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The oral subacute toxicological activity of hydroalcoholic extract of *Moringa oleifera* Lam. *(Moringaceae)* growing in South West of Burkina Faso in Wistar rats

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ABSTRACT

Acute and Subacute toxicity investigations were carried out to evaluate the safety of *M. oleifera* leaf used in the treatment of various diseases and in nutrition. Five groups of 10 rats of both sex (5rats/sex) were constituted. The first group served as control and received distilled water 10mL/kg/ day while groups II, III, and IV respectively received orally and daily the ethanolic extract of *Moringa oleifera* at doses 200, 400 and 800mg/kg bw for 4 weeks. The last group (V) considered as satellite group received also received the EEM 800mg/kg daily and orally for 4 weeks. At the end of the test period (4 weeks), the animals of this last group were observed without any treatment for 2 weeks again before their sacrifice in order to study the persistence or the disappearance of possible toxic effects of the extract. The rat was segregated according to gender and housed in cages of 5 rats. In Subacute toxicity investigated with Wistar rats, no mortality was recorded during the experimentation period. Moreover, there was no significant change in weight gain, relative organ weight, or hematological and serum chemical parameters except in a group of female animals where we noticed a reversible decrease in serum ALAT level (at 400mg/kg), total cholesterol (at 400mg/kg) and LDL level (at 800mg/kg) compared to ED group. The histopathological examination had shown some differences between the treated group and the control group that cannot be considered treatment-related.

Keywords: *Moringa oleifera*, Toxicity, Phytochemical, Wistar rat.

INTRODUCTION

traditional medicinal practices are most often the preliminary health care system usable for indigenous peoples, especially in nomadic communities. **[1]** However, some herbal medicines, including herbal supplements or so-called botanicals, have drug-like effects that can be dangerous. Indeed, a number of phytochemicals called secondary plant metabolites exist to protect plants from predators such as insects and animals. These secondary metabolites which act on animal's cells and tissues may have beneficial or detrimental effects on the physiology of the human organ. [2, 3]. *Moringa oleifera* Lam. (*M. pterygosperma* Gaertn.) is a fast-growing small tree native to the sub-Himalayan tracts of Northern India, Pakistan, Asia Minor, Africa, and Arabia which has been spread worldwide in tropical and subtropical countries [4, 5]. All parts of this plante have been used in traditional medicine in Burkina Faso for the treatment of various diseases [6]. Moringa oleifera leaf has been widely studied for chemical composition [4, 7-9], antioxidant [7-11], anti-diabetic [14, 15], hypotensive [16-18], antibacterial and antifungal [19, 20] activity, etc. Many studies are also available on the toxicity of seeds but very little data is available on that of the leaves. This study aims to assess the phytochemical profile as well as the possible subacute toxicological effects of *Moringa oleifera* leaf extract in Wistar rats.

MATERIAL AND METHODS

Plant material and extraction

Fresh Moringa oleifera leaves were harvested in southwestern Burkina Faso. The plant was identified at the InfoBio Center of Joseph Ki Zerbo University where a supporting specimen n°16869 was deposited. The leaves were dried at room temperature and powdered. The powder obtained was macerated at room temperature in a 70° ethanol solution (proportion 10%, g/v) for 72 hours. Then, the macerate was filtered and concentrated using a rotary evaporator under reduced pressure in order to extract the ethanol part. The remaining aqueous part was first dried at room temperature under ventilation. Then, the pasty rest obtained was dried in an oven at 40°C. The yield was 16.09%.

