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Role of Peroxisome Proliferator-activated Receptor Gamma Agonist on Hepatic Oxidative Stress and Insulin Resistance in High Fat Diet Induced Diabetes

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Abstract

Peroxisome proliferator-activated receptor-γ agonists have beneficial effects in the oxidative stress pathway by improving the endothelial function. Obesity, a major contributor of insulin resistance has a significant probability of type 2 Diabetes Mellitus, elicits oxidative stress that exacerbates the onset and progression of hepatotoxicity. The prospective of the research is to investigate the role of PPAR-γ agonist on insulin resistance and hepatocellular damages due to obesity and diabetes.

Albino Wistar (n=40) was categorized into 5 groups; Group I: Rats fed on a normal rat diet; Group II: High fat diet (HFD) induced obese rats (fed on HFD for 8 weeks); Group III: HFD fed rats treated with Rosiglitazone (3 mg/kg) for 7 days; Group IV: T2DM rats induced by HFD and low dose of Streptozotocin (i.p. 35 mg/kg); Group V: T2DM rats treated with Rosiglitazone (3 mg/kg) for 7 days. Insulin resistance was assessed by Serum insulin level and HOMA-IR. Hepatic oxidative stress was estimated by MDA, SOD, Catalase activity and plasma Paraoxonase -1 level. Obesity and T2DM caused a significant raised insulin resistance. There is marked increased MDA and decreased Catalase, SOD and plasma paraoxonase-1 activity. PPAR-γ agonist treatment decreased the insulin resistance in both obesity and T2DM rats and reversed and restored antioxidant status.

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The results divulge that PPAR-γ agonist not only reversed the effect of HFD and T2DM but also impede deleterious effects on hepatocellular damages due to insulin resistance and oxidative stress.

Keywords: Hepatotoxicity; High fat diet; Insulin resistance; Oxidative stress; PPAR-γ agonist; Paraoxonase-1.

1. Introduction

Diet has been considered as the significant contributor in the etiology of obesity [1]. Obesity-related excessive adiposity is considered as a major contributor for several metabolic disorders, like increased systemic inflammation, cardiovascular risk [2], arthritis, insulin resistance [3] and especially correlate with Type 2 Diabetes. The Pathogenic basis of Type 2 Diabetes is gradually decreased insulin action (insulin resistance) and unable the β-cells to restore insulin resistance that facilitate hyperglycemia and other metabolic disturbances [4].

Epidemiological studies linked obesity with increased production of reactive oxygen species (ROS) that cause endothelial dysfunction [5]. It has marked influence on β-cells, skeletal muscles and hepatocytes thus exacerbate insulin resistance [6, 7]. Various studies showed that oxidative stress have a critical role in the prognosis and pathogenesis of insulin resistance [8], which has been widely estimated in research by using the HOMA-IR (homeostasis model assessment-estimated insulin resistance) [9].

There is considerable evidence that diabetes mellitus is strongly correlated with access production of free radicals and ultimately leads to damage oxidative stress. Another cause of increasing reactive oxygen species including hydrogen peroxide and superoxide dismutase is NADPH oxidase which is activated as consequences of increasing inflammatory cytokines [10]. Activity of serum PON1 that is an important emerging marker of oxidative stress is modulated both by diet and oxidative stress [11], and reported previously to be significantly reduced in obesity and by elevated level of oxidative stress [12]. Several evidences showed that obesity may leads to fatty liver which correspond to lipid accretion in the liver and exhibit increased lipid peroxidation in liver and a defect in anti-oxidative pathways [13]. Peroxisome proliferator-activated receptor gamma (PPAR gamma), is one of the important nuclear receptor. It has previously been known as an important curative and therapeutic target for the cure of metabolic disorders and has a potential role to combat oxidative damages through distinct mechanism. PPAR-γ have effective role in regulation and differentiation of adipocytes and lipid metabolism. Thiazolidinediones (TZDs) are PPAR- γ agonist, the class of drug that are insulin sensitizer and acts by activating PPAR-γ, and shown to ameliorate endothelial function of human and animals. It has a key role in FFA transportation and in the conversion of FFAs to triglycerides [14, 15]. However, brief researches available about the potential role of PPAR-γ related effects on hepatic antioxidant status in rat model of high fat diet induced diabetes mellitus. In this study we aimed to assess the contribution of insulin resistance, hepatic oxidative stress, Paraoxonase 1 activity and to identify the underlying mechanism and effects of Rosiglitazone in animal model of high fat diet induced obesity and type 2 diabetes mellitus.

