DOI: https://doi.org/10.53350/pjmhs2023175113

ORIGINAL ARTICLE

Vasorelaxant Properties of *Moringa oleifera*leaf extract: An in-vitro study on mice blood vessels

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ABSTRACT

Background: *Moringa oleifera* is a plant rich in bioactive compounds, including flavonoids, polyphenols, and alkaloids, which have been shown to have numerous health benefits. In recent years, several studies have investigated the potential of *Moringa oleifera* in the treatment ofhypertension and diseases of vascular dysfunction.
Aim: To observe the effect of *M. oleifera* leaf extract on contractility of aortic tissue of mice, *in vitro.*
Methods: An animal-based in-vitro experimental study was carried out in Pharmacology Department of CMH Lahore Medical College and Institute of Dentistry from February 2022 to March 2023. Mice were euthanized with ketamine. Aortic tissue was dissected and mounted in the organ bath. For evaluation of vasodilatory effect, cumulative dose-response curve of *M. oleifera* leaf extract was obtained using increasing doses of the extract against1 µM adrenaline and 80 mM KCl, in group I and group II, respectively.Response was obtained through Powe rLab (AD Instruments, Australia).
Results: A consistent decrease was recorded against adrenaline and KCl-induced contractions in aorta, resulting in vasodilation. IC50 was 0.068 mg and 0.161 mg, in group I and II, respectively. Difference between IC50 of both groups was statistically significant (p-value = 0.0008).
Conclusion: Leaf extract of *Moringa oleifera* exerts smooth muscle relaxant effectin blood vessels, most likely through inhibition of calcium influx, mediated through Gq-signalling pathway as well as depolarization-dependent voltage operated calcium channels.
Keywords: *Moringa oleifera*, *dose-response relationship, trachea, aorta, calcium channels*

INTRODUCTION

Hypertension is a condition characterized by chronic blood pressure elevations above 130mmHg systolic and 80mmHg diastolic. Hypertension can be further classified into two subtypes, primary hypertension, accounting for 90% of adult cases, and secondary hypertension, the remaining 10%. The likelihood of developing hypertension increases with age, and it is suggested that one in every three individuals above the age of 45 years is hypertensive[1].

Worldwide, 31.1% of the population (1.39 billion) was found to be hypertensive in 2010[2]. The condition is widespread in Pakistan, afflicting 46.2% of the adult population. Highest prevalence was seen in Punjab (49.2%), followed by Sindh (46.3%), Baluchistan (40.9%), and Khyber Pakhtunkhwa (33.3%)[3].

The pathophysiology of hypertension revolves around arterial blood pressure changes. Arterial blood pressure can be measured through changes in peripheral resistance and cardiac output. Therefore, factors that alter either of these two variables can lead to hypertension.Although an increase in vascular stiffness is a normal, physiological, reaction to blood pressure elevations around the body, prolonged vasoconstriction, or 'stiffness', as in hypertension, is problematic as it can cause vessel wall weakness and other similar pathologies. Therefore, heightened vascular responsiveness is the pathophysiological basis of primary hypertension[4].

Moringa oleifera, also known as horseradish tree or drumstick tree, is a perennial, fast growing and drought-tolerant plant. It is abundantly grown in Pakistan, tropical India, Africa, tropical America, Sri Lanka, Mexico, Malaysia and the Philippine Islands[5]. All parts of the plant including leaves, bark and roots possess medicinal and nutritional properties. Due to its numerous benefits, it is famously called the "Miracle tree"[6].

Multiple studies have been done recently to scientifically evaluate the benefits of *M. oleifera.* In scientific research, it has demonstrated antioxidant, anti-inflammatory, antidepressant, hypoglycemic and antihyperlipidemic effects[7-10]. Several herbal remedies have been used for alleviation of blood pressure. These include garlic, ginger, celery, basil, flaxseed, black cumin, and fish oil. Their molecular make up bestows upon them pharmacodynamic properties useful for the treatment of hypertension[11].

Moringa oleifera has been widely used for treatment of hypertension, in Ayurvedic medicine. While the exact mechanism of action is difficult to isolate and test, there are studies that merit the compound being the topic of further study and exploration[12]. Despite widespread use of *M. oleifera*as herbal medicine, there is limited scientific evidence available for its pharmacological properties. Results of study could provide a cost effective and safe adjunct option for alleviation of asthma and hypertension.