Subacute toxicity

Animals and experimental design

Healthy Wistar rats of both sexes weighing between 93-153g were used for the study. Animals were randomly divided into groups of 10 animals (five males and five females). Animals were segregated according to gender and housed in cages, 5 rats in one cage. Animals of the group I served as control, received 10mL/kg/ day of distilled water. Those of groups II, III, and IV respectively received orally (by gavage) and daily the ethanolic extract of *Moringa oleifera* 200, 400 and 800mg/kg bw for 4 weeks. The last group (V) considered as satellite group received also the EEM 800mg/kg daily and orally. During the testing periods, animals were supplied with water and food ad libitum. The body weight of animals was evaluated twice a week during the experience. At the end of the experience, all animals except those of group V were anesthetized decapitated with diethyl ether and blood samples were collected with or without anticoagulant. Blood with the anticoagulant was used immediately for the determination of hematological parameters, while blood without the anticoagulant was centrifuged at 3000rpm for 15min at 4°C. The serum obtained was stored at -4 °C for biochemical parameters. The animals of group V remaining were observed without any treatment for 2 weeks again before their sacrifice. The same parameters as previously mentioned were recorded in other to study the persistence or the disappearance of possible toxic effect the extract. The rats were dissected and different organs were excised and weighed for recording absolute organ weights. The relative organ weights were calculated against terminal body weights for every animal. For histopathology, the specimens of different organs were fixed in10% and preserved in 10% neutral buffered formalin solution. The specimens of liver, kidney, ovary with oviduct, pancreas, testis, were stained with hematoxylin and eosin stain, following the standard laboratory procedures. The stained sections were examined under microscope for any cellular damage or change in morphology.

Biochemical, hematological and tissue analysis

The determination of serum level of some biochemical parameters was performed using standardized enzymatic colorimetric methods by measurement of the optical density of the reaction products at the corresponding wavelength with a spectrophotometer (Genesys 20Thermo Spectronic). Then, total cholesterol (CT), triglycerides (TG) and HDL cholesterol (HDL-C) level were determined according to the manufacturer protocols described by Biolabo assay kits (Maizy, France). C-HDL concentration in plasma was determined by the enzymatic method based on specific precipitation of VLDL and LDL in the presence of magnesium ions. Cypress diagnostics kits (UV kinetic test IFCC. HBE07 Code) were used for the measurements of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while creatinin, and total bilirubin (TB) were determined using Fortress diagnostics kits (United Kingdom). The Ham screen 18 Haematology analyzer was used to determine hematological parameters including red blood cell (RBC), granulocyte differentiation (GR), white blood cell (WBC), platelets (PLT), lymphocyte counts (LY), hematocrit (Hct), Hemoglobin (Hgb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), the white blood cells count (WBC) and monocyte (MO).

Statistical analysis

The results are presented as the mean ± standard error of the mean. The comparison between means was made using a one-way ANOVA, followed by Tukey's post-test using Graph Pad Prism software version 5.03 (Graph Pad Software, San Diego, California, USA). The difference was considered significant if the P value is greater than 0.05

