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Acute toxicity and effects of methanolic extract of *Moringa oleifera* flower on haematological parameters of Wistar rats

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Abstract

Methanolic extract of *Moringa oleifera* flowers was investigated for its acute toxicity and effects on haematological parameters of Wistar rats. The acute toxicity study to determine the LD₅₀ was evaluated using Lorke's method. It involves first and second phases with six rats each phase. The rats were divided into three groups of two rats each of the phase. In the first, they were administered 10, 100 and 1000 mg/kg of the extract intraperitoneally while in the second phase, they were administered 1600, 2900 and 5000 mg/kg and then observed for 24 hours. The haematological study was carried out according to standard procedures. Twenty (20) rats were divided into 4 groups with five rats in each group. The rats in groups B, C and D were treated orally with the methanolic extract of *Moringa oleifera* flowers using 200 mg/kg, 400 mg/kg, and 800 mg/kg respectively for 21 days while group A that served as control were administered sterile water for injection at 0.5 ml/kg. Blood sample was taken from all the rats and used to establish the baseline values prior to treatments with the extract. Blood samples were collected at the end of every week and analyzed for haematological values. The administration of the extract at the dose of 5000 mg/kg intraperitoneally to the rats resulted in death but was safe at the dose of 2900 mg/kg. This indicated that the LD₅₀ is below 5000 mg/kg. There were significant increases ($p < 0.05$) in red blood cells, haemoglobin concentration and the packed cell volume values in all treated groups when compared to the baseline values (control group). There were also significant ($p < 0.05$) decrease and variation in the neutrophilic values and an increase in the lymphocytic values of the differential leukocytes counts. This research has revealed that the methanolic extract of *Moringa oleifera* flowers increased significantly the red blood cells, packed cell volume, haemoglobin concentration and lymphocytosis in Wistar rats.

Keywords: Acute toxicity, Haematological parameters, *Moringa oleifera* flowers, Albino rats

Introduction

Moringa oleifera is a vegetable belonging to the order and family *Brassica* and *Moringaceae* respectively. The family consists of 13-14 known species worldwide (Khawaja *et al.*, 2010). *Moringa oleifera* has a height of about 10 -12 m and 4 cm in diameter. The bark is whitish grey with purplish or greenish white young shoots, flowers are bisexual and surrounded by 5 unequal thinly veined yellowish white petals. Pods are pendulous and have a three-sided brownish color splitting lengthwise into three when dry. The seeds contain about 20 seeds embedded in the pith and are about 1 cm in diameter having three whitish papery wings (Ramasubramania *et al.*, 2017). *Moringa oleifera* can withstand severe drought and mild frost conditions and hence vastly cultivated worldwide (Lakshmipriya *et al.*, 2016). It can be grown in any tropical and subtropical regions of the world. It can thrive in sandy or loamy soil with a fairly acidic to alkaline pH and a net rainfall of 250 – 3000mm (Thurber & Fahey, 2010). It can be preserved for a long time through drying or freezing without proportionate loss of nutrients (Lakshmipriya *et al.*, 2016). *Moringa oleifera* differs in nutrient composition at different locations. The tree grown in India has slightly different nutritional components than that grown in Nigeria (Aslam *et al.*, 2005; Asante *et al.*, 2014). It has earned the name miracle tree among its various users due to its many healing abilities (Ahmad *et al.*, 2014). *Moringa oleifera* has been used for many centuries and in many cultures for the treatment of various ailments such as anaemia, blackheads, chest congestion, bronchitis (Khawaja *et al.*, 2010; Singh & Sharma, 2012); it has also been used as an anti-inflammatory, anti-hypertensive, antiepileptic, anti-diabetic (Paliwal *et al.*, 2011) and as a hepato protective agent (Huang *et al.*, 2012). The leaves are rich in minerals, vitamins and other essential phytochemicals (Lakshmipriya *et al.*, 2016). Extracts from the leaves are used to stimulate lactation and increase breast milk production in nursing mothers. The seeds, a natural coagulant is used extensively in treatment of water (Lakshmipriya *et al.*, 2016). Moringa leaf is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more potassium than bananas and 25 times more iron than spinach (Rockwood *et al.*, 2013). An overdose of *moringa* may cause high accumulation of iron which can cause gastrointestinal distress and haemochromatosis. Therefore, a daily dose of 70g of moringa is suggested to be tolerable and prevents over accretion of nutrients (Asiedu-Gyekye *et al.*,

2014). This study aimed at investigating the acute toxicity and effects of methanolic extract of *Moringa oleifera* flowers on haematological parameters of Wistar rats.

