



## ANTI-HYPERTENSIVE EFFECTS OF LYCOPENE ON FRUCTOSE INDUCED HYPERTENSIVE RATS.

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### ABSTRACT

Based on the ethno pharmacological use of lycopene in the treatment of hypertension, this study was designed to evaluate its antihypertensive effects in rats. Rats were divided into 5 groups (n=6). Group I was considered as normal control, II received lycopene (30 mg/kg p.o.), III received fructose (10 % w/v p.o.), IV lycopene + fructose (30 mg/kg p.o.+10 % w/v p.o.) and captopril (20 mg/kg p.o.) for 6 week. After 24 h animals were separated and invasive BP (Carotid artery cannulation) and ECG was measured by AD

instruments data acquisition system. After cannulation the animals were connected to the data acquisition system and recorded the BP and ECG. After the experiment the rats were sacrificed using ketamine (100 mg/kg) overdose. From the results of this study, it was concluded that Lycopene has shown significant decrease in systolic, diastolic and mean arterial blood pressure when given to the hypertensive rat. Lycopene has given significant changes in QRS and RR interval segments of the ECG of hypertensive rat.

**KEYWORDS:** Lycopene, Hypertension, ECG, Carotid Artery Cannulation.

### INTRODUCTION

Cardiovascular disease (CVD) has become a ubiquitous cause of morbidity and a leading contributor to mortality in most countries.<sup>[1,2]</sup> Cardiovascular disease (CVD) remains the principle cause of death in the both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year.. The World Health Organization (WHO) predicts the deaths due to circulatory system to double between 1985 and 2015.<sup>[3]</sup>

CVD is a group of problems that occur when the heart and blood vessels are not working the way they should. CVD include coronary heart disease (CHD, heart attacks), rheumatic heart disease, peripheral artery diseases, congenital heart disease and heart failure. The major causes of CVD are tobacco use, alcohol consumption, physical inactivity and an unhealthy diet.<sup>[4]</sup> Diabetes mellitus and hypercholesterolemia are well established risk factors for coronary artery disease.<sup>[5]</sup> Vascular disease include retinopathy, nephropathy, peripheral vascular disease, stroke and coronary artery disease.<sup>[6]</sup>

**Hypertension** is the most common chronic disease in developed societies, affecting >25% of adults.<sup>[7]</sup> The Joint National Committee defines resistant hypertension as failure to achieve goal BP (<140/90 mm Hg for the overall population and <130/80 mm Hg for those with diabetes mellitus or chronic kidney disease) when a patient adheres to maximum tolerated doses of 3 antihypertensive drugs including a diuretic.<sup>[8]</sup> This definition does not apply to patients who have been recently diagnosed with hypertension.<sup>[9]</sup>

In 90–95% of cases of hypertension, there is no underlying medical illness to cause high blood pressure. This is termed “essential” hypertension because it was once erroneously believed that this was an “essential” compensation mechanism to maintain adequate circulation. The precise etiology of essential hypertension is currently unknown. Genetic factors clearly play a part as the condition clusters in families, with hypertension being twice as common in subjects who have a hypertensive parent. Genetic factors account for about one-third of the blood pressure variation between individuals, although no single gene appears to be responsible except in some rare conditions such as polycystic kidney disease and other metabolic conditions such as Liddle's syndrome.

Hypertension is more common in black people of African Caribbean origin, who are also at particular risk of stroke and renal failure. Hypertension is exacerbated by other factors, for example, high salt or alcohol intake or obesity.<sup>[10]</sup> Although many drugs are available in allopathic medicine to treat hypertension, they produce systemic side effects or exhibit tolerance upon chronic use. To overcome this problem nowadays herbal drug are more tested as compared to their synthetic counterparts.<sup>[11]</sup>

**Lycopene** is a carotenoid that is present in tomatoes, processed tomato products and other fruits. It is one of the most potent antioxidants among dietary carotenoids. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a

decreased risk of chronic diseases, such as cancer and cardiovascular disease. Serum and tissue lycopene levels have been found to be inversely related to the incidence of several types of cancer, including breast cancer and prostate cancer. Although the antioxidant properties of lycopene are thought to be primarily responsible for its beneficial effects, evidences suggest that other mechanisms may also be involved. In this article we outline the possible mechanisms of action of lycopene and review the current understanding of its role in human health and disease prevention.<sup>[12]</sup>

The fruit and the vegetable diet as well as the combination diet reduced clinical and ambulatory blood pressure in hypertensive and normotensive subjects more so than a control diet.<sup>[13]</sup> A 6- month primary care intervention, aiming to increase fruit and vegetable intake to five servings a day in hypertensive subjects, revealed increases in  $\alpha$ - and  $\beta$ -carotene, lutein,  $\beta$ -kryptoxanthin, and vitamin C and decreases in systolic and diastolic blood pressure.<sup>[14]</sup> It has been known that increase in dietary carbohydrate intake can raise blood pressure in experimental animal. The increased intake of either sucrose or fructose has shown to enhance the development of either spontaneous hypertension or salt hypertension in rats.<sup>[15,16,17]</sup> Thus, the action of lycopene on fructose induced Hypertension in normotensive rat has been carried out.

