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RESEARCH ARTICLE

Antihypertensive effects of *Moringa oleifera* leaf extract Lam. (Moringaceae) in NG-nitro-L-arginine-methyl ester-induced hypertensive rats

Adam Gbankoto[1], Mariette Sindete[1], Marius Adjagba[2], Machioud Maxime Sangare[3], Eugene Selidji Attakpa[3], Bonaventure Awede[4]

[1]Laboratory of Experimental Physiology and Pharmacology, Faculty of Sciences and Technology, University of Abomey Calavi, Benin, [2]Unit of Human Biology, Faculty of Sciences Health, University of Abomey Calavi, Benin, [3]Laboratory of Molecular Physiopathology and Toxicology, Faculty of Sciences and Technology, University of Abomey Calavi, Benin, [4]Unit of Physiology, Faculty of Health Sciences, University of Abomey Calavi, Benin

Correspondence to: Adam Gbankoto, E-mail: qgoba@yahoo.fr

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ABSTRACT

Background: The use of natural products such as *Moringa oleifera* (MO) for the improvement of resistance artery relaxation is a potential strategy for the prevention and treatment of hypertension. **Aims and Objective:** The present study was designed to evaluate the protective effect of MO hydro-ethanol extract on NG-nitro-L-arginine-methyl ester (L-NAME)-induced hypertension and the toxicity profile in Wistar rats and shrimp larvae. **Materials and Methods:** An experimental hypertensive model was established by administration of L-NAME at 40 mg/kg body weight (BW) by oral gavages to male Wistar rats for 2 weeks and treated with hydro-ethanol extract of MO leaves and losartan. The control group received distilled water. L-NAME group was treated with L-NAME and distilled water. L-NAME-Losartan group was treated with L-NAME and losartan at 100 mg/kg BW. L-NAME-MO group was treated with L-NAME and the hydro-ethanol extract of MO leaves at 500 mg/kg BW. Blood pressure (BP) was measured by tail cuff method. *In vitro* toxicity study of the extract was performed on *Artemia salina* larvae using a biological test for the determination of shrimp lethality. A 14 days acute oral toxicity study was evaluated in female rats on a single dose of 2000 mg/kg BW of hydro-ethanol extract of MO. **Results:** L-NAME treated rats developed significantly increased BP from 110.20 ± 4.46 mmHg to 159.60 ± 4.38 mmHg. Concurrent oral treatment with hydro-ethanol extract of MO leaves decreased the high BP from 159.60 ± 4.38 mmHg to 102.40 ± 5.07 mmHg. No harm was observed at the tested doses *in vitro* and *in vivo* toxicity studies. **Conclusion:** These findings suggest that MO leaves may be potentially useful as a natural product against hypertension.

KEY WORDS: *Moringa oleifera*; Hypertension; NG-Nitro-L-Arginine-Methyl Ester; Wistar Rat; Toxicity

INTRODUCTION

Hypertension is a public health burden globally and is one of the major risk factors for cardiovascular morbidity and mortality[28] which is defined as blood pressure (BP) greater than or equal to 140/90 at two or more records in two different ambulatory office visits.[2] Risk factors include age, obesity, poor diet, sex, family history, increased sodium intake, excessive alcohol consumption, smoking, metabolic syndrome, and obesity.[2] Hypertension accounts for approximately 9.4 million deaths worldwide[29] and is a major risk factor for several cardiovascular diseases such as stroke, coronary heart disease and stroke, heart failure, left ventricular hypertrophy, and valvular heart disease.[2,4,28] It significantly increases the morbidity and mortality of the adult population

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and represents a significant financial burden for the health system.[3] Peripheral vascular resistance is a determinant of BP and is determined by the contractile state of vascular smooth muscle, particularly in arterioles.[33] Endothelial dysfunction resulting from nitric oxide (NO) deficiency[7] and adrenergic-mediated overactive vasoconstriction contributes to increase peripheral vascular resistance, which leads to elevated BP, which is characteristic of hypertension.[4] In fact, it has been found that the vascular endothelium of hypertensive patients produces less NO. NO released by endothelial cells is an important regulator of vascular function. NO is synthesized from the amino acid L arginine catalyzed by NO synthase (NOS). Therefore, chronic administration of NG-nitro-L-arginine-methyl ester (L-NAME), a non-specific inhibitor of NO, causes high BP in rats.[7]

NO is synthesized from the amino acid L arginine catalyzed by NOS. In addition to acting directly on vascular smooth muscle cells to induce relaxation, NO also inhibits adrenergic neurotransmission and suppresses the nicotinamide adenine dinucleotide phosphate superoxide anion.[4] The animal model of hypertension induced by L-NAME acts mainly by causing NO deficiency and oxidative stress. The inhibition of NO synthesis by L-NAME has many features that reflect human idiopathic hypertension such as reactive oxygen species -induced cardiovascular disorders, inflammation, activation of the sympathetic and renin-angiotensin nervous systems, and as cardiovascular remodeling.[4] The L-NAME-induced hypertension is, therefore, an appropriate model for testing the vascular protection effects of antihypertensive in the context of the NO deficit.[7]

