

Antihypertensive activity of *Moringa oleifera* seeds hydroalcoholic extract on high sodium diet-induced hypertension in Wistar Rat

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Abstract

Our objective was to study the effect of hydroalcoholic extract of *M. oleifera* seeds on high blood pressure in Wistar rat. After 21 days of a high sodium diet, the value of systolic blood pressure and diastolic blood pressure increased from $87.2 \pm 1.96 / 58 \pm 0.89$ mm to $200.8 \pm 0.49 / 151.2 \pm 1.2$ mm Hg ($p < 0.05$). Administered orally, the extract reduces the high blood pressure of hypertensive rats. It returns to its normal value after 19 days for the control batch, versus 9, 6, and 3 days for the animals treated with the extract at doses 100, 200, and 400 mg/kg ($P < 0.05$). The extract also has a diuretic activity; the 24 h urinary volume increases from 4.8 ± 0.14 ml in control group, versus 6.85 ± 0.1 , 8.3 ± 0.13 and 10.7 ± 0.5 ml in groups treated with the extract at doses 100, 200 and 400 mg/kg ($p < 0.05$), while natriuresis increases from 2.06 ± 0.05 mEq/L in control group, versus 2.92 ± 0.05 ; 6.82 ± 0.11 and 10.73 ± 0.17 mEq/L in animals treated with the extract at doses 100, 200 and 400 mg/kg ($p < 0.05$). *In vitro*, it relaxes the isolated aorta contracted with norepinephrine at the concentration of 10^{-5} M in the bath, with EC_{50} equal to 2.06 mg/ml ($p < 0.05$). These results show that the hydroalcoholic extract of *M. oleifera* seeds has antihypertensive activity through its diuretic and vasodilator effects.

Keywords: *Moringa oleifera*; Hypertension; Diuretic; Vasodilation; Rat

1. Introduction

All over the world cardiovascular diseases have remained one of the leading causes of death, hypertension being the most common and the main contributor to the pathogenesis of the risk of heart, brain, kidney and other diseases [1]. According to a WHO report in 2021 [2], about 1.28 billion patients suffer from hypertension in the world. With its incidence of around 28.5 % in urban areas in Madagascar, it becomes a public health challenge [3, 4].

Hypertension is caused by increased cardiac output and/or increased peripheral resistance. If blood pressure is consistently above 140/90 mmHg and the risk of other problems is high, medicines will be offered to lower it. Several types of medicine can be used to help control high blood pressure. They can be used separately or in a combination of different medicines. They can dilate the vessels or reduce the heartbeat; diuretics are also useful in treatment of high blood pressure by flushing excess water and salt from the body [5, 2].

On top of the synthetic drugs developed for the treatment of hypertension, using herbal medicines is still important because of their ease of availability and cost effectiveness. Some of them have been found useful in the management of cardiovascular disease and lowering of blood pressure. The following examples, have been widely used for the treatment of hypertension in traditional medicine: garlic [6, 7], *Fraxinus excelsior* (Oleaceae, vern. Ash) [8], *Lepidium sativum* L (Brassicaceae; vern. garden cress) [9], *Anacardium occidentale* (Anacardiaceae, vern. Cashew nut) [10],

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Lantana Camara (Verbenaceae, vern. camara) [11], *Saccharum officinarum* (Fary), *Zea mays* (Poaceae; vern. maize) [12], *Bidens pilosa* (Asteraceae, vern. cobblers pegs) [11], and *Moringa oleifera* (Moringaceae, vern. moringa) [13].

Moringa is a nutrient-packed superfood that comes from the *M. oleifera* tree in India. Almost all parts of this tree are edible and have therapeutic properties [14]. Traditional use of *M. oleifera* seeds evidence suggests that it is associated with reduced problem of hypertension in the northern part of Madagascar. Despite its traditional usage, there is a lack of data to support the antihypertensive properties of the seeds. Therefore, the aim of the present study was to investigate the hypotensive effect of *M. oleifera* seeds hydroalcoholic extract, in Wistar rat. Since anti-hypertensive activity might be due to its vasodilation or diuretic activities, its effect on diuresis and blood vessels are investigated in this work.

2. Material and methods

2.1. Drugs and Chemicals

Norepinephrine, and all chemicals used in this study were of analytical grade; Norepinephrine was purchased from Sigma Chemical Co. (St. Louis, MO), while ingredients used to prepare the bath for isolated organ (NaCl, KCl, MgCl₂, Na₂HPO₄, NaHCO₃ et Glucose) were purchased from MERCK (39 Rte Industrielle de la Hardt, 67120 Molsheim, France) and (CaCl₂) was purchased from LABOSI (141 Rue de JAVEL, Paris 75).

