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A Study of The Cardio-Protective Effect of Pioglitazone on Isoprenaline- Induced Myocardial Infarction in Male Albino Rats

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ABSTRACT

Background: Isoprenaline (ISO) in large doses induces morphological and functional alterations in the heart leading to myocardial necrosis. It also produces excessive free radicals resulting from oxidative metabolism of catecholamine. There are increasing evidences that cardiotoxicity of ISO occurs because of generation of free radicals and oxidative stress.

Objective: To investigate the protective effect of pioglitazone on the outcome of isoprenaline (ISO) induced myocardial infarct-like lesions in rats.

Materials and methods: 50 male Wistar albino rats (150-200gm) were selected for this study. The rats were divided into five groups each consisted of 10 rats. This study was conducted at Department of Pharmacology, Faculty of Medicine, Al-Azhar University (Assiut).

Results: Results showed that pretreatment with pioglitazone significantly decreased the activity of LDH, CK, TNF alpha, IL 6 and the levels of CTnI in serum of ISO-induced rats. Our results showed that pretreatment with pioglitazone significantly ($p<0.01$) decreased the level of MDA compared with ISO alone-induced rats.

Conclusion: Pioglitazone has a valuable protective role against myocardial infarction through its action as peroxisome proliferator activated receptor gamma (PPAR-γ) agonist.

Keywords: Pioglitazone, Isoprenaline, Myocardial infarction, Male albino rats.

INTRODUCTION

Despite great efforts during the last decades, cardiovascular diseases (CVDs) remain the major cause of death worldwide, increasing their prevalence every year [(1)]. Myocardial infarction (MI) is a cardiovascular disease that occurs when the blood supply to a part of the heart is interrupted, causing death to the heart tissue. It is a complex phenomenon affecting the mechanical, electrical, structural, and biochemical properties of the heart [(2)]. Rates of death from ischemic heart disease have declined in most high income countries, although cardiovascular disease still accounted for 1 in 3 of all deaths in the USA in 2013 [(3)]. Ischemic heart disease is becoming a more common cause of death in the developing world. For example in India, ischemic heart disease had become the leading cause of death by 2004 accounting for 1.46 million deaths (14% of total deaths) and deaths due to ischemic heart disease were expected to double during 2005–2015 [(4)].

ISO, a β adrenergic agonist, has been reported to induce infarct-like lesions in rats and other animal species [(5)]. It has been shown to exhibit many metabolic and morphological aberrations in the heart tissue of experimental animals similar to those seen in humans with myocardial infarction [(6)]. Injected isoprenaline undergoes auto-oxidation resulting in the generation of free radicals that stimulate lipid peroxidation, which causes destruction and damage to the myocardial cell membrane. Inflammation is a key process involved in mediating myocardial tissue damage through the release of proteolytic enzymes [(7)].

PPAR-γ plays an important role in regulation of adipocyte differentiation and insulin resistance. In addition, recent large clinical studies have demonstrated that a Peroxisome PPAR-γ agonist had beneficial effects not only on glycemic control but also in preventing atherosclerotic disease [(8)]. Increasing evidence has demonstrated that PPAR-γ plays important roles in the immune system, since PPAR-γ is expressed in inflammatory cells such as macrophages, T cells, B cells, and dendritic cells [(9)].

Pioglitazone is a thiazolidinedione with PPAR-γ agonist activity. It was proved that treatment with pioglitazone therapy resulted in a significantly greater reduction in blood glucose level. It can improve hypertriglyceridemia and hypercholesterolemia [(10)]. Additionally, it plays an important role in suppression of inflammation in intestinal ischemia as a PPAR-γ agonist. Pioglitazone (10 mg/kg) is effective in reducing ventricular arrhythmias, creatine kinase-MB release and restoring energy production [(11)].

Bisphenol, A diglycidyl ether (BADGE), is a synthetic substance used in the production of polycarbonate and industrial plastics. Competition-radioligand-binding studies showed this compound to be a ligand for PPAR-γ with micromolar affinity. BADGE has been reported to act as PPAR-γ antagonists [(12)].

