Trop J Nat Prod Res, October 2022; 6(10):1695-1700 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antihypertensive Effect of *Moringa oleifera* (Moringaceae) Methanolic Leaf Extract (MoMLE) on *Cricetomys gambianus* (Muridae)

Elijah C. Odii[a,f], Joseph O. Okoro[a,f]*, Augustine N. Okorie[b], Chinweike N. Asogwa[a], Christian O. Chukwuka[g], Charles O. Okoye[a,e,f], Emmanuel S. Okeke [c,d,e,f]*, Vincent C. Ejere[a], Joseph E. Eyo[a]

[a]*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, 410001, Enugu State, Nigeria.*
[b]*Department of Pharmacology, University of Nigeria, Nsukka, Enugu State, Nigeria.*
[c]*Department of Biochemistry, University of Nigeria, Nsukka, 41000, Enugu State, Nigeria*
[d]*Natural Science Unit, SGS, University of Nigeria Nsukka, 410001 Enugu State, Nigeria*
[e]*School of Environment and Safety Engineering, Jiangsu University, 212013 P.R. China*
[f]*Organization of African Academic Doctors (OAAD), Off Kamiti Road, P. O. Box 25305000100, Nairobi, Kenya*
[g]*Department of Zoology, University of Otago, Dunedin, New Zealand*

ARTICLE INFO

Article history:
Received 18 March 2022
Revised 07 October 2022
Accepted 11 October 2022
Published online 01 November 2022

ABSTRACT

Cardiovascular diseases are on the rise around the world and have become some of the leading causes of death. To enhance their chances of survival and effectively manage costs, indigenous communities in developing countries have sought natural remedies from plants and food materials within their reach. This established preference has seen *Moringa oleifera* garner special attention due to the praised effects of its leaves and other parts. The focus of this study was to investigate the antihypertensive activity of crude MoMLE (CME) and five solvent fractions of the same using *C. gambianus* and elucidate the effect of their separate interactions with atropine. Moringa oleifera (MO) leaf was collected from the University of Nigeria, Nsukka; *C. gambianus was* obtained from Okposi-Okwu, Ebonyi State; and antihypertensive activity was assessed using a blood pressure transducer following established methods. The result showed that CME and other fractions had a dose-dependent negative inotropic and chronotropic effect on *C. gambianus* as follows: N-Hexane fraction (NHF)-19.0 8.1 mmHg, chloroform fraction (CLF)-15.0 3.0 mmHg, ethyl acetate fraction (EAF)-22.3 10.7 mmHg, acetone fraction (ACF)-19.7 7.2 mmHg, and methanol fraction (MEF)-25.7 1.2 mmHg. Similar activity was observed for acetylcholine (-42 ± 4.4 mmHg) which was used as a reference drug (positive control). The study revealed that MoMLE has a hypotensive effect with methanol fraction (MEF) having the second highest hypotensive (-25.7 ± 1.2 mmHg) activity after CME with a 28.7 ± 4.2 mmHg lowering effect. Atropine interaction blocked the extracts as well as acetylcholine, suggesting a muscarinic-receptor mediated action.

***Keywords*:** Acetylcholine, Antihypertensive, *C. gambianus*, Hypotensive effect, *Moringa oleifera*

Introduction

Elevated knowledge of natural products and technological manipulations has resulted in advances in health care and management.[1] The increase in the discovery of potential medicinal plants and concurrent screening of their biological activities provides helpful information to enable patients and physicians to make wise decisions.[2] These medicinal plants, which can be in the form of plant extract (either as a standardized extract or in pure form), have paved the way to a wide range of opportunities in drug discoveries as a result of their unlimited availability and the unmatched diversity of their chemical constituents.[3] Consequently, communicable diseases are beginning to lose relevance to lifestyle-associated diseases such as hypertension. Hypertension, termed 'silent killer' by epidemiologists, is a commonest cardiovascular disease affecting up to one billion people worldwide.[4]

*Corresponding authors. E mail: joseph.okoro@unn.edu.ng; emmanuel.okeke@unn.edu.ng
Tel: +2347033065065; +2348035277554