RESULTS

Subacute toxicity

Effects of ethanolic extract of serum chemistry

Table 1: Subacute effect of EEM on serum chemistry

	Male				
	Experimental Groups				
	DW (control)	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**SAT mg/kg**
ALAT (UI/L)	3.27±0.4	4.08±0.55	2.10±0.4	2.92±0.49	2.77±0.59
ASAT (UI/L)	9.80±0.7	6.42±0.67**	6.07±0.68**	3.65±0.11***	6.85±0.59*+
CREAT. (mg/dL)	1.39±0.08	1.63±0.06	2.09±0.35	1,68±0,1	2.46±0.57
BILI (mg/dL)	0.10±0.01	0.09±0.01	0.09±0.01	0.09±0.01	0.16±0.03*+
CT (mg/dL)	82.49±3.67	80.72±5.43	71.09±3.08*	64.39±2.52	66.18±4.86
HDL (mg/dL)	19.34±0.34	17.10±1.2	21.74±1.32	22.13±2.02	16.41±2.56
LDL (mg/dL)	48.53±2.09	49.54±5.86	36.93±3.49	24.24±4.7*	27.64±5.36
TRIG (mg/dL)	73.12±11.84	70.40±7.15	62.08±10.87	90.08±17.3	110.66±9.7
PROT (mg/dL)	7.66±0.07	7.42±0.3	7.53±0.1	7.50±0.12	10.80±0.24
	Female				
ALAT (UI/L)	0.73±0.28	3.15±0.9	2.45±0.34	1.75±0.32	3.21±0.65
ASAT (UI/L)	5.02±0.75	4.96±0.57*	4.47±0.75	5.10±0.93	6.07±0.6*
CREAT (mg/dL)	1.44±0.14	1.82±0.11	1.86±0.1	1.58±0.09	1.78±0.14
TBL (mg/dL)	0.11±0.01	0.08±0.01	0.08±0.01	0.09±0.00	0.12±0.01
CT (mg/dL)	66.99±5.76	64.76±5.31	55.90±5.39	68.92±6.16	51.24±4.55
HDL (mg/dL)	17.57±1.29	18.06±1.44	17.35±0.84	18.13±1.26	19.24±1.48
LDL (mg/dL)	34.16±5.15	35.44±4.1	27.61±5.23	34.91±3.81	21.19±3.53
TG (mg/dL)	76.32±15.48	56.32±6.02	54.72±7.59	79.36±13.59	54.08±13.5
TP (mg/dL)	8.19±0.04	7.82±0.15	8.02±0.11	7.84±0.11	10.05±0.23

ED or Ctrl: Corresponds to the control group treated with distilled water (1mL / 100g), the other groups received the doses 200, 400 and 800 mg/ kg of EEM.; Sat: satellite group, animals also receiving the highest dose (800mg/ kg) and leave under observation (after 4weeks of treatment) for two weeks without treatment in order to observe possible reversibility. ALT: alanine aminotransferase; ASAT, aspartate aminotransferase; TP, total protein; TBL, total bilirubin; CRE, creatinine; CT, total cholesterol; TG, triglycerides; HDL, high density lipoprotein; LDL low density lipoprotein; * P <0.05, ** p <0.01, *** p <0.001 significant difference compared to control group (ED) of the same sex. + P <0.05 significant difference compared to the opposite sex group; n=5.

Hematological data

Table 2: hematological data for oral subacute treatment of rats with ethanolic extract of *M. oleifera.*

	Male				
	Experimental Groups				
Parameters	**DW (control)**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**SAT (800mg/kg)**
GB (10^3/mm^3)	9.240±1.758	9.06±0.453	9.787±1.098	10.467±2.088	11.100±1.060
GR (10^6/mm)	5.837±0.914	4.780±0.405	4.770±0.726	4.950±0.317	3.667±0.381
HGB (g/dl)	13.760±0.090	13.667±0.767	13.367±1.071	13.550±0.541	11.367±1.112
HCT (%)	38.667±4.372	41.933±1.551	41.333±2.028	41.333±1.333	33.333±4.410
PLA (10^3/mm)	261.667±61.344	245.667±21.620	291.333±29.168	311.333±75.757	230.667±47.079
CCMH (g/dl)	31.100±1.000	30.733±1.732	28.767±0.667	27.733±2.829	36.047±3.424
VMP (10^3/m^3)	12.600±1.422	22.570±4.83	10.767±1.638	15.610±2.022	18.233±0.667
LYM (%)	37.233±9.483	21.570±8.680	36.400±9.739	32.173±3.703	35.633±2.603
MON (%)	6.333±2.624	6.39±2.970	13.633±2.404	22.133±5.988	22.633±5.044
GRA (%)	43.767±10.219	39.733±9.648	40.333±5.185	43.433±7.025	46.433±4.667
	Female				
GB (10^3/mm^3)	9.973±1.447	11.267±0.967	7.092±2.127	8.660±3.163	12.133±1.271
GR (10^6/mm)	4.480±1.036	4.627±0.307	4.488±1.082	4.250±0.544	4.027±0.242
HGB (g/dl)	11.247±0.485	13.000±0.200	11.280±0.902	12.467±0.917	11.800±0.727
HCT (%)	35.133±1.097	39.333±0.333	34.766±0.577	37.000±1.528	34.333±2.295
PLA (10^3/mm)	223.333±17.938	219.667±17.704	210.929±19.099	229.000±35.698	331±82.291
CCMH (g/dl)	31.6±0.40	24.433±11.407	24.419±1.453	34.400±3.350	32.667±2.875
VMP (10^3/m^3)	30.867±6.960	15.267±2.530	9.866±2.004	12.310±0.010	21.900±6.678
LYM (%)	34.900±2.173	33.833±0.584	34.048±3.645	31.30±0.920	32.933±1.136
MON (%)	21.300±5.393	8.163±2.741	11.491±2.019	9.453±1.769	13.233±2.750
GRA (%)	38.633±10.718	27.067±3.379	38.860±3.493	38.367±5.011	38.100±3.821

