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Pharmacological Role Of Propylthiouracil In Heavy Metal Induced Ischemia Reperfusion Injury Of Heart

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Abstract
Arsenic toxicity is posing great risk to the world population. Arsenic has been reported to cause various organ damage including blood vessels and heart. Ischemic heart disease is the leading cause of death worldwide. It has been reported that arsenic is associated with the generation of oxidative stress and cardiac impairment but its role in ischemic preconditioning has not been studied yet. It has been long hypothesized that arsenic & cadmium may contribute to the pathogenesis of CVD via the mitochondrial permeability transition due to upregulation of the caveolin protein The present study has been designed to investigate the beneficial role of an antithyroid drug, propylthiouracil in arsenic and cadmium induced-attenuation of ischemic preconditioning (IPC) and ischemic postconditioning (I post). Isolated normal arsenic and cadmium treated rat hearts were subjected to global ishchemia for 30 min, followed by reperfusion for 120 min. Myocardial infarct size was assessed macroscopically using triphenyltetrazolium chloride staining. Coronary effluent was analyzed for Lactate dehydrogenase and Creatine kinase release to assess the extent of cardiac injury. The oxidative stress in the heart was assessed by measuring thiobarbituric acid reactive substances (TBARS), superoxide anion generation and reduced form of glutathione (GSH) in normal arsenic and cadmium treated rat hearts. The arsenic and cadmium treated rat hearts showed enhanced I/R -induced myocardial injury and a high degree of oxidative stress as compared with normal rat hearts subjected to I/R. Four episodes of IPC and I-Post (5 min each) afforded cardioprotection against I/R- induced myocardial injury in normal rat hearts as assessed in terms of improvement in coronary flow rate and reduction in myocardial infarct size, levels of LDH, CK-MB and oxidative stress. On the other hand, IPC and I-Post mediated myocardial protection against I/R were abolished in arsenic and cadmium treated rat hearts. Propylthiouracil an antithyroid drug in a dose of (5mg/kg/day p.o.) for 21 days did not affect the cardioprotective effects of IPC in normal rat hearts but its treatment markedly restored the cardioprotective potential of IPC in arsenic treated rat hearts.

Introduction
Cardiovascular disease (CVD) is an important public health problem, since the last decay, the main causes of CVD death are high blood pressure, smoking, diabetes mellitus, elevated cholesterol levels, and obesity or being overweight.

However, the other causes directly linked to environmental pollutants including polyfluoroalkyl chemicals, acrylamide, and so on as a result of their extensive use in industries, homes, agriculture, and medicine, heavy metals are widely distributed throughout the environment (liu et al 2021). It is reported that most heavy metals are highly toxic. Besides ingestion and inhalation, they can also be absorbed through the skin, causing heavy health effects when in contact with humans.

Arsenic and other (often co-occurring) toxic metals have also been suggested as independent cardiovascular disease risk factors. Lead exposure is strongly linked to an increased risk of hypertension. It can interfere with the renin-angiotensin system, which regulates blood pressure.

Chronic exposure can lead to atherosclerosis (hardening and narrowing of the arteries), increasing the risk of coronary artery disease, heart attacks, and strokes. Lead induces oxidative stress by generating reactive oxygen species (ROS), damaging endothelial cells and contributing to vascular inflammation. Although chronic exposure to arsenic, particularly in drinking water, is associated with an increased risk of ischemic heart disease, hypertension, peripheral artery disease, oxidative stress and inflammation, which contribute to endothelial dysfunction and atherogenesis.

QT interval prolongation on the electrocardiogram, increasing the risk of ventricular arrhythmias and sudden cardiac death (Binu, Priya, Abhilash, Vineetha, & Nair, 2018).

Ischemia-reperfusion injury (IRI) Ischemia-reperfusion injury (IRI) in the heart is a complex pathophysiological process that occurs when blood supply returns to the heart tissue (reperfusion) after a period of ischemia (lack of blood flow). While reperfusion is essential for the survival of ischemic tissue, it can paradoxically cause additional injury to the heart. Here's an overview of the mechanisms, consequences, and potential therapeutic strategies for ischemia-reperfusion injury in the heart (Li, et al., 2017):

Mechanisms of Ischemia-Reperfusion Injury
Mitochondria are particularly susceptible to damage from ROS and calcium overload. Mitochondrial permeability transition pores (mPTP) can open, leading to loss of mitochondrial membrane potential, release of pro-apoptotic factors, and cell death (li et al 2017). Reintroduction of oxygen during reperfusion leads to the rapid production of reactive oxygen species (ROS). ROS cause oxidative damage to lipids, proteins, and DNA, leading to cell death and tissue injury (wang et al 2011). Ischemia disrupts cellular calcium homeostasis, leading to an accumulation of calcium in the cytosol and mitochondria.

Reperfusion exacerbates calcium overload, triggering mitochondrial dysfunction and activation of calpain and other proteases, which can lead to cell death (scichtiano et al 2021). Reperfusion induces an inflammatory response characterized by the activation of endothelial cells, leukocytes, and platelets. Pro-inflammatory cytokines, such as TNF-α and IL-6, contribute to further tissue damage (crurciru et al 2020). Ischemia and reperfusion damage the endothelial lining of blood vessels, impairing vasodilation and increasing vascular permeability. This contributes to edema, leukocyte adhesion, and thrombosis (cry et al 2020). The combined effects of oxidative stress, calcium overload, and mitochondrial dysfunction can lead to both apoptotic (programmed) and necrotic (uncontrolled) cell death (Darcy et al 2019).

Ischemic Preconditioning:
Ischemic preconditioning (IPC) is the phenomenon whereby an organ supposedly becomes more resistant to IRI following multiple reversible episodes of ischemia and reperfusion. This phenomenon was first described by Murry et al., who studied the hearts of seven anesthetized dogs preconditioned with four 5-minute circumflex occlusions, each separated by five minutes of reperfusion, followed by a sustained 40-minute occlusion. 3 Brief periods of ischemia followed by reperfusion before a prolonged ischemic event can make the heart more resistant to subsequent IRI (ventrago brovo 2020).

Hypothyrodism
Thyroid hormones have a variety of effects on the cardiovascular system that can greatly impact cardiac function. Hypothyroidism is associated with decreased cardiac output due to impaired relaxation of vascular smooth muscle and decreased availability of endothelial nitric oxide. This produces a cascade effect of increased arterial stiffness that leads to increased systemic vascular

resistance. On a molecular level, these alterations result from reduced expression of sarcoplasmic reticulum Ca2+-ATPase and increased expression of phospholamban, which inhibits ATPase. Thyroid hormones also impact the renin-angiotensin-aldosterone system. Renin substrates are synthesized in the liver under the stimulus of T3. Thus, in a hypothyroid state, diastolic blood pressure increases, pulse pressure narrows, and renin levels decrease (Udovcic, Pena, Patham, Tabatabai, & Kansara, 2017).

