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Original Article

Anti-hypertensive Effect of *Abelmoschus Esculentus* (Okra) Seed Extracts in Fructose-induced Hypertensive Rats

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Abstract

The aim of this study was to investigate the protective activity of ethanolic *Abelmoschus esculentus* seed extract (EAESE) in fructose-induced Hypertensive rat model. Even though several synthetic drugs have been discovered to treat hypertension, it is important to observe the contribution of the daily diet in easing the hypertension and hence the possible therapeutic activity of the seeds from the fruit was studied. The rats were divided randomly into 4 groups (n=6). Group I was considered as the Normal control. Group II animals were treated with only 10% w/v Fructose solution for 6 weeks (42 days). Group III animals were treated with 10% w/v Fructose solution for 6 weeks (42 days) and EAESE (150 mg/kg bw p.o) after 3 weeks i.e. from the 22nd day till the end of the study period (42nd day). Group IV animals were treated with 10% w/v Fructose solution for 6 weeks (42 days) and Enalapril (10 mg/kg bw p.o) after 3 weeks i.e. from the 22nd day till the end of the study period (42nd day). Physiological parameters ECG, heart rate and blood pressure were measured and recorded by the invasive method and the Serum parameters: Total Cholesterol and Triglyceride were measured.

Result: The ECG pattern and Heart rate was improved in the EAESE treated Hypertensive rats. Remarkable reduction in the Blood pressure [systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP)] levels were observed along with a significant reduction in the Total cholesterol and Triglyceride levels.

Conclusion: From the results obtained, it can be suggested that the ethanolic extract of Abelmoschus esculentus seeds has the potential antihypertensive activity as well anti-hyperlipidaemic activity.

Introduction

Hypertension is high blood pressure, that is, a resting systemic pressure consistently above the normal range (90 to 120/60 to 80 mmHg). Clinicians now consider 125 to 139/85 to 89 mmHg to be pre-hypertension (stage before the onset of hypertension). Although hypertension often produces no symptoms, the long-term consequences may be very serious. Chronic hypertension has greatest effects on the main arteries and structure and functioning of heart. Chronic hypertension has its greatest effects on the

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arteries and on the heart. Although the walls of arteries are strong, hypertension weakens them and contributes to arteriosclerosis. Deaths due to hypertension arise from cerebrovascular and cardiovascular complications such as stroke, end-stage renal disease, congestive heart failure, myocardial infarction and cardiac arrest (1). Hypertension is directly responsible for 57% of all stroke related deaths and 24% of all coronary heart disease deaths. Based on the reports of a statistical analysis of clinical trials report, it is observed that the risk is reversible with a relatively modest reduction in blood pressure of 10-6 mm/Hg associated with a 38% reduction in stroke and 16% reduction in coronary events, while a 5-mmHg reduction in blood pressure is associated with a 25% reduction in risk of renal failure (2).

Despite various advances in the field of medicine, it is projected that 1.56 billion people will suffer from hypertension by 2025, which thereby demonstrates the pressing need for the development of novel therapies in the treatment of this pathological condition (3). Lifestyle measures are recommended for all patients, including those with normal blood pressure and those who have a higher risk and require drug treatment include smoking cessation, weight reduction, physical exercise, reduction of excessive alcohol intake (particularly for binge drinking). Although several drugs are available in the market today for treating hypertension, they have been observed to produce a systemic side effect or known to exhibit tolerance upon chronic use. In the last 2 decades, plants referred as crude drugs, have remained historically important as sources of novel compounds with potentials of being channeled into drug pipelines for the development of safe, efficacious, and economically-effective antihypertensive drugs. In sub-Saharan Africa, the initial ethnopharmacological surveys have identified over 100 species of plants with antihypertensive activity in animals and humans (4, 5). To overcome these arising problems nowadays, herbal drugs usage has increased and are more under trials as compared to their synthetic counterparts. The pods of *Abelmoschus esculentus* have long been used as a vegetable and a source of dietary medicine. Pharmacological studies have revealed that okra/bhindi possesses antioxidant, neuroprotective, antidiabetic, antihyperlipidemic, anti-depressant, analgesic and anti-fatigue activity (6, 12). The extracts of the seeds and its derivatives such as quercetin and rutin are known to exhibit a hypolipidemic effect which can be attributed for reducing the risk involved in the cardiovascular diseases such as hypertension. And flavonoids which constitutes a major part of the phytoconstituents found in the seeds and the pods, is found to have a critical role in preventing cardiovascular disease, as it stimulates formation of nitric oxide, increase vasodilatation, and reduce endothelial dysfunction, thereby reducing blood pressure.