Therefore, the objective of this study was to demonstrate the vasorelaxant effect of *Moringa oleifera* leaf extract on aortic tissue of mice and elucidate the possible underlying mechanism.

METHODOLOGY

An experimental study was carried out in Pharmacology Department of CMH Lahore Medical College and Institute of Dentistry from February 2022 to March 2023, after receiving ethical approval from the institutional review board of CMH Lahore Medical College and Institute of Dentistry, Lahore (ERC no. 609/ERC/CMH/LMC). Adult healthy male Swiss albino mice, weighing 25-35 grams, were included in the study. Animals with any visible sign of disease were excluded. Sample size was calculated with WHO formula.The study done by Dabire *et al* was used as reference for calculation of sample size[13]. According to the formula, sample size was calculated to be 1, but keeping in view the similar studies, a sample size of 6 was used in each group.

Moringa oleifera dried leaves (800g) was purchased from the local market. The plant was verified from Botany Department, Government College University (GCU), Lahore (Voucher number: GC-Herb-BOT-3785).The dried leaves (100g) were boiled, twice, in onelitre distilled water (DW) for 40 min, followed by filtration

Received on 02-04-2023
Accepted on 10-05-2023

through Whatman filter paper no. 1. A total of 800 grams of plant material was used. The filtrate was concentrated using a rotary evaporator to produce a powdered crude extract, which was stored in a clean, air-tight and light-protected container at 4ºC until further use.It was dissolved in distilled water before administration[12].

Animals were anesthetized with ketamine. Thoracotomy was performed and viscera were carefully removed. Underlying aorta was identified, removed, and placed in a Krebs solution. The aorta was cut into rings (2-3 mm length) which were mounted, through metal hooks, and connected to isometric force transducer with a thread, in a 25 ml organ bath, containing oxygenated Krebs solution at 37°C. The aortic tissue was equilibrated in Krebs solution for one hour. The basal tension was monitored and adjusted to 2 g. Adrenaline (1 µM) or KCl (80 mM) was used to induce a sustained vascular contraction. It was equilibrated for 30 minutes, followed by cumulative addition of increasingdoses of the extract. Isometric responses were recorded through PowerLab (AD Instruments, Australia) data acquisition system[14].

For the evaluation of antihypertensive effect, animals were divided into two groups, with six animals in each group, as follows;

Group A: Cumulative dose-response curve of *M. oleifera* leaf extract was obtained using increasing doses of the extract,starting from 1µg, 2ug, 4ug, 8ug, 16ug and so on, up to a maximum dose of 100 mg,in the presence of fixed concentration of 1 µMAdrenaline.

Group B: Cumulative dose-response curve of *M. oleifera* leaf extract was obtained using increasing doses of the extract,starting from 1µg, 2ug, 4ug, 8ug, 16ug and so on, up to a maximum dose of 100 mg, in the presence of fixed concentration of 80 mM KCl.

GraphPad Prism (version 8.0.1) was used for statistical analysis. The collected data waspresented as mean ± standard error of mean (SEM). For the purpose of standardized comparisons, the data was normalized and expressed in terms of percentage inhibition. The number of animals was expressed as *n*. Non-linear regression was applied for analysis of thedose-response curves. Half-maximal inhibitory dose (IC50 values) with 95% confidence intervals (CI) were calculated. IC50 of different groups were compared to test the significance of difference between the groups. $p < 0.05$ was considered as significant.

RESULTS

The leaf extract consistently decreased the Adr-induced contractions in aorta, resulting in vascular smooth muscle relaxation (Fig. 1a). Cumulative dose-response curve was obtained using increasing doses of the extract, in the presence of fixed concentration of 1 µM adrenaline (Fig. 1b). IC50 was 0.068 mg, with 95% CI (0.0476 to 0.0955).