2.2. Plant material used and preparation of extract

Dried seeds of *M. oleifera* were collected in September 2019 from the northern part of Madagascar. It was identified and authenticated at the Botany department of the "Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar". A voucher specimen has been deposited in the Pharmacology Department, Faculty of Sciences, University of Antananarivo, Madagascar for future reference [15]. The aqueous ethanol (60:40) extract of *M. oleifera* seeds was prepared by the cold maceration process. It was filtered through Whatman grade 1 filter paper, and centrifuged at 3 000 rpm. The supernatant was concentrated with a rotary evaporator (Evapotec®), under reduced pressure.

2.3. Phytochemical Analysis

Phytochemical analysis was conducted to detect the main secondary metabolites in the hydro alcoholic extract of *M. oleifera* seeds. This test is based on reaction between specific reagents and the correspondent chemical family. The reaction intensity depends on the quantity of the compounds in the extract [16].

2.4. Animal Material

Male Wistar rats aged 6-8 weeks and weighing 150-160 g were randomly selected from the colony of the Pharmacology Animal House, Faculty of Sciences, University of Antananarivo, Madagascar. Every effort was made to minimise animal suffering and to reduce the number of animals used. They were exposed to a daily 12-hour light/dark cycle. They were maintained at room temperature (25 ± 3 °C) with free access to a standard diet (animal food LFL 1420®) and tap water.

2.5. Hypertension Induction

The anti-hypertensive activity of *M. oleifera* seeds was evaluated in high sodium diet induced hypertensive male Wistar rats. They were given a mixture of NaCl-feed for livestock 8% (w:w) and free access to tap water until their blood pressure raised until steady state [17]. Every morning, at the same time, animal's blood pressure was measured with a tail cuff sphygmomanometer, in a quiet place, and high sodium diet was stopped when the blood pressure was maximal [18].

Hypertensive animals were divided into 4 groups of 6: 1 control and 3 treated with the extract. Every morning after blood pressure monitoring, animals of control group received 10 ml/kg of distilled water, and the rats of the other groups received the extract at 100, 200 and 400 mg/kg, dissolved in 10 ml/kg of distilled water, by oral route, until the blood pressure returns to its normal value [19].

2.6. Evaluation of the extract diuretic activity

Since diuretics are used in high blood pressure treatment, the extract was tested for diuretic activity, using the method described by Kau *et al.* [20]. After checking the urinary excretion in rats of Wistar strain, the animals with the best urine volume were selected and divided into 4 groups of 6 animals each. The first group was used as control and given distilled

water (10 ml/kg) and the rest received the extract at doses of 100, 200 and 400 mg/kg, by oral route. The animals were individually placed in standard metabolic cages and deprived of food and water during 12 hours before the experiment.

Fasted animals received 50 ml/kg of water. After 30 minutes, the rats of the control group received 10 ml/kg of distilled water, while the rest received the extract at different doses and put in individual metabolic cages for 24 h. Urine excretion was measured at 24 hours in all groups. As diuretic action is subjected to variability, diuretic activity was calculated, and urine concentration of Na⁺, K⁺ were measured by flame spectrophotometry (SYSTONIC®) as described by the user instruction manual of the material [20].

$$\text{urinary excretion} = \frac{\text{total urine output}}{\text{total fluid administered}} \times 100$$

- 80 % < EUV < 100 % : no diuretic activity
- 100 % < EUV < 130 % : low diuretic activity
- 130 % < EUV < 150 % : mild diuretic activity
- 150 % > EUV : high diuretic activity

2.7. Evaluation of the extract activity on isolated trachea

Vasodilators are part of medicines used in the treatment of high blood pressure. The potential vasodilator activity of *M. oleifera* extract was assessed on its effect on trachea contracted by norepinephrine.

Rat of Wistar strain was used in this experiment. The rat was euthanized with 100 mg/kg of phenobarbital, injected by intramuscular route. A thoracotomy was practised. The internal viscera were pulled aside, to expose the aorta which was cut close to the heart and dissected as far as possible. Then, the isolated organ was transferred to a Petri dish containing a Krebs solution at room temperature. Surrounding fats and connective tissues were removed and the organ was cut into rings of 3 mm. Threads were tied to each end of the rings, then it was put in an isolated organ bath containing Krebs solution, maintained at 37°C and aerated with carbogen. One end was attached to the tissue holder in the organ bath, and the other end was connected to an isometric transducer (UF1 Force Sensor®) with a tension of 1.5 g. The preparation was allowed to equilibrate for 30 min, during which, the bath was renewed 3 times. After equilibration, norepinephrine was injected in the bath to check the organ viability and to sensitize it. The preparation was rinsed and let to equilibrate for 20 minutes, during which the bath was renewed twice before injecting norepinephrine in a cumulative manner until maximal contraction of the organ. At that point, the extract was injected in the bath in a cumulative manner until complete relaxation of the organ [21].