Data are expressed as mean±SEM, n=5; no significant difference between the different groups of animals of both sexes subjected to the oral subacute test using EEM.

Subacute effects of ethanolic extract of M. oleifera in relative body weight gain and relative organs mass in wistar rat.

Table 3: effects of ethanolic extract of *M. oleifera* in relative body weight gain in Wistar rat

	Male				
	Experimental Groups				
Weeks	**ED**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**SAT (800mg/kg)**
W0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
W1	17.77±2.52	19.45±1.03	16.21±3.36	16.88±2.30	13.34±1.30
W2	49.03±4.67	45.72±2.53	37.57±4.96	45.67±5.20	42.39±1.03
W3	59.51±6.62	56.19±4.28	51.82±7.20	60.20±7.32	54.76±2.59
W4	73.44±8.31	67.24±4.11	60.30±8.16	67.59±7.30	58.86±5.73
	Female				
W0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
W1	15.86±2.80	11.79±1.07	14.16±3.10	16.58±2.64	16.98±5.06
W2	33.25±4.04	34.61±1.94	34.58±6.21	42.44±7.26	30.61±2.45
W3	41.78±4.63	44.66±3.94	39.56±7.39	53.80±9,21	40.96±3.54
W4	50.42±4.63	48.42±4.34	45.40±7.91	59.49±8,84	44.94±4.14

The data expressed in this table are the mean of weight gain ±SEM. Weight gain= $[(P0-P_{Sn})/P0]*100$; P0: initial weight; Psn: Weight at the nth week; Wn = nth week; n= 5. No significant difference was noticed between the different groups in male and female animals. Similarly, no difference in weight gain was observed between males and females. Furthermore, we did not record any mortality or morbidity during this experiment. n=5

Table 4: effects of ethanolic extract of *M. oleifera* on relative organs mass in Wistar rat

	Female				
	Experimental Groups				
Organs	**ED**	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**SAT (800 mg/kg)**
Kidney	0.620±0.009	0.618±0.019	0.621±0.018	0.616±0.009	0.616±0.009
Spleen	0.260±0.017	0.335±0.013	0.550±0.149	0.316±0.029	0.316±0.029
Liver	3.270±0.051	3.525±0.075	3.372±0.218	3.581±0.086	3.581±0.086
Testicle	1.182±0.018	1.102±0.034	1.342±0.058	1.277±0.084	1.262±0.109
	Females				
Kidney	0.593±0.027	0.610±0.017	0.639±0.033	0.637±0.011	0.661±0.037
Spleen	0.283±0.014	0.372±0.059	0.320±0.014	0.349±0.049	0.289±0.025
Liver	3.426±0.099	3.641±0.177	3.390±0.163	3.844±0.214	3.953±0.216
Ovary	0.072±0.003	0.087±0.017	0.074±0.010	0.074±0.007	0.063±0.003