The aim of the present study was to evaluate the protective effects of pioglitazone on cardiac marker enzymes [creatine kinase (CK) and lactate dehydrogenase (LDH)], troponin-I, inflammatory markers (IL-6 and TNF-alpha) and antioxidant

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parameters (MDA, SOD and GSH) in isoprenaline-induced myocardial infarcted rats.

MATERIALS AND METHODS

Drugs and chemicals: Isoprenaline (ISO), Pioglitazone, Bisphenol A diglycidyl ether (BADGE)

Animals: 50 male Wistar albino rats (150-200 gm) were selected for this study. This study was conducted at Department of Pharmacology, Faculty of Medicine, Al-Azhar University (Assiut). The rats were divided into five groups each one consisted of 10 rats:

Group I (Control group) received no medication and was given free access to food and water.

Group II (pioglitazone group) received pioglitazone orally (10 mg/kg) once daily for 5 days [(11)].

Group III (Isoprenaline group) received subcutaneous injection of isoprenaline hydrochloride dissolved in saline (100 mg/kg s.c) once daily for two successive days to induce infarction [(13)].

Group IV (Isoprenaline + pioglitazone group) received pioglitazone (10 mg/kg) once daily for 5 days and isoprenaline (100 mg/kg s.c) once daily after 1 h of pioglitazone administration on the last day of the treatment period and the next day [(13)].

Group V (Isoprenaline + Pioglitazone + BADGE group): This group was treated by PPAR-γ antagonist and BADGE. It was dissolved in minimal volume of ethanol, diluted with saline and injected intraperitoneally (i.p) at a dose of 30 mg/kg given 30 min before pioglitazone injection (10 mg/kg orally) once daily for 5 days. For induction of infarct like lesion, rats received isoprenaline (100 mg/kg s.c) after 1 h of pioglitazone administration on the last day of the treatment period and the next day [(13)].

Procedures:

1-Induction of myocardial infarction (MI): It is now well recognized that ISO in large dose produces MI. ISO, a β-adrenergic agonist, causes oxidative stress in the myocardium resulting in gross and microscopic infarction in rat's heart muscle. It has been reported that ISO produces free radicals and stimulates lipid peroxidation, which is a causative factor for irreversible damage to the myocardial membrane. Isoprenaline (100 mg/kg) dissolved in saline was subcutaneously injected to rats at an interval of 24 hrs for 2 days. ISO-induced MI was confirmed by elevated levels of serum creatine kinase (CK), troponin-I, serum aspartate transaminase (AST) and lactate dehydrogenase (LDH) in rats [(13)].

2- Drug administration: Pioglitazone received orally 10 mg / kg once daily for 5 days [(11)].

3- Collection of samples: At the end of the experimental period, after 12 hrs of second ISO injection overnight- fasted rats were anaesthetized with urethane (1.5 g/kg i.p.) for ECG monitoring [(14)]. Thereafter, blood samples were collected via cardiac puncture for serum separation and estimation of troponin, cardiac marker enzymes such as serum level of creatine kinase (CPK) and lactate dehydrogenase (LDH) as well as inflammatory markers such as tumor necrosis factor- α (TNF-α) and interleukin-6 (IL-6). The rats were then sacrificed by decapitation and their hearts were rapidly isolated. The injury extension was measured in the excised hearts. Portions of the heart tissues were used to determine the oxidative stress as malondialdehyde (MDA) and antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSH).

ECG monitoring:

The anesthetized rats were placed in the supine position on a board and ECG was recorded continuously with standard artifact free lead II (right forelimb to left hind limb). Needle electrodes were inserted subcutaneously into the paw pads of each rat, and connected to Biocare ECG 101 (Shenzhen Biocare Electronics Co., Ltd., China). The ECG was measured to determine the duration and amplitude of the P wave, the QRS complex, and the ST segment alterations [(13)].