Fig. 1a: Effect of *M. oleifera* extract on aorta pre-treated with 1 uM Adrenaline (n=6)

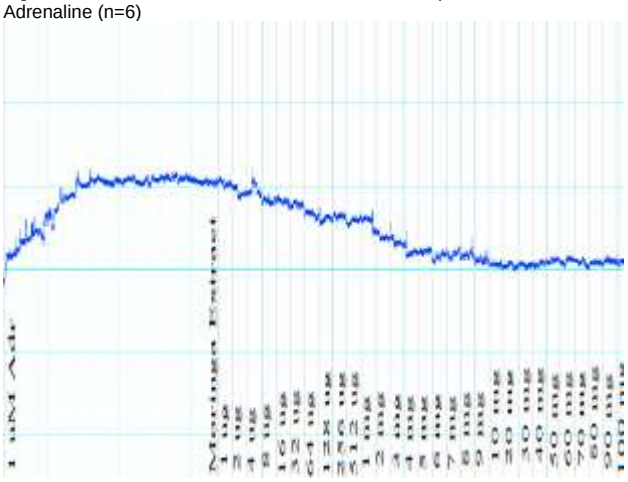
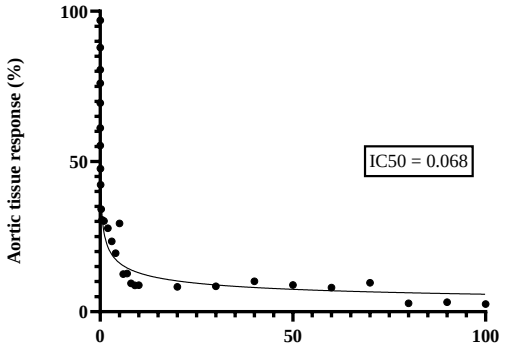


Fig. 1b: Effect of *M. oleifera* extract on aorta pre-treated with 1 uM Adrenaline (n=6)



Similarly, the leaf extract consistently decreased the KCl-induced contractions in aorta, resulting in vasodilation (Fig. 2a). Cumulative dose-response curve was obtained using increasing doses of the extract, in the presence of fixed concentration of 80 mM KCl (Fig. 2b). IC50 was 0.161 mg, with 95% CI (0.110 to 0.231).

Fig. 2a: Effect of *M. oleifera* extract on aorta pre-treated with 80 mM KCl (n=6)

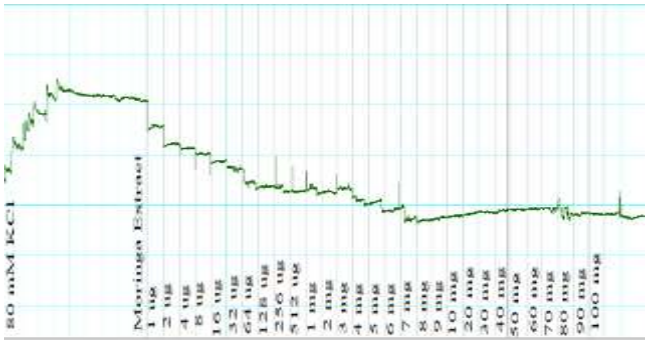
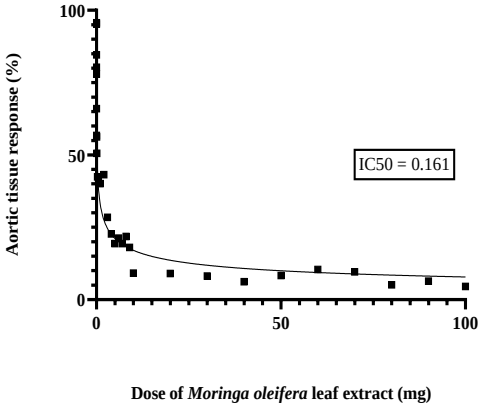


Fig. 2b: Effect of *M. oleifera* extract on aorta pre-treated with 80 mM KCl (n=6)



IC50 of aortic tissue response, produced by the *M. oleifera* leaf extract against Adr-induced contractions, was compared with the effect produced against KCl-induced contractions, through non-linear regression analysis. The difference between the IC50 of both groups was found to be statistically significant (p=0.0009), as shown in Table 1.

Table 1: Non-linear regression analysis of effect of *M. oleifera* extract against Adrenaline and KCl induced aortic contraction (n=6)

Parameters	Group I (Adrenaline 1 uM)	Group II (KCl 80 mM)
IC50	0.068	0.161
95 % CI (IC50)	0.0476 to 0.096	0.110 to 0.231
Hill Slope	-0.382	-0.383
R squared	0.818	0.793

P value 0.0009

DISCUSSION

Moringa oleifera is a plant that has been used for medicinal purposes for centuries. Several studies have shown that *Moringa oleifera* possesses a wide range of pharmacological properties, including anti-inflammatory, antioxidant, antidiabetic, anticancer and immunomodulatory effects[15]. In recent years, there has been growing interest in investigating the potential effects of Moringa oleifera on blood vessels.