2.0. Material & Methods

2.1- Animals

Adult albino Wistar rats of either sex, weighing (180-250 gm), were employed in the present study (procured from IBRI Bareilly U.P. India). They were maintained on a standard laboratory pellet chow diet (Kisan Feeds Ltd, Chandigarh, India) and water ad libitum. The animals were exposed to 12 h light and 12 h dark cycle. The experiments were conducted between 09:00 and 18:00 in a semi-soundproof laboratory. The protocol of the study was duly approved by IAEC and care of the animals was taken as per CPCSEA, Government of India.

2.2- Drug and chemicals

Propylthiouracil was a gift from macleods pharmaceutical ltd , Arsenic trioxide, Zinc sulfate solution, Greiss reagent, Nitrobluetetrazolium (NBT), Diethylenetriaminepentaacetic acid (DTPA), Pyridine, Dithiobisnitrobenzoate solution, Eserine solution, , Thiobarbituric Acid, n-butanol pyridine mixture, 1,1,3,3- tetramethoxypropane, cyclosporin A (mPTP inhibitor) , daidzein , Trichloro acetic acid, Disodium hydrogen phosphate, Lowry's reagent, Folin- ciocalteu all these chemicals are obtained from sigma Aldrich , sisco research laboratory and CDH.

2.3. Drug Administration

Propylthiouracil, and arsenic trioxide was suspended in CMC (0.5%) and administered orally. Dose of propylthiouracil was selected to be 5mg/kg orally (Hasan et al., 2013). Dose and dosage schedule of arsenic (2 mg/kg, orally daily for 30 days) were selected on the basis of previous research (Mershiba et al., 2013). daidzein at a dose of 25 mg kg−1 bodyweight for 3 days (jia et al 2023). All the chemicals were freshly prepared and were analytical grade.

2.5- Isolated heart preparation

Rats were administered heparin (500 IU/L, i.p) 20 min prior to sacrificing the animal by cervical dislocation. Heart was rapidly excised and immediately mounted on Langendorff's apparatus Isolated heart was retrogradely perfused at constant pressure of 80 mmHg with Kreb's-Henseleit (K-H) buffer (NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5mM; $MgSO_4$ .7H_2O 1.2mM; KH_2PO4 1.2mM; glucose 11mM), pH 7.4, maintained at 37°C bubbled with 95% O_2 and 5% CO_2 . Flow rate was maintained at 7-9ml/min. using Hoffman's screw. The heart was enclosed in double wall jacket and the temperature was maintained at 37°C by circulating water. Global ischemia was produced for 30 min by blocking the inflow of K-H solution followed by 120 min. of reperfusion. Coronary effluent was collected before ischemia, immediately, 5 min. and 30 min. after reperfusion for estimation of lactate dehydrogenase (LDH) and creatine kinase (CK-MB) (Saini et al., 2011).

2.6-Assessmentofmyocardialinfarctsize

Infarct size was measured by macroscopic method using TTC-staining dye and the infracted area reported as the percentage of total ventricular area. Hearts were removed from the Langendorff's apparatus, the auricles and the root of the aorta were excised, and the ventricles were frozen. These were then sliced into uniform sections of 2-3 mm thickness and incubated in 1% triphenyltetrazolium chloride, at 37°C in 0.2M Tris buffer (pH 7.4), for 20 min. TTC was converted to red formazone

pigment by reduced nicotinamide adenine dinucleotide (NADH) and dehydrogenase enzyme and, therefore, stained the viable cells deep red, while the infracted cells remained unstained or dull yellow. The ventricular slices were placed between two glass plates and a transparent plastic grid with 100 squares in 1 cm ^{2} was placed above it. The average area of each slice was calculated by counting the number of squares on either side. Similarly numbers of square falling over non-stained dull yellow area were counted. Infarct size was expressed as percentage of average ventricular area of both slides of slice. Whole of ventricle slices were weighed, infracted dull yellow part was dissected out, weighed and infarct size was expressed as a percentage of total ventricular weight (Yadav et al., 2011)

2.7- Estimation of Lactate dehydrogenase (LDH)

LDH was estimated in coronary effluent collected immediately and 30 min after reperfusion using 2, 4- DNPH method as described by King, 1959. Lactate dehydrogenase catalyzes the reduction of pyruvate by NADH to form lactate and NAD+. The catalytic concentration is determined from the rate of decrease of NADH measured at 340 nm (Taliban et al 2010).

2.8. Estimation of creatine kinase (CK-MB)

CK-MB was measured in coronary effluent after stabilization and 5 min after reperfusion using modified method of Hughes, 1962. The principle is based upon the phenomenon of immunoinhibiting whereby antibody developed against CK-M monomer renders CK-MM activity and inhibits activity of CK-MB by 50%. The CK method is used to estimate CK-B activity quantitatively, then CK-MB activity is obtained by multiply CK-MB activity by two (xing et al 2022).

2.9- Biochemical parameters

2.9.1- Collection of Blood sample

Blood samples for biochemical estimation were collected by retroorbital bleeding. The blood was kept at room temperature for 30 min and then centrifuged at 4000 rpm for 15 min to separate serum which was then used for biochemical estimation. After retro-orbital bleeding, animals were sacrificed by cervical dislocation; and heart tissue was carefully removed for the estimation of superoxide anion, and various biochemical estimations.

The removed hearts were homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer and centrifuged at 3000 rpm for 15 min to obtain the clear supernatant. This clear supernatant was removed carefully from the centrifugation tube and it was then used for different biochemical estimations.

2.9.2- Measurement of thyroid hormones (T_4)

Plasma T_4 quantitative measurements were performed with ELISA, using kits obtained from S.R Diagnostic Patel Nagar Meerut (No 1100 for total T_4), as previously described (Mourouzis et al., 2005). T_4 levels were expressed as ng/ml of plasma. Absorbance measurements were performed at 450 nm with Tecan Genios ELISA reader (Tecan, Austria).

2.9.2- Estimation of super oxide anion generation

The superoxide anion was determined spectrophotometrically at 540 nm. Briefly, the heart was cut into transverse section and placed in 5 ml of K-H solution buffer containing 100 mmolL^{-1} of nitroblutetrazolium (NBT) and incubated at 37ºC for 1.5 h. NBT reduction was stopped by addition 5mL of 0.5 molL-1 HCl.

