Original Article

Role of phosphoinositide-3-kinase/AKT activation mediated by inhibition of protein tyrosine phosphate-1B inhibitor in cardioprotection

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ABSTRACT

Background: Dyslipidemia is a downregulator of phosphoinositide-3-kinase (PI3K)/AKT-mediated cell survival pathway and reduces the preconditioning potential in ischemic-reperfusion (I/R) injured rat heart ultimately leads to cardiac cell death. **Objective:** The present study was designed to explore and confirm the pharmacological perspective of sodium orthovanadate (SOV), an inhibitor of tyrosine phosphate-1B protein against ischemic-reperfusion (I/R) injury in dyslipidemic rat. **Martials and Methods:** The dyslipidemic rodent hearts were isolated and fixed on Langendorff's device, exposed to 30 miniature reperfusion. Ischemic preconditioning was started by four episodes of 5 min ischemia and 5 min reperfusion. Infarction was assessed using triphenyltetrazolium chloride staining, and coronary effluent was inspected for lactate dehydrogenase (LDH) and creatinine kinase-MB discharge to decide the degree of myocardial damage. The cardiac discharge of nitric oxide (NO) was assessed by determining the discharge of nitrite in coronary emission. **Results:** Pharmacological preconditioning with SOV significantly re-imposed I/R mediated myocardial infarction in dyslipidemic rat heart when compared with dyslipidemic and I/R control group. In this study, the statistical data show the significant values $P < 0.05$. However, in observed results, cardioprotection was abolished by perfusion of BEZ-235, a PI3K/AKT pathway inhibitor noted in terms of myocardial injury events such as a rise in myocardial infarct mass, tumor necrosis factor-alpha, LDH, CK-MB, and also decrease in the release of NO. **Conclusion:** Hence, it stated that SOV repairs impaired cardioprotective effect in dyslipidemic rat heart maybe because of activation of the PI3K/AKT cell survival pathway which was confirmed using PI3K/AKT inhibitor BEZ-235.

Keywords: Dyslipidemia, ischemic reperfusion injury, ischemic preconditioning, phosphoinositide-3-kinase, protein kinase-B

INTRODUCTION

Ischemic heart disease has been reported as a leading source of ill health and death rates globally.[1,2] The ischemic state is produced by obstruction of blood vessels which leads to an inadequate amount of oxygen and nutrients to myocardium tissue whereas reperfusion in the ischemic state is necessary to rehabilitate the damaged myocardium.[3] Though sudden reperfusion of the damaged heart can lead to a dysfunction known as ischemic reperfusion (I/R) injury.[4,5] Ischemic preconditioning (IPC) a potent endogenous defensive mechanism in which short intermittent episodes of ischemia and reperfusion previously sustained ischemic insult improves lenience of heart against I/R-induced injury.[6] Various mechanisms such as activation of phosphoinositide-3-kinase (PI3K)/AKT,[7] phosphorylation of endothelial nitric oxide synthase (eNOS),[8,9] generation of NO in endothelial cells as with sickle cell anemia,[10-12] and initial of the mitochondrial-KATP channel[13] contribute to vascular and cardiac protection.[14]

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The previous studies conducted on several conditions such as myeloproliferative disorders,[15] sickle cell anemia[16,17] and veno-occlusive disease,[18,19] have shown that endothelial damage and microparticles generation impair NO production and favor vasoconstriction.[14] Furthermore, some researchers observed that cardioprotection mediated through IPC does not protect myocardium in various diseases such as hypertension, hyper homocysteinemia, hyperlipidemia, heart failure, and diabetes mellitus.[20,21]

Protein tyrosine phosphatase (PTP) is an enzyme that functions in a unit with tyrosine kinases to regulate intracellular signaling pathways.[22] Several members of the PTP family link to human disease predisposition such as PTP-1B and inhibition of these enzymes may represent effective palliative therapy.[23] Tyrosine-protein phosphatase inhibition has been intensively investigated as an important therapeutic target against metabolic syndrome and has been documented to improve hepatic insulin sensitivity, reduce liver triglycerides (TG), and cholesterol levels, and protects against endoplasmic reticulum stress.[24] Several cellular events such as damage to cardiomyocyte, endothelial cells, and vascular smooth muscle can be observed after local or global ischemia, which can be terminated after the administration of sodium orthovanadate (SOV) at the time of reperfusion which can further defend myocardium from ischemic insult.[25,26] I/R injury post-global ischemia damage to the cardiomyocytes, vascular endothelial cell, and post-SOV administration at reperfusion time thus protects the myocardium from I/R insult. Therefore, pharmacological interventions of SOV can restore the cardioprotection through activation of the PI3K/AKT pathway.[27,28]

Dyslipidemia is recognized as a noticeable risk factor for cardiovascular diseases.[29] Dyslipidemia subsequently results in atherosclerosis, ischemia, myocardial infarction, and heart attack.[30] It is a metabolic idiosyncrasy that leads to a stubborn rise in the plasmatic concentration of cholesterol and TG. Dyslipidemic state processed enhancement of PTP-1B level and mostly investigated in the negative regulation of tyrosine kinase receptors (TKRS).[31] PTP represents an important target to treat cardiovascular complications. Preconditioning mediated cardioprotective mechanism mostly altered in a dyslipidemic state and is one of the major factors for therapeutic exploration of IPC.[32] Therefore, the current study investigated the myocardial preconditioning potential of PTP-1B inhibitor SOV in dyslipidemic rat heart.

MARTIALS AND METHODS

Experimental animals

Wistar rats of either sex, weighing 180–220 g were used in the current research. Rodents were received from Central Animal House of ISF College of Pharmacy, Moga, and are kept in the group of three in polypropylene cages. Cages are provided with a layer of husk. Standard conditions of light and dark have been followed, with the proper facility of feed and water. Rats were acclimatized to research conditions and equipment before performing actual experiments. The exploratory convention was checked on and endorsed by the Regulation Committee (IAEC) (ISFCP/IAEC/CPCSEA/Meeting No: 21/2018/Protocol No: 351) and was passed out in understanding with rules of Indian National Science Institute (INSA) for the use and care of testing animals.

Drugs and chemicals

SOV and BEZ-235 were obtained from Sigma-Aldrich Ltd., Bengaluru, India. Triphenyltetrazolium chloride (TTC) was acquired from CDH Pvt. Ltd., New Delhi, India. The lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), serum total cholestero (TC), high-density lipoprotein (HDL), and TG kits were procured from Coral Clinical System, Goa, India. All other reagents which have been used in biochemical tests were freshly prepared. Unless stated, all other chemicals and biochemical reagents were freshly prepared.

