera* (*M. oleifera*) is the most widely cultivated species of a monogeneric family, the moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicine and industrial uses (2). Commonly referred to as drumstick tree from the appearance of long, slender, *Moringa oleifera* is known by various names in English , French and local African, Asian and South American languages. English: Horseradish tree, radish tree, Benoil tree, Drumstick, Miracle tree ,mother's Best friend ,west Indian ben , Never die , *Moringa* Nut. FRENCH: Benaile , Benzolive.

In Nigeria, the fulanis call it Guwara, Konamarade, Rinimaka, Habiwal hausa; the Hausas call it Zogalla, Baganuwar, Maka,zongallangandi/Bagaruwar, Rimi tuwara, masar ,shipka hali,shuka halimka,Barambo,koraukin zaila,Rimin nacara. The Igbos call it Odudu Oyibo, Okwe Oyibo, Uhe, Ikwe beke okwe olu ,okochi egbu, okughara ite and the Yorubas call it Ewe ile, Ewe Igbale, Adagba, Malonye. (3).

The seeds of *Moringa oleifera* are produced annually in the tropical and sub tropical countries of Asia and Africa. *Moringa oleifera* seeds are obtained from the pods of the *Moringa oleifera* trees,fresh and raw moringa seeds are quite tender but as soon as they get dried ,they become hard and start resembling small beans. Herbal products are perceived by the public as being natural, healthful and free from side effects. Most people believe that herbal drugs are cheap, locally available and have no side effects or potential risk due to their natural origins and are often considered as food supplements not drugs (4). These products are actually self-prescribed by the consumers and there is lack of control and review in terms of dose, manner, efficacy and frequency of administration. Medicinal plants serve as a significant alternative source of drugs for majority of the population due to lack of access to orthodox healthcare facilities and poverty especially in African and underdeveloped countries leading to indiscrimate use when there is inadequate clinical and laboratory diagnosis or control(5). The phytochemicals may be natural to the plant but toxic to the human body. The increased uses of these plants and their products have resulted in concerns over both the efficacy and safety of the product. The mineral content of Moringa Oleifera products show variation in composition with changes in location (6), (7).Hematological variables have been known as good predictors of the physiological status of animals and its

changes are important as diagnostic tool to evaluate their responses to various physiological situations(8). The assessment of haematological parameters is a biomarker for evaluating the haematotoxic potential and medicinal property of the extract in the area of pharmacognosy (9).

Moringa oleifera seeds are attributed with medicinal properties and high nutritional values among other industrial uses. Some local scientific publications reveal inconsistencies in findings especially in Nigeria from different geographical locations. The phytochemical and proximate analysis too varied accordingly, which is responsible for the effect of the substance. Dike and Luteino ,(11) observed that there was no significant effect of the extract of *Moringa oleifera* seeds on Haemoglobin, Red Blood Cells, Packed cell volume ,MCV,MCHC of albino wistar rats, however ,there was significant increase in the total white blood cell(TWBC),platelets and monocytes. The concentration of 1600mg/kg induced portal cellular infiltration, periportal congestion and hydropic degeneration of hepatocytes in the liver as well as cortical congestion and intestitial haemorrhages in the kidney (10).

The phytochemical, proximate and elemental analysis of *Moringa oleifera* seeds also suggests its pharmacological and nutritional potentials for human and other animal uses. Kawo *et al, (12)* reported that the seed powder has the following proximate composition Nitrogen, crude protein, tannins, alkaloids, saponins and other predominate mineral elements like Aluminum, Calcium, potassium, phosphorus, sodium, manganese, Bromine, Iron Chromium, Arsenic, Lanthanum, Samarium, Rubidium, Scandium, thorium and Zinc found in Kano though Manganese, Iron, Chromium, Arsenic, thorium and Zinc were quantifiably found beyond detectable limits (BDL). For many trace elements the margin of safety between beneficial and harmful is narrow (13). Uptake of heavy metals by plant from soil and contamination of food by metals during harvesting, transportation, storage, marketing and processing stages are the major sources of heavy metals in food (14). Heavy metals are very harmful because of their non-biodegradable nature, long biological half lives and their potential to accumulate in different body parts. Most of the heavy metals have damaging effects to man and animals because there is no good mechanism for their elimination from the body. Presently heavy metals are commonly found because of their excessive use in individual application (15).

