HEXANE AND METHANOL EXTRACTS OF *Trigonella foenum-graecum* L. SEEDS: HYPOTENSIVE EFFECTS ON NORMOTENSIVE CATS

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ABSTRACT

Trigonella foenum-graecum L. (Fenugreek) contains a broad spectrum of therapeutic properties: galactagogue and antidiabetic, among others, in traditional medicine practice. The present research was conducted to determine the effects of hexane and methanol extracts of *Trigonella foenum-graecum* L. seeds on blood pressure. Intravenously administered doses (0.1 mg/ml, 1 mg/ml, 10 mg/ml, and 100 mg/ml) of the extracts to anaesthetized normotensive cats were used for the studies. Organ bath connected to Ugo Basile twin-channel recorder (Model 7090). The blood pressure parameters were recorded on the filter paper of the micro-dynamometer.The results of the study produced a decrease in mean diastolic, systolicand mean arterial pressures in a graded dose-response manner, but the decrease was significant as on diastolic blood pressure was 14.50 ± 0.50 mmHg (hexane extract) and 13.50 ± 2.50 mmHg (methanol extract), while the dose of Ach 10µg/ml produced a fall in mean diastolic, systolic, and mean arterial pressure of 13.5 ± 2.50 mmHg, 22 ± 4.0 mmHg, and 16.3 ± 2.5 mmHg, respectively. Atropine (25 mg/ml) at all tolerable doses did not block the hypotensive effect of both extracts but rather potentiated the effect of the extracts. Results of the studies indicated that the seeds of *Trigonella foenum-graecum* L. has hypotensive properties and, therefore, lends pharmaco-physiological credence to folkloric and ethnomedicinal use of the plant in the management and/or control of high blood pressure in some rural communities.

Keywords: fenugreek, hypotensive properties, high blood pressure, *Trigonella foenum-graecum L.*
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INTRODUCTION

As many herbs have been used locally to lower high blood pressure, including Radish *Rauwolfia serpentina, Sesamum Indicum, Solanum sisymbriifolium*, and others, [1]. Cardiovascular diseases (CVD) remain the leading cause of disease burden in the world. The CVD burden continues its decades-long rise for almost all countries outside high-income countries [2, 3]. One of the main factors contributing to disability, mortality, and morbidity in the population through heart attacks and strokes is persistently high blood pressure. It is the most prevalent chronic illness that people worldwide deal with [4, 5].

B-carotene, vitamin C, and vitamin E antioxidants protect and restore the arteries [6, 7, 8]. An annual plant in the Fabaceae family, *Trigonella foenum-graecum* L. is commonly referred to as fenugreek in English, methi in India, hilba in Arabic. The plant, which has tiny round leaves and is grown as a semi-arid crop all over the world, is frequently used as an ingredient in Indian cuisine [9, 10]. Fenugreek seeds are known to contain many medicinal properties, including cardiotonic, hypoglycaemic, diuretic, antiphlogistic, and hypotensive agents [9, 11]. Additionally, a more recent study suggests that *Trigonella foenum-graecum* L. has antioxidant, antihypertensive, anticaking, and anti-inflammatory properties [12, 13, 14].

High arterial pressure is a significant public health concern. Even though it is frequent, asymptomatic, and easily detectable, if untreated, it frequently results in deadly complications [15]. Due to the lack of socioeconomic resources, low-income individuals-particularly rural residents in developing nations-have turned more and more to herbal remedies to manage high blood pressure and the difficulties that come with it [16]. This research was conducted to determine the effects of hexane and methanol extracts of *Trigonella foenum graecum* L. on blood pressure.

MATERIALS AND METHODS

Plant materials

Trigonella foenum-graecum seeds were purchased from a farm in Tudun Wada, Kaduna North Local Government of Kaduna State, Nigeria. Authentication and identification were done by Malam Musa of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A specimen was deposited and voucher number 494 was assigned for future reference.

Drugs and chemicals

Thiopental sulfate, Atropine sulfate 25mg/ml, Promethazine maleate, heparin, Methanol 95% v/v (2.5 L), tween 80, dried seeds of *Trigonella foenum-graecum* L. (140 g).