Materials and Methods

Experimental Animals:

The study protocol was approved by the Institutional Animal Ethics Committee of PES College of Pharmacy, Bangalore, India, which conforms to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (IAEC Reg. No.-PESCP/IAEC/26/2015). 24 Male Albino Wistar rats weighing about 110-150g were used in the current experimental study. The animals were obtained from AditaBiosys, Pvt. Ltd. Bangalore, India. and were kept at standard laboratory conditions under natural light and dark cycles, a constant room temperature (25±2°C). The animals were fed chow pellet diet and drinking water *ad libitum*.

Chemicals:

Fructose and Ethanol was obtained from Hi-Media laboratories (P) Ltd, Mumbai. Enalapril of Cadila Pharmaceuticals was procured from a local Pharmacy. Heparin was obtained Nirlife, Nirma Ltd, India. Xylazine was obtained from Sanex drugs and chemicals Pvt. Ltd. Normal saline of Eurocare healthcare Pvt Ltd was obtained from a local Pharmacy and Ketamine hydrochloride manufactured by Neon Laboratory ltd. Mumbai was obtained from Excelcare Hospital, Bengaluru, India.

Biochemical Kits:

HDL and Total Cholesterol kits used in the study for HDL and Cholesterol estimations were obtained from ERBA Diagnostics Mannheim GmbH.

Collection of plant material and extract preparation:

The *Abelmoschus esculentus* seeds were collected from the local market in the month of September 2016. The plant seeds (bhindi seeds) were identified and authenticated by a reputed taxonomist of Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. About 117.5 g of the coarse powder was taken in a Whatman Filter paper thimble weighing 5 g and was extracted by continuous hot percolation in a soxhlet apparatus at a temperature 70-80°C, of 1000 ml capacity using 70% v/v ethanol. The extraction procedure was continued for 72 hours and the hydro-alcoholic extract thus obtained was allowed for re-distillation to remove the excess quantity of ethanol from the extract and to concentrate the product into dry mass (6). A brownish golden extract (13.1 g) was obtained and preserved in an airtight container in cool temperature.

Dose Selection:

From earlier literature review, the therapeutic dose of *Abelmoschus esculentus* seed extract was found to be 100 mg/kg and 200 mg/kg, p.o. and hence a medium dose of 150 mg/kg was selected for the present study owing to the preference of using a single dose for the experiment (7, 12, 15).

Experimental Protocol:

Albino Wistar rats were used for the study. 24 rats were randomly divided into 4 groups with 6 animals each and the study was conducted for 6 weeks (42 days). Group I (normal control) received chow pellet diet and normal drinking water throughout the study period till the 42nd day. Group II animals received Fructose at a dose of 10% w/v as a solution replacing normal drinking water throughout the study period of 6 weeks or 42 days along with chow pellet diet. Group III animals received Fructose at dose of 10% w/v as a solution instead of normal drinking water for 42 days and EAESE at a dose of 150 mg/kg p.o for 3 weeks (21 days) from the 22nd day of the study period till the end of the study period i.e. the 42nd day. Group IV animals received Fructose at dose of 10% w/v as a solution instead of normal drinking water for 42 days and Enalapril at a dose of 10 mg/kg bw p. o for 3 weeks (21 days) from the 22nd day of the study period till the end of the study period i.e. the 42nd day.