2. Methods

2.1. Animals

Forty male Wistar rats, (180-200 g body weight), were procured from ICCBS (International Centre for Chemical and Biological Sciences), University of Karachi. The rats were maintained at an ambient temperature (23 ± 4°C) with 12-h light-dark cycles and habituated to housing conditions for 1 week before experiments. Rats had free access to the standard rat diet and water. Experiment was conducted according to institutional ethical guidelines.

2.2. Preparation of High Fat Diet

High fat diet was prepared by mixing following ingredient according to the method of Khalifa et al.2009 [16], 7g wheat flour, 30 g of Casein, 6 g common salt, 4g bran, 10 g glucose, 3 % vitamin mixture and 40 g raw melted beef fat to make palettes of 12 gram. Almost 54 % of the daily ingested calories from this diet were from fat contents.

2.3. HFD/STZ Animal model of Type 2 diabetes

HFD/STZ animal model mimicking human type 2 diabetes was designed according to Luo et al., 1998 [17], briefly rats were fed on High fat diet for 4 weeks and injected a single low dose of Streptozotocin (35 mg/kg) and continued feeding on HFD for next 4 weeks. High fat diet for 8 weeks has been used to model the Insulin resistance and metabolic syndrome that mimics human type 2 diabetes. Streptozotocin did not destroy all of the β-cells but killed some of them to renders the rats become hyperglycemic [18, 19]. Blood glucose level was regularly monitored. The animals having blood glucose level more than 250 mg/dl confirming type 2 Diabetes mellitus were included in experiment.

2.4. Study design

Rats were categorized into five groups (n = 8): Group I, normal control group: fed with a standard diet, Group II, Obese control group: fed with HFD for 8 weeks, Group III, Obese + RSG treated group: received HFD for 8 weeks and treated with Rosiglitazone (3 mg/kg body weight) prepared in aqueous solution, daily for last 7 days using an intra-gastric tube, Group IV, Diabetic control group: received HFD for 8 weeks and a single i.p injection of Streptozotocin (35mg/kg body weight). Group V, Diabetic + RSG treated group: fed with HFD for 8 weeks and single i.p injection of Streptozotocin (35mg/kg body weight) treated with Rosiglitazone (3 mg/kg body weight) in aqueous solution daily for last 7 days by an intra-gastric tube. The study design was approved by the Institutional Animal Ethics Committee.

Animals from all experimental groups were weighed and their food intake with normal activity was regularly monitored per week.

2.5. Sample Collection

After 24 hours of last dose of treated groups, all the animals were decapitated by cervical dislocation blood samples were collected in a heparinized tube, centrifuged to collect plasma and stored at −70°C for biochemical analysis. Liver was excised and rinsed with ice chilled buffer saline (pH 7.0) weighed and stored at −20°C for further biochemical estimations.

2.6. Anthropometrical determinations

Anthropometrical determination was carried out by measuring the body weight and body length of individual animal.

Body mass index (BMI) was calculated as; body weight (g) / length^2 (cm^2)

Lee index was estimated as; cube root of body weight (g) / nose-to-anus length (cm)

The difference in body weight of each rat was calculated and expressed as a percentage change according to the following:

%change in body weight = final body weight- initial body weigh /initial body weight ×100 [20]

2.7. Estimation of blood glucose

Weekly random and final fasting blood glucose levels were measured using the Glucometer (EZ II) from the tail vein.

2.8. Estimation of serum insulin level

Serum insulin conc. was estimated by using ELISA kit [21].

2.9. HOMA-IR determination

HOMA-IR was used to evaluate insulin resistance (serum insulin (μU/ml) × plasma glucose (mmol/ l)/22.5) [9].

2.10. Assessment of Hepatic Oxidative Stress

2.10.1. Preparation of post mitochondrial supernatant

Liver from each rat was removed and washed with ice-chilled buffer saline. Each liver was minced, and homogenized with 10% (w/v) chilled 0.1 M sodium phosphate buffer, pH 7.4. The homogenate is then centrifuged at 1,000 rpm for 10 min at 4°C. The supernatant was used to estimate Malondialdehyde (MDA). 10μl BHT ((butylated hydroxytoluene) (0.5M in acetonitrile) was added to the portion of homogenate for the assessment of Lipid peroxides to prevent the homogenate from oxidation and stored at -70ºC. To get post-mitochondrial supernatant (PMS), homogenates was again centrifuged at 12,000 rpm for 20 min at 4°C which was used to assay CAT and SOD [22].

