Sub-acute Toxicity StudyofDefatted *Moringa oleifera* Seed MealAdministrationon Wistar Rats

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Abstract: The study was conducted to evaluate the clinico-pathological changes associated with sub-acute administration of defatted Moringa oleifera seed meal (DMOSM) in Wistar rats. Eight adult male Wistar rats were divided into two groups of 4 rats. Group I rats served as the control while group II were administered DMOSM at 480 mg/kg for 28 days. Rats were weighed on days 0, 7, 14, 21 and 28 respectively. Extraction of Moringa oleifera seeds was done using the non-solvent mechanical cold press method and phytochemical analysis of the defattedMoringa oleifera seed cake was performed using standard procedures.Blood samples were collected from sacrificed animals at day 28 and analyzed for haemato-biochemical parameters. Tissue sections of the liver, kidney and brain were harvested at necropsy and processed for histopathological studies using standard methods. Results showed that 81.71 g oil was extracted and 441.04 g of the seed cake was obtained from 522.750 g of Moringa oleifera ground seed using non-solvent mechanical cold press method. Phytochemical compounds present in the DMOSM were alkaloids, reducing sugars, cardiac glycosides and saponins. There was a significant reduction ($P < 0.05$) in the mean body weight of rats administered DMOSM at days 21 (153.5±6.82) and 28 (155.8±6.37) compared to the control (185.0±6.25) and (191.4±6.04) respectively.Also, significant ($P < 0.05$) reduction in serum creatinine in the test rats (25.25±1.93) compared to the control (64.00±11.60) as well as significant ($P < 0.05$) increase serum phosphate level also in the test group (4.64±0.46) compared to the control (3.27±0.24) were observed. Results obtained showed that defatted Moringa oleifera seeds meal possess phytoconstituents with useful and antinutrients properties. There were no remarkable toxic effects on most parameters investigated except those reflecting reduction in body weight of the rats. The seeds are safe if defatted and processed adequately as feed supplements.

Index Terms - Creatinine, Moringa oleifera seeds, Non-solvent extract, Phosphate, Phytochemicals.Wistar rats.

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1. INTRODUCTION

There is evidence that the use of extracts from plant species possess properties that are safe for human health [1 Ali *et al.,* 2004; 2 Akinnibosun*etal.,* 2008 and 2009]. *Moringa oleifera* is a plant that have diverse uses. The seeds of this plant has been extensively studied and shown to possess antioxidant [3 Paliwal*et al*., 2011; 4 Ogbunugafor*et al*., 2011], nutritional [5 Compaore*et al.,* 2011; 6 Ben Salem *et al*., 2008], antimicrobial [7 Karthy*et al*., 2009; 8 Abdulmoneim and Abu, 2011; 9 Manivasagaperumal*et al*., 2012], Biosorption [10 Sahabi*et al*., 2014; 11 Alves *et al*., 2010; 12 Marques *et al.,* 2012], coagulation for water purification [13 Jadhav*et al*., 2008; 14 Ali *et al*., 2009; 15 Michael, 2010] and host of other important bioactive properties. But with the increasing awareness on the health benefits of *Moringa oleifera*, and the reported medicinal potentials of all parts of the plant, some level of caution must be exercised to avert the possible toxic effect of the plant, particularly, the seeds which are now eaten raw for the purported health benefits. Therefore, the current study was conducted to evaluate the clinico-pathological effects of sub-acute administration of defatted *Moringa oleifera* seed meal on Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and identification of the plant material

Moringaoleifera is a plant commonly grown in most parts of Nigeria. The seeds of *Moringa oleifera* were collected from Ruma, Batsari Local Government Area of Katsina state and were authenticated by a taxonomist at the herbarium of Department of Biological Sciences, Ahmadu Bello University, Zaria, and given a voucher number, 571.

2.2 Extraction of *Moringa oleifera*

The fresh seeds were allowed to dry in a shed under room temperature for two weeks. The dried seeds of the plant were pulverized to powdered specimen using a mortar and pestle. Exactly 522.750 g of the powdered seeds was weighed out and utilized for non-solvent extraction.

2.2.1 Mechanical Cold Press Extraction

The method of [16 Fils (2000)] was using for the non-solvent extraction with slight modification. Briefly, the 522.750 g of powdered *M. oleifera* seeds was mixed with 500 ml of lukewarm deionized water and the mixture made into paste by stirring with glass rod. The paste material was then transferred into an oven and left for 5 minutes at 40^0C to remove the water content, after which it was transferred into and tied in a clean cheese cloth. This was then pressed using the mechanical oil press. The oil was in this way extracted

and the defatted cake was left in the cheese cloth. The defatted cake in the cheese cloth was kept on a shelf for 3 days to dry at room temperature and then removed and stored.