Data are expressed as mean of relative organ weight±SEM, n=5; relative organ weight= $\frac{m}{M}x100$; m: weight of organ; M: weight of animal; No significant difference was observed in the relative weight of organs in different groups of male and female animales

Phytochemical analysis

Table 5: Phytochemical screening of EEM

Structural Group	**Result**
Volatil oil	+
Galli tannins	+++
Catecho tannins	-
Phenolic compounds	+++
Poly-Uronides	+
Poly-oses	++
Saponins	++
Reducing substances	++
Anthocyannes	-
Anthraquinones	++
Anthracenic glycosides	-
Fatty acids	+
Coumarins	-
Alkaloids	-
Flavonoids	++
Sterols	+++
Triterpenes	-
Legend: **-**: None; **+:** weak; **++:** Abundant; **+++:** very abundant.	

DISCUSSION

Serum analysis of some biochemical parameters such as transaminases provides information on cell integrity and liver function [21]. Our results showed no variation in the activity of alanine aminotransferase between control group and those subjected to different doses of EEM. Similarly, there was no increase in both aspartate aminotransferase activity and alanine aminotransferase activity. On the other hand, a significant decrease was observed in the level of AST. In satellite animals group this decrease was reversible. In the cells, ALT is located preferentially in the liver cells whereas ASAT is encountered in the cells of many organs such as the liver, kidneys, and pancreas. [22]. Transaminases, ALT and AST are involved in the metabolism of amino acids and carbohydrates. These enzymes are distributed in different animal tissues with a predominance and specificity of ALT activity in hepatic tissue [23]. Elevation of ALT and serum AST activity may occur in conditions of alteration of liver cell membrane permeability, circulatory hypoxia, or exposure to toxins and toxemia, following an inflammation, in case of metabolic disorders or the proliferation of hepatocytes. The liver plays a very important role in the metabolism of food as well as in the detoxification of the body. Increased AST and ALT activities have also been associated with lesions and intestinal, renal, or pancreatic lesions [24-27, 22]. The decrease in serum AST level in our study may be perceived as a beneficial protective effect of EEM on these organs' targets. The Benzylcarbamothioethionates isolated from the roots of *M. oleifera* showed no significant variation in hematological parameters as well as in hepatic and renal function (ALT, AST, creatinine, and bilirubin) [28]. Serum parameters of renal function such as serum creatinine, proteinemia, and bilirubinemia are not affected by different doses of EEM in male and female Wistar rats. The leaves of *Moringa* are known for their antioxidant property and their abundance of polyphenolic compounds, and varied vitamins whose importance in the maintenance of the organism is no longer to be demonstrated [7, 20]. They are known to be non-toxic as evidenced by the LD_{50} in acute toxicity. Lipid profile parameters indicated a decrease in CT at 400mg /kg and LDL at 800mg /kg in males. No significant variation was observed in females. The analysis of the histological sections seems to indicate lesions for certain intermediate doses in particular the dose of 400mg /kg in the spleen and the kidney in the female Wistar rat. Paradoxically, the highest doses of 800 mg /kg showed no signs of a lesion in the same organs. The same result is observed in groups of male animals. In addition, the analysis of serum parameters, as previously mentioned, revealed no evidence of toxicity in all groups of animals treated; Which corroborates the histological analyzes of high doses and low and medium doses. From the above, it can be concluded that the alterations observed are due to artifacts. On the other hand, hypotension was observed during iv administration in normotensive rats. In addition, vasodilatation beyond the baseline of vascular tension was also observed [29]. Thus, *Moringa oleifera* leaf product should be administered with caution.

CONCLUSION

The present study indicated that the ethanolic extract of *Moringa oleifera* did not induce either acute toxicity in mice or subacute toxicity after 28 days of repeated administration in rats. Thus, Nevertheless, *Moringa oleifera* leaf products should be administrated with caution because of the risk of hypotension.

Conflict of Interest

None declared.

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