Effects of Water Crude Leaf Extract of *Moringa Oleifera* Lam (Moringaceae) on Normotensive Rat Blood Pressure and Isolated Duodenum

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Somé A. A., Belemtougri R. G., Ouedraogo Y., Traoré A. and Ouedraogo S.

1 UFR of Life and Earth Sciences, Laboratory of Animal Physiology, University of Ouagadougou BP 7021, Burkina Faso.
2 Research Institute of Health Sciences, Centre of National Research Sciences and Technology (C.N.R.S.T) BP 7192 Ouagadougou, Burkina Faso.

Abstract

*Moringa oleifera* is used in folk medicine for the treatment of many ailments among which, hypertension and intestinal disorders. The aim of this study was to investigate the hypotensive and/or antihypertensive activity of *Moringa oleifera* leaf extract on normotensive anaesthetized rats by direct measure of the arterial pressure and in the other hand, to determine its antispasmodic effects on isolated rat duodenum. Intravenous administration of aqueous leaf extract of *Moringa oleifera* (EAMO 5mg/kg - 75 mg/kg body weight), caused a transitory dose-dependent fall in the mean arterial blood pressure of normotensive anaesthetized rats (p< 0.05). Moreover, hypertensive arterial blood pressure induced by phenylephrine 100µg/kg rat body weight, was completely abolished by aqueous extract of *Moringa oleifera* 10mg-40mg/kg body weight. The studies on isolated rat duodenum smooth muscles demonstrated that the aqueous extract of *Moringa oleifera* produced a dose dependent relaxation of these muscles (p<0,05). The extract decreases the contractile effects of Ach on these tissues doses dependently. These results indicate that aqueous leaf extract of *Moringa oleifera* possesses hypotensive, antihypertensive activity on normotensive anaesthetized rats and antispasmodic effects on isolated duodenum smooth muscle. This result can explain the traditional use of *Moringa oleifera* leaf extracts in the treatment of hypertensive arterial blood pressure, diarrhea and dysentery.

Keywords: *Moringa oleifera*, rat blood pressure, rat duodenum, hypertension, antispasmodic.
Introduction

*Moringa oleifera* Lam (Moringaceae) is a tree which can reach eight to twenty meters originated from India. It is naturalized in tropical and subtropical countries and is utilized in traditional medicine because of its therapeutic and nutritional properties (Anwar *et al.*, 2007). In the plateau central of Burkina Faso, *Moringa oleifera* is used to treat many ailments among which hypertension and gastrointestinal disorders (Nacoulma-Ouedraogo, 1996).

Hypertension affected many people through the world and caused 6% of death every year (Culter *et al.*, 2008). Fourcade *et al.*, (2007) estimated that the prevalence of hypertension will reach 29.5% in 2025. In sub-saharian countries, hypertension will affect 150 million of persons in 2025.

In Burkina Faso, no serious statistics exist but Niakara *et al.*, (2003) indicated that the prevalence was 23% in Ouagadougou, the principal town of Burkina Faso.

In developing countries, gastrointestinal disorders caused diarrhea and death among the young children. Thapar and Sanderson, (2004) estimated that 2.5 million of children died every year of diarrhea.

The aim of this study is to investigate the hypotensive and/or antihypertensive activity of *Moringa oleifera* leaf extract on normotensive anaesthetized rats by direct measure of the arterial pressure and in the other hand, to determine its antispasmodic effects on isolated rat duodenum.

Materials and Methods

**Plat Collection**

Fresh leaves of *Moringa oleifera* were collected from the campus of University of Ouagadougou in July 2011. The plant was identified by the taxonomist Professor Millogo-Rasolodimby, Department of Botany, University of Ouagadougou. A voucher species has been deposited in this Department.

**Preparation of the Extract**

The leaves were shade dried and powdered. Forty grams of powder were first macerated in deionized water with shaking for 24h at room temperature. The resulting extract was filtered through muslin cloth to retain great particles and then filtered again on colon tube containing hydrophile cotton. The extract was freezed for 24h and then lyophilized to give brown powder which was utilized for this study.