Experimental protocol: The protocol comprises eight groups consist of six rat hearts (n = 6) [Figure 1]

Group 1: (Sham control; n = 6): Isolated heart preparation of normal rat was allowed to soothe for 10 min and then perfused unceasingly with K-H (Krebs-Henseleit) buffer solution for 190 min without exposing them to global ischemic-reperfusion.

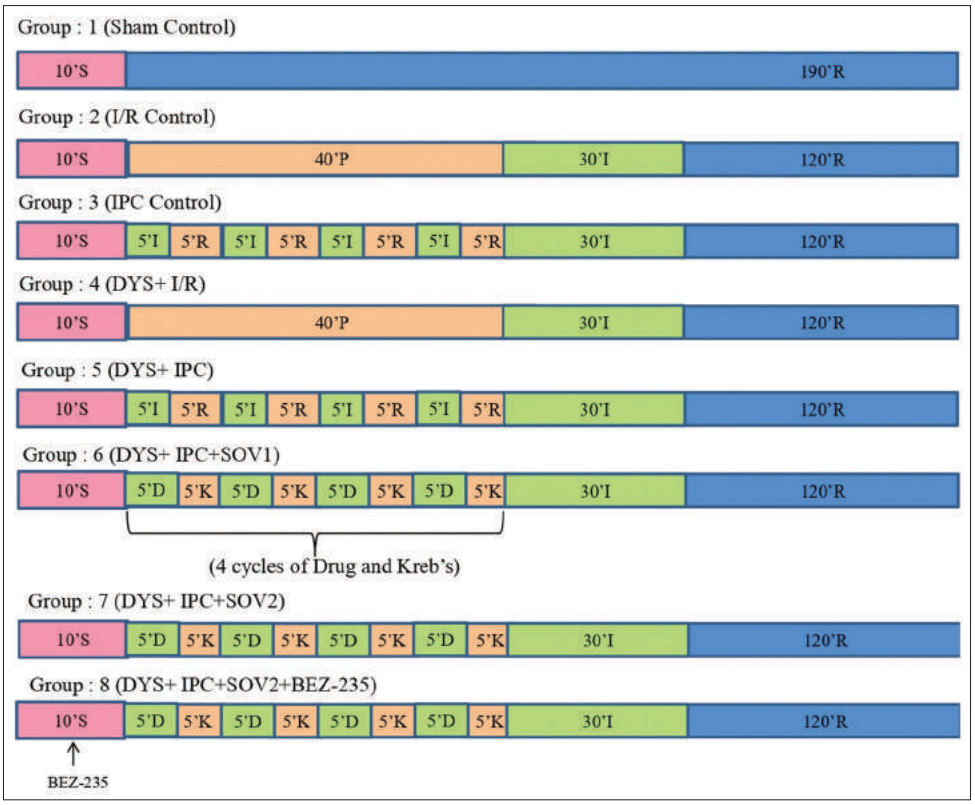
Group 2: (Ischemia-reperfusion in normal rat heart; n = 6): Isolated heart of normal rat was allowed to stabilize for 10 min and was perfused for 40 min K-H buffer solution. Then, it was exposed to 30-min global ischemia tracked by 120 min of reperfusion.

Group 3: (Ischemia-preconditioning in normal rat heart; n = 6): Isolated heart of normal rat was allowed to stabilize for 10 min and then imperiled to four episodes of IPC, each cycle composed of 5-min global ischemia followed by 5 min reperfusion with K-H solution. Then it was subjected to 30-min global ischemia followed by the 120 min of reperfusion.

Group 4: (Ischemic reperfusion in dyslipidemic rat heart; n = 6): Isolated dyslipidemic heart of rat was permitted to calm for 10 min and was perfused for 40 min with K-H buffer solution. Then, it was subjected to 30-min global ischemia followed by a 120 min of reperfusion.

Group 5: (IPC in dyslipidemic rat heart; n = 6): Isolated preparation of rat heart from dyslipidemic rat was allowable to stabilize for 10 min and exposed to four episodes of IPC, each cycle composed of 5-min ischemia tailed by 5-min reperfusion with K-H buffer solution. Then, it was exposed to 30-min global ischemia followed by a 120 min of reperfusion.

Group 6: (Preconditioning with SOV [1 mM/L] in dyslipidemic rat heart): Isolated preparation of dyslipidemic heart of rat was allowed to soothe for 10 min and after stabilization of isolated dyslipidemic rat's heart was subjected to four episodes of pharmacological preconditioning, each episode embraced of 5-min perfusion with SOV (1 mM/L) solution tracked by 5-min reperfusion with K-H solution. Then, the preparation was subjected to 30-min global ischemia monitored by the 120 min of reperfusion.



Figures 1: Experiment protocol schedule

Group 7: (Preconditioning with SOV [2 mM/L] in dyslipidemic rat heart): Isolated dyslipidemic rat heart preparation was allowed to alleviate for 10 min and post-stabilization of isolated dyslipidemic rat heart was allowed to four cycles of pharmacological preconditioning, every episode comprised of 5 min perfusion with SOV (2 mM/L) solution followed by 5-min reperfusion with K-H solution. Then, isolated preparation was subjected to 30-min global ischemia followed by 120 min of reperfusion.

Group 8: (Preconditioning with SOV [2 mM/L] and BEZ-235 [20 uM/L] in dyslipidemic rat heart): Isolated heart preparation was perfused with BEZ 235 (20 mM/L) for 10 min and post stabilization of isolated dyslipidemic heart of rat was subjected to four sequences of pharmacological preconditioning, every episode composed of 5-min perfusion with SOV (2 mM/L) solution followed by 5-min reperfusion with K-H solution. After that, isolated preparation was subjected to 30-min global ischemia followed by a 120 min of reperfusion.

Induction of experimental dyslipidemia

The animals (rats) were divided into six groups containing six subjects per group, except three groups that were feed on a normal chow diet. A high-fat diet (HFD) for 8th weeks was given to animals. HFD was prepared using ingredients (g/kg): Powdered NPD 300, Lard 275, Casein 200, Cholesterol 10, Vitamin and mineral mix 60, dl-methionine 03, Sodium chloride, and Sucrose 150. The dyslipidemia was confirmed by body weight and lipid biomarkers. The hearts were isolated after 8 weeks of HFD and were mounted on the Langendorff apparatus. All the parameters were assessed afterward [Figure 2].

