IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 5 Ver. IV (Sep. - Oct.2016), PP 74-77
www.iosrjournals.org

Toxicological Studies on the Ethanol Extract of Moringa Oleifera Seeds.

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Abstract: *The use of Moringa oleifera seed as an ingredient in the formulation of nutraceuticals has gained prominence in recent times. This study investigated the acute and sub-acute effect of the ethanol extract of M. oleifera seed. For the acute toxicity study a total of 17 mice were used, and the LD_{50} of the extract calculated using the geometric mean method. The sub-acute test involved 15 rats, separated into three groups of five rats each. Two of the groups were orally administered 100 and 200 mg kg^{-1} of the extract for 28 days, whereas the control received normal saline. Serum samples from the rats were subjected to hematology and LFT analyses using auto-analysers. Result of the acute toxicity study showed the extract to have LD_{50} of 284.84 mg kg^{-1}. For the sub-acute studies, there was no significant ($p>0.05$) perturbation of the LTF biomarkers assayed when compared to the control. The haematological indices also showed no significant ($p>0.05$) difference between test and control. However, the platelet count was significantly ($p<0.05$) elevated in the test (200 mg kg^{-1}) compared to the control; with values of 504.75 ± 71.32 and 165.00 ± 84.69 (10^9 L^{-1}), respectively. This result further reinforces the use of M. oleifera in medicine and diet.*

Keywords*: Moringa oleifera, nutraceuticals, ethanol extract, toxicology, Wister rats, haematology.*

I. Introduction

The use of natural products to fortify food in order to boost nutritional quality or to achieve a therapeutic target has seen a global rise within the past decade [1]. These products called nutraceuticals, herbals, dietary supplements or functional foods basically serve to improve health or minimize the risk of disease through prevention [2,3]. Most of these nutraceuticals often contain bioactive agents that are antioxidants, omega-3 PUFAs, probiotics among others [2].

Moringa oleifera Lam (Moringaceae), colloquially called "drumstick," is a food plant commonly cultivated in many countries of the tropics and subtropics, and highly valued for its nutritional and medicinal qualities [4, 5, 6]. There are several anecdotal and empirically-validated claims as regard the effectiveness of different parts (leaves, seeds, pods, roots, flowers) of the plant in curing a plethora of ailments [6]. The past two decades have recorded a substantial rise in interest on the plant, and as such a proliferation of its use, especially as nutraceuticals or herbal formulation. Nigeria in particular has recorded highest interest in the plant over the past decade (Google Trends, as at August 2016). Today, it is commonplace to see various herbal formulations peddled by road side vendors or marketed by drugs stores purported to contain parts of *M. oleifera* in Nigeria. However, as these herbal products fortified with *M. oleifera* parts are often not screened to ascertain their toxicological properties and safe-dose levels, they often pose health risks to unsuspecting users. Although Nigeria has a food and drug regulatory agency (NAFDAC-modeled after the Food and Drug Administration of the USA) whose purview it is to ensure the safety of such preparations, nonetheless, several of these formulations are not toxicologically screened for adverse side effects [7,8]. This research therefore investigated the toxicological impact (acute and sub-acute) of the extract of *M. oleifera* seeds on experimental animal models in a bid to provide empirical data on its toxicological properties. This work is an extension upon the works done by other researchers [9,10] who assessed the toxicological effect of the aqueous extract of the leaves and seeds, respectively.

II. Materials and Method

2.1 Collection and authentication of plant material

Fresh *Moringa oliefera* pods were collected from Saminaka, Kaduna state, and was identified and authenticated by a taxonomist with the Department of Botany, Nnamdi Azikiwe University, Awka.

2.2 Preparation of plant material

The pods were broken to expose the seeds, hulled and crushed using a mortar and pestle. The seeds were ground into a fine powder with an electronic blender (BLG-555 Binatone, India).

2.3 Extraction of plant material

The extraction procedure of plant materials as indicated by Harborne [11], and Culei [12] were employed. A quantity (200 g) of M. *oleifera* seed was macerated in 500 ml of ethanol (80 %) for 72 h by shaking the mixture at regular intervals. The mixture was filtered using Whatman No. 1 filter paper in a Buchner funnel. The filtrate obtained was left to evaporate at room temperature away from direct sunlight for 48 h. This was subsequently lyophilized (Yorke, Scientific Industries PVT, India) to obtain the crude extract which was refrigerated at 4^oC until needed.