## METHODS

**Experimental animal:** Groups of 6 male Wistar rats weighing 210-250 g were used. They were housed two per cage on a 12-h light 12-h dark cycle and were allowed free access to standard laboratory diet (Purina rat chow) and drinking fluid. Drinking fluid consisted either of tap water and 10% w/v fructose solution according to study protocol.<sup>[18]</sup>

The experimental protocols were approved by the Institutional Animal Ethics Committee (Ref No. PESCP / IAEC/ 01 /2014. Dated 25-01-2014) and conducted according to CPCSEA (Reg No- 600/PO/ERe/S/02/CPCSEA validity 12-7-2017) guidelines, Govt. of India.

**Ethical considerations:** All efforts were made to minimize animal suffering and to reduce the number of animals used. The animals received human care and all the experiments were conducted strictly in accordance with the approved guidelines by the “Institutional Animal Ethics Committee” (IAEC), PES College of Pharmacy, Bangalore regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals

(CPCSEA) according to Government of India accepted principles for lab animal's use and care.

**Selection of the dose:** The Lower dose (10 mg/kg) and the higher dose (50 mg/kg) of lycopene were selected as per the previous study performed by the other researchers on lycopene and median dose i.e.30 mg/kg was selected for study protocol.<sup>[19]</sup> The rats were divided into five groups containing six animals each.

**Procedure:** The first group (normal control group) provided with standard rodent diet and water ad libitum for 6 weeks. 10% fructose as a drinking water was provided to induce hypertension to the group II for a period of 6 weeks. Lycopene 30mg/kg in water by oral gavaging route was provided to the group III. Lycopene 30mg/kg in water by oral gavaging route along with 10 % fructose in drinking water were provided to the group IV for a period of 6 weeks. Group V was provided with captopril 30 mg /kg as a standard anti-hypertensive drug along with 10 % fructose in drinking water for a period of 6 week.

At the end of the dosing period, post 24 h animals were separated and invasive BP and ECG was measured by the Power lab instruments and lab chart software.

### **Recording of BP**

Rats were anaesthetized with Ketamine (50 mg/kg) and Xylazine (10 mg/kg) and upon verifying appropriate depth of anaesthesia; the animals were intubated and then transferred to a rodent ventilator. The depth of anaesthesia in the rats was assessed by monitoring the rate and depth of respiration, as well as by assessing the heart rate using ECG electrodes. The rat was provided with a circulating warm water blanket to maintain the body temperature during the surgical intervention. The surgical site was prepped by clipping hair and disinfecting the skin with betadine scrub, followed by 70% ethanol application. A blunt dissection was made along the superficial cervical platysma muscle and the carotid artery was isolated from the sheath after reflecting the salivary glands and overlying musculature.

The pressure sensor was pre-warmed before being inserted into the artery. The tip of the transducer was positioned 1–2 mm distal to the electromagnetic flow probe, which was positioned around the ascending aorta. A midline skin incision was made 1 cm rostral to the manubrium and extending to the xiphoid. The incision was extended into the thoracic cavity, avoiding damage to the internal mammillary artery. The carotid artery was located near the

vagus nerve. One side of the carotid artery, along with the vagus nerve, was separated from the adjacent connective tissue. The carotid artery was separated from the vagus nerve using a small needle, and the cephalic end of the blood vessel was tied and the cardiac end was clamped with a bulldog clip for cannulation.





**Fig.No.1: Pictures showing Carotid Artery Cannulation**

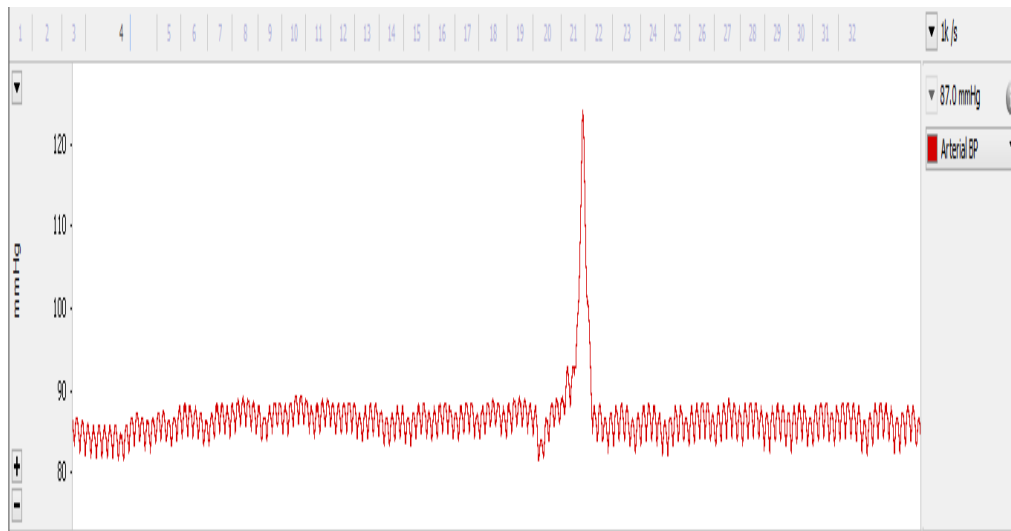
The blood vessel was cannulated using a cannula pre-filled with heparinised normal saline (0.5IU /ml). The other end of the cannula was connected to a three-way stopcock/saline filled tuberculin syringe. Then the carotid artery cannulation site was tied with a thread without obstructing the blood flow in the carotid cannula. After cannulation, the bulldog clamp at the cardiac end of the blood vessel was released slowly, ensuring that there was no bleeding at the cannulation site. The three-way stopcock was connected to the pressure transducer and a syringe filled with heparinised saline. The pressure transducer of the data acquisition system converts BP into an electrical signal which can be read by data acquisition system with the help of lab tutor software.<sup>[21]</sup>