Materials and Methods

Collection, identification and processing of the plant material

Fresh *Moringa oleifera* flowers were collected from Mairi village, Jere Local Government Area of Borno State, Nigeria. Jere is one of the local governments that constitutes the Borno state with vast arable land used for agriculture. It is located on latitude 11.9°N and Longitude 13.2°E (Geody, 2020). The plant part was identified to be from *Moringa oleifera* by a taxonomist from the Department of Biological Sciences, University of Maiduguri. The flowers were air-dried in a room for two days, grounded into powder using pestle and mortar and then stored in a plastic container. The *Moringa oleifera* flower sample was extracted using reflux method of extraction where by the powdered plant extract (600 g) was refluxed with 50% w/v methanol at different temperature range with repeated solvent evaporation followed by condensation. A total of 220.32 g was obtained after the extraction. This was transferred to an air-tight container, properly labeled and stored in a refrigerator at 4°C.

Experimental animals

All the rats were handled according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS & ICLAS, 2012). A total of 32 Wistar rats of both sexes used for the experiments were obtained from the animal house, Department of Veterinary Pharmacology and Toxicology, University of Maiduguri. The rats were kept in plastic cages and were allowed to adjust to the laboratory environment for a period of two weeks before the commencement of the experiment. They were fed grower's mash (composing the following nutrients per gram: dry matter 896.7 g, crude protein 615.3 g, crude fat 190.0 g, crude ash 64.9 g, calcium 17.6 g, phosphorus 12.1 g, fibre 26.5 g, other essential minerals and amino acid 128.5 g) and water provided *ad libitum*.

Ethical statements

All the rats were handled according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS & ICLAS, 2012).

Acute toxicity study (LD_{50})

The acute toxicity study was evaluated using the procedure described by Lorke's method (Lorke, 1983). It involves phases 1 and 2 studies. Twelve Wistar rats were divided into two groups of six rats each. The rats were weighed, and the extract was intraperitoneally administered at a limit dose of 1000 mg/kg and 5000 mg/kg in the first and second phases. The rats were then observed for 24 hours for signs of toxicity (weakness, staggering and recumbency) or mortality.

Experimental design and treatments

Twenty rats divided into 4 groups of five rats each (A, B, C and D) were used. Group A rats were used as the control group while B, C and D were treated with the extract. Administration of the extract was done at the concentration of 200 mg/ml daily for 21 days. Extract doses used on the rats in groups B, C and D were 200 mg/kg, 400 mg/kg and 800 mg/kg respectively. Body weights of the animals (rats) were obtained prior to extract administration, ranging from 133 g to 196 g.

Haematology

Blood samples (1ml each) were collected from the tail vein of the rats at 7 days' interval within 3 weeks of extract administration in a sample bottle containing EDTA anti-coagulant. The blood samples obtained were used to determine packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), haemoglobin concentration (Hb) and differential leukocyte count (DLC). PCV was measured by micro haematocrit reader using capillary tube while RBC and WBC were measured using Neubauer counting chamber. Differential Leucocyte Count (DLC) were manually measured by making a thin blood film and staining it with Leishman's stain, while Hb concentration was determined by Shali's method (Lewis *et al.*, 2006). Blood samples were obtained for another two weeks following the withdrawal of the extract to determine the effect of the extract withdrawal on the haematological parameters.

Statistical analyses

The data obtained from the study were analyzed and expressed as mean \pm standard deviation (SD) using One Way Analysis of Variance (ANOVA) in GraphPad statistical package (2003). $P \leq 0.05$ was considered statistically significant.