2.11. Estimation of Malondialdehyde (MDA)

The Malondialdehyde (MDA) level in tissue homogenate is the measure of lipid peroxidation. The principle of the method is based on determination of thiobarbituric acid (TBA) reacting substance according to Okhawa et

al., 1979 by measuring absorbance of the pink color produced by the interaction of TBA with MDA at 530 nm on Schimadzu UV Spectrophotometer. Values were expressed as nmol/ml [23].

2.12. Estimation of Catalase

Catalase activity in liver homogenate was assayed by the method of Sinha [24]. The activity was calculated as μM / g of tissue.

2.13. Estimation of Superoxide dismutase

Level of SOD was measured by the method of Kono, 1978 in the cell free supernatant. Percent inhibition in the rate of NBT reduction in the reaction mixture was recorded per minutes spectrophotometrically by Schimadzu UV Spectrophotometer at 560 nm [25].

2.14. Estimation of Plasma Paraoxonase-1 (PON1)

Paraoxonase-1 was assayed via commercially available Kit that uses ELISA established on biotin binary antibody sandwich technology. (Shanghai Yehua Biological Tecnology Co.)

2.15. Statistical analysis

Results were expressed as mean ± S.D. Statistical analysis was carried out by SPSS program, version 10 (SPSS Inc, USA), values of $p<0.05$ were considered as statistically significant.

3. Results

3.1. Anthropometrical determination in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

Table 1 showed the anthropometrical parameters of control, HFD fed, and T2DM groups and effect of PPAR-γ agonist (RSG) treatment among these groups. A Significant ($p<0.05$) increased % change in body weight was observed in animal fed on HFD for 8 weeks which reduced significantly ($p<0.001$) after the treatment of PPAR-γ agonist (RSG) as compared with control. However T2DM rats showed significant ($p<0.05$) reduction in body weight when compared to both control and HFD fed obese group. Marked increased BMI was observed in HFD fed obese rats when compared with control ($P<0.001$) which decreased after RSG treatment ($p<0.05$). T2DM rats showed significant ($p<0.05$) increased BMI as compared with control. Treatment of T2DM with RSG significant ($p<0.05$) decreased BMI. Lee index was significantly ($p<0.01$) increased in HFD fed obese rays as compared with control. Treatment of HFD with RSG decreased lee index significantly ($p<0.01$) as compared with HFD. T2DM showed increased ($p<0.05$) lee index as compared with control and decreased ($p<0.001$) in HFD which decreased ($p<0.001$) after RSG treatment. The weights of liver were significantly ($p<0.001$) increased among all the groups as compared to control as shown in table 1.

Table 1: Anthropometrical parameters and Organ weight in High Fat diet fed and Type 2 Diabetes mellitus rats with and without PPAR-γagonist (Rosiglitazone) treatment

Parameters	Control	HFDa	HFD+RSGa,b	T2DMa,b,	T2DM+RSGa,b,c
Initial Body Weight (g)	184.34 ± 25.52	202.08 ± 25.39	208.81 ± 29.08	195.63 ± 36.98	202.75 ± 22.37
Final Body Weight (g)	201.23 ± 18.05	240.25 ± 14.02	238 ± 30.91	222.75 ± 35.62	227.58 ± 29.51
Body Weight Gain (g)	16.89 ± 11.46	38.17 ± 11.89	29.19 ±6.31	27.12 ± 12.70	24.83 ± 12.51
% Change in body weight	9.85 ± 7.12	19.74 ± 8.02 *	14.16 ± 3.22 ***	14.80 ± 8.44 *, #	12.19 ± 5.82 **, #
Food Consumption (g)	68.04± 6.84	76.07± 6.89	75.42 ±7.24	72.90± 6.62	73.77± 5.45
BMI (g/cm2)	0.471 ± 0.06	0.604 ± 0.06 ***	0.512 ± 0.08 #	0.550 ± 0.07 *	0.456 ± 0.06 # # #, µ
Lee index (g / cm)	0.351 ± 0.02	0.394 ± 0.03 **	0.352 ± 0.02 # #	0.376 ± 0.02 *, # # #	0.333 ± 0.02 ***, µµµ
Liver Weight (g)	5.28 ± 0.46	7.08 ± 0.511 ***	6.082± 0.265 ***	6.60 ± 0.874 **	6.37 ± 0.361 ***, ##
Liver Weight Index(g/Kg of Body Weight)	26.239	29.498	28.676	29.643	28.012

Data are presented as mean ±SD; n=8,

***P< 0.001, **P< 0.01,*P< 0.05 with Control, ###P<0.001

##P<0.01, #P< 0.05 with HFD (High Fat Diet group)