2.2.2 Identification of phytochemical groups in the defatted *Moringaoleifera* (MO) seed meal (DMOSM)

The DMOSM was tested for various classes of compounds using the methods described by [17 Trease and Evans (1996)]. The compounds that were tested for included Alkaloids (Dragendoff's test), Steroids (Salkowski test), Tannins (Lead subacetate test), Anthraquinones (Bontrager test), Cardiac glycosides (Keller-Kiliani test), Flavonoids (Sodium hydroxide test) and Saponins (Frothing test), which were reported to have biological activities on animal tissues [18 Kapadia *et al.*, 1978; 19 Okwu and Josiah, 2006; 20 Calderón-Montaño*et al*., 2011].

2.3 Acute Toxicity Study

The acute toxicity study lethal dose (LD50) for DMOSM was carried out as described by [21 Locke (1983). A total of 12 rats were used for the acute study. The first phase involved nine rats divided into 3 groups with 3 rats in each group. The rats were dosed with 10 mg/kg, 100 mg/kg and 1000 mg/kg of DMOSM respectively, once, orally, for the first phase and observed for 48-72 hours for neurological, behavioural changes and or, mortality. The second phase involved three rats which were divided into 3 groups with 1 rat in each group. The rats were dosed 1600 mg/kg, 3200 mg/kg and 4800 mg/kg respectively, once, orally. The rats were then observed for 48-72 hours for any sign of toxicity or mortality. A dose of 480 mg/kg (one tenth of the highest dose, 4800 mg/kg) was then selected for the sub-acute toxicity study based on the absence of observable signs of toxicity and mortality at the highest administered dose.

2.4Experimental site

This study was carried out in the Experimental Animal room of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

2.4.1Experimental animals

A total of 8 apparently healthy 8 to 10-week old Wistar rats (*Rattusnovergicus*) were obtained from the Laboratory Animal Unit of National Institute for Trypanosomosis Research, Kaduna,Kaduna State. They were kept in steel cages in the experimental animal room of the Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, at an average room temperature of around 27°C and under 12/12-hour light dark cycle. The rats were allowed to acclimatize for fourteen days in the experimental animal room before the commencement of the experiment. All animals were handled in accordance with the standard guide for the care and use of laboratory animals [22 NRC, 1996].

2.5Therapeutic effect of feeding defatted *Moringaoleifera* seed meal

The therapeutic effect of defatted MO seed meal was tested. This agent was selected following reported findings on its medicinal and nutritive values, as well as its availability.

2.5.1 Preparation of defatted *Moringa oleifera* seed meal

Defatted *Moringa oleifera* seed cake was ground to powder form using mortar and pestle, and then sieved using 200 micron pore size. Exactly 5 g of the fine powder of the defatted *Moringa oleifera* seed cake was dissolved in 20 mls of distilled water to give the 250 mg/ml concentration of the seed meal used for this study. The respective concentrations were prepared daily and administered to the experimental rats at 480 mg/kg body weight.

2.6Experimental Design

Eight (8) wistar rats were randomly divided into 2 groups of 4. All the rats were fed daily with pelleted growers' marsh (Vital Feeds Ltd®, Jos) and water provided *ad libitum*. The grouping was as follows:

Group I: Rats served as negative control and received distilled water via drinkers daily for 28 days.

Group V: Rats in this group received defatted *Moringa oleifera* seed meal at a dose of 480mg/kg by oral gavage daily for 28 days.

2.7Blood sampling for haematological and biochemical analyses

About 7 ml blood was collected via jugular venesectionfrom the 8 rats following light ketamine/xylaxine anaesthesia. About 2 ml of this blood was dispensed into sterile sample bottles containing Na EDTA and used for haematological studies, while 5 ml was dispensed into sterile sample bottles without anticoagulant and then centrifuged to harvest serum for biochemical analysis.

2.7.1 Haematological screening

Packed cell volume (PCV %), Haemoglobin content (Hb g/dl), Total leukocyte count (TLC), Mean Cell Volume (MCV), Red Blood Cell (RBC) Count, total and differential leukocyte count were carried out as described by [23 Jain (1993)].