The heart was minced and homogenized in a mixture of 0.1molL^{-1} NaOH and 0.1% SDS in water containing 40 mgL^{-1} diethylentriaminepentaaceticacid. The mixture was centrifuged at 20,000g for 20 mins, the resultant pellet was resuspended in 1.5 mL of pyridine and kept at 80^{0}C for 1.5 h to extract formazon. The mixture was centrifuged at l0, 000 g for 10 min and the absorbance of the formazon was determined spectrophotometrically at 540 nm.
The amount of reduced NBT was calculated using the following formula,
Amount of reduced NBT = A x V / (T x M x ε x l)
where A is the absorbance, V is the volume of pyridine, T is the time for which the rings was incubated with NBT, M is the blotted wet mass of the heart, ε is the extinction coefficient (0.72 l/mmolmm^{-1}), and l is the length of the light. Results were expressed as reduced NBT picomoles per min per mg of wet tissue (Balakumar et al., 2008; wang et al., 1998)

2.9.4- Estimation of thiobarbituric acid reactive substances (TBARS)
The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in heart was performed. 0.2 ml of supernatant of homogenate was pipette out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid and the volume was made up to 4 ml with distilled water.

The test tubes were incubated for 1 h at s95^{0}C, then cooled and added 1 ml of distilled water followed by addition of 5 ml of n-butanolpyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 g for 10 min.

The absorbance of developed pink color was measured spectrophotometrically. A standard calibration curve was prepared using 1-10nM of 1,1,3,3 -tetramethoxypropane. The TBARS value was expressed as nanomoles per gram of wet tissue wt (Okhawa et al., 1979; Balakumar et al., 2008).

Preparation of Reagents
Preparation of Sodium Dodecyl Sulphate Solution 810 mg of sodium dodecyl sulphate was dissolved in 10 ml of distilled water.
Preparation of 30% Acetic Acid Solution
30 ml of acetic acid was diluted to 100 ml with distilled water and pH was adjusted to 3.5 with saturated solution of sodium hydroxide using pH meter.
Preparation of 0.8% Thiobarbituric Acid Solution
400 mg of thiobarbituric acid was dissolved in 50 ml of warm distilled water.
Preparation of 15:1 v/v n-butanol-pyridine mixture
90 ml of n-butanol was mixed with 6 ml of pyridine.
Preparation of 1 Nm 1, 1, 3, 3-Tetramethoxy Propane
0.82 ml of standard 1, 1, 3, 3-tetramethoxy propane was diluted to 5 ml with distilled water to make 1 M solution. 1 ml of this dilution was further diluted to 10 ml with distilled water and this dilution process was further repeated for eight times to get 1 nM 1, 1, 3, 3-tetramethoxy propane.

2.9.5- Estimation of reduced glutathione (GSH)
The reduced glutathione (GSH) content in heart was estimated spectrophotometrically at 412 nm. Briefly, the supernatant of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 1000 g for 10 min at 4^{0}C. The supernatant obtained (0.5 ml) was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25 ml of 0.001 M freshly prepared DTNB [5, 5`-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added and absorbance was noted spectrophotometrically at 412 nm. A standard curve was plotted using 5-50µM of reduced

form of glutathione and results were expressed as micromoles of reduced glutathione per gm of wt tissue weight (Balakumar et al., 2008; Beutler et al., 1963).
Preparation of 10% Trichloroacetic Acid
10 g of trichloroacetic acid was dissolved in 100 ml of distilled water.
Preparation of 0.3 M Disodium Hydrogen Phosphate
4.26 g of anhydrous disodium hydrogen phosphate was dissolved in 100 ml distilled water.
Preparation of 5, 5`-Dithiobis (2-Nitrobenzoic Acid) In 1% Sodium Citrate
7.92 mg of 5, 5`-dithiobis (2-nitrobenzoic acid) was dissolved in 20 ml of 1% sodium citrate.
Preparation of 100 µM of Reduced Glutathione 6.14 mg of reduced glutathione was dissolved in 200 ml distilled water.

2.10- Experimental Protocol
In total 54 wistar rats of either sex were employed in the study which were divided into 9 groups of 6 animal each

Group I-Normal control group
Isolated rat heart was perfused continuously for 200 min without subjecting them to global ischemia and reperfusion.

Group II- Vehicle control group
Rats were administered CMC (0.5%) for 30 days at day 31 these rats were sacrificed and heart was isolated and perfused for 200 min with K-H solution after 30 min of stabilization

Group III-Ischemia reperfusion injury group
Isolated rat heart preparation after 10 min of stabilization was perfused for 50 min with K-H buffer solution. Then the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion.

Group IV-Ischemic Preconditioning group
After 10 min of stabilization, the isolated normal rat heart was perfused for 10 min with K-H solution. The heart was then subjected to four episodes of 5 min global ischemia, followed by 5min of reperfusion to produce IPC. After four episodes of IPC, the heart was subjected to 30 min of global ischemia, followed by 120 min of reperfusion

Group V- Arsenic + Ischemia reperfusion injury group
Rats were administered arsenic trioxide (2mg/kg/day p.o.) for 30 days, at day 31 these rats were sacrificed by cervical dislocation and the heart was isolated rat heart preparation after 10 min of stabilization was perfused for 50 min with K-H buffer solution. Then the preparation was subjected to 30 min global ischemia followed by 120 min of reperfusion

Group V- Arsenic + Ischemic preconditioning group
Rats were administered arsenic trioxide (2mg/kg/day p.o.) for 30 days, at day 31 these rats were sacrificed by cervical dislocation and the heart was isolated and after 10 min of stabilization the arsenic treated rat heart was perfused with K-H solution for 10 min. The heart was then subjected to IPC as in group 7. After four episodes of IPC, the heart were subjected to 30 min of global ischemia, followed by 120 min of reperfusion.

