Sub-acute Toxicity Study of Defatted *Moringa oleifera* Seed Meal Administration on Wistar Rats


**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 1 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 defatted *Moringa oleifera* seed cake was performed using standard procedures. Blood samples were collected from sacrificed animals at day 28 and analyzed for haematobiological 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 et al., 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 Karthikey et al., 2009; 8 Abdulmoneim and Abu, 2011; 9 Manivasagaperumal et al., 2012], Biosorption [10 Sahabit 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**

*Moringa oleifera* 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 used 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°C 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 *Moringa oleifera* (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.

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

2.4.1 Experimental animals
A total of 8 apparently healthy 8 to 10-week old Wistar rats (*Rattus norvegicus*) 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].

2.5 Therapeutic effect of feeding defatted *Moringa oleifera* 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.

2.6 Experimental 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 480 mg/kg by oral gavage daily for 28 days.

2.7 Blood sampling for haematological and biochemical analyses
About 7 ml blood was collected via jugular venesection from 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
distilled water 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 (Ca²⁺), phosphate (PO₄⁻), chloride (Cl⁻) and bicarbonate (HCO₃⁻) using the automated
Audiocombanalysyer (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 glycosides at various concentrations as shown in table 1. These phytoconstituents possess diverse potentials as pharmaceutics and nutritional supplements [25 Auwalet al., 2010; 26 Kakengiet al., 2007; 27 Kasololet al., 2010; 28 Olugbemiet 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; 31Zadeet al., 2013]. The presence of these secondary metabolites from different extracts of the seeds of Moringa oleifera has been reported [32Ajibadeet al., 2012; 33Zadeet al., 2013].

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>−</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>−</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>−</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>−</td>
</tr>
</tbody>
</table>

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).
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;

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I (control)</th>
<th>II (administered DMOSM 480 mg/kg orally)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>40.50±1.32</td>
<td>44.25±1.84</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.48±0.44</td>
<td>14.70±0.61</td>
</tr>
<tr>
<td>RBC (10⁶/µL)</td>
<td>6.80±0.29</td>
<td>7.38±0.30</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>59.65±0.96</td>
<td>60.08±1.87</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.84±0.31</td>
<td>19.83±0.72</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.27±0.02</td>
<td>33.22±0.02</td>
</tr>
<tr>
<td>WBC (× 10³/µL)</td>
<td>6.85±1.14</td>
<td>8.28±0.75</td>
</tr>
<tr>
<td>NEU</td>
<td>23.00±5.15</td>
<td>22.50±1.32</td>
</tr>
<tr>
<td>LYMP</td>
<td>76.50±5.01</td>
<td>76.50±1.19</td>
</tr>
</tbody>
</table>

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

Acknowledgment

The authors are grateful to the staff of Clinical Pathology Laboratory, Department of Veterinary Pathology, Ahmadu Bello University, Zaria; Pharmacognosy Research Laboratory, Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria and Multi-purpose Laboratory of National Research Institute for Chemical Technology, Zaria for their technical assistance. Authors are also thankful to the Director General, Raw Material Research and Development Council, Abuja, Nigeria for financial support.

Conflict of Interest

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

References


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