Blood sampling and serum processing:

Blood sampling was done on 43rd day, 24 hours after the last treatment on 42nd day. Blood was withdrawn from all the animals of each group using capillary tubes of 0.1 mm diameter by retro-orbital plexus puncture under light ketamine (40 mg/kg i.p) anesthesia, transferred to the Eppendorf tubes and then centrifuged at 3000 rpm for 15 min (8). The separated serum was then used for the estimations of Total cholesterol and Triglyceride using standard Erba kit method.

Blood pressure and ECG measurement:

The Blood pressure in each group was determined by invasive method on the 44th day, 24 h after blood withdrawal, by carotid artery cannulation. Overnight fasted (minimum period of 8–10 h) rats were used in the experiment. The animals were anaesthetized with ketamine (60 mg/kg, i.p. and xylazine (16 mg/kg, i.p.). The reflexes of the animals were checked before performing the experiment and were then placed on a suitable rodent surgical table or a flat movable surface (9).

All the surgical instruments were sterilized with 70% alcohol before use. Then the fur and the skin were surgically removed using scissors and forceps. Layer by layer the muscles above the trachea was removed. Carotid artery is situated adjacent to the vagus nerve. Left side of the trachea was surgically exposed, to locate the left common carotid artery and the vagus nerve. The artery was separated from the adjacent nerves and the tissues, using surgical tweezers and forceps. The artery was then staged on a stainless-steel stage and the cephalic end of the blood vessel was tied with help of surgical silk thread of size 1.0. The cardiac end was clamped with a bulldog clip for

cannulation. Then the artery was incised using a microsurgical scissor and cannulated using the rubber catheter which was prefilled with heparinized saline (0.5 IU/ml). The other end of the cannula was connected to a three way stop cock/or saline filled 2.5 ml syringe. Then finally the cannulated site of the artery was tied with the silk thread, without obstructing the blood flow from the carotid artery to the catheter. After cannulation, the bulldog clamp at the cardiac end of the blood vessel was released slowly, ensuring that there is no bleeding at the cannulation site.

The three-way stopcock was connected to the pressure transducer and a syringe filled with heparinized saline. The pressure transducer of the data acquisition system converts BP into an electrical signal.

Statistical Analysis:

Results are expressed as Mean ± Standard deviation. Statistical significance was assessed using oneway ANOVA analysis of variance followed Bonferroni's test comparison of selected pair of columns. *P*<0.05 was considered statistically significant.

Results

Effect of EAESE on ECG:

Group II has shown significant decrease in the P wave duration, an insignificant rise in the QRS complex duration as well as a mildly significant decrease in T wave duration and a highly significant increase in the RR interval duration. The hypertensive rats of Group III treated with EAESE after the induction of hypertension have shown a significant rise in the P wave duration a moderately significant rise in QRS complex duration and a significant recovery in the T wave duration as well as in the duration of the RR interval when compared with the group II hypertensive animals.

Graphical representation of effect of EAESE on ECG on normal and treated:

Effect of EAESE on Blood Pressure:

Blood Pressure in group II has spiked tremendously due to the induction of hypertension after treatment with fructose. The hypertensive rats of Group III treated with EAESE after the induction of hypertension have shown a significant decrease in Systolic arterial pressure or SAP, Diastolic arterial pressure or DAP and Mean arterial pressure or MAP. The similar significant reduction SAP, DAP and MAP is also observed in the hypertensive rats of Group IV treated with Enalapril.

Effect of EAESE on Serum Parameters- Lipid Profile:

Group II rats showed a significant increase in the cholesterol and Triglyceride level after being treated with Fructose for 6 weeks and compared with the normal group. Group III has shown a significant decrease in the cholesterol and Triglyceride level when it is compared with the group II animals. This may be attributed to the potential anti-hyperlipidemic activity of EAESE. Group IV animals treated with Enalapril have also shown significant decrease in their cholesterol and triglyceride levels when compared with the group II animals treated only with fructose.

TABLE I: Effect of EAESE treatment on ECG of normal and treated rats.