Group VI- PTU treated + Ischemic preconditioning group

Rats were administered normal saline alone for 9 days and with PTU treatment (5mg/kg/day p.o.) from day 10th to 30th days. At day 31 these rats were sacrificed by cervical dislocation and the heart was isolated and after 10 min of stabilization was perfused for 10 min with K-H solution. The heart was then subjected to IPC as in group 7. .After four episodes of IPC, the heart were subjected to 30 min of global ischemia, followed by 120 min of reperfusion

Group VI- PTU treated arsenic trioxide + Ischemic preconditioning group
Rats were administered arsenic trioxide (2mg/kg/day p.o.) alone for 9 days and with PTU treatment (5mg/kg/day p.o.) from day 10th to 30th days. At day 31 these rats were sacrificed by cervical dislocation and the heart was isolated and after 10 min of stabilization was perfused for 10 min with K-H solution. The heart was then subjected to IPC as in group 7. .After four episodes of IPC, the heart were subjected to 30 min of global ischemia, followed by 120 min of reperfusion

Group VII- PTU treated arsenic trioxide + Daidzein +Ischemic preconditioning group
Rats were administered arsenic trioxide (2mg/kg/day p.o.) alone for 9 days and with PTU treatment (5mg/kg/day p.o.) from day 10th to 30th days and daidzein at a dose of 25 mg/ kg/ bodyweight for last 3 days. At day 31 these rats were sacrificed by cervical dislocation and the heart was isolated and after 10 min of stabilization was perfused for 10 min with K-H solution. The heart was then subjected to IPC as in group 7, After four episodes of IPC, the heart were subjected to 30 min of global ischemia, followed by 120 min of reperfusion

2.11- Statistical analysis
All values were expressed as mean ± standard deviation (S.D). Statistical analysis was performed using Sigma stat Software. The values of infarct size, LDH and CK-MB and T4 were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test as a post hoc test. Value of $P < 0.05$ was considered to be statistically significant

Induced cardiac injury by Arsenic and Cadmium
Rats were administered arsenic trioxide (2mg/kg/day p.o.) for 30 days (yia et al 2019) and cadmium chloride (2.5mg/kg/day p.o.) for 30 days for respective group to generate cardiotoxicity, at day 31 these rats were sacrificed by cervical dislocation and the heart is isolated and after 10 min of stabilization was perfused for 40 min with Kreb's Henseliet solution (NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5mM; $MgSO_4.7H_2O$ 1.2mM; KH_2PO4 1.2mM; glucose 11mM). The heart was then subjected to 30 min of ischemia followed by 120 min of reperfusion.

Result & Discussion
Effect of 21 days administration of Propylthiouracil on Serum T4 level
The T4 levels in nM for normal v/s PTU treated hypothyroid rat were found to be 46.47±0.73 v/s 19.23±0.39

Effect on Myocardial infarct size
Global ischemia for 30 min followed by 120 min of reperfusion markedly increased the myocardial infarct size as compared to normal control group. Four episodes of IPC before ischemia and four episodes of I-Post after ischemia significantly reduced the I/R induced increase in myocardial infarct size in normal rat heart.

However induction of cardiotoxicity with arsenic significantly attenuated the myocardial infarct size i,e. abolishes the cardioprotective effect of ischemic preconditioning in normal rat heart. Treatment

with antithyroid drug propylthiouracil markedly decrease the myocardial infarct size in normal rat heart subjected to I/R but has no additional effect on IPC.

However, PTU treatment along with daidzein significantly abolish the restored effect of IPC in arsenic and treated rat heart. See fig no.1,

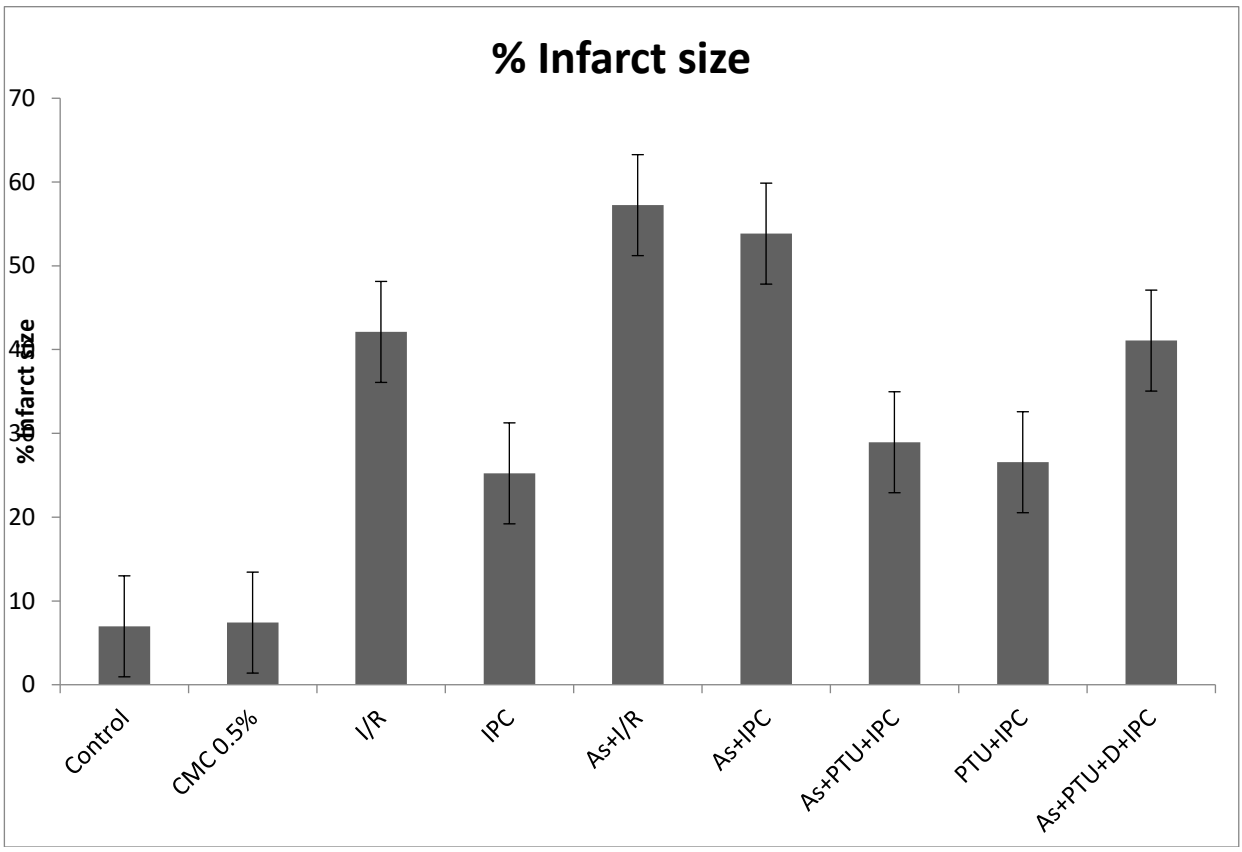

Figure 1. Myocardial infarct size in normal group

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *^{a}p<0.05 vs control: ^{b}p<0.05vs I/R respectively ^{c}p<0.05vs IPC:, ^{d}p<0.05vs PTU+IPC:

Effect on LDH and CK-MB in coronary effluent

Global ischemia for 30 min followed by 120 min of reperfusion markedly increased the release of LDH and CK-MB as compared to normal control group. Four episodes of IPC before ischemia ischemia significantly reduced the I/R induced increase in the release of LDH and CK-MB in coronary effluent in normal rat heart.