Living Organisms require varying amount of heavy metals such as Iron, Cobalt, Copper, Manganese, Molybdenum and Zinc while other heavy metals such as Mercury (Hg), Cadmium (CD), Plutonium, Lead (Pb), Nickel (N_1), Chromium (Cr) are toxic metals that have no known vital or beneficial effect on organisms and their accumulation overtime in the bodies of animals or man can cause serious illness. The presence of such reportedly harmful elements such as Arsenic, Chromium and Thorium (a radioactive element) beyond detectable limit as reported by Kawo *et al, (12)* in Kano in excess above critical levels in *Moringa* plant product poses danger for consumption. Katsayal et al (16) also reported the presence of harmful elements like Samarium (69 ± 30) and Rubidium (122 ± 0.00) in the leaves of *Moringa Oleifera* collected from Dambo village of Sabon Gari LGA of Kaduna State. The methanol extract of *Moringa oleifera* seed was reported to possess some potent hypotensive principles (17). Anhwange *et al, (18)* also reported that the *Moringa oleifera* seeds contain hydrogen cyanide 0.58mg/100g, as cyanide inhibits cytochrome thus halting electron transport, oxidative phosphorylation and aerobic glucose metabolism resulting in the buildup of lactate

and increased concentration of oxygenated hemoglobin. Increased oxyhaemoglobin in the venous circulation reflects that oxygen is not being utilized in the peripheral tissue. Most serious consequences of oxidative phosphoxylation inhibition are relative to neurological and cardiovascular problem, neurological sequelue, respiratory arrest, arrhythmia and cardiac failure induced (19). Possible causes of acute toxic signs and mortality may contribute to the blood circulatory failure induced by acute sudden hypotension and also may be due to tissue anoxia. However, according to Oguojinmi *et al,* (20) the phytochemical screening of *Moringa oleifera* seeds collected from Ibadan in Oyo State after methanol extraction revealed the presence of alkaloid, Glucoside, flavonoid, saponin and steroid ring. Adoum *et al*, (21) reported that variations in the phytochemistry/phytochemical screening outcome may occur due to the degree of the concentration of the phytoconstituents or fractionated portions of the *Moringa oleifera* seeds present based on geographical location the plant is found and even the extraction solvent used. The presence of these chemical constituents is an indication that the plant if properly screened could yield drugs of pharmaceutical significance. Phytochemicals are chemical compounds that are naturally found in plant. They are responsible for the colour and organoleptic properties of the plant (22). It is also referred to as those chemicals that may have biological significance but are not established as essential nutrients in plants (23).

However, the results of the safety or toxicity studies of *Moringa oleifera* seeds extract have been contradictory a study by Faizi *et al* ,(17) stated that seeds extract up to 3g/kg caused neither behavioural change nor lethal effect in mice. Ajibade *et al* (10) however observed renal hemorrhages, hydropic degeneration of hepatocytes, decrease in platelet count, monocytes and total white blood cells in rats treated with *Moringa oleifera seeds extract*. Aggregation of bile canaliculi around the portal vein in liver of albino wistar rats receiving the extract for 21 days as well as significant increases in tissue enzymes when 1 to 10 mg/ml of seeds extract was used were also observed as reported by Oluduro *et al* ,(24)..

The findings of this study will benefit the consumers and the society at large of the *Moringa oleifera* seed due to its common use in water purification and consumption being an edible medicinal seed by providing scientific data based on safety and some haematological properties evaluation in Enugu.