2.7.2 Biochemical analysis

The serum samples were collected from the clotted blood by centrifuging at 3000 g for 15 minutes. The sera were carefully harvested into appropriately labeled plastic tubes and analyzed immediately. Serum samples were used for measuring the concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, blood urea nitrogen (BUN), albumin, sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), phosphate (PO_4^-), chloride (Cl^-) and bicarbonate (HCO_3^-) using the automated

Audiocombanalyser (Bayer Express Plus, Bayer Germany, Serial Number 15950) in the Chemical Pathology Laboratory of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

2.8 Gross and Histopathological studies

Following collection of blood samples at the end of the study, the experimental rats were sacrificed by severing the jugular vein under light anaesthesia using ketamine/xylaxine. Postmortem examination was carried out on each of the rats. Specimens from liver, kidney and brain were collected from the sacrificed rats and preserved in 10% buffered neutral formalin for histopathology using the method described by[24 Bancroft and Cook (1994].

2.9 Statistical analysis

Statistical values were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed by student's t-test using GraphPad Prism version 5 and unpaired post hoc test. Values of $P < 0.05$ were considered to be significant**.**

3. Results and Discussion

3.1 Percentage yield of defatted Moringa oleifera seed meal (DMOSM) and Phytochemical Analysis

Following the non-solvent mechanical extraction using 522.750 g of *Moringa oleifera* ground seed, 441.04 g of the seed cake was obtained, implying that 81.71 g oil was extracted.The results of the qualitative phytochemical analysis on the defatted *Moringa oleifera* seed meal (DMOSM)revealed the presence of reducing sugar, alkaloid, saponins and cardiac glycoside at various concentrations as shown in table 1.These phytoconstituents possess diverse potentials as pharmaceutics and nutritional supplements [25 Auwal*et al*., 2010;26 Kakengi*et al*., 2007; 27 Kasolo*et al*., 2010; 28 Olugbemi*et al*.,2010]. The seeds also contain antinutrients such as saponins, tannins, phytates*etc* that have deleterious effects on appetite, nutrient utilization and coagulation of erythrocytes [19Okwu and Josiah, 2006; 29Gauthaman and Adaikan, 2008; 30Singh and Gupta, 2011; 31Zade*et al*., 2013]. The presence of these secondary metabolites from different extracts of the seeds of *Moringa oleifera* has been reported [32Ajibade*et al*., 2012;33Zade*et al*., 2013].

Phytochemical	Result
Alkaloid	+
Reducing sugar	+
Saponins	+
Tannins	–
Anthraquinones	–
Flavonoids	–
Cardiac glycosides	+
Steroids	–

Table 1: Phytochemicals present in M. oleifera seeds
Key: where –ve: absence, +ve: present

3.2 Clinical observations in the acute toxicity study

There was no respiratory distress, salivation or change in hair coat appearance during the acute toxicity study. Similarly, no mortality or changes in behaviour and or nervous signs were observed in the rats administered DMOSM at 10, 100, 1000, 1600, 3200 and 4800 mg/kg body weight body weight respectively.

3.3 Weight changes

The mean body weight of the rats in the test group at days 21 and 28 were (153.5±7.95), (155.8±6.37) while those of the control group was (185.0±6.25) and (191.4±6.04) respectively as shown in fig. 1 below:The administration of defatted *Moringa oleifera* seed meal at 480 mg/kg body weight for 28 days resulted in significant ($P < 0.05$) reduction in the mean body weight of the rats in the test group on days 21 (153.5±6.82) and 28 (155.8±6.37) compared to the control group at the respective days (185.0±6.25) and (191.4±6.04).

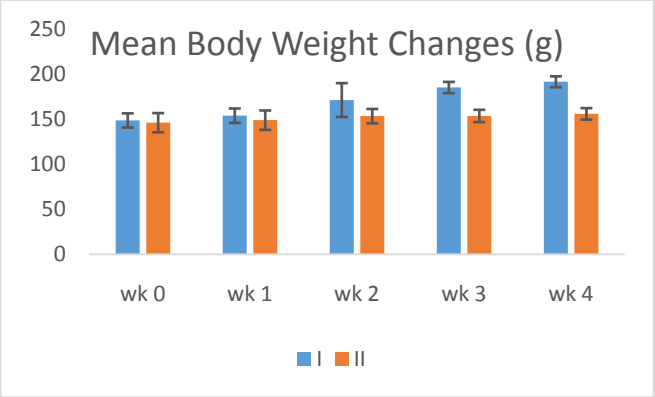

Fig. 1.*Shows the mean difference in weight of rats in the experimental groups after 28 days of administration. P < 0.05 is considered to be significant.*
Key: *I (control group), II (group administered DMOSM) 480 mg/kg orally*

3.4 Haematology

The results (mean ± SEM) of the haematological parameters (packed cell volume, haemoglobin concentration, red blood cell count, total leukocytes, neutrophils, lymphocytes and total protein) for the test group was not significantly different (P < 0.05) compared to the control group at the end of the experiment as shown in table 2 below;

