METABOLIC AND HAEMODYNAMIC EFFECTS OF PP-28 IN FRUCTOSE-FED WISTAR RATS.

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**Summary**

Chronic fructose treatment in rats has repeatedly been shown to elevate blood pressure in association with insulin resistance, hyperinsulinemia and hyperlipidemia. The purpose of the current study was to investigate the effect of a newly synthesized β blocker PP-28 {1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-ol-oxalate.} on blood pressure, heart rate, plasma glucose, insulin, cholesterol, LDL, HDL, VLDL, and triglycerides levels in rats with fructose induced hypertension. Male Wistar rats weighing 180-200 g were divided into five groups of 8 animals each. Control groups were given ordinary drinking water ad libitum throughout the whole treatment course and the remaining groups were given 10% fructose solution to drink ad libitum for nine weeks. After nine weeks, the fructose-treated animals were assigned the following treatment regimens: fructose-fed, fructose plus PP-28 (3, 10 and 30 mg/kg, p.o.) and fructose plus atenolol (10 mg/kg, p.o.). The animals received these treatment regimens orally for the next two weeks. Fructose-fed rats showed significant increase in blood pressure, plasma glucose, insulin, LDL, VLDL, and triglycerides when compared to control groups but not in cholesterol and HDL level and two week after the treatment with PP-28 (10 and 30 mg/kg,p.o.) significantly reversed the high Blood pressure, Heart rate, TG,LDL and VLDL level significantly in fructose feeding rat.

In conclusion, PP-28 was able to prevent BP elevation as well as TG, LDL and VLDL level in fructose-fed rats.

**Keyword:** Fructose induced Hypertension, Hyperinsulinemia, Hypertriglyceridemia.

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Introduction

Clinical studies have demonstrated an association between hypertension, insulin resistance, and abnormal plasma lipid profile, findings that have been commonly referred to as syndrome X (1). Diseases of the cardiovascular system implicate lifestyle. Non-genetic Environmental factors, such as diet can produce hypertension. 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 (2). It has been demonstrated that hypertension develops when normal rats are fed a fructose-enriched diet (3, 4). The fructose-induced hypertensive rat is a diet induced model of hypertension; where in feeding normal wistar rats a fructose enriched diet, results in marked insulin resistance, hyperinsulinemia and elevated blood pressure (5). Insulin resistance and hyperinsulinemia are common findings in patients with essential hypertension (6). These defects in glucose metabolism are associated with a high atherogenic risk profile, and recent evidence suggests that they may play a role in the development of hypertension, dyslipidemia, and atherosclerosis (7, 8).

Insulin resistance has also been documented in several models of experimental hypertension, including the fructose hypertensive rat. The fructose rat model represents an acquired form of systolic hypertension, in which the rise in blood pressure (BP) is not genetically determined but is diet induced. Although the precise mechanism by which hypertension develops in fructose-fed rats has not been defined, it has been proposed that the rise in BP is secondary to the development of insulin resistance and hyperinsulinemia (9). Demonstration of an adverse effect on serum lipids by some antihypertensive agents has focused attention on the actions of antihypertensive drugs other than their blood pressure-lowering properties. Although not proved, it has been suggested that the adverse effect on lipids may offset some of the beneficial effects of reducing cardiovascular risk with antihypertensive drug treatment (10).

During the past few years, studies demonstrated that most antihypertensive agents modify insulin sensitivity in parallel with alterations in the atherogenic lipid profile. $\alpha_1$-Blockers and angiotensin converting enzyme inhibitors were shown to either have no impact on or even improve insulin resistance and the profile of atherogenic lipids, whereas most of the calcium channel blockers were found to be metabolically inert. The diuretics and $\beta$-adrenoreceptor antagonists further decrease insulin sensitivity and worsen dyslipidemia. The mechanisms by which $\beta$-adrenoreceptor antagonist treatment exert its disadvantageous effects are not fully understood, but several possibilities exist: significant body weight gain, reduction in enzyme activities (muscle lipoprotein lipase and lecithin cholesterol acyltransferase), alterations in insulin clearance and insulin secretion, and, probably most important, reduced peripheral blood flow due to increase in total peripheral vascular resistance. Although conventional $\beta$-blocker treatment was able to take care of the former, the latter got worse; the newer vasodilating $\beta$-blocker generation seems to be capable of successfully treating both of them. (11).

