Antihypertensive Effects of *Crossopteryx febrifuga* Leaf Methanol Extract in Adrenaline-Induced Hypertension in Wistar Rats

[1]Isah Otaru Audu, [2]MacDonald Idu, [2]Odaro Timothy and **[3]Fabian C. Amaechina**

[1]Department of Botany, Federal University Lokoja, Kogi State, Nigeria.
[2]Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.
[3]Department of Pharmacology and Toxicology, University of Benin, Benin City, Edo State, Nigeria.

Correspondence: isah.audu@fulokoja.edu.ng

Abstract

The antihypertensive activity of Crossopteryx febrifuga leaf was conducted on Wistar rats using invasive method with the aid of sphygmomanometer and Ugo basile pressure transducer coupled with Ugo basile unirecorder. A concentration of 2.5 mg/ml was made by taking 1 ml from the solution and added to 9 ml of distilled water. From the resulting concentration, graded doses were calculated. After 20 minutes of rat's stabilization, the extract was intravenously administered at (5, 10, 20) mg/kg through the caudal vein. Adrenaline was utilized as hypertension inducing agent while amlodipine and ramipril were used as standard drugs. The extract reduced the systolic blood pressure from 151, 150 and 152 to 124, 126 and 135 respectively mmHg at a span of 0 to 100 minutes. On diastolic blood pressure, the test extract reduced the pressure from 96, 93 and 92 to 87, 82 and 85 respectively over 100 minute period. On mean arterial blood pressure (MABP), the extract reduced MABP from 119, 118 and 119 to 99, 98 and 100 mmHg respectively over 100 minute period. On heart rate, the extract however, reduced heart rate from 359, 358 and 359 beat/min to 338, 339 and 340 beat/min respectively over the same period ($P<0.05$). The extract significantly reduced systolic, diastolic and mean arterial blood pressure as well as heart rate. Going by the results, the methanol extract of C. febrifuga therefore, has antihypertensive property that was revealed via significant reduction in Systolic Blood Pressure and Diastolic Blood Pressure.

Keywords: Antihypertensive, Blood pressure, *Crossopteryx febrifuga*, Invasive method, Wistar rat

1.0 Introduction

Crossopteryx febrifuga (Afzel. Ex.G.Don) Benth., is a plant species commonly called ordeal tree. Mature form normally has rough or cracked bark. Fruit is ovoid in shape, green when unripe and pink when ripe. Leaf is compound pinnate, petiolate and not serrated. It is a deciduous savannah tree distributed across West Africa and in particular, the North Central States of Nigeria where the traditional medical practitioners claim to use it for managing/treating hypertension, fever, stomach ache and general body pain. Some scientific investigations such as anti-plasmodial, analgesic, anti-inflammatory and antipyretic activities of *C. febrifuga* on rodents have been reported by Salawu *et al.* (2008). In addition, other previous studies done on *C. febrifuga* using crude methanol extract revealed that it contains biologically active substances with potential values in the treatment of trypanosomiasis, malaria and *Staphylococcus aureus* infection (Hostettmann *et al*., 2000; Yusuf *et al*., 2004).

Hypertension is described as persistent rise in blood pressure to a level greater than 130/80 mmHg (Salem *et. al*., 2022). Thus, it is a chronic medical condition in which the blood pressure (BP) in the arteries is elevated. In the last three decades, a lot of concerted efforts have been channeled into research for local plants with hypotensive and antihypertensive therapeutic values. The hypotensive and antihypertensive effects of some of these medicinal plants have been validated and others disproved (Lee *et al*., 2008). Traditional knowledge needs to be coupled with modern medicine and more scientific research needs to be done to verify the effectiveness, and elucidate the safety profile of such herbal remedies for their antihypertensive potential.