Laboratory tools and equipment
21-G butterfly cannula, Pressure transducer, Ugo Basile twin channel recorder, thermometer, mercury sphygmomanometer, Petri-dish, Ringer-Locke solution, glass cannula, force-displacement transducer, thermostat water bath, Mattler Toledo Weighing Scale, mortar, pestle, water bath, beaker, dessert spoon, Soxhlet apparatus, timble, grease, distilled water, anti-bubble chips, separating funnel, cotton wool, evaporating dish, Viking Joncod electric milling machine (Model: 9809005), cages and feeders.

Methanol extraction
Using a mortar and pestle, the dried seeds of *Trigonella foenum-graecum* L. were ground into a powder and sieved. The plant material about 140 g of powdered seed sample that had been weighed was added to a separator funnel. After covering the plant with a suitable amount of 95% v/v methanol, the plant was emptied and given another methanol wash after standing for a full day. The filtrate was transferred onto an evaporating dish and heated to around 60°C on a water bath, causing all of the water molecules to evaporate and leaving behind a dark brown residue of extract. The extract residue (approximately 78 g) was transferred into an appropriate container. The extraction technique was adapted from the maceration method [17].

Hexane extraction
Using a Viking Joncod electric milling machine (Model: 9809005), the dried seeds of *Trigonella foenum-graecum* L. were ground into a fine powder. A Soxhlet device was used to extract the 140 g of pulverized seed. The oil from the seed was extracted using a specific glass vessel with repeated washings and percolation with hexane under reflux [18].

Acute toxicity study
The lethal dose (LD_{50}) of the plant extract was determined using the method of using 13 rats (19). A total of nine rats were grouped into 3 groups of 3 each for the first phase of the LD_{50}. Group 1 was administered intraperitoneally with a dose of 1000 mg/kg of body weight, Group 2 was given 100 mg/kg and Group 3 was given 10mg/kg. Four rats were grouped into four (one for each) for the second phase of the LD_{50}, each was administered intraperitoneally with 140 mg/kg, 225 mg/kg, 370 mg/kg, and 600 mg/kg per group respectively. The median lethal dose (LD_{50}) was calculated using the second phase.

Cat blood pressure studies
There were four cats included in the present in vivo studies. Thiopentane sodium was injected intraperitoneally into the cat at a dose of 50 mg/kg to induce anaesthesia. After 20 minutes of administration, the animal passed out. It was then appropriately restrained on the dissecting table by having its limbs tied with thread to prevent the animal from moving around throughout the experiment. A peripheral ligature was used to tie off the exposed left femoral vein. Additionally, a plastic 21-G butterflycannula was used to expose and cannulate the right internal carotid artery to measure blood pressure using a Ugo Basile twin-channel recorder (Model 7090). After the heparinized normal saline was administered, the basal blood pressure was measured using a micro-dynamometer filter paper to prevent. The cannula, which was placed just above it in the left femoral vein, was used to give the medications and extract. The machine's speed and sensitivity were 95mm per minute and 1 mV, respectively. Standard medications (Ach and Adrenaline) were the initial group of pharmaceuticals recorded, followed by graded dose responses for each extract (0.1 ml, 0.4 ml, and 0,8 ml, respectively), and the dose that had the greatest impact was selected as the experimental dose. Following each use of the extract and medication, adequate flushing was performed until the blood pressure returned to normal. Systolic and diastolic blood pressure changes are measured in centimetres (1 cm), which equates to a 10mmHg change in pressure in a glass sphygmomanometer. The diastolic pressure on the tracing is represented by the values from the baseline to the lowest border, while the systolic pressure is represented by the data from the baseline to the top border [20, 21, 22].The research was carried out according to Ahmadu Bello University Guides on Animal Use.

Drug interaction studies
The observed effect of the methanol and hexane extracts of *Trigonella foenum-graecum L.* was similar to that of acetylcholine; therefore, the mechanism of action of acetylcholine was blocked with atropine sulfate, and interaction was attempted using a high volume of methanol extract, i.e., 0.8 ml of 100 mg/ml, which produced the highest observed action.

Statistical analysis
Using SPSS version 20, the gathered data were analysed and presented as mean ± SEM. Using a one-way ANOVA of variance and dependent Paired t-test, all analyses of significance were conducted; values with a p-value of 0.05 or higher were deemed significant.

RESULTS

Acute toxicity study (LD_{50})
Using Lorke's approach, acute research on albino rats using hexane and methanol extracts of *Trigonella*

foenum-graecum L. revealed levels exceeding 5000 mg/kg [19].