However induction of cardiotoxicity with arsenic significantly attenuated the decrease in the release of LDH and CK-MB i,e. abolishes the cardioprotective effect of ischemic preconditioning in normal rat heart. Treatment with antithyroid drug propylthiouracil markedly decrease the level of LDH and CK-MB in coronary effluent in normal rat heart subjected to I/R but has no additional effect on IPC.

However, PTU treatment significantly restored the effect of IPC and I-Post in arsenic and cadmium treated rat heart. See fig no. 2

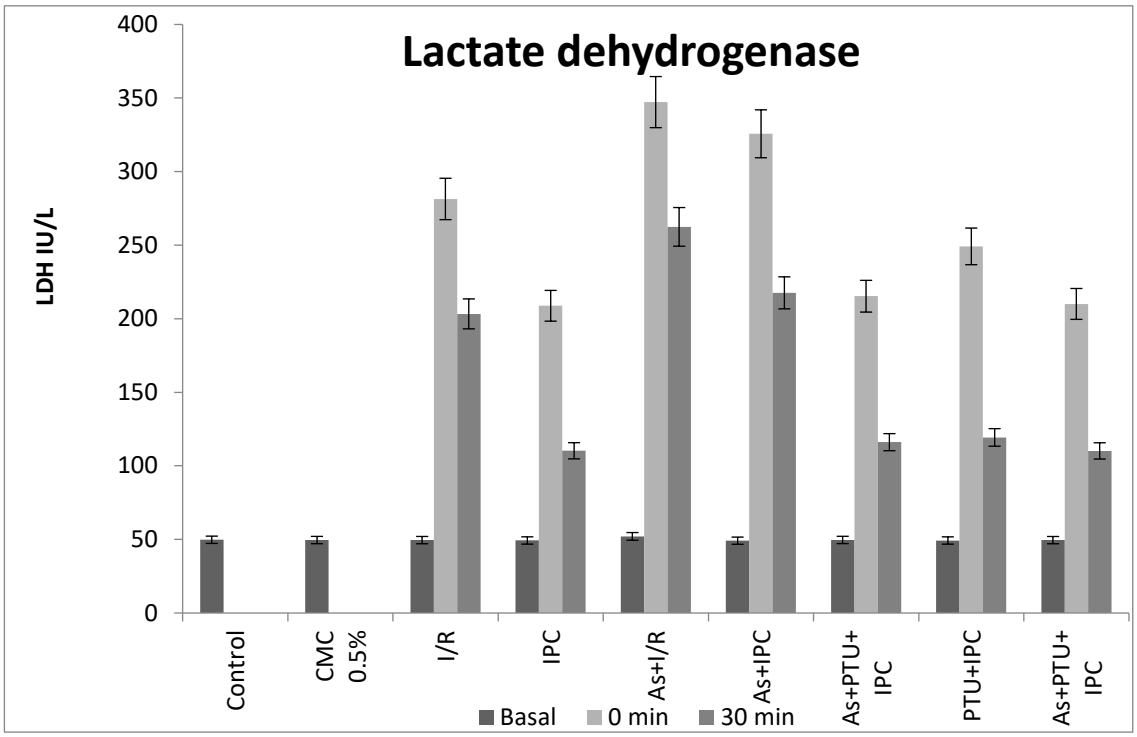

Figure 2: Level of lactate dehydrogenase (LDH) in coronary effluent

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *[a]p<0.05 vs control basal , 0 minute, 30 min respectively: [b]p<0.05vs I/R basal , 0 minute, 30 min respectively [c]p<0.05vs IPC: basal , 0 minute, 30 min respectively, [d]p<0.05vs PTU+IPC: basal , 0 minute, 30 min respectively

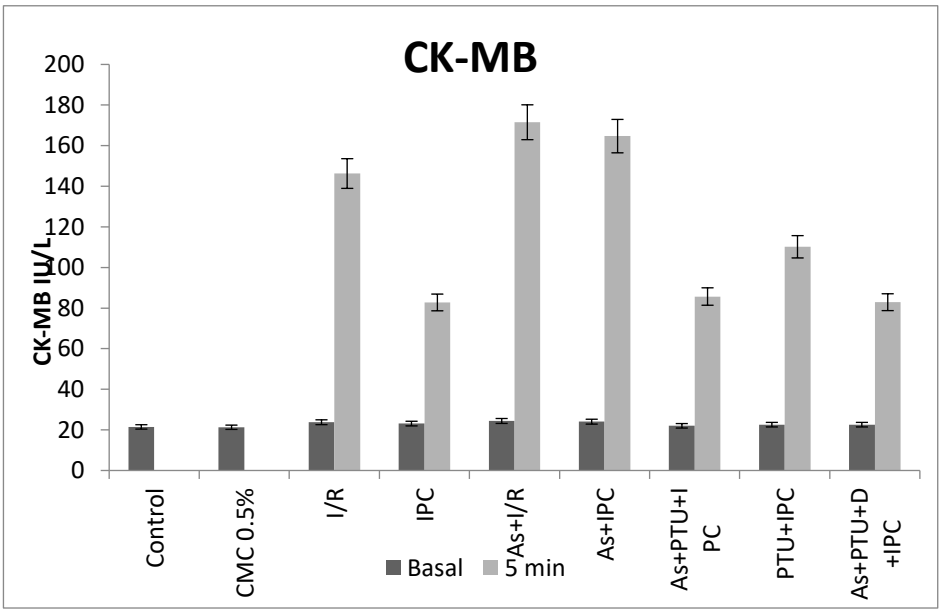

Figure 3: Level of Creatinine kinase (CKMB) in coronary effluent

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *[a]p<0.05 vs control basal , 5 minute, respectively: [b]p<0.05vs I/R basal , 5 minute, respectively: [c]p<0.05vs IPC: basal 5 minute, respectively:, [d]p<0.05vs PTU+IPC: basal , 5 minute, respectively:

Effect on coronary flow rate

Global ischemia for 30 min followed by 120 min of reperfusion markedly decreased the coronary flow rate as compared to normal control group. Four episodes of IPC before ischemia significantly increased the I/R induced decrease in coronary flow rate in normal rat heart. However induction of cardiotoxicity with arsenic and cadmium significantly attenuated the increase in coronary flow rate i,e. abolishes the cardioprotective effect of ischemic preconditioning and postconditioning in normal rat heart. Treatment with antithyroid drug propylthiouracil markedly increase the coronary flow rate in normal rat heart subjected to I/R but has no additional effect on IPC. However, PTU treatment significantly restored the effect of IPC and I-Post in arsenic and cadmium treated rat heart as shown by marked increase in coronary flow rate. See fig no. 3