Group (n=6)	*Treatment*	*P wave duration (sec)*	*QRS complex duration (sec)*	*T wave duration (sec)*	*RR interval duration (sec)*
I	Normal Control	0.02317±0.002317	0.02917±0.002994	0.03383±0.002041	0.1425±0.005128
II	Fructose	0.01717±0.002858**[a]	0.02517±0.002401	0.02667±0.02667*[a]	0.2655±0.008479***[a]
III	EAESE + Fructose	0.02417±0.002858***[b]	0.0325±0.004889**[b]	0.03883±0.004997***[b]	0.1490±0.006542***[b]
IV	Enalapril + Fructose	0.02633±0.002944***[b]	0.0265±0.003987	0.03583±0.002401**[b]	0.1688±0.007055***[b]

ECG was expressed in wave duration i.e. seconds in each group and the values were expressed as Mean±SD (n = 6) animals in each group. *a P<0.05, **a P<0.001 and ***a P<0.0001, is considered as significant when compared to the normalgroup. **b P<0.001 and ***b P<0.0001 is considered as significant when compared to Fructose group done by Bonferroni's test comparison of selected pair of columns.

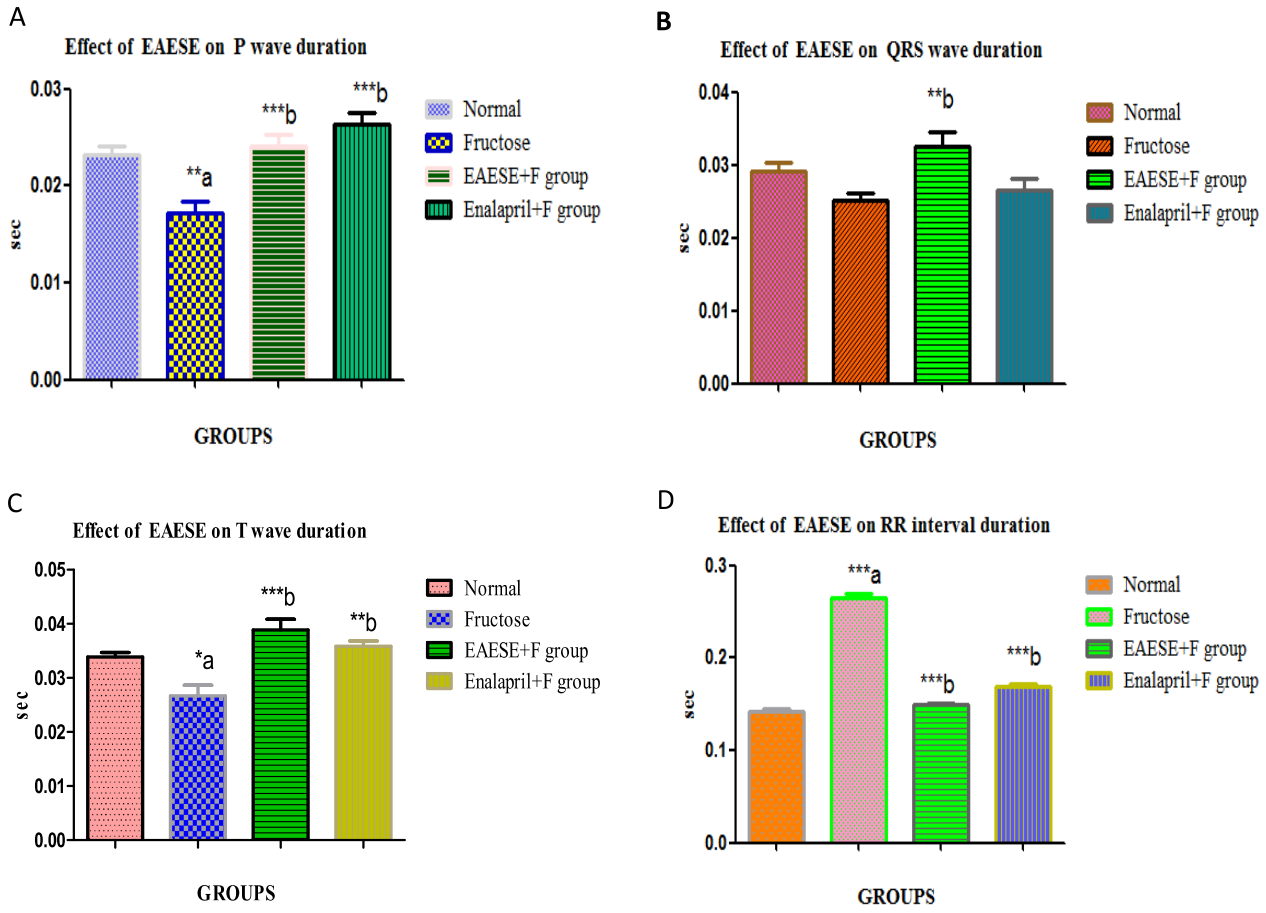

Fig. 1 : Graphs showing the effect of EAESE on ECG (A- P wave, B- QRS wave, C- T wave & D- RR interval wave). Effect Data expressed as Mean±SD (n = 6, Wistar rats) in each group. *a P<0.05, **a P<0.001 and ***a P<0.0001, when compared to the normalgroup. **b P<0.001 and ***b P<0.0001, compared to Fructose group done by Bonferroni's test comparison of selected pair of columns.

TABLE II : Effect of EAESE on blood pressure of normal and treated rats.

Group (n=6)	*Treatment*	*SAP in mm Hg*	*DAP in mm Hg*	*MAP in mm Hg*	*Heartrate in beats/min*
I	Normal Control	111.1±8.229	98.68±9.896	102.4±9.866	421.2±14.93
II	Fructose	174.1±8.079***a	165.7±7.676***a	168.0±7.762***a	226.3±7.074***a
III	EAESE + Fructose	102.2±2.896***b	94.25±2.645***b	96.29±1.134***b	402.7±18.38***b
IV	Enalapril + Fructose	100.8±11.63***b	89.87±7.270***b	93.27±8.547***b	355.7±14.24***b

