Original Article

Beneficial effects of co-administration of PPAR-γ agonist with melatonin on cardiovascular complications associated with diabetes

Abstract

Aim: Effect of PPAR-γ agonists, Thiazolidinediones, in combination with melatonin was studied for cardiovascular complications associated with diabetes. **Materials and Methods:** Diabetic rats were treated with combination of pioglitazone (10 mg/kg/day p.o.) or rosiglitazone (5 mg/kg/day p.o.) with melatonin (10 mg/kg/day p.o.) for 7 weeks. The biochemical parameters, serum glucose, triglyceride, total cholesterol, HDL-cholesterol, AST, LDH and LDL-cholesterol levels were evaluated at the end of 7 weeks. Also cardiovascular parameters like atherogenic index, blood pressure, hisotology of heart were done. **Results and Conclusions:** The combination treated groups, pioglitazone plus melatonin (PM) and rosiglitazone plus melatonin (RM), showed significant decrease in the blood pressure when compared with diabetic control group. The level of cell injury markers AST and LDH was normalized in the combination groups PM and RM along with significant decrease in the atherogenic index. In the biochemical parameters, the serum glucose, triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol levels were significantly lowered in the combination groups. There was significant inhibition of the myonecrosis, reduction in the infiltration and inflammatory cells, and vacuolar changes when compared with diabetic control. The combination treated groups also proved to be effective in normalizing the levels of SOD, GSH, catalase, and LPO in heart homogenates when compared with diabetic control as well as pioglitazone, rosiglitazone, and melatonin-alone-treated groups. Hence, it may be concluded that the combination of PPAR-γ agonists, thiazolidinediones, with melatonin may be beneficial in the treatment of diabetes-induced cardiovascular complications.

Key words:
Cardiomyopathy, diabetes mellitus, hypertension, melatonin, oxidative stress, PPAR-γ agonist

Introduction

It is well known that diabetes and hypertension are major risk factors in the development of nephropathy, retinopathy, and specifically cardiomyopathy progressing to myocardial infarction.[1] In type 1 and 2 diabetic patients, diabetic cardiomyopathy progresses to congestive heart failure. There is an increase in the risk of heart failure in diabetic patients.[2-6] Nearly 80% of the deaths associated with diabetes are due to cardiac complications.[7]

Hyperglycemia plays an important role in the development and progression of diabetic complications, including endothelial cell dysfunction.[8-10] In diabetes, increased adrenergic activity, myocardial ischemia/functional hypoxia, activated cardiac renin-angiotensin system, and elevated glucose level lead to oxidative stress, production of reactive oxygen and nitrogen species, and abnormal gene expression leading to myocardial cell death.[11]

The hypertension associated with diabetes mellitus leads to pathological changes resulting in a particular array of events including hemodynamic alterations and structural changes

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Yogesh Suresh Ahire, Mahesh M. Ghaisas[1], P. R. Dandawate, S. P. Gandhi

Department of Pharmacology, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, [1]Indira College of Pharmacy, Tatawade, Pune, Maharashtra, India

Address for correspondence:
Mr. Yogesh Suresh Ahire,
Department of Pharmacology, KBHSS Trust's,
Institute of Pharmacy, Malegaon, Maharashtra, India.
E-mail: ysahire@hotmail.com

such as basement membrane thickening, extracellular matrix protein deposition, fibrosis, myocyte hypertrophy, cell necrosis,[12-16] activation of redox transcription factor, and nuclear factor-kB (NF-kB) which increase oxidative stress.[17] Last step in the sequence of diabetes-induced hypertension is vasoconstriction.[18]

Controlling blood pressure is positively beneficial in diabetes associated with hyperlipidemia, which has a deteriorating influence on myocardial membrane function.[19,20]

Alloxan-induced diabetic rats produce cardinal signs of diabetes mellitus such as polydipsia, polyphagia, increase in blood pressure, decrease in heart rate, and loss of body weight.[21-23] PPAR-γ and its ligands or agonists have blood pressure-lowering action in patients or animal models of diabetes/metabolic syndrome, but this condition is uncertain in patients or animal models of non-diabetes/metabolic syndrome.[24] PPAR-γ activators (pioglitazone and rosiglitazone) exhibit antioxidant property against alloxan-induced diabetes in rat.[25]

On the other hand, PPAR-γ activators may trigger an aggravation of congestive heart failure,[26,27] which is counterintuitive with respect to the cardiovascular preventive potential of these drugs. PPAR-γ agonist, thiazolidinediones, show fluid retention effect as a consequence of their insulinomimetic action on the kidney rather than a negative inotropic effect. Thus, caution has been urged in the use of thiazolidinediones in diabetic patients with advanced heart failure, even though these agents may have cardiovascular protective properties in patients with less advanced cardiovascular disease.[28,29]

Melatonin, synthesized by pineal gland,[30] is involved in the regulation of the cardiovascular system by influencing the arterial pressure and smooth muscle tone.[31] Melatonin administration was reported to reverse the development of hypertension in pinealectomized rats,[32] and intravenous injection of melatonin was observed to reduce blood pressure without changing the cardiac output.[33] Melatonin is reported to have antihypertensive effects in spontaneously hypertensive rats[34,35] and in humans as well.[36,37] In patients with coronary heart disease, nocturnal serum melatonin levels were seen to be more than five times lower than in controls.[37] As a nutritional supplement, melatonin might be better accepted for long-term use than prescription drugs.[34] Hence, the aim of this study was to evaluate the effects of PPAR-γ agonist–melatonin combination on cardiovascular complication associated with diabetic rat.