2.4 Animal Handling and Preparation

Seventeen Swiss mice were used for the acute toxicity investigation, while a total of 15 male Wister albino rats were used for the sub-acute toxicity studies. The animals were purchased from the zoological garden of Faculty of Pharmaceutical Sciences, of Nnamdi Azikiwe University Awka, housed in well ventilated cages, and allowed 7 days for acclimatization before the commencement of investigation.

2.5 Acute Toxicity Study

The two-step, geometric-mean method as described by Lorke [13] was used to obtain the LD_{50} of the extract. In the first phase 9 mice, divided into three groups, were administered extract doses of 10, 100, and 1000 mg/kg b.wt intraperitoneally. The treated animals were monitored for 24 h for mortality and abnormal behaviors. In the second phase, 8 mice dived into four groups were administered 200, 400, 800, and 1600 mg/kg b.wt of the extract, and the treated animals again monitored for 24 h. The LD_{50} was calculated as the geometric mean of the lowest dose causing death and the highest dose not resulting in death.

2.6 Sub-acute Toxicity Study

Fifteen Wister rats were used for this, in conformation with OECD's guideline 407 (repeat-oral dose toxicity study)[14]. The animals were divided into three groups of five rats per group; two groups served as test and were orally administered 200 and 400 mg/kg b.wt of the extract for 28 days, while the third group (control) was administered normal saline. On the 29^{th} day, the animals were anaesthesized with chloroform and blood samples collected via cardiac puncture for biochemical and haematological analyses.

2.6.1 Biochemical and Haematological Analysis

Blood samples collected from the rats were centrifuged at 3000 rpm (Rotofix 3A, Hettich Instruments, USA) to obtain the serum that were used for biochemical assay for; alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, protein, albumin and alkaline phosphatase. The analysis was carried out with a clinical chemical autoanalyzer (Mindray BS 200, Canada). For haematological studies, haemaglobin (HGB), haematocri (HCT), White blood cells (WBC) , Mean corpuscular haemaglobin (MCHC), Mean corpuscular volume (MCV) and platelet (PLT) counts were conducted using an auto haematology analyzer (Mindray BC-6800, Canada).

2.7 Statistical Analysis

Collected data were subjected to one-way analysis of variance (ANOVA) for comparison using the SPSS software (Version 15, SPSS Inc, Chicago, USA). Results were expressed as mean ± SEM and variations were considered significant when $P < 0.05$.

III. Results

The results of the acute and sub-acute toxicity study of the ethanolic extract of *M. oleifera* are presented below. Value are presented as mean ± SEM where applicable

3.1 Acute Toxicity Study

Table 1: Showing death pattern of Swiss mice upon administration of graded- dose of *M. oleifera* seed extract.

Dose (mg/kg b.wt)	Death pattern within 24 h
Stage I	
10	0/3
100	0/3
1000	2/3
Stage II	
200	0/2
400	½
800	2/2
1600	2/2

LD_{50} = 282.84 mg/kg b.wt

The LD_{50} was calculated as the geometric mean of the lowest dose causing death and the highest dose not resulting in death.

3.2 Sub-Acute Toxicity Study

The results of the biochemical and haematological assays are presented below

Table 2: Effect of ethanolic extract of *M.oleifera* seeds on the activity (U/L) of liver enzymes and other liver function indices.

Parameter	**Control**	**Test**	
		100 mg/kg	**200 mg/kg**
AST	170.20 ± 48.8	166.60 ± 42.11	69.00± 4.09
ALT	77.00 ±9.74	76.00 ± 12.40	92.40 ± 7.93
ALP	221.80± 40.22	186.80 ± 15.81	211.25 ± 7.93
Total Bilirubin (mg/dL)	2.62 ± 0.34	2.54 ± 0.22	2.93 ± 0.44
Conjugated bilirubin (mg/dL)	1.80 ± 0.34	1.96 ± 0.37	2.37 ± 0.32
Unconjugated bilirubin (mg/dL)	0.82 ± 0.33	0.58 ± 0.05	0.53 ± 0.16
Total protein (g/dL)	7.24 ± 0.28	7.56 ± 0.31	6.74 ± 0.65
Albumin (g/dL)	4.12 ± 0.20	3.80 ± 0.09	3.57 ± 0.24
Globulin (g/dL)	3.12 ± 0.27	3.66 ± 0.33	2.90 ± 0.24

The table above shows the effect of ethanol extract of *M. oleifera* seeds at doses of 100 and 200 mg/kg b.wt on various liver function parameters. There was no significant difference (P> 0.05) between the test and control rats in all the indices measured. *Values are expressed as mean ± SEM*

Table3: Effect of ethanolic extract of *M. oleifera* seed on haematological indices.

