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A COMPARATIVE STUDY OF ROSIGLITAZONE AND PIOGLITAZONE TO EVALUATE THE CARDIOVASCULAR EFFECTS IN HIGH FRUCTOSE DIET INDUCED HYPERTENSIVE RATS

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ABSTRACT

Thiazolidinediones (TZDs) were widely used for the treatment of type 2 diabetes. Recent studies have shown that TZDs have paradoxical effects on cardiovascular diseases. The objective of the present study was to investigate the effect of TZDs (Rosiglitazone and Pioglitazone) in High Fructose Diet (HFD) induced hypertension in rats. HFD was given for 14 weeks. After 8 weeks of hypertension induction period, treatment phase was started with Rosiglitazone (ROSI 10 and 30 mg/kg, p.o.) and Pioglitazone (PIO 10 and 30 mg/kg, p.o.) to the respective groups which were continued till 6 weeks. Systolic Blood Pressure (SBP) was measured weekly and serum glucose, triglyceride, cholesterol and HDL-C were measured at the end of study period. In HFD fed rats hypertension was observed after 8 weeks. Treatment with the test drugs significantly reversed the changes in serum enzyme levels as well as SBP made by HFD feeding compared to the control group. The study concludes that TZDs possess antihypertensive effect as exhibited in the present experimental settings.

INTRODUCTION: The thiazolidinediones, pioglitazone and rosiglitazone were introduced into global markets in 1999–2000, but the debate about the risks and benefits of the use of thiazolidinediones (rosiglitazone and pioglitazone) to treat diabetes is still going on. However, recent evidence suggests that these drugs may be associated with an increased risk of cardiovascular events [1], leading to heightened controversy regarding the appropriate role of thiazolidinediones in the treatment of diabetes.

When thiazolidinediones were first introduced, they held great promise in reduction of cardiovascular risk among people with type 2 diabetes mellitus. However in addition to improved glycemic control without hypoglycemia, thiazolidinediones decreased insulin resistance, a key risk factor for cardiovascular disease [2].

Along with evidence of benefit on surrogate cardiovascular markers such as lipids, blood pressure, inflammatory biomarkers, endothelial function and fibrinolytic status led to the widespread use of these drugs [3].

In the past few years, studies have been done on both the glitazones and they uncovered both the beneficial and detrimental aspects of these controversial drugs. Some reviewers have concluded that rosiglitazone caused more fatal outcomes than pioglitazone [4].

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In September 2010, the European Medicines Agency suspended the use of rosiglitazone. In the United States the drug is available on a restricted basis. India banned the production and import of diabetes drug Rosiglitazone in October 2010. However the other Thiazolidinedione, Pioglitazone is still in medical practice for diabetes management.

Considering these information and lack of sufficient data from the chronic animal studies to support the clinical findings about the TZDs, the comparative effects of both thiazolidinediones were examined in HFD rats for assessment of cardiovascular risk factors.

MATERIAL AND METHODS

1. **Drugs and Chemicals:** All chemicals were procured from standard companies and were of analytical grade. Food grade fructose was obtained from SRS scientific, Bangalore. Rosiglitazone and Pioglitazone were obtained as generous gift samples from Dr. Reddy's Lab Ltd and Dekka India respectively. Enzokit Glucose (GOD/POD) has been procured from Ranbaxy Fine Chemicals Limited (RFCL) Diagnostics Division and for the lipid profile parameters the kits were procured from Swemed Diagnostics. Double-distilled water was used for all biochemical measurements.

2. **Animals:** Eight week-old healthy, laboratory bred, male Wistar rats weighing 180 ± 10 gm were maintained under standard laboratory conditions such as temperature 22-25^{o} C, 12 hour light / dark cycle and provided water and pellet food *ad libitum*. The experiments were conducted in CPCSEA (Committee for the Purpose Of Control and Supervision of Experiments on Animals, Chennai, India) approved animal house after obtaining the prior approval from the Institutional Animal Ethics Committee (IAEC).

3. **Diet and treatment / Experimental groups:**

a. Groupings: 4 weeks old 72 male Wistar rats were randomly divided into 12 groups of 6 animals each that received the treatments as follows:

Group 1	Control (Normal saline)
Group 2	Rosiglitazone (10 mg/kg/day p.o)
Group 3	Rosiglitazone (30 mg/kg/day p.o)
Group 4	Pioglitazone (10 mg/kg/day p.o)
Group 5	Pioglitazone (30 mg/kg/day p.o)
Group 6	HFD fed rats
Group 7	HFD + Rosiglitazone (10 mg/kg/day p.o)
Group 8	HFD + Rosiglitazone (30 mg/kg/day p.o)
Group 9	HFD + Pioglitazone (10 mg/kg/day p.o)
Group 10	HFD + Pioglitazone (30 mg/kg/day p.o)

b. Induction of Hypertension [5]: All the animals were fed with fructose diet for complete duration of the protocol (14 weeks) except the control animals. After 8 weeks of induction period the animal's blood pressure reading was taken. Animals which were showing significant increased SBP compared to control was considered as hypertensive and used for further study.

c. High Fructose Diet (HFD) [6]: The fructose diet was prepared fresh everyday based on the method of *Sleder and team (1981)*. The composition is given below.