Materials and Methods

Chemicals

Pioglitazone, rosiglitazone, and melatonin were obtained from Themis Lab. Ltd., Mumbai; Glenmark Pharmaceuticals Ltd., Nasik; and Arati Bulk Manufacturer Pvt. Ltd., Mumbai, India, respectively. Other chemicals and kits for biochemical estimations were obtained from Biolab Diagnostic Pvt. Ltd., India.

Animals

Wistar rats of either sex weighing (200–250 g) were used for study and were kept in animal house at 26±2°C with relative humidity 44–56% along with light and dark cycle of 12 h, respectively. Institution Animal Ethics Committee (198/99/CPCSEA) approved the experimental protocol. Animals were provided with standard diet and water *ad libitum*. The food was withdrawn 18–24 h before the start of the experiment.

Experimental induction of diabetes

Diabetes was induced by a single intraperitonial injection of alloxan monohydrate in citrate buffer (pH 4.5) at a dose of 140 mg/kg body weight of the rat.[38] The diabetic state was confirmed 48 hours after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study.[39,40]

Experimental protocol

The animals were divided into nine groups (n = 6).

- Normal control (NC): The animals of this group were nondiabetic and received 1% CMC (1 ml/kg/day, p.o.) for 6 weeks

  The animals of the following groups were diabetic:
- Diabetic control (DC): received 1% CMC (1 ml/kg/day, p.o.) for 6 weeks
- Losartan (LR): received losartan (2 mg/kg/day, p.o.) for 6 weeks
- Pioglitazone (PIO): received pioglitazone (10 mg/kg/day, p.o.) for 6 weeks
- Rosiglitazone (ROSI): received rosiglitazone (5 mg/kg/day, p.o.) for 6 weeks
- Melatonin (MEL): received melatonin (10 mg/kg/day, p.o.) for 6 weeks
- Pioglitazone plus melatonin (PM): received pioglitazone (10 mg/kg/day, p.o.) plus melatonin (10 mg/kg/day, p.o.) for 6 weeks
- Rosiglitazone plus melatonin (RM): received rosiglitazone (5 mg/kg/day, p.o.) plus melatonin (10 mg/kg/day, p.o.) for 6 weeks.

Biochemical parameters from blood

Blood was withdrawn under light ether anesthesia by puncturing retro-orbital plexus using fine glass capillary and collected in the Eppendorf tubes. Estimation of serum glucose (GOD/POD method), HDL-cholesterol, total cholesterol (COD/POD method), triglyceride (GPO/POD method), AST, and LDH was done using standard diagnostic kits from Biolabs Diagnostic Pvt. Ltd., India. LDL-cholesterol was calculated using Freidewald's formula.[41]

Atherogenic index

Atherogenic index was calculated by using the following formula[42]

$$\text{Atherogenic Index} = \left\{\frac{\text{Total Cholesterol}}{\text{HDL-cholesterol}} - \text{HDL-cholesterol}\right\}$$

Study of hemodynamic parameters

At the end of the 6-week treatment, blood pressure was measured according to the procedure described by Balaramam *et al*.[43] Rats of all the experimental groups were anesthetized by urethane (1.75 gm/kg, i.p.), and the temperature was maintained at 37°C throughout the experiment. The left carotid artery was cannulated with polyethylene tube which was filled with 0.9% v/v heparinized saline. Mean, systolic and diastolic blood pressure, and heart rate were directly measured at left common carotid artery using a precalibrated pressure transducer SS13L connected to Biopac MP-30 data acquisition system (BIOPAC system, Inc., CA).

Study of biochemical parameters from heart

At the end of the experimental period, five animals from each group were killed with overdose of urethane. The heart was removed and weighed immediately. Heart homogenates were prepared in cold 50 mM Tris buffer (pH 7.4) using Remi homogenizer so that clear homogenate was formed. The supernatant was used for the estimation of GSH, malondialdehyde (MDA),[44,45] superoxide dismutase (SOD),[46] and catalase.[47-49]

Histopathological studies

Heart was isolated from a single animal from each group, washed with distilled water, and kept in 10% formalin solution and sent to local pathology laboratory for histopathological studies.

Statistical analysis

The results were expressed as mean±SEM and statistically analyzed by analysis of variance followed by Dunnett test, with a level of significance set at $P<0.05$.