Citation: Odii EC, Okoro JO, Okorie AN, Asogwa CN, Chukwuka CO, Okoye CO, Okeke ES, Ejere VC, Eyo JE. Antihypertensive Effect of *Moringa oleifera* (Moringaceae) Methanolic Leaf Extract (MoMLE) on *Cricetomys gambianus* (Muridae). Trop J Nat Prod Res. 2022; 6(10):1695-1700. http://www.doi.org/10.26538/tjnpr/v6i10.23

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

It continues to be the leading cause of global disease and mortality, accounting for approximately 9.4 million deaths per year.[5,6] Hypertension, commonly referred to as high blood pressure (HBP), is characterized by an abnormal rise in blood pressure (≥140/90 mmHg) as against the normal of ≥120/80 mmHg, establishing high-risk factor for stroke, arteriosclerosis, end-stage renal disease and myocardial infarction.[7] Recent studies from various researchers from Nigeria have reported major target organ complications associated with hypertension namely, left ventricular hypertrophy (LVH),[8] diastolic dysfunction,[9] ischaemic heart disease (IHD), congestive heart failure (CHF), stroke, and renal failure.[10] Hypertension is the most common cause of sudden death in developing countries, according to studies conducted in several centres.[11] This medical condition is most often diagnosed in older people in Nigeria. In a cohort study at Ibadan involving 613 Nigerians aged 65-110 years (398 women and 215 men), cardiovascular disease was found to be the most common ailment in this group, with hypertension (27.8%) being the most common diagnosis.[12] Hypertension was also the commonest condition associated with dementia in Nigeria. It has been reported as the commonest health condition among senior executives and army recruits. According to autopsy findings, hypertension was the leading cause of sudden death naturally. The most common cause(s) of "sudden natural death" was/were cardiovascular disease(s), with complications of hypertension accounting for the majority of deaths, according to a survey of 876 consecutive coroner autopsies in Ibadan, Nigeria.[13] Furthermore, it has been established that 3% of individuals living with hypertension in Nigeria die yearly, with a population at risk of 7%.[14] According to the

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

World Health Organization, suboptimal blood pressure (115 mmHg) causes % of cerebrovascular disease and 49 per cent of ischemic heart disease, with no gender disparity. Additionally, high blood pressure is the leading cause of death worldwide. Despite advances in the usage of orthodox medicine in the treatment and control of high blood pressure, hypertensive agents (such as calcium-channel blockers, diuretics, rennin-angiotensin system blockers and β-blockers,) have several side effects such as angioedema, dry coughing and reduced renal function [15], therefore, there is need for the use of ethnomedicine as a substitute for the management of HB. One of the established relevant alternatives is leaf extract of *M. Oleifera* which is popularly used by local communities in various regions of the world. For thousands of years, man has used natural plant products to cure various diseases, either as a crude extract or pure form.[16] The tree of Moringa has spread across the tropical and sub-tropical world, adapting to local conditions and resulting in numerous varieties. As a result, localized studies are required to test the therapeutic benefits, nutritional benefits, and physiological effects in the various areas where a variant of the tree is found.[17] This study investigated the antihypertensive effects of MoMLE harvested in the University of Nigeria, Nsukka, Enugu State, Nigeria.

Materials and Methods

Experimental animals

Sibling *C. gambianus* (African giant rat), weighing 1.2 ± 0.30 kg, collected from the wild at Okposi Okwu, Ohaozara in Ebonyi State, Nigeria and reared in the Genetics and Animal Breeding House, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka was used. The *C. gambianus* had free access to standard feed and clean water. They were housed in standard stainless wire cages under 10-12hr/day photoperiod, 25 degrees Celsius temperature and fed with rodent diet and water *ad libitum*. All animal experiments were conducted according to the Committee's guidelines for Control and Supervision on Experiments on Animals.[18] The faculty ethical committee approved the experimental animal usage with ethical clearance number UNN/FBS/EC/1069.