2.8. Statistical analysis

Data were expressed as mean ± standard error of the mean. The comparisons between the groups were performed by analysis of variance (ANOVA) followed by t test of Student. Differences between pairs of means were considered significant when the probability 'p' was less than 5 % (p < 0.05).

3. Results

3.1. Results of phytochemical screening

Phytochemical screening carried out on *M. oleifera* extract indicate the presence of phenolic compounds, flavonoids, tannins, leucoanthocyanins, alkaloids and steroids in it.

3.2. Effect of the extract on high sodium diet induced blood pressure

After 21 days of high sodium diet, significant elevations in systolic and diastolic blood pressure were observed in rat. Animals blood pressure rose from 87.2 ± 1.96 / 58 ± 0.89 mm to 200.8 ± 0.49 / 151.2 ± 1.2 mm Hg (p < 0.05). Oral administration of *M. oleifera* extract, once a day brings the blood pressure to its normal value after 9, 6, and 3 days in animals treated with the extract at doses of 100, 200, and 400 mg/kg, versus 19 days for the control group (p < 0.05) (Figures 1 a and b). These results demonstrate the anti-hypertensive activity of *M. oleifera* hydroalcoholic extract.

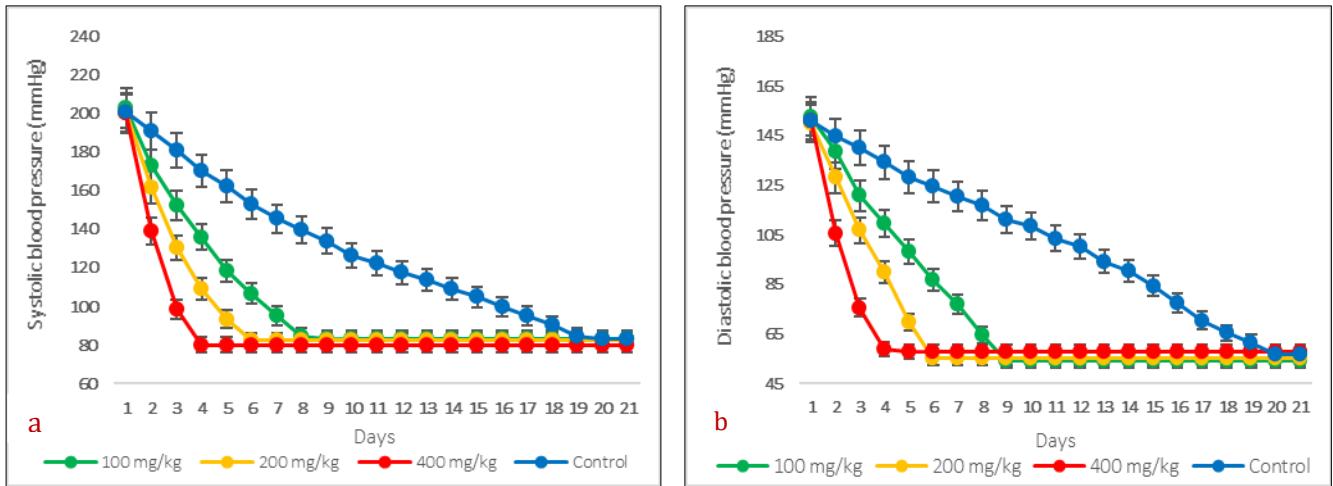


Figure 1 Reduction of systolic (a) and diastolic (b) blood pressure in hypertensive induced control group and hypertensive animals treated with the extract, administered orally, at doses of 100 , 200 and 400 mg/kg (mean ± e.s.m; n = 6; p < 0.05)

3.3. Diuresis activity of *M. oleifera* hydroalcoholic extract

Administered orally, at doses of 100, 200 and 400 mg/kg, *M. oleifera* hydroalcoholic extract increases the urinary volume of rat in a dose dependant manner. It is 4.8 ± 0.14 ml in control group, versus 6.85 ± 0.1 , 8.3 ± 0.13 and 10.7 ± 0.5 ml in animals treated with the extract at doses of 100, 200 and 400 mg/kg (p < 0.05) (Figure 2). It indicates that *M. oleifera* hydroalcoholic extract possesses diuretic activity. After calculation EUV is equal to 115.38 %, a value between 100 % and 130 %. With this value, *M. oleifera* hydroalcoholic extract, at the doses used in this work is considered as having low diuretic activity.