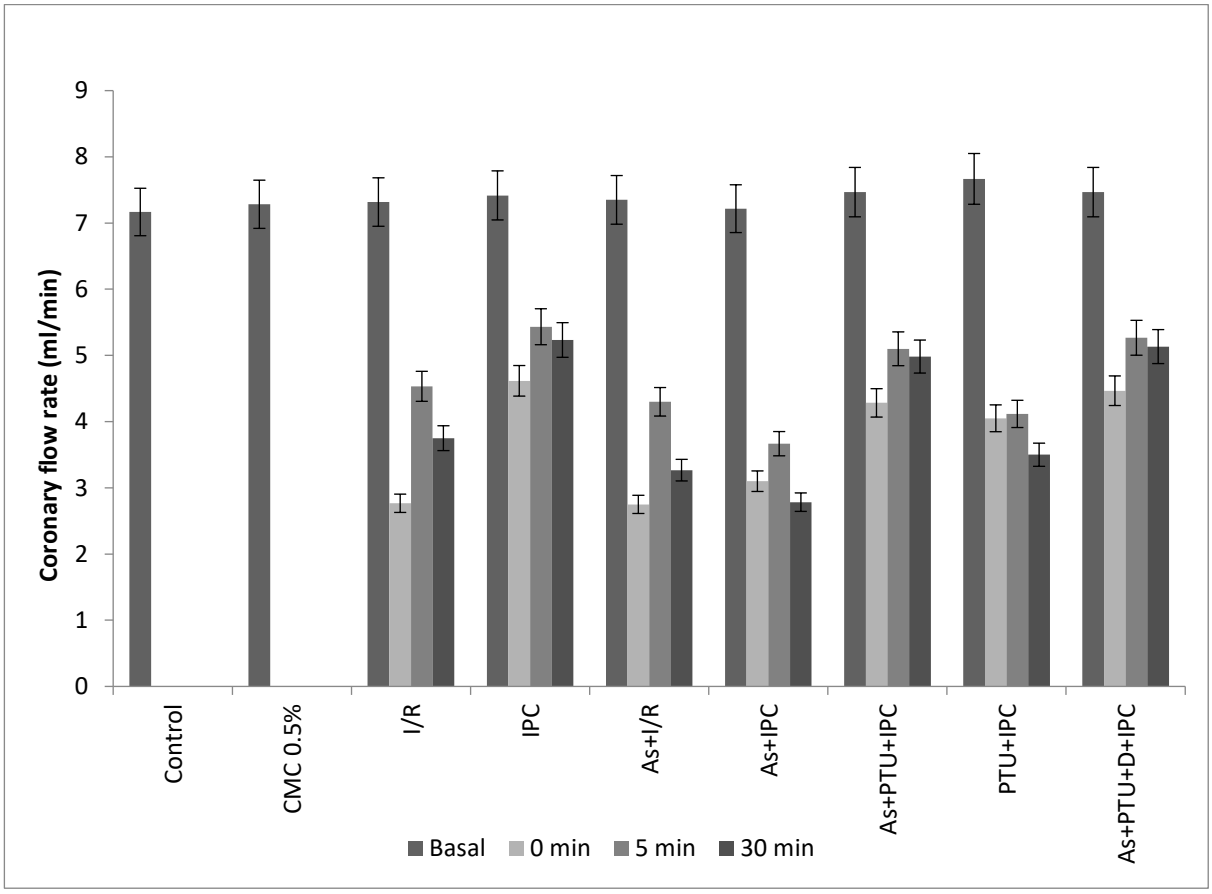

Figure 4: Level of coronary Flow rate (ml/min) in coronary effuent

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *^{a}p<0.05 vs control basal , 0 minute, 5 minute, 30 min respectively: ^{b}p<0.05vs I/R basal , 0 minute, 5 minute, 30 min respectively ^{c}p<0.05vs IPC: basal , 0 minute, 5 minute, 30 min respectively, ^{d}p<0.05vs PTU+IPC: basal , 0 minute, 5 minute, 30 min respectively

Effect on oxidative stress

Global ischemia for 30 min followed by 120 min of reperfusion markedly increases the oxidative stress as compared to normal control group as assessed in terms of increase in TBARS, superoxide anion generation and decrease in GSH. Four episodes of IPC before ischemia and four episodes of I-Post after ischemia significantly decrease the level of TBARS and superoxide anion generation and

increase the level of GSH. However induction of cardiotoxicity with arsenic and cadmium significantly attenuated the decreased oxidative stress i,e. abolishes the cardioprotective effect of ischemic preconditioning and post conditioning. Treatment with antithyroid drug propylthiouracil markedly decrease the oxidative stress in normal rat heart

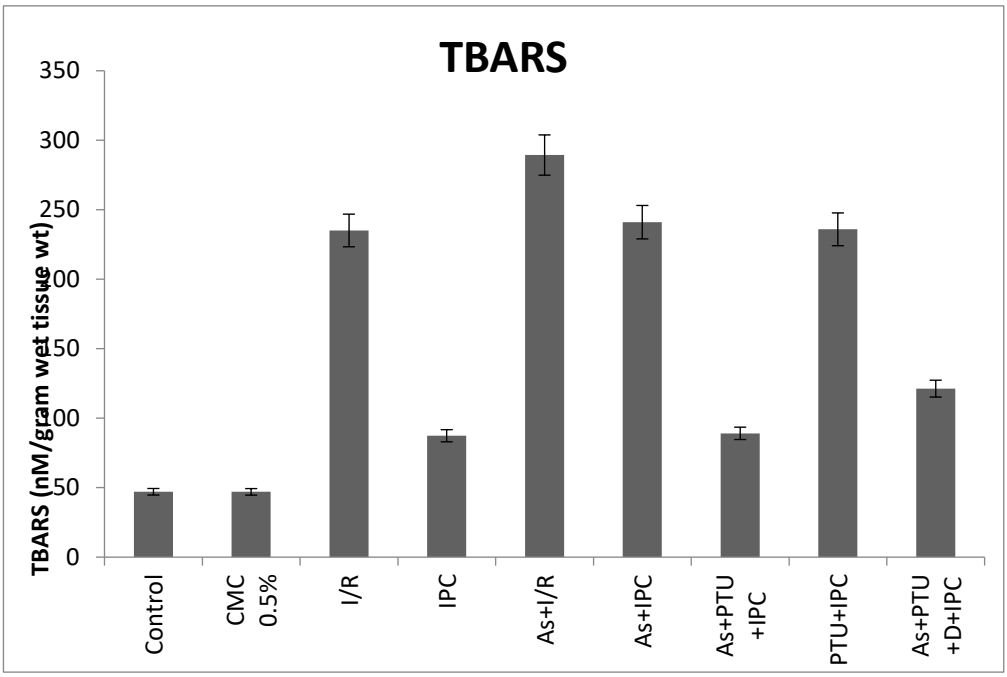

Figure 5. **Level of TBARS in coronary effluent**

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *^{a}p<0.05 vs control: ^{b}p<0.05vs I/R respectively ^{c}p<0.05vs IPC:, ^{d}p<0.05vs PTU+IPC:

