ISSN: 2754-4516

Journal of Medicine and Healthcare

SCIENTIFIC Research and Community

Research Article

Open Access

Possible Role of Ppar-Alpha Agonist in Attenuated Cardioprotective Effects of Ischemic Postconditioning in Diabetic Rat Heart

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ABSTRACT

Background: It has been accounted for that infarct size reduction and cardioprotective effects of Ischemic Postconditioning (IPOC) have been abrogated in few pathological conditions including diabetes. Peroxisome Proliferator-Activated Receptor-Alpha (PPAR-Alpha) agonist is known to have cardioprotective effect. Therefore, the current examination researched the possible role of PPAR-Alpha Agonist in Attenuated Cardioprotective Effects of Ischemic Postconditioning in Diabetic Rat Heart.

Materials and Methods: Rats were injected Alloxan Monohydrate (150/mg/kg/i.p) single dose to produced diabetes. Isolated Langendorff's perfused normal and diabetic rat hearts were subjected to global ischemia for 30 min followed by reperfusion for 120 min. Coronary effluent was analyzed for Lactatedehydogenase (LDH) and Creatine Kinase (CK) release to evaluate the extent of cardiac injury. The oxidative stress in heart was evaluated by measuring Thiobarbituric Acid Reactive Substances (TBARS), superoxide dismutase (SOD) generation and reduced form of glutathione.

Result: In the current investigation, Ischemia-reperfusion (I/R) induced oxidative stress by increasing TBARS, superoxide anion generation and the decreased form of glutathione in normal and diabetic rat heart. Moreover, I/R induced myocardial injury, was evaluated in terms of increase in, LDH and CK release in coronary effluent, and reduction in coronary flow rate in normal and diabetic rat heart. The diabetic rat heart showed enhanced I/R induced myocardial injury with high extent of oxidative stress as compared with normal rat heart subjected to I/R Six episodes of IPOC afforded cardioprotection against I/R induced myocardial injury in normal rat heart as assessed in terms of improvement of coronary flow rate and decrease of LDH, CK and oxidative stress. On other hand, IPOC mediated myocardial protection against I/R injury was nullified in diabetic rat heart. Fenofibrate (5μM), a selective agonist of PPAR alpha, its administration markedly restored the Cardioprotective potential of IPOC in diabetic rat heart.

Conclusion: The current investigation presumed that, the high degree of oxidative stress produced in diabetic rat heart during reperfusion and resulting inactivation of PPAR alpha receptor might be responsible for abolishing the Cardioprotective potential of IPOC against I/R induced myocardial injury.

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Received: May 23, 2022; **Accepted:** June 07, 2022; **Published:** June 10, 2022

Keywords: Diabetes, Ischemic Postconditioning, Ischemia Reperfusion Injury, PPAR Alpha

Introduction

Cardiovascular disease (CVD) is the main source of death comprehensively, representing 17.8 million passing for each year [1-3]. Myocardium ischemic harm is the vast majority of the causing of incapacity and passing in edified around the world [4]. Myocardial Ischemia is a condition in which restriction in blood supply to heart tissue which results in decrease oxygen and nutrient supply that is essential to keep tissue alive. The reperfusion of coronary blood flow is essential to avoid the myocardial damage in an ischemic myocardium condition. Ischemia-reperfusion (I/R) injury is the reperfusion of ischemic myocardium that is frequently followed harmful changes in myocardial tissues [5, 6]. Reperfusion therapy in myocardial ischemia leads to myocardial I/R injury followed by excessive reactive oxygen species (ROS) generation, inflammatory reactions, mitochondrial dysfunction and cell death [7]. It is noted that various cellular mechanisms in I/R injury like involved calcium overload, oxidative and nitrosative stress, mitochondrial dysfunction, activation of protein kinases, Adenosine Triphosphate (ATP) depletion, Mitochondrial Permeability Transition Pore (MPTP) opening, activation of Mitogen Activated Protein Kinases (MAPK) and Janus Kinase (JNK), down regulation of PPAR alpha [4, 8-11]. Ischemic Postconditioning (IPOC) was first proposed in 2003 by Vinten-Johansen's group as an endeavor to offer an intercession which could be applied at the time of myocardial reperfusion to ensure patients determined to have an AMI (Acute Myocardial Injury). IPOC is episodes of brief ischemia and reperfusion cycles that are applied instantly at the area of the ischemic organ after ischemia and onset of reperfusion that is results in reduced infarction in myocardial ischemia [12]. IPOC effectively reduces the neural