Measurement of extension of cardiac injury:

The excised beating heart was submerged in cold (8^{o}C) 30 mmol KCl to achieve diastolic arrest. The right ventricle and both atria were excised to isolate the left ventricle (the septum and free wall). The left ventricle was then sectioned by a sharp surgical scissor into transverse slices, each of about 1.5 mm thickness. The slices were stained in a 1.5% triphenyltetrazolium chloride (TTC) (MP biomedical, France) in phosphate buffer, PH 7.4, for 10-15 minutes at 37^{o}C. The TTC stain formed red color precipitates in the presence of an intact dehydrogenase enzyme system. Areas of necrosis lost dehydrogenase activity and therefore failed to stain **(Sharma and Singh 2000; Vivaldi *et al.* 1985).** The slices were washed with saline and then clear glass plates were placed over both sides of each slice. Epicardial and endocardial outlines as well as the TTC stained and non- stained areas were traced on clear plastic sheets. The plastic sheets were then fixed on an E.C.G paper and the small squares occupying the stained and non-stained areas were counted giving each in mm^2. The sum of the stained and the non-stained areas give the surface area of the whole heart slices. The surface area of the whole left ventricle was calculated by adding the surface areas of all cardiac sections measured on E.C.G paper. The surface area of the injured tissue of the whole heart was obtained by adding the surface area of the injured tissue in all cardiac slices and the injury extension was calculated as percentage of the sum of the injured areas to the sum of surface areas of all the slices [(15)].

Biochemical analysis

The serum was separated by centrifugation (5000 rpm for 5 min) and used for biochemical analysis. Cardiac marker enzymes such as CPK and LDH were detected using Stanbio CK-MB diagnostic kit (USA).

In addition inflammatory markers such as TNF-α and IL-6 were determined by the ELISA technique using standard kits (Ray Biotech, Inc., USA). The sensitivities of the methods as stated in the instructions of enzyme immunoassay kits are 12 pg/ml for IL-6 and 11.2 pg/ml for TNFα. The intraassay coefficient of variation for the measurements was < 5%. Portions of the heart tissue were homogenized in a saline solution (0.9%) and centrifuged at 3000 rpm for 15 min, the supernatant was kept at – 20 ºC and used to determine the oxidative stress as level of MDA [16] and antioxidant enzymes such as SOD and GSH [17].

Biochemical measurements:

1-Assay of cardiac marker enzymes:

a- Estimation of serum lactate dehydrogenase (LDH).

b- Estimation of serum creatine kinase.

c- Estimation of serum Troponin-I (CTnI).

2- Estimation of the level of reduced glutathione (GSH) in the heart.

3- Estimation of the level of superoxide dismutase (SOD) in the heart.

4- Estimation of the level of malondialdehyde (MDA) in the heart:

5- Estimation of the level of inflammatory markers.

Ethical consent:

An approval of the study was obtained from Al-Azhar University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean ± standard error (S.E). Statistical analysis of the difference between groups was performed by using One –way analysis of variance (ANOVA) followed by Tukey-Kramer test for differences between means. P value < 0.05 was considered significant.

RESULTS

The levels of cardiac enzymes (CPK and LDH) in the plasma showed a significant rise (P<0.05) in the ISO group, ISO + PIO group and ISO + PIO + BADGE group compared to the control group. Pretreatment with pioglitazone significantly reduced the cardiac enzymes to near normal values. On the other hand, BADGE pretreatment in the rats that received pioglitazone and subjected to infarct-like lesion significantly increased (P<0.05) the plasma levels of cardiac enzymes that still showed significant reduction (P<0.05) as compared to the isoprenaline group (Table 1).