Results

Mortality was observed at the dose of 5000 mg/kg but no death at 2900 mg/kg of the methanolic flower extract of *Moringa oleifera*. The LD_{50} therefore is 3807.9 mg/kg since the highest dose that did not kill the rats is 2900 mg/kg, while the least dose that killed is 5000 mg/kg. The mean RBC counts of rats treated orally with the methanolic extract of *Moringa oleifera* flowers were presented in Table 1. The result indicated a significant ($p < 0.05$) increase in the RBC values with doses 200 mg/kg, 400 mg/kg and 800 mg/kg during weeks one, two and three of extract administration as compared with the control. These significant ($p < 0.05$) increase in the mean RBC values was maintained up to two weeks after withdrawal of the extract with all the doses administered compared to the mean values for the control group.

The mean total white blood cell counts (WBC) of rats treated with methanolic extract of *Moringa oleifera* flowers is presented in Table 2. The result indicated a significant ($p < 0.05$) increase with doses of 400 mg/kg and 800 mg/kg, while no significant ($p > 0.05$) increase with dose of 200 mg/kg during weeks one and two. At week three, all the administered doses produced significant ($p < 0.05$) increase in WBC values compared to the values obtained from the control group. Significant increase ($p < 0.05$) was persistent one week after withdrawal of the extract with all doses administered. There was no significant ($p > 0.05$) increase two weeks after withdrawal of the extract with all the doses. The mean haemoglobin concentration of albino rats treated with methanolic extract of *Moringa oleifera* flower is presented in

Table 1: Effects of graded doses of the methanolic extract of *Moringa oleifera* flowers on the red blood cell of Wistar rats

Extract dose (mg/kg)	Weeks of Extract Administration			Weeks of Extract Withdrawal	
	1	2	3	1	2
	Mean \pm SD ($\times 10^{12}$ /L)			Mean \pm SD ($\times 10^{12}$ /L)	
Control	6.02 \pm 0.14 ^b	6.12 \pm 0.10 ^b	6.27 \pm 0.19 ^b	6.07 \pm 0.08 ^b	6.02 \pm 0.16 ^b
200	7.68 \pm 0.13 ^a	7.80 \pm 0.07 ^a	7.90 \pm 0.07 ^a	6.84 \pm 0.20 ^a	6.28 \pm 0.19 ^a
400	7.82 \pm 0.36 ^a	8.08 \pm 0.13 ^a	8.34 \pm 0.11 ^a	7.14 \pm 0.13 ^a	6.28 \pm 0.08 ^a
800	7.92 \pm 0.83 ^a	8.16 \pm 0.16 ^a	8.58 \pm 0.08 ^a	7.25 \pm 0.19 ^a	6.40 \pm 0.07 ^a

Key: Columns with different letter superscripts a,b are significantly different ($p < 0.05$)

Table 2: Effects of graded doses of the methanolic extract of *Moringa oleifera* flowers on the white blood cells of Wistar rats

Extract dose (mg/kg)	Weeks of Extract Administration			Weeks of Extract Withdrawal	
	1	2	3	1	2
	Mean ± SD ($\times 10^9/L$)			Mean ± SD ($\times 10^9/L$)	
Control	8.82 ± 0.21 ^b	8.97 ± 0.30 ^b	8.91 ± 0.19 ^b	8.90 ± 0.15 ^b	9.00 ± 0.33
200	10.02 ± 0.28 ^b	10.18 ± 0.46 ^b	11.98 ± 1.04 ^a	9.64 ± 0.47 ^a	8.94 ± 0.79
400	10.08 ± 0.31 ^a	12.16 ± 1.06 ^a	13.42 ± 0.14 ^a	9.84 ± 0.16 ^a	9.24 ± 0.33
800	11.06 ± 0.21 ^a	12.70 ± 0.63 ^a	13.88 ± 0.19 ^a	10.16 ± 0.18 ^a	9.66 ± 0.35

Key: Columns with different letter superscripts a,b are significantly different ($p < 0.05$)

Table 3: Effects of graded doses of the methanolic extract of *Moringa oleifera* flowers on the haemoglobin concentration of Wistar rats