cell death or apoptosis, cerebral edema and decrease infarct size, improve cerebral circulation, relieve inflammation, oxidative stress and attenuating the mitochondrial calcium overload [13, 14]. IPOC has been noted to diminish I/R-induced myocardial injury by activation of phosphatidyl inositol-3 kinase/protein kinase B (PI3K/Akt) and endothelial nitric oxide synthase (eNOS) and consequent release of nitric oxide (NO) have been suggested to be implicated in the cardioprotective effect of IPOC [14]. Various cellular pathways and molecular pathways that are involved in cardioprotective effects of IPOC include activation of Janus Kinase/signal transducer and activator of transcription 3 (JAK/STAT3), inhibition of MPTP opening, Oxidative stress, phospha tidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) [15]. However, the cardioprotective and infarct size limiting impacts of IPOC has been abrogated in some pathological conditions such as obesity, heart failure, hyperlipidemia, cardiac hypertrophy, ageing, hypertension, diabetes, hyperglycemia and Hyperhomocysteinemia [17-19]. Diabetes mellitus is one of the major health concerns all around the world today [20, 21]. Diabetes is leads to highly risks of cardiovascular diseases and events [22]. Diabetes mellitus develops because of aggravations of metabolism in the body as the consequence of outright or relative nonappearance insulin or insulin obstruction which at last prompts adjustments in metabolism of supplements carbohydrates, amino acids, and fats [23]. Diabetes activates nuclear transcription factor kappa B, activation of PKC, downregulation of eNOS, NO production, PI3K/Akt pathway, increase in MAPK, and up-regulation of reactive oxygen species (ROS), oxidative stress, including Angiotensin II mediated activation of Janus activated kinase 2 (JAK2) and downregulation of PPAR alpha [11,24,25]. PPAR alpha is a ligand-activated transcription factor that it is belonging to the nuclear receptor superfamily [26]. PPAR alpha is involved in the pathogenesis of various cardiovascular diseases. PPAR alpha agonists are lowering the triglycerides level and blood sugar and treat the symptoms of Diabetes. The three PPAR isoforms, PPARα (NR1C1), PPARβ/δ (NR1C2), PPARγ (NR1C3), SHARE 60% to 80% of basic homology and show a particular tissue articulation design yet can apply comparative or diverse physiological capacities. Fenofibrate is selective agonist of PPAR alpha [26, 27]. Fenofibrate is also known as 2-4[(4-chlorobenzoyl) phenoxy]-2-methylpropanoic acid, 1-methylethyl ester.

Therefore, the present study has been designed to investigate the possible effect of Fenofibrate, a selective agonist PPAR alpha in abrogated cardioprotective effects of IPOC in diabetic rat hearts subjected to I/R.

Materials and Methods

The experimental protocol used in the present study was duly approved by the Institutional Animal Ethics Committee (IAEC) (1380/PO/Re/S/10/CPCSEA). Sprague Dolly (SD) Rat of either sex, weighing 180-220gm was employed in the present study. They were maintained on a standard laboratory diet and tap water ad libitum. They were maintained under standard condition of relative humidity, 12 hours light-dark cycle, adequate ventilation and ambient room temperature. The experiment was conducted in a semi sound proof laboratory.

Drugs and Chemicals

All the medication arrangements were newly arranged before use. Alloxan Monohydrate was purchased from Explicit Chemicals and Fenofibrate was purchased from Sai Healthcare (Baddi). Every single other substance/reagents utilized in the examination were of analytical grade.

Alloxan Induced Diabetes

The healthy rats were utilized to actuate diabetes after acclimatization time of 10 days. The animals were infused with newly prepared aqueous solution of Alloxan Monohydrate at a portion of 150mg/kg/i.p. The 10% of dextrose was controlled orally for 7 days to battle the quick hypoglycaemia that could happen. The serum glucose was watched 72h after Alloxan administration. The serum glucose level was watched for 7 days once in a day. The rats demonstrated serum glucose level running from 180-220 mg/dl following 7 days and same range of animals was chosen for the investigation.

Dose of Alloxan Monohydrate

The single dose of Alloxan Monohydrate (150 mg/kg i.p) administered 1st day of the protocol and was dissolved in 0.9% normal saline (NS).

Dose of Fenofibrate

Langendorff's infusion of Fenofibrate was given in the dose (5 μM) for 10 min in the present study.

Assessment of Blood Glucose Level

Assessment of blood glucose level was done by glucometer.

Isolated Rat Heart Preparation

Rat was heparinized (500 I.U., i.p.) and was sacrificed by cervical dislocation. The heart was quickly excised and promptly mounted on Langendorff mechanical assembly (Langendorff, 1895). The hearts was enclosed in a double walled jacket and the temperature of which kept at 37°C by circulating warm water. The preparation was perfused with Krebs Henseleit (K-H) solution (NaCl 118 mM; KCl 4.7 mM; CaCl2 2.5 mM; MgSO4.7H2O 1.2 mM; NaHCO3 25mM; KH2PO4 1.2 mM; C6H12O6 1 mM) of pH 7.4, maintained at 37°C and bubbled with 95% O2 and 5% CO2.

Estimation of Coronary Flow Rate

The coronary flow rate was kept up at around 7 ml/min by keeping the perfusion pressure at 80 mmHg. Global ischemia was produced for 30 min by obstructing the inflow of physiological solution and was followed by reperfusion of 120 min. The coronary flow rate was noted at basal (before global ischemia), 0 min (at the onset of reperfusion), 5 min, 30 min and 120 min of reperfusion.