Medicinal plants have been used as a natural source of biologically active compounds with beneficial effects, which can be used to improve human health, and their use is increasingly recognized to prevent and treat certain diseases, including hypertension.[4,20] Some of these biologically active compounds have beneficial effects, which can be used to improve human health. One of these medicinal plants is *Moringa oleifera* (MO). MO is a tropical plant belonging to the family Moringaceae (genus *Moringa*). It is the most cultivated species in the family and is the most widely used[9] because of its phytochemical and pharmacological properties related to human health. Indeed, MO is able to grow in hot, humid and dry environments, including less fertile soils.[20] This plant family is known to possess hypotensive, anticancer, and antibacterial activity.[9] Almost every part of MO tree has enormous properties in medicine, nutrition, water treatment, and a host of human activities or other industrial purposes.[35] The whole plant possesses antimicrobial activity and is also used for the treatment of rheumatic conditions, ascites, diabetes, dyslipidemia, infections, cancer, asthma, and venomous bites and for enhancing cardiac function.[18,39] Pharmacological studies have demonstrated that MO leaf extract has anticonvulsant properties as well as antioxidant, antidyslipidemic, antihyperglycemic, and anticancer activities.[11,36] The leaves exhibit strong hypotensive, diuretic, and spasmolytic effects and have been seen to be useful against inflammation and scurvy.[18] It also rich in a number of vitamins, minerals, and other more common phytochemicals such as carotenoids, including carotene or provitamin.[9] Plant roots have been used as carminatives, anthelmintics, and diuretics and for treating intermittent fever, epilepsy, and chronic rheumatism.[18] It is also known to alter the blood parameters.[9] The plant's seeds have been used for purgation, in fever, and against inflammatory conditions.[18] Although levels of these phytochemicals (bioactive compounds) were higher in the leaves than the seeds, mineral content of MO showed variations in composition with changes in location.[9] The present study was designed to evaluate the protective effect of MO hydro-ethanol extract on L-NAME-induced hypertension and the toxicity profile in Wistar rats and shrimp larvae.

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of MO were collected from the area in Houedo, Atlantique Department of Southern Benin. Authentication was carried out at the National Herbarium of Benin, University of Abomey Calavi, where voucher sample was deposited (YH 239/HNB). Leaves were gently washed twice under running tap water and then washed again in distilled water to remove the sand and dried at room temperature for 14 days, and crushed into coarse powder with electric grinder (Flour mills Nigeria, El Motor N°1827).

Preparation of Plant Extract

Two hundred and fifty grams of powder of MO leaves were extracted with 3 ml × 500 ml of the ethanol water cosolvent system (70:30) for 72 h by maceration with continuous shaking. Macerate was filtered on cotton and Whatman No. 1 filter paper (Whatman International Ltd.; Maidstone, England), then concentrated using a rotary evaporator (Heidolph efficient Laborota 4000) at 40°C under reduced pressure. The extract was then stored in light-resistant bottles and later refrigerated awaiting further work after determining the yield according to the formula: % Yield of extraction = (weight of extract/weight of initial powder taken for extraction) × 100.[30]

Phytochemical Analysis of MO Leaves

The phytochemical screening to identify the major compounds present in MO leaves was carried out according to the classical method of Houghton and Raman.[14] Identification tests of these extracts were carried out to detect various phytoconstituents present in MO leaves, such as alkaloids

(Mayer test), tannins (ferric chloride test), flavonoids (Shinoda test), triterpenoids (Liebermann–Burchard test), saponins (foam test), and phenols (ferric chloride test).

Experimental Animals

Twelve-week old healthy Wistar rats (*Rattus norvegicus*) of both sexes weighing 180 ± 20 g were used. Both sexes were maintained separately in standard cages with top grill, and the selected female rats were nulliparous and non-pregnant. Each animal was assigned a unique identification number. The animals were sourced and kept in the laboratory animal room of the Teaching and Research Unit in Human Biology of the Faculty of Health Sciences (FSS) of Cotonou, University of Abomey Calavi, Benin. The rats were kept in a controlled environment at ambient temperature with 12/12 h light natural and dark cycles throughout the experiment. They were fed with standard rat pellet food (Complete Food, Group Veto Services S. A., Benin) and drinking water *ad libitum* during the experiment. Brine shrimp eggs marketed by German company (JBL GmbH D-67141 Neuhofem) were used to assess cytotoxicity. The protocol was conducted in accordance with the institutional guidelines and Ethics Committee on research of the Institute of Applied Biomedical Sciences of Cotonou, Benin, under the number December N°075/CER/ISBA-2015.

L-NAME-induced Hypertension in Male Wistar Rats and Treatment

L-NAME-induced hypertension was established by the administration of L-NAME in male Wistar rats with normal BP.[42] The rats were divided into two major groups, namely the normal control group and the hypertensive group L-NAME. The normal control group received distilled water orally for 2 weeks. The hypertensive group received L-NAME (40 mg/kg body weight [BW])[32] orally for 2 weeks.[17] To monitor development of hypertension during L-NAME administration, systolic BP (SBP) in conscious rats was measured before (baseline) and thrice a week during the treatment period using non-invasive tail-cuff BP method with the CODA™ 20942 device (Kent Scientific Corporation, USA).[3,37] Hypertensive rats were assigned into four groups of five animal per group which were administered by gavages once daily for 2 weeks distilled water, MO extract or losartan as follows: Control group: Normal rats administered distilled water; L-NAME Group: L-NAME hypertensive rats administered distilled water; L-NAME-Losartan Group: Hypertensive rats administered losartan at 100 mg/kg of BW; and L-NAME-MO Group: Hypertensive rats administered hydro-ethanol extract of MO leaves at 500 mg/kg BW.