Extracts

Moringa oleifera leaves were harvested in April 2020 from the staff quarters of the University of Nigeria, Nsukka, Enugu State, Nigeria, identified[19] and authenticated by a curator in Department of Plant Science and Biotechnology (PSB) where a voucher specimen, number UNN/Herb/16C, was deposited. The leaves were shade-dried for 3 days to a constant weight and pulverized using an electric blender. The maceration method was employed in extraction.[20] The crude methanolic extract (MoMLE) was fractionated using five solvents (n-Hexane, chloroform, ethyl acetate, acetone, and methanol in increasing order of polarity. The percentage yield was obtained, and phytochemical analysis was carried out following the standard methods.[21,22] An acute toxicity test was carried out according to Lorke.[23]

Antihypertensive Activity

Antihypertensive activity of CME and fractions of MO was assayed using a modified method[24,25] The animal was measured (1.2kg) and then anaesthetized intraperitoneally with 50mg/kg pentobarbitone injection. Incisions were made on the left hind limb and neck region to reveal the carotid artery and the femoral vein, respectively. The carotid artery and the femoral vein were cannulated for administration of various samples and point of connection of UGO BASILE blood pressure (BP) transducer, respectively. In contrast, the trachea was cannulated to avoid death due to nasal mucous blockage.

Heparin (0.2mls) was administered through the femoral vein to prevent blood clotting exposure to atmospheric oxygen, then the baseline BP/heart response was established. Standard preparations of adrenaline 1µg/ml and acetylcholine 1µg/ml were administered through the femoral vein as negative and positive controls, respectively; followed by replicates of varying doses of methanol extract and fractions of *M. oleifera*; flushed with normal saline after every administration to clear the effect of the preceding drug. The cannula attached to the carotid artery was connected to a BP transducer, which monitors changes in blood pressure and heart rate on the UgoBasile double-channel recorder. Drug interaction was also done with atropine to establish a mechanism of action using rabbit jejunum.

Statistical analysis

The analysis of variance (ANOVA) analyzes for significant differences ($p<0.05$) between the mean values. The mean reduction in blood pressure elicited by crude methanol extract, acetone fraction, ethyl acetate fraction, N-hexane fraction, chloroform fraction and the methanolic fraction was compared. The same was done between crude methanol extract and the standard drug acetylcholine. The statistical package for the social sciences (SPSS) version 17 was used for all analyses.

Results and Discussion

Phytochemical and toxicity

Phytochemical composition varied with the solvent of extraction (Table 1). CME had the highest composition of phytochemicals compared to all other fractions. Although alkaloids and flavonoids were the most abundant in CME, other phytochemicals identified from the qualitative phytochemical screening were terpenoids, flavonoids, alkaloids, saponins, glycosides, resins, steroids and tannins, which also varied with the solvent of extraction.

Table 1: Phytochemicals of crude methanol extract and fractions of *Moringa oleifera* leaves

	Qualitative Phytochemical Compositions					
	Crude Methanol Extract (CME)	**n-Hexane (NHF)**	**Chloroform Fraction (CLF)**	**Ethyl-acetate Fraction (EAF)**	**Acetone Fraction (ACF)**	**Methanol Fraction (MEF)**
Alkaloids	+	+	-	+	-	+
Flavonoids	+	-	-	+	+	+
Glycosides	+	+	+	-	+	+
Steroids	+	+	-	+	+	+
Saponins	+	+	+	+	-	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	-	-	+
Resins	+	+	-	+	-	-

-Absent, +=Present

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

This agrees with the report of Mulat *et al*.[26] who investigated phytochemicals constituents of *M. oleifera* using similar solvents and reported the presence of these phytochemicals. Krishnaiah *et al*.[27] also studied phytochemical constituents of six medicinal plants including *M. oleifera* and gave similar report. All other phytochemicals were moderately present in CME. Saponin was present only in small quantities in MEF, while the resin was absent. CLF and ACF had the least number of phytochemicals; four phytochemicals were lacking in both. CLF lacked alkaloids, flavonoids, steroids, and resins, while ACF lacked alkaloids, saponin, terpenoid, and resins. All animals showed usual activity for the acute toxicity study, with no observable death or gross behavioural changes (such as aggression or apathy) for 48 hours (Table 1).