Blood pressure studies

The results of the present study have shown that the hexane and methanol extracts of *Trigonella foenum-graecum* L. demonstrate a blood pressure-lowering effect in normotensive cats, where a statistical difference of P≤0.05 was considered significant as obtained from the microdynamometer (Table 1 & 2). The study evaluated the effects of *Trigonella foenum-graecum* L. seeds on blood pressure in normotensive cats. Intravenously administered doses resulted in a significant decrease in diastolic and systolic pressures, with a significant decrease at 0.8 ml compared to acetylcholine, indicating that the plant has a blood pressure-lowering effect.

Table 1: Comparison of blood pressure among normal basal rhythm, acetylcholine, and hexane extract of *Trigonella foenum graecum* L.

B. P (mmHg)	Basal Rhythm	Ach 10µ/ml	Hexane extract 1 mg/ml	10 mg/ml	100 mg/ml
Systolic B.P	27.00 ± 1.00	22.00 ± 4.00^{S}	29.50 ± 2.50^{NS}	29.50 ± 2.50^{NS}	29.50 ± 2.50^{NS}
Diastolic B.P	25.50 ± 1.50	13.50 ± 2.50^{S}	22.50 ± 4.50^{NS}	24.50 ± 5.50^{NS}	14.50 ± 0.50^{S}
PulsePressure	1.50 ± 0.50	13.50 ± 1.50^{NS}	10.00 ± 3.00^{NS}	5.00±1.50^{NS}	6.50±1.50^{NS}
M.A.B.P	26.00 ± 1.00	16.30 ± 2.30^{S}	24.60 ± 2.80^{NS}	25.85 ± 0.85^{NS}	18.00 ± 0.00^{NS}

S = Significant P ≤ 0.05 NS= Not Significant P ≥ 0.05 (ANOVA) n=3. MABP –Mean Arterial Blood Pressure. BP – Blood Pressure.

Table 2: Comparison of blood pressure amongnormal basal rhythm, acetylcholine, and methanol extract of *Trigonella foenum graecum* L.

B. P (mmHg)	Basal Rhythm	Ach 10µ/ml	Methanol extract 1 mg/ml	10 mg/ml	100 mg/ml
Systolic BP	27.00 ± 1.00	22.00 ± 4.00^{S}	16.50 ± 1.00^{NS}	19.50 ± 2.50^{NS}	22.50 ± 2.50^{NS}
Diastolic BP	25.50 ± 1.50	13.50 ± 2.50^{S}	16.00 ± 4.50^{NS}	19.50 ± 5.50NS	13.50 ± 0.50^{S}
PulsePressure	1.50 ± 0.50	13.50 ± 1.50^{NS}	9.00 ± 1.00^{NS}	5.00±1.50^{NS}	4.50 ± 1.50^{NS}
M.A.B.P	26.00 ± 1.00	16.30 ± 2.30^{S}	16.50 ± 2.80^{NS}	19.80 ± 1.15^{NS}	16.20 ± 1.10^{NS}

S = Significant P ≤ 0.05 NS= Not Significant P ≥ 0.05 (ANOVA) n=3. MABP –Mean Arterial Blood Pressure. BP – Blood Pressure.

DISCUSSION

Pharmacologically, conventional medications cause hypotension through a variety of methods. Certain hypotensive medications, such as beta-receptor antagonist propranolol, may directly affect the heart and reduce the force and rate of myocardial contraction. Alternatively, hypotensive medications may relax the vascular smooth muscle and reduce peripheral resistance, such as alpha-adrenoceptor antagonists like prazosin and calcium ion channel blockers like nifedipine.

The Acute Toxicity Study on *Trigonella foenum-graecum* L. (fenugreek) seeds found that the plant has an LD_{50} greater than 5000 mg/kg, making it safe for consumption [20] also found no fatal effects after 90 days of administration; but scientific research reported slight signs of toxicity at doses of 3000 mg and 5000 mg/kg. Overall, the plant's safety and safety have been confirmed [24].

The study found a significant decrease in blood pressure in intravenously administered graded doses of fenugreek extracts, contrary to previous reports [21, 22], who reported significant falls in blood pressure at lower doses with similar chemical constituents.

Many chemical components found in fenugreek, such as ascorbic acid, tetracyclic compounds, antioxidants such as polyphenols, and anti-cholesterol terpenes, may be the cause of the blood pressure-lowering effects seen in plant extracts. Their pharmacological activities explain these extracts' medicinal uses in African traditional medicine. The findings are consistent with other studies on the hypotensive properties of *Phyllanthus amarus* [21] and *Cnidoscolus aconitifolius* [25]. The powerful vasodilation effect may be caused by blood vessel smooth muscle relaxation.