µµµP<0.001, µµP<0.01, µP<0.05with DM (Diabetes Mellitus group),

P > 0.05 (Non-Significant)

Table 2: Blood glucose, serum Insulin level and HOMA-IR in High Fat diet fed and Type 2 Diabetes mellitus rats with and without PPAR-γagonist (Rosiglitazone) treatment

Parameters	Control	HFD a	HFD+ RSG a, b	T2DMa,b	T2DM+RSGa,b,c
BLOOD GLUCOSE mg/dl	74.011 ± 2.80	89.084 ± 3.82 ***	83.038 ± 2.25 ***, ###	472.117 ± 33.17 ***, ###	276.643 ±21.04 ***, ###, μμμ
SERUM INSULIN LEVEL mIU/ml	9.028 ± 1.07	13.426 ± 1.99***	9.954 ± 0.358 ###	10.648 ± 3.063	9.722 ± 0.621 ###
HOMA-IR	1.641 ± 0.22	3.006 ± 0.457***	2.057 ± 0.074 ***, ###	12.842 ± 4.348 ***, ###	6.470 ± 0.686 ***, ###, μμ

Data are presented as mean ±SD; n=8,

***P< 0.001, **P< 0.01,*P< 0.05 compared with control

# P<0.05 compared with HFD

μμμP<0.001, μμP<0.01 as compared with T2DM (Type 2 Diabetes mellitus)

P>0.05(Non- Significant)

3.2. Blood Glucose Level in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

Increased level of Blood Glucose (p<0.001) were observed in HFD fed obese rats as compared to control. Treatment with PPAR-γ agonist (RSG) showed a significant (p<0.001) effect on Blood Glucose as compared to HFD fed obese rats. T2DM rats showed markedly increased Blood Glucose level (<0.001) as compared with control and High fat diet fed obese rats, which significantly reduced (p<0.001) by the treatment of PPAR-γ agonist (RSG). (Table 2)

3.3. Serum Insulin Level and HOMA-IR in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

Significantly increased serum insulin level and HOMA-IR (p<0.001) were observed in HFD fed obese rats as compared to control. Treatment of HFD fed rats with PPAR-γ agonist (RSG) showed a significant (p<0.001) decreased in serum insulin level and HOMA-IR. T2DM rats showed markedly increased HOMA-IR (<0.001) as compared with control and High fat diet fed obese rats, which significantly reduced (p<0.001) with the treatment of PPAR-γ agonist (RSG). (Table 2)

3.4. Hepatic Lipid Peroxidation in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

Hepatic tissue MDA levels were significantly ($p<0.001$) increased in HFD fed rats as compared with control. Treatment with PPAR-γ agonist (RSG) reduced hepatic tissue MDA levels significantly ($p<0.001$). T2DM (Diabetes mellitus) rats show markedly increased in hepatic tissues MDA level ($p<0.01$) as compared to HFD fed obese rats and decreased significantly ($p<0.001$) when treated with PPAR-γ agonist (RSG).

3.5. Hepatic Catalase activity in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

High fat diet fed obese rats showed nonsignificantly decreased hepatic tissues Catalase activity as compared with control (Figure 2). T2DM rats showed significant ($p<0.01$) decrease in Catalase activity as compared to HFD fed obese rats which markedly enhanced with the treatment of PPAR-γ agonist ($p<0.01$).

3.6. Hepatic Superoxide dismutase in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

SOD level in the hepatic tissues of experimental animals is depicted in Figure 3. High fat diet fed obese rats shows reduced level of hepatic SOD activity ($p<0.05$) as compared to control (Figure 3). Treatment with PPAR-γ agonist (RSG) shows significant ($p<0.001$) increased in SOD activity in the Liver. T2DM showed markedly decreased in hepatic SOD level as compared to control ($p<0.001$) as well as compared to HFD fed rats (0.01).

3.7. Serum Paraoxonase-1 activity in Liver Homogenate in Control, HFD, T2DM and treatment of PPAR-γ agonist (RSG) on HFD and HFD+Streptozotocin induced T2DM

High fat diet fed obese rats shows reduced level of serum PON-1 activity ($p<0.05$) as compared to control (Figure 4).Treatment with PPAR-γ agonist (RSG) showed non significant changes in PON-1 activity.T2DM showed decreased PON-1 activity which increased by the treatment of PPAR-γ agonist (RSG).