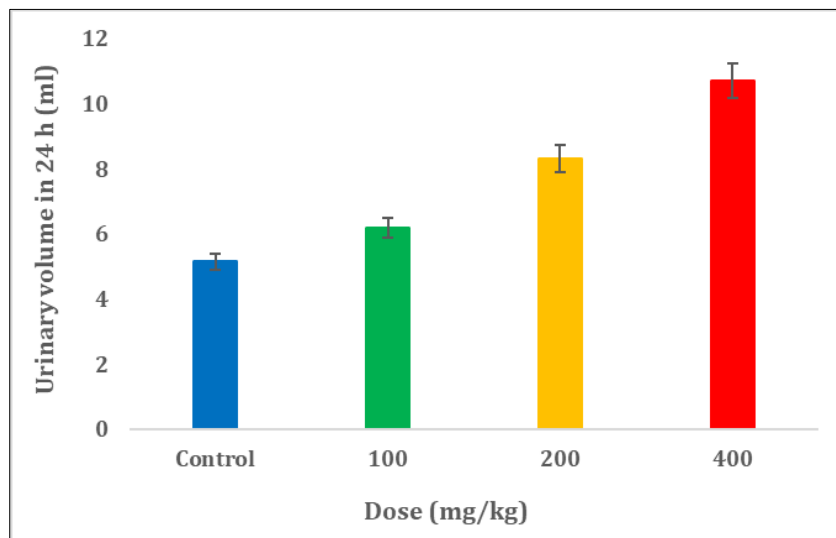


Figure 2 Urinary volume of rat in control group and those treated with *M. oleifera* hydroalcoholic extract, administered orally, at doses of 100, 200 and 400 mg/kg , after 50 ml/kg hydric load (mean ± e.s.m; n = 6; p < 0.05)

3.4. Natriuretic activity of *M. oleifera* hydroalcoholic extract

From the result it can be observed that hydroalcoholic extract of *M. oleifera* seeds increases sodium output in the treated animals when compared to control group. This effect is dose dependent, it is 2.06 ± 0.05 mEq/L in control group, versus 2.92 ± 0.05 ; 6.82 ± 0.11 and 10.73 ± 0.17 mEq/L in animals treated with the extract at doses 100, 200 and 400 mg/kg. These results indicate the natriuretic activity of *M. oleifera* seeds (Figure 3) (p < 0.05).

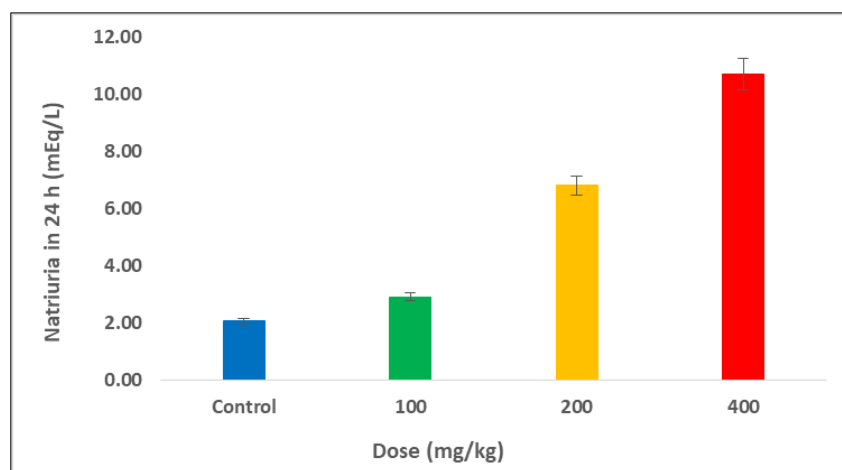


Figure 3 Effects of the hydroalcoholic extract of *M. oleifera*, administered orally, at doses of 100, 200, and 400 mg/kg, compared to control group on natriuretic activity from 24 h of urine (mean \pm e.s.m; n = 6; p < 0.05)

3.5. Effect of the extract on isolated aorta contracted with norepinephrine

Injected in the bath containing aorta, norepinephrine contracts 100 % the organ at the concentration of 10^{-5} M in the bath. The amplitude of contraction diminished in the presence of the extract in the bath. The decrease is concentration dependant. After regression calculation, EC_{50} is equal to 2.06 mg/ml (p < 0.05) (Figure 4). These results exhibit significant vasodilation of *M. oleifera* hydroalcoholic extract.