Hypertension is today one of the principal health problems in the world and a major cause of cardio-vascular deaths in various communities worldwide. It can also lead to other conditions such as congestive heart failure, kidney disease, and blindness (Holm *et al*., 2006; Oparil *et al*., 2018; Wang and Zhang, 2021). It is classified as either *primary* or essential (with no known etiology) or

secondary arising from preexisting condition (Yaxley and Thambar, 2015). About 90 to 95% of cases fall under primary hypertension, which refers to high blood pressure for which no medical cause can be found (Carretero *et al.*, 2000). The remaining 5 to 10% of cases, called *secondary* hypertension, are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system (Beevers *et al.*, 2001). Persistence of hypertension is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure (Pierdomenico *et al.*, 2009). Elevation of arterial blood pressure leads to shortened life expectancy. Both dietary and lifestyle changes as well as medicines can improve blood pressure control and decrease the risk of associated health complications (Okello, 2020).

Hypertension is usually classified based on the systolic and diastolic blood pressure. Systolic blood pressure is the blood pressure in vessels during a heartbeat, usually 120 mmHg in normal person. Diastolic blood pressure is the pressure between heartbeats, usually 80 mmHg in normal person. A systolic or diastolic blood pressure measurement higher than the accepted normal values for the age of the individual is classified as hypertension (Dodt *et al*., 2009).

The long historical utilization of plants in the management of ailments, including hypertension, has demonstrated their efficacy and safety (Aiyeloja *et al*., 2005). Considering the ravaging effect of hypertension, searching for alternative drug of low cost, easy accessibility and minimal side effects from plants with antihypertensive therapeutic value is a necessity. With the aforementioned various challenges in the application of modern medicine for managing hypertension, there is need to search for alternative or natural sources from plants that are cost effective and have minimal side effects through ethnobotany. This research report is first of its kind to provide scientific information on antihypertensive activity of *C. febrifuga*.

2.0 Materials and Methods

2.1 Collection and preparation of plant material

Crossopteryx febrifuga leaves were collected at Zango Daji, Adavi Local Government Area, Kogi State, Nigeria. The plant was identified and authenticated at the Herbarium Unit of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. It was assigned the voucher specimen, UBHdt/217. The leaves were placed under shade, air dried and ground into powder using mechanical grinder. The powdered sample (150 g) was extracted using 500 ml of methanol in Soxhlet apparatus and the extract was concentrated to dryness and weighed.

2.2 Experimental animals

Healthy Wistar rats weighing between 180 g to 200 g were obtained from the Animal House of Faculty Life Science and kept in the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria under standard environmental conditions (free aeration, free access to food and water *ad libitum*) and acclimatized for 7 days. The ethical committee in the Faculty of Life Sciences certified the use of animals of this study with ethical number; LS19027.

2.3 Evaluation of antihypertensive activity of methanol extract of *Crossopteryx febrifuga* in rats

The invasive method described by Amaechina and Omogbai (2007) and Idu *et al*. (2008) was adopted with slight modification.

The rat was fully anaesthetized with a combination of both urethane (1250 mg/kg) and thiopentone (20 mg/kg).

Rat was affixed to a dissecting table and its neck region was cleared of fur, dissected and opened up. The tail vein was cannulated with 23G scalp vein needle for intravenous administration of drug while the trachea was located and cannulated with a narrow polythene tube to aid respiration. The carotid artery was located, cleared of connective tissue, separated from the vagus nerve and cannulated with polythene tube for blood pressure

measurement. The end of the carotid artery leading to the head of the rat was lighted to prevent back flow of blood. Polythene plastic tube was used to cannulate the carotid artery. This was connected to the Ugo Basile pressure transducer (Ugo Basile Bentley Trantec Pressure Transducer, Model: 800; No: 62327) which was connected to Ugo Basile Unirecorder (Ugo Basile Uni-channel Recorder, Model: 7040) for blood pressure measurement. The rats were induced with hypertension using adrenaline (at 6 mg/kg).

Extract of 0.25 mg from *C. febrifuga* was measured and dissolved in 0.5 ml of Tween 20 and then mixed with 9.5 ml volume of distilled water. Definite concentration of 2.5 mg/ml was obtained by taking 1 ml from the solution and added to 9 ml of distilled water from which graded doses were calculated. These doses were administered to rats of known weights intravenously through the tail vein.