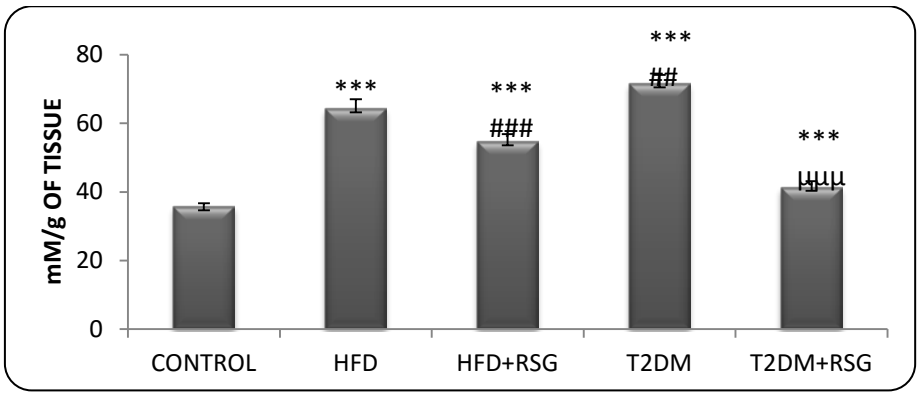

Figure 1: Effect of PPAR-γ agonist (RSG) treatment on hepatic Lipid peroxidation in high fat diet fed and Streptozotocin induced diabetic rats.

***P< 0.01 with Control

###P<0.001, ##P<0.01, with High Fat Diet,

µµµ P<0.001 with T2DM

n=8, values represents the mean ±SD.

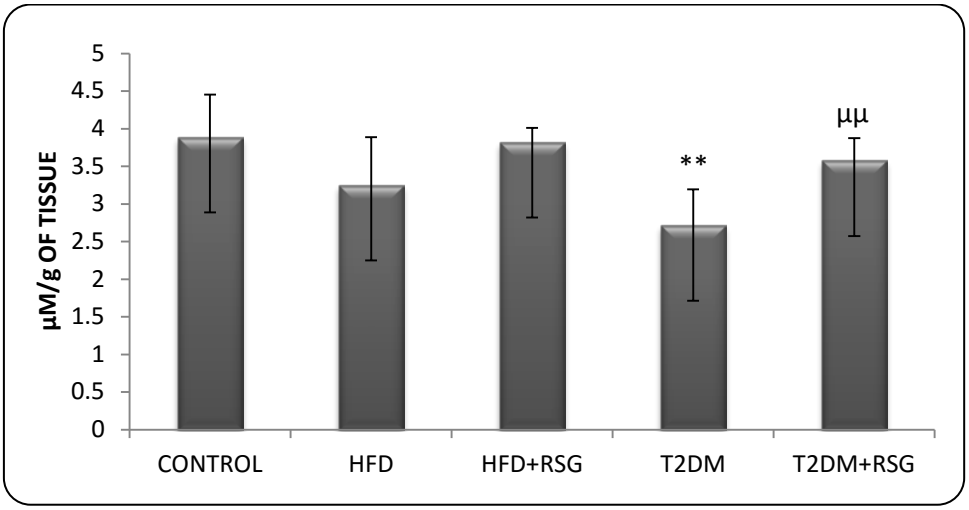

Figure 2: Effect of PPAR-γ agonist (RSG) treatment on hepatic Catalase activity in high fat diet fed and Streptozotocin induced diabetic rats.

**P< 0.01 with Control

µµP<0.01, with T2DM

n=8, values represents the mean ±SD.

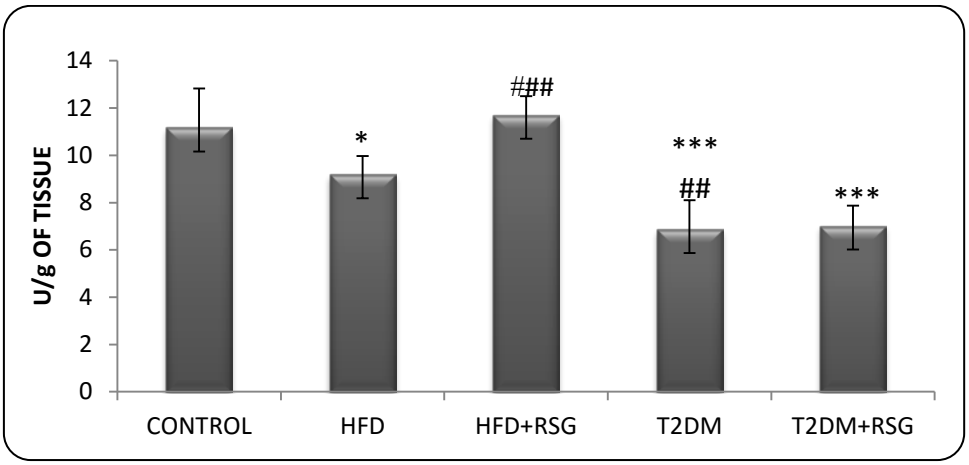

Figure 3: Effect of PPAR-γ agonist (RSG) treatment on hepatic Superoxide dismutase activity in high fat diet fed and Streptozotocin induced diabetic rats.