**Recording of ECG:** The animal was restrained by ketamine 30 mg/kg ip and rested down to the surgical table. Then the positive electrode was attached to the surface of left limb, the negative electrode was attached to the surface right limb and the earth electrode was attached to the surface of the posterior limb of the animal. The electrodes were then attached to the transducer which was connected to the data acquisition system. The ECG was recorded in the system with the help of lab tutor software.

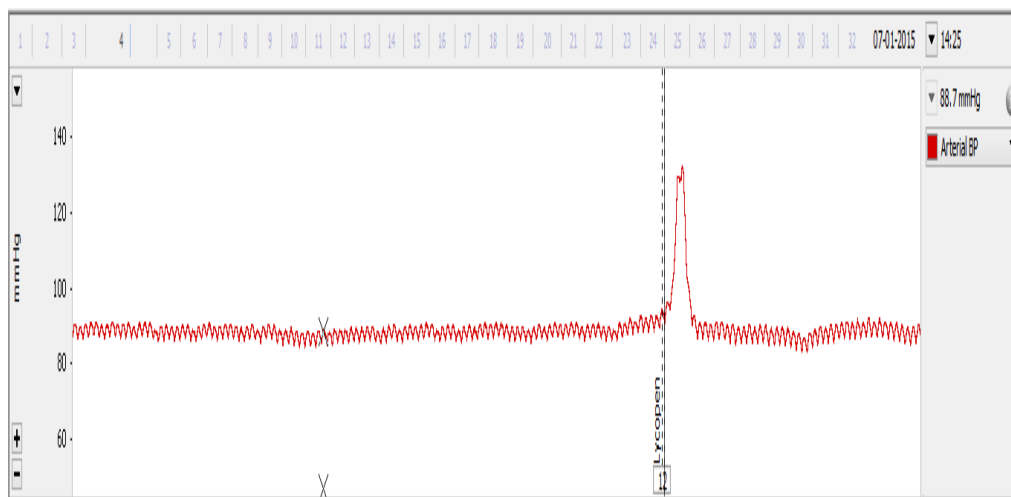
## RESULTS

### Blood Pressure

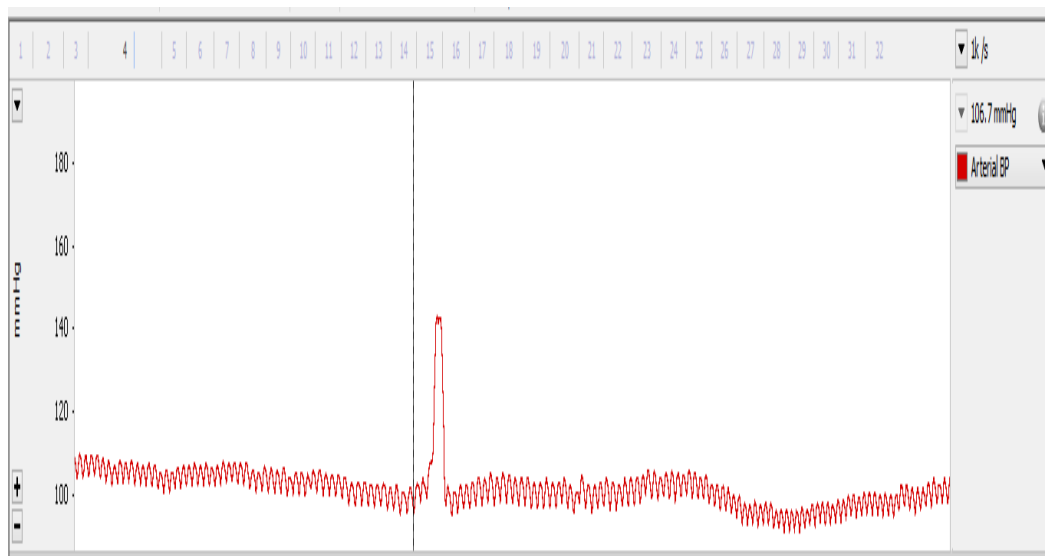
As discussed in the methodology part, all rats were subjected for recording for Blood pressure and ECG. All the results showed that the Fructose group had significant effect in inducing the Blood pressure in normotensive rat. While other group did not show the significant statistics.