Table (1): Effect of pioglitazone on the activity of (CPK and LDH) associated with isoprenaline-induced infarct-like lesion in rats and the role of PPAR-ɣ

	Control group	**Pioglita-zone group**	**ISO group**	**ISO + Pioglita-zone group**	**ISO + Pioglita-zone + BADGE group**
CPK (U/l)	681.4 ± 2.9	679.5 ± 3.2	1011.3 ±7.3*	743.3 ± 9.3*†	983.1 ± 4.0*† ‡
LDH (U/l)	2156 ± 5.2	2160 ± 7.1	8234 ± 3.9*	3642 ± 4.6*†	7454 ± 1.9*† ‡

Data is expressed as mean ± standard deviation (n = 10 *per* group). P < 0.05 is significant tested by using One-way analysis of variance (ANOVA) and Post Hoc multiple comparisons (LSD). *P< 0.05 *vs.* control group; †P < 0.05 *vs*. ISO group; ‡ P < 0.05 vs. ISO+ Pioglitazone group.

As regards pro-inflammatory cytokines, TNF-α and IL-6 were increased significantly (P<0.05) in the ISO group, ISO + PIO group and ISO + PIO + BADGE group as compared to the control group. However, these cytokines showed significant reduction in the ISO-injected rats pretreated with pioglitazone (P<0.05) compared to the ISO group. Furthermore, the pretreatment with BADGE in the rats that received pioglitazone and subjected to infarct-like lesion produced significant elevation (P<0.05) of pro-inflammatory cytokines that still revealed significant reduction (P<0.05) as compared to isoprenaline group (Table 2).

Table (2): Effect of pioglitazone on the activity of pro-inflammatory cytokines (TNF-α and IL-6) associated with isoprenaline-induced infarct-like lesion in rats and the role of PPAR-ɣ

	Control group	**Pioglitazone group**	**ISO group**	**ISO + Pioglita-zone group**	**ISO + Pioglita-zone + BADGE group**
TNF-α (pg/ml)	80.4 ± 0.43	79.6 ± 0.52	132.0 ± 0.12*	91.1 ± 0.90*†	124.5 ± 0.41*† ‡
IL-6 (pg/ml)	41.5 ± 0.57	39.8 ± 0.78	126.4 ± 0.61*	57.0 ± 0.24*†	119.2 ± 0.74*† ‡

Data is expressed as mean ± standard deviation (n = 10 *per* group). P < 0.05 is significant tested by using One-way analysis of variance (ANOVA) and Post Hoc multiple comparisons (LSD). *P< 0.05 *vs.* control group; †P < 0.05 *vs*. ISO group; ‡ P < 0.05 vs. ISO+ Pioglitazone group.

As regards levels of cTnI in serum, it was increased significantly (P<0.05) in the ISO group, ISO + PIO group and ISO + PIO + BADGE group as compared to

the control group. However, these levels showed significant reduction in the ISO-injected rats pretreated with pioglitazone (P<0.05) compared to the ISO group. Furthermore, the pretreatment with BADGE in the rats that received pioglitazone and subjected to infarct-like lesion showed significant elevation (P<0.05) of levels of cTnI that still revealing significant reduction (P<0.05) as compared to isoprenaline group (Table 3).

Table (3): Effect of pioglitazone on the level of cardiac troponin-I (cTnI) associated with isoprenaline-induced infarct-like lesion in rats and the role of PPAR-ɣ

	Control group	**Pioglita-zone group**	**ISO group**	**ISO + Pioglita-zone group**	**ISO + Pioglita-zone + BADGE group**
Troponin I	0.158± 0.027	0.149± 0.016	2.35± 0.015*	0.86± 0.058*†	1.96± 0.067*†‡

Data is expressed as mean ± standard deviation (n = 10 *per* group). P < 0.05 is significant tested by using One-way analysis of variance (ANOVA) and Post Hoc multiple comparisons (LSD). *P< 0.05 *vs.* control group; †P < 0.05 *vs*. ISO group; ‡ P < 0.05 vs. ISO+ Pioglitazone group.

