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Research Article

EFFECT OF MELATONIN, PIOGLITAZONE AND THEIR COMBINATION ON FRUCTOSE INDUCED-INSULIN RESISTANT DIABETES IN RATS.

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ABSTRACT

Present study investigated the effects of melatonin, pioglitazone and their combination on fructose induced insulin resistant diabetes in rats. Administration of fructose 10% w/v ad libitum in a feeding bottle for 20 days produced insulin resistant diabetes, dyslipidemia and oxidative stress. Increase in serum glucose, insulin, total cholesterol, triglycerides and decrease in HDL levels were observed. In addition to insulin resistance, diabetes induced oxidative stress was observed by increase in TBARS and decrease in GSH levels. Treatment with pioglitazone (5mg and 10mg/kg/day p.o.), melatonin (100µg and 200µg/kg/day i.p.) and pioglitazone (5mg/kg/day p.o.) in combination with melatonin (100µg.kg/day i.p.) were started in insulin resistant diabetic rats after 20 days of fructose administration. The treatment with pioglitazone and melatonin alone and their combination attenuated fructose-induced insulin resistant diabetes as observed by a decrease in serum glucose, serum insulin and lipid levels. Further these results demonstrated that serum glucose, insulin and lipid levels were significantly lowered ($p<0.05$) in combination group as compared to fructose treated groups as well as with individual groups of pioglitazone and melatonin. The combination group improved the levels of TBARS and GSH also. These effects are probably due to reduction of insulin resistance via decrease in oxidative stress and control of hyperglycemia

Key words: *Insulin resistant diabetes, Lipid peroxidation, Melatonin, Pioglitazone*

INTRODUCTION

Diabetes mellitus is a complex metabolic syndrome characterized by absolute insulin deficiency or development of insulin resistance that leads to hyperglycemia and an altered glucose, fat and protein metabolism [1]. It is heterogeneous primary disorder of carbohydrate metabolism with multiple etiologic factors that generally involves absolute or relative insulin deficiency or insulin resistance or both leading to many long term complications [2].

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Insulin resistance diabetes is a condition where the insulin, becomes less effective at lowering blood glucose [3]. Certain cell such as fat and muscle cells require insulin to absorb glucose. When these cells fail to respond adequately to circulating insulin, blood glucose levels rise.The liver helps to regulate glucose levels by reducing its secretion of glucose in the presence of insulin. Insulin resistance in fat cells reduces the normal effects of insulin on lipids and results in reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Diabetes induces oxidative stress also that contributes to the insulin resistance. Thus treatment with anti oxidants can be used to reduce oxidative stress induced insulin resistance [4]. Melatonin

chemically known as N-acetyl-5-methoxytryptamine is secreted into the blood by the pineal gland in the brain[5]. Many biological effects of melatonin are produced through activation of melatonin receptors, while others are due to its role as a pervasive and powerful antioxidant, with a particular role in the protection of nuclear and mitochondrial DNA [6]. Melatonin has also been found to decrease the blood glucose levels and reduce the serum lipid levels by reducing insulin resistance [7], enhancing the glucose uptake in skeletal muscles in vitro via IRS-1/ PI-3- kinase. Insulin receptor subsrate-1/ Protein kinase-3 pathway stimulates glucose transcription and lipid lowering activity. Ghaisas *et al* have also found that the melatonin is effective in dexamethasone induced insulin resistance in mice. Pioglitazone belongs to the Thiazolidinedione class of drugs which selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α [8]. It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. As a result, pioglitazone reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose, insulin and glycated haemoglobin in the bloodstream [9]. Pioglitazone decreases the level of triglycerides and increases that of high-density lipoproteins HDL [10]. However, pioglitazone has been reported to have uncertain cardiovascular side-effects like fluid retention, peripheral edema and some other complications. Therefore, Pioglitazone has been used in combination with other anti-diabetic agents to improve its efficacy but the combination available limits its optimal use leading to introduction to the insulin therapy. Therefore, there is need to study new combination. The combination with melatonin has not been used so far due to insufficient studies in this regard. Hence, the same has been assessed in the present study.

