# PROTECTION AGAINST ENDOTHELIAL DYSFUNCTION BY PIOGLITAZONE AND IRBESARTAN IN FRUCTOSE-FED DIABETIC RATS

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## ABSTRACT

The protective effects of metformin, pioglitazone, irbesartan, ramipril; and their combinations against oxidative stress and endothelial dysfunction in the aortic tissues in type-2 diabetic rats were investigated. Metformin (350 mg/kg, p.o.), pioglitazone, irbesartan, and ramipril (10 mg/kg, p.o.); and their combinations were administered for a period of 6 weeks after induction of type-2 diabetes by fructose (66% w/v, p.o.) in rats. The effects were examined on body weight, serum glucose, triglyceride, cholesterol, blood pressure (BP) and heart rate. At the end of treatments, vascular reactivity was tested with catecholamines. Acetylcholine and sodium nitroprusside-induced vasorelaxation was measured on isolated rat aortas. The oxidative stress indices were determined. Chronic treatment with drugs significantly decreased weight gain, serum glucose, triglyceride, cholesterol levels; normalize the heart rate and BP in fructose fed rats. All the treatments significantly reduced the pressor response to catecholamines. The significant improvement in the relaxant response to acetylcholine and sodium nitroprusside was obtained on isolated aortas. Furthermore, treatments were effective in restoring defensive antioxidant enzymes. Metformin, pioglitazone, irbesartan, and Ramipril; and their combinations were able to reverse oxidative stress and vascular endothelial dysfunction. Combination of pioglitazone with irbesartan has shown better ameliorating potential.

**Keywords:** Acetylcholine, aorta, Oxidative stress, Sodium nitroprusside, vascular reactivity.

## INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin [1].There is a strong association between insulin resistance, hypertension, glucose intolerance, low high density lipoprotein (HDL) -cholesterol and raised very low density lipoprotein (VLDL) – triglycerides [2].Insulin resistances associated with many risk factor abnormalities that commonly precede the development of hyperglycemia and these typically include obesity, dyslipidaemia characterized by high triglyceride, elevated blood pressure, oxidative stress, endothelial dysfunction [3]. Oxidative stress is an increase in the steady-state levels of reactive oxygen species and may occur as a result of increased free radical generation and/or decreased anti-oxidant defense mechanism. It has been shown that, when the activities of superoxide dismutase (SOD, which capture $O_2\bullet$) and catalase (CAT, which capture $H_2O_2$) were maintained, the endothelial function was not altered even in cases of hyperglycemia [4].Insulin resistance leads to abnormalities in regulatory mechanism of blood pressure resulting into cardiovascular complications in type-2 DM [5].The dyslipidaemia is a central player in the development of atherosclerosis and other cardiovascular complications in the setting of insulin resistance in Type-2 DM [6].

Endothelium produces a number of vasodilator substances including prostacycline and nitric oxide (NO); they balance the action of vasoconstrictors such as endothelin and angiotensin II.NO is a key signaling molecule that causes vascular relaxation, inhibits vascular smooth muscle cell growth [7]. It activates soluble guanylate cyclase by binding to the heme group in the enzyme and enhances cGMP production which helps in vasodilatation [8].The increases in BP and triglyceride level in type-2 diabetes are secondary to the hyperinsulinemia, and then a drug intervention that reverses these effects should also attenuate the hypertension and cardiovascular complications [9, 10]. Rodents fed fructose or enriched diets can develop hypertension that is also related to insulin resistance and hyperinsulinemia. The fructose fed hypertensive rat model represents experimentally acquired

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form of systolic hypertension, wherein feeding normotensive rats a fructose enriched food results in high insulin plasma, insulin resistance, hypertriglyceridemia and eventually hypertension [11]. A drug that modulates endothelial functions can significantly alter morbidity and mortality associated with endothelial dysfunction [12].Antidiabetic drugs like metformin (biguanides) and pioglitazone (thiazolidinediones) are known to be effective in increasing the insulin sensitivity and thereby reduce hyperinsulinemia [13, 14].While the antihypertensive drugs like irbesartan (angiotensin II receptor antagonist) and ramipril (angiotensin converting enzyme (ACE) inhibitor) have action on vascular endothelium by normalizing the antioxidant and vasodilatory mechanism in hypertension [15, 16].

