EVALUATION OF SAPONIN RICH FRACTION OF *TRIGONELLA FOENUM GRAECUM* FOR ANTIHYPERTENSIVE ACTIVITY

Arun Theerthahalli Sudheendra[1], Rekha Shenoy[2], Ashok D Taranalli[3]

[1] Lecturer, Dept of Pharmacology
MCOPS, Manipal- 576 104, Fax: +918202571998 Tel: +918202922482, e-mail: arun.ts@manipal.edu

[2]Lecturer, Dept of Pharmacology
MCOPS, Manipal- 576 104

[3] Professor and Head
Dept of Pharmacology
KLES's college of Pharmacy, Belgaum- 590 010

[1]Corresponding author

Summary

To separate the saponin rich fraction of *Trigonella foenum graecum* (TFG) seeds and to evaluate its influence on blood pressure in normal and hypertensive rats.
The ethanolic extract of TFG seeds was fractionated with cold diethyl ether to obtain saponin rich fraction. Albino rats of Wistar strain of either sex weighing between 200 to 250 g were used for the hypertension models. Hypertension was induced by 1K-1C Goldblatt method. Systolic BP was measured by Harward non invasive BP apparatus and Biopack data acquisition system.
The treatment was given as a single dose at two different dose levels of 100 mg/kg and 200 mg/kg bodyweight. The saponin rich fraction of TFG seeds showed significant, dose dependent antihypertensive activity. The extract did not show any observable decrease in blood pressure in normal non hypertensive rats.
The results demonstrate the effectiveness of TFG saponins as a potential treatment for hypertension.

Keywords: 1K- 1C Goldblatt, fenugreek, Non invasive BP

Introduction

Hypertension is the most common complex human disease which has become a major health problem. The antihypertensive therapy has remarkably improved in last 50 years. Despite recent advances in understanding and treating hypertension, its prevalence continues to rise (1, 2). The recent reports suggest that the saponin extract obtained from various plants have shown significant hypotensive activity comparable to enalapril and furosemide (3, 4, 5, 6). Scientific evaluation of existing herbal medicine can be a good strategy to identify potential antihypertensive drugs. The seeds of Trigonella foenum-graecum (Family: Leguminosae) (TFG) have long been known as folk medicine for the treatment of hypertension. However, there is no scientific evidence to substantiate the folklore claims of Trigonella foenum-graecum (fenugreek) against its diverse use. Thus the present study has been undertaken.

Materials and methods

Materials

The fenugreek seeds were procured from local market of Anavatti, Shimoga district, Karnataka and were authenticated by Professor V S Salimath, Dept of Botany, R L Science Institute, Belgaum, Karnataka.
Enalapril maleate (Envas, 10 mg, Cadila Health Care Limited, Ahmedabad) was procured from the hospital pharmacy at KLES Hospital and Research Centre, Belgaum.
The required chemicals of analytical grade were obtained from Loba chemicals, Mumbai

Extraction and preparation of saponin rich fraction

About 150 grams of powdered seeds were packed in the thimble of a soxhlet extractor and subjected to 95% ethanol which was continued for about 20 cycles. The temperature was maintained at 60°C. The extract obtained was distilled and later evaporated to get a thick mass. The extract was concentrated to dryness. The residue was dissolved in minimum amount of methanol and diluted with cold diethyl ether to precipitate the crude glycosidic mixture. This process is repeated several times and the yellow precipitate is collected by filtration (3, 5). Foam test and haemolysis tests were performed to confirm the presence of saponins in the extract.

Animals

Animal care and handling was done according to the statutory guidelines issued by the CPCSEA, Govt. of India. Approval was obtained from the Institutional Animal Ethics Committee for the conduct of the study. Healthy inbred albino rats of wistar strain of either sex were used for the hypertension models. They were maintained under controlled conditions of temperature (23±2^{o}C), humidity (50±5%) and light (14 and 10 h of light and dark, respectively) at the animal house. The animals were provided with food and water *ad libitum*. One animal was housed in each polypropylene cage containing paddy husk as bedding. The surgical intervention was carried out under ketamine anesthesia (10mg/kg)

Induction of Experimental Hypertension

Experimental hypertension was induced by 1kidney-1clip-Goldblatt method. Albino rats of wistar strain weighing 200-250 g were anesthetized, left kidney was partially stenosed by slipping a U-shaped silver clip over the renal artery. Right kidney was removed after tying the renal pedicle. The skin incisions were closed. The surgery was performed under aseptic conditions and topical antibiotic was applied to prevent the infection of the wounds.

Rats were placed in the individual cages post surgery. After 4 weeks the animals with systolic blood pressure higher than 150 mm Hg were selected for the experiment (7). A total of 10 hypertensive rats were allocated for each treatment group. In 04 weeks duration, animals were trained with animal restrainer. The training time was matched with that of the actual measurement time. This was performed to avoid stress to the animals during restrained period and its effect on the outcome.

Measurement of Blood pressure

The systolic blood pressure was measured by tail-cuff method by Harward non invasive BP apparatus. The data was acquired by Biopack data acquisition system.