Similarly, cardiac MDAwas significantly increased (P<0.05) with a parallel significant decrease in SOD and GSH content (P<0.05) in the ISO group, ISO + PIO group and ISO + PIO + BADGE group as compared to the normal rats. Pretreatment with pioglitazone resulted in significant decrease in MDA (P<0.05) and a significant increase in SOD and GSH content (P<0.05) as compared to the isoprenaline group. Similar to other parameters, the BADGE pretreatment abolished the protective effect of pioglitazone in rats subjected to infarct-like lesion. There was a significant decrease (P<0.05) in cardiac MDA, while there was a significant increase (P<0.05) in SOD content and a non-significant increase (P>0.05) in GSH content comparing the ISO + PIO + BADGE group to the ISO group (Table 4).

Table (4) Effect of pioglitazone on the activity of MDA, SOD and GSH associated with isoprenaline-induced infarct-like lesion in rats and the role of PPAR-ɣ

	Control group	**Pioglita-zone group**	**ISO group**	**ISO + Pioglit a-zone group**	**ISO + Pioglita-zone + BADGE group**
MDA (nmol/g tissue)	115.1 ± 1.3	113.8 ± 3.9	157.8 ± 6.5*	126.5 ± 7.1*†	149.9 ± 5.2*†‡
SOD (IU/mg protein)	7.4 ± 0.93	7.0 ± 0.49	3.1 ± 0.12*	6.2 ± 0.61*†	3.6 ± 0.40*†‡
GSH (IU/mg protein)	4.6 ± 0.82	4.8 ± 0.63	1.2 ± 0.60*	3.5 ± 0.34*†	1.7 ± 0.57*‡

Data is expressed as mean ± standard deviation (n = 10 *per* group). P < 0.05 is significant tested by using One-way analysis of variance (ANOVA) and Post Hoc multiple comparisons (LSD). *P< 0.05 *vs.* control group; †P < 0.05 *vs*. ISO group; ‡ P < 0.05 vs. ISO+ Pioglitazone group.

Pioglitazone pretreatment significantly decreased the injury extension (P<0.05) as compared to the isoprenaline group. Moreover, BADGE pretreatment in rats receiving pioglitazone and undergone infarct-like lesion significantly increased the injury extension (P<0.05) that was still reduced (P<0.05) as compared to isoprenaline group. Isoprenaline injection induced infarct-like lesion represented by ST segment elevation, and a decrease in R wave amplitude as compared to the normal group. Pretreatment with pioglitazone resulted in a reduction in the ST segment elevation with an increase in R wave amplitude as compared to the isoprenaline group. The BADGE pretreatment abolished the protective effect of pioglitazone in rats subjected to infarct-like lesion (Table 5).

Table (5) Effect of pioglitazone on extension of cardiac injury and ECG parameters in isoprenaline-induced infarct-like lesion in rats and the role of PPAR-ɣ

	Control group	**Pioglita-zone group**	**ISO group**	**ISO + Pioglita-zone group**	**ISO + Pioglita-zone + BADGE group**
Infarction size (% LV)	0.0	0.0	36.4 ± 2.7*	19.3 ± 3.1*†	28.8 ± 4.3*†‡
ST elevation	Normal ECG	Normal ECG	5.3± 1.02mm pathological Qwave	1.15± 0.411mm	3.9± 0.94mm

Data is expressed as mean ± standard deviation (n = 10 *per* group). P < 0.05 is significant tested by using One-way analysis of variance (ANOVA) and Post Hoc multiple comparisons (LSD). *P< 0.05 *vs.* control group; †P < 0.05 *vs*. ISO group; ‡ P < 0.05 vs. ISO+ Pioglitazone group.

DISCUSSION

Serum CK, and LDH are well known markers of myocardial infarction. When myocardial cells are damaged or destroyed due to deficient oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture, which results in leakage of enzymes. These enzymes enter into the blood stream thus increasing their concentration in the serum **(18)**.

The present study revealed that ISO treatment results in a marked elevation in the levels of serum cardiac marker enzymes including LDH and CK.