Therefore, we initiated long-term antidiabetic (metformin and pioglitazone) and antihypertensive (irbesartan and ramipril) treatments and their combinations in fructose-fed rats and evaluated their effects on cardiovascular complications associated with type-2 DM.

## MATERIAL AND METHODS
### Experimental Animals

Male Wistar strain rats (150-200 g) were used for the study. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature $25\pm2ºC$, 12:12 h L: D cycle and $50\pm5\%$ RH with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Each group consisted of five (n=5) animals. All the experiments were carried out during the light period (08:00-16:00 h). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee of M.V.P.S College of Pharmacy, Nashik approved the protocol of the study (CPN/IAEC/2010/04).

### Drugs and Chemicals

Metformin, pioglitazone and ramipril(Glenmark Pharmaceuticals Ltd., Nashik, India), irbesartan (Matrix Laboratories Pvt. Ltd., Nashik, India) were used. The drug solutions of metformin and ramipril were freshly prepared in distilled water, whereas suspension of irbesartan and pioglitazone were made in distilled water using 0.5% carboxy methyl cellulose. Biochemical kits for glucose, triglyceride and cholesterol (Auto Span, Surat, India) were used. All the chemicals used were of analytical grade and purchased from standard manufacturers.

### Fructose-induced type-2 DM in rats

Rats were administered with fructose (66% solution, 5 ml/kg/day, p.o., for 6 weeks) to induce type-2 DM [17].The animals showing fasting blood glucose level more than 200 mg/dl and BP over 145 mmHg were selected for the study.

### Experimental Design

The animals were randomly assigned to seven groups (n = 5). Group I - control (vehicle, 5 ml/kg, p.o.), Group II - fructose ( 66% w/v p.o), Group III – fructose + irbesartan (66% w/v p.o,+ 10 mg/kg, p.o), Group IV - fructose + ramipril (66% w/v p.o + 10 mg/kg, p.o), Group V -fructose + pioglitazone (66% w/v p.o + 10 mg/kg, p.o),Group VI -fructose + metformin (66% w/v p.o + 350 mg/kg, p.o), Group VII - fructose + pioglitazone + irbesartan, Group VIII - fructose + metformin + ramipril. The drugs were administered daily for a period of 6 weeks after induction of diabetes.

### Experimental Method

After induction of type -2 DM, metformin, pioglitazone, irbesartan and ramipril and their combinations were administered for a period of 6 weeks. The body weight of each group rats was measured before start of treatment and thereafter weekly of drug treatments. Using tail-cuff method, systolic blood pressure and pulse rate were recorded weekly during the treatments on Power-lab data acquisition system (AD Instruments, Australia). Blood samples were collected through retro-orbital plexus under ether-anesthesia weekly for determination of serum glucose, triglyceride and cholesterol levels. At the end of treatments, blood pressure was determined by invasive method and vascular reactivity was tested with adrenaline (Adr), noradrenaline (NA) and phenylephrine (PE). Thoracic aortas were dissected out and used for detection of antioxidant and vasorelaxation studies. Acetylcholine (ACh) and sodium nitroprusside (SNP)-induced vasorelaxation were determined on isolated rat aortas [18].

### Biochemical investigation
### Estimation of serum glucose, triglyceride and cholesterol levels

For the estimation of serum glucose, triglyceride and cholesterol levels standard biochemical kits were used.

### Vascular reactivity to catecholamines

After the completion of treatment schedule, rats from each group were anesthetized with ketamine and xylazine (75 and 15 mg/kg, i.p. resp.). Right jugular vein was cannulated with fine polyethylene catheter for the administration of drugs. Blood pressure was recorded from left common carotid artery using pressure transducer by direct method on Power-lab data acquisition system. Heparinised saline (100 IU/ml) was filled in the transducer and in the fine catheter cannulated to the carotid artery to prevent clotting. After 30 min of stabilization, mean change in BP to Adr (1 µg/kg), NA (1 µg/kg) and PE (1 µg/kg) were recorded [19].

### *In-Vitro* Study: ACh and SNP-induced vasorelaxation on isolated rat aortas

Immediately after completion of vascular reactivity studies, rats were sacrificed by cervical dislocation.