Extract dose (mg/kg)	Weeks of Extract Administration			Weeks of Extract Withdrawal	
	1	2	3	1	2
	Mean ± SD (g/dl)			Mean ± SD (g/dl)	
Control	10.44 ± 1.59 ^b	10.54 ± 1.51 ^b	10.96 ± 1.44 ^b	10.96 ± 1.32 ^b	10.44 ± 0.77 ^b
200	12.74 ± 0.42 ^a	13.60 ± 0.14 ^a	13.72 ± 0.10 ^a	11.56 ± 0.77 ^b	11.12 ± 0.65 ^b
400	13.00 ± 0.18 ^a	13.78 ± 0.10 ^a	13.92 ± 0.17 ^a	12.36 ± 0.43 ^b	11.80 ± 0.52 ^a
800	13.24 ± 0.16 ^a	13.78 ± 0.10 ^a	14.00 ± 0.14 ^a	12.96 ± 0.16 ^a	12.56 ± 0.26 ^a

Key: Columns with different letter superscripts a,b are significantly different ($p < 0.05$)

Table 4: Effects of graded doses of the methanolic extract of *Moringa oleifera* flowers on the packed cell volume of Wistar rats

Extract dose (mg/kg)	Weeks of Extract Administration			Weeks of Extract Withdrawal	
	1	2	3	1	2
	Mean ± SD (%)			Mean ± SD (%)	
Control	40.6 ± 0.89 ^b	41.2 ± 0.83 ^b	41.2 ± 0.83 ^b	41.4 ± 0.54 ^b	41.4 ± 0.54 ^b
200	45.8 ± 1.30 ^a	46.4 ± 0.54 ^a	46.2 ± 0.44 ^a	44.6 ± 0.54 ^a	42.8 ± 0.83 ^a
400	46.6 ± 0.54 ^a	47.6 ± 0.54 ^a	47.4 ± 0.54 ^a	44.8 ± 0.83 ^a	43.8 ± 0.44 ^a
800	47.6 ± 0.54 ^a	47.6 ± 0.54 ^a	47.8 ± 0.44 ^a	45.4 ± 0.54 ^a	44.4 ± 0.54 ^a

Key: Columns with different letter superscripts a,b are significantly different ($p < 0.05$)

Table 3. Significant ($p < 0.05$) increase occurred with doses of 200 mg/kg, 400 mg/kg and 800 mg/kg during weeks one, two and three of extract administration. The significant increase persisted one week after extract withdrawal with dose of 800 mg/kg. Significant increase was also seen two weeks after the withdrawal of the extract with doses of 400 mg/kg and 800 mg/kg compared to the mean values of the control.

The mean packed cell volume (PCV) of albino rats treated with methanolic extract of *Moringa oleifera* flower is presented in Table 4. The result indicated significant ($p < 0.05$) increase with all the doses (200 mg/kg, 400 mg/kg and 800 mg/kg) during weeks one, two and three compared to the mean values obtained from the control group. PCV of all treated groups remained significantly ($p < 0.05$) higher than the

untreated control up to two weeks post withdrawal. The significant ($p < 0.05$) increase persisted at week one and two of the extract withdrawal. The differential leukocyte count (DLC) of rats treated with various doses of the methanolic extract of *Moringa oleifera* flowers is presented in Table 5. Following treatment with the extract at 200 mg/kg, 400 mg/kg and 800 mg/kg, there was a significant ($p > 0.05$) decrease in the neutrophils during the first, second and third weeks at doses 200 mg/kg and 400 mg/kg. At the dose of 800 mg/kg, there was a significant ($p < 0.05$) rise in the neutrophils at week one, followed by a significant decrease at week two and three. At First week after withdrawal of the extract, there was a slight increase with dose of 400 mg/kg followed by a significant ($p < 0.05$) increase with 400 mg/kg and 800 mg/kg dose at week two of extract withdrawal.