Results

Effect of combination of PPAR-γ agonists with melatonin on biochemical parameters

Diabetic control group exhibited significant hyperglycemia and dyslipidemia ($P<0.01$) when compared with normal control. The serum glucose, triglyceride, total cholesterol, LDL-cholesterol, AST and LDH levels were increased and HDL-cholesterol level was decreased significantly ($P<0.01$) in the diabetic control group when compared with normal control [Figures 1-7]. In the combination groups (PM and RM), there was significant decrease in the serum glucose, triglyceride, and total cholesterol levels when compared with diabetic control ($P<0.05$), when compared with PIO and ROSI groups. The combination groups (PM and RM) showed significant reduction ($P<0.05$) in the triglyceride and cholesterol levels. The combination groups (PM and RM) showed significant decrease ($P<0.01$) in the serum glucose level when compared with MEL group.

In the combination groups, PM showed significant decrease in the serum triglyceride, total cholesterol ($P<0.01$), LDL-cholesterol ($P<0.05$), and increase in the HDL-cholesterol ($P<0.05$) when compared with the MEL-treated group. HDL-cholesterol level significantly increased in combination groups (PM and RM) when compared with diabetic control, PIO, and ROSI groups ($P<0.01$) as well. The AST and LDH enzyme levels were significantly increased in the diabetic control group ($P<0.01$) when compared with normal control group. The combination groups (PM and RM) showed significant decrease in AST and LDH levels ($P<0.05$) when compared with diabetic control and PIO, ROSI, and MEL groups as well.

Effects of combination of PPAR-γ agonists with melatonin on haemodynamic parameters

Administration of alloxan resulted in significant increase ($P<0.01$) in the systolic, diastolic mean blood pressure, and significant decrease in heart rate when compared with normal control [Figures 8 and 9]. The PIO-, ROSI-, and MEL-treated groups showed significant decrease ($P<0.05$) in systolic, diastolic, and mean blood pressure when compared with diabetic control. The heart rate in diabetic control group was significantly increased which was significantly decreased in LR-treated group only. PM- and RM-treated groups did not show significant change in heart rate when compared with diabetic control group.

The combination groups (PM and RM) showed significant decrease ($P<0.01$) in systolic, diastolic, and mean blood pressure when compared with diabetic control.

In the diabetic control group, there was significant increase ($P<0.01$) in the atherogenic index when compared with normal control group. In the combination groups (PM and RM), there was significant decrease ($P<0.01$) in the atherogenic index when compared with diabetic control, as well as PIO, ROSI, and MEL groups, respectively [Figure 10].

Effect of combination of PPAR-γ agonists with melatonin on sod, cat, gsh, and Lpo levels in heart homogenate of diabetic rats

In diabetic control group, there was significant decrease in the levels of SOD, CAT, and GSH ($P<0.01$) and significant increase ($P<0.01$) in the LPO level when compared with normal control group [Figures 11-14]. The combination groups (PM and RM) showed significant increase in levels of SOD, CAT, and GSH ($P<0.01$) and significant decrease ($P<0.01$) in the LPO level when compared with diabetic control group. PM-treated group showed significant increase

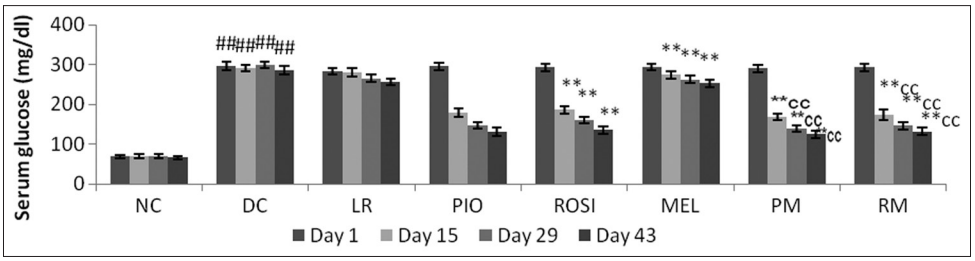

Figure 1: Effect of combination of PPAR-γ agonists with melatonin on serum glucose level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus Melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control. $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{a}P<0.05$ when compared with pioglitazone; $^{b}P<0.05$ when compared with rosiglitazone. $^{c}P<0.05$; $^{cc}P<0.01$ when compared with melatonin