Thoracic aorta from the arch down to diaphragm was isolated and was placed in Krebs solution of composition (mM), NaCl: 118.4; KCl: 4.7; $CaCl_2$: 2.5; $KH_2PO_4$: 1.2; $MgSO_4$: 1.2; $NaHCO_3$: 25.0, Glucose: 11.0, at $37^{°}C$ and aerated with 95% $O_2$ and 5% $CO_2$. The rings of 3mm length were prepared and mounted in organ bath containing 15ml of Krebs solution. The contractions were recorded by a force transducer (Power-Lab, AD Instruments). The resting tension of 1 g was applied to the preparation and equilibrated for 90 min before the experiment with exchange of bathing solution every 15 min. After the equilibration, the rings were exposed to $1 \times 10^{-6}$ M PE for pre-contraction. When the contractile response of PE was plateaued, ACh or SNP were added in a cumulative fashion for endothelium dependent vasorelaxation studies respectively [20].The concentrations of ACh and SNP were made in range of $1 \times 10^{-9}$ to $1 \times 10^{-4}$ M. To verify the integrity of smooth muscle in thoracic aortas, SNP-induced vasorelaxation (a NO donor) was investigated which causes aortas to relax due to endothelial nitric oxide [21].

### Estimation of antioxidant activities

Thoracic aorta catalase (CAT) activity was assessed spectrophotometrically according to the method of Luck [22]. Super oxide dismutase (SOD)content in thoracic aorta was estimated spectrophotometrically following the reduction of nitroblue tetrazolium chloride (NBT) which was inhibited by the superoxide dismutase and measured at 560 nm [23]. Lipid peroxidation (LPO) content in thoracic aorta was determined by thiobarbituric acid reaction with lipid peroxide end-product malondialdehyde(MDA). The concentration of MDA was estimated as thiobarbituric acid-reactive substances (TBARS) by spectrophotometrical assay [24]. The content of reduced glutathione (GSH) was assessed spectrophotometrically using Ellman's reagent. The yellow color developed was read immediately at 412 nm [25].The protein content was evaluated by spectrophotometer according to the method of Lowery [26].The accumulation of nitrite in the supernatant, an indicator of the production of NO, was determined with Greiss reagent and the absorbance at 543 nm was determined spectrophotometrically [27].

### Statistical Analysis

Results are expressed as mean ± SEM, and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability level less of 0.05 was considered statistically significant.

## RESULTS
### Effect on body weight

Before induction of diabetes, rats had a mean body weight of 207 ± 2.2 g. Fructose induced significant increase in body weight ($P<0.001$) to 314 ± 2.8 g when compared with control. Irbesartan and ramipril administration for 6 weeks had a non significant effect on body weight, whereas rats treated with metformin and pioglitazone and their combination with irbesartan and ramipril produced significant reduction in body weight gain when compared with fructose treated rats ($P<0.001$) (Table 1).

### Effect on biochemical parameters

Fructose treated rats showed significant ($P<0.001$) rise in serum glucose, cholesterol and triglycerides levels compared to control. Long term treatment with only metformin and pioglitazone and in combination with irbesartan and ramipril significantly ($P<0.001$) reduced elevated serum glucose, cholesterol and triglyceride levels compared to fructose treated rats (Table 1).

### Effect on heart rate and blood pressure

The control group showed normal blood pressure and heart rate. Fructose treated group showed a marked increase in blood pressure and decrease in heart rate relative to control rats ($P<0.001$). Administration of metformin and pioglitazone and combination with irbesartan and ramipril, for a period of 6 weeks exhibited a significant ($P<0.001$) reduction in blood pressure compared to fructose treated rats. All the treatments completely restored the heart rate to normal except ramipril (Table 2).

### Effect on vascular reactivity to catecholamines

The control group showed normal response to Adr (1 µg/kg), NA (1µg/kg) and PE (1µg/kg) in vascular reactivity, whereas fructose treated rats showed a significant ($P<0.001$) elevation in mean change in BP to Adr, NA and PE. Administration of metformin and pioglitazone and in combination with irbesartan and ramipril significantly ($P<0.01$) reduced the mean change in BP to Adr, NA and PE compared to fructose treated rats. Pioglitazone and irbesartan combination exhibited more significant ($P<0.01$) reduction to mean change in BP (Table 3).