After equilibrating for 20 minutes, the methanol extract was intravenously injected at graded doses of (5, 10, 20) mg/kg. Ramipril and amlodipine at 5 mg/kg were administered as standard drugs. Change in both systolic and diastolic blood pressures as well as heart rate were recognized as variance between the steady state value before and the peak readings after injection of the extract. Arterial blood pressure was left to return to basal level prior to administering the next dose. Mean arterial blood pressure (MABP) was calculated as the diastolic blood pressure (DBP) + 1/3 pulse pressure (PP) (systolic blood pressure (SBP) - diastolic blood pressure (DBP)).

During the experiment, normal saline was infused periodically to compensate for loss of fluid and in order to keep the temperature of the rat body maintained, an overhead lamp was put on.

3.0 Results and Discussion

Antihypertensive effect of methanol extract of *Crossopteryx febrifuga* in hypertensive Wistar rats

The results show that methanol extract of *C. febrifuga* exhibited a dose dependent reduction in blood pressure at 5, 10 and 20 (mg/kg) in adrenaline induced hypertension. The extract produced a significant decrease in systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate. Recorded tracings demonstrated the depressions resulting from the extract action in both systolic and diastolic blood pressures (Figure 3.1).

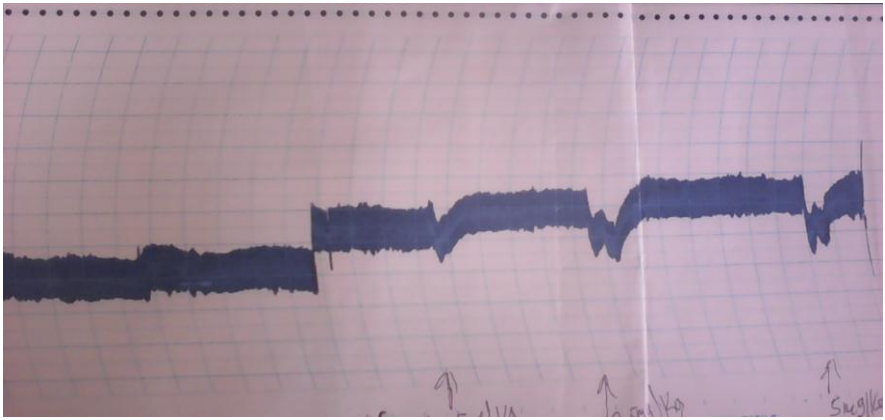

Figure 3.1: Effect of methanol extract of *Crossopteryx febrifuga* on adrenaline induced hypertensive rat (sample of tracing)

The extract at 5, 10 and 20 mg/kg reduced the systolic blood pressure from 151, 150 and 152 to 124, 126 and 135 mmHg at a span of 0 – 100 minutes while the positive controls; amlodipine and ramipril both reduced systolic blood pressure from 151 and 153 to 121 and 120 mmHg respectively (Figure 3.2).

On diastolic blood pressure, normal control remain same, negative control remain high, positive controls amlodipine and ramipril both reduced diastolic blood pressure from 93 and 90 to 82 and 81 mmHg respectively. The test extract at 5, 10 and 20 mg/kg

reduced diastolic blood pressure from 96, 93 and 92 to 87, 82 and 85 respectively over 100 minute period (Figure 3.3).

On mean arterial blood pressure (MABP), as usual the normal and negative controls remain almost at same level throughout the experiment. The positive control; amlodipine and ramipril reduced MABP from 119 and 118 to 98 and 97 respectively. The test extract at 5, 10 and 20 mg/kg reduced MABP from 119, 118 and 119 to 99, 98 and 100 respectively over 100 minute period (Figure 3.4).

