RESEARCH ARTICLE

Antihypertensive Effect of *Trigonella Foenum-Graecum* (Fenugreek) Seed Extract in Wistar Albino Rats

Dr. Prativa sahoo[1*], Prof. Dr. Ashok Kumar Panigrahi[2], Dr. Parthsarathi Das[3], Dr. Bhabagrahi Rath[4]

[1]Assistant Professor, Department of pharmacology, Pabitra Mohan Pradhan Medical college and Hospital, Talcher, Odisha, India
[2]Professor, Department of pharmacology, Veer Surendra sai Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha, India
[3]Assistant Professor, Department of pharmacology, Government Medical College and Hospital, Sundargarh, Odisha, India
[4]Professor and Head, Department of pharmacology, Veer Surendra sai Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha, India
Corresponding Author: Dr. Prativa sahoo*

ABSTRACT
Introduction: Hypertension (HTN) is an important public health problem in both economically developed and developing nations[1].it is one of the risk factors for cardiovascular mortality. Fenugreek (*Trigonella foenum-grecum*) seeds have been shown to antihypertensive effect.
Materials and methods: Following institutional animal ethics committee permission, the study was conducted in male wistar rats (150 -250 gm). the model was developed using a high fat (vanspati ghee:coconut oil, 3:1) oral diet along with 25% fructose (high sugar) diet (HFHS) added in distilled water over a period of 6weeks. enalapril (10mg/kg/day) was administered 3weeks after initiation of HFHS diet, 2ml of aqueous extract of fenugreek seed with enalapril(5mg/kg/day) and 2ml of aqueous extract of fenugreek seed continued for another 3weeks.the systolic BLOOD pressure was measured by tail cuff method by Harvard non-invasive B.P apparatus.
Results: The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. Mean and standard deviation (SD) was calculated for quantitative variables. The results were expressed as mean ± SE**.** the results were analyzed by ANOVA followed by tukeys multiple comparison test. There was a highly significant ($p<0.001$) reduction in mean blood pressure in both groups as compared to standard and control group**.**
Conclusion: TFG seed powder showed significant antihypertensive effect which is comparable to that of enalapril the result of our study also revealed that combination of 2ml of fenugreek with half effective dose of enalapril (10 mg /kg) has got beneficial effect on Systolic Blood pressure evaluated.
Keywords: Hypertension, *Trigonella foenum-grecum(*TFG), Enalapril, High fat and high sugar (HFHS), Blood pressure, fenugreek.
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INTRODUCTION

Hypertension (HTN) is an important public health problem in both economically developed and developing nations[1]. As per World Health Organization report, about 40% of people aged more than 25 years had hypertension in 2008[2]. Worldwide, 7.6 million premature deaths (about 13.5% of the global total) were attributed to high blood pressure. About 54% of stroke and 47% of ischemic heart disease worldwide were attributable to high blood pressure[3]. Hypertension has been associated with increased risk of coronary artery disease and is an independent risk factor for cardiovascular and cerebrovascular diseases.[4,5]

To reduce the risk associated with hypertension, the development of several drugs e.g., β blocker, Ca channel blocker, ACE inhibitors, ARB blocker etc. have been adopted intensively in India which has many side effects.

Fructose is widely present in numerous foods. It has been commonly used as a sweetener and promoted as being useful for weight reduction, exercise endurance and diabetes[6]. It has been demonstrated that hypertension develops when normal rats are fed with a fructose-enriched diet[7].

Fenugreek has not been highly explored for its therapeutic profile in hypertension. Therefore, the objective of our current study was to investigate its antihypertensive activity using fructose-induced hypertensive models in rats.

Trigonella foenum-graecum. L. (Leguminosae) is an annual herb, commonly known as fenugreek, is native to the area