Table 5: Effects of graded doses of the methanolic extract of *Moringa oleifera* Flowers on the differential leukocyte count (DLC) Wistar rats

Parameters	Extract dose(mg/kg)	Weeks of Extract Administration			Weeks of Extract Withdrawal	
		1	2	3	1	2
		Mean \pm SD (%)			Mean \pm SD (%)	
Neutrophils (%)	Control	2734 \pm 1.78 ^a	2655 \pm 1.51 ^a	2566 \pm 0.83 ^a	2598 \pm 1.09	2646 \pm 1.14
	200	2585 \pm 1.30 ^b	2341 \pm 1.58 ^b	2419 \pm 1.30 ^b	2544 \pm 0.89	2610 \pm 1.64
	400	2560 \pm 0.89 ^b	2480 \pm 1.94 ^b	2281 \pm 0.70 ^b	2617 \pm 1.14	2790 \pm 2.16
	800	3141 \pm 1.51 ^b	2590 \pm 1.51 ^b	2470 \pm 1.09 ^b	2580 \pm 0.54	2801 \pm 1.73
Eosinophils (%)	Control	353 \pm 0.70	396 \pm 0.48	410 \pm 0.54	357 \pm 0.15	377 \pm 0.20
	200	503 \pm 0.08	468 \pm 0.54	645 \pm 0.77	405 \pm 0.44	376 \pm 0.67
	400	497 \pm 0.54	632 \pm 0.44	671 \pm 0.70	375 \pm 0.67	407 \pm 0.54
	800	551 \pm 0.37	659 \pm 0.22	722 \pm 0.44	427 \pm 0.44	405 \pm 0.30
Basophils (%)	Control	71 \pm 0.44	72 \pm 0.44	71 \pm 0.83	89 \pm 0.42	72 \pm 0.44
	200	80 \pm 0.44	81 \pm 0.44	168 \pm 0.54	77 \pm 0.44	89 \pm 0.33
	400	101 \pm 0.70	122 \pm 0.70	188 \pm 0.54	98 \pm 0.89	92 \pm 0.66
	800	111 \pm 0.70	127 \pm 0.44	167 \pm 0.44	102 \pm 0.31	97 \pm 0.22
Lymphocytes (%)	Control	5557 \pm 1.58 ^b	5723 \pm 1.30 ^b	5720 \pm 0.83 ^b	5732 \pm 0.54 ^b	5778 \pm 0.83
	200	6693 \pm 0.83 ^a	7146 \pm 1.48 ^a	8530 \pm 0.83 ^a	6497 \pm 0.54 ^a	5739 \pm 2.38
	400	6854 \pm 0.70 ^a	8755 \pm 1.58 ^a	10011 \pm 0.54 ^a	6593 \pm 1.00 ^a	5821 \pm 2.34
	800	7631 \pm 0.70 ^a	9055 \pm 0.54 ^a	10327 \pm 0.54 ^a	6848 \pm 0.54 ^a	6221 \pm 2.50
Monocytes (%)	Control	122 \pm 0.23	126 \pm 0.54	142 \pm 0.61	125 \pm 0.54	126 \pm 0.09
	200	160 \pm 0.54	144 \pm 0.76	216 \pm 0.44	135 \pm 0.23	125 \pm 0.82
	400	144 \pm 0.41	175 \pm 0.12	240 \pm 0.33	138 \pm 0.54	129 \pm 0.54
	800	174 \pm 0.79	206 \pm 0.15	222 \pm 0.54	164 \pm 0.07	116 \pm 0.44

Key: Columns with different letter superscripts a,b are significantly different ($p < 0.05$)
SD = Standard Deviation

All the values of the treated groups were comparable with the values of untreated control group at dose of 200 mg/kg after withdrawal of the extract.

Eosinophils, basophils and monocytes were not significantly affected by the treatment of the extract with all the doses throughout the weeks of treatment and after the withdrawal of the extract. There was a significant ($P < 0.05$) increase in the mean lymphocyte count during the first, second and third week of treatment with doses of 200 mg/kg, 400 mg/kg and 800 mg/kg compared with the control. During the first week after withdrawal of the extract, there was significant increase with all administered doses and none during week two.