Isolated rat heart preparation

Heparin (500 international units (IU)/L, i.p.) was administered 20 min before sacrificing the animal by cervical dislocation. The heart was quickly excised and immediately mounted on Langendorff's apparatus (Digital Langendorff's system, Radnoti LLC, Monrovia, USA).[33,34]The double-walled jacket was used for enclosing the heart; the temperature was maintained to 37.8°C. The preparation was retrogradely perfused at constant pressure (by a peristaltic pump) with Krebs-Henseleit (K-H) buffer of pH 7.4, bubbles with 95% O_2 and 5% CO_2. Global ischemia was produced for 30 min by closing the inflow of K-H solution which was followed by the 120 min of reperfusion. Coronary effluent was collected before ischemia, 0, 5, and 30 min after reperfusion; for LDH and CK-MB estimation.

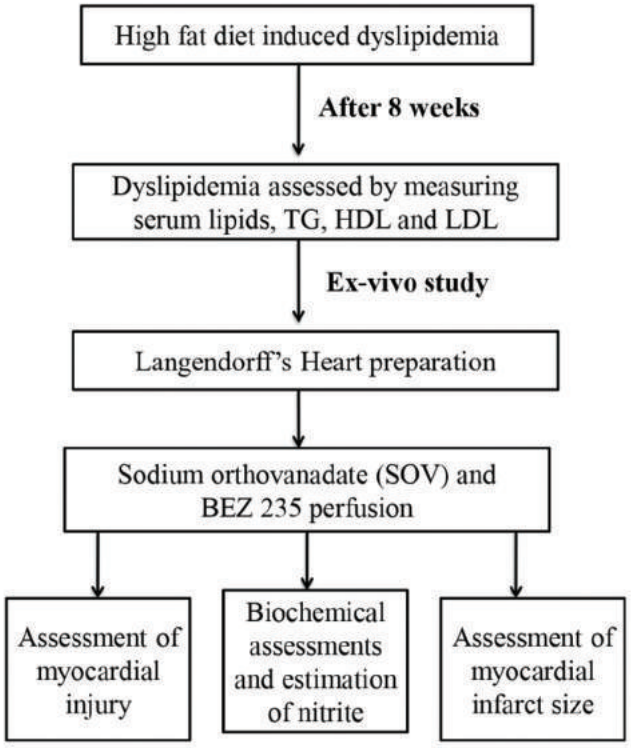
IPC

Isolated heart of rat was exposed to four short-term episodes of I/R (every episode composed of 5 min ischemia followed by 5-min perfusion) after the stabilization period of 10 min. Then, the heart was subjected to 30-min ischemia followed by the 120-min reperfusion.

Assessment of dyslipidemia

Measurement of body weight

The body weights of animals of all groups were measured every week. Initial body weight was the same for all animals. However, after 6



Figures 2: High-fat diet induced dyslipidemia

weeks; HFD groups showed a significant rise in weight as compared to control.

Estimation of lipid biomarkers

After 6 weeks, the blood was collected by retro-orbital puncture post-inhalation anesthesia. The blood was allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 4000–5000 rpm for 15 min and analyzed for serum cholesterol, HDL, and TG levels using Coral Clinical Systems (diagnostic kits), Goa, India.

Estimation of myocardial injury

The release of LDH and CK-MB in coronary effluents is considered as the extent of myocardial injury. These were assessed by commercially available kits (Coral Clinical System, Goa, India). Values were expressed in IU/l.

Assessment of LDH release

LDH was estimated after stabilization at 0 and 30 min after reperfusion by modified International Federation of Clinical Chemistry (IFCC) method using a Coral diagnostic kit of Coral Clinical System, Goa, India; at 340 nm spectrophotometrically (UV-1700 Spectrophotometer, Shimadzu, Japan).

Estimation of CK-MB release

CK-MB release was estimated after stabilization and 5 min after reperfusion by the modified IFCC method using the Coral diagnostic kit of Coral Clinical System, Goa, India; spectrophotometrically at 340 nm (UV-1700 Spectrophotometer, Shimadzu, Japan).

Assessment of myocardial infarct size

The heart was removed from Langendorff's apparatus. The atria and root of the aorta were expunged. At −20°C Ventricles were frozen and then sliced into uniform sections of 1–2 mm thickness. The uniform slices were incubated in 1% TTC for 30 min at 37°C in 0.2 M Tris-chloride buffer (prepared by dissolving 7.27 g of Tris (hydroxymethyl) methylamine and 5.27 g of sodium chloride in water, pH was adjusted to 7.4, finally diluting up to 1000 ml with distilled water).[35] NADH and dehydrogenase enzyme converted TTC into red formazone pigment and therefore, the viable cells were stained brick red. The enzyme and cofactor were absent in infracted cells, thus remain unstained or dull yellow. The ventricular slices were placed between two glass plates. A transparent plastic grid with 100^2 in 1 cm^2 was placed above it. The average area of the ventricular slice was calculated by the squares counting method. Similarly, numbers of square falling over the non-stained dull yellow areas were counted. Infarct size was expressed as a percentage of average ventricular volume.[36]

Estimation of tumor necrosis factor-alpha (TNF-α) levels

TNF-α kit (RayBio, Rat TNF-alpha ELISA kit protocol) was used to assess the TNF-α level through a microtiter plate readable at 450 nm. TNF-α concentrations were calculated by plotting the standard curve.

Biochemical assessment of oxidative stress markers

Estimation of nitrite concentration

The aggregation of nitrite within the supernatant was estimated using Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric corrosive). As described equal volumes of supernatant and Griess reagent were blended and then ruminated for 10 min at room temperature within the dark and the absorbance decided at 546 nm spectrophotometrically. The concentration of nitrite within the supernatant was decided from sodium nitrite standard bend and communicated as μmol/mg protein.

Estimation of serum thiobarbituric acid reactive species (TBARS) level

TBARS, a marker of lipid peroxidation, was done with the help of Kumar *et al*. 2009.[37] 0.2 ml of supernatant homogenate was pipette out in a test tube, taken after by expansion of 0.2 ml of 8.1% sodium dodecyl sulfate. 1.5 ml of 30% acetic acid (pH 3.5) and 1.5 ml of 0.8% of thiobarbituric acid were added and the volume was made up to 4 ml with refined water. The test tubes were incubated for 1 h at 95°C, then cooled and include 1 ml of distilled water taken after by expansion of 5 ml of an n-butanol-pyridine blend (15:1 v/v). The test tubes were again centrifuged at 4000 g for 10 min and hence pink color produced. The absorbance was measured spectrophotometrically (UV-1700 spectrophotometer, Shimadzu, Japan) at 532 nm. A standard calibration curve was arranged using 1–10 nM of 1, 1, 3, 3-tetra methoxypropane. The TBARS concentration was communicated as nanomoles per gm of wet tissue weight.