**Author for Correspondence: akhiprativa@gmail.com*

Table 1. Chemicals and Drugs

SN	Chemicals / drugs	Source	Nature of use
1	D Fructose extra pure	SD Fine chemicals (Mumbai, India)	For Induction of Hypertension and Hyperlipidemia.
2	High fat and High Sugar diet	Prepare in the Lab	For Induction of Hypertension and Hyperlipidemia.
3	Enalapril	Glenmark Pharmaceuticals (Mumbai, India)	Standard Drug
4	EDTA	MERCK Ltd	Anticoagulant
5	Distilled Water	Prepare in the Lab	Vehicle for Drug

from the eastern Mediterranean to Central Asia and Ethiopia and is cultivated in Pakistan, India, and China[8]. The seeds are hot with a sharp, bitter taste. They are used for their carminative, tonic, laxative, and expectorant properties. They possess antipyretic, anthelminthic, appetite stimulant[9], aphrodisiac[10], antifatigue[11], hypoglycemic activities[12,13]. Fenugreek seeds and leaves are also said to have antidiabetic[14], antiulcer[15], hypolipidemic, antihypertensive properties[16]. Lipid lowering effects of fenugreek have been attributed to Naringenin, a flavonoid having significant hypocholesterolemic effects[17]. Yet there is paucity of data regarding hypolipidemic and antihypertensive effect of fenugreek seed in experimental animals.

MATERIALS AND METHODS

The present study had been undertaken to evaluate antihypertensive effect of *Trigonella foenum-graecum* (fenugreek) seed extract in High fat and high sugar diet induced hypertension in wistar albino rats. The study was approved by institutional animal ethics committee (I.A.E.C), VIMSAR Sambalpur (approval no. VIREC – 02/2020) and the experiment was carried out during the period November 2019 to October 2021 as per CPCSEA Guidelines.

Animals

Adult wistar albino male rats weighing between150-250 gm were used and maintained in central animal house, department of pharmacology, VSS Medical college, Sambalpur, Odisha.

They were housed in clean polypropylene cages (four rats/ cage) and maintained under controlled room temperature (25 ± 10^0 C) and with relative humidity of 45-55% under 12:12hr light and dark cycle. They were provided with standard lab diet and water ad libitum and kept for 1 week to acclimatize with laboratory condition before starting the experiment.

Plant Material

The *Trigonella foenum-graecum* (fenugreek) seed was purchased from local market burla, sambalpur, odisha.

Preparation of aqueous extract of fenugreek seeds

The fenugreek aqueous extract made according to Noor[18] by soaking 10g fenugreek seeds in 250ml of boiled de-ionized water for 1hr. The extract left for whole night and then filtered and completed to 250ml de-ionized water (4%).[18]

Apparatus

1. Oral feeding tube

Table 2. Experimental Study Design of hypertensive effect of administration of aqueous extract of fenugreek seed

Groups	No. of rats	Drugs and duration
I	6	Distilled water×6 weeks
II	6	HFHS×6 weeks
III	6	HFHS×6 weeks. After 3 weeks of HFHS, 1ml aqueous extract of fenugreek seed×3 week
IV	6	HFHS×6 weeks. After 3 weeks of HFHS, 2ml aqueous extract of fenugreek seed ×3week

Table 3. Experimental Study Design of hypertensive effect

Groups	No. of rats	Drugs and duration
I	6	Distilled water ×6 weeks
II	6	HFHS ×6 weeks
III	6	HFHS ×6 weeks. After 3 weeks of HFHS, Enalapril (10 mg/kg/day) ×3week
IV	6	HFHS ×6 weeks. After 3 weeks of HFHS, 2ml aqueous extract of fenugreek seed + enalapril 5mg/kg body wt ×3week

2. CODA Multi channel, Computerised, Non-invasive blood pressure system for mice & rats- Kent Scientific corporation, Torrington, CT, USA. It has the following parts –

- CODA Controller
- Power supply for CODA Controller
- CODA Controller Stand
- Occlusion cuff
- VPR cuff
- Cuff replacement bladder
- Replacement o rings
- Rodent holders
- Animal warming platform
- Infrared thermometer
- Computer

Experimental Protocol

The experimental work was done in two parts:

Part -1: -To get effective conc. of aqueous extract of fenugreek seed

Part -2: Anti-hypertensive effect

Induction of hypertension – High fat and high sugar diet induced hyperlipidemia and hypertension[22]

HFHS constitute high fat (vanaspati ghee: coconut oil= 3:1) oral diet along with 25% fructose (high sugar) added in distilled water[22].

Hypertension was induced experimentally in male Wistar rats (150–250 g) by high-fat diet or by giving 25%fructose solution to drink ad libitum for 6 weeks. Fructose solution was prepared every 2 days by dissolving the fructose in distilled water. Ordinary tap water was given to control animals to drink throughout the whole experimental period (Vogel, 2002)

Part -1

Effective conc. of aqueous extract of fenugreek seed

Twenty-four healthy male wistar albino rats weighing 150-250 gm were taken and randomly divided into four groups(n=6)/ All the standard drugs were administered orally, once daily during the study period.