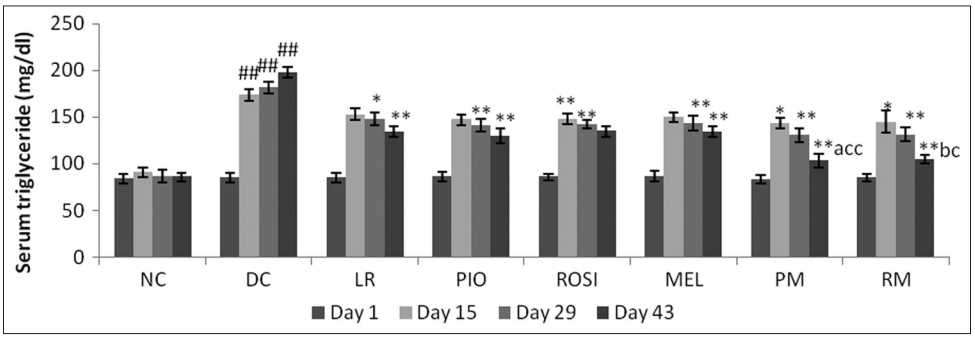

Figure 2: Effect of combination of PPAR-γ agonists with melatonin on serum triglyceride level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control. $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{a}P<0.05$ when compared with pioglitazone; $^{b}P<0.05$ when compared with rosiglitazone. $^{c}P<0.05$; $^{cc}P<0.01$ when compared with melatonin

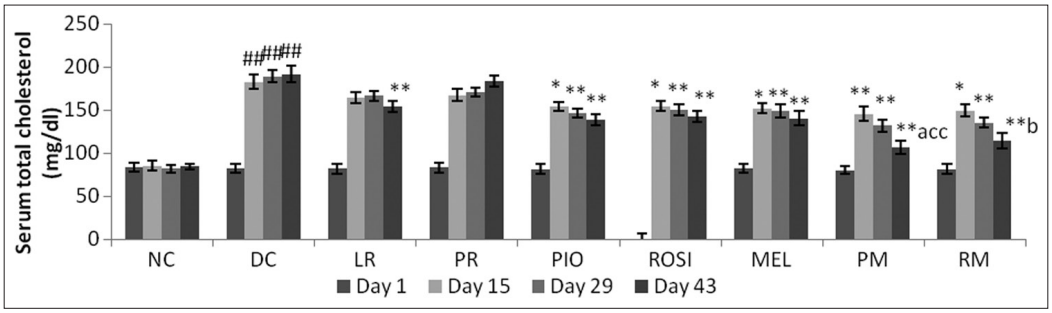

Figure 3: Effect of combination of PPAR-γ agonists with melatonin on serum total cholesterol level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone and melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{a}P<0.05$ when compared with pioglitazone; $^{b}P<0.05$ when compared with rosiglitazone. $^{cc}P<0.01$ when compared with melatonin

in levels of SOD, CAT, and GSH and significant decrease in LPO (P<0.05) when compared with PIO and MEL groups. RM-treated group showed significant increase in levels of SOD, CAT, and GSH and significant decrease in the LPO (P<0.05) when compared with ROSI group.

Discussion

This study was undertaken to evaluate the effect of combination of PPAR-γ agonist, thiazolidinediones (pioglitazone and rosiglitazone) with melatonin in the diabetic rats. In the cardiac tissue, there is an enhanced oxidative stress which can increase the propensity to diabetic complications.[50-52] Therefore, the parameters of oxidative defense, biochemical parameters, and the markers of oxidative stress were evaluated in the heart of normal control and diabetic rats after the treatment with combination of PPAR-γ agonist thiazolidinediones (pioglitazone and rosiglitazone) with melatonin for 7 weeks in alloxan-induced diabetic rats.

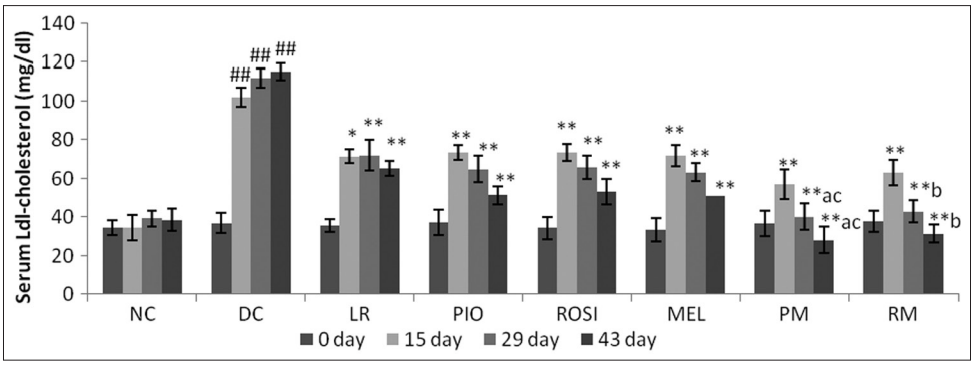

Figure 4: Effect of combination of PPAR-γ agonists with melatonin on serum LDL-cholesterol level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{a}P<0.05$ when compared with pioglitazone, $^{b}P<0.05$ when compared with rosiglitazone. $^{c}P<0.05$ when compared with melatonin