Material and Methods

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young Wistar rats of either sex weighing about 230-260 were employed. Rats were fed on standard chow diet (Ashirwad Industries, Ropar, India) and water *ad libitum*. They were acclimatised in animal house and were exposed to normal day and light cycle. Insulin resistance diabetes was induced by 10% w/v fructose administration *ad libitum* in a feeding bottle dissolved in distilled for 20 days. The pathological changes start after 13 days of fructose administrstion and insulin resistance diabetes develops after 20 days of fructose administration [11-12]. The animals were divided into seven groups each of six rats of either sex. Group I: Normal control group fed on standard chow diet, Group II: Diabetic control administered with fructose 10% w/v *ad libitum* in feeding bottle for 20 days; Group III: Pioglitazine (5mg/kg/day p.o) treated diabetic rats. Group IV: Pioglitazone (10mg/kg/day p.o.) treated diabetic rats. Group V: Melatonin (100 µg/kg/day i.p) treated diabetic rats. Group VI: Melatonin (200 µg/kg/day i.p.) treated diabetic rats. Group VII: Melatonin (100 µg/kg/day i.p.) + Pioglitazone (5mg/kg/day p.o) treated diabetic rats. Treatment with pioglitazone, melatonin and their combination was given for 7 days started after 20 days of fructose administration. At the end of 27 days of experiment blood samples were collected after overnight fasting and analysed. Serum glucose levels were determined by GOD-POD method [13-14] using commercially available kit (coral clinical system, Goa, India). Serum insulin levels were determined using electrochemiluminescence immunoassay. The total cholestrol was determined by CHOD-PAP method [15] using commercially available kit (coral clinical system, Goa, India). The serun triglyseride was determined by glycerophosphate oxidase peroxidase GOD-PAP method[13-16] using commercially available kit (coral clinical system, Goa, India).The HDL was determined by PEG

precipitation method using commercial available kit (Coral clinical system, Goa, India).The serum TBARS, an index of lipid peroxidation was estimated according to the method described[17]. The GSH level in the serum was estimated by the method as described [18]. Body weight of rats was determined at the beginning of the experiment and at the end. Results were expressed as mean ± standard deviation (S.D.). Differences between groups were calculated by the means of one way ANOVA followed by Tukey's multiple comparison test and $p \leq 0.05$ were accepted to be statistically significant.

RESULTS

A significant increase in body weight was observed on fructose treated diabetic group. Increase body weight was not reduced by administration of pioglitazone, melatonin or their combination in diabetic rats significantly. A significant increase in serum concentration of glucose was noted in diabetic rats when compared with age matched normal rats. Treatment with pioglitazone (5mg & 10mg/kg/day, 7 days), melatonin (100µg & 200 µg /kg/day *i.p*, 7 days) and pioglitazone (5mg/kg/day) plus melatonin(100 µg/kg/day i.p,) for 7 days significantly decreased ($p \leq 0.05$) serum glucose when compared with diabetic control (table 1)

A significant increase ($p \leq 0.05$) in serum triglycerides and serum total cholesterol and significant decrease in HDL level was noted in diabetic rats when compared with age matched normal rats. Treatment with Pioglitazone (5 mg/Kg/day 7days) did not significantly reduced serum triglycerides and serum total cholesterol and did not increase serum HDL significantly when compared with diabetic rats. However, treatment with Pioglitazone (10mg/kg/day 7days) significantly reduced ($p<0.05$) serum triglycerides and serum total cholesterol and significantly increased serum HDL when compared with diabetic rats. Treatmant with melatonin (100µg/kg i.p., 7 days) significantly reduced ($p<0.05$) serum triglycerides and serum total cholesterol but serum HDL was not significantly increased with melatonin with this dose when compared with diabetic rats. However, treatment with melatonin (200µg/kg i.p., 7 days) significantly reduced ($p<0.05$) serum triglycerides and serum total cholesterol and significantly increased serum HDL when compared with diabetic rats. Treatment with concurrent administration of Pioglitazone (5mg/kg/day, 7 days) plus melatonin (100µg/kg/day i.p.,7days) significamtly decreased ($p<0.05$) serum glucose, triglycerides and serum total cholesterol and significantly increased the serum HDL when compared with diabetic rats. Results are given in table 1.