Assessment of Myocardial Injury

The I/R -induced myocardial injury was evaluated by assessing the release of lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) in the coronary effluent [28].

Estimation of Ldh and Ck-Mb

The myocardial injury was evaluated by estimating the release of LDH in coronary effluent using the commercially available enzymatic kits. LDH was measured in the coronary effluent using the UV-kinetic method, which is depends on the principle that LDH catalyzes the oxidation of lactate to pyruvate joined by the concurrent decrease of NAD to NADH. LDH activity in the serum is proportional to the increase in absorbance due to the reduction of NAD into NADH. The LDH activity was measured using the following formula: LDH activity = ΔAbs/min × 3376. In CK-MB. The myocardial injury was evaluated by estimating the release of CK-MB in coronary effluent using the commercially available enzymatic kits [17]. The CK-MB was estimated in the coronary effluent using the immunoinhibition method, which is depends on the principle that the CK-M fractions of the CK-MM and the CK-MB in the sample are totally hindered by anti CK-M

agglutinating sera present in the reagent. CK-MB activity was calculated using the following formula: Activity of CK-MB (IU/L) = ΔAbs/min × 6666.

Assessment of Oxidative Stress Parameters
The tissue from freshly excised heart was minced and homogenized in 0.1M ice cold phosphate buffer (pH 7.4) using a homogenizer. The clear supernatant of the homogenate was used to estimate the thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD) and glutathione (GSH) levels in the tissue [17].

Estimation of Thiobarbituric Acid Reactive Substances
The tissue lipid peroxidation reaction was evaluated by assessing thiobarbituric acid reactive substances (TBARS) by the method of Wass et al. (1966) with certain alterations. Briefly, 500µl homogenate was added to equal amount of the same buffer, and incubated for 2 hrs at 37°C. After incubation, 1 ml of 10 % trichloroacetic acid (TCA) was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (1 ml) was added to 1 ml of 0.67% TBA, solution and boiled for 15 minutes on water bath. The samples were cooled in tap water and 1 ml of distilled water was added. The color intensity was determined at 532 nm. TBA reacting compound was expressed as nM of malonaldehyde (MDA)/mg of tissue [29].

Estimation of Glutathione
The diminished glutathione (GSH) level was evaluated by the method of Ellman, 1959. Briefly, the tissue homogenate in 0.1M phosphate buffer pH 7.4 was blended in with equivalent volume of 20% trichloroacetic acid (TCA) containing 1mM (Ethylene diamine tetra acetic acid) EDTA to precipitate the tissue proteins. The blend/mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200 rpm. The supernatant (200 µl) was then moved to another set of test tubes and included 1.8 ml of the Ellman's reagent (DTNB: 5, 5'-dithio bis-2-nitrobenzoic acid) (0.1mM) was prepared in 0.3M phosphate buffer with 1% of sodium citrate solution. At that point, all the test tubes make up to 2ml volume. The absorbance of the solution was measured at 412 nm against blank and the amount of reduced GSH was expressed as nM/mg of tissue [28, 29].

Assessment of Superoxide Dismutase (Sod)
Superoxide dismutase (SOD) activity was measured according to a method described by Misra and Fridovich (1972), by following spectrophotometrically the auto-oxidation of epinephrine at pH 10.4. In this method, supernatant of the tissue was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and the reaction was started by the addition of 0.02 ml (−)-epinephrine. After 5 min, the absorbance was measured at 480 nm. The activity of SOD was expressed as percent activity of vehicle-treated control [29, 30].

Experimental Protocol
Eight groups were employed in the current investigation and each group included six animals. In all groups, isolated perfused rat heart was allowed to stabilize for 10 min by perfusing with K-H solution.

Group I Normal Control: Isolated normal rat heart was perfused for 160 min using K-H solution after 10 min of stabilization.

Group II Ischemia-Reperfusion (I/R) Control: Normal rat heart after 10 min stabilization was subjected to 30 min of global ischemia. The heart was then perfused for 10 min using K-H solution followed by 120 min of reperfusion.

Group III Ischemic Postconditioned (IPOC): Isolated normal rat heart were subjected to 30 min of global ischemia after 10 min stabilization followed by six cycles of ischemia and reperfusion of 10 sec each. After six episodes of IPOC, the heart was subjected to 10 min perfusion with K-H solution followed by 120 min of reperfusion.

Group IV Diabetic (D) Control: Isolated diabetic rat heart was perfused for 160 min using K-H solution after 10 min of stabilization.

Group V D-I/R Control: Isolated diabetic rat heart after 10 min of stabilization was subjected to 30 min of global ischemia. The heart was then perfused for 10 min with K-H solution followed by 120 min of reperfusion.