Discussion

The intraperitoneal administration of methanolic extract of *Moringa oleifera* flowers to Wistar rats at 5000 mg/kg resulted in death of the Wistar rats. This shows that the LD₅₀ of the extract is 3807.9 mg/kg. Any substance whose LD₅₀ in rats is between 500 to 1000 mg/kg is described as moderately toxic (Gerald & Victor, 2014), while the one above 5000 mg/kg is less toxic.

The twenty-one-day administration of graded doses of the methanolic extract of *Moringa oleifera* flowers to Wistar rats have shown some beneficial effects on the haematological parameters. The results of the haematological study revealed a significant increase ($p < 0.05$) in RBC values of the treated rats. This is synchronous with the folkloric use of the plant to correct anaemia (Ramachandran *et al.*, 1980; Mazumder & Gupta, 1999).

A sufficient amount of RBC count after days of administration of a test substance is indicative of the positive effect of the test substance which signifies erythrocyte production and oxygen utilization (Akintunde *et al.*, 2017). Substances with haematinic properties are known to stimulate an increased production of RBC and improve the values of haemoglobin and the PCV. The PCV is an indicator of the volume and percentage of red cells in blood and also an index of toxicity. Any reduction in its concentration in the blood usually suggests the presence of toxic factors (haemagglutinins) which have adverse effects on blood formation (Oyawoye & Ogunkunle, 1998). There were significant increases ($p < 0.05$) in the PCV values and these increases were

observed in all the administered doses. The improvement in the values of RBC, haemoglobin and the PCV of the treated animals obtained in this study may be due to the presence of iron and copper in *Moringa oleifera* flowers (Busani *et al.*, 2011).

The administration of the extract at all doses did not elicit appreciable changes in the WBC. *Moringa oleifera* flowers as revealed in this study is therefore said to have low immune potentiating effects as compared with the leaves and seeds. Previous reports described the plant leaves as an immune builder (Ramachandran *et al.*, 1980). Ezejindu *et al.* (2015) also reported the impact of aqueous extract of *Moringa oleifera* leaves on the blood parameters of Wistar rats and found that *Moringa oleifera* raised the WBC count. The WBC are part of blood cells which stimulate cell-mediated immunity and help B-cells to make antibodies that fight against antigens. This might be attributed to the high concentration of flavonoids and saponins in the methanolic extract of *Moringa oleifera* leaves, which are known to have profound effects on the function of the immune and inflammatory cells (Evans, 2006). According to Lee *et al.* (2003), flavonoids in the seeds of *Moringa oleifera* may be the reason for the leucocytosis observed, since flavonoids were reported to increase intracellular vitamin C synthesis, leucocytosis, decrease capillary permeability, fragility and also have antioxidants property. The differential leucocyte counts as independently assessed revealed a significant decrease in the neutrophils count in week one of extract administration at the dose of 200 mg/kg and 400 mg/kg. However, it increases abruptly at the doses of 800 mg/kg in week one and decreases again in weeks two and three at the same dose. This decrease and variation in the neutrophil level in this current study might be due to stress subjected to the Wistar rats during the administration of the extracts (Drue Jr *et al.*, 2018).

Significant increase ($p < 0.05$) in the lymphocytes count was observed all through the weeks of extract administration. Drue Jr *et al.* (2018) reported that administration of moringa oleifera tea can influence stressed induced variation to circulating neutrophils but not lymphocytes. This research work is in agreement with the results of Onuoha & Ala (2020), who experimented with the aqueous leaf extracts of *Tithonia diversifolia* and *Moringa oleifera*. The PCV, haemoglobin (Hb), platelets, RBC, and WBC values were significantly different ($p < 0.05$) at 200 mg/kg of both extracts administered solely and in combination therapy when compared with the control group. This showed that the extracts of both plants were

effective and can positively alter the blood profile of experimental animals.

In conclusion, this research has shown that the methanolic extract of *Moringa oleifera* flowers at a recommended dose of either 200 mg/kg, 400 mg/kg or 800 mg/kg increased significantly the red blood cells, packed cell volume and haemoglobin concentration count in Wistar rats.

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Nil

Conflict of Interest

The authors declare that there is no conflict of interest.

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