### *In-vitro* studies - Effect on vascular endothelial function
### Effect on ACh and SNP-induced relaxation of rat aorta pre-contracted with PE ($1 \times 10^{-6}$M)

ACh and SNP ($10^{-9}$ M to $10^{-4}$ M) induce relaxation on rat aortas, pre-contracted with PE ($1 \times 10^{-6}$M).The aortas of control group animals showed normal relaxant response to cumulative doses of ACh and SNP. This signifies normal endothelial function. The aortas from fructose treated rats showed significant ($P<0.001$) impairment in relaxation with cumulative doses of ACh and SNP. Metformin, pioglitazone, irbesartan, and ramipril administration for a period of 6 weeks showed significant ($P<0.001$) improvement in relaxation, which indicates improvement in endothelial function in diabetic rats. Improvement in relaxation was most significant ($P<0.001$) with combination of pioglitazone and irbesartan (Figure 1a-b).

### Effect on antioxidant levels in rat aorta

The antioxidant levels were determined by estimating SOD, CAT, GSH and LPO in rat aorta. The

contents of SOD, CAT and GSH which are the most important antioxidants were markedly reduced in fructose treated rats. Fructose administration significantly (*P*<0.01) enhanced MDA, an oxidative stress and lipid peroxidation marker levels in fructose fed diabetic rats compared to control rats. On the other hand treatment with metformin, pioglitazone, irbesartan, ramipril and their combinations significantly (*P*<0.01) increased SOD, CAT and GSH levels and decreased LPO levels compared to fructose treated rats (Table 4).

**Effect on NO levels in rat aorta**

The NO levels were significantly (P<0.001) lower in fructose fed diabetic rats compared to control group. Treatment with metformin, pioglitazone, irbesartan, and ramipril; and their combinations significantly (P<0.01) increased NO levels compared to fructose treated rats (Table4).

**Table 1. Effect of metformin, pioglitazone, irbesartan, and ramipril; and their combinations on body weight and biochemical parameters in fructose fed diabetic rats**

| Groups | Body Weight (g) | Glucose (mg/dl) | Cholesterol (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|---|
| Control | 207 ± 2.2 | 99 ± 3.3 | 62 ± 2.1 | 96 ± 3.3 |
| Diabetic | 314 ± 2.8[###] | 224 ± 5.1[###] | 187 ± 3.4[###] | 219 ± 6.5[###] |
| Irbesartan | 297 ± 1.9 | 189 ± 3.4[***] | 169 ± 1.6[**] | 196 ± 3.4[***] |
| Ramipril | 298 ± 3.4 | 210 ± 3.2 | 173 ± 1.8[*] | 192 ± 3.6[**] |
| Pioglitazone | 257 ± 3.4[***] | 121 ± 2.0[***] | 88 ± 2.0[***] | 103 ± 4.4[***] |
| Metformin | 237 ± 2.5[***] | 114 ± 4.1[***] | 81 ± 2.8[***] | 141 ± 5.0[***] |
| Pioglitazone + Irbesartan | 242 ± 7.8[***] | 120 ± 4.0[***] | 91 ± 2.6[***] | 94 ± 2.6[***] |
| Metformin + Ramipril | 253 ± 9.5[***] | 126 ± 2.2[***] | 79 ± 3.8[***] | 151 ± 2.6[***] |

Each value represents mean ± SEM (n=5).
[#]Diabetic group compared to control.[*] Treatment groups compared to diabetic group.
(One-way ANOVA followed by Dunnett's test). [###, ***]*P*< 0.001.

**Table 2. Effect of metformin, pioglitazone, irbesartan, and ramipril; and their combinations on cardiovascular parameters in fructose fed diabetic rats**

| Groups | Heart rate (Beats/min) | NIBP(mm/Hg) | IBP(mm/Hg) |
|---|---|---|---|
| Control | 340 ± 4.8 | 111 ± 1.2 | 105 ± 1.9 |
| Diabetic | 291 ± 3.6[###] | 148 ± 1.6[###] | 167 ± 1.3[###] |
| Irbesartan | 310 ± 2.0[**] | 116 ± 1.4[***] | 130 ± 1.3[***] |
| Ramipril | 300 ± 3.0 | 121 ± 0.9[***] | 119 ± 0.9[***] |
| Pioglitazone | 314 ± 3.4[***] | 128 ± 1.1[***] | 132 ± 1.0[***] |
| Metformin | 317 ± 2.2[***] | 120 ± 1.0[***] | 127 ± 1.1[***] |
| Pioglitazone + Irbesartan | 320 ± 1.8[***] | 112 ± 6.2[***] | 122 ± 2.5[***] |
| Metformin + Ramipril | 315 ± 3.6[***] | 124 ± 1.0[***] | 129 ± 1.4[***] |