Group VI D-IPOC: Isolated diabetic rat heart was subjected to 30 min of global ischemia after 10 min stabilization followed by six cycles of ischemia and reperfusion of 10 sec each. After six episodes of IPOC, the heart was subjected to 10 min perfusion with K-H solution followed by 120 min of reperfusion.

Group VII Fenofibrate Treated D-I/R Control: After 10-min stabilization, the isolated diabetic rat heart was subjected to 30 min of global ischemia, followed by infusion with Fenofibrate for 10 min. The heart was then subjected to 120 min of reperfusion.

Group VIII Fenofibrate Treated D-IPOC: Isolated diabetic rat heart was subjected to 30 min of global ischemia after 10 min stabilization followed by six cycles of ischemia and reperfusion of 10 sec each. After six episodes of IPOC, the heart was subjected to 10 min infusion with Fenofibrate followed by 120 min of reperfusion.

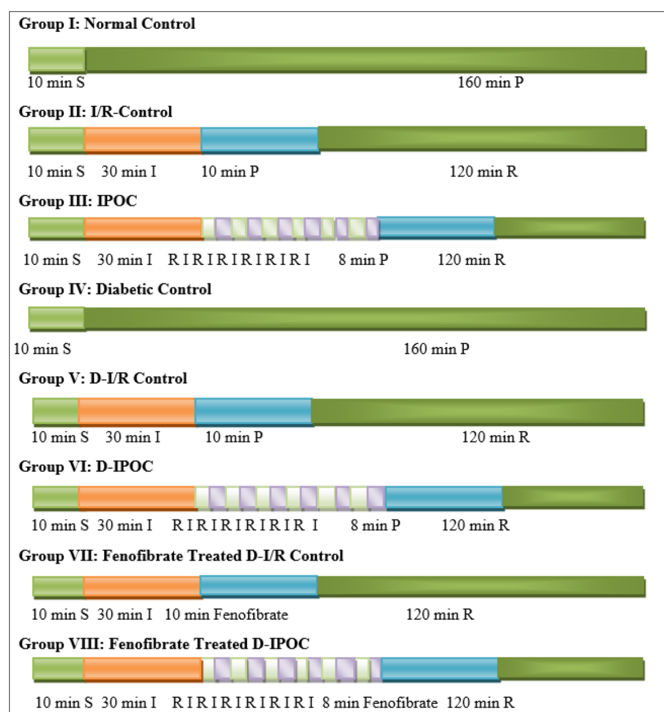

Figure 1: Diagrammatic Representation of Experimental Protocol

S indicates stabilization; I indicate global ischemia; P indicates perfusion with K-H solution; R indicates reperfusion with K-H solution; I/R indicates Ischemia-Reperfusion; Ischemic Postconditioned indicates Ischemic Postconditioned normal rat heart; D Ischemic Postconditioned indicates Ischemic Postconditioned diabetic rat heart.

Study Protocol

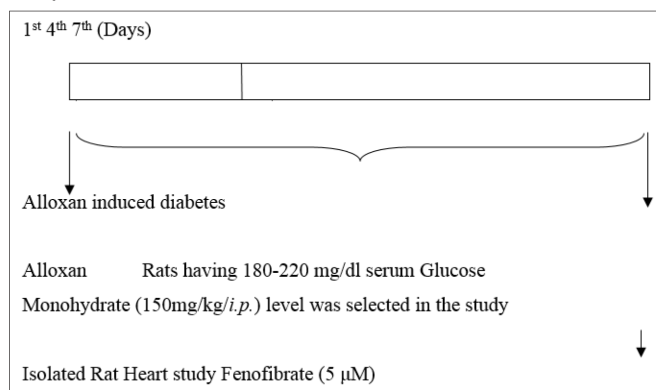

Figure 2: Diagrammatic Representation of Study Protocol

Statistical Analysis

All results were expressed as the mean ±SEM. Results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test and $p<0.05$ was considered to be statistically significant.

Results

Alloxan Induced Diabetes

Rats were injected with Alloxan Monohydrate (150mg/kg/day, i.p). Those rats having blood glucose level above 180mg/dl were selected for the present study.

Effect of Alloxan Monohydrate on Blood Glucose Level (end day of the Experimental Protocol)

Groups	Normal	IR Control	IPOC Control	Diabetic Control	Diabetic IR	Diabetic IPOC	Fenofibrate Diabetic IR	Fenofibrate Diabetic IPOC
Blood Glucose Level (mg/dl)	106±2.13	100.66 ±0.763	110.4 ±2.09	416 ±7.50	390. ±4.58	362.75 ±9.74	447.4 ±15.39	438.5 ±22.84

The diabetic rats were selected for the perfusion at Langendorff Apparatus.