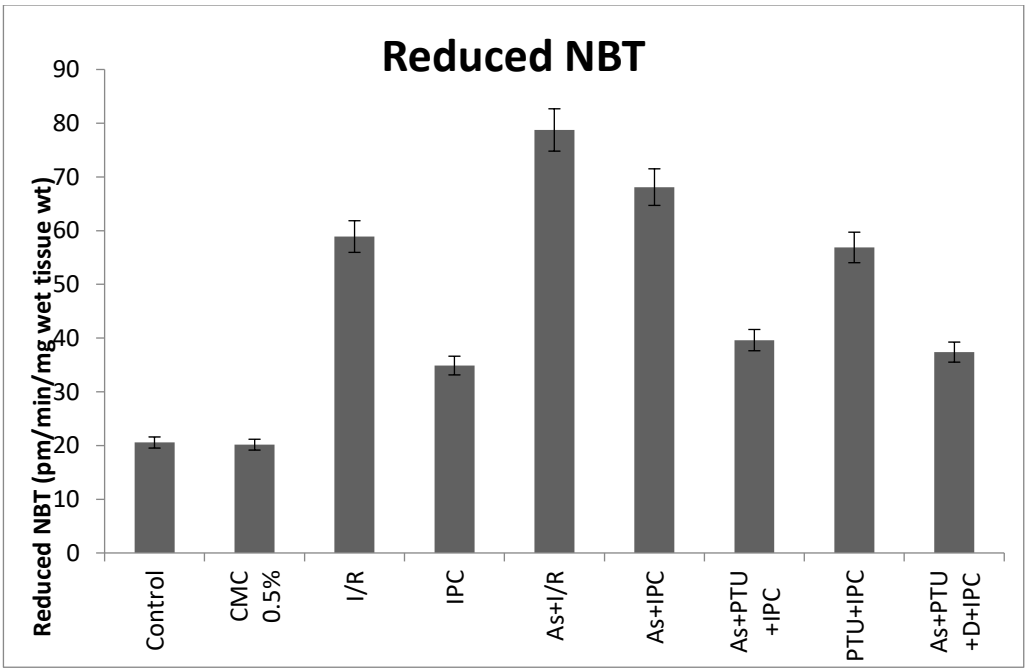

Figure 6. Level of reduced NBT in coronary effluent

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *[a]p<0.05 vs control: [b]p<0.05vs I/R respectively [c]p<0.05vs IPC:, [d]p<0.05vs PTU+IPC

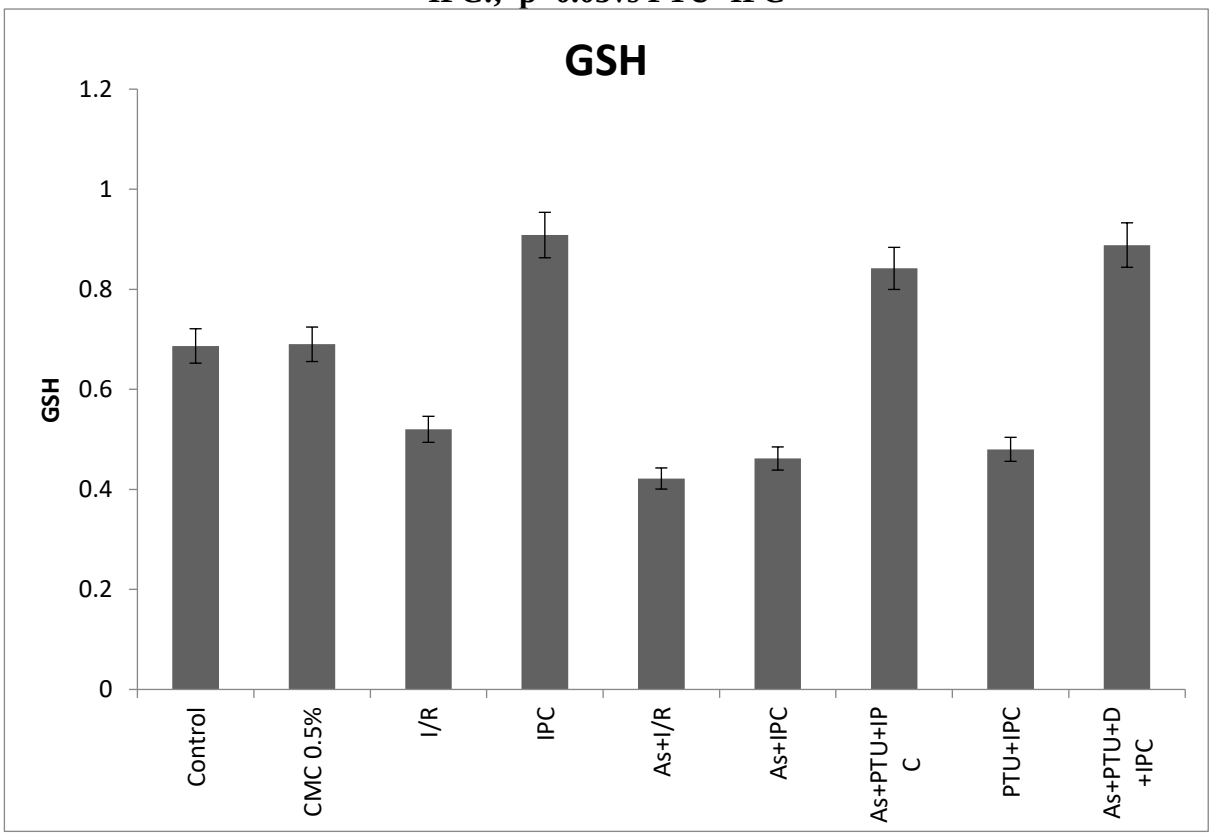

Figure 7. **Level of GSH in coronary effluent**

Note : CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *[a]p<0.05 vs control: [b]p<0.05vs I/R respectively [c]p<0.05vs IPC:, [d]p<0.05vs PTU+IPC