These advantages have initiated the search for some novel potential $\beta$-blocker of greater selectivity toward $\beta_1$-receptor which also improves insulin sensitivity and dyslipidemia. We have been involved in development of new $\beta$-blockers for past few years (12). Recently we develop new $\beta$-blocker of same series PP-28 chemically known as 1-tert-butylamino-3-[3-(tert-buty lamino-methyl)-phenoxy]-propan-2-oloxlate.
In this research, PP-28 chemically known as 1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-oloxolate. (Figure 1), was synthesized from Tert-butyl amine, which was combined with aryloxyproanol (the basic structure with β-blocking activity). Basically, this study was aimed to investigate its efficacy in Hypertension and metabolic abnormalities induced by a high fructose diet in normal rats.

![Chemical structure of PP-28](image)

1-tert-butylamino-3-[3-(tert-butylamino-methyl)-phenoxy]-propan-2-ol-oxalate.

Figure1: Chemical structure of PP-28.

**Material and methods**

**Animals**

Male Wistar rats weighing 180-200 g were used in this study; they were obtained from National Toxicology Centre, Pune, India. The rats were housed two per cage in an environmentally controlled room with a temperature of $25^\circ \pm 2^\circ \text{C}$ and relative humidity $55 \pm 5\%$ under a 12-h light:12-h dark cycle and free access was allowed to normal rat chow and tap water.

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India.

**Fructose-induced hypertension and effect of PP-28**

Forty eight male wistar rats were divided into six groups of 8 animals each. Control groups were given ordinary drinking water ad libitum throughout the whole treatment course and the remaining groups were given 10% fructose solution to drink ad libitum. Nine weeks later, the fructose-treated animals were assigned the following treatment regimens: fructose-fed, fructose plus PP-28 (3, 10 and 30 mg/kg, p.o.) and fructose plus atenolol (10 mg/kg, p.o.). The animals received these treatment regimens for the next Two week.
Blood Pressure Measurement Procedure

Wistar rats were anaesthetized with urethane (1.25 g/kg, i.p.). Trachea and carotid artery were cannulated with the help of polyethylene catheter containing heparin dissolved in isotonic saline. Body temperature was maintained at 37°C with a help of overhead tungsten lamp. The cannulated artery was connected to pressure transducer (SS13L, BIOPAC Systems, Inc., Santa Barbara, CA) for measurement of MAP.

Stainless steel needles of gauge 26x1/2 inch were inserted subcutaneously on the flexor aspect of limbs. ECG (Lead II) was connected using electrode lead set (SS2L, BIOPAC Systems, Inc., Santa Barbara, CA). Pressure transducer and electrode lead set were connected to four-channel physiological data acquisition system (MP 30, BIOPAC Systems, Inc., and Santa Barbara, CA). MAP and ECG were digitally recorded. Measurement of heart rate was done from R-R interval of ECG recording.

Oral Glucose Tolerance Test

At the end of the 2-week period of treatment, plasma insulin and glucose concentrations were measured in response to an oral glucose tolerance test. Animals were fasted overnight and given only water to drink. On the morning of the test, a control blood sample (0.4 mL) was drawn from the tip of the tail and vehicle, PP-28 (3, 10 and 30 mg/kg, p.o.) and atenolol (10 mg/kg, p.o.) administered orally. After one hour of drugs administration, blood samples were collected, then each animal received an oral glucose load of 2 g/kg of body weight by oral gavage. Additional blood samples were drawn at 15, 30, 60, 120 and 180 min after oral glucose load. All samples were immediately centrifuged and stored at –20°C until the assay for plasma concentrations of glucose and insulin.

Biochemical Measurements

Concentrations of plasma glucose, cholesterol, LDL, HDL, VLDL, and triglycerides were measured in plasma samples at the end of the experiment using a Hitachi 902 fully automated random access biochemistry analyzer Roche. Plasma insulin was determined with a ELISA (linco research).