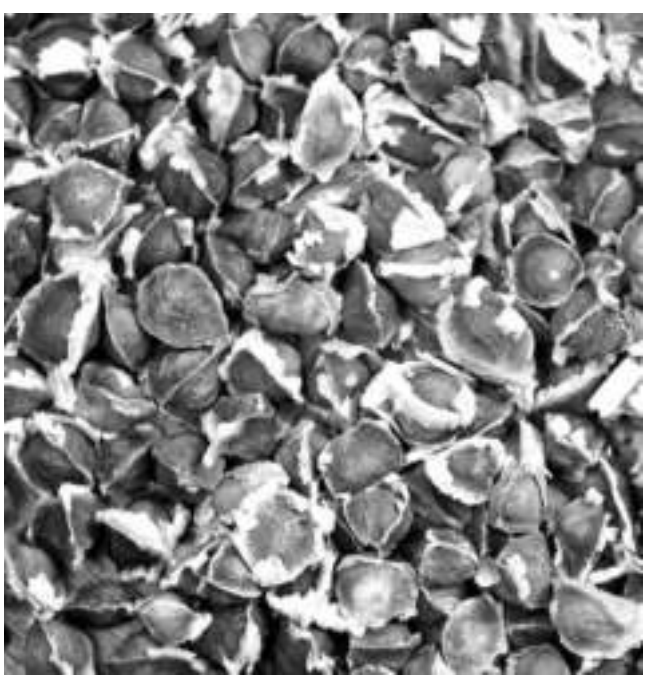

Figure 1: Moringa oleifera seeds

Materials and Methods

Preparation of plant extract

The seeds of *Moringa oleifera* collected from Trans Ekulu ,Enugu were authenticated at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka by Professor M.O Nwosu with voucher number 16c. Three hundred and eight six grams (386g) powder of *Moringa oleifera* were obtained after air drying and pulverizing before the extraction with methanol as solvent. The seed extract of *Moringa oleifera* was concentrated by removing the remaining solvent (method) in porcelain dishes by evaporation invacuo. The residue weighing 24.6g (a yielding of 6.4%) was reconstituted in 400mls of distilled water to give a final concentration of 61.5mg/ml.

Experimental animals and design

Thirty healthy albino Wistar rats weighing (120-170) g, aged 2-3 months of both sexes obtained from the Physiology Department, College of Medicine and kept in the Animal House, University of Nigeria, and Enugu Campus (UNEC) were used for the study. The rats were fed with top super starter feeds and water *ad libitum*. Rats were chosen as the experimental animal for the study because toxic substances readily produce demonstrable effects in rats (25). The rats were allowed a period of acclimatization to laboratory conditions, the rats were randomly divided into five groups (A, B, C, D and control E). A varied dosage of the seed extract at 25mg/kg, 50mg/kg, 100mg/kg and 125mg/kg were administered orally to rats in groups A, B, C and D respectively. The control (group E) rats were not treated with *Moringa oleifera* seed extract but continued only on super starter feeds and water for the period of the study. The phytochemical screening of *Moring oleifera* seed was done at Projects Development Agency/Institute (PRODA) Emene, Enugu and shown in the result of Table 5.

Acute toxicity study:

The LD_{50} was determined to be greater than 5000mg/kg following the guideline of OECD (26).

Clinical toxicity symptoms such as respiratory distress, salivation, weight loss and change in appearance of hair were not observed at any period of the experiment.

Sample collection

On days eight and fifteen of the extract treatment each rat was bled 4mls of blood through the orbital sinus into k_3EDTA bottle for haematological analysis using micro-capillary tube. Moreover, on day fifteen the rats were dissected after sample collection to remove the liver, kidney and heart which were transferred into 10% buffered formalin for tissue processing and histological analysis as described by Culling, (27) , Lilhe (28) .