Non-invasive Tail-cuff BP Measurement

BP was measured in conscious rats, using non-invasive tail-cuff plethysmography (CODA™ 20942 Non-Invasive BP System, Kent Scientific Corporation, USA).[3,37] The following programmable settings were done for the device to give reproducible BP measurements: Ten preliminary unrecorded measurements were done to warm up and give a waveform after placing each rat into the device; each session included two sets of 10 measurements, 10 unrecorded sessions, and 10 actual recorded session; rats were kept in a restrainer placed at 37°C for 15 min on a thermostatically regulated warming plate with mini-cuffs fix around the tail to detect the artery pulsations. SBP and diastolic BP were measured thrice a week.

Toxicity Test on *Artemia salina*

The larval toxicity of the hydro-ethanol extract of MO leaves was evaluated according to an adaptation of the method described by Ahouansou *et al*. and Duarte *et al*.[5,10] 10 mg of the eggs of *A. salina* were incubated in 100 ml of seawater. After 48 h of incubation with continuous stirring, the larvae were collected using a Pasteur Pipette and dissolved in dimethyl sulfoxide. A series of 10 dilutions from 25 mg/ml to 0.049 mg/ml (25.00, 12.500, 6.250, 3.125, 1.562, 0.781, 0.390, 0.195, 0.097, and 0.049 mg/ml) of hydro-ethanol extract of MO leaves was prepared. A colony of 16 larvae was introduced into each solution. All solutions, including the control, were allowed to stir continuously at room temperature for 24 h, after this, the number of surviving larvae is counted for each solution and the calculated mortality at each concentration. In cases where control deaths were detected, the percentage of mortality (% M) was calculated as follows: $\% M = [(X–Y)/X] \times 100$ with X = Percentage of survival in the control and Y = Percentage of survival in the treatment.[1] The data (dose-response) were transformed to logarithmic, and the LD_{50} was determined by linear regression. To assess the degree of toxicity from LD_{50} lethal dose values, we used the correspondence table prepared by Mousseux.[43]

Acute Oral Toxicity Test

The oral acute toxicity study of MO leaves extract was carried out in accordance with Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals Section 4, n° 423 limit test, adopted in 2001[24] 9 females rats, nulliparous and nonpregnant were grouped into three groups of three rats each and kept in their cage for 5 days acclimatization.[19,25] After acclimatization period, all groups of the rats fasted overnight before extract administration. After the fasting period (12 h), the first experimental group received a single dose of 300 mg/kg BW of the freshly prepared extract; single dose of 2000 mg/kg BW of the freshly prepared extract was administered to each rat of Group 2; control group treated with vehicle, 10 ml/kg BW of distilled water to establish a comparative control group according to the OECD guideline. All substances were administered orally using an oropharyngeal metal cannula. After administration, the

rats have further fasted for 4 h, all animals were observed individually to monitor physical and behavioral alteration providing special attention to first 30 min, 2 h, 4 h, 6 h, 10 h, and 24 h after exposure and then twice daily for mortality and morbidity until the end of treatments. In addition, consideration was given for observations of convulsions, tremors, diarrhea, salivation, lethargy, sleep, and coma. The food consumption recorded daily while the BW of animals recorded at the end of each week and the percentage of BW change calculated according to the equation (BW at the end–initial BW/initial BW) × 100.[34] At the end of treatment, the rat was fasted overnight, but the animals had free access to water. They were then euthanized by chloroform inhalation in a saturation closed jar containing cotton wool soaked in chloroform for blood collected by retro-orbital puncture with the help of capillary tubes early in the day for biochemical analysis. After exsanguinations, the liver and kidneys of the animals were carefully removed, weighed and rinsed in 0.9% sodium chloride solution and fixed in 10% buffered formalin for histological study.[15] The relative weight of the organs was also calculated using the formula: Relative weight = (Weight of the organ/BW of the animal on sacrifice day) × 100.[34]

Statistical Analysis

The data were expressed as mean (±standard error of the mean). The averages were analyzed using analysis of one-way analysis of variance and supplemented by Student's *t*-test. The post-test analysis was performed using Dunnett's multiple comparison tests to determine significant differences in all parameters. Values were considered significantly different when $P < 0.05$. The analysis and construction of the graphics were done using the GraphPad Prism software version 6.00 (GraphPad Prism Software, Inc., San Diego, California).

RESULTS

Plant Extract and Phytochemical Analysis of MO Leaves

The yield of the hydro-ethanol extract of MO leaves after drying was 11.04%. The phytochemical screening showed the presence of alkaloids, tannins, flavonoids, triterpenoids, steroids, anthocyanins, and mucilages [Table 1].

Effect of MO Leaves Extract on L-NAME-induced Hypertension in Male Wistar Rats

Daily administration of L-NAME caused a significant increase in mean arterial pressure from 110.20 ± 4.46 mmHg to 159.60 ± 4.38 mmHg. This pressure decreased significantly ($P < 0.05$) after the losartan administration (88.20 ± 5.76 mmHg) as well as after hydro-ethanol extract of MO administration (102.40 ± 5.07 mmHg) [Figure 1].