Data Analysis

Data in table and fig. are expressed as mean ± S.E.M. Significant differences were determined by the independent and paired student’s t- test in unpaired and paired samples, respectively. Whenever a control group was compared with more than one treated groups one-way analysis of variance followed by the Dunnett test used for analysis. Values for P≤0.05 were considered significant. Analysis of data and plotting were done with the aid of software (InStat®, Version 3.0 and Graph Pad PRISM®, Version 4.0, San Diego, CA, USA) run on Windows operating system.

Results

Systolic Blood Pressure and Heart Rate
The effects of fructose, PP-28 and atenolol on MAP are shown in Fig.1 and values are given in table 1. Fructose treatment was associated with a significant increase (P ≤ 0.001) in MAP. Two week treatment with the PP-28 and Atenolol blocked the elevation of blood pressure in fructose-treated rats and provoke a decline toward control values. PP-28 showed dose dependent decrease in MAP in fructose fed rats. The antihypertensive activity is in following order atenolol > PP-28. No significant difference in the heart rate was observed in animals treated with fructose alone or fructose plus PP-28 (3 and 10 mg/kg) compared with the control diet but showed significant decrease at 30 mg/kg (P ≤ 0.01) when compared with fructose alone and treatment with atenolol also showed significant decrease in heart rate when compared with fructose alone (P ≤ 0.01).

Fig 1. Mean Arterial pressure (MAP) and heart rate of rats drinking ordinary tap water or 10% fructose after 7 days treatment with PP-28 (10 mg/kg) and Atenolol (10 mg/kg). N = 8 rats per group. Data are expressed as mean ± S.E.M. ###P < 0.001 as compared to control group, **P < 0.01 and ***P < 0.001 as compared to fructose treated group.
Glucose Tolerance Test

After 2-week administration of PP-28 and atenolol, OGTT was performed. Among the groups, plasma glucose and insulin levels at 0 time were different but not significantly. Glucose challenge significantly increased the Plasma glucose and insulin levels upto 120 min in fructose fed rats treated with vehicle when compared with control animals, while PP-28 treated groups not showed any significant change in plasma glucose and insulin levels compared with vehicle group at any time point (Data not shown).

Biochemical Parameters

Table 1 shows values of plasma concentrations of glucose, insulin, cholesterol, LDL, HDL, VLDL, and triglycerides levels in control rats, fructose-fed and all treatment rats after 12 hours fasting. After 9 weeks, fructose-fed rats showed significant increased in plasma glucose level (P < 0.05) when compared with control rats, none of the treatment groups had significantly different plasma glucose levels when compared with fructose fed rats. Treatments with PP-28 showed dose dependent decrease in plasma glucose level when compared with fructose-fed rats but not significantly. Plasma insulin levels were increased significantly (P < 0.001) by the high fructose diet, and this effect persisted in rats treated with the PP-28 and atenolol. Treatments with PP-28 showed dose dependent decrease in plasma insulin level when compared with fructose-fed rats but not significantly.

Fructose feeding led to significant increase in plasma triglycerides level when compared with control group (P < 0.001) and Treatments with PP-28 (30 mg/kg, p.o.) showed significant decrease in plasma triglycerides when compared with vehicle group (Fig 2). Cholesterol levels were not affected by fructose feeding and treatments with PP-28 and atenolol did not significantly decreases the cholesterol level in blood plasma when compared with vehicle group.