Effect of Ipoc in I/R-Induced Oxidative Stress in Normal and Diabetic Rat Heart

The lipid peroxidation estimated in terms of TBARS was noted to be elevated essentially in normal and diabetic rat heart exposed to 30 min of global ischemia and 120 min of reperfusion (Fig. 3). Then again, SOD and the diminished form of GSH were seen as diminished in normal and diabetic rat heart exposed to I/R (Fig. 4, 5). Besides, diabetic rat heart indicated high oxidative stress when contrasted with normal rat heart subjected with I/R (Fig. 3-5). Six episodes of IPOC particularly lessened the I/R-induced oxidative stress in normal rat heart as surveyed as far as decrease in TBARS and subsequent expanded SOD and diminished form of GSH. However, IPOC intervened decrease in oxidative stress against I/R was uniquely canceled in diabetic rat heart which indicated high extent of oxidative pressure (Fig. 3-5).

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= $p<0.05$ vs Normal Control; b= $p<0.05$ vs I/R Control; c=$p<0.05$ vs D-Control; d=$p<0.05$ vs D-IPOC; e=$p<0.05$ vs Fenofibrate treated D-I/R; f=$p<0.05$ vs D-IPOC

Figure 3: Effect of Fenofibrate and IPOC in I/R induced increase in TBARS Level

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= $p<0.05$ vs Normal Control; b= $p<0.05$ vs I/R Control; c=$p<0.05$ vs D-Control; d=$p<0.05$ vs D-IPOC; e=$p<0.05$ vs Fenofibrate treated D-I/R; f=$p<0.05$ vs D-IPOC

Figure 4: Effect of Fenofibrate and IPOC in I/R induced reduced form of GSH Level

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= p<0.05 vs Normal Control; b= p<0.05 vs I/R Control; c=p<0.05 vs D-Control; d=p<0.05 vs D-IPOC; e=p<0.05 vs Fenofibrate treated D-I/R; f=p<0.05 vs D-IPOC

Figure 5: Effect of Fenofibrate and IPOC in I/R induced decreased superoxide dismutase generation (SOD)

Effect of Ipoc in I/R-Induced Myocardial Injury in Normal and Diabetic Rat Heart

Global ischemia followed by reperfusion essentially expanded LDH and CK-MB levels in coronary effluent in normal and diabetic rat heart (Fig. 6, 7). Greatest arrival of LDH was noted immediately after reperfusion, while peak arrival of CK-MB was noted at 5 min of reperfusion. In addition, diabetic rat heart indicated enhanced myocardial injury when contrasted with normal rat heart subjected with I/R. The IPOC managed cardioprotection in normal rat heart by essentially constricting I/R-induced myocardial injury as evaluated as far as decrease in LDH and CK-MB levels (Fig. 6, 7). In any case, the IPOC interceded cardioprotection against I/R-injury was extraordinarily nullified in diabetic rat heart.

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= p<0.05 vs Normal Control; b= p<0.05 vs I/R Control; c=p<0.05 vs D-Control; d=p<0.05 vs D-IPOC; e=p<0.05 vs Fenofibrate treated D-I/R; f=p<0.05 vs D-IPOC

Figure 6: Effect of Fenofibrate and IPOC in I/R induced increased in LDH Level

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= p<0.05 vs Normal Control; b= p<0.05 vs I/R Control; c=p<0.05 vs D-Control; d=p<0.05 vs D-IPOC; e=p<0.05 vs Fenofibrate treated D-I/R; f=p<0.05 vs D-IPOC

Figure 7: Effect of Fenofibrate and IPOC in I/R induced increased in CK-MB Level

Effect of Ipoc and Fenofibrate on Coronary Flow Rate (Ml/Minute) In Normal and Diabetic Rat Heart

Global ischemia followed by reperfusion fundamentally diminished the measure of coronary perfusate in normal and diabetic rat heart (Table 4). In addition, the diabetic rat heart indicated marked decrease in coronary perfusate when contrasted with normal rat heart subjected to I/R. The IPOC fundamentally improved the coronary flow rate in normal rat heart. Then again, the IPOC has neglected to improve the coronary flow rate in diabetic rat heart subjected to I/R. Treatment with Fenofibrate (5 µM) markedly improved the coronary flow rate.