Table 1: Phytochemical finding of *Moringa oleifera* leaves powder

Metabolites	Tests performed	Inference
Alkaloids	Mayer	+
	Dragendorff	+
Tannins	Ferric chloride	+
Flavonoids	Shinoda	+
	Ferric chloride	+
Triterpenoids	Liebermann–Burchard	+
Steroids	Liebermann–Burchard	+
Anthocyanins	Shinoda	+
Saponins	Foam	−
Cyanogenic derivatives	Guignard	−
Mucilages	Precipitation	+
Reducing compound	Fehling liquor	−
Quinoid derivatives	Bornträger	−
Leucoanthocyanins	Shinoda	−
Coumarin	Ammonia	−

(+) indicate the presence of the compounds, (−) indicate the absence of the compounds

Effect of MO Leaves Extract on *A. salina*

Larval mortality is noticeable from 0.095 mg/ml with a 50% lethal concentration (LC_{50}) of 0.78 mg/ml extract [Figure 2]. This concentration shows no toxicity to *A. salina* larvae because it is >0.1 mg/ml (LC_{50} >0.1 mg/ml) according to the LC_{50} correspondence scale and larval toxicity.[43]

Effect of MO Leaves Extract on BW and Food Intake in Acute Oral Toxicity Test

No clinical symptoms such as behavioral and neurological changes were detected during the experiment. No mortality was observed within 4 h of continuous observation and also after 24 h. There was also no lethal effect observed after the administration of the extract for the experimental period of 14 days. During the 2nd week of the assay, there was an insignificant decrease in food consumption in treated rats [Figure 3]; this decrease was not observed in control group. However, a gradual increase in BW [Figure 3] was observed in both the control and treatment groups during the test.

Effect of MO Leaves Extract on Biochemical Parameters in Acute Oral Toxicity Test

Tests carried out on biochemical parameters reveal no significant variation in the rate of creatinine, protein, urea, electrolytes (sodium, potassium, and chloride ions), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) enzymes compared to controls. A significant increase in blood glucose was observed for treated lots followed by a significant decrease in total cholesterol for the batch treated at 2000 mg/kg BW [Table 2].

Effect of MO Leaves Extract on BW Gain, Absolute, and Relative Organ Weight in Acute Oral Toxicity Tests

The administration of the extract to female rats did not result in any significant difference ($P > 0.05$) in the percentage weight gain of the treated group compared to the control group as well as the absolute weight of the kidneys and liver [Table 3]. No significant difference was observed for the relative weight of kidneys and liver for treated groups compared to control [Figure 4].

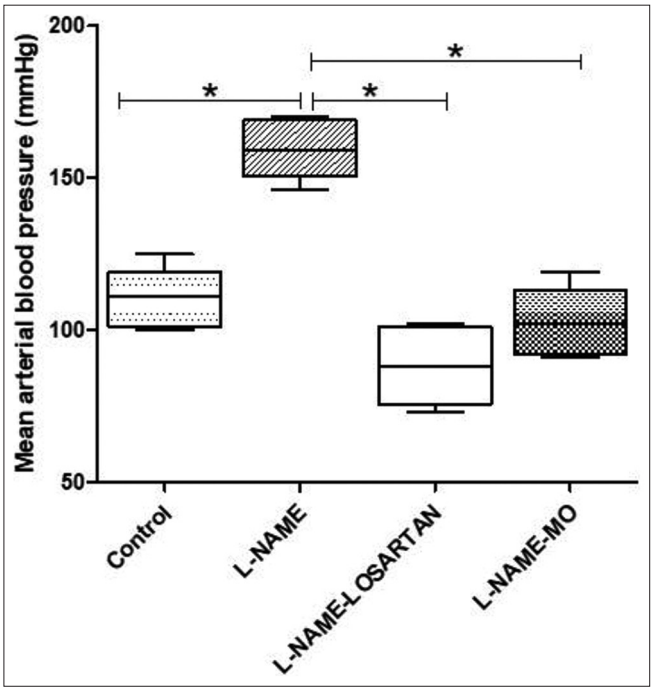

Figure 1: Effect of hydro-ethanolic extract from *Moringa oleifera* (MO) leaves on mean arterial pressure. The control group received 10 ml/kg body weight (BW) distilled water from day 1 to day 28. NG-nitro-L-arginine-methyl ester (L-NAME) group was treated with L-NAME at 40 mg kg day BW from day 1 to day 14 and distilled water at 10 nil kg day BW from day 15 to day 28. L-NAME-Losartan group was treated with L-NAME at 40 mg kg day BW from day 1 to day 14 and with losartan at 100 mg kg day of BW from day 15 to day 28. L-NAME-MO group was treated with L-NAME at 40 mg kg/day BW from day 1 to day 14 and with hydro-ethanolic extract of MO leaves at 500 mg/kg/day BW from day 15 to day 28. Values are means of five replicates ± standard error of the mean: Data shown as box plots representing medians with 25th and 75th percentiles and whiskers illustrating 10th and 90th percentiles of mean arterial blood pressure of rats, *$P < 0.05$

Effect of MO Leaves Extract on Histopathological Changes in Acute Oral Toxicity Test

The histology of the liver cross-section of a control group [Figure 5] shows normal hepatocytes (H) bays arranged radially around a centrilobular vein, sinusoidal capillaries (S), and a granular cytoplasm which is comparable to those of the group treated with extract of MO at 2000 mg/kg BW. The renal cortex cross-section of a control group [Figure 5] shows the presence of glomeruli with Bowman's capsule, Bowman's space, and well-defined convoluted tube. There were no changes in the group treated with MO extract at 2000 mg/kg BW.