**Animals**

Rats of either sex were used for these experiments. All of them were purchased from local market of Ouagadougou. The animals were kept at 22 ± 5°C and submitted to a 12h light/dark cycle with free access to food and water. Twelve hours before experimentation, the food was withdrawn, but water remained available *ad libitum*.

**Blood Pressure Study**

The rats were anaesthetized with urethane (40%) at dose of 1g/kg intraperitoneally and fixed in supine position on dissecting table. The right jugular vein and the left carotid artery were exhibited and cannulated with catheters PE10 and PE50 respectively toward the heart. The two catheters were filled with heparinized saline solution (125 UI/ml) for further injection to prevent intravascular blood clotting. Body temperature was maintained by using overhead lamp. All animal procedures were strictly with national laws and guidelines.

**Experimental Protocol**

The left carotid cannulated, the blood pressure from the carotid was recorded using an Elcomatic EM 750 SER n°2203 transducer connected to a blood pressure amplifier unit (Harvard Transducer Amplifier). The amplifier was then connected to an oscillograph (Harvard Student Oscillograph). After a 45 min equilibration period, the baseline blood pressure was recorded before samples were injected at doses indicated. The interval between injections was usually 15 min after the blood pressure was returned to control baseline.

**Antispasmodic Study**

The rats were killed by cervical dislocation. A portion of duodenum was removed and placed in Tyrode’s solution at room temperature. The connective tissue was removed and the duodenum
was then suspended in a 20 ml organ bath containing Tyrode’s solution of the following composition (mM): NaCl, 136.7; KCl, 2.69; CaCl₂, 1.8; MgCl₂, 0.1; NaH₂PO₄, 0.04; NaHCO₃, 11.9 and Glucose, 5.5 and adjusted at pH 7.4 with Tris. The solution was continuously maintained at 37°C and aerated by air. A load of 1g was applied and a 60 min equilibration period was allowed, during which the physiological solution was changed every 15 min to protect the organ against interfering metabolites (Altura and Altura, 1970).

At the end of equilibration period, acetylcholine (Ach 10⁻⁴M) was applied to assess the tissue viability. The extracts were added directly to the organ bath in volumes not exceeding 5% of bath volume. The blocking effect of atropine and extracts were investigated on Ach induced contractions of the duodenum using a 10 min contact. Isotonic contractions were recorded using Harvard isotonic transducer and displayed on a Harvard Student Oscillograph pen recorder device.

**Drugs**

Drugs used were acetylcholine chloride (Ach), atropine sulphate, phenylephrine (Phe) and sodium chloride (NaCl). All these drugs were obtained from sigma (Sigma Chemical Company, USA). All drug solution was freshly prepared.

**Statistical Analysis**

Results were expressed as mean ± SEM of five observations. Student t-test was used to test for significant difference between the means and p < 0.05 was considered as significance.

**Results**

**Effect of Extract of M. Oleifera on Normotensive Rat Blood Pressure**

Intravenous administration of 0.1 ml of NaCl (0.9%) did not cause any significant change in blood pressure (fig.1a).

However, intravenous injection of *M. oleifera* decreased the blood pressure in dose dependent manner (5 to 70 mg/kg) (fig.1d, e, f, g) and reversible fashion, similar to that of acetylcholine (Ach) (fig.c). However administration of Phe increased blood pressure (fig.1b). To attempt to indicate at least one of the mechanisms underlying the plant action, interactions were done with some reference drugs.

**Fig. 1:** Recordings of Mo at different doses in rat blood pressure in comparaison with Ach and Phe. NaCl serves as control. Mo = Moringa oleifera, Ach = Acetylcholine, Phe = Phenylephrine.

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Interaction Phenylephrine (Phe) *M. Oleifera*

To verify the antihypertensive effect of the extract, Phe a α1 adrenergic agonist was utilized. Then administration of Phe at the dose of 100 µg/kg induced increase of the blood pressure. When the plateau was reached, cumulative injection of *M. oleifera* from 10 to 40 mg/kg decreased the blood pressure induced by Phe. It is interesting to observe that at the dose of 40 mg/kg the extract of *M. oleifera* decreased the blood pressure to 100% (fig. 2).