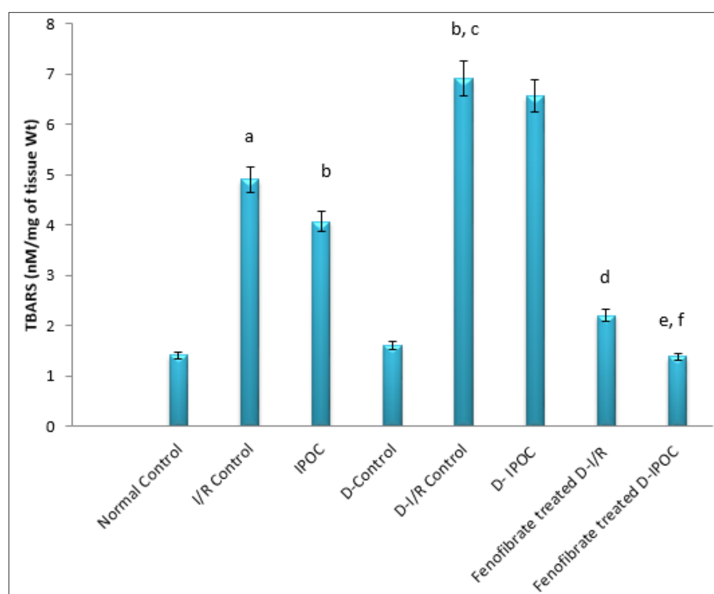

Table 1: Effect of Fenofibrate and IPOC in I/R induced increase in TBARS Level

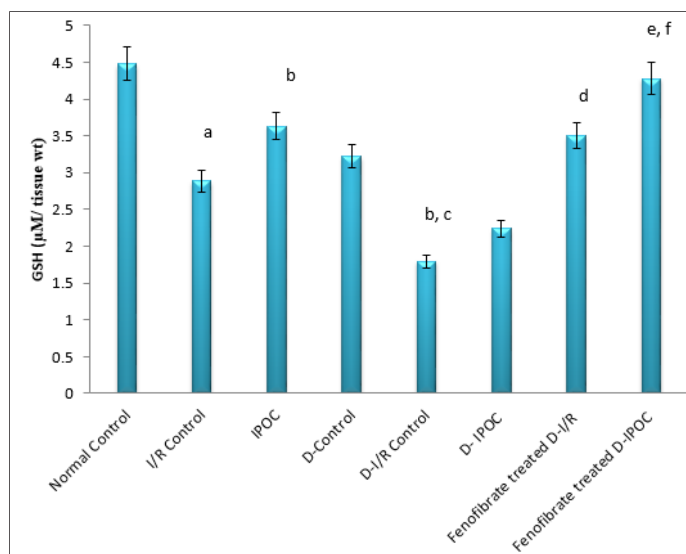

Table 2: Effect of Fenofibrate and IPOC in I/R induced reduced form of GSH Level

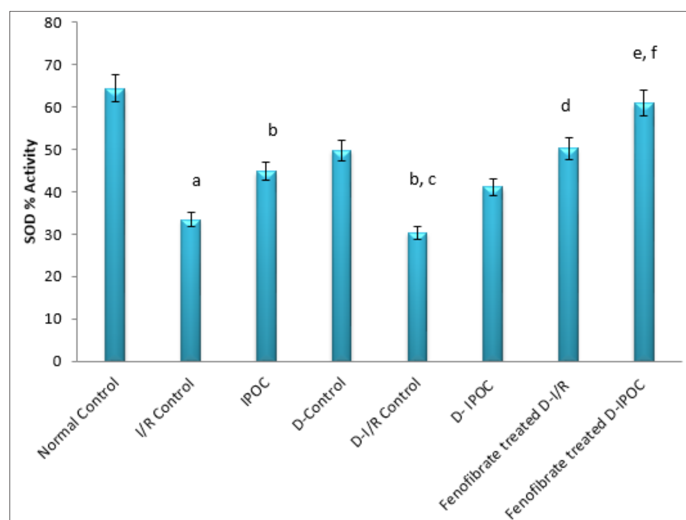

Table 3: Effect of Fenofibrate and IPOC in I/R induced decreased superoxide dismutase generation (SOD)