***P< 0.001, *P< 0.05 with Control

###P<0.001, ##P<0.01 with High Fat Diet

n=8, values represents the mean ±SD.

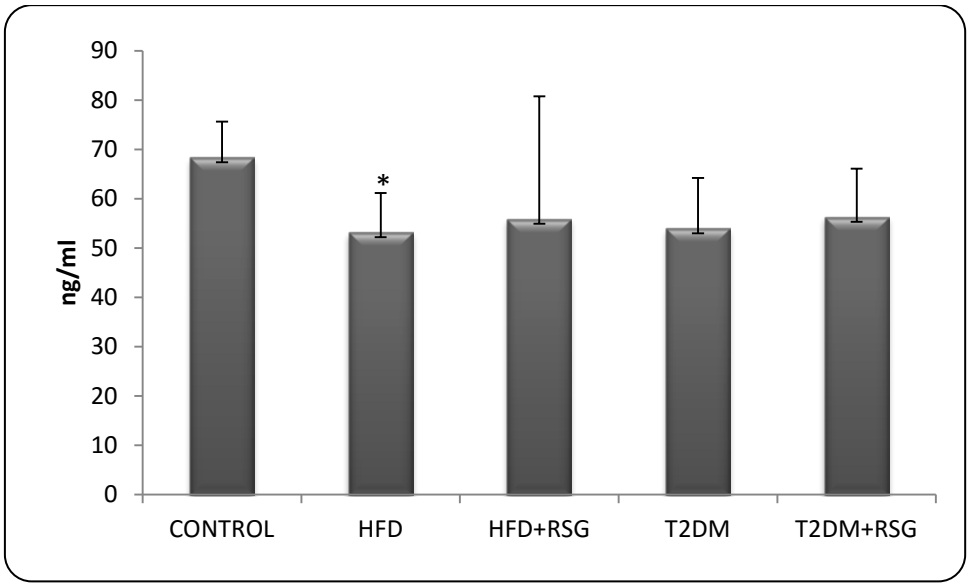

Figure 4: Effect of PPAR-γ agonist (RSG) treatment on serum Paraoxonase-1 activity in high fat diet fed and Streptozotocin induced diabetic rats.

*P< 0.05 with Control

n=8, values represents the mean ±SD.

4. Discussion

The present study has demonstrated an animal model of obese type 2 diabetes to investigate the effects of PPAR-γ agonist (Rosiglitazone) treatment on the alterations in oxidative enzymes and antioxidant markers due to obesity and obesity induced type 2 diabetes. High fat diet or calories rich diet and less physical activity is the significant cause of increasing obesity and type 2 Diabetes worldwide. High fat diet cause increased accumulation of lipid in visceral adipose tissues. It has been previously validated that liver and adipose tissues in collaboration maintain the glucose and lipid homeostasis by secreting several hormone and other factors [26-28]. Any disturbance in these tissues collaborations may cause insulin resistance and other metabolic disorders [29]. It has been reported that there is up-regulation of reactive oxygen species production and oxidative stress in liver and adipose tissue before the insulin resistance through discrete mechanism. [30]. Increase oxidation of free fatty acid can lead to increase reactive oxygen species production. In normal condition the antioxidant defense mechanism can alter these harmful changes, but in pathological condition the antioxidant defense system become weak and it cannot compete with the metabolic alterations hence, oxidative stress is initiated.

High fat diet leads to insulin resistance which is the major cause of diabetes type II. Hyperglycemia also triggers the oxidative stress related tissues damages which is characterized by unbalanced reactive oxygen species. Oxidative unbalanced in hepatic cells may cause structural damage to the hepatic tissues and may play a pivotal role in the genesis of the diabetic chronic liver disease.