Statistical analysis

The results were expressed as mean±SD. One-way ANOVA followed by Tukey's multiple comparison tests was used to analyze the percent (%) infarct size, TNF-α, and TBARS. Two-way ANOVA followed by following Bonferroni was utilized to analyze body weight, lipid

profile, CK-MB, LDH, nitrite, and coronary flow levels. $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of HFD treatment on body weight

HFD treatment for 8 weeks produced a significant promotion in body weight during HFD treated animals (342.3±15.72) as compared with normal chow diet animals (201.7±9.070). These values signify $P < 0.05$ as compared to 0 week [Figure 3].

Effect of HFD treatment on lipid profile (TC, TG, LDL, and HDL)

A HFD formed a significant rise in TC (203.7±17.77) and TG level (134.0±8.944) in the HFD treated animals correspondingly as linked to animals treated with normal chow diet (65.67±10.07), (50.33±9.41).

HFD produced a significant upsurge in the serum levels of LDL (163.7±12.09) whereas, a remarkable decrease in the levels of HDL (27.00±3.286) was detected in the HFD treated animals as compared to the normal diet treated animals (58.00±9.633), (61.33±5.750), These values signifies $P < 0.05$ as compared to 0 week [Figure 4].

Effect of preconditioning on cardiac enzymes

Effect of SOV on LDH level in IPC mediated dyslipidemic rat heart

Global ischemia for 30 min monitored by 120 min of reperfusion remarkably elevates the LDH level when associated with a sham control group. Perfusion with PTP-1B inhibitor SOV in dyslipidemic rat heart decreases LDH release, which was reversed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four IPC episodes notably reduced I/R mediated increased LDH release in the normal heart of rat, whereas, there were no significant alterations were found in LDH enhanced of dyslipidemic rat heart. The decreased level of LDH in association with IPC was expressively restored by SOV mediated reperfused dyslipidemic rat heart. Although, perfusion with BEZ-235 in the stabilization phase markedly enhanced the LDH level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 5].

Effect of SOV on CK-MB level in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min followed by a 120 min of reperfusion remarkably elevates the CK-MB level when compared with sham control. Furthermore, perfusion with PTP1B inhibitor SOV in dyslipidemic rat heart decreases the release of CK-MB, which was effectively reversed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four IPC episodes notably summarized I/R mediated increased secretion of CK-MB in the normal heart, whereas, there was no significant alteration which was found in CK-MB levels of the dyslipidemic heart. Furthermore, IPC associated decrease level of CK-MB was expressively restored in SOV-reperfused dyslipidemic heart. However, perfusion with BEZ-235 in the stabilization part markedly enhanced the CK-MB level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 6].

Effect of SOV on infracts size in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min tracked by 120 min of reperfusion remarkably elevates the infarct size when related to sham control. Perfusion with PTP1B inhibitor SOV in dyslipidemic rat heart decreases the infarct size, which was reversed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four IPC episodes notably condensed I/R mediated increased infarct size in normal heart, whereas, there was no significant alteration which was found in the infarct size of dyslipidemic rat heart. In addition, IPC associated declined infarct size was expressively refurbished in SOV-reperfused dyslipidemic heart. However, perfusion with BEZ-235 in the stabilization stage, markedly enhanced the infarct size level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$), Figure 7a and b.

Effect of SOV on coronary flow in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min followed by a 120 min of reperfusion remarkably elevates the coronary flow level when compared with

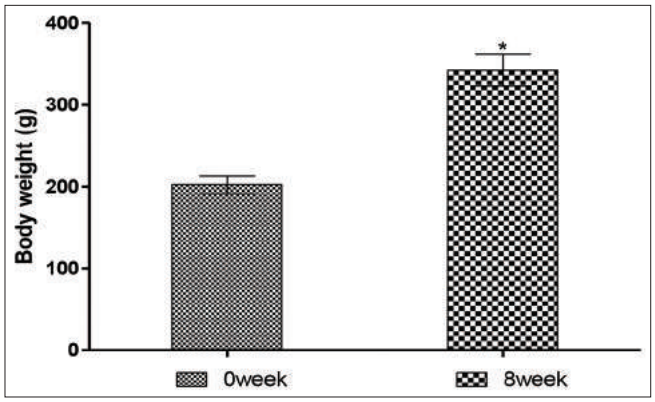


Figure 3: Effect of High-fat diet on body weight. Values were expressed as mean±SD ($n = 6$). *signifies $P < 0.05$ as compared to 0 week (two-way ANOVA followed by Bonferroni's multiple comparison test)

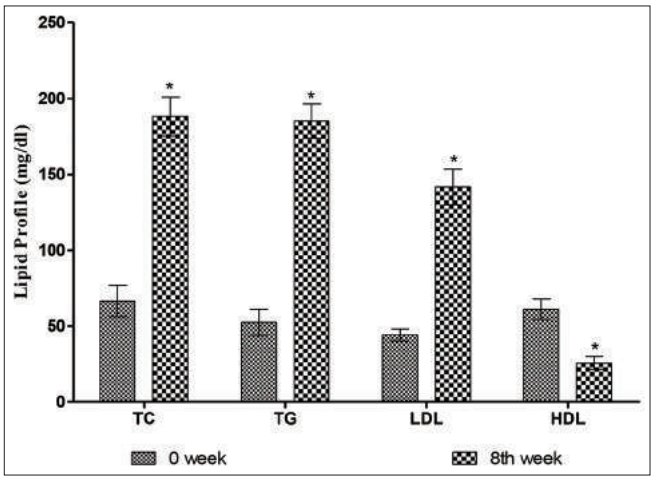


Figure 4: Effect of high-fat diet on lipid profile (total cholestero, triglycerides, low density lipoprotein, high-density lipoprotein). Values were expressed as mean±SD ($n = 6$). *signifies $P < 0.05$ as compared to 0 week (two-way ANOVA followed by Bonferroni's multiple comparison test)