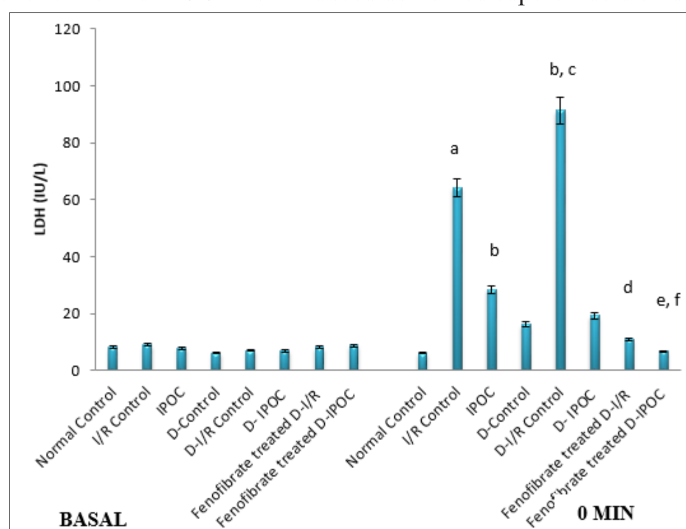

Table 4: Effect of Fenofibrate and IPOC in I/R induced increased in LDH Level

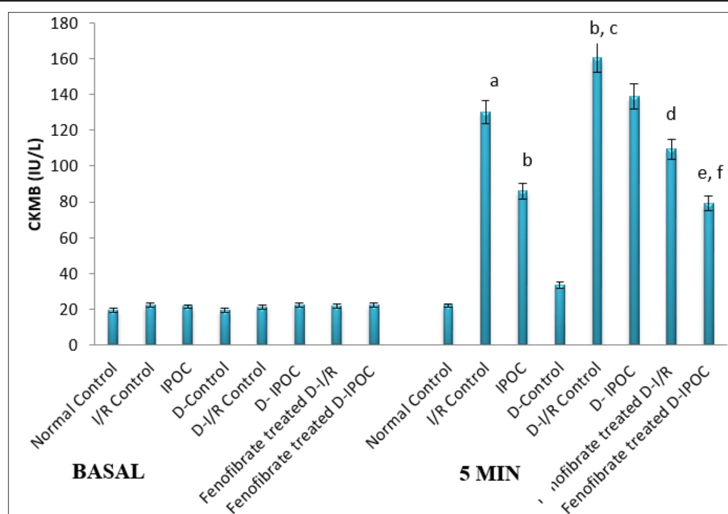

Table 5: Effect of Fenofibrate and IPOC in I/R induced increased in CK-MB Level

Table 4: Effect of Fenofibrate and Ipoc on Coronary Flow Rate (Cfr) (Ml/Min)

S.N.	Groups	Basal	0 Min	5 Min	30 Min	120 Min
1	Normal Control	7.5±0.707	7.4±0.649	7.5±0.9403	6.7±0.8803	6.15±0.841
2	I/R Control	5.966±0.775	2.7±0.960	2.2±1.0016	1.733±0.683	1.133±0.504[a]
3	IPOC	7.52±0.231	6.84±0.183	6.64±0.1077	6.44±0.0927	6.24±0.074[b]
4	Diabetic Control	6.733±0.866	6.3±0.8544	5.066±1.7835	4.133±1.8888	3.533±1.707
5	Diabetic -I/R Control	6.766±1.010	5.066±1.2004	4.733±1.0806	4.233±1.125	3.366±1.18[b,c]
6	Diabetc-IPOC	7.9±0.4708	6.725±0.3010	6.575±0.225	6.2±0.1224	6.025±0.2393
7	Fenofibrate Treated diabetic-I/R Control	7.54±0.16	5.7±0.884	5.22±0.854	4.44±0.812	3.62±0.731[d]
8	Fenofibrate Treated Diabetic-IPOC	7.05±0.405	6.225±0.475	5.75±0.695	5.1±0.665	5±0.070[e,f]

Values are expressed as Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison tests, a= $p<0.05$ vs Normal Control; b= $p<0.05$ vs I/R Control; c=$p<0.05$ vs D-Control; d=$p<0.05$ vs D-IPOC; e=$p<0.05$ vs Fenofibrate treated D-I/R; f=$p<0.05$ vs D-IPOC

Discussion

Diabetes mellitus develops because of aggravations of metabolism in the body as the consequence of outright or relative nonappearance insulin or insulin obstruction which at last prompts adjustments in metabolism of supplements carbohydrates, amino acids, and fats [23]. In Diabetes, activation of nuclear transcription factor kappa B, activation of PKC, downregulation of eNOS, NO production, PI3K/Akt pathway, increase in MAPK, and up-regulation of reactive oxygen species (ROS), oxidative stress, including Angiotensin II mediated enactment of Janus activated kinase 2 (JAK2) and downregulation of PPAR alpha [11,24,25,31].

Alloxan Monohydrate induced diabetes has been widely used in an animal model for the new experimental research. It has been noted that the Alloxan administration starts the production of ROS, including superoxide radical (O2), hydroxyl radical (OH) and hydrogen peroxide (H2O2), which harm, and later destroy, the cells [32]. Alloxan is chemically known as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione [33, 34]. In our study, administration of Alloxan Monohydrate 150 mg/kg lead to elevation in blood glucose level and it was previously reported [32, 35]. Alloxan not showed effective results to the animals so repeated dose of Alloxan was given to few animals for better results [36]. In this way, the observed marked enhancement in myocardial injury in diabetic rat heart might be because of the development of high extent of oxidative stress. This dispute is supported by the fact that a marked enhancement in lipid peroxidation and subsequent reduction in glutathione reductase and superoxide dismutase were noted in diabetic rat heart when compared with normal rat heart subjected to I/R. The increase in CK-MB isoenzyme has been shown to be related strongly with infarct size [37]. LDH is an enzyme which increases in myocardial infarction after reperfusion because of sustained ischemic injury [38]. Ischemia/Reperfusion (IR) injury fundamentally causes the increase of enzymes involved in myocytes injury including CK-MB and LDH. Leakage of CK-MB isoenzyme and LDH from myocardial tissues to blood is indicator of acute myocardial infarction [39]. In the present study, 30 min of ischemia followed by 120 min of reperfusion produced myocardial injury was analyzed in terms of raise release of LDH and CK-MB in the coronary effluent, which was consistent with earlier reports [40]. The maximal arrival of LDH was noted immediately after reperfusion whereas the peak release of CK-MB was seen after 5 min of reperfusion, which are in accordance with earlier investigations [40]. In the present study, a significant

decreased coronary flow rate and marked increased release of LDH and CK-MB were noted in diabetic rat heart as compared with the normal rat heart subjected to I/R.