DISCUSSION

Plants were used during the age for cure and treatment of diseases since the start of humankind.[21] There contain a large number of bioactive compounds that have been shown to be beneficial and that have biological activities such as anti-inflammatory, antihypertensive, anticancer, antimicrobial, antioxidant, and healing properties. Medicinal plants have played a key role as a source of innumerable new drugs because of easy availability and less toxic effects.[31] This study investigated the antihypertensive effect of MO hydro-ethanol extract in L-NAME-induced hypertensive rats and its effects on the toxicity profile in Wistar rats and shrimp larvae. Hypertension is established according to the protocol of Adjagba *et al*.[3] and Lawson *et al*.[17] The phytochemical analysis was carried out on the powders of the leaves of MO by implementing the Houghton and Raman method,[14] the toxicity of hydro-ethanol extract was checked *in vitro* on shrimp larvae according to the method of Ahouansou *et al*.[5] and *in vivo* on females Wistar rats as per the OECD 2001

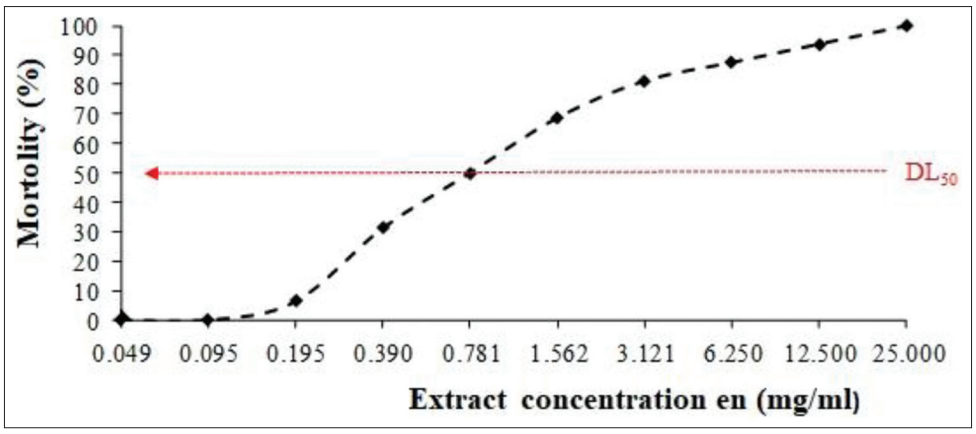

Figure 2: Mortality rate of *Artemia salina* larvae after 24 h exposure to different concentrations of hydroethanolic extract of *Moringa oleifera* leaves

Table 2: Biochemical parameters of female rats in an acute oral toxicity test

Parameters	Control	300 mg/kg BW	2000 mg/kg BW
Glucose (g/L)	0.51±0.02	0.84±0.06*	0.77±0.07*
Urea (g/L)	0.57±0.03	0.6±0.02	0.72±0.03
Creatinine (mg/L)	6.78±0.12	6.1±0.14	7.2±0.28
Proteins (g/L)	63±7.07	63±2.82	60.5±0.70
Sodium (mEq/L)	136.5±9.19	139±1.41	134±5.60
Potassium (mEq/L)	4.5±0.70	5.1±0.14	4.8±0.28
Chloride (mEq/L)	103.5±0.70	97.5±3.53	101.5±2.12
Total cholesterol (g/L)	0.71±0.05	0.69±0.14	0.52±0.03*
AST (UI/L)	301±4.24	297.5±3.53	291±1.41
ALT (UI/L)	33±4.24	32±1.41	34.5±2.12

Values are mean±standard error of the mean (n=3), AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. *P<0.05 significantly different from the control group. BW: Body weight

Table 3: Effect of *Moringa oleifera* leaves extract on BW gain and absolute organ weight in acute oral toxicity tests

Variables	Control	Single dose (mg/kg BW)	
		300	2000
Weight gain (%)	10.83±1.55	8.70±1.36	10.22±0.4
Absolute liver weight (g)	6.93±0.15	7.26±0.6	7.76±0.28
Absolute kidneys weight (g)	1.43±0.15	1.60±0.2	1.73±0.15