Each value represents mean ± SEM (n=5).
[#]Diabetic group compared to control.[*] Treatment groups compared to diabetic group.
(One-way ANOVA followed by Dunnett's test). [###, ***]*P*< 0.001, [##,**]*P*< 0.01.

**Table 3. Effect of metformin, pioglitazone, irbesartan, and ramipril; and their combinations on vascular reactivity to Adr, NA and PEin fructose fed diabetic rats**

| Groups | Adrenaline (mm/Hg) | Noradrenaline (mm/Hg) | Phenylephrine (mm/Hg) |
|---|---|---|---|
| Control | 33.0 ± 0.7 | 42.0 ± 1.3 | 49.4 ± 1.0 |
| Diabetic | 65.4 ± 1.6[###] | 76.6 ± 1.4[###] | 87.8 ± 1.7[###] |
| Irbesartan | 41.2 ± 0.7[***] | 50.6 ± 0.4[***] | 57.0 ± 0.3[***] |
| Ramipril | 41.6 ± 0.4[***] | 55.2 ± 0.3[***] | 59.0 ± 0.3[***] |
| Pioglitazone | 38.2 ± 1.4[***] | 52.6 ± 1.2[***] | 56.0 ± 1.3[***] |
| Metformin | 41.2 ± 1.4[***] | 56.4 ± 1.6[***] | 62. 0 ± 0.8[**] |
| Pioglitazone + Irbesartan | 36.0 ± 0.7[***] | 48.0 ± 0.8[***] | 53.0 ± 0.7[***] |
| Metformin + Ramipril | 43.4 ± 0.6[***] | 54.4 ± 0.6[***] | 59.8 ± 1.4[***] |

Each value represents mean ± SEM (n=5).
[#]Diabetic group compared to control.[*] Treatment groups compared to diabetic group.
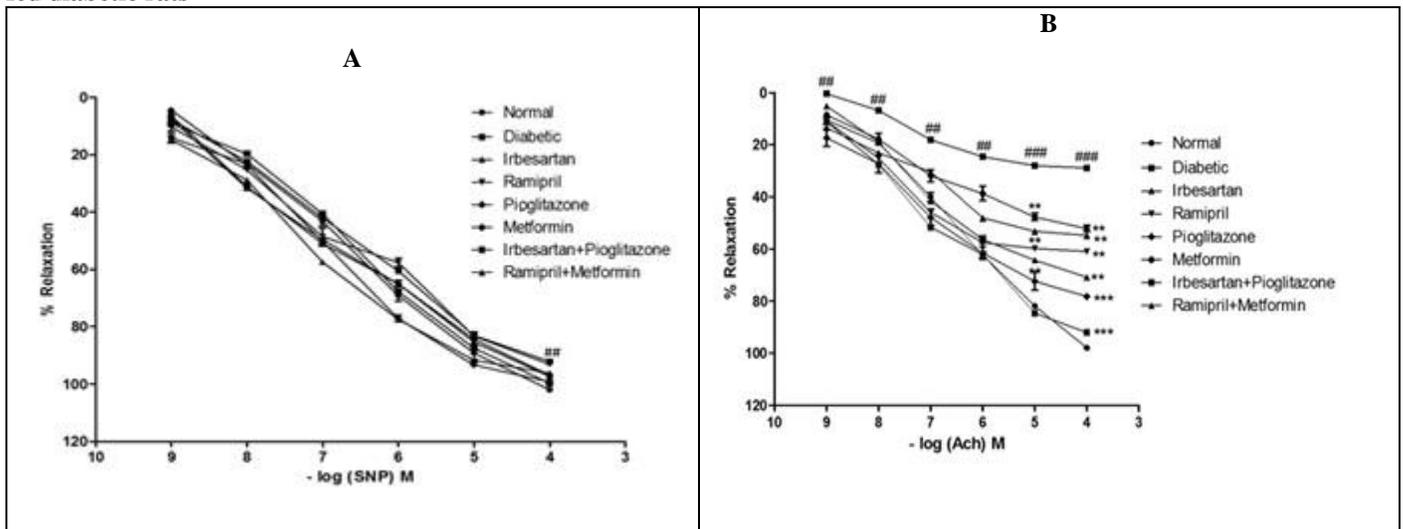(One-way ANOVA followed by Dunnett's test). [###, ***]*P*< 0.001, [##,**]*P*< 0.01.