On heart rate, the normal and negative control remain virtually same throughout the test. The positive controls; amlodipine and ramipril reduced heart rate from 360 and 361 to 320 and 338 respectively. The extract at 5, 10 and 20 mg/kg however, reduced heart rate from 359, 358 and 359 beat/min to 338, 339 and 340 beat/min respectively at a period of 0 - 100 minutes (Figure 3.5). Amlodipine, ramipril and the extract reduced all the parameters significantly ($P<0.05$).

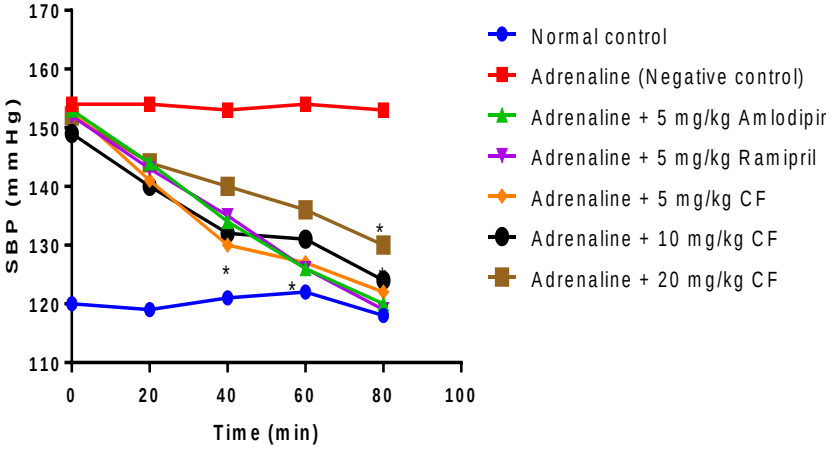

Figure 3.2: Effect of ME of *C. febrifuga* on SBP in hypertensive rats

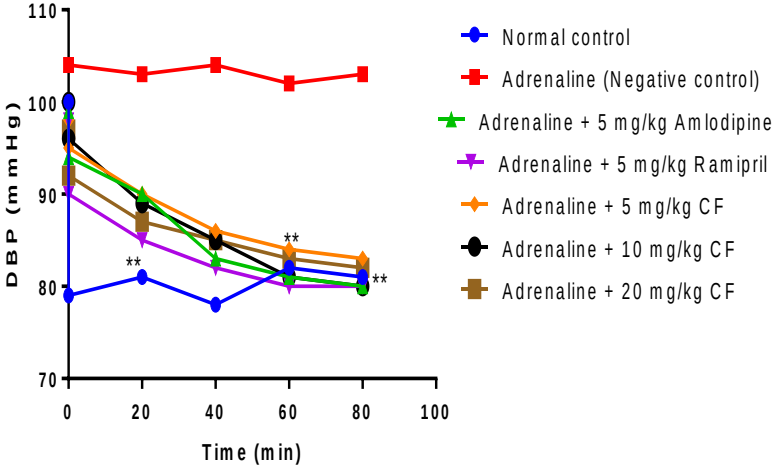

Figure 3.3: Effect of ME of *C. febrifuga* on DBP in hypertensive rats

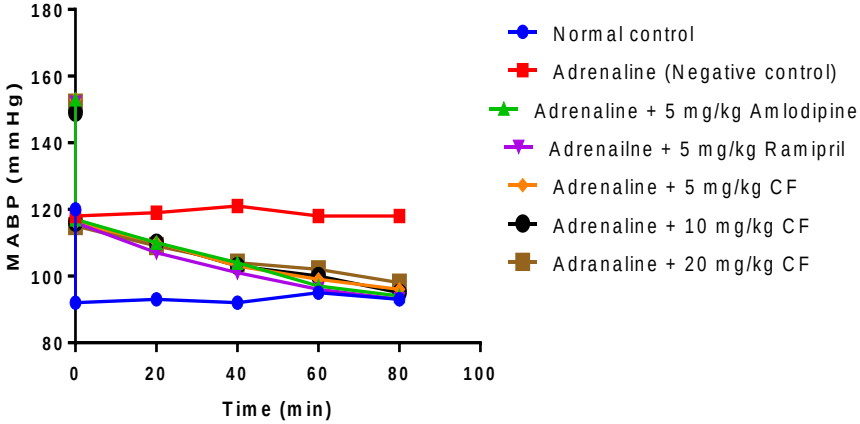

Figure 3.4: Effect of ME of *C. febrifuga* on MABP in hypertensive rats.