Thirty healthy male wistar albino rats weighing 150-250 gm were taken and randomly divided into four groups(n=6) and one extra group (n=6) All the standard and test drugs were administered orally, once daily during the study period.

Drug Administration

Enalapril maleate (Envas, 10mg) was used as test drug and the dose was chosen 5 mg/kg/day and 10 mg/kg/day. The test drug was dissolved in distilled water for injection and administered intraperitoneally. The standard drug was administered through oral route.

The animals were weighed. Following this, only those rats who develop hypertension (BP=>130/80 mm Hg) were grouped, continued with the HFHS diet and the standard and test drugs were administered for the next 3 weeks as per the following study design.

Figure 1. CODA multichannel, computerised, non-invasive blood pressure system for mice & rats

Measurement of blood pressure- by non-invasive (indirect) method

For arterial blood pressure measurement using the tail-cuff method, rats were trained for at least 1 week until the blood pressure were steadily recorded with minimal stress and restraint. The first cardiovascular parameters were discarded and the mean of five or six subsequent measurements were recorded.

Measurement of systolic blood pressure

The systolic blood pressure is measured by tail cuff method by Harvard non-invasive B.P. apparatus. NIBP System was used for the measurement of blood pressure of rat. Rear gate of animal holder was removed, the nose cone close to front of holder was slid and thumb nut was tightened. Rat was placed into the holder. The animal was allowed to enter freely into the holder (a little force sometimes was used) if necessary. The rear gate was replaced on the holder and tightened the thumb nut. The animal holder was placed in the V groups of the animal warming platform. Then the nose cone was adjusted so that the animal's movement was limited and the animal appeared comfortable.

The occlusion cuff was slid upward near to the base of the tail (it should slide freely) but was fitted closely when not pressurised (care was taken not to force the cuff further if any resistance encountered during sliding of the cuff). It was positioned as close to the base of tail as possible without force or pressure. The occlusion cuff was secured in tubing inside one of the groups located in the back of the red fastener on top of thew holder, which was keeping the cuff in position.

Then the VPR cuff was slid up the tail, with larger diameter end first, till it reached reaching the occlusion cuff without any resistance (no force should be applied). Tail swelling on any part can be sensed the VPR cuff. The position of VPR cuff was maintained by tubing inside the group situated opposite to that for the occlusion cuff.

The animal was placed in the holder at least 10-15 mins prior to BP measurement for acclimatization so that an accurate pressure measurement could be obtained. After acclimatization, the system was started and all the parameters were recorded by using CODA software installed in the computer.

Cardiovascular parameters (systolic, diastolic, mean blood pressure) were measured weekly for 6 weeks by indirect non-invasive tail-cuff method using Harward non-invasive BP apparatus.

Figure 2. VPR cuff

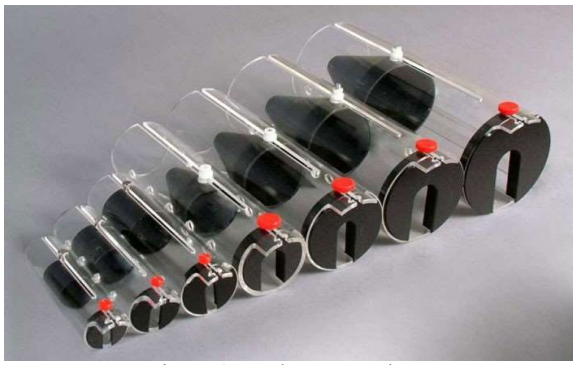


Figure 3. Rodent Restrainer

Table 4. Comparison of mean systolic blood pressure before and after giving different treatment

Animals No	2ml/kg b.w. aqueous extract of TFG groups		Standard Drug (Enalapril malaete) 10 mg/ kg body weight		Combined effective conc. of aqueous extract of fenugreek seed +enalapril 5mg/kg body wt ×3week	
	Before	After	Before	After	Before	After
1	165	131	160	90	150	115
2	218	174	180	122	145	105
3	155	125	145	85	154	118
4	168	130	153	93	164	135
5	170	140	170	97	156	128
6	180	150	158	99	148	125
P value	P<0.001		P<0.001		P<0.001	

Table 5. Effect of TFG and enalapril on blood pressure in HFHS induced Hypertensive rats