BW: Body weight

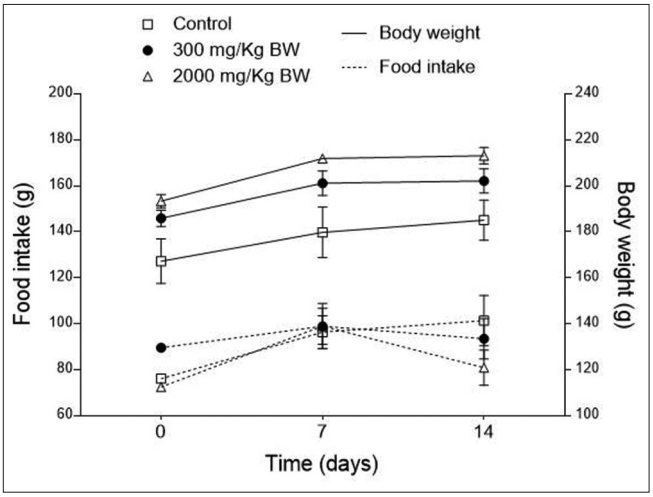

Figure 3: Effect of hydroethanolic extract from *Moringa oleifera* leaves on food consumption and body weight of control and treated female Wistar rats at days 0: 7 and 14 following the acute toxicity test. The first experimental group received a single dose of 300 111 g kg body weight (BW) of the extract; the second experimental group received a single dose of 2000 mg kg BW of the extract and the control group received 10 ml kg BW of distilled water. Values are means of three replicates ± standard error of the mean; 110 significant differences were observed compared to the control

protocol. Oral administration hydro-ethanol extract of MO leaves did not present acute toxicity in rats and shrimp larvae and have significantly reduced BP in L-NAME-induced hypertensive rats.

Phytochemical analysis of extracts from plants is a preliminary step and of great importance. In the present work, major groups of chemical compounds have been characterized. These are alkaloids, tannins, triterpenoids, flavonoids, steroids, anthocyanins, and mucilages. Indeed, tannins intervene between others in the inhibition of certain enzymes such as 5-lipo-oxygenase and angiotensin-converting enzyme (ACE) inhibitors. These molecules may increase the bioavailability of nitrogen mono-oxide (NO) by providing surplus L-arginine.[13] Anthocyanins are known for their anti-edematous activity, their involvement in reducing the permeability of blood capillaries.[8] Mucilage about them is polysaccharides. Triterpenoids have cytostatic properties, antiviral, analgesic, and anti-inflammatory.[8] These results agree partially with those of Bassey *et al*.[6] who detected alkaloids, anthraquinones, flavonoids, triterpenoids, and tannins; Divi[35] have revealed that the presence of alkaloids, flavonoids, steroids, tannins, and saponins[26] revealed the presence of alkaloids, tannins, flavonoids, and steroids. The results of this authors show similarity to the tannins; this resemblance is due to a specific property to the plant. Other chemical compounds differ from author to author. This difference can be explained by noticed the adopted screening method, the geographical location of the species, the country of collection, harvest season, the leaf stage of maturation, and the physicochemical characteristics of soils.

Our results showed that L-NAME at a dose of 40 mg/kg BW induced significant hypertension in rats. The interaction L-NAME-Losartan on BP of rats showed a significant reduction in hypertension induced by L-NAME. This opposition losartan in the antihypertensive action of L-NAME has already been observed.[3] It is well known that the antihypertensive action of losartan is due to inhibition of the angiotensin-I-converting enzyme to angiotensin II at the level of the renin-angiotensin-aldosterone.[40] This inhibition suppresses thereby producing angiotensin II that

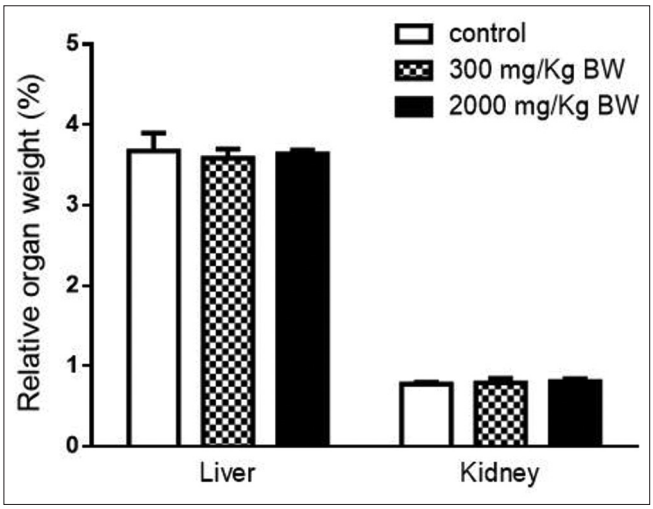

Figure 4: Effect of hydroethanolic extract from *Moringa oleifera* leaves on relative organ weight of control and treated female Wistar rats following the acute toxicity test. The first experimental group received a single dose of 300 mg/kg body weight (BW) of the extract; the second experimental group received a single dose of 2000 mg/kg BW of the extract and the control group received 10 ml kg BW of distilled water. Values are means of three replicates ± standard error of the mean; no significant difference was observed compared to the control

promotes arterial vasoconstriction. The interaction L-NAME hydro-ethanol extract of MO also showed a significant reduction in hypertension induced by L-NAME. Thus, the similarity between the effects of hydro-ethanol extract of MO and those on L-NAME-induced hypertension, let suggest a mechanism of muscle relaxation and a decrease in peripheral resistance, causing vasodilatation and a fall in arterial pressure. Antihypertensive power of the extract would be primarily due to the presence of flavonoids, alkaloids, and tannins which are phenol compounds whose antioxidant and antiradical power is well known.[17] These observations are in accordance with the result of previous studies that reported that methanol and ethyl acetate extracts of MO have significantly reduced SBP in L-NAME-induced hypertensive mice[45] These results have been observed by Aekthammarat *et al*.[4] who were showed that L-NAME-treated rats developed significantly increased BP and heart rate. Concurrent oral treatment with MO could decrease the high BP and tachycardia in a dose-dependent manner.[4] The effect of L-NAME on arterial pressure at the same dose has previously been reported by Tata *et al*.[32] who were showed also that BP increased progressively in all animals from