**Table 4. Effect of metformin, pioglitazone, irbesartan, and ramipril; and their combinations on levels of SOD, CAT, GSH, LPO and NO in fructose fed diabetic rats**

| Groups | SOD (% inhibition of control) | CAT (μmol of $H_2O_2$ decomposed / min / mg protein ) | GSH (μmol of GSH / mg protein) | LPO (nmol of MDA / mg protein) | NO (μmol /ml) |
|---|---|---|---|---|---|
| Control | 99.8 ± 0.1 | 5.70 ± 0.1 | 4.74 ± 0.2 | 11.33 ± 0.5 | 75.0 ± 2.2 |
| Diabetic | 81.7 ± 0.8[##] | 2.40 ± 0.6[##] | 1.29 ± 0.1[##] | 17.21 ± 0.2[##] | 38.0 ± 2.1[###] |
| Irbesartan | 90.8 ± 0.3[**] | 3.19 ± 0.4 | 2.49 ± 0.4 | 16.02 ± 0.5 | 70.0 ± 3.1[**] |
| Ramipril | 88.5 ± 0.1[*] | 3.01 ± 0.2 | 2.09 ± 0.8 | 15.26 ± 0.7 | 62.0 ± 4.1[**] |
| Pioglitazone | 94.2 ± 0.1[**] | 4.17 ± 0.9 | 3.91 ± 0.7[**] | 11.37 ± 0.6[**] | 69.0 ± 3.2[**] |
| Metformin | 91.8 ± 0.1[**] | 3.64 ± 0.1 | 2.79 ± 0.5 | 13.38 ± 0.3[**] | 64.0 ± 3.7[**] |
| Pioglitazone + Irbesartan | 96.9 ± 0.4[**] | 5.05 ± 0.9[**] | 4.68 ± 0.1[**] | 10. 70 ± 0.2[**] | 74.0 ± 2.6[**] |
| Metformin + Ramipril | 93.8 ± 0.2[**] | 3.82 ± 0.8 | 3.72 ± 0.7[**] | 12.78 ± 0.8[**] | 71.0 ± 2.1[**] |

Each value represents mean ± SEM (n=5).
[#]Diabetic group compared to control.[*] Treatment groups compared to diabetic group.(One-way ANOVA followed by Dunnett's test). [###, ***]$P< 0.001$, [##,**]$P< 0.01$, [#,*]$P< 0.05$.

**Figure 1. Effect of metformin, pioglitazone, irbesartan, and ramipril; and their combinations on a) SNP-induced relaxation of rat aorta and b) Acetylcholine-induced relaxation of rat aorta pre-contracted with phenylephrine in fructose fed diabetic rats**



Each value represents mean ± SEM. (n=5).
[#]Diabetic group compared to control.[*] Treatment groups compared to diabetic group.
(One-way ANOVA followed by Dunnett's test). [###, ***]$P< 0.001$, [##,**]$P< 0.01$, [#,*]$P< 0.05$.

## DISCUSSION

Insulin resistance and hyperinsulinemia are the principal features of metabolic syndrome. This increased circulating level of insulin is the main factor causing cardiovascular complications of metabolic syndrome (5). Insulin resistance causes enhancement in sympathetic activity resulting in net rise in BP. Insulin has vasodilatory action mainly in skeletal muscles, vascular bed, probably by an endothelium-dependent mechanism. However, the hormone also has pressor effects mainly *via* stimulation of the sympathetic nervous system and enhancement of renal sodium absorption. Insulin resistance causes blunting of

these effects leading to impaired vasodilator and enhanced sympathetic activation resulting in net rise in blood pressure. The increased sympathetic outflow results in salt and water retention and rise in BP [28].