Animals No	Control	Standard	Test Drug	Standard Drug (Enalapril malaete) 10 mg/ kg body weight	Combined effective conc. of aqueous extract of fenugreek seed +enalapril 5mg/kg body wt ×3week
1	110	160	131	90	115
2	120	200	174	122	105
3	125	150	125	85	118
4	115	165	130	93	135
5	118	170	140	97	128
6	122	175	150	99	125

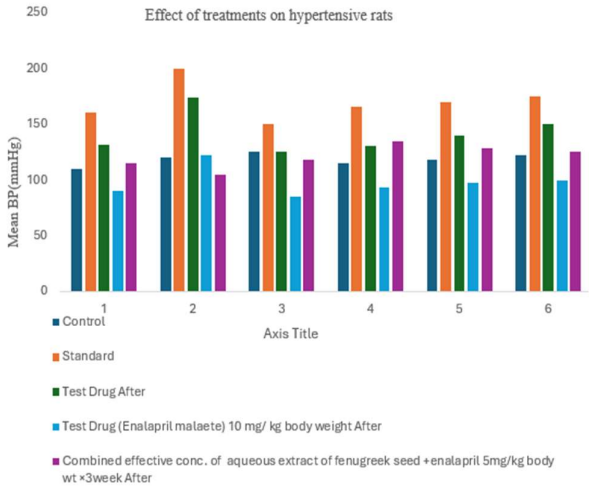
Statistical analysis

Results were analysed by paired t test. Mean Blood pressure before and after treatment were compared for 1ml and 2ml/kg b.w. groups. One-Way ANOVA followed by Post-Hoc Tukey's multiple comparison test was applied to compare with control, standard (enalapril group) and grade the activity across the treatments. The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. Mean and standard deviation (SD) was calculated for quantitative variables. The results were expressed as mean ± SE.

Observation and Results

Results were analyzed by paired t test. Mean Blood pressure before and after treatment were compared for 1ml and 2ml groups.

The blood pressure was measured before dosing and 0.5 hour to 1hour post dose. The results observed were statistically significant p<0.001 improvement with increase in dose of fenugreek 1ml & 2ml respectively. There was a significant decrease in mean blood pressure in 2ml aqueous extract of TFG group as compared with 1ml aqueous extract of TFG group. Following this, then the mean blood pressure ± SEM of control, standard, 1ml aqueous extract of TFG, 2ml aqueous extract of TFG analyzed by ANOVA followed by tukeys multiple comparison test. There was a highly significant (p<0.001) reduction in mean blood pressure in both groups as compared to standard and control group.



Graph 1. Influence of different treatment on rat blood pressure

Results were analyzed by paired t test. Mean Blood pressure before and after treatment were compared for 2ml/kg b.w. aqueous extract of TFG groups, Enalapril 10 mg/ kg body weight treated group and the group treated with combination of 2ml/kg b.w aqueous extract of fenugreek seed + enalapril 5mg/kg body wt. Each animal served its own control. The mean systolic blood pressure significantly (p<0.001) reduced in 2ml/kg b.w. aqueous extract of TFG groups, Enalapril 10 mg/ kg body weight treated group and the group treated with combination of 2ml/kg b.w aqueous extract of fenugreek seed + enalapril 5mg/kg body wt However, the mean systolic blood pressure reduction in Enalapril treated group was more than the combined extract treated group.

One-Way ANOVA followed by Post- Hoc Tukey's multiple comparison test was applied to compare with standard (Enalapril group) and grade the activity across the treatments. The mean systolic BP significantly reduced in test drugs (p<0.001) reduced in 2ml/kg body.wt. aqueous extract of TFG groups , Enalapril 10 mg/ kg body weight treated group and the group treated with combination of 2ml/kg body.wt aqueous extract of fenugreek seed + enalapril 5mg/kg body wt However the mean systolic blood pressure reduction in Enalapril treated group was more than the combined extract treated group and which is also showed highly significant reduction (p<0.001) in comparison with control and standard group.

DISCUSSION

The present study had been undertaken to evaluate the antihypertensive effect of aqueous extract of *Trigonella foenum-graecum* (fenugreek) seed in wistar albino rats in high fat and high sugar fed animals. The effect of the FGS in different doses like 1ml,2ml per kg BW, were studied by estimating lipid profile and systolic blood pressure and compared with that of standard drug atorvastatin and enalapril maleate respectively. The test drug fenugreek was obtained from local market, burla and other drugs, chemicals and vehicles used in this study were procured from different pharmaceutical sources.