HDL levels were not affected by fructose feeding; Treatments with PP-28 increases HDL level but not significantly when compared with vehicle group but atenolol decreases the HDL level when compared with vehicle group. LDL and VLDL level increases significantly in Fructose-fed animals (P < 0.01 and P < 0.001) when compared to control rats. Treatments with PP-28 (30 mg/kg, p.o.) significantly decrease the VLDL (P < 0.01) level in plasma when compared with vehicle group.
Fig 2. Plasma Triglycerides (m mol/L) and Cholesterol (m mol/L) of rats drinking ordinary tap water or 10% fructose after 7 days treatment with PP-28 (3, 10, 30 mg/kg, p.o.) and Atenolol (10 mg/kg, p.o.). N = 8 rats per group. Data are expressed as mean ± SEM. ###P < 0.001 as compared to control group, *P < 0.05 and **P < 0.01 as compared to fructose treated group.
Table 1. Effect of PP-28 and atenolol on Mean arterial blood pressure, heart rate, plasma levels of glucose, insulin, cholesterol, triglycerides, HDL, LDL and VLDL in Fructose-fed rat.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fructose + Vehicle</th>
<th>Fructose + PP-28 (3 mg/kg)</th>
<th>Fructose + PP-28 (10 mg/kg)</th>
<th>Fructose + PP-28 (30 mg/kg)</th>
<th>Fructose + Atenolol (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>94 ± 4.30</td>
<td>155 ± 3.7###</td>
<td>149 ± 3.0</td>
<td>140 ± 2.4*</td>
<td>111 ± 2.1$</td>
<td>105 ± 4.5$</td>
</tr>
<tr>
<td>Heart rate (Beats per min)</td>
<td>309 ± 11.6</td>
<td>338 ± 13.1</td>
<td>326 ± 10.8</td>
<td>306 ± 10.1</td>
<td>282 ± 5.2**</td>
<td>279 ± 7.5**</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>98 ± 10.0</td>
<td>140 ± 8.0³</td>
<td>130 ± 8.0</td>
<td>125 ± 7.0</td>
<td>120 ± 11.0</td>
<td>140 ± 8.0</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.2 ± 0.20</td>
<td>3.8 ± 0.3###</td>
<td>3.6 ± 0.20</td>
<td>3.2 ± 0.4</td>
<td>2.5 ± 0.20</td>
<td>3.7 ± 0.60</td>
</tr>
<tr>
<td>Cholesterol (m mol/L)</td>
<td>1.31 ± 0.10</td>
<td>1.57 ± 0.04</td>
<td>1.51 ± 0.07</td>
<td>1.4 ± 0.04</td>
<td>1.25 ± 0.08</td>
<td>1.6 ± 0.08</td>
</tr>
<tr>
<td>Triglycerides (m mol/L)</td>
<td>0.80 ± 0.01</td>
<td>1.47 ± 0.03###</td>
<td>1.45 ± 0.02</td>
<td>1.41 ± 0.02</td>
<td>1.30 ± 0.03*</td>
<td>1.52 ± 0.07</td>
</tr>
<tr>
<td>HDL (m mol/L)</td>
<td>0.38 ± 0.01</td>
<td>0.35 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.39 ± 0.01</td>
<td>0.31± 0.02</td>
</tr>
<tr>
<td>LDL (m mol/L)</td>
<td>0.38 ± 0.02</td>
<td>0.58 ± 0.04###</td>
<td>0.55 ± 0.02</td>
<td>0.55 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>VLDL (m mol/L)</td>
<td>0.36 ± 0.03</td>
<td>0.66 ± 0.04###</td>
<td>0.59 ± 0.04</td>
<td>0.55 ± 0.04</td>
<td>0.48 ± 0.02**</td>
<td>0.55 ± 0.02</td>
</tr>
</tbody>
</table>

Data are given as Mean ± S.E.M. for eight animals per group. #P <0.05, ##P <0.01 and ###P <0.001 as compared to control group, *P <0.05, **P <0.01 and $P <0.001 as compared to fructose treated group.

Discussion

The results of this study indicate that insulin resistance, hyperinsulinemia, increase in mean blood pressure and hypertriglyceridemia develop when normal rats are fed a high fructose in drinking water, as has been seen previously (13; 14). In addition, we have shown that these metabolic changes are associated with an increase in LDL and VLDL level in blood plasma. Several anti-hypertensive drugs effectively prevent and reverse the increase in blood pressure induced by high fructose diets (15). The results of this study demonstrate that 9 weeks of high fructose feeding in rats resulted in a hypertensive state that was normalized by two week treatment with the PP-28 (30 mg/kg, p.o.) and atenolol (10 mg/ kg,p.o.). The results of our study confirm that fructose feeding can induce hypertension in normal wistar rats. Plasma insulin as well as glucose level were significantly higher in these rats from the controls, suggesting that the
rats were insulin-resistant which was found previously (16). Previous study favors the hypothesis that hypertriglyceridemia, insulin resistance or both, contribute to the development of hypertension in these rats (17).