Antihypertensive effects

Intravenous injection of anaesthetized *C. gambianus* with 5mg/ml of CME and fractions of *M. oleifera* elicited various levels of BP reduction, which varied with the solvent of extraction (Table 2).

On the administration of a standard preparation of acetylcholine 1µg/ml, a negative inotropic response of -42.0 ± 4.4 mmHg (Fig. 1a) was elicited, defining the direction of reduction (positive control). In comparison, adrenaline showed a positive inotropic response of 24.0 ± 5.3 mmHg (Fig. 1b) on the myocardium, thereby establishing the direction of increased pressure (negative control). CME showed the highest hypotensive action of -28.7 ± 4.2 mmHg followed by MEF -25.7 ± 1.2 mmHg, while ACF yielded -22.3 ± 10.7 mmHg, all in the same direction with acetylcholine (Fig. 1c-1e). EAF, NHF and CLF elicited -19.7 ± 7.2 mmHg, -19.3 ± 8.1 mmHg and -15.0 ± 3.0 mmHg respectively (Table 2). CME 10 mg/ml and various fractions of *M. oleifera* elicited varying degrees of BP reduction. The tracings are shown in Figure 1f – 1h, revealing a left-wards movement from the points of administration of the treatments over the time that the treatment effect wanes (3 minutes) and the system was flushed with normal saline for another effect to be observed. CME, CLF, and EAF all elicited a negative inotropic response of 28.0 mmHg, ACF (23.0 mmHg) and MEF (20.0 mmHg) being the lowest reduction (Figure 2).

Fig. 1a:

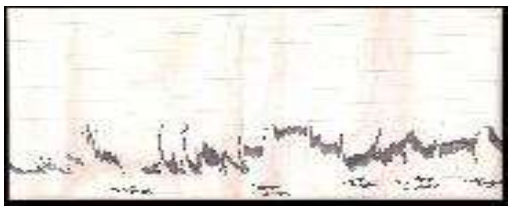

Fig. 1b:

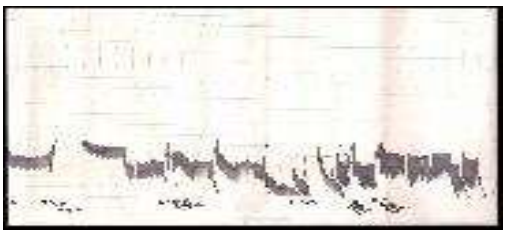

Fig. 1c:

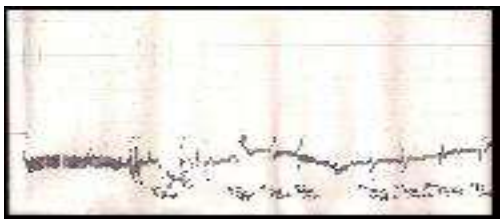

Fig. 1d:

Fig. 1e:

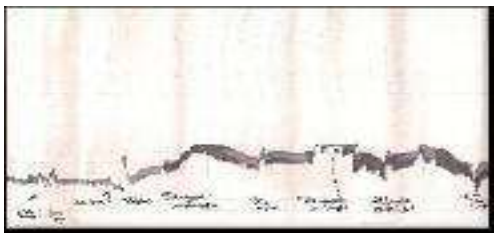

Fig. 1f:

Fig. 1g:

Fig. 1h:

Figure 1a-h: Tracing of baseline and pressure reduction on the administration of 1µg/ml of acetylcholine, b: Tracing of pressure variation on the administration of adrenaline 1µg/ml and 5 mg/ml of *M. oleifera* (CME, NHF, CLF replicated), c: Tracing of pressure variation on the administration of 5 mg/ml of *M. oleifera* (ACF, EAF, CME replicated). CDM- cardiac massage, d: Tracing of pressure variation on the administration of adrenaline, 5 mg/ml of *M. oleifera* (CME, NHF replicated), e: Tracing of pressure variation on the administration of 5 mg/ml of *M. oleifera* (CLF, ACF, EAF, MEF replicated, f: Tracing of pressure variation on the administration of 10 mg/ml of *M. oleifera* (CME, NHF, CLF, EAF replicated), g: Tracing of pressure variation on the administration of 10 mg/ml of *M. oleifera* (ACF, MEF replicated), h: Tracing of pressure variation on the administration of 10 mg/ml of *M. oleifera* (ACF, MEF, replicated). End of tracing – ET.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Acute toxicity test

Animal Groups	Dosages (mg/kg) CME	No. of deaths
Day 1 (0-24h)		
	10	None
	100	None
	1000	None
Day 2 (0-24h)		
	1600	None
	2900	None
	5000	None

Table 3: Antihypertensive effects of Crude methanol extract (CME) and fractions of *M. oleifera* leaves (5 mg/kg) on *C. gambianus*

Treatments	Mean response ± SD (mmHg)
Acetylcholine	42.0 ± 4.4
Adrenaline	24.0 ± 5.3
Crude Methanol Extract	28.7 ± 4.2
Fractions	
N-Hexane Fraction	19.3 ± 8.1
Chloroform Fraction	15.0 ± 3.0
Ethyl-acetate Fraction	19.7 ± 7.2
Acetone Fraction	22.3 ± 10.7
Methanol Fraction	25.7 ± 1.2

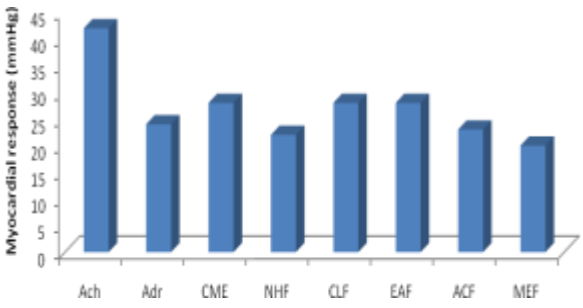

Figure 2: Antihypertensive Effects of Crude Methanol Extract (CME) and Fractions of M. oleifera (10 mg/ml) on C. gambianus. Key: Ach – Acetylcholine, Adr – Adrenaline, CME – Crude Methanol Extract, NHF – n-Hexane Fraction, CLF – Chloroform Fraction, EAF – Ethyl Acetate Fraction, ACF – Acetone Fraction and MEF – Methanol Fraction

The decrease in BP due to CME and fractions of *M. oleifera* 5 mg/ml compared with the lowering achieved by a standard preparation of acetylcholine (Table 3) and an inter-comparison between CME and fractions of *M. oleifera* is made. The negative inotropic response elicited by acetylcholine 42.0 ± 4.4 mmHg was significantly higher ($p<0.05$) than all the fractions and CME. There was no significant difference between the effects elicited by CME 28.7 ± 4.2 mmHg and other fractions except CLF 15.0 ± 3.0 mmHg, which was significantly ($p<0.05$) lower than CME in reduction potentials. At 10mg/ml, acetylcholine was still significantly more effective in its antagonistic effect on myocardium pressure output (Figure 3). An interaction of acetylcholine and atropine produced a more negative inotropic effect than acetylcholine alone (Fig. 4). It also blocked the negative inotropic responses elicited by CME and fractions of *M. oleifera* on the myocardium of anaesthetized *C. gambianus*. The extract only (red bars) produced greater negative inotropic responses than the fractions and CME when interacted with atropine (blue bars) (Figure 4). The tracing recorded on the record sheet is presented (Figure 5a). The illustration revealed that acetylcholine had its usual agonist effect on muscarinic tissue contraction on the rabbit jejunum. CME and all fractions produced similar results on the rabbit jejunum (Figure 5b and 5c). Alkaloids isolated from *Thalictrum revolutum* have been shown to possess hypotensive activity[28] and muscarinic alkaloids isolated from elsewhere have a hypotensive effect similar to the use of acetylcholine.[29] Tannins isolated from traditional Chinese herbs have also been reported to have an antihypertensive effect on angiotensin-converting enzymes as e5-specific inhibitors.[30] Saponins isolated from tea-leaf have been documented to have a spontaneous hypotensive effect on hypertensive rats.[31] Therefore, the presence of glycosides, alkaloids, tannins, and saponins may be implicated in the hypotensive potential recorded in this study. Considering the safety levels of the extract, animals showed usual activity with no observable death or gross behavioral changes (such as aggression or apathy) for 48 hours, even at 5000 mg/kg of the test animal, and this is in agreement with the work done by Diallo et al. [32], who reported that the LD50 of the extracts of *M. oleifera* leaves is more than 5,000 mg kg^{-1}. They used the Organization for Economic Cooperation and Development's (OECD) Limit Test technique, primarily used when the investigator has knowledge showing that the test substance is likely to be non-toxic or of low toxicity.[33] The acute oral toxicity analysis result shows that the extracts of *M. oleifera* leaves are non-toxic and healthy in an oral formulation at the low dose tested.