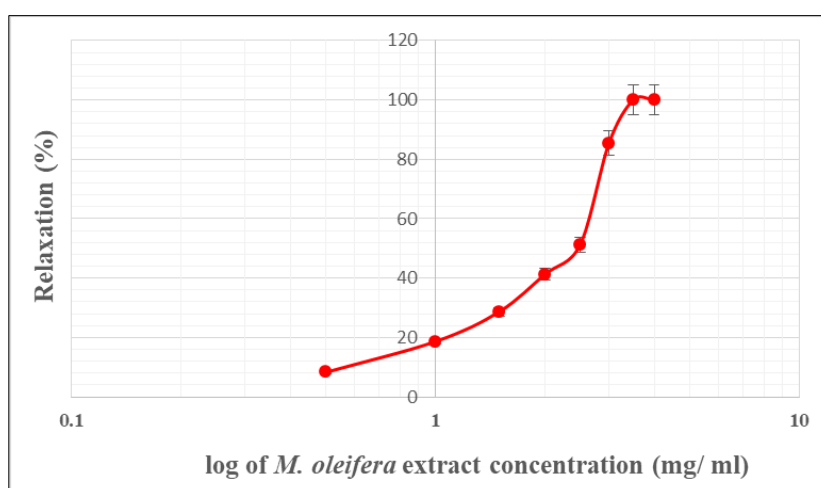


Figure 4 Relaxation of isolated aorta contracted with norepinephrine at 10^{-5} M in the bath, in the presence of the extract, injected in the bath in a cumulative manner (mean \pm e.s.m; n = 6; p < 0.05)

4. Discussion

This study aimed to investigate the antihypertensive effects of *M. oleifera* seeds hydroalcoholic extract in high sodium diet-induced hypertensive in Wistar rat. Since diuretics and vasodilators are part of medicines used in the treatment of high blood pressure, we investigated the effect of this extract on diuresis and blood vessel. Results obtained showed that chronic high consumption of sodium increases blood pressure. These findings are in agreement with previous studies demonstrating that high sodium diet is one of the major risk factors in the development and maintenance of hypertension. High sodium intake and the increase in blood pressure levels are related to water retention, increase in systemic peripheral resistance, alterations in the endothelial function, modification in sympathetic activity, and in the autonomic neuronal modulation of the cardiovascular system [22]. Our results disclose that the extract is efficient as antihypertensive agent by significantly and dose-dependently decreasing blood pressure in sodium induced hypertensive rats. These results suggest that *M. oleifera* extract have an antihypertensive effect that could be due to an action on peripheral resistance. Investigation carried out on its diuretic activity have shown that it is a natriuretic.

Which means, it reduces water retention and natremia, resulting in reduction of peripheral resistance. On the other hand, *in vitro* investigations indicate that it also dilates the isolated aorta contracted with norepinephrine. Which might be due to its action as calcium channel antagonist, or through nitric oxide production. The vasorelaxant function of the endothelium is in great part mediated by the production of nitric oxide (NO). Excessive salt consumption is known to be associated with increased tissue production of reactive oxygen species which damages the endothelium, reducing the NO production [23].

Treatment with the hydroalcoholic extract reversed the adverse effect of prolonged sodium intake suggesting its ability to improve the endothelial function. This potential may be due to the presence of secondary metabolites like flavonoids, alkaloids and phenols which are able to stimulate the production of nitric oxide and to scavenge superoxide anion produced during the high sodium diet [24]. Since it has a low diuretic activity, and possesses a vasodilating property, we suggest that its antihypertensive activity could be due to its vasodilating activity or the combination of those two activities.

5. Conclusion

Hydroalcoholic extract of *M. oleifera* seeds exhibited an antihypertensive action in high sodium diet induced-hypertension in rat. It significantly increases diuresis and sodium excretion. It also dilates the aorta contracted by norepinephrine. These findings indicate that *M. oleifera* seeds' antihypertensive action may be related to its diuretic and vasodilating effects. These properties justify the empirical use of the seeds of *M. oleifera* in the treatment of hypertension. More studies need to be done to identify the active compounds in the extract and their exact mechanism of action.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Statement of ethical approval

All procedures and protocols involving animals and their care were conducted in accordance with institutional guidelines and approved by the Ethics Committee of Faculty of Sciences, University of Antananarivo, Madagascar (Reg. No.13/2022).

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