Cardiac-specific troponins accurately distinguish skeletal from cardiac muscle damage. The troponins are now considered the preferred biomarker for diagnosing MI. Elevated troponin levels predict the risk of both cardiac death and subsequent infarction [19]. In our study we observed increased levels of cardiac troponin (CTnI) in serum of ISO-treated rats. Results of the present study are in line with those reported by **Chikku and Rajamohan** [20]. They found that ISO-treated rats showed significant ($P<0.05$) increased activity of LDH, CK, AST, ALT and increased concentration of cardiac troponins in the serum compared to normal control rats. **Kumaran and Prince** [21] showed that the activity of serum CK was considerably ($P<0.05$) increased in ISO-treated rats compared to normal control rats. The increased activity of this enzyme in serum might be due to ISO-induced myocardial necrosis. They also reported that Rats treated with ISO showed considerable ($P<0.05$) elevation in the levels of serum cTnI compared to normal control rats. The observed increased levels of cardiac troponin I might be due to ISO-induced cardiac damage. **El-Gohary and Allam** [13] found that ISO-treated rats showed significant ($P<0.05$) increased activity of LDH, CK, IL 6 and TNF alpha.

Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ) plays an important role in regulation of adipocyte differentiation and insulin resistance [22]. In addition, recent large clinical studies have demonstrated that PPAR-γ agonist has beneficial effects not only on glycemic control but also in preventing atherosclerotic disease [23]. Increasing evidence has demonstrated that PPAR-γ plays important roles in the immune system, since PPAR-γ is expressed in inflammatory cells such as macrophages, T cells, B cells, and dendritic cells [24]**.** We found that PPAR-γ activation lead to cardioprotective effect, and this result is in agreement with results obtained by [13]**.** In our study, results showed that pretreatment with pioglitazone significantly decreased the activity of LDH, CK TNF alpha and IL 6 in serum of ISO induced rats. These results are in agreement with the results obtained by other researchers e.g. **El-Gohary and Allam** [13] showed that activities of LDH, CK TNF alpha and IL 6 in serum decreased in pioglitazone-pretreated ISO-induced rats probably due to the protective effect of pioglitazone on the myocardium as a PPAR gamma agonist, which reduced the extent of myocardial damage induced by ISO and thereby restricting the leakage of these enzymes from myocardium.

Pretreatment with pioglitazone also decreased the size of infarction and this could be due to the reduction of the degree of damage in the myocardium by pioglitazone thereby preventing leakage of myocardial enzymes and inflammatory markers. In our study results showed that pretreatment with pioglitazone significantly decreased the activity of LDH, CK TNF alpha and IL 6 and the levels of CTnI in serum of ISO-treated rats.

Lipid peroxidation, a type of oxidative deterioration of polyunsaturated fatty acids, has been linked with altered membrane structure and enzyme inactivation. Lipid peroxidation is associated with a variety of chronic health problems, such as cancer, ageing and atherosclerosis [25]**.** The results of our study showed that ISO treatment resulted in an increase in the levels of lipid peroxidation products e.g. malondialdehhyde (MDA) in the heart. Results of the present study are in line with those reported by **Chikku and Rajamohan** [20]. They found that ISO administration is associated with increased levels of lipid peroxidation as evidenced by increased levels of MDA in the heart. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage and this leads to oxidative damage of cell components like proteins, lipids and nucleic acids.

Our results showed that pretreatment with pioglitazone significantly ($p<0.01$) decreased the level of MDA compared to ISO alone-treated rats. Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The equilibrium between antioxidants and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like myocardial infarction, the generation of reactive oxygen species can dramatically upset this balance with an increased demand on the antioxidant defense system. Free radical scavenging enzymes such as superoxide dismutase (SOD) and glutathione reductase (GRd) are the first line of cellular defense against oxidative injury. These enzymes are lowered due to enhanced lipid peroxidation [19]**.** Superoxide radicals generated at the site of damage in myocardial infarction modulates superoxide dismutase resulting in the lowered activities of the enzyme and accumulation of superoxide anion, which also damages the myocardium [19]**.**