This study demonstrates that high fat diet and high fat diet induced Type 2 diabetes remarkably increased glucose level, serum insulin level, HOMA-IR, BMI (Table 1 and Table 2) and production of Lipid peroxidation (Figure 1) along with the reduction of antioxidant enzymes activity like Catalase (Figure2), SOD (Figure 3) in liver and Paraoxonase-1 activity in Plasma (Figure 4). Rosiglitazone treatment led to improved Glycemic control, insulin resistance and BMI in both HFD and T2DM as showed in the Table 1 and Table 2. High fat diet increase the Lipid peroxidation as compare to control which decreased significantly after treatment with RSG. (Figure 1) It has been reported that , Rosiglitazone, a Peroxisome proliferator-activated receptor- gamma agonist (PPAR-γ agonist) is an anti-diabetic drug that have imperative role in the regulation of lipid and glucose metabolism, inflammatory, and vascular responses as describe in previous studies [31]. It significantly decreased blood glucose level in type 2 diabetes and also decreased serum insulin level and hence enhanced insulin sensitivity as shown in Table 2. RSG has potential anti-hyperglycemic activity that attenuates the oxidative damages cause by hyperglycemia in hepatic tissues. CAT is ubiquitously present in a wide range in all types of cells where it protect against peroxidation. Due to unbalanced glucose level there is increased generation of reactive oxygen species in the cell that may lead to decrease Catalase activity as shown in Figure 2 which reverse after the treatment of Rosiglitazone. While the activity of Superoxide dismutase is also decrease due to obesity and diabetes type II, which enhanced by the treatment of RSG as shown in Figure 3.

The increased in PON-1 activity was suggested to have increased protection from oxidation (Figure 4). The treatment with Peroxisomes proliferator activated receptor gamma agonist (Rosiglitazone) alters these oxidative enzymes and has potentially beneficial effects that may improved insulin sensitivity and protects the hepatic tissue from oxidative damages.

5. Conclusion

The findings of the study are of merit in revealing that PPAR-γ agonist has great potential to reverse the oxidative stress caused by obesity and type 2 diabetes mellitus induced by high fat diet in rats. The results have implications in the use of PPAR-γ agonists in human for protecting against High fat diet and type II diabetes induced hepatotoxicity. In future studies genetic evaluation are needed to demonstrate the efficacy of the Rosiglitazone for the prevention and treatment of T2DM. Greater understanding of the exact mechanism of this pleiotropism will open the way for new therapeutics for the prevention of diabetic complications.

References

[1]. B. E. Levin. “Factors promoting and ameliorating the development of obesity.” Physiol Behav, vol. 86, pp. 633–639, 2005.

[2]. A. H. Berg, P. E. Scherer. “Adipose tissue, inflammation, and cardiovascular disease.” Circ Res, vol.

96: pp. 939–949, 2005.

[3]. R. S. Ahima. “Adipose tissue as an endocrine organ.” Obesity, vol. 14, pp. 242-249, 2006.

[4]. H. E.Lebovitz and M. A. Banerji. “Treatment of insulin resistance in diabetes mellitus.” Eur J Pharmacol., vol.490, pp. 135–46, 2004.

[5]. O. Galili, D. Versari, K. J. Sattler et al,. “Early experimental obesity is associated with coronary endothelial dysfunction and oxidative stress.” American Journal of Physiology—Heart and Circulatory Physiology, vol. 292 (2), pp. H904–H911, 2007.

[6]. S. Cinti, G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, S. Wang, M. Fortier, A. S. Greenberg and M. S. Obin. “Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans.” J Lipid Res, vol. 46, pp. 2347-2355, 2005.

[7]. M. F. Gregor, L. Yang, E. Fabbrini, B. S. Mohammed, J. C. Eagon, G. S. Hotamisligil and S. Klein. “Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss.” Diabetes, vol.58, pp. 693-700, 2009.

[8]. S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda and I. Shimomura. “Increased oxidative stress in obesity and its impact on metabolic syndrome.” J Clin Invest. 114: 1752-1761, 2004.

[9]. D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher, R. C. Turner. “Homeostasis model assessment: insulin resistance and betacell function from fasting plasma glucose and insulin concentrations in man.” Diabetologia, vol. 28, pp. 412– 419, 1985.

[10]. W. Dröge. “Free radicals in the physiological control of cell function.” Physiol Rev, vol. 82:pp. 47–95, 2002.

[11]. L. G. Costa, A. Vitalone, T. B. Cole, C. E. Furlong. “Modulation of paraoxonase (PON1) activity.” Biochem Pharmacol, vol. 69, pp. 541–50, 2005.

[12]. A. Mertens, P. Verhamme, J. K. Bielicki et al. “Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis.” Circulation, vol. 107, pp. 1640–1646, 2003.

[13]. K. Begriche et al. “Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it.” Mitochondrion, vol. 6, pp. 1–28, 2006.