Hematological Studies

The samples collected into *K_3EDTA* bottles were properly labeled and mixed on a mechanical mixer before analysis for haematological parameters using a Haematology auto-analyzer BC 3200 mindray. The Mindray BC3200 was standardized using routine quality control samples for low, normal and High commercially prepared controls for precision and accuracy following manufacturers guidelines.

Statistical analysis

The data were statistically analyzed by one way *ANOVA (Analysis Of Variance)*. Comparison between treatment and control groups were made by Duncan, Turkey/Hsd, Sheffe and Bonferroni, multi-comparism tests used for indication of any significant differences among and between groups of animals. Differences observed were considered significant at p value

less than 0.05 ($P<0.05$)

Results. The phytochemical screening of ethanol extract in table 1a and 1b showed the absence of flavonoid , steroid and phenol while the AAS quantification of chemical elements revealed only the presence of Manganese, Zinc , Potassium , Calcium and Sodium in increasing proportion respectively by parts per million .

Table 2 from the study revealed that on day 8 *Moringa Oleifera* seeds extracts administration in albino wistar rats indicated that the Red Blood Cells (RBC), Haemoglobin (HB) / Haematocrit (HCT/PCV) and Total white blood cells count were statistically increased and decreased respectively compared to the control at the concentration of 25mg/kg.

Table 1a: The phytochemical screening of the *Moringa Oleifera* seed

Parameter	Water/Aqueous	Ethanol	Ethyl acetate	N- Hexane
Glucoside	~~III~~	~~III~~	~~III~~	~~I~~
Alkaloid	-	~~II~~	~~I~~	-
Saponin	-	-	~~III~~	~~III~~
Flavonoid	-	-	~~II~~	-
Steroid	-	-	-	~~III~~
Phenol	-	-	-	-
Tannin	-	~~I~~	~~I~~	-

Key symbol indicators: (-) Absent; (~~I~~) Present ;(~~II~~) Moderately Present ;(~~III~~) Highly Present

Table 1b: Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy(Parameters)	CU	Zn	Cd	Cr	Ca	K	Mn	Pb	Na
Parts Per Million(PPM)/Values	Nil	0.60	Nil	Nil	2.27	1.24	0.04	Nil	14

Table 2: Effects of methanol extract of *Moringa oleifera* seed extract on Hematological parameter of rats after seven days of administration

Parameter Concentrations GROUPS(A-E)	LYMPH	EOSINO	HCT %	RBC	MCHC	HB	MCH	MCV	PLAT	TWBC	NEUT%
Control(E)	42.47 ±6.58	7.24 ±1.68	29.33 +3.67	12.63 ±3.33	17.3 ±0.75	59.7 ±5.8	815.67 ±302.7	15.43 ±1.53	24.67 ±0.58	73.67 ±3.27	5.00 ±
25mg/kg (A)	48.78* ±2.31	8.94* ±0.31	31.94 ±0.37	16.07* ±1.28	17.35 ±0.28	54.64 ±1.34	815.00 ±110.54	9.87* ±0.68	11.5 ±1.28	72.5 ±971	5.40 ±2.6
50mg/kg (B)	38.5 ±1.57	6.91 ±0.45	32.04 ±0.47	12.36 ±0.55	17.72 ±0.81	55.48 ±2.89	672.6 ±6943	11.5 ±1.28	75.8 ±6.22	60.8 ±9.0	5.00 ±2.92
100mg/kg (C)	40.74 ±2.66	7.42 ±0.43	31.86 ±0.54	13.00 ±0.94	17.48 ±0.75	55.02 ±2.22	726.8 ±131.1	13.72 ±11.92	12.14 ±1.38	63.8 ±9.09	3.60 ±2.51
125mg/kg (D)	40.06 ±5.37	7.02 ±0.86	31.8 ±0.42	12.78 ±1.80	18.14 ±0.53	57.12 ±1.82	3.00 ±0.37	12.14 ±1.38	32.8 ±19.5	14.14 ±1.38	5.5 ±3.54