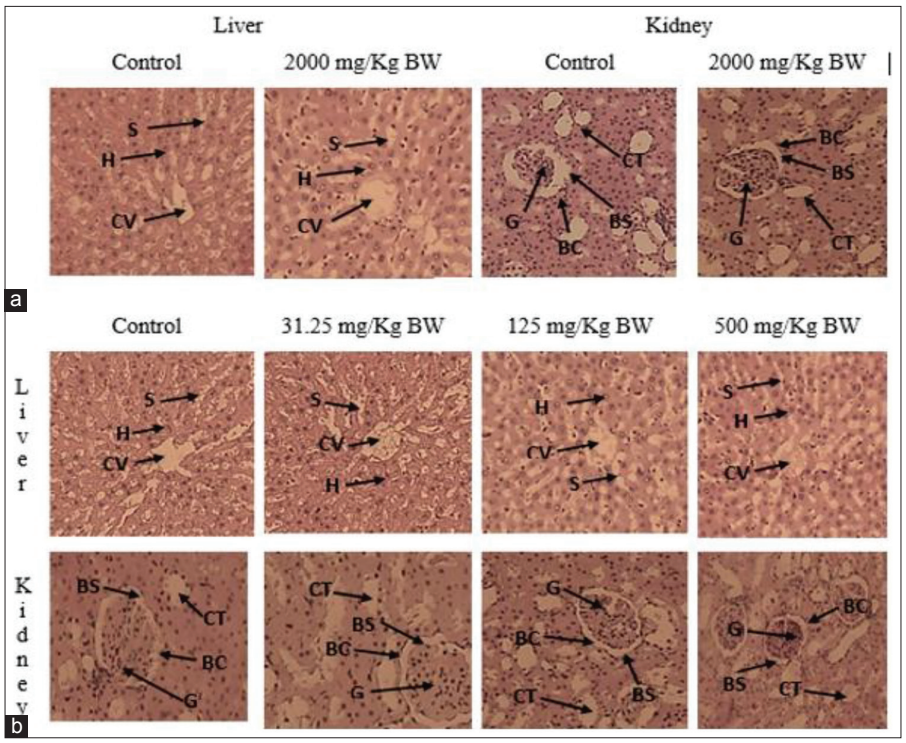

Figure 5: Photomicrographs (hematoxylin and eosin × 400) of histoarchitecture of kidney and liver of rats. Effect of *Caesalpinia bonduc* root extract on histopathological changes of the liver and kidney of female rats in acute toxicity test of 14 days (a) and male rats in subacute oral toxicity test 28 days (b). The control group received dimethyl sulfoxide. The experimental groups received a single dose of 2000 mg/kg of body weight (BW) (a) and repeated dose of 31.25 mg/kg; 125 mg/kg; and 500 mg/kg of BW (b) for 28 days of ethanolic extract of CB root. Liver: Control (a) group liver section showed the normal appearance of hepatocytes (H). Centrilobular vein (CV). Sinusoids (S) are preserved and ordered. No morphological difference was observed for 2000 mg/kg BW (a) when compared with the control. Control (b) group liver section showed the normal appearance of hepatocytes (H). CV. Sinusoids (S) are preserved and ordered only that the sinusoids were less developed at the levels of the groups treated with 125 and 500 mg/kg BW compared to the controls. Liver of 31.25 mg/kg BW shows no morphological difference when compared with the control. Kidneys: Renal glomeruli (G) and convoluted tube (CT) without alterations are visible in control and extract treated groups. Control (a) and treated group (a) showing no difference in morphology glomeruli (G). Tubules (CT). Bowman's capsule and Bowman's space

the 2nd week of L-NAME treatment. Chronic treatment of L-NAME produces hypertension in animals by deficiency of NO, endothelial dysfunction, and complete loss of response to acetylcholine (ACh)-induced relaxation in major blood vessels.[44] ACE is a dipeptidyl carboxypeptidase that plays a significant role in rennin-angiotensin-aldosterone system, by converting the precursor angiotensin I into angiotensin II which is the peptide responsible in triggering BP increasing mechanisms.[2] Arginase is another crucial enzyme that helps to regulate NO levels by competing with endothelium NOS for L-arginine.[2] As such, increased arginase activity and expression have been implicated in much vascular pathology such as hypertension.[2] ACh acts as neurotransmitter and vasorelaxant in the cardiovascular system, thus affecting vascular tone and BP. ACh signal transduction is associated with enhancement of NO production in endothelial cells and cardiomyocytes.[2] Skeletal and cardiac muscles cells also contain an enzyme called acetylcholinesterase (AChE). This enzyme rapidly breaks down ACh into the compounds acetate and choline, terminating the action of the neurotransmitter. NO causes vasodilatation by stimulating vascular smooth muscle soluble guanylate cyclase to convert guanosine -5-triphosphate to cyclic guanosine monophosphate (cGMP), which leads to a reduction in intracellular calcium concentration.[2] cGMP is degraded by cGMP-specific phosphodiesterase-5 (PDE-5), cGMP-binding PDE-5, and intracellular concentrations of cGMP and is tightly controlled by this enzyme through a number of negative feedback mechanisms.[2] Inhibitors of PDE-5 increase the intracellular concentration of cGMP, with the consequence that NO-mediated cellular responses, such as vascular smooth muscle relaxation, are promoted.[2] Odunbajo *et al*.[23] reported that extracts of MO and nutmeg seeds inhibited ACE, AChE, PDE-5, and arginase activities in dose-dependent manner in rat penile tissue. Furthermore, the work of Oboh *et al*.[22] demonstrated the correlation between phenol constituents from MO leaves and the *in vitro* inhibition of key enzymes (ACE and arginase) linked with erectile dysfunction.