Composition of HFD

INGREDIENTS	g/kg
Fructose	660
Casein	100
Lard	80
Zn Carbonate	0.04
Vitamin mix	5
Mineral mix	5
Cellulose	150

d. Treatment: Treatment with the respective drugs and doses were started from the 9th week onwards and were given for 6 weeks.

- Rosiglitazone (10 and 30 mg/kg/day p.o.) [7,8]
- Pioglitazone (10 and 30 mg/kg/day p.o.) [9,10]

The drugs (ROSI & PIO) used for treatment was suspended in 1%w/v CMC Suspension. The suspension prepared was thoroughly mixed using cyclo mixer.

e. **Sample collection and preparation:** After the experimental period, the blood was withdrawn from the retro orbital vein of the rats under light anesthesia and analyzed for serum glucose and lipid profile. The dilution of serum sample was done using potassium phosphate buffer pH 7.4 (100mol/L).

f. **Recording Systolic Blood Pressure (SBP) [11, 12]:** The blood pressure was recorded every week in conscious rats non-invasively by tail cuff method. In the present study, the SBP was recorded in conscious rats by using non invasive blood pressure recorder (Power Lab, Australia). The equipment makes use of piezoelectric sensor which is basically a pulse transducer.

g. **Statistical analysis:** Data was presented as Mean ± S.E.M.Data obtained from various groups was subjected to one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. The value of $p \leq 0.05$ was taken to imply statistical significance.

RESULTS:

1. **Effect on lipid profile and glucose levels:** The effects of treatment on lipid profile and glucose levels in control and HFD fed group of rats at the end of the experimental period i.e. 14 weeks were given in **Table 1**. The rats fed with HFD showed increased glucose, cholesterol and triglyceride levels as compared to control rats. These rats also showed decreased HDL-C content as compared to control rats. ROSI and PIO administered HFD-fed rats' registered near-normal levels of all the parameters except cholesterol.

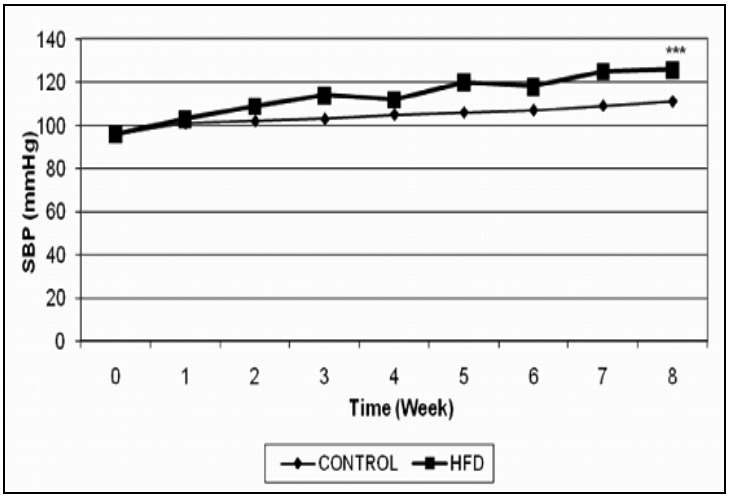
TABLE 1: EFFECT OF TZDS TREATMENT ON SERUM GLUCOSE AND LIPID PROFILE IN HFD DIET INDUCED HYPERTENSIVE RATS.

Groups (n=6)	Glucose (mg/dl)	TG (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)
Control	57.09±1.19	64.89±1.37	67.45±1.46	27.86±0.58
ROSI 10	60.96±0.79	69.46±1.11	66.71±1.43	26.64±0.70
ROSI 30	61.63±1.19	60.97±0.62	64.44±3.01	26.40±0.75
PIO 10	62.19±0.52	64.67±3.38	60.42±0.89	24.00±0.97
PIO 30	62.20±1.68	58.07±2.66	61.14±0.77	24.41±0.44
HFD	78±2.31[a]	101.93±1.86[a]	81.54±0.95[a]	17.36±0.72[a]
HFD+ ROSI 10	64.94±2.13**	75.42±1.48***	79.33±0.97	21.52±0.45*
HFD+ ROSI 30	65.71±3.19***	69.24±1.3***	75.03±0.77	21.97±0.64*
HFD+ PIO 10	67.99±2.64*	69.96±1.53***	79.23±1.15	22.11±0.72**
HFD+ PIO 30	63.50±0.89***	65.86±1.54***	75.95±1.31	22.45±0.63**

All values are expressed as Mean ± SEM, Statistical Analysis: One-way ANOVA followed by Tukey's Multiple Comparison Test ***-p<0.001 as compared with control rats.