The blood pressure was expressed in mm of Hg in every group and each value were expressed as Mean±SD (n = 6) animals in each group. ***a p<0.0001 is considered as significant when as compared to normal group; ***b p<0.0001 is considered as significant when as compared to Fructose group done by One-way ANOVA followed by Bonferroni's test comparison of selected pair of columns.

TABLE III : Effect of EAESE on Total Cholesterol and Triglyceride of normal and treated rats.

Group (n=6)	*Treatment*	*Total cholesterol(mg/dl)*	*Triglycerides (mg/dl)*
I	Normal Control	62.40±8.531	18.23±3.463
II	Fructose	74.68±3.615**a	29.43±5.772***a
III	EAESE + Fructose	46.40±4.777***b	16.63±4.770***b
IV	Enalapril + Fructose	42.08±7.108***b	14.65±2.889***b

Total cholesterol and Triglyceride was expressed in mg/dl in each group and the values were expressed as Mean±SD (n = 6) animals in each group. **a P<0.001 and ***a P<0.0001, is considered as significant when the column is compared to the normal groupand ***b P<0.0001 is considered as significant when the columns are compared to Fructose group done by Bonferroni's test comparison of selected pair of columns.

Discussion

In the present research work, Hypertension is caused due to hyperinsulinemia, hypertriglyceridemia and hyperglycemia. The increase in blood pressure following fructose treatment apparently confirms that fructose-induced hypertension in rats is a relatively mild hypertension in comparison with the genetic hypertension, the renovascular, or DOCA salt-induced (deoxycorticosterone acetate) hypertension in rats. The blood pressure in this study was measured by invasive method i.e. by carotid artery cannulation using fluid filled catheters. Hence, this method is focused on measuring the Central Blood Pressure or the systemic blood pressure instead of the peripheral blood pressure, as it is measured directly by cannulating the carotid artery originating from the aorta.