	Grouping	
Parameters	**I**	**II**
PCV (%)	40.50±1.32	44.25±1.84
Hb (g/dl)	13.48±0.44	14.70±0.61
RBC (10^6/μL)	6.80±0.29	7.38±0.30
MCV (fl)	59.65±0.96	60.08±1.87
MCH (pg)	19.84±0.31	19.83±0.72
MCHC (g/dl)	33.27±0.02	33.22±0.02
WBC (× 10^3/μL)	6.85±1.14	8.28±0.75
NEU	23.00±5.15	22.50±1.32
LYMP	76.50±5.01	76.50±1.19

Table 2: Key*; RBC = Red blood cell count, Hb = Haemoglobin, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, WBC = Total white blood cell count, NUE = Neutrophil, LYMP = Lymphocyte*

3.5 Clinical Biochemical Parameters

The only significant changes (P < 0.05) observed in the biochemical parameters at the end of the study were decrease in serum creatinine (25.25±11.60) and increase in serum phosphate (4.64±0.46) concentrations when it was compared with those of the control groups (64.00±11.60) and (3.27±0.24) respectively as shown in table 3 below; The remarkable decrease in serum creatinine level may be attributed to the antinutrients present in the seeds of *Moringa oleifera* which probably affected the appetite of the rats or impaired nutrient uptake and utilization evidenced by the reduction in mean body weight. This effect could not be associated with renal or hepatic insufficiency because the associated biochemical parameters investigated as well as postmortem examination were negative for signs of toxicity. The decrease in serum creatinine concentration observed in this study corroborate those of other authors [32, 34, 35]. The significant (P < 0.05) increase in serum phosphate concentration in the test rats (4.64±0.46) when it was compared to the rats in the control group (3.27±0.24) may be due to the reported high phosphorus in the seeds of this plant [25, 36] or as a result of the myodegenerative changes.

	Grouping	
Parameters	Group I	Group II
ALT (IU/L)	132.5±35.41	129.5±4.44
AST (IU/L)	158.8±25.31	209.0±22.87
ALP (IU/L)	839.3±22.38	750.8±82.81
Albumin (g/dl)	37.00±1.47	37.75±1.03
Creatinine (mg/dl)	64.00±11.60[a]	25.25±1.93[b]
Urea (mg/dl)	8.75±0.53	11.30±1.08
TP (g/dl)	6.45±0.21	6.65±0.30
Na	159.0±3.00	159.5±5.12
Cl	114.8±2.14	120.8±3.59
K	5.80±0.43	6.23±0.33
HCO_3	20.75±3.25	24.50±4.01
Ca	2.17±0.31	1.97±0.12
PO_4	3.27±0.24[a]	4.64±0.46[b]

Table 3: *Means of biochemical parameters in the experimental groups*

Key*: ALT = Alanine amino transferase, AST = Aspertate amino transferase, ALK = Alkaline phosphatase, TP = Total protein, Na^+ = Sodium, K = Potassium, Cl^- = chloride, $HC0_3$ = Bicarbonate, Ca^{2+} = Calcium ion, $P0_4$ = Phosphate*

Superscripts with different alphabets (a, b) are statistically significant, where as those with similar alphabets (a, a or b, b) are not statistically different.

3.6 Gross and Histopathological Findings

At post mortem, there were no gross lesions observed on the carcass, also no microscopic lesions were observed in tissue sections of the liver, kidney and brainof the rats administered defatted *Moringa oleifera* seed meal at the end of the study (day 28).

The results of this study agrees with those of other authors that the seeds of *Moringa oleifera* are relatively safe and can be utilized as feed/food supplement in the diet of animals and man [37,38, 39, 40].

4. Conclusion and Recommendation

The result of this study revealed that defatted *Moringa oleifera* seed meal contain both useful and deleterious secondary metabolites. The antinutrients present in the seeds of *Moringa oleifera* may be responsible for the reduction in body weight evidenced by low serum levels of creatinine and high serum phosphate concentration which are indicative of myodegenerative changes/ muscle atrophy. Defatted *Moringa oleifera* seed meal did not produce any remarkable haematotoxic, hepatotoxic, nephrotoxic or neurotoxic effects following acute and sub-acute administration. It also did not produce any gross or microscopic lesion in the tissues investigated at the selected dose and duration of study, and therefore, can be said to be relatively safe as feed/food supplement for animal and human consumption. However,we recommend adequate processing of the seeds through boiling and or roasting to minimize the deleterious effect of the antinutrients present in the seeds of this plant. This negative effect of the plant with respect to body weight reduction could be positively explored in obese conditions in future research.

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6. Conflict ofInterest

The authors declare that there is no conflict of interest associated with this study.

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