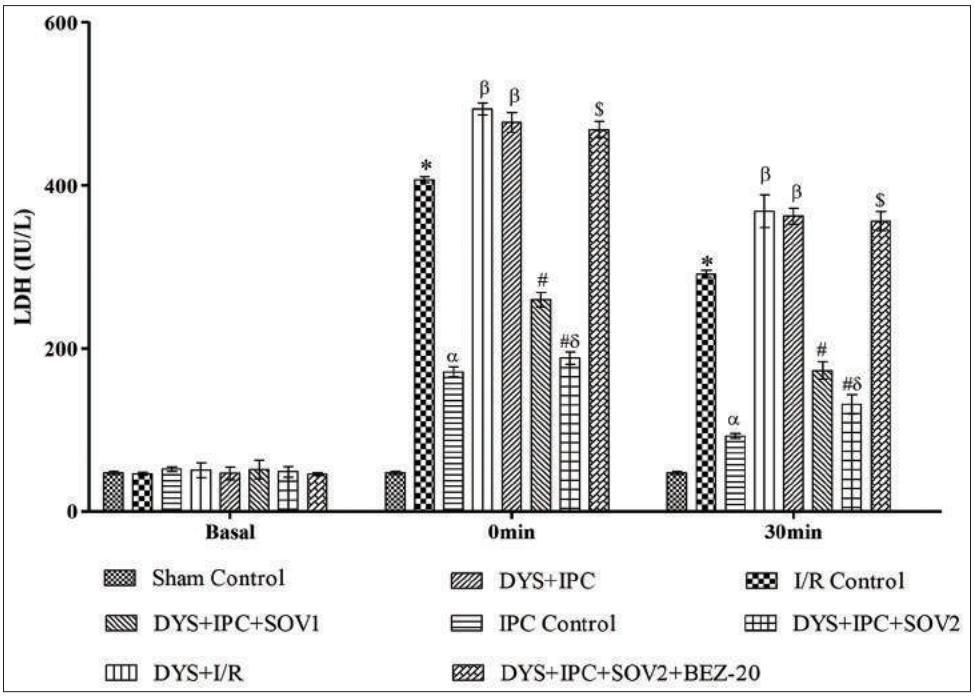


Figure 5: Effect of sodium orthovanadate on lactate dehydrogenase level in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as mean±SD ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (two-way ANOVA followed by Bonferroni's multiple comparison test)

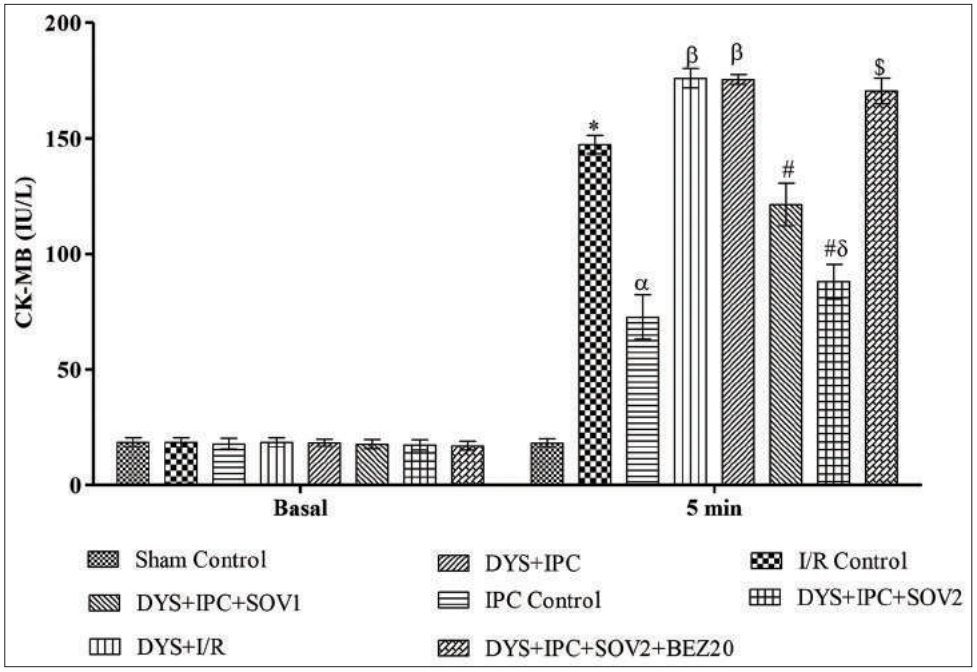


Figure 6: Effect of sodium orthovanadate on creatine kinase-MB level in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as are mean±SD ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (two-way ANOVA followed by Bonferroni's multiple comparison test)

perfusion of BEZ in association with SOV ($P < 0.05$). Four episodes of IPC notably increase the I/R mediated reduced level of nitrite in the normal heart of rat, whereas, no significant alteration was found in nitrite levels of dyslipidemic rat heart. IPC associated increase level of nitrite was expressively restored in SOV-reperfused dyslipidemic heart. Nevertheless, perfusion with BEZ-235 in the stabilization phase markedly attenuated the nitrate level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 8].