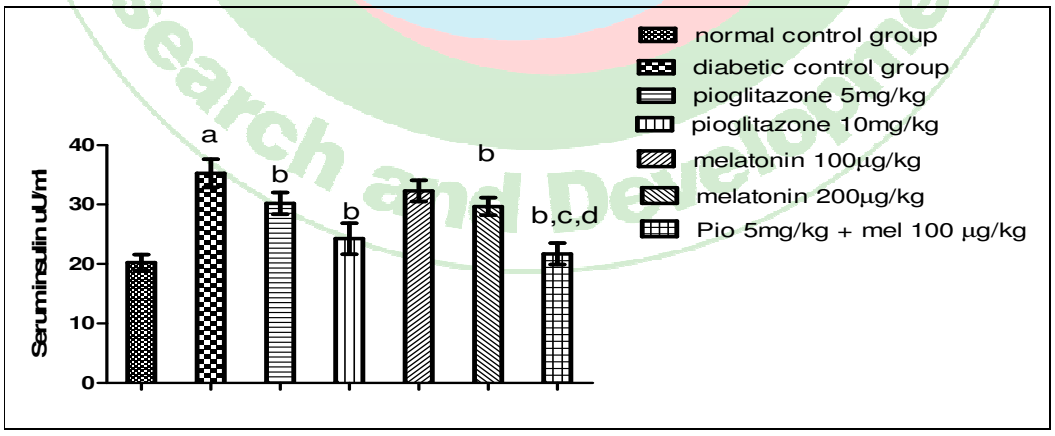

Fig1: Serum insulin levels before and after various treatments

Table 1. Effect of various pharmacological interventions on body weight, serum glucose, serum HDL, serum total cholesterol, serum TBARS, serum GSH, and serum triglycerides.

Groups	*Body weight (grams)*	*Serum glucose (mg/dl)*	*Serum HDL (mg/dl)*	*Serum total cholesterol (mg/dl)*	*Serum triglycerides (mg/dl)*	*TBARS (nmol/ml)*	*GSH (nmol/ml)*
Normal control	187.5±17.2	88.1±8.1	39±2.6	127.8±10.4	107.6±14.4	3.9±0.3	4.1±0.2
Diabetic control	232.5±17.2[a]	145.1±10.3[a]	25±2.4[a]	192.8±11.3[a]	184.8±10.5[a]	6.01±0.26[a]	1.51±0.2
Pioglitazone (5mg/kg/day)	224.1±23.5	112±9.6[b]	27.5±2.9	178±10.5	171.1±11.7	5.3±0.27[b]	2.15±0.2
Pioglitazone (10mg/kg/day)	210 ±38.2	95.3± 6.2[b,c]	32.2 ±2.6[b]	166.3±11.5[b]	153.8 ±10.9[b]	4.9±0.33[b,c]	2.94±0.3
Melatonin (100µg/kg/day)	207.5±18.6	133.2±8.6[b]	28.3±2.2[b]	168±12	165±10.2	5.1±0.46	2.52±0.3
Melatonin (200µg/kg/day	200±20.7	120.6±12.6[b,d]	33.6±2.7[b]	158±9.7[b,c]	148±8.4[b,c]	4.67±0.34[b]	3.05±0.34
Pioglitazone (5mg/kg/day)+ Melatonin (100µg/kg/day)	195±28.1	94±10.2[b,c,d]	35±1.7[b,c,d]	149±7.3[b,c,d]	131±8.6[b,c,d]	4.20±0.27[b,c,d]	3.69±0.44

All values expressed as mean ± S.D. a=p< 0.05 vs. normal control; b= p< 0.05 vs. diabetic control; c=p<0.05 vs. pioglitazone 5mg/kg; d=p<0.05 vs. melatonin 100µg/kg

