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Review