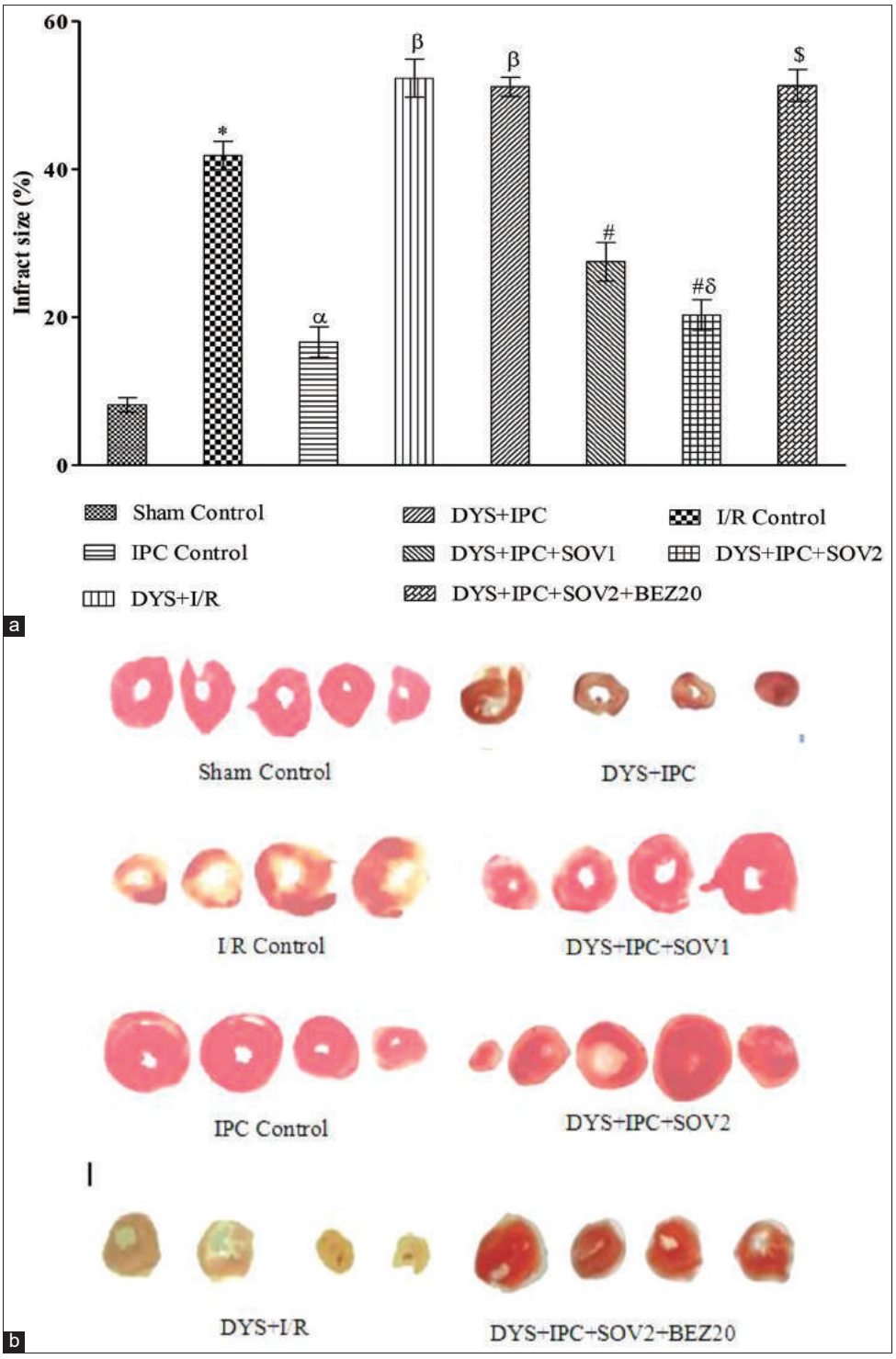


Figure 7: (a) Effect of sodium orthovanadate on infracts size level in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as mean±S.D ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (one-way ANOVA, followed by Tukey's test). (b) Effect of sodium orthovanadate on infracts size level in TTC stained heart sections

Effect of SOV on TBARS in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min followed by a 120 min of reperfusion remarkably elevates the TBARS level when compared with sham control. Furthermore, perfusion with PTP1B inhibitor SOV in dyslipidemic rat heart decreases the production of TBARS, which was opposed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four episodes of IPC notably reduced the I/R mediated increased release of TBARS in the normal heart of rat, whereas, there was no significant alteration which was found in TBARS levels of the dyslipidemic heart. Besides, IPC

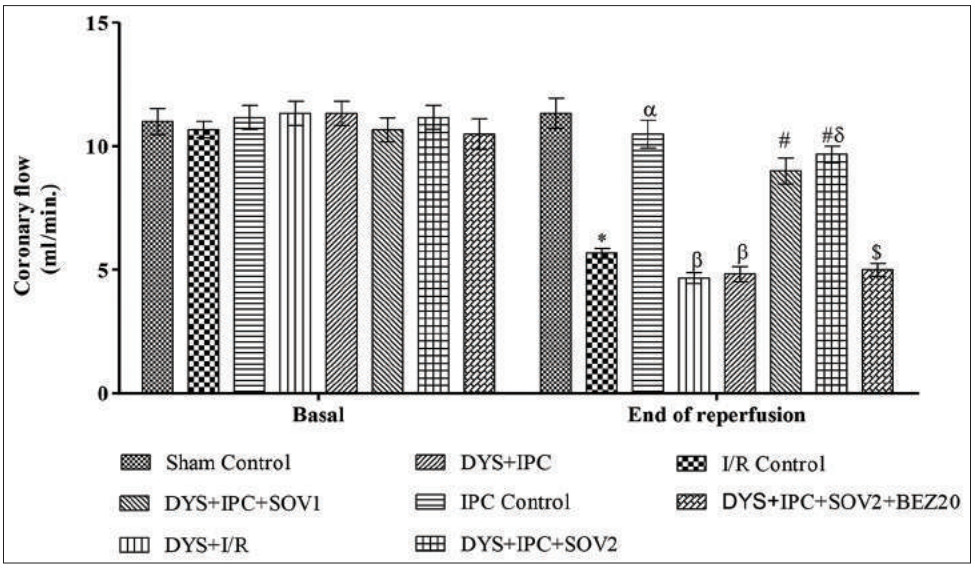


Figure 8: Effect of sodium orthovanadate on coronary flow in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as mean±SD ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (two-way ANOVA, followed by Bonferroni's multiple comparison test)

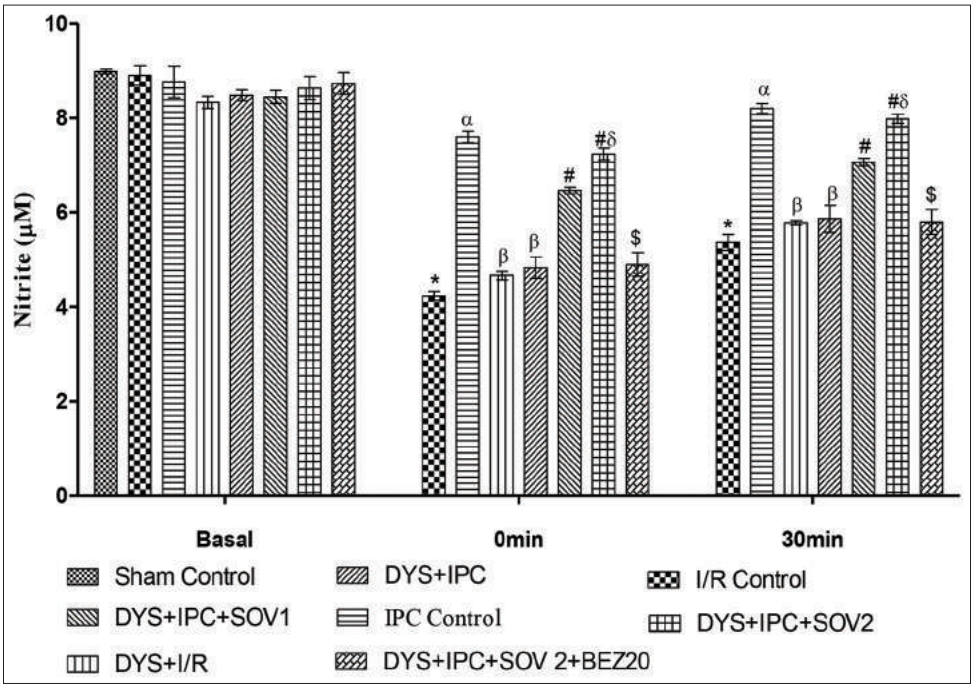


Figure 9: Effect of sodium orthovanadate on nitrite in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as mean±S.D ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (two-way ANOVA, followed by Bonferroni's multiple comparison test)

associated diminish the level of TBARS was expressively restored in SOV-reperfused dyslipidemic rat heart. Although, perfusion with BEZ-235 in the maintenance phase markedly enhanced the TBARS level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 9].