Drug Administration

The saponin rich fraction was tested at two dose levels of 100 mg/kg and 200 mg/kg body weight. Enalapril maleate (Envas, 10mg) was used as standard drug and the dose chosen was 3mg/kg. The test drug was dissolved in water for injection and administered intra peritoneally. The standard drug was administered through oral route.

Statistical analysis

Results were analyzed by paired t test. Mean Blood pressure before and after treatment were compared for 100 mg/kg and 200mg/kg b.w. groups. 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 statistical analysis was performed using the Graphpad Prism, version 4.00 for Windows, GraphPad Software, San Diego California USA. The results were expressed as mean ± SE.

Results

The blood pressure was measured before dosing and 0.5 hour to 1hour post dose. To get the reasonable inference, at least five readings were taken for each rat and average was considered for the recordable measurement. For the standard drug the blood pressure measurement was performed one hour after the drug administration. Significant reduction in systolic blood pressure was observed in hypertensive rats treated with 100 mg/ kg and 200mg/kg b.w. of the saponin rich fraction and enalapril. Enalapril treated group showed drastic reduction in systolic blood pressure and the effect was long lasting. Each animal served its own control. The results observed were statistically significant at $p<0.05$ for 100mg/kg b.w. and $p<0.0001$ for 200mg.kg b.w. saponin rich fraction of TFG groups and 3 mg/kg b.w enalapril group. The results were expressed as mean ± standard error and tabulated in table-01. The saponin rich fraction showed decrease in blood pressure in a dose dependent manner (Figure-01). On the normal non hypertensive rats, the TFG saponins did not show observable reduction in the blood pressure.

Table 1: Effect of saponin rich fraction and enalapril on blood pressure in 1K-1C Goldblatt Hypertensive rats

Treatment	Test Drug (Saponin Rich Fraction)				Enalapril Maleate	
Dose	100mg/kg (ip)		200mg/kg (ip)		3mg/kg (po)	
	Before Treatment	After treatment	Before Treatment	After treatment	Before Treatment	After treatment
Mean ±SEM*	178.4 ±11.79	159.3 ±11.76	169.9 ±7.74	117.9 ±6.90	162.2 ±5.25	73.86 ±5.28
'p' value	<0.05		<0.0001		<0.0001	

*n=10

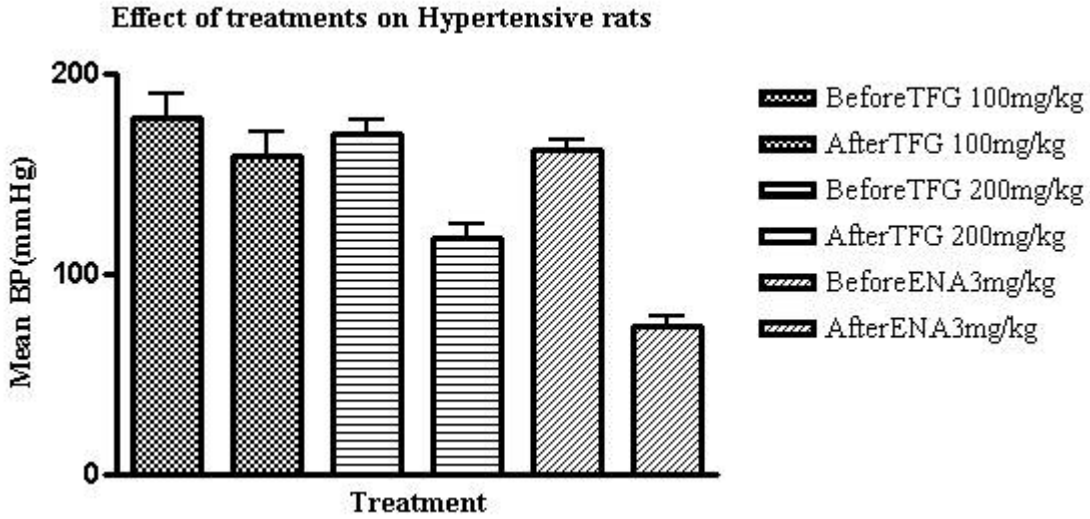

Figure 1: Influence of TFG saponins on rat blood pressure

Discussion

The saponins are naturally occurring surface-active glycosides. They are mainly produced by plants. Saponins constitute a heterogeneous group of substances. Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances (8). Hence the present study was undertaken to verify whether fenugreek saponins could influence blood pressure. The pharmacological data of the present study clearly shows that the saponin rich fraction effectively brought down the systolic blood pressure. However, the reduction in blood pressure was not as long lasting as enalapril. The reduction in the blood pressure was specific to hypertensive rats. The saponin fraction showed good antihypertensive activity in a dose dependent manner on 1K-1C Goldblatt hypertensive rats. To evaluate and confirm the actual mechanism of action, further work needs to be done on purification, characterization of TFG saponins and identification of specific saponin responsible for antihypertensive activity. Evaluation of TFG seems to be an interesting approach to treat the co morbidity.

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