* = p value <0.05, as p<0.05 is considered Significant

Table 3: Effect of Methanol extract of *Moringa oleifera* seed extract on Hematological parameters of rats on day 15

Parameters GROUPS (A-E) CONCENTRATION	HCT	RBC	MCHC	HB	MCH	MCV	PLAT	TWBC	NEUT	LYMPH	EOSINO
Control(E)	43.13 ±4.58	7.48 ±0.84	30.8 ±0.63	13.32 ±1.55	17.75 ±0.63	67.63 ±12.97	874.83 ±109.97	7.73 ±1.39	28 ±5.05	73.67 ±3.21	3± 0.00
25mg/kg (A)	45.46 ±4.33	7.76 ±0.62	31.04 ±1.2	13.98 ±1.66	17.9 1.26	55.88 ±6.04	852.0 ±129.23	8.12 ±2.36	28 ±4.47	69.8 ±5.36	9.5 ±0.71
50mg/kg (B)	46.88 ±4.79	8.27 ±9.16	31.08 ±0.81	14.63 ±1.83	17.63 ±0.64	56.85 ±2.41	1154.25 ±243.42	8.58 ±2.36	30.75 ±6.42	68.2 ±2	5.0 ±0.0
100mg/kg (C)	46.12 ±4.76	7.79 ±0.75	31.52 ±1.15	14.24 ±1.76	18.18 ±0.63	59.24 ±1.84	859.00 ±234.41	7.10 1.19	22.6 6.58	68.0 ±8.76	4.50 ±0.00
125mg/kg (D)	23.73 ±4.56	6.70 ±1.78	28.17 ±4.74	12.50 ±3.24	18.63 ±0.13	57.49 ±3.450	815 ±332.4	10.4 ±3.4	24.67 ±0.58	73.8 ±5.97	5.00

Table 4: Effect of *Moringa oleifera* seed extract on the mean weights of the albino Wistar rats

CONCENTRATION (mg/kg)	Group E CONTROL	Group A 25mg/kg	Group B 50mg/kg	Group C 100mg/kg	Group D 125mg/kg
WEIGHTS (g)					
Mean Weight before treatment (g)	135	135	149	136	147
Mean Weight after treatment (g)	143.3	148	176	164	176
% difference (%)	**7.9**	**12.3**	**25.6**	**26.6**	**27.5**

Table 3 from the study showed that on day 15, there was no statistical significance between the groups compared to the control group (E) for all the parameters examined. However, there were mean value increases in the following parameters. HCT/PCV, RBC, HB and platelets except the total white blood cell count which was reduced (but not statistically) on day fifteen from day eight.

However, the mean values of some parameters between days 8 and 15 of experimental period were changed. The mean values of HCT, RBC increased at concentrations of (50-125) mg/kg i.e. (groups B-D) while Hemoglobin was also increased at the concentration of 50-100mg/kg (Group B-C) whereas the Platelet counts increased across the different concentration doses (25-125mg/kg) on day 15 from day 8 of the extract administration.

Table 4 shows the nutritional effect of the extract *Moringa oleifera* seeds (MOS) on the body weight of the rats. It was observed that the higher the concentration of extract of the MOS given the greater the weight gain percentage difference obtained across the groups (A-D) studied. This is an indication that the *Moringa oleifera* seed extract possesses high nutritional values when compared to the control (group E).It was observed that the higher the concentration of extract of the *Moringa Oleifera* seed given the greater the weight gain percentage difference obtained across the groups (A-D) studied compared to the control group(group E).