Effect of SOV on TNF-α in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min followed by a 120 min of reperfusion remarkably elevates the TNF-α level when compared with sham control. Furthermore, perfusion with PTP1B inhibitor SOV in dyslipidemic rat heart decreases the release of TNF-α, which was reversed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four episodes of IPC notably reduced I/R mediated increased release of TNF-α in the normal heart of rat, whereas, no significant alteration was found in TNF-α levels of dyslipidemic rat heart. Furthermore, IPC associated drop of TNF-α was expressively re-established in SOV-reperfused dyslipidemic rat heart. However, perfusion with BEZ-235 in the stabilization phase markedly enhanced

the TNF-α level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 10].

Effect of SOV on TNF-α in IPC mediated dyslipidemic rat heart

Global ischemic for 30 min followed by a 120 min of reperfusion remarkably elevates the TNF-α level when compared with sham control. Furthermore, perfusion with PTP1B inhibitor SOV in dyslipidemic rat heart decreases the release of TNF-α, which was reversed by the perfusion of BEZ in association with SOV ($P < 0.05$). Four episodes of IPC notably reduced I/R mediated increased release of TNF-α in the normal heart of rat, whereas, no significant alteration was found in TNF-α levels of dyslipidemic rat heart. Furthermore, IPC associated drop of TNF-α was expressively

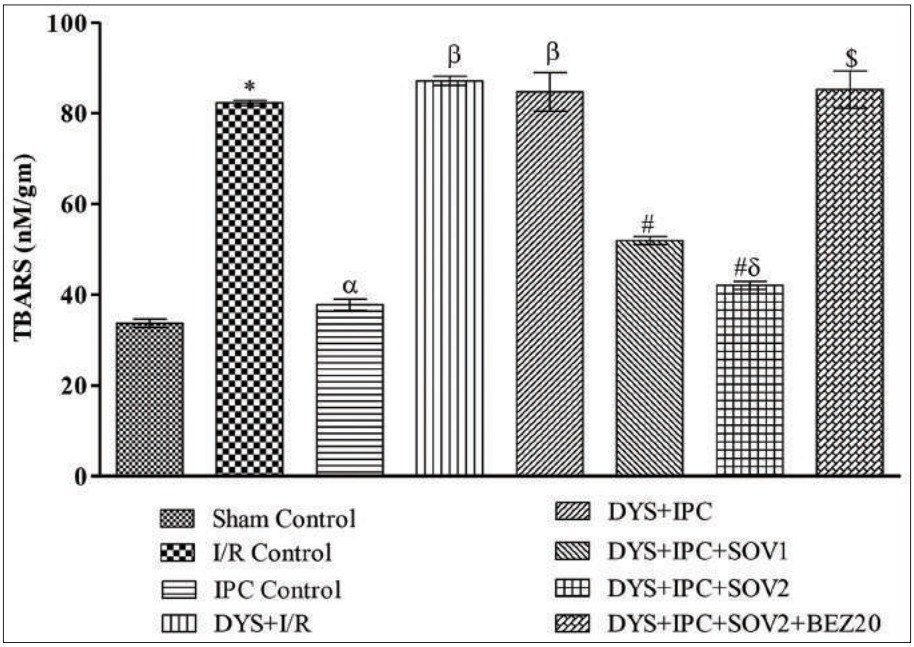


Figure 10: Effect of sodium orthovanadate on thiobarbituric acid reactive substances in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were stated as mean±SD ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (one-way ANOVA, followed by Tukey's test)

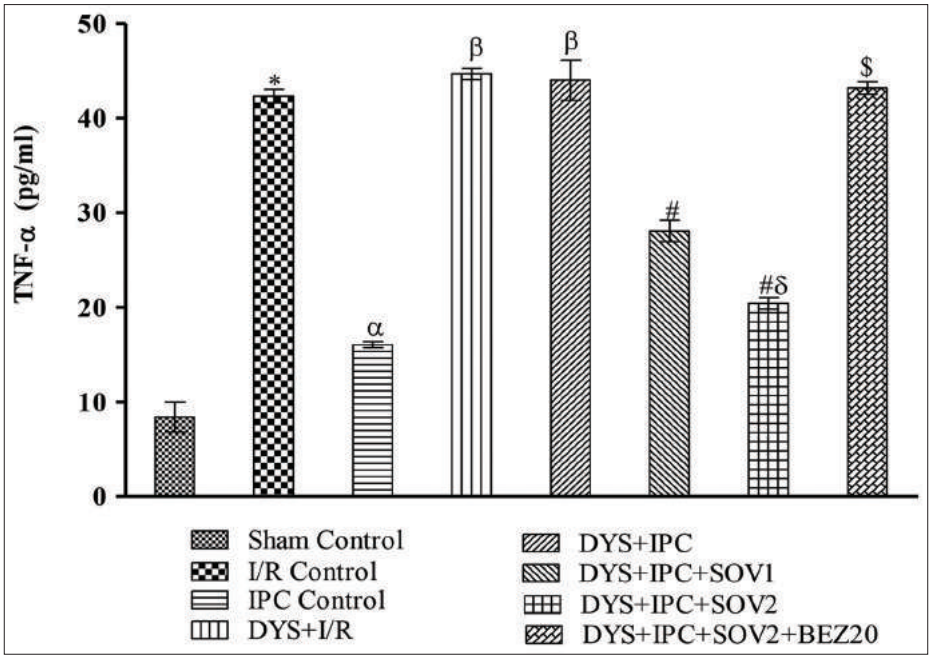


Figure 11: Effect of sodium orthovanadate on tumor necrosis factor -α in ischemic preconditioning (IPC) mediated dyslipidemic rat heart. Values were expressed as mean±SD ($n = 6$). *signifies $P < 0.05$ versus sham control; $^{\alpha}P < 0.05$ versus I/R control; $^{\beta}P < 0.05$ versus IPC control; $^{\#}P < 0.05$ versus DYS+IPC; $^{\#\delta}P < 0.05$ versus IPC+DYS+SOV1; $^{\$}P < 0.05$ versus IPC+DYS+SOV2 (one-way ANOVA, followed by Tukey's test)

re-established in SOV-reperfused dyslipidemic rat heart. However, perfusion with BEZ-235 in the stabilization phase markedly enhanced the TNF-α level in association with SOV perfused dyslipidemic rat heart ($P < 0.05$) [Figure 11]

DISCUSSION

IPC consists of decreasing infarct and offers cardioprotection to the myocardium.[38,39] Principally, it was detected that four intermittent episodes of 5-min ischemia followed by 5-min reperfusion before prolonged ischemic insult could decrease infarct size by 75% against ischemia reperfusion-induced injury in normal rat myocardium.[40] With the help of advanced technology, various efforts made to categorize endogenous mechanisms of preconditioning and its cardioprotective effects by pharmacological agents.