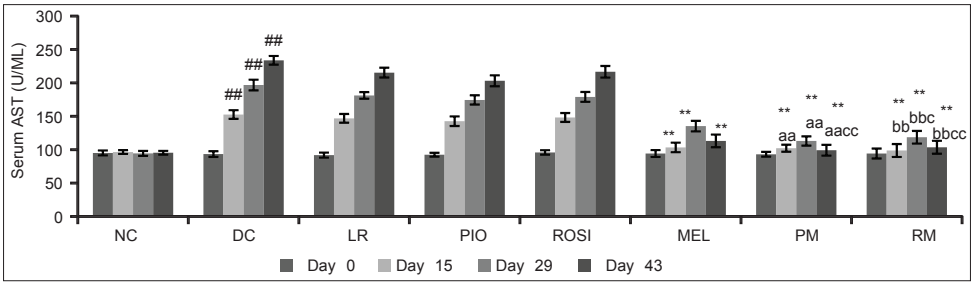

Figure 5: Effect of combination of PPAR-γ agonists with melatonin on serum AST level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone; $^{bb}P<0.01$ when compared with rosiglitazone. $^{cc}P<0.01$ when compared with melatonin

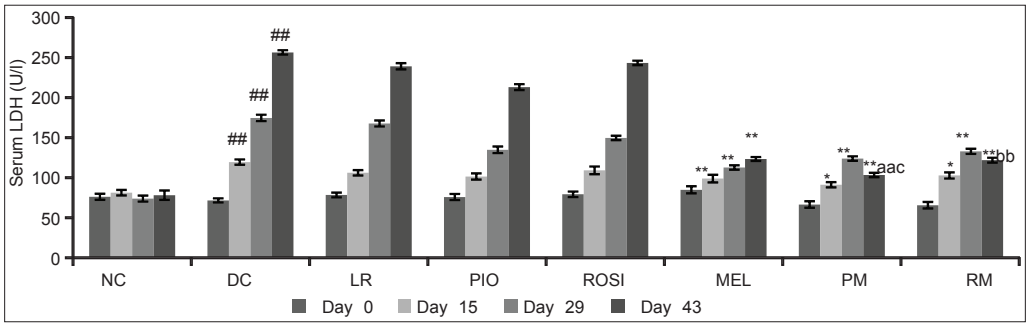

Figure 6: Effect of combination of PPAR-γ agonists with melatonin on serum LDH level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone; $^{bb}P<0.01$ when compared with rosiglitazone. $^{c}P<0.05$ when compared with melatonin

In this study, rats in the diabetic control group had characteristic hyperlipidemia, significantly decreased HDL-cholesterol level, and significant increase in the atherogenic index and hypertension, which lead to atherosclerosis and coronary heart disease.[53-60] Diabetic control rats also showed decrease in the heart rate and increase in blood pressure and levels of AST and LDH, which are markers of cell injury.[61,62]

The treatment with combination of PPAR-γ agonists thiazolidinediones (pioglitazone and rosiglitazone) with melatonin showed significant decrease in the serum glucose. The probable mechanism behind decrease in serum glucose level may be due to increase in the glucose uptake via translocation of GLUT-4 by thiazolidinediones, pioglitazone, and rosiglitazone,[63] as well as by melatonin,[64] along with the increased insulin sensitivity

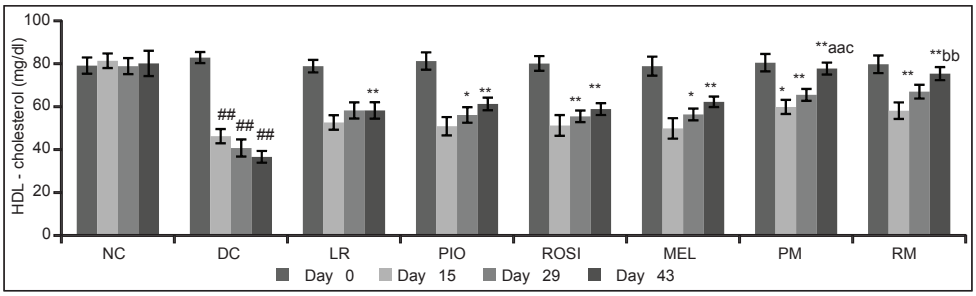

Figure 7: Effect of combination of PPAR-γ agonists with melatonin on serum HDL-Cholesterol level in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone; $^{bb}P<0.01$ when compared with rosiglitazone. $^{c}P<0.05$ when compared with melatonin

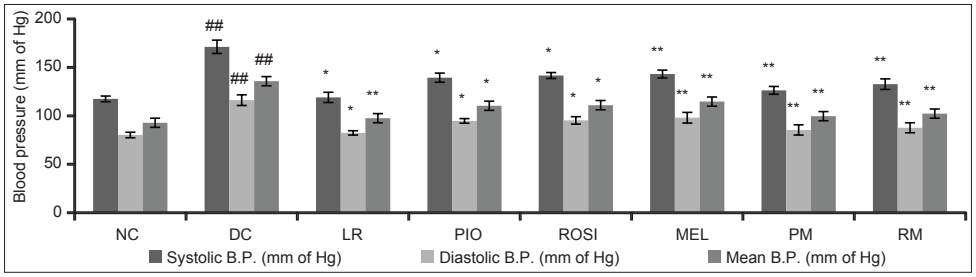

Figure 8: Effect of combination of PPAR-γ agonists with melatonin on blood pressure in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control