Normal rats of group I exhibited a standard ECG pattern, whereas the animals treated with fructose alone in the Group II for 6 weeks, showed a significant decrease in the P wave duration and QRS complex duration, which indicates enlargement of the atria and due to the compromised ionotropic effect. In the group III rats, after being treated with EAESE and induced hypertension with fructose, showed a significant recovery in the duration of P wave duration, QRS complex duration by showing a significant rise in the time interval. Time taken for the T wave and RR interval has also decreased along with a significant rise in the Heart rate for animals treated with EAESE, which indicates the protective activity of the extract against Hypertension. This can be attributed to the presence of polyphenols and iso-quercetin derivatives, as antioxidants, which have proved to have a curative effect on hypertension (10). In group IV rats as well there has been an overall protection in the ECG pattern, by showing increase in the P wave and QRS complex wave duration, and significant decrease in the T wave and RR interval duration was observed in Enalapril treated group. Heart rate improved considerably as compared to the group II animals. It can be owed to the prevention in ECG alteration and bradycardia due to the role in oxidative stress and antioxidant defense mechanism (11).

The blood pressure of normal group was found out to be in the normal specified range of 80-129 mm Hg for SAP and 91 mm Hg for DAP. When compared with the group II rats treated with Fructose only, for 6 weeks, exhibited a high elevation in the SAP, DAP and MAP which is due to the change in the diet of the animals. High fructose diet induces insulin resistance, leading to hyperinsulinemia and overactivation of sympathetic nervous system, which in turn increases the level of Catecholamines, resulting either in vasoconstriction or endothelial dysfunction, thereby leading to Hypertension. Heart rate in the Group II rats are observed to show decrease in their values, this is because, although elevated Heart rate is associated with increased risk of Hypertension, it holds true in only increasing Peripheral Blood Pressure. Whereas it appears to have an inverse relationship between Heart Rate and Central Blood Pressure. Therefore, this varying relationship is an important consideration to be made while administering antihypertensive medications that effect Heart rate. Group III hypertensive rats when treated with EAESE and compared with the Group II, shows reinstatement in the SAP, DAP and MAP values. This is directly related to the presence of the phytoconstituents, polyphenols, flavonoids and isoquercetin derivatives which has proven antihypertensive activities (13, 14). Heart Rate was also stabilized to normal range due to the improvement in the cardiac performance. Group IV hypertensive animals treated with Enalapril showed a tremendous recovery in the SAP, DAP and MAP values. This is due to its specific activity as Angiotensin Converting Enzyme-Inhibitor, which prevents the conversion of Angiotensin I into Angiotensin II thereby preventing the mechanisms involved in increasing blood pressure such as increased sympathetic activity, aldosterone secretion, ADH (Antidiuretic Hormone) secretion, and arteriolar vasoconstriction, which leads to increase in blood pressure. Therefore, it shows a remarkable activity in restoring the cardiac activity.

The Total cholesterol and triglyceride of the all the 4 groups were compared. And group II has shown a significant increase in the TC and TG in the serum owing to the evidence that elevation of arterial pressure can precipitate coronary thrombosis by causing rupture of the surface of the atherosclerotic

plaque. Hence it is important to monitor the levels of cholesterol and triglyceride in hypertensive experimental animals or human subjects (12). Group III animals have shown a significant decrease in the TC and TG levels. This activity can be attributed to Mucilage, found in okra, is responsible for washing away toxic substances and bad cholesterol, which loads the liver and maintaining the cholesterol level of the body due to the presence of pectin in the fruit and is also due to the proven antihyperlipidemic activity of EAESE (12). In group IV, the levels of TC and TG decreased significantly when compared with the group II rats, this is due to the antihypertensive effect of Enalapril, which prevents the precipitation of HTN or elevated Cholesterol levels.

Conclusion:

EAESE has proven its high therapeutic efficacy as an Anti-hypertensive drug, a considerable reduction of blood pressure parameters SAP, DAP and MAP was observed in fructose-induced hypertensive rats. It has improved the cardiac functioning and heart rate. Further, it decreased the levels of Total cholesterol and Triglyceride in the hypertensive rats.

The exact mechanism behind the activities shown by this extract could not be elucidated through this in *vivo* study, hence, future studies involving the recognition of the pathway and the mechanism of the drug is certainly warranted.

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