In both experimental and clinical findings, IPC is found to be as an endogenous cardioprotective phenomenon[41-43] through endogenous release of mediators that triggers several signaling mechanisms including the activation of phosphatidylinositol-3 kinase (PI3K)/Akt pathway, generation of NO, activation of mito-KATP channels, and inhibition of the opening of mPTP. Many pharmacological interventions such as an adenosine receptor agonist (2-chloro-N6-Cyclopentyl adenosine), opioid receptor agonist (TAN-67), bradykinin, and erythropoietin, PTEN phosphatase and tensin homolog inhibitors (SF1670), atrial natriuretic peptide, a dopamine agonist (Z1046) produce IPC-like cardioprotection.

In the present study, in both normal and dyslipidemic rat hearts when subjected to experimental I/R induced injury; all the above changes were assessed. IPC reduced the myocardial injury, represented the release of LDH, CK-MB, TBARS, and TNF-α in coronary effluent and in the myocardial infarct size in normal rat heart as compared with I/R injured rat heart.

Dyslipidemia consequently results in atherosclerosis, coronary heart disease (angina pectoris, myocardial infarction or heart attack), and hypertension because of boosted cholesterol levels in sarcolemmal and mitochondrial membranes. The HFD induced dyslipidemia which exhibited raised serum lipids; TGs, clogged arteries, and endogenous cell mediators production in a dyslipidemic state amplifies the myocardial ischemic injury.[44]

In the present study, we also found the elevated levels of LDH, CK-MB, TBARS, TNF-α, and myocardial infarct size and decreased nitrite level in dyslipidemic preconditioned heart. The release of various mediators (eNOS, nucleotidase, peroxynitrite, tetrahydrobiopterin, and superoxide anion) in the dyslipidemic states which distress the preconditioning cascade.[45]

The previous steady finding shows that declined myocardial NO concentration, excessive generation of reactive oxygen species (ROS), enhanced the activation of apoptotic caspase-3, impaired activation of mito-KATP channels and inhibition of mPTP subsidize toward the elimination of the cardioprotective effect of IPC in dyslipidemic rat heart.[46] Among all, because of excessive ROS production a major component of the attenuation of the cardioprotective effect of IPC in dyslipidemic rats.[47,48]

However, few researchers have been found that dyslipidemia mediated enhancement of PTPase level which tends to the downregulation of PI3K/Akt pro-survival pathway, responsible for cell survival. PTP-1B; a non-receptor type of PTPs, is widely investigated in the opposite regulation of TKRS which further downregulates PI3K/Akt signaling and represent as an important target to treat cardiovascular morbidity.[49]

Among the current efforts and interest from pharmacologists, it is feasible that more effective and selective PTP modulators can appear either natural resources or resulting from the structural development of the natural product. The drug discovery in PTPase is a tricky region to work with various pharmaceutical companies and the educational laboratories are focusing their investigation on the way to the development of potential PTP modulators that would show to be a target for the management of the metabolic disorder such as diabetes, dyslipidemia, hypercholesterolemia, hyperhomocysteinemia, and hypertension. One of the specific inhibitors of PTP-1B, SOV exerts ameliorative effects in dyslipidemia.[50] Therefore, we envisage finding out the role of PTP1B inhibitor SOV on the cardioprotective potential of preconditioning in the dyslipidemic heart. In our study, we observed that pharmacological intervention of PTP1B inhibitor (SOV) at 1 mM/L and 2 mM/L[51] perfusion, respectively, was created significant protection (decreased the release of LDH, CK-MB, and reduction in myocardial infarct size) against I/R induced myocardial injury in dyslipidemic rat heart. Whereas, during 10-min stabilization, these effects were further abolished with the use of BEZ-235; a PI3K/Akt pathway inhibitor further results in enhancement of infarct size and increase the production of CK-MB and LDH levels.

Oxidative stress is an indicator of increased lipid peroxidation. The lipid peroxidation measured in terms of TBARS was distinguished to be increased because of I/R induced injury in both normal and dyslipidemic rat hearts.

Myocardial ischemia is caused due to generation of pro-inflammatory cytokine marker; TNF-α, foremost to myocardial dysfunction.[52] Myocytes, mast cells, macrophages, and vascular smooth muscle cells produced TNF-α locally. The generation of TNF-α in I/R induced injury is probably because of the inflammatory process during I/R period and reducing the contractility functions and serving as an initiator for the progression of cardiac cell injury.

The CFR was found to be diminished against ischemic reperfusion (I/R) in normal and dyslipidemic hearts, as compared to basal values. CFR also declined in preconditioned dyslipidemic hearts. Whereas, pre-treatment with SOV before 30-min ischemic insult restored CFR in the dyslipidemic heart in dose graded manner.

All these findings showed that the administration of SOV with preconditioning restore the diminished cardioprotective effect of preconditioning alone in dyslipidemic rat heart and BEZ-235

restricted the cardioprotective effect of SOV when both were used with the combination in dyslipidemic rat heart. The present study shows that the impaired cardioprotective mechanism mediated through IPC in dyslipidemic rat heart may be due to overexpression of PTP-1B which ultimately downregulates the PI3K/AKT signaling and leads to cardiac cell death. Along with it, a result of the current study exposed that SOV may be shown a significant role in cardioprotection by stimulating the PI3K/Akt signaling pathway. Further, research can be directed on pharmacological preconditioning in different pathological states or in aged subjects for it to be rendered clinically applicable.

CONCLUSION

The present study shows that attenuation of the cardioprotective effect of IPC in the dyslipidemic rats maybe because of the overexpression of PTP, which leads to cardiac injury. Based on our observed results, we can confirm that SOV (PTP1B inhibitor) in association with IPC which protects the cardiac cell death from dyslipidemia mediated myocardial injury.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this work.

Shivani Verma: Research Scholar (Performs cardiac surgeries and biochemical estimations).

Himanshi Khera: Research Scholar (Perform infarct size of heart and data interpretation).

Anupam Awasthi: Co-Guide (Mentor to perform surgeries and biochemical estimations).

Dr. Sidharth Mehan: Guide (Original hypothesis and experiment protocol generator).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The exploratory convention was checked on and endorsed by the Regulation Committee (IAEC) (ISFCP/IAEC/CPCSEA/Meeting No: 21/2018/Protocol No: 351) and was carried out in understanding with the rules of INSA for the use and care of testing animals.

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