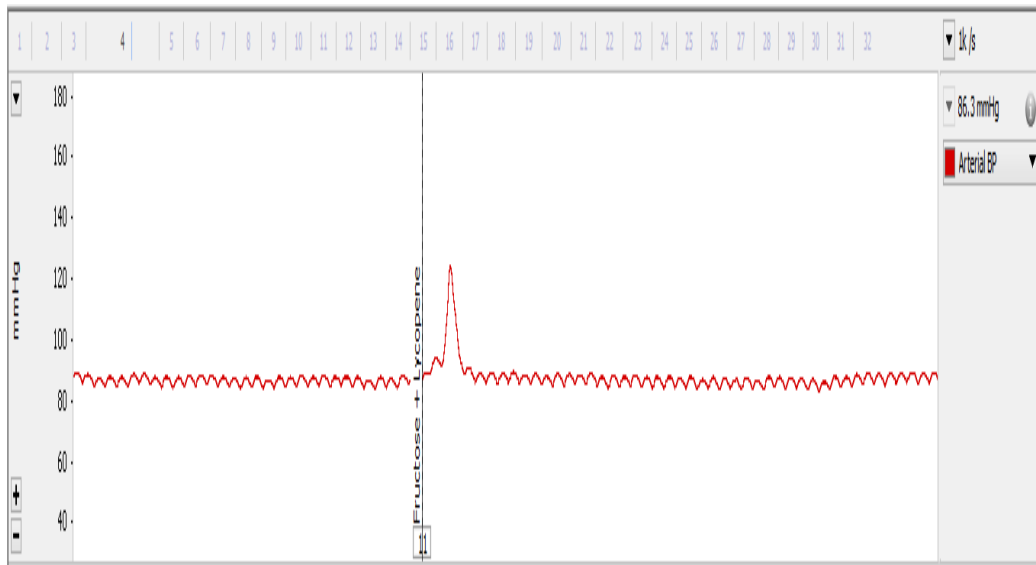
**Fig 2. Control group Blood Pressure graph.**



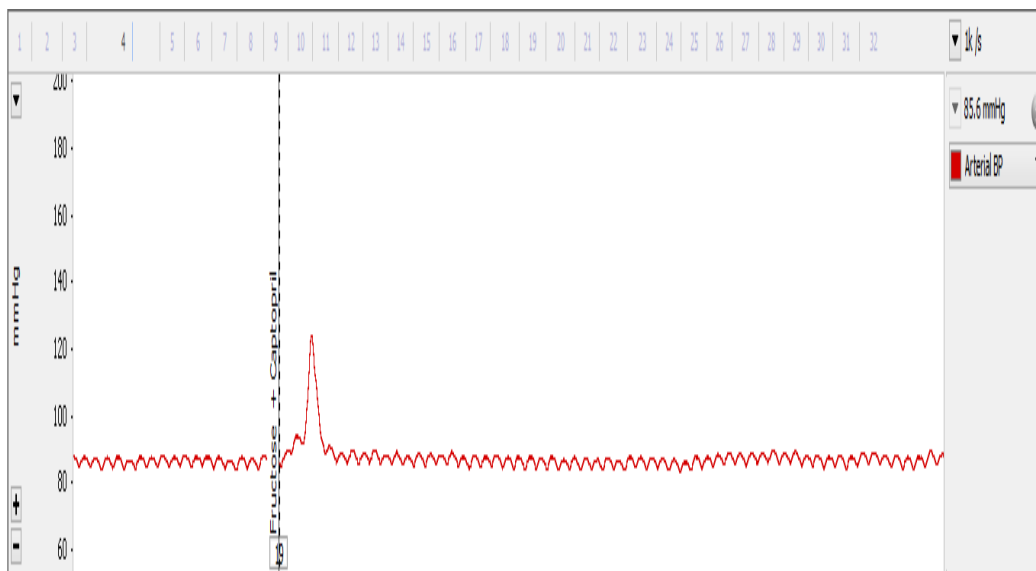
**Fig 3. Lycopene group Blood pressure graph.**



**Fig 4. Fructose group Blood pressure graph.**



**Fig 5. Fructose + Lycopene group Blood pressure graph.**



**Fig 6. Fructose + Captopril Blood pressure graph.**

**Table No.1: The effect s of vehicle, Lycopene, fructose and captopril on rat invasive BP**

Group No. (N=6)	Treatment	Systolic BP (mm of Hg)	Diastolic BP (mm of Hg)	Mean Arterial BP (mm of Hg)
1.	Control	122.15 ± 2.090	84.93 ± 2.792	97.33 ± 1.769
2.	Lycopene (30 mg/kg po)	123.36 ± 5.889	84.85 ± 4.698	98 ± 5.452
3.	Fructose (10% w/v)	144.1 ± 3.655 ****	94.5 ± 4.945 **	111.03 ± 4.466 ***
4.	Fructose (10% w/v) + Lycopene (30 mg/kg po)	123.81 ± 3.230****	83.58 ± 3.757*	96.48 ± 2.617***
5	Fructose (10% w/v) + Captopril (25 mg/kg po)	123.58 ± 1.915***	84.88 ± 3.156*	97.78 ± 2.353**