CONCLUSION

The experiment reveals *Trigonella foenum-graecum* hypotensive properties, making it suitable for folkloric and ethnomedical use in managing high blood pressure in rural communities. The extracts' hypotensive effect may be due to myocardial

depression and muscarinic receptor-mediated relaxation.

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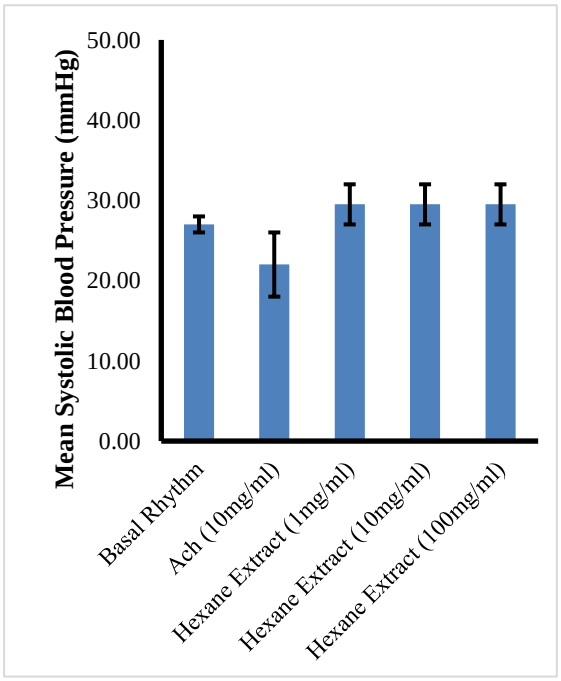


Figure 1: Mean Systolic Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Hexane Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies. *denotes a statistically significant difference.

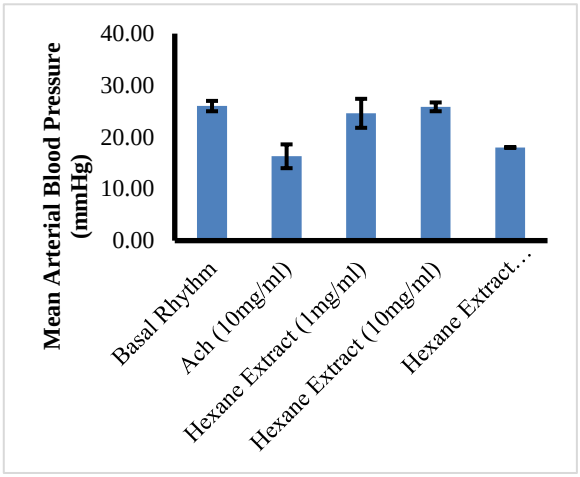


Figure 2: Mean Diastolic Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Hexane Extract of *Trigonella foenum graecum* L. One Way ANOVA in Cat Blood Pressure Studies. *denotes a statistically significant difference.

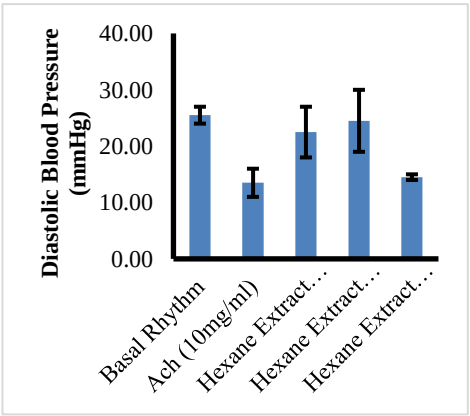


Figure 3: Mean Pulse Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Hexane Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies. *denotes a statistically significant difference.

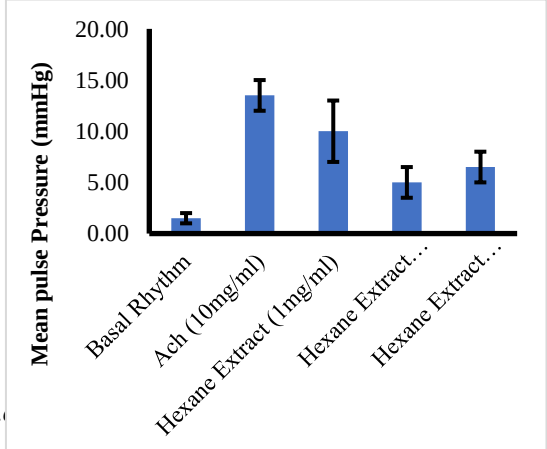


Figure 4: Mean Arterial Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Hexane Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies. *denotes a statistically significant difference.