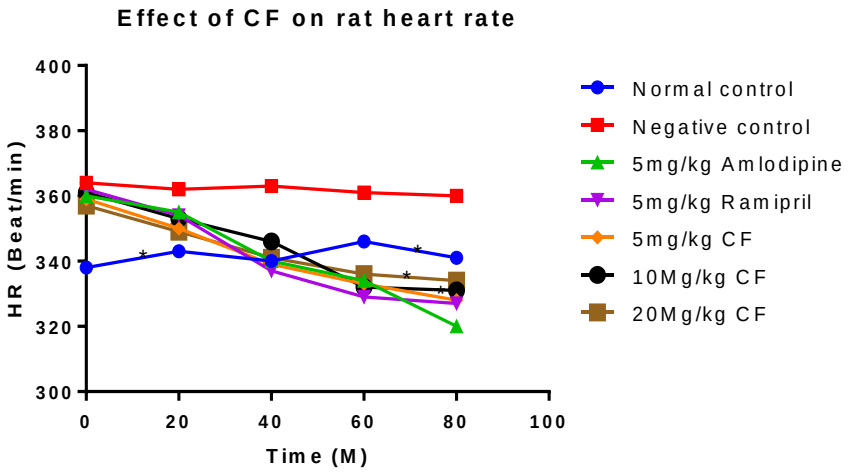

Figure 3.5: Effect of ME of *C. febrifuga* on HR (Beat/min) in hypertensive rats

The effective doses were determined by taking the one-tenth of the value of lethal dose (LD_{50}) in accordance with Wang and Zhang (2021). The extract reduced the systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate. This pattern of action exhibited by the methanol extract of *C. febrifuga* in all the parameters evaluated is in agreement with the earlier reports of Muhammad *et al*. (2016) and Alamgeer *et al*. (2013) on aqueous-methanol extract of *Sonchus asper* and aqueous-methanol extract of *Berberis orthobotrys* respectively in rats.

It is suggestive that the interaction between the extract and adrenaline on the adrenergic receptor agonist cause the receptor inhibition and subsequently, reduction in blood pressure. This observation is in line with those made by Pierre *et al*. (2011) on total aqueous extract of *Justicia secunda*, Fatehi-Hassanabad *et al*. (2005) on *Berberis vulgaris* and Yu *et al*. (2004) on *Stephania tetranda*. The pattern of antihypertensive activity of *C. febrifuga* leaf methanol extract was similar to that of *Nauclea latifolia* root ethanol extract (Nworgu *et al*., 2008; Odey *et al*., 2012) which share

the same family, Rubiaceae and it is possible that both plant species share common phytochemical constituents that may be responsible for showing similar biological activities.

Basically, the regulation of blood pressure is hinged on either vascular or cardiac activity or both. Therefore, the effect of *C. febrifuga* leaf methanol extract could be on one or both factors. This action indicates that the plant extract possibly possesses antihypertensive ingredients in the form of vasodilator or depressor, the identity and mechanism of which need to be ascertained. Furthermore, isolation of the specific active ingredients and the pharmacological screening is required to adequately provide better information on its mechanism of action. The level of potency demonstrated by the methanol extract of *C. febrifuga* leaf in reducing blood pressures and heart rate justified the plant's folkloric utilization in the treatment of hypertension by some locals of the North Central States of Nigeria.

Conclusion

C. febrifuga leaf methanol extract demonstrated significant reduction in blood pressures that is comparable to the effect of the amlodipine and ramipril. Going by this result, the methanol extract of *C. febrifuga* has antihypertensive property that is most perhaps expressed through blocking of voltage operated calcium channel (VOCC) and store operated calcium channel (SOCC) or angiotensin converting enzyme inhibition.

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