2. **Effect on Blood Pressure:** All the animals were fed with HFD *ad libitum* and weekly systolic blood pressure (SBP) was measured. 8th week blood pressure data showed significant increase in SBP in HFD fed rats compared to that of control rats fed with normal chow diet.

   Animals which were showing significant increased SBP compared to control was considered as hypertensive and used for further study (**Graph 1**).



GRAPH 1: INDUCTION OF HYPERTENSION BY HFD FEEDING IN RATS. All values are expressed as Mean ± SEM, Statistical Analysis: One-way ANOVA followed by Tukey's Multiple Comparison Test ***-p<0.001 as compared with control rats.

The HFD fed hypertensive rats which were selected for the later stage of experiment was having significant ($p<0.001$) elevated SBP when compared with the control group of rats. Treatment with ROSI 10 & 30 mg/kg, PIO 10 & 30 mg/kg in NPD fed rats showed no significant change in SBP when compared to normal control rats. The SBP of HFD fed rats treated with ROSI10 & 30 mg/kg, PIO 10 & 30 mg/kg showed significant ($p<0.001$) reduction in SBP compared to hypertensive condition (**Table 2**).

TABLE 2: EFFECT OF TZDS TREATMENT ON SYSTOLIC BLOOD PRESSURE (SBP)

Groups	SBP (mmHg)
Control	116±1.39
ROSI 10	110±1.08
ROSI 30	109±0.79
PIO 10	118±1.17
PIO 30	116±0.86
HFD	134±1.07[a]
HFD+ ROSI 10	104±1.61***
HFD+ ROSI 30	101±1.54***
HFD+ PIO 10	103±1.93***
HFD+ PIO 30	108±1.80***

All values are expressed as Mean ± SEM, Statistical Analysis: One-way ANOVA followed by Tukey's Multiple Comparison Test a-$p<0.001$ as compared with control rats, ***-$p<0.001$ as compared with HFD fed rats.

DISCUSSION: The results of our study showed that a fructose enriched diet for 8 weeks lead to significant increase in systolic blood pressure (SBP) [Graph 1]. Treatment with different doses of the test drugs (ROSI and PIO) significantly reduced this elevated SBP in rats.

It is generally agreed that fructose loading induces insulin resistance and glucose intolerance [13]. The present study showed that there was significant increase in serum glucose levels in Wistar rats following fructose treatment for 8 weeks. Administration of ROSI and PIO showed significant decrease in glucose level indicating the insulin sensitizing activity of these drugs [14]. Insulin resistance and hyperinsulinemia could be viewed as permissive, representing changes that predispose an individual to high blood pressure, but not that necessarily cause hypertension by themselves [15].

Although we had not measured serum insulin levels, but serum glucose levels remained high, suggesting that the rats might be insulin-resistant [16].

Increased insulin resistance and hyperinsulinemia were linked to the development of hypertension [17]. Hyperinsulinemia is caused by increased insulin secretion in response to glucose which results from hyperactivity of the pancreatic cells to glucose and an activation of sympathetic nerves [18]. It, therefore, seems that our test drugs (ROSI and PIO) exert its antihypertensive effect by improving insulin sensibility as observed with earlier studies in thiazolidinedione [19.20].

Hypertriglyceridemia in fructose-treated rats has been demonstrated by several workers. In our study, serum triglyceride and cholesterol levels were significantly increased in fructose fed rats. Hypertriglyceridemia has been proposed to be caused by either increased hepatic secretion of very-low-density lipoprotein-triglyceridc (VLDL-TG) or a decreased removal of triglyceride-rich lipoprotein from the circulation [21]. In the present study, the fructose fed rats treated with test drugs produced a marked fall in serum triglyceride levels but not in the cholesterol levels. Similar finding was also observed with the other TZDs [22].

Blood pressure lowering effect has been reported with the TZDs administration in a variety of settings characterized by normal as well as reduced insulin action on glucose metabolism. Specifically, TZDs have lowered blood pressure in several models characterized by insulin resistance: obese Zucker rats [23], Dahl salt-sensitive rats [24], fructose-fed rats [25], and humans with obesity, impaired glucose tolerance, or NIDDM [26, 27, 28]. In each of those settings, the blood pressure lowering effect was associated with an improvement in insulin action, suggesting that the two effects of TZDs might be linked mechanistically.

CONCLUSION: This study has shown the anti-hypertensive effect of ROSI and PIO using the high fructose diet fed rat model. Further studies are required to establish the true mechanism(s) underlying the antihypertensive effects of ROSI and PIO.

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