Experimental animals

Male wistar albino rats have been selected for this study as rats are these animals are small in size, oral feeding, handling, collecting blood and drug administration in rats are relatively easy. Rats are also relatively resistant to infection and can withstand longer duration of experimentation.

Standard drug and vehicle

In this study Enalapril, an ACE inhibitor was selected as standard drug because the experimental models used were hypertensive wistar, distilled water was used as vehicle as all the drugs and fructose get fully dissolved in distilled water.

Dose and route of administration

The dose and route of fructose administration for induction of hypertension were selected as per the previous published articles and Vogel 2002. The constituent of HFD were followed as per the procedure described by Noor[18]. The dose of Enalapril was selected as per the previous articles. The doses of fenugreek were selected by pilot study. All the drugs and vehicles are administered by oral route with the help of oral feeding cannula for rats. But the Enalapril administered by intraperitoneal route.

Experimental models

HFHS model was used in this present study as it is a combination of high fat (vanaspati ghee: coconut oil= 3:1) and high fructose (25%) fed induced hypertension in rats. There are different ways to induce experimental hypertension has been widely used, as it gives a clue about the role of dietary changes in hypertension, which has become an important factor of the modern lifestyle.

The mechanism of fructose-induced hypertension is still not clear. Recent studies have shown that a high-fructose diet is associated with increased blood pressure in rats[19,20,21]. Several studies have demonstrated that chronic fructose feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia, hyperglycemia, and hypertriglyceridemia in a relatively short time in normal rats[22,23,24]. These metabolic changes lead to essential hypertension[25]. Hyperinsulinemia could activate the sympathetic system, which in turn could elevate the BP[27]. An impaired response to endothelium-dependent vasodilators in fructose-fed rats has also been demonstrated[27]. A growing body of evidence indicates that locally generated vasoactive substances such as angiotensin-II (Ang-II) and nitric oxide (NO) are important determinants of the natural history of vascular disease. Recent evidence suggests that endothelial NO production could be decreased in fructose-fed rats at both renal[28] and vascular levels[29].

Endothelial dysfunction is a devastating condition which is associated to induce various disorders such as atherosclerosis, hypertension, diabetes mellitus etc[30]. The essential oil obtained from fenugreek in combination with other essential oils has been employed to reduce systolic blood pressure in spontaneously hypertensive rat[31]. The aqueous and benzene extract of fenugreek has been found to show diuretic activity in a dose dependent manner by increasing the volume of urine and natriuretic activity by increasing the levels of Na+/K+ ions ratio in Wistar rats; which can be employed to treat hypertension[32,33]

CONCLUSION

The present piece of work has been undertaken to evaluate the hypotensive and hypolipidemic effect of aqueous extract of Fenugreek (*Methi, Trigonella foenum-graecum*) in high fat and high sugar fed induced hypertensive models

The animals were fed with high fat high sugar diet ((vanaspati ghee: coconut oil= 3:1) oral diet along with 25% fructose (high sugar) added in distilled water for development of hypertension.

TFG seed powder in different doses was administered orally to both normal, hypertensive rats for a period of 21 days.

BP was measured.

The result of this study revealed that the aqueous extract of *Trigonella foenum-graecum* did not alter the BP in normal rats but showed improvement in hypertensive animals.

TFG seed powder showed significant antihypertensive effect which is comparable to that of enalapril. This effect may be due to saponin rich fraction present in fenugreek.

Positive presence of saponin, alkaloid, 4 hydroxyleucine in TFG seed may be responsible for the hypotensive activity.

The result of our study also revealed that combination of 2ml of fenugreek with half effective dose of enalapril (10 mg /kg) has got beneficial effect on Systolic Blood pressure evaluated.

The present study showed the potency aqueous extract of *Trigonella foenum-graecum* seed to ameliorate the hypertensive in HFHS induced hypertensive rats. Therefore, it can be concluded that aqueous extract of TFG seed may be a potent drug, for treatment and prevention of hypertension used alone or in combination with other antihypertensive. But the standard drugs enalapril is more potent in comparison with aqueous extract of TFG. However further chemical and pharmacological intervention is required to isolate the active phytoconstituents responsible for these effects and establish its use in future.

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