This study investigated the effects of *Moringa oleifera* leaf extract on contractility of blood vessels of mice, in-vitro. In the present study, *M. oleifera* caused a relaxant effect against adrenaline and KCl induced aortic contraction. The difference between the IC50 of both groups was found to be statistically significant (p=0.0009). Several studies have investigated the effect of Moringa oleifera on blood vesselsin animal models. One such study, conducted in vivo, established that crude methanolic and ethyl-acetate extracts of *Moringa oleifera*caused a significant reduction in blood pressure of hypertensive mice. This antihypertensive effect was attributed to inhibition of angiotensin-converting enzyme (ACE) and increased synthesis of nitric oxide[16]. Another study demonstrated the blood pressure lowering effect*Moringa oleifera*in human population. [17] Recentstudies have found that Moringa oleifera extract increases the expression of endothelial nitric oxide synthase (eNOS), which is an enzyme that produces nitric oxide. Nitric oxide is a molecule that causes vasodilation by activating guanylyl cyclase, resulting in increased cGMP that causes myosin-light chain dephosphorylation[18].

The present study explored the vasorelaxant effect of *M. oleifera*, through endothelium-independent pathways. Therefore, vasodilatory effect of *M. oleifera* leaf extract was observed against adrenaline and high KCl induced contractions. Adrenaline stimulates $alpha_1$-adrenergic receptors located on the vascular smooth muscle cells (VSMCs). This triggers a cascade of events that ultimately leads to vasoconstriction. Specifically, adrenaline activates the Gq protein, which in turn activates an enzyme called phospholipase C. Phospholipase C then cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 triggers the release of intracellular calcium from sarcoplasmic reticulum (SR), followed by calcium influx through receptor-operated calcium channels (ROCCs), which leads to the activation of myosin light chain kinase (MLCK) and subsequent phosphorylation of the myosin light chain. This activation of the myosin light chain leads to the contraction of the smooth muscle cells, resulting in vasoconstriction[19]. On the other hand, when high KCl (80mM)is added to the extracellular fluid surrounding the smooth muscle cells, it increases the concentration of potassium ions outside the cell, which causes depolarization. This depolarization triggers the opening of voltage-gated calcium channels in the cell membrane, allowing an influx of calcium ions into the cell.The increase in intracellular calcium concentration triggers the contraction of the smooth muscle cells, causing vasoconstriction[20].

In this study, *Moringa oleifera* leaf extract decreased *both* the adrenaline and KCl triggered vasoconstriction, indicating that a final common pathway must be involved, such as blockade of calcium channels. Another possible mechanism could be activation of potassium channels, causing hyperpolarization of smooth muscle cell membrane, with subsequent closure of voltage-operated calcium channels[21].

Phytochemical analysis has revealed that tannic acid, isoquercetin, and cathechin are the predominant phenols in *M. oleifera*. These compounds produce strong smooth muscle relaxation, and most likely responsible for the vasorelaxant effect of the plant[12]. Endothelium-dependent vasorelaxant effect of *M. oleifera* leaf extract has already been documented through increased activity of eNOS and subsequently raised levels of nitric oxide[18]. The endothelium-independent action is proposed to bemediated by the blockade ofthe voltage and/or receptor operated calcium channels, limiting the entry of extracellular calcium into the smooth muscle cells, as well as inhibiting the release of intracellular calcium from the sarcoplasmic stores.

This study had a few limitations. Firstly, scarcity of resources did not allow performance of phytochemical analysis. Therefore, the effect of crude extract was explored and the active substanceresponsible for the relaxant effect could not identified. Secondly, effect of *M. oleifera* leaf extract against low KCl was not included in study design, and hence, was not elucidated. Further studies are suggested to demonstrate the presence or absence of potassium-channel opening activity of the leaf extract.

CONCLUSION

Leaf extract of *Moringa oleifera* exerts vasorelaxant effect, most likely through inhibition of calcium influx, mediated through Gq-signalling pathway as well as depolarization-dependent voltage operated calcium channels.

Conflict of Interest: NO conflict of interest.
Supporting agency: No funds received.

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