Test for determining the toxicity of substances on *A. salina* shrimps is considered an important tool for the preliminary evaluation of the pharmacological activity of plant extracts. It has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides, and cytotoxicity testing of dental materials.[41] Our results show the safety of the hydro-ethanol extract of MO leaves on *A. salina* shrimp larvae. Given the correlation between shrimp larvae, 9PS and 9KB cells (human nasopharyngeal carcinoma), on the one hand, and A-549 cells of lung carcinoma and HT-29 cells of carcinoma of the colon, on the other hand,[41] we can confirm subject to more in-depth studies that the hydro-ethanol extract of MO leaves requires clinical safety at the doses studied. This similarity of results was observed by Rafsanjani *et al*.[47] who worked on the ethanol extract from *Maringa lifer* leaves. These results are in contradiction with those of Rocha-Filho *et al*.[46] who specified that the unlimited use of MO extract should be avoided due to its toxicity to *A. salina*. This dissimilarity can be explained by the material (the flower) that they used.

A rise rate of the renal and hepatic enzymes after administration of the substances may occur when the liver or the kidney suffers damage.[38] The ALT (alanine transaminase) is an enzyme produced by the liver cells (hepatocytes). The ALT levels in the blood increase when hepatocytes are damaged or destroyed at a faster rate than normal.[38] In our case, no significant increase in levels of this enzyme is observed in the rats administered hydro-ethanol extract of MO. The AST (aspartate transaminase) is an enzyme similar to ALT but which is not present specifically in the liver. This enzyme is also present in other organs such as muscle and heart. The above observations are substantially the same with respect to the change in AST levels of treated rats. In many cases of liver inflammation, ALT levels and AST are high.[27] Renal function as measured by laboratory tests, which include creatinine and uric acid. The content of creatinine in blood is the most wide measurement of kidney function. After administration of the products, kidneys play a key role in the elimination of organic waste and in regulating BP.[27] Any imbalance in kidney function can be life threatening. The extract tested had no significant effect on blood creatinine levels. Electrolytes maintain osmotic gradients of body fluids and cellular hydration.[27] The stable serum electrolyte level observed indicates good reabsorption suggesting normal functioning of the renal tubules. This similarity of result was observed by Acuram *et al*.[45] who worked on the root. This contributes to the balance of cellular homeostasis. The study of biochemical parameters revealed that the hydro-ethanol extract of the leaves of MO has no significant effect on the liver and kidneys to the studied dose. This is confirmed by the relative weight of its organs, which provides information on the evolution of the organ compared to the whole body and the structure of their histological sections which have a normal structure with no lesion. These results agree with those of several authors. Uwaifo *et al*.[35] established in the tests carried out on rats than the ethanol extract of the leaves of MO was nontoxic. This is explained well through the results of the screening when the identified molecules (tannins, anthocyanins, triterpenoids, and alkaloids) were found to have no indication of toxicity.[16] The absence of toxic compounds such as derivatives cyanogenic the quinone derivatives guarantees a certain safety of sheets used as vegetable or other in the treatment of high BP without risk of toxicity to the consumer.

This study discovered the acute profile of leaves of MO whom can be beneficial for the management of BP. This method is a step-by-step procedure that begins with the maximum dose of 2000 mg/kg BW and then depending on the mortality and/or morbidity of the animals, is lowered to 300, 50, or 5 mg/kg BW doses to allow a judgment of the test substance's acute

toxicity and provides information on short-time toxicity level of the test extract which helps in the selection of doses for repeated oral toxicity study. The findings of this study greatly help the researchers to provide useful evidence for further work. Our study has several limitations; first, we limited ourselves to the assessment of acute toxicity without expanding our subchronic toxicity assessment study. We were able to identify several biologically active compounds but without quantifying them. In this model of L-NAME-induced arterial hypertension in rats, we did not discuss the mechanism of action of our plant extract.

CONCLUSION

This study demonstrated the antihypertensive properties of the hydro-ethanol extract of MO plant used in traditional medicine in Benin. The results reveal that the antihypertensive effects are due in part to a vasodilator action. Furthermore, vasodilator properties of the extract were conferred on it by the presence of numerous secondary metabolites and their safety has been proven.

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