Hyperinsulinemia and insulin resistance could induce elevation of blood pressure levels via a variety of mechanisms including sodium–water retention, sympathetic nerve stimulation, changes in transmembrane ion traffic, and direct stimulation of smooth muscle cell growth (13). Moreover, other reports have shown that reducing insulin levels in these rats leads to a reduction in blood pressure and correction of other metabolic abnormalities (18, 19, 20). The mechanism by which fructose leads to insulin resistance is unclear. Also, fructose diets have been found to activate sympathetic nervous system activity and elevate blood pressure in rats (21; 22) and Sympathoexcitation also may play an early and integral role in the final expression of elevated plasma insulin levels and blood pressure in rats fed a high fructose diet (23). Plasma Norepinephrine has been found elevated in sucrose- and fructose-fed rats, and it has been proposed to account for hypertension and insulin resistance because of its vasoconstrictor activity (24). The possible interrelationship between these changes has not been explored. For example, one might speculate that the primary alteration induced by sucrose feeding is activation of sympathetic nervous system activity. This change might then lead to hypertension, and since catecholamines may oppose insulin action, a state of Insulin resistance could develop. Alternatively, it is possible those alterations in insulin action are the primary consequences of sucrose feeding and that the other effects of sucrose are secondary. Indeed, at this point it is unclear if all the known effects of sucrose are actually causally connected. Insulin sensitising activity has been reported to reduce plasma insulin levels leading by the same occasion to a reduction of plasma glucose levels and blood pressure (25).

In our study, the PP-28 (30mg / kg,p.o.) reduces plasma glucose as well as plasma insulin level in fructose-fed animals suggesting that the mechanism by which contribute to lowering blood pressure also related to insulin sensitivity. Where as atenolol 10 mg/kg did not decreases the blood plasma glucose and insulin level. Hypertriglyceridemia in fructose-treated rats has been demonstrated by several workers. In our study, plasma triglyceride levels were significantly increased in fructose fed rats when compared with control animals and significantly decreased with two weeks treatment of PP-28 (30 mg/kg, p.o.) where as we did not found any significant change in plasma cholesterol level across any group. In human being triglycerides were also invariably increased by atenolol, whereas the effect on cholesterol has been variable and usually not statistically significant (10). Hypertriglyceridemia has been proposed to be caused by either increased hepatic secretion of very-low-density lipoprotein-triglyceride (VLDL-TG) or a decreased removal of triglyceride-rich lipoprotein from the circulation (14). Treatment with PP-28 increase the HDL and decreases the LDL VLDL, level in blood plasma but not significantly when compared to fructose fed rat. Where as atenolol has variable effect. It is known that high-fructose feeding leads to hypertension by several mechanisms including sodium retention, fluid volume expansion, and stimulation of the sympathetic nervous system, vascular small muscle proliferation and increase in cytosolic Ca ion (14). PP-28 and atenolol reduced the extent of development of hypertension induced by the high fructose-diet. Various study suggest that fructose feeding is responsible for activation of sympathetic nervous system activity which is inhibited by PP-28 and atenolol. The results of this study cannot explain the precise mechanism (s) of action of these drug, yet these results suggest that PP-28 can play a major role in the mechanisms underlying the pathogenesis of fructose-fed hypertension as...
demonstrated by the beneficial effects on blood pressure. On the other hand, PP-28 also improving insulin sensitivity increases HDL and decreases VLDL and TG level in blood plasma.

Conclusion

In conclusion, this study has shown the antihypertensive effect of the PP-28 using the fructose drinking rat model. These results may lend further support to mount up evidence that the PP-28 if taken in sufficient quantities could conceivably be beneficial in the attenuation and prevention hypertension and hyperinsulinemia and dislipidimia induced by high fructose diets. Further studies are required to establish the mechanism(s) underlying the antihypertensive effects of PP-28.

References