In this study, crude methanol extract and fractions of *M. oleifera* obtained using N-Hexane, chloroform, ethyl acetate, acetone, and methanol have been used as a possible agent for managing hypertension using *C. gambianus* as an animal model. The study has demonstrated that CME and fractions of *M. oleifera* leaves have hypotensive properties due to their negative inotropic effect on the heart, confirming their wide usage in traditional medicine. The antihypertensive effect of CME in the present study agreed with Iwu's[34] report on the hypotensive potency of Moringa leaves. The M2 receptor is the most common type of muscarinic receptor found in the hearts of various mammalian species.[35] Negative chronotropic and inotropic responses were induced by stimulation of these M2 receptors. This happens when the CME or fractions of *M. oleifera* move through the femoral vein, bearing spent blood from the body back to the lungs, re-oxygenation and then to the heart for pumping. When it gets to the heart, the M2 receptors are immediately stimulated, and there is an inhibition of the contraction of the myocardium. This reduces the cardiac output, and BP produces total peripheral resistance and cardiac output. Blood pressure reduction of CME and its fractions happened similarly to the use of acetylcholine but with a significant ($p<0.05$) difference between their effect and that elicited by acetylcholine. This could be attributed to acetylcholine being a specific blocker of muscarinic receptors and is already in a refined state while the extracts used are in their crude form. These effects were opposite to that produced by adrenaline which was used as a reference drug that causes positive inotropic and chronotropic effects. This agreed with the study of Fahey, who assessed the prophylactic and therapeutic properties of *M. oleifera* leaves extract and reported its antihypertensive properties.[36] There was no significant ($p>0.05$) difference between the effects elicited by CME and other fractions except CLF, which was significantly lower in reduction potentials. This could be as a result of the absence of some major phytochemicals (alkaloids and flavonoids) and the presence only in a small quantity of two others (glycoside and saponin) reported having antihypertensive effects.[37] An interaction of atropine and the extracts showed that atropine inhibits their effects on the heart. This suggested that it is muscarinic-receptor mediated. In the anaesthetized *C. gambianus*, atropine blocked the hypotensive effects of both acetylcholine and CME and fractions of *M. oleifera* in the same fashion. However, the blockage of acetylcholine was more effective since atropine is a specific blocker of acetylcholine. The tracing obtained using the BP transducer also reveals a negative effect on the heart.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