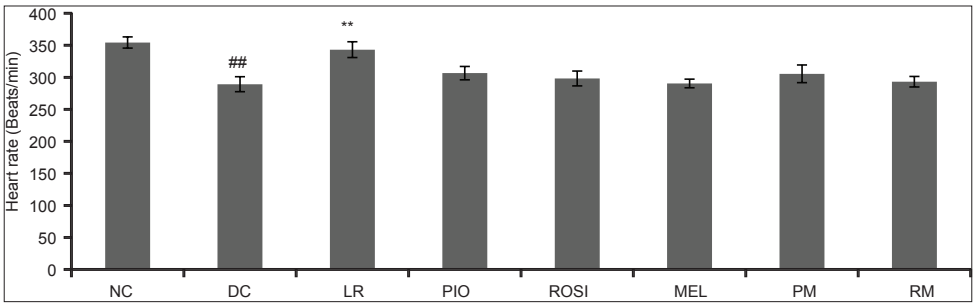

Figure 9: Effect of combination of PPAR-γ agonists with melatonin on heart rate in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$ when compared with diabetic control

by thiazolidinediones[65] which leads to increased glucose uptake in cells.

The combination groups (PM and RM) showed decrease in cholesterol, triglyceride, LDL-cholesterol levels, and atherogenic index, when compared with the individual PIO-, ROSI-, and MEL-treated groups, which may be due to the reduction in the expressions of leptin and TNF-a in the cells.[66] In addition, thiazolidinedione's direct effect on lipoprotein lipase and the fatty-acid protein aP2, genes of fatty-acid metabolism, may lead to increase in levels of lipoprotein lipase in fat cells[65] causing increased uptake of triglycerides by fat and thereby improve the insulin signaling in muscles and liver.[66] Melatonin decreases Δ-5 desaturase activity in liver microsomes leading to decreased hyperlipidemia in diabetic rats.[67]

The combination of pioglitazone plus melatonin and rosiglitazone plus melatonin exhibited control over blood pressure because of the combined effect of these drugs; however, the results obtained were not significant when compared with the individual PIO, ROSI, and MEL groups. Thiazolidinediones decrease blood pressure, but the exact mechanism by which they decrease the blood pressure is unknown; however, the probable mechanism may be due

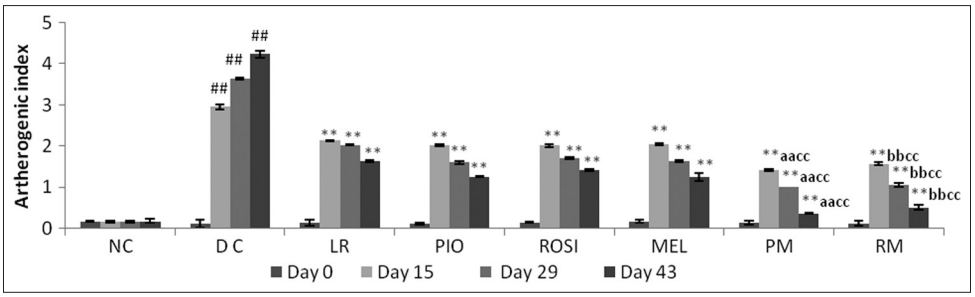

Figure 10: Effect of combination of PPAR-γ agonists with melatonin on atherogenic index in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=6), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$, when compared with pioglitazone. $^{bb}P<0.01$, when compared with rosiglitazone. ^{cc} < 0.01 when compared with melatonin

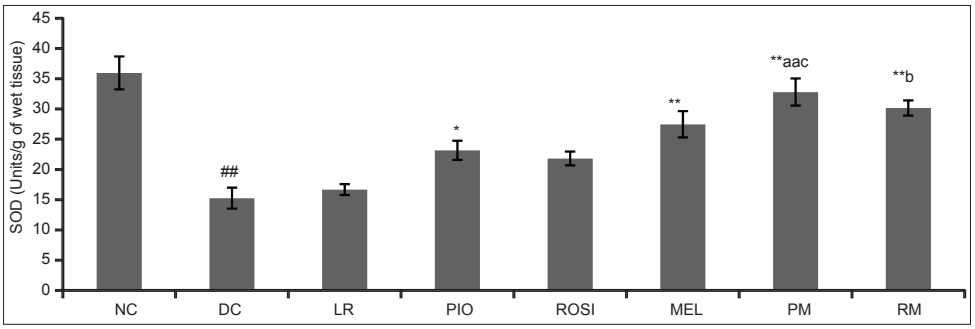

Figure 11: Effect of combination of PPAR-γ agonist melatonin on levels of SOD level in heart homogenate in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are expressed as mean±SEM. (n=5), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone, $^{b}P<0.05$ when compared with rosiglitazone. $^{c}P<0.01$ when compared with melatonin