![Fig. 2: Recording showing relaxant effect of Mo at different doses on hypertensive effect induced by Phe on rat blood pressure in vivo.](image1)

Antispasmodic Activity

The extract of *M. oleifera* failed to display significant activity on rat duodenum basal tone when applied to the organ bath. Then we utilized Ach, a well known spasmogen on rat duodenum to research some antagonistic effect. Ach induced concentration dependent contraction of duodenum, reaching the maximum within few seconds of contact (fig. 3a). *M. oleifera* at the concentration of 2 mg/ml in the organ bath inhibited the duodenum contraction induced by Ach with reducing maximum amplitude to 66.04% (fig. 3b). The curve of Ach was shifted to right in a no competitive manner. Atropine, a muscarinic receptor antagonist inhibited the tension developed by Ach with maximum amplitude decrease to 44.36%. It shifted also to right the curve of Ach in no competitive manner (fig. 3b).

![Fig. 3a: Recording showing effect of Ach at different doses ([Ach] x 10^-8 M) on rat isolated duodenum. Ach = Acetylcholine.](image2)
Fig. 3b: Effect of Mo and Atr on contraction induced by Ach on rat isolated duodenum.

Ach = Acetylcholine, Atr = atropine, Ach+EAMO = Acetylcholine + aqueous extract of *Moringa oleifera*, Ach + Atr = Acetylcholine + Atropine.

**Discussion**

The regulation of blood pressure is complex and involves many factors among which the substances released by sympathetic and parasympathetic neurons. The present results showed that the hypotensive effect produced by leaf extract of *M. oleifera* in anaesthetized normotensive rats were in dose dependent manner. These results were similar to that induced by Ach (fig. 1c).

According to Cowley (1984), the antihypertensive substances lower blood pressure by interfering with mechanisms involved in the regulation of blood pressure. Parasympathetic neuron stimulation resulted in bradycardia and hypotension due to Ach release and its effect on heart and vascular smooth muscle (Ajagbonna et al., 2001). The pharmacological agents that augment Ach action at neuroeffector sites will lower the blood pressure (Adeneye et al., 2006). Our result is similar to those obtained for the aqueous extract of *Laurelia sempervirens* on rat (Schmeda-Hirschmann et al., 1994), the aqueous leaf extract of *Lantana camara* on rabbit (Belemtougri et al., 2001) and the aqueous leaf extract of *Sclerocarya birrea* on rat (Belemtougri et al., 2007). In this condition, it was interesting to use atropine to research muscarinic receptor implication. Indeed, Gilani et al., (1994) showed that hypotension induced by pure compounds isolated from leaves of *M. oleifera* did not involve muscarinic receptors.

Some components such as niazinin A, niazinin B, niazimicin and niaziminin A+B were isolated from leaves of *M. oleifera* and showed hypotensive and bradycardic activities on rat (Gilani et al., 1994). These compounds could be present in our extract and showed the results observed in our experimentation. Some antihypertensive agents reduced the sympathetic nervous system activities by stimulating structures which inhibited sympathetic tonus. Such stimulation could be done through adrenergic receptors (Giroud et al., 1980; Kanagy, 2005). The use of phenylephrine (Phe), a $\alpha_1$ adrenergic receptor agonist was used to research the antihypertensive effect of the extract, also to see if...
these receptors were involved in the hypotension induced by *M. oleifera*. Indeed, Phe induced vascular smooth muscle contraction by increasing intracellular calcium through calcium voltage dependent channels and its release from intracellular pools (Graham *et al*., 1996). The calcium released linked to calmodulin and allowed activation of myosin kinase which phosphorylated myosin and increased vascular tone. The inhibition of the contraction induced by Phe by *M. oleifera* suggested a calcium antagonism that led to vasorelaxation and blood pressure lower. The future use of α1-adrenergic receptor inhibitor must confirm this hypothesis.

The hypotensive effect induced by *M. oleifera* could be due to the presence of flavonoids. Indeed, many researchers indicated that flavonoids isolated from other medicinal plants possessed anti-calcium effects on different isolated organs (Reveluela *et al*., 1997; Gilani *et al*., 2007; Belemtougri *et al*., 2007) and showed hypotensive and vasodilator activities (Ajay *et al*., 2003). According to Kasolo (2010), flavonoids are present in the leaves of *M. oleifera* and it was possible that these compounds were present in our extract and lowered the blood pressure in vivo in our experiment.