The serum insulin concentration increased significantly (p<0.05) in diabetic rats when compared with age matched normal rats. Treatment with pioglitazone (5mg & 10 mg/kg/day, 7 days) significantly decreased (p<0.05) serum concentration of insulin level when compared with diabetic rats. Treatment with melatonin (100µg & 200 µg /kg/day *i.p.*, 7 days) significantly decreased (p<0.05) serum concentration of insulin levels when compared with diabetic rats. Treatment with pioglitazone (5mg/kg/day, 7 days) plus melatonin (100ug/kg/day *i.p.*, 7 days) also significantly decreased serum concentration of insulin level when compared with diabetic control rats. (Fig 1)

A significant increase in serum TBARS was noted in diabetic rats when compared with normal rats. Treatment with pioglitazone (5mg & 10mg/kg/day, 7 days treatment with melatonin (200ug/kg/day i.p., 7 days) and concurrent administration of pioglitazone (5mg/kg/day *p.o.*, 7 days) plus melatonin (100ug/kg/day *i.p.*, 7 days) significantly decreased the TBARS when compared to diabetic rats. However, melatonin (100ug/kg/day *i.p.*, 7 days) did not affect significantly on TBARS. A significant decrease (p<0.05) in serum GSH levels was noted diabetic rats when compared with age matched normal rats. Treatment with pioglitazone (5mg & 10mg/kg/day, 7 days treatment with melatonin (100ug & 200ug

/kg/day *i.p.*, 7 days) and concurrent administration of pioglitazone (5mg/kg/day, 7 days) plus melatonin (100µg/kg/day i.p., 7 days) has significantly increased GSH when compared with diabetic group.

DISUCUSSION

The present study focused on some of the adverse effects induced by the long term administration of fructose, such as insulin resistance, metabolic dyslipidemia and oxidative stress. Increase of blood glucose level has been used as a marker of hyperglycemia and increase in serum insulin has been documented to be index of insulin resistant diabetes [19]. In the present study serum glucose and serum insulin levels were noted to be significantly increased in fructose induced diabetic rats as compared to normal rats. It has been documented that any defect in the insulin signaling cascade at the multiple steps through alterations in the protein levels and activities of the signaling molecules, enzymes, and transcription factors as a result of insulin resistance [20]. Oxidative stress has been assessed in terms of change of TBARS and GSH values. High levels of total cholesterol, triglycerides and low level of HDL have been used as a marker of dyslipidemia [21]. Lipoprotein lipase (LPL) in the vascular endothelium is involved in the breakdown of triglycerides into free fatty acids. It has been reported that decreased release of lipoprotein lipase is associated with VED leading to hypertriglyceridemia and decreased HDL levels without affecting serum cholesterol levels, which contributes to insulin resistant diabetes [22]. A strong correlation between dyslipidemia and insulin resistant diabetes has been reported [23]. Fructose-induced diabetes is often associated with hypercholesterolemia and hypertriglyceridemia [24]. This contention is supported by the results obtained in the present study in fructose induced diabetes i.e. increased serum triglycerides, serum cholesterol and leading to development of insulin resistant diabetes. Clinically, Pioglitazone has been well reported to decrease serum glucose, insulin and lipid levels in diabetic patients [25- 27]. Therefore, Pioglitazone has been employed as a standard drug in the present study.

In the present study biochemical parameters like serum glucose, insulin and lipids were significamtly lowered ($p<0.05$) in the combination group as compared to fructose treated groups as well as with individual groups of pioglitazone and melatonin. The combination group improved the levels of TBARS and GSH also. This could be due to reduced insulin resistance with pioglitazone and antioxidant effect of melatonin. These results are supported by the contention that melatonin has been reported to possess hypoglycemic effect in diabetic rats through restoration of insulin resistance, enhancing glucose uptake by skeletal muscle via IRS-1/ PI-3-kinase pathway and stimulation of glucose transport [28] and lipid lowering activity [29]. Improved levels of TBARS and GSH are due to antioxidant effect of melatonin. Pioglitazone decreases serum glucose insulin and lipid levels in diabetic patients. Improvement in reducing the insulin resistance and increase in levels of antioxidant enzymes such as GSH, SOD, Catalase in the liver with melatonin in combination with thiozolidenediones has also been reported [30]. In view of the synergistic effect of pioglitazone and melatonin in reducing the insulin resistance, dislipidemia and oxidative stress induced by fructose administration warrants further studies.

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