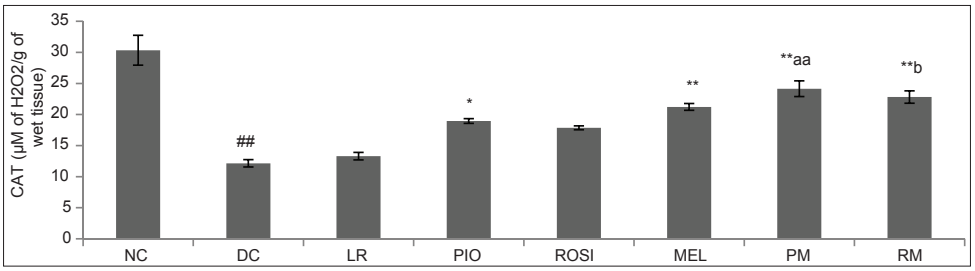

Figure 12: Effect of combination of PPAR-γ agonists with melatonin on levels of Catalase level in heart homogenate in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=5), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone, $^{b}P<0.05$ when compared with rosiglitazone

to vasodilation of blood vessels and increased blood flow to organs and decrease in insulin resistance.[65] Melatonin reduces blood pressure as a consequence of various actions including a direct hypothalamic effect, lowering of catecholamine levels, relaxation of the smooth muscle wall, and, most importantly, as a result of its antioxidant properties.[31,68]

In this study, atherogenic index was decreased by the combination of pioglitazone plus melatonin and rosiglitazone plus melatonin. The pioglitazone and rosiglitazone both showed antiatherosclerotic effects, which were probably mediated by decreased P-selectin, VCAM-1, and ICAM-1 expression within the atherosclerotic plaque.[65,69,70] In MEL group, reduction in the atherogenic index, reduction in total cholesterol levels, and improved HDL-cholesterol levels might be mechanisms behind a decrease in the atherogenic index.[68]

The levels of GSH, CAT, and SOD in heart homogenate of diabetic-treated groups were significantly decreased, and LPO level was significantly increased in diabetic group, but in combination groups PM and RM there was a significant

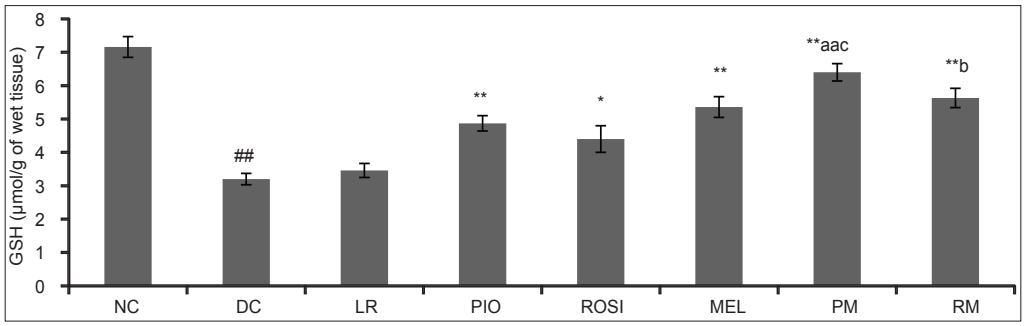

Figure 13: Effect of combination of PPAR-γ agonists with melatonin on GSH level in heart homogenate in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=5), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone. $^{b}P<0.05$ when compared with rosiglitazone. $^{c}P<0.05$ when compared with melatonin

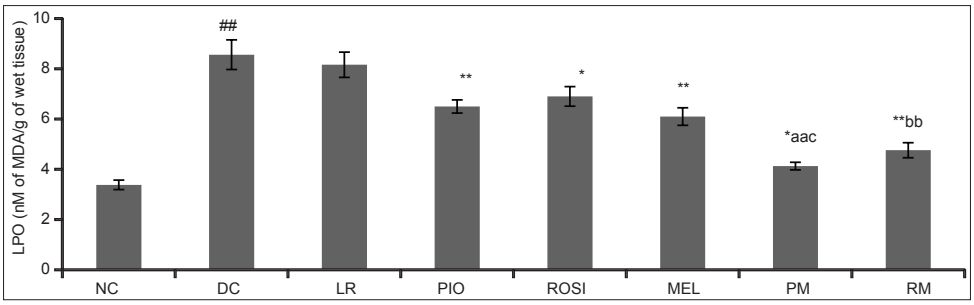

Figure 14: Effect of combination of PPAR-γ agonists with melatonin on LPO level in heart homogenate in diabetic rats. NC = normal control, DC = diabetic control, LR = losartan, PIO = pioglitazone, ROSI = rosiglitazone, MEL = melatonin, PM = pioglitazone plus melatonin, RM = rosiglitazone plus melatonin. Results are presented as mean±SEM (n=5), ANOVA followed by Dunnett test. $^{\#\#}P<0.01$ when compared with normal control; $^{*}P<0.05$, $^{**}P<0.01$ when compared with diabetic control. $^{aa}P<0.01$ when compared with pioglitazone. $^{bb}P<0.01$ when compared with rosiglitazone. $^{c}P<0.05$ when compared with melatonin