[14]. K. K. Naka, K. Papathanassiou, A.Bechlioulis et al. “Rosiglitazone improves endothelial function in patients with type 2 diabetes treated with insulin.” Diabetes and Vascular Disease Research. 8 (3): 195–201, 2011.

[15]. J. Tian., W. T. Wong, X. Y. Tian, P. Zhang, Y. Huang, and N. Wang. “Rosiglitazone attenuates endothelin-1-induced vasoconstriction by upregulating endothelial expression of endothelin b receptor.” Hypertension, vol. 56 (1), pp. 129– 135, 2010.

[16]. M. M. Khalifa, S. A. Mangoura, M. A. El-Moselhy and M. E. El-Daly. “The effectiveness of beta Adrenoreceptor Agonists on Progression of Insulin Resistance and type II Diabetes in High Fat Diet-Fed rats.” Saudi Pharmaceutical Journal, vol. 17 (1), pp. 19-28, 2009.

[17]. J. Luo, J. Quan, J. Tsai, C. K. Hobensack, C. Sullivan, R. Hector, G. M. Reaven. “Nongenetic mouse models of non-insulin-dependent diabetes mellitus.” Metabolism, vol.47, pp. 663–668, 1998.

[18]. S. Lenzen. “The mechanisms of alloxan- and streptozotocin-induced diabetes.” Diabetologia 51, 216-226, 2008.

[19]. T. A. Lutz and S. C. Woods. “Overview of animal models of obesity.” Curr Protoc Pharmacol, Chapter 5, Unit 5.61, 2012.

[20]. L. L. Bernardis. “Prediction of carcass fat, water and lean body mass from Lee’s nutritive ratio in rats with hypothalamic obesity.”Experientia, vol. 26, pp. 789–90, 1970.

[21]. W. G. Reaves “Insulin antibody determination in theoretical and practical considerations.” Diabetologia, vol. 24, pp. 399−403, 1983.

[22]. M. S. Khan, M. K. A. Khan, M. H. Siddiqui, J. M. Arif. “An in vivo and in silico approach to elucidate the Tocotrienol-mediated fortification against infection and inflammation induced alterations in antioxidant defense system.” European Review for Medical and Pharmacological Sciences, vol. 15, pp. 916-930, 2011.

[23]. H. Ohkawa, N. Ohishi, K. Yagi. “Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.” Anal Biochem, vol. 95, pp. 351-358,1979.

[24]. K. A. Sinha. “Colorimetric assay of Catalase.” Anal Biocehm, vol. 47, pp. 389-394, 1972.

[25]. Y. Kono. “Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase.” Arch Biochem Biophys, vol.186, pp.189-195, 1978.

[26]. T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, et al. “Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase.” Nat Med, vol. 8, pp. 1288–1295, 2002.

[27]. M. Watanabe, S. M. Houten, C. Mataki, M. A. Christoffolete, B. W. Kim, H. Sato, N. Messaddeq, J. W. Harney, O. Ezaki, T. Kodama, K. Schoonjans, A. C. Bianco, J. Auwerx. “Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation.” Nature, vol.439, pp.484–489, 2006.

[28]. K. Uno, H. Katagiri, T. Yamada, Y. Ishigaki, T. Ogihara, J. Imai, Y. Hasegawa, J. Gao, K. Kaneko, H. Iwasaki, H. Ishihara, H. Sasano, K. Inukai, H. Mizuguchi, T. Asano, M. Shiota, M. Nakazato, Y. Oka. “Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity.” Report. Science, vol. 312 (5780), pp. 1656-1659, 2006.

[29]. M. A. Herman., B. B. Kahn. “Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony.” J Clin Invest, vol. 116, pp. 1767–1775, 2006.

[30]. N. Matsuzawa-Nagata , T. Takamura , H. Ando, S. Nakamura, S. Kurita, H. Misu, T. Ota, M. Yokoyama, M. Honda, K. Miyamoto, S. Kaneko. “Increased oxidative stress precedes the onset of high-fat diet–induced insulin resistance and obesity”. Metabolism, vol. 57 (8), pp. 1071–1077, 2008.

[31]. A. Rull, B. Geeraert, G. Aragonès, R. Beltrán-Debón, E. Rodríguez-Gallego, A. García-Heredia, J. Pedro-Botet, J. Joven, P. Holvoet, and J. Camps. “Rosiglitazone and Fenofibrate Exacerbate Liver Steatosis in a Mouse Model of Obesity and Hyperlipidemia. A Transcriptomic and Metabolomic Study.” J. Proteome Res, vol.13 (3), pp. 1731–1743, 2014.

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