Discussion

The finding from the phyto-constituents and proximate analysis of the *Moringa oleifera* seeds in our locality-Enugu revealed that the absence of flavonoid which increases intracellular

vitamin C synthesis ,decrease capillary permeability fragility and antioxidant property thereby enhancing leucocytosis and immunity effect. The absences of Steroid and Phenol also from the finding reveal the decrease or poor stimulation of the bone marrow for the production of blood cells and antioxidant property respectively. The absence of heavy/harmful metals like Chromium, Lead and Cadmium in this study illustrates its good use as an edible plant (12) .

This finding from the phytochemistry seems to be in disparity with the study done by Ogunjinmi *et al* ,(20) reported the presence of flavonoid and steroid with methanol extraction and absence of Tannin and alkaloid using both methanol and Ethyl acetate in Ibadan and kawo *et al* (12) reported the absence of saponin using ethanol as the extraction solvent and harmful elements like Arsenic,Chromium, and Thorium beyond detection limits and others like Samarium,Rubidium ,Scandium,Bromine and Lantharium in quantifiable values from moringa seeds in Kano. The phytochemistry also varies from the analysis reported by Auwal *et al* (29) of the aqueous extract of moringa seeds showing the presence of carbohydrate,tannin,saponin,alkaloid,cardiac glycosides,anthraquinones and flavonoids in Sokoto. The differences in phytochemistry and proximate chemical element composition could be attributed to the geographical location,part of the plant examined,season or climate , other environmental factors significantly influence the phytochemicals and nutrient contents of the plant and in nature of the product ,dosage and fractionated portions of the Moringa Oleifera Seeds that different solvents have different extraction capabilities and spectrum of solubility for the phytoconstituents (,31,21).

The significant increase in some parameters : Red Blood Cells (RBC), Haemoglobin (HB) and Haematocrit (HCT/PCV) suggests that the extract contain some bioactive constituents or phytoconstituents which could boost haemapoietic activities/effect. This is an indication that the extract enhances blood production especially the Red Blood Cells (RBC) when consumed within certain limits, this observation is in agreement with the findings by Adedapo *et al* (32), Hisman *etal,(33).* (Secondary) Metabolites present in the phytoconstituents contain polyphenols such as Tannin in the *Moringa Oleifera* seeds extract which may be responsible for the haematopoietic activity and it shown to extert protective effect on chemical induced haemolysis. (34), (35). This study also shows that the extract did not induce obvious toxic substances that can cause an anemia condition in rats as the HB, HCT/PCV and RBC were not reduced significantly which agrees with the report of Ajibade *et al* (10). The decreased total white blood cell count(TWBC) agrees with the study by Dike and Luteino (11) and Ajibade *et al*,(10) . The decreased total white blood cell count might have resulted from the suppression of Leucopoiesis in the bone marrow and may have consequential effect on the immune system according to Afolayan and Yakubu (36) and also impair inflammatory process . The suppressive effect of the seed extract of this plant on leucocytes may be due to the effect of some of the phytochemicals such as alkaloid which may supports the growth and differentiation of some cells in the bone marrow {(37),(34)}

The findings with respect to the hematological effect of the methanol extract of *Moringa oleifera* seeds produce in albino wistar rats after fourteen days of its administration agreed with the reports of Ajibade *et al (10)* , Dike and Luteino (39) in their separate studies discovered that there was no significant effect of the extract of moringa oleifera seeds on

Haemoglobin, Red Blood Cells, Packed cell volume ,MCV,MCHC of albino wistar rats. This finding may be attributed the absence of some phytochemicals: Phenols, Flavonoid and Steriod in the extract of Moringa Oleifera seeds which confers antioxidant effect and stimulation of the bone marrow for the blood cells production in Haemopoiesis (38).The absence of significant negative change or decrease in these parameters /indices suggest that the extract does not possess toxic substances that can cause an anemia in the rats.

This result differs from the finding of Auwal *et al* (29) who found that aqueous extract of *Moringa oleifera* seed increased total white blood cells count, Haemoglobin, Haematocrit and Red Blood Cells.

Declarations

Competing interest: The Author declares that there are no conflicts of interest.

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