The elevation in lipid peroxidation has been recommended to be indicator of oxidative stress [41]. Glutathione and superoxide dismutase forms an essential part of defense against elevated oxidative stress and is usually reduced in oxidative stress. Lipid peroxidation estimated as far as TBARS was noted to be expanded and the glutathione level and superoxide dismutase was diminished because of I/R. This proposes the improvement of I/R-induced oxidative stress, which might be liable for the prominent I/R-induced myocardial injury in the current examination.

IPOC has been very much recorded to create myocardial insurance against I/R-induced myocardial injury [42]. Ischemic postconditioning is an episode of brief ischemia and reperfusion cycles that are applied right away at the zone of the ischemic organ after reperfusion that is brings about decreased infarction in myocardial ischemia [12]. Cardioprotective phenomenon of ischemic postconditioning that reduces the oxidative stress, inhibiting the apoptotic and necrotic cell death ultimately protect the myocardium form lethal ischemia reperfusion injury and attenuating the mitochondrial calcium overload [13]. IPOC effectively reduces the neural cell death or apoptosis, cerebral edema and decrease infarct size, improve cerebral circulation, and relieve inflammation, and oxidative stress [14]. IPOC has been noted to decrease I/R-induced myocardial injury by actuation of phosphatidylinositol-3 kinase/protein kinase B (PI3K/Akt) and endothelial nitric oxide synthase (eNOS) and resulting arrival of nitric oxide (NO) have been recommended to be embroiled in the cardioprotective impact of IPOC [14]. Various cellular pathways and molecular pathways that are involved in cardioprotective effects of IPOC include initiation of Janus Kinase/signal transducer and activator of transcription 3 (JAK/STAT3), inhibition of MPTP opening , Oxidative stress, phosphatidylinositol 3-kinase (PI3K), extracellular signal-regulated kinase (ERK) [15,16].

In the current study, six episodes of 10 sec ischemia followed by reperfusion for 10 sec, effectively postconditioned the normal rat heart as showed by a significant diminished ischemia-reperfusion induced arrival of LDH and CK-MB and oxidative stress. In any case, the cardioprotective effect of IPOC was not seen in diabetic rat heart with high degree of noted oxidative stress. In this manner, it is firmly recommended that the high degree of oxidative stress created in diabetic rat heart may be liable for the observed confusing impact of IPOC. The effect of IPOC abolished in D-IPOC. But in this case, the effect of IPOC is not abolished in D-IPOC in LDH parameter and it might be due to practical error. Treatment with Fenofibrate (5μM) significantly restored the cardioprotective effect of IPOC in diabetic rat heart. Fenofibrate is selective agonist of PPAR alpha [26]. Fenofibrate is also called as 2-4[(4-chlorobenzoyl) phenoxy]-2-methylpropanoic acid, 1-methylethyl ester. Thus, it is recommended that PPAR alpha inactivation during reperfusion of ischemic myocardium may play a critical role in the constriction of cardioprotective capability of IPOC in diabetic rat heart. PPAR alpha agonists are lowering the triglycerides level and blood sugar and treat the symptoms of Diabetes. The three PPAR isoforms, PPARα (NR1C1), PPARβ/δ (NR1C2), PPARγ (NR1C3), SHARE 60% to 80% of essential homology and show a particular tissue articulation design however can apply comparable or distinctive physiological capacities Studies proposed that diabetes downregulates eNOS and lessens the age and bioavailability of NO by expanding the oxidative pressure [27]. Since, Fenofibrate has reestablished the cardioprotective impact of IPOC in diabetic rat heart might be proposed that PPAR alpha inactivation in diabetic rat heart may be capable to annul the cardioprotective potential of IPOC. Thus, it could be suggested that generation of high amount of ROS in diabetic rat heart during reperfusion may inactivate PPAR alpha, which may abolish the cardioprotective effect of IPOC. This conflict is upheld by the outcomes gotten in the current investigation that treatment with Fenofibrate has reestablished the cardioprotective properties of IPOC in diabetic rat heart.

Based on above discussion, it might be presumed that the high extent of oxidative stress delivered in diabetic rat heart during reperfusion and subsequent downregulation of PPAR alpha might be responsible to annul the cardioprotective capability of IPOC against I/R-induced myocardial injury.

Conclusion

The current investigation showed that, the high degree of oxidative stress produced in diabetic rat heart during reperfusion and resulting inactivation of PPAR alpha receptor might be responsible for abolishing the Cardioprotective potential of IPOC against I/R induced myocardial injury.

Acknowledgement

The authors thank Department of Pharmacology, Rayat Bahra Univeristy, Kharar, Punjab India and Aakash Institute of Medical Sciences, Nalagarh, HP India, for all the support to complete this study.

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