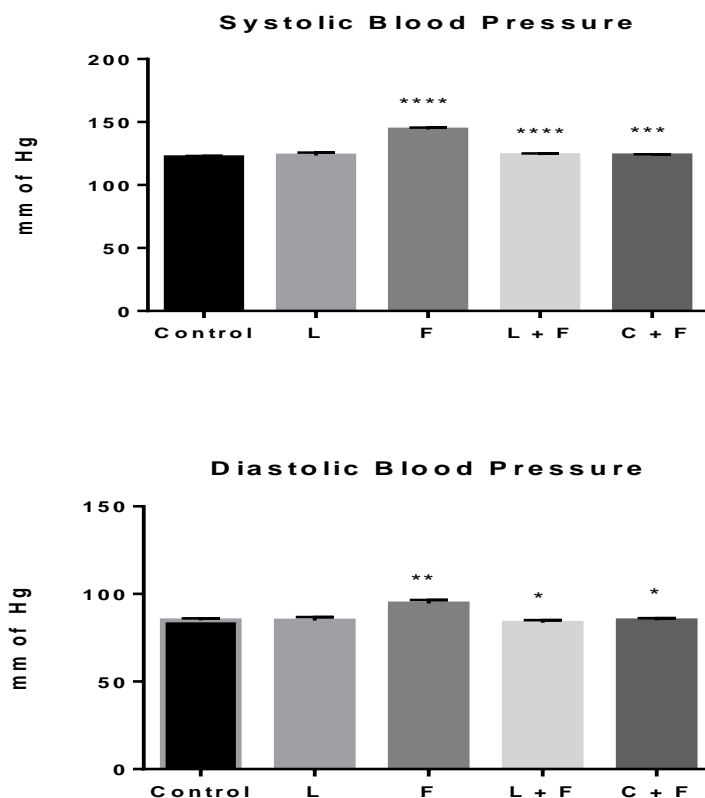
The blood pressure was expressed in mm of Hg in every group and each values were expressed as Mean  $\pm$  SEM (n = 6) animals in each group.  $p < 0.05$ ,  $p < 0.001$ ,  $p < 0.0001$  as compared to control group by One way ANOVA followed by Dunnett's multiple comparison test.

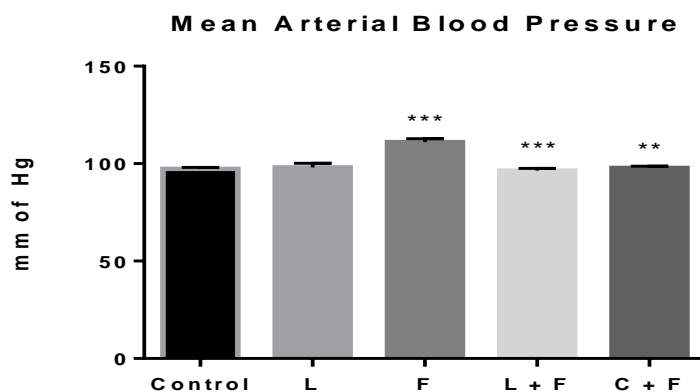
Lycopene group had shown the no significant statistics when compared to control group in systolic, diastolic and mean arterial blood pressure which proved that lycopene did not have any direct effect on blood pressure but maintain the blood pressure in normotensive rat.

Fructose group had shown very significant elevation in blood pressure in Systolic, diastolic and mean arterial Blood pressure ( $p < 0.0001$ ,  $p < 0.01$  and  $p < 0.001$ ) respectively when compared to the control group, as it had induced the hypertension in normotensive rat.

Lycopene along with Fructose showed significant values in systolic, diastolic and mean arterial blood pressure.

Captopril along with Fructose also had shown significant statistics in all the parameters of blood pressure blood pressure.





## ECG

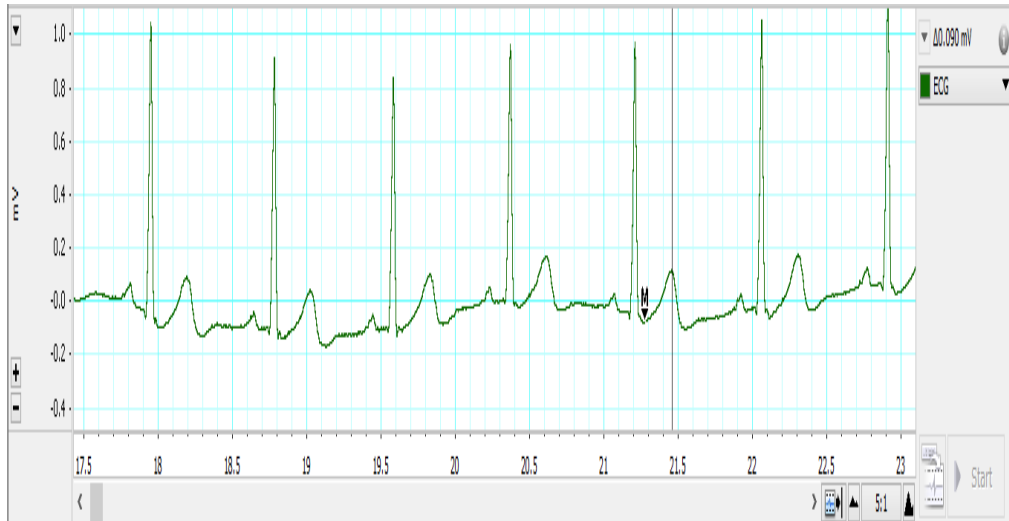
**Table No. 2: Showing the effects on ECG in vehicle, Lycopene, fructose and captopril treated rats**