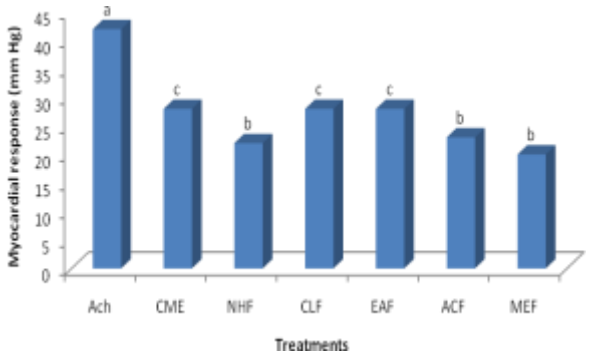

Figure 3: Antihypertensive Effects of (CME) and Fractions of *M. oleifera* (10 mg/kg) compared with acetylcholine.
Bars with different alphabets on top are significantly different ($p < 0.05$). (Ach – Acetylcholine, Adr – Adrenaline, CME – Crude Methanol Extract, NHF – n-Hexane Fraction, CLF – Chloroform Fraction, EAF – Ethyl Acetate Fraction, ACF – Acetone Fraction and MEF – Methanol Fraction).

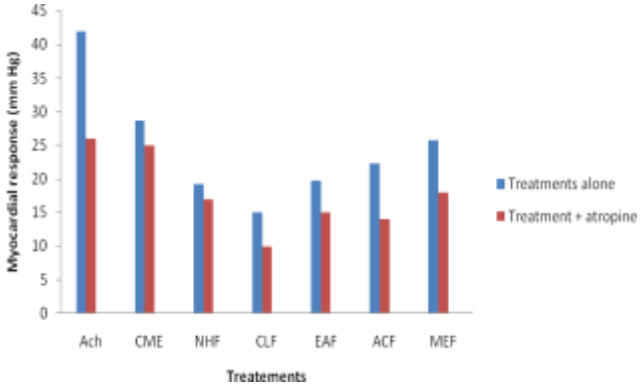

Figure 4: Effects of interactions of acetylcholine, CME, and fractions of *M. oleifera* with atropine.
(Ach – Acetylcholine, Adr – Adrenaline, CME – Crude Methanol Extract, NHF – n-Hexane Fraction, CLF – Chloroform Fraction, EAF – Ethyl Acetate Fraction, ACF – Acetone Fraction, and MEF – Methanol Fraction.

Fig. 5a:

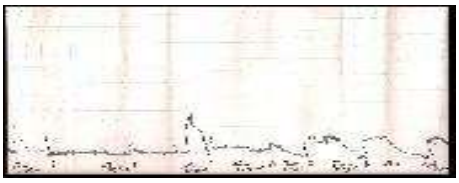

Fig. 5b:

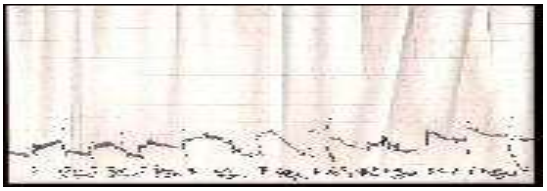

Fig. 5c:

Figure 5a-c: Tracing of pressure variation on the administration of 5 mg/ml of *M. oleifera* and atropine interaction (replicated), b: Tracing of effects of acetylcholine, adrenaline and *M. oleifera* Leaves (*5* mg/ml) on rabbit jejunum, c: Tracing of relaxation effect of *M. oleifera* leaves on rabbit jejunum.

Conclusion

The study has demonstrated the antihypertensive efficacy of fractions of methanolic extract of *M. oleifera* using *C. gambianus*. From the results obtained, it is necessary to conduct a similar study using human populations since *M. oleifera* is widely accepted and consumed either as food for its high nutritional qualities or as medicine for the many claims of its prophylactic properties. This is important because extrapolating from the rat to the human heart can be difficult due to the relative abundance of 1B- rather than 1A-adrenoceptors in the rat heart and the overall expression of functional cardiac 1-adrenoceptors in rats being far higher than in humans. The consumption of *M. oleifera*, either dried or fresh, as food or medicine, is encouraged, and it presents a cheap means of managing the devastating effects of hypertension.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors are truly grateful to all the staff of Animal Genetics and Breeding Laboratory of the Departments of Zoology and Environmental Biology and Pharmacology, University of Nigeria, Nsukka, Enugu State, Nigeria, for the provision of laboratory space, equipment, reagents, and for the care and management of the animal model used for this study.

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