In the present study, isoproterenol injection resulted in significant alterations in ECG patterns such as ST segment elevation coupled with marked decrease in R wave amplitude that reflect isoprenaline-induced infarct-like lesion. ECG pattern alterations by isoprenaline were previously demonstrated by other investigators [26, 27]**.** In addition to the measurement of injury extension, ECG abnormalities were the main criteria used for the diagnosis of infarct-like lesion. These alterations could be due to the consecutive loss of cell membrane potential in the injured myocardium as a result of oxidative stress [28]**.** It was reported that ST elevation correlates well with the leak of creatine kinase from the myocardium [29]. Administration of pioglitazone for 5 days before induction of infarct-like lesion resulted in amelioration of cardiac injury specifically there was improvement in ECG

parameters. These findings are in agreement with the results obtained by **El-Gohary and Allam [13].** Isoproterenol injection was seen to result in significant alterations in ECG patterns such as ST segment elevation coupled with marked decrease in R wave amplitude that reflect isoprenaline-induced infarct-like lesion, and administration of PPAR gamma agonist decrease the cardiac injury. Administration of pioglitazone for 5 days before induction of infarct-like lesion resulted in amelioration of cardiac injury. Specifically, there was improvement in cardiac injury extension of ISO-injected rats pretreated with pioglitazone. These findings are in line with a recent study of **El-Gohary and Allam [13]** and **Abood and Elshal [14]**, who reported a cardio-protective effect against MI through PPAR gamma stimulation based on amelioration of cardiac injury

In our study we found that pharmacological inhibition of PPAR- ɣ avtivation using BADGE led to elevation of cardiac enzymes, inflammatory markers, alteration of ECG parameters and increasing of cardiac injury extension. These results are in agreement with the results of **El-Gohary and Allam [13]** who found that pharmacologic inhibition of PPAR- ɣ using BADGE abolished PPAR- ɣ agonist-mediated cardioprotection, supporting the involvement of PPAR- ɣ in protective action of PPAR- ɣ agonists.

There are previous studies that reported the protective action of pioglitazone on the heart **[11].** To the best of our knowledge, our study reports for pioglitazone that essentially involves PPAR- ɣ activation for its cardioprotective effect, as pharmacologic inhibition of PPAR- ɣ using BADGE abolished pioglitazone-mediated cardioprotection, supporting the involvement of PPAR- ɣ in protective action of pioglitazone. To explain the protective effect of pioglitazone on isoprenaline-induced infarct-like lesion we measured plasma TNF-α and IL-6, as markers for inflammation, together with cardiac MDA, SOD and GSH content, as markers for the oxidative state. The down regulation of inflammatory cytokines demonstrated by pioglitazone administration in ISO-injected rats denotes that pioglitazone exerts its protective effect, at least in part, by an anti-inflammatory action. The reduction of inflammatory cytokines as evidenced in our study has previously been reported by **El-Gohary and Allam [13]** and **Arnson *et al.* [30].**

We revealed that pioglitazone treatment reduced ISO-induced infarct-like lesion through PPAR- ɣ activation. The PPAR- ɣ agonists are well documented to protect against IRI in various tissues through down-regulation of molecular pathways like nuclear factor kappabeta, thromboxane synthase, monocyte chemoattractant protein-1, inducible nitric oxide synthase, and fibronectin **[31].** Moreover, the PPAR- ɣ agonists are proposed to inhibit Jun N-terminal kinase phosphorylation that belongs to mitogen-activated protein kinase and production of endothelin-1 that are produced in response to stress stimuli including cytokines and are responsible for cellular apoptosis and damage **[32].** There are previous studies that reported the protective action of PPAR- ɣ agonist on the heart **[14, 33].**

To the best of our knowledge, our study reports for the first time that pioglitazone essentially involved PPAR- ɣ activation (for its cardioprotective effect, as pharmacologic inhibition of PPAR- ɣ using BADGE) abolished pioglitazone-mediated cardio protection, supporting the involvement of PPAR- ɣ in protective action of pioglitazone.

CONCLUSION

Pioglitazone has a valuable protective role against myocardial infarction through its action as PPAR-gama agonist.

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