No.	Treatment	P wave (sec)	QRS complex (sec)	QT interval (sec)	RR interval (sec)	ST height (mV)
1.	Control	0.0766 ± 0.0081	0.0983 ± 0.0075	0.2693 ± 0.0283	0.84933 ± 0.0047	0.1923 ± 0.0025
2.	Lycopene (30 mg/kg po)	0.1011 ± 0.0050 **	0.1238 ± 0.0162 *	0.2498 ± 0.0236	0.8363 ± 0.0120	0.161 ± 0.0176 *
3.	Fructose (10% w/v)	0.0285 ± 0.0038 ****	0.0241 ± 0.0021 ****	0.0423 ± 0.0021 ****	0.3776 ± 0.0041 ****	0.1248 ± 0.0026 ****
4.	Fructose (10% w/v) + Lycopene (30 mg/kg po)	0.0253 ± 0.0013	0.0278 ± 0.0014 *	0.0413 ± 0.0013	0.2813 ± 0.0061 ****	0.1245 ± 0.0051
5.	Fructose (10% w/v) + Captopril (25 mg/kg po)	0.024 ± 0.0014	0.0223 ± 0.0012	0.042 ± 0.0021	0.316 ± 0.0107 ***	0.1423 ± 0.0023

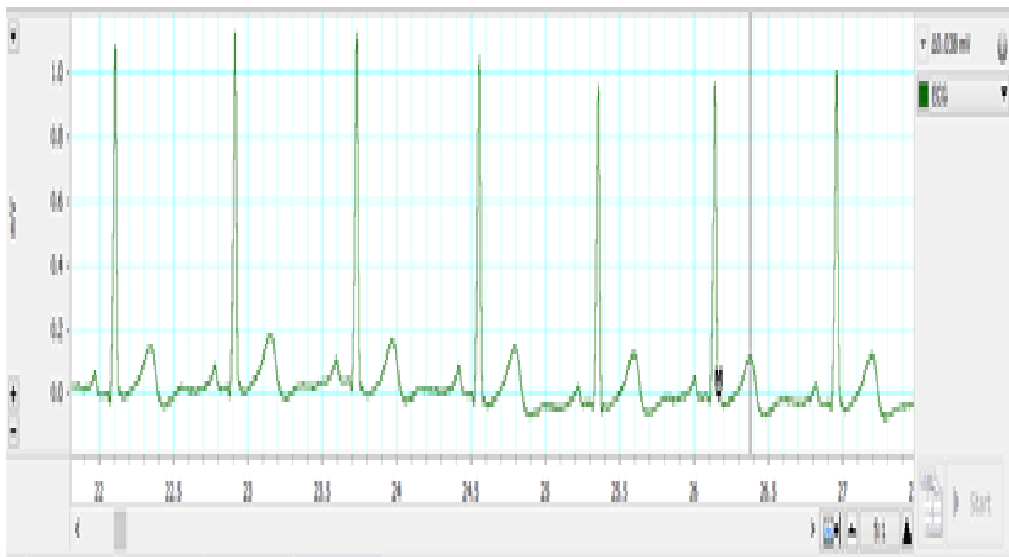
The ECG was expressed in second in every group and each values were expressed as Mean ± SEM (n = 6) animals in each group. p<0.05, p<0.01 p<0.001, <p0.0001 as compared to control group by One way ANOVA followed by Dunnett's multiple comparison test. Lycopene group had shown significant values in P wave (p<0.01) and QRS Complex (p<0.05) but not in the QT interval and RR interval. It had shown the significant values in ST height (p<0.05) when compared to control group.

Fructose had shown very significant values in all the parameters (p<0.0001) when compared to control group. Fructose along with Lycopene had shown significant values in RR interval (p<0.0001) and QRS complex (p<0.05) when compared to Fructose group.

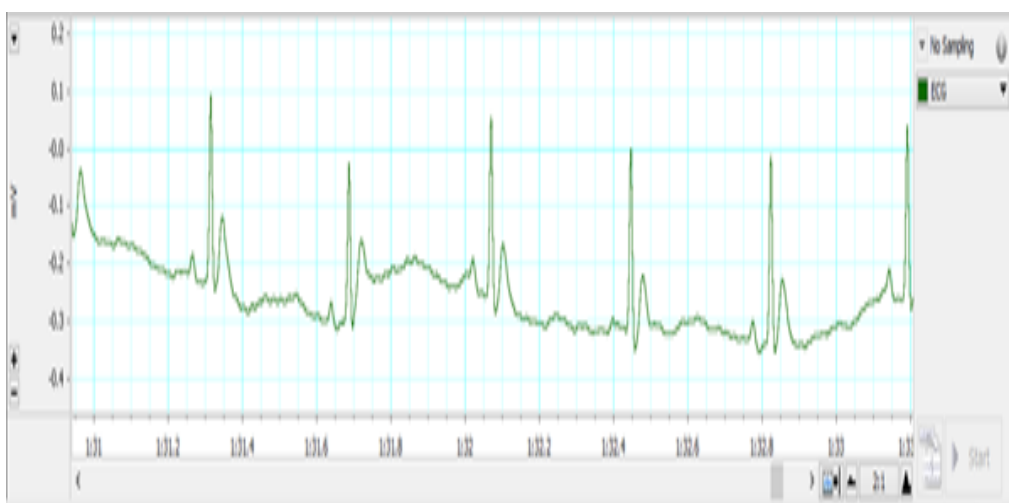
Fructose along with Captopril had shown very significant values RR interval (p<0.001) when compared to Fructose group.