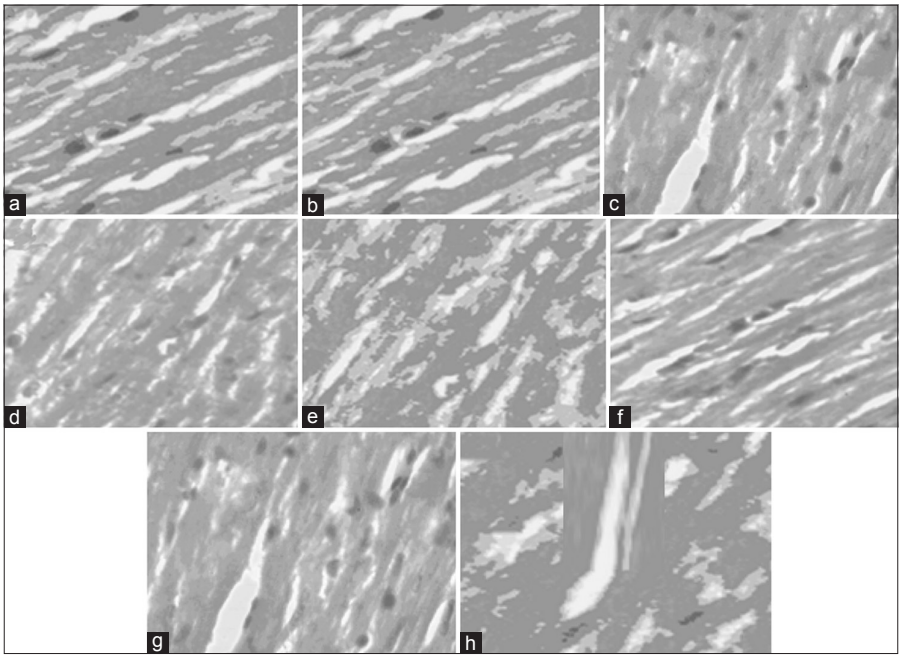

Figure 15: Effect of combination of PPAR-γ agonists with melatonin on histopathological studies of heart in diabetic rats. (a) Normal control (1% CMC, 1 ml/kg, p.o.). (b) Diabetic control (1% CMC 1 ml/kg, p.o.). (c) LR (losartan 2 mg/kg, p.o.) (d) PIO (pioglitazone 10 mg/kg, p.o.). (e) Rosiglitazone (5 mg/kg, p.o.). (f) MEL (10 mg/kg, p.o.). (g) PM (pioglitazone10 mg/kg, p.o. plus melatonin 10 mg/kg p.o.). (h) RM (rosiglitazone 5 mg/kg, p.o. plus melatonin 10 mg/kg, p.o.) (Stains: H and E, ×100)

increase in the levels of GSH, CAT, and SOD and significant decrease in the LPO level in heart homogenate, because of antioxidant effect of melatonin and PPAR-γ agonist thiazolidinediones (pioglitazone and rosiglitazone). The antioxidant effect observed in the combination groups may be the combined effect of the PPAR-γ agonists (pioglitazone and rosiglitazone) which cause reduction of AGE's formation hence decreasing the oxidative stress[71,72] and the potent free radical scavenging activity of melatonin.

In this study [Figure 15], histopathological examination of the diabetic control group showed significant myocardial damage, presence of focal myonecrosis with myophagocytosis and lymphocytic infiltration, and infiltration of inflammatory cells (myocarditis) in the subendocardial region.[73,74] The combination groups (pioglitazone plus melatonin and rosiglitazone plus melatonin) significantly inhibited the infiltration of inflammatory cells and showed that improvement in the myocardium also decreased the focal myonecrosis. The observed recovery of myocardial cells may be due to the anti-inflammatory and antioxidant effect of PPAR-γ agonist thiazolidinediones (pioglitazone and rosiglitazone) and melatonin.[69,71,72,75]

In summary, the results suggest that the combination of pioglitazone plus melatonin and rosiglitazone plus melatonin was effective in restoration of biochemical and histopathological alterations and may prevent the cardiovascular complications in alloxan-induced diabetic rats. Thus, thiazolidinediones with melatonin can be considered as potential drug candidates for cardiomyopathy arising from metabolic disorder such as diabetes. However/ further study would be needed to support and encourage our preclinical hypothesis.

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