In the present investigation, it has been found that the peculiar signs produced by oral administration of 66% w/v fructose for 6 weeks were similar with those reported earlier [29]. Fructose-fed rats showed significant increase in body weight. Both metformin and pioglitazone and their combination with irbesartan and ramipril were able to reduce fructose induced gain in body weight. The serum glucose levels of fructose fed rats were significantly increased, indicating hyperglycemia. Administration of

metformin and pioglitazone and their combination with irbesartan and ramipril significantly attenuated the hyperglycemia induced by fructose. The serum triglyceride and cholesterol levels were also significantly high in fructose fed rats. Long term treatment with metformin and pioglitazone restored the triglyceride and cholesterol levels in fructose-fed animals. Irbesartan and ramipril were also able to normalize the elevated serum cholesterol and triglyceride levels significantly.

Chronic treatments with all the drugs restored the heart rate to normal compared to fructose fed rats. The BP was significantly elevated in fructose fed group after 6 weeks when measured by both indirect and direct method. Metformin, pioglitazone, irbesartan, ramipril and their combinations significantly reduced elevated BP in fructose-fed hypertensive rats. Irbesartan and pioglitazone combination showed better ameliorating effect than rest of all. The effect of metformin, pioglitazone, irbesartan and ramipril were studied on the vascular reactivity to the various catecholamines such as Adr, NA and PE. The significant increase in pressor response to Adr, NA and PE in the fructose treated rats was observed. This increase in pressor response might be due to presence of hypertension in fructose treated rats. All the treatments significantly reduced the increase in pressor response to Adr, NA and PE in fructose treated group. The most significant reduction in pressor response was observed with pioglitazone and irbesartan combination.

Endothelial dysfunction is a feature of metabolic syndrome, there is decreased production of endothelium derived vasodilator NO, and the impaired endothelium-mediated relaxation results in both human and animal models after diet induced hyperinsulinemia [30].The normal relaxant response was observed to cumulative doses of ACh and SNP on isolated aortas from control group. Whereas relaxant response to cumulative doses of ACh and SNP was significantly reduced on aortas isolated from fructose treated group. This finding is consistent with those reported earlier indicating presence of endothelial dysfunction. Chronic treatment with all the drugs significantly improved the relaxant response to ACh and SNP on aortas isolated from fructose treated hyperinsulinemic group. The combination of pioglitazone and irbesartan exhibit the most significant improvement among all the treatments. Their ability to decrease the pressor response attributes to their capacity to restore the elevated blood pressure in fructose fed rats.

Oxidative stress is one of the major reasons of endothelial dysfunction in metabolic syndrome. The oxidative stress increases in type-2 diabetes which is indicated by the reduction in the levels to innate reducing proteins like SOD, CAT and GSH. The reduction in these substances courses the lipid oxidation and the lipid peroxidation increases. Significant increase in reactive oxygen species production in both endothelial and smooth muscle cells through auto-oxidation of glucose reduces synthesis of NO and accelerates inactivation of NO [21]. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense against ROS by lowering the steady state level oxygen [23]. CAT is a hemeprotein, localized in the microperoxisomes. It reduces hydrogen peroxide produced by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisome [22].GSH is a major non-protein thiol and endogenous antioxidant that counters balance free radical mediated damage [25]. Oxidative stress is associated with peroxidation of cellular lipids, which is determined by measurement of TBARS. The concentration of LPO products may reflect the degree of oxidative stress [24].

The contents of SOD, CAT, GSH and NO were significantly decreased whereas the LPO levels were significantly increased in fructose fed rats indicating increase in oxidative stress in type-2 DM. Treatment with metformin, pioglitazone, irbesartan, ramipril and their combinations for a period of 6 weeks significantly increased SOD, CAT, GSH and NO levels and reduced the LPO compared to fructose fed rats, it implies that the treatments reduced the oxidative stress in diabetic animals. The reduction in oxidative stress was most significant with pioglitazone and irbesartan combination.

**CONCLUSION**

Metformin, pioglitazone, irbesartan, and ramipril; and their combinations have cardiovascular protective effects in rat model of fructose-induced metabolic syndrome. Pretreatment with these drugs for a period of 6 weeks restored the cardiovascular, biochemical parameters and endothelial function. Thus, the study concluded that the combination of pioglitazone with irbesartan could be better in ameliorating the cardiovascular complications such as endothelial dysfunction associated with the type-2 diabetes as compared to their individual effect.

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