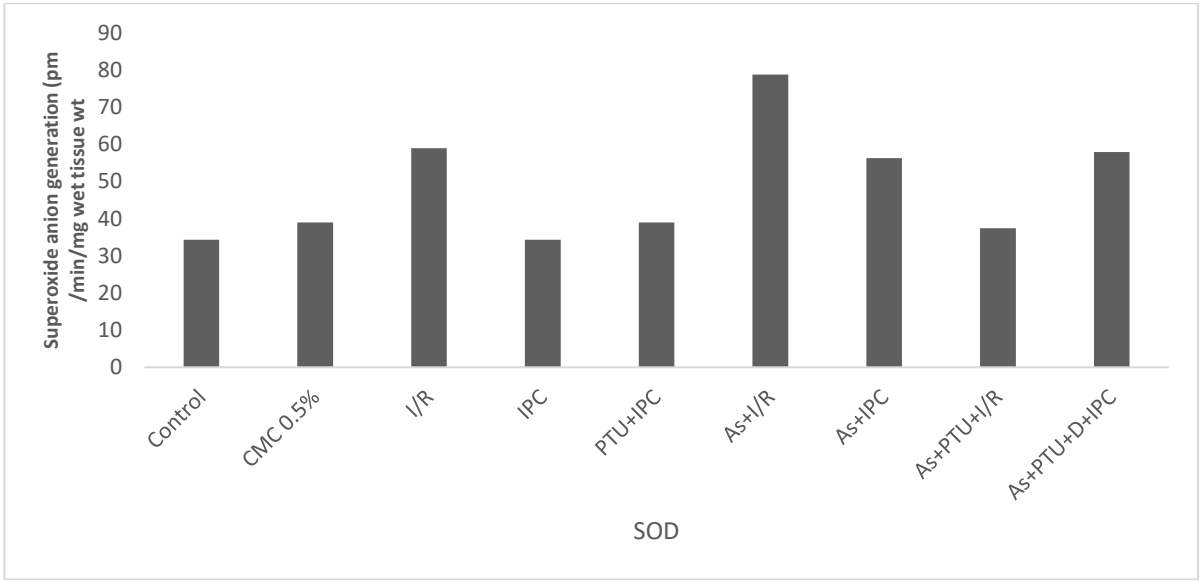

Figure 8. Level of S Superoxide anion generation (pm /min/mg wet tissue wt.) in coronary effluent

Note: CMC(Carboxy methyl cellulose), I/R (Ischemia reperfusion injury), IPC(Ischemic Preconditioning), As(Arsenic), PTU(propylthiouracil), D (Daidzein): Value were expressed in mean ±SD for each group (n=6) *[a]p<0.05 vs control: [b]p<0.05vs I/R respectively [c]p<0.05vs IPC:, [d]p<0.05vs PTU+IPC

Discussion

The magnitude of CK-MB elevation has been shown to correlate strongly with infarct size. LDH is an enzyme that increases in myocardial infarction after reperfusion, which may be due to sustained ischemic injury, the increase in infarct size and the release of LDH and CK-MB are an index of I/R induced myocardial injury (Singh et al., 2008). The maximal release of LDH was noted immediately and 30 min after reperfusion whereas the peak release of CK-MB was noted 5 min after reperfusion which are in agreements with the results of previous studies (Taliyan et al., 2011). In the present study I/R has shown a significant increase in cardiac markers like LDH and CK-MB and myocardial infarct size with a high degree of oxidative stress and significant reduction in coronary flow rate. Preconditioning has significantly attenuated the I/R induced increase in LDH, CK-MB, infarct size and oxidative stress and decrease in coronary flow rate. Administration of arsenic trioxide has significantly impaired the preconditioning and induced protection of myocardium by I/R injury.

propylthiouracil (selective thyroperoxidase inhibitor) has significantly restored the protective effect of preconditioning and postconditioning in I/R injury of heart which has impaired by administration of arsenic. The mechanism underlying ischemia reperfusion injury involves, upregulation of the caveolin protein that indirectly increased resistance to the opening of mitochondrial permeability transition pores (mPTP) (Pantos et al., 2003c

Treatment with propylthiouracil (PTU), selective thyroperoxidase inhibitor (5mg/kg/day P.O.) did not alter the cardio protective effect of IPC in normal rat hearts but PTU treatment significantly restored the cardioprotective effect of IPC in arsenic treated rat hearts subjected to I/R. In this regard, sudden reoxygenation occurring when the vessels are opened causes considerable cell injury produced by the action of oxygen-derived reactive species, generated mainly by mitochondria (García 2005; Munzel 2010), associated to the Ca^{2+} overload which contributes to the myocardial insult. Increasing evidence suggests that heart injury by reperfusion results from mitochondrial Ca^{2+} overload, oxidative stress, adenine nucleotide depletion, elevated phosphate concentration, and depolarization which in turn induce the increase of nonspecific permeability (Halestrap et al., 2003). As reported, mitochondria from hypothyroid rats are resistant to permeability transition induced by Ca^{2+} (Edmundo et al., 2011). In this sense, myocardial tissue from PTU hypothyroid rats is also resistant to the damage exerted by reperfusion after an ischemic period (Bobadilla et al., 2001).

Daidzein is a caveolin inhibitor that potentially abolish the PTU treated rat hearts shows marked reduction in myocardial injury as shown by the decrease in LDH and CK-MB, decrease in infarct size and oxidative stress and increase in coronary flow rate in ischemia reperfusion induced rat heart. But preconditioning exerts no additional effect in PTU treated hypothyroid rat hearts.

Conclusion

The attenuated cardioprotective effect of ischemic preconditioning and by arsenic trioxide treated rat heart subjected to ischemia reperfusion was restored by the administration of propylthiouracil the mechanism involve in the cardioprotective, was the upregulation of the caveolin cellular protein. That inversely give the resistance to the mitochondrial permeability transition pore (mPTP), that increase the ca^{+2} load to the myocardium cell Experimental HT models are described as a reduction of Ca2+ transport in cardiac cell mitochondria and suggest decreased uniplex expression, low [Ca2+]c, preventing high resting Ca2+, and as a cooperative activator when [Ca2+]c increases, ensuring rapid mitochondrial Ca2+ accumulation, thus stimulating different metabolic pathways

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