It has been shown that tryptophan conversion by an enzyme indoleamine 2-3 dioxygenase to kynurenine led to vasorelaxation and blood pressure lowering in spontaneous hypertensive rats (Wang *et al*., 2010; Passos *et al*., 2012). The leaves of *M. oleifera* which contained tryptophan, it was possible that the metabolite of this amino acid could be involved in the decrease of blood pressure observed in our assay.

Our result on isolated duodenal muscle showed that the extract of *M. oleifera* failed to show any significant effect on spontaneous contractions. Indeed, it has been reported that the smooth muscle tone was influenced by many mediators among which Ach, a parasympathetic neurotransmitter. Many muscarinic receptors were present on intestinal smooth muscle (Radenkovic *et al*., 2006) and five subtypes, namely M1, M2, M3, M4 and M5 have been identified (Tobin *et al*., 2009). However M2 and M3 receptors were involved in intestinal smooth muscle contraction (Matsui *et al*., 2002; Takeuchi *et al*., 2005; Unno *et al*., 2005). The muscarinic receptor stimulation led to activation of protein G which increased the activity of membrane bound phospholipase C enzyme and generation of inositol triphosphate (IP3) and diacylglycerol (DAG). These two substances released in the cytoplasm will interact with the receptor on intracellular calcium store sites and cause the release of intracellular calcium store or calcium influx into the cell (Bolton *et al*., 1999; Kirschstein *et al*., 2009). Therefore, the extract of *M. oleifera* can act in this way by blocking a cascade of reactions which lead to calcium release and then inhibited the contractions induced by Ach. Anticholinergic substance like atropine is known to block muscarinic receptors, thereby causing relaxation in spontaneous contraction of smooth muscles (Joan and Palmer, 1995). Our result is similar to that of atropine on contraction induced by Ach on duodenal smooth muscle, suggesting that the extract of *M. oleifera* could interact on muscarinic receptors. It has been reported that some components isolated from the leaves of *M. oleifera* exhibited spasmylytic activity on guinea pig ileum (Gilani *et al*., 1994) and this result is in a good agreement with our experimental investigation on rat duodenal muscle.

It has been reported that flavonoids were isolated from the leaves of *M. oleifera* and they could play an important role on the relaxation on intestinal smooth muscle. Indeed, flavonoids isolated from other medicinal plants showed spasmylytic activities on smooth muscles preparations. Then, the main flavonoid as quercetin, isolated from the leaves of *Psidium guajava* exhibited spasmylytic effect on isolated intestinal muscle (Lutterodt, 1989; Lozoya *et al*., 1990). According to Sachez *et al*., (1994), the flavonoids exhibited their effects by blocking calcium influx through calcium type L channel. Mehmod *et al*., (2011) showed that the flavonoids isolated from the aqueous extract of *Phyllanthus emblica* exhibited antispasmodic activity by blocking muscarinic receptor and calcium channels. On the other hand, tannins which exist in the leaf extract of *M. oleifera* could play an important role on the smooth muscle relaxation. Indeed, it has been reported that it has spasmylytic effect on smooth muscle cells (Tona *et al*., 1999).
Conclusion

Our results showed that the extract of *M. oleifera* exhibited hypotensive and/or antihypertensive activity on rat blood pressure in vivo. Its effect on Phe induced hypertension on rat blood pressure was confirmed on rat duodenum where it induced relaxation suggesting involvement of α1 adrenergic receptor on vascular smooth muscle. Further investigations will be needed to precise the mechanisms of action on smooth muscles and the chemical characterization of the active principles which causes relaxation on mammalian smooth muscles. Indeed, it is well known that blood pressure is the product of peripheral resistance and cardiac output. Some experiments will be done on rat aortic rings and atria to confirm or not the involvement of these organs. The observed effects seem to justify its traditional use on the management of many diseases such as hypertension and gastrointestinal disorders.

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