Parameter	**Control**	**100 mg/kg**	**200 mg/kg**
WBC (10^9/L)	6.62 ± 1.65	8.92 ± 1.83	10.40±0.96
HGB (g/dL)	11.00 ± 0.82	11.66 ± 1.40	12.60±0.54
RBC (10^{12}/L)	6.54 ± 0.43	7.02 ± 0.77	7.24±0.23
HCT (%)	35.78 ± 2.65	37.16 ± 4.11	39.37±1.94
MCV (fL)	54.60 ± 0.58	52.90 ± 0.68	54.70±0.99
MCH (pg)	16.72 ± 0.21	16.44 ± 0.33	17.52±0.24
MCHC (g/dL)	30.68 ± 0.10	31.18 ± 0.45	32.10±0.32
RDW-SD (fL)	30.10 ± 0.49	30.68 ± 0.58	30.63±0.62
RDW-CV (%)	15.14 ± 0.36	16.14 ± 0.34	15.57±0.32
PLT (10^9/L)	165.00 ± 84.64	172.30 ± 62.14	*504.75 ± 71.32

The above table compares the effect of ethanolic extract of *M. oleifera* on heamatolical parameters at doses of 100 and 200 mg/kg against a control. There was no significant difference (p >0.05) in the parameters assayed, except for platelets which was markedly elevated (p< 0.05) in the group administered 200 mg/kg. (*) signifies that value differ significantly from control.

IV. Discusion

Acute toxicity studies on the ethanolic extract of *M. oleifera* seed showed the seeds to possess a relatively low lethal dose (282.84 mg kg^{-1}) when administered intraperitoneally to Swiss albino mice. This result is collaborated by the result of a previous study carried out by Ferreira *et al.*,[15] who reported an LD_{50} of 446.5 mg kg^{-1} when water extract of *M. oleifera* seed was administered to mice. However, Ajibade *et al.*,[16] obtained a much higher median LD_{50} of 3873 mg/kg when they administered methanol extract of the *M. oleifera* seeds to mice. The low LD_{50} obtained in this study may be as a result of the route of administration; unlike orally administered agents, intraperitoneally administered agents do not undergo extensive metabolism as a result of first-pass effect. Again, orally administered agents often fail to be absorbed efficiently in the gastrointestinal tract as a result of interactions with other chemicals therein.

The non significant effect of the extract on liver function indices in the sub-acute toxicity study implies that the extract does not exert any hepatotoxic effect upon prolonged exposure to sub-lethal doses. This result is supported by the result of Ajibade *et al.*,[16], who reported that the methanol extract of *M. oleifera* seed at doses of 400 and 800 mg kg^{-1} body weight did not affect the levels of liver function enzymes and biochemicals in rats. However, they observed a marginal increase in the levels of ALT and AST at concentrations upward of 1600 mg/kg in rats.For most of the haematological parameters that were determined, there was no significant difference between the test and control subjects. However, there was a significant increase in the level of platelets of the test group administered 200 mg kg^{-1} body weight of the extract as compared to the control group. Nonetheless, this elevated count (504.75×10^9 L^{-1}) is still within the reference range for platelet ($200\text{-}1000 \times 10^9$ L^{-1}) in rats as noted by Pass *et al.*,[17]. The thrombocytosis induced by the extract may be as a result of increased platelet production by the bone marrow or less likely, by a decrease in platelet clearance by the spleen. The alkaloid content of the seed may be responsible for is thrombocytic property; vincristine, an alkaloidal drug found in several plants, has been shown to transiently increase platelet count [18].

V. Conclusion

The use of herbal preparations and other forms of allopathic therapy in healthcare management is on the rise globally. However, there is a very serious health risk associated with the use of these forms of medicine, especially in developing countries where they are often poorly prepared with little or no factual investigation carried out to ascertain their efficacy and safe-dose levels. The findings of this study indicate that the seed of *M. oleifera* may not pose any serious challenge when administered orally as it did not perturb the levels of key biological markers used in assessing liver and haematological functions. However, caution should be taken if the seed's extract is to be administered systemically, as this research showed that LD_{50} in mice is quite low when administered systemically.

Acknowledgment

We wish to thank the laboratory personnel of Glanson Laboratory, Awka for helping with the haematolgical analysis. Thanks also to Prof. Izundu I of Botany Department, Nnamdi Azikiwe University Awka, for identifying the seed samples used for the study.

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