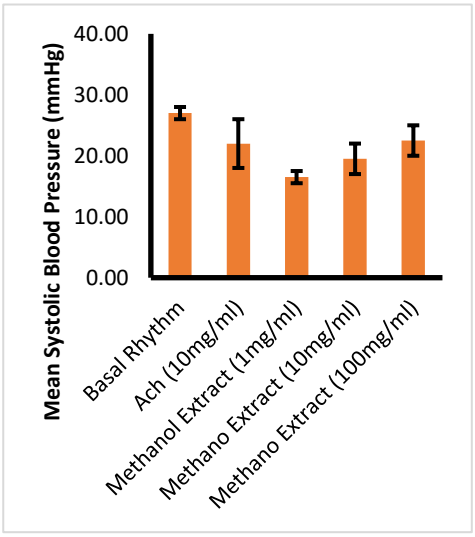


Figure 5: Mean Systolic Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Methanol Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies.

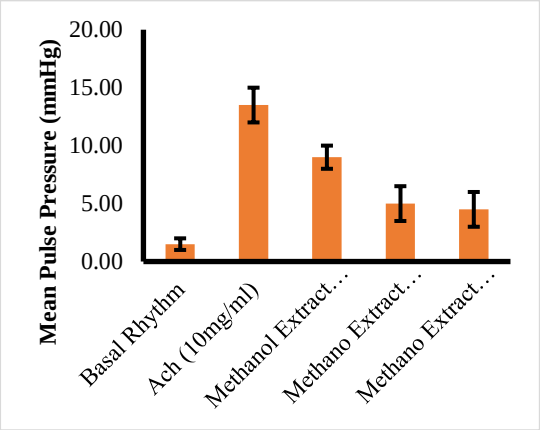
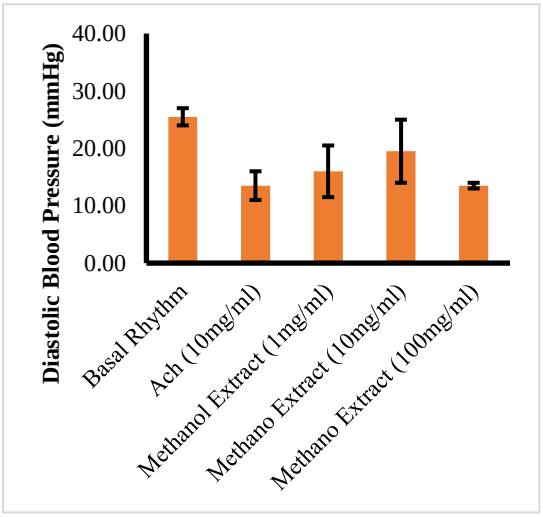


Figure 6: Mean Diastolic Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Methanol Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies.

Figure 7: Mean Pulse Pressure (mmHg) for



Acetylcholine (Ach) 10µg/ml, Methanol Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies. *denotes a statistically significant difference.

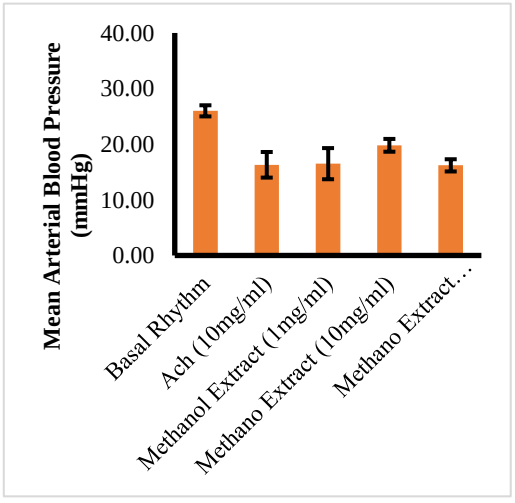


Figure 8: Mean Arterial Blood Pressure (mmHg) for Acetylcholine (Ach) 10µg/ml, Methanol Extract of *Trigonella foenum-graecum* L. One Way ANOVA in Cat Blood Pressure Studies.