**Fig No. 7. Control group ECG.**



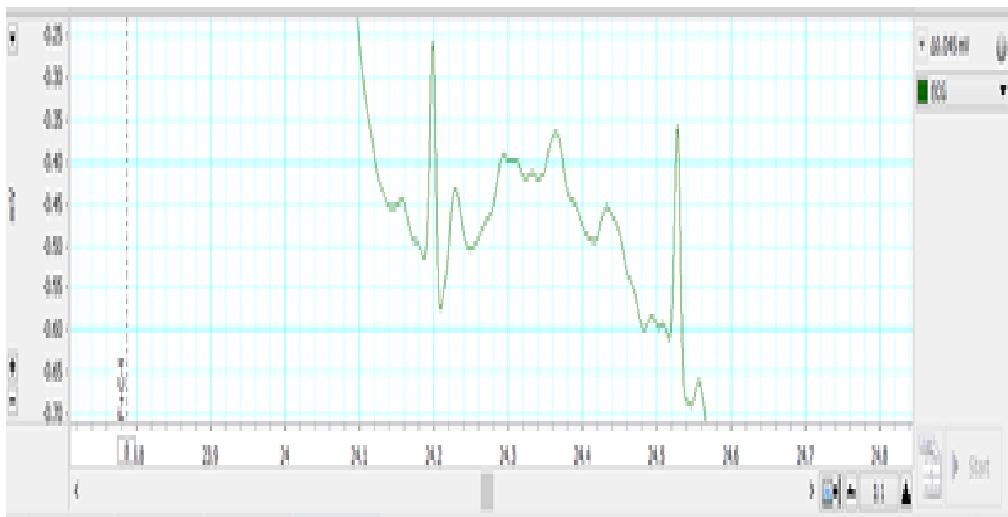
**Fig No. 8. Lycopene group ECG.**



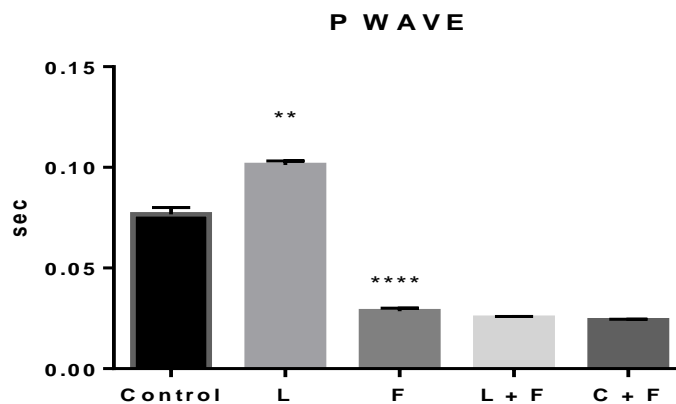
**Fig No. 9. Fructose group ECG.**

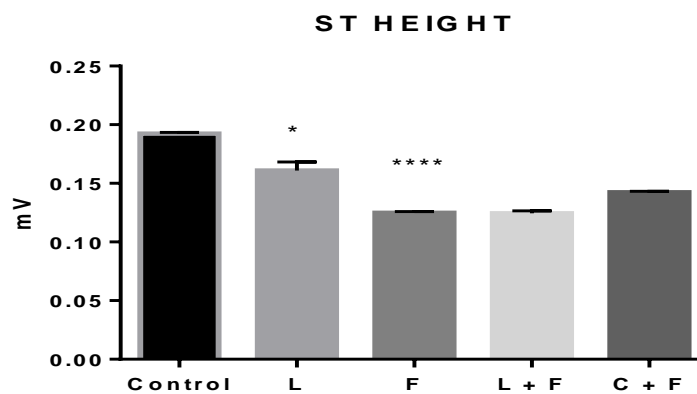
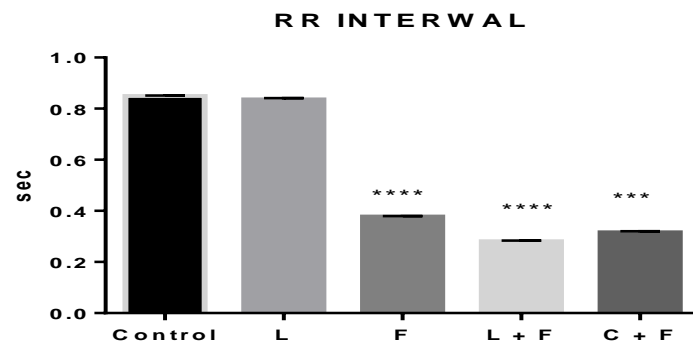
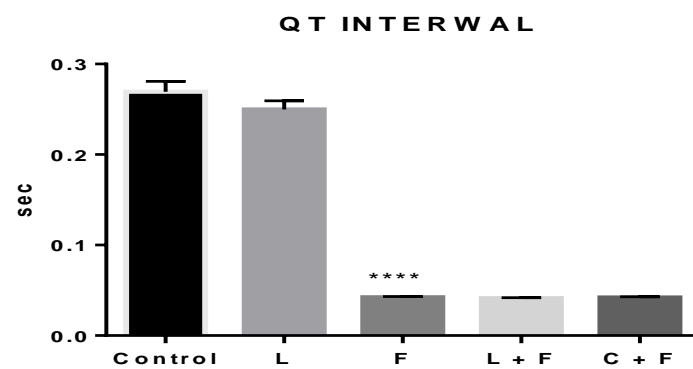
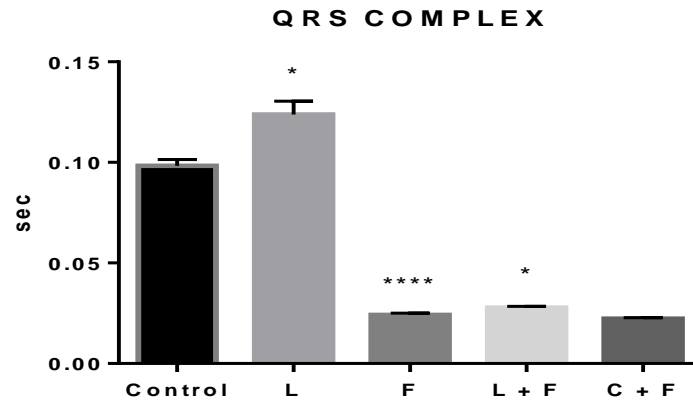


**Fig No. 10. Fructose + Lycopene group ECG.**



**Fig No. 11. Fructose + Captopril group ECG.**





## DISCUSSION

The use of herbs and other natural products as remedies has gained popularity and scientifically proved.<sup>[22]</sup> The present study was designed to evaluate the role of Lycopene on experimentally induced hypertension in rats. As hypertension is a second most cause of the death in western world, the natural drug which has lesser side effects than allopathic drug should be preferred to the maintenance of the same.

In present study, Fructose induced hypertension was assigned to evaluate the anti-hypertensive effect of lycopene in normotensive albino wistar rats. The daily dose of fructose 10 %w/v in drinking water, which is equivalent to a diet containing 48-57% (by calories) fructose, for one week or longer appeared to be most suitable for rapid induction hypertension. Hypertension is precipitated due to occurrence of hyperinsulinemia, hyperglycemia and hypertriglyceridemia. The present study also revealed that the maximum increase in systolic blood pressure following fructose treatment was 20-25 mmHg.. These findings agree with those reported previously.<sup>[23]</sup> It is, thus, apparent that fructose-induced hypertension in rats is a relatively mild hypertension in comparison with the genetic hypertension in SHR<sup>[24,25]</sup> and the renovascular<sup>[26]</sup> or DOCA-induced hypertension in rats.<sup>[27,28]</sup> The present findings that fructose treatment in rats was associated with a decrease in food intake agree with those reported previously.<sup>[29,30]</sup> The decrease in food intake in these animals was compensated for by the increase in intake of the fructose solution as shown by the actual caloric intakes as well as the body weight.

The present study found that the significant increase in blood pressure following fructose treatment occurred. The present study did not attempt to explore the mechanisms of fructose-induced hypertension. However, these results, coupled with the previous findings that fructose-induced hypertension occurred in streptozotocin-induced diabetic rats in the presence of hypoinsulinemia,<sup>[27]</sup> suggest that, in addition to hyperinsulinemia, other mechanisms that have not been thoroughly studied may also contribute to the production of fructose-induced hypertension.

The measurement of Blood pressure in the experimental animal was carried out by invasive method i.e. cannulation of carotid artery, as it gives significant measurement of blood pressure than non-invasive method i.e. tail cuff method and many others. The ECG was taken to support the further evidence to assist the anti-hypertensive effect of Lycopene. The blood pressure result has revealed that lycopene does not have the direct effect on

hypertension but can be useful for the maintenance. At the same time, lycopene has depicted the evidence having direct but not the significant effect on ECG. Moreover, study has revealed that lycopene in hypertensive rat has very significant effect on various ECG wave i.e. P wave, QRS complex, QT interval, RR interval and ST segment.

### CONCLUSION

From the results of this study it is concluded that Lycopene (30 mg/kg) does not have any effect on the blood pressure. The ECG of Lycopene (30mg/kg) has significant effect on P wave, QRS complex and ST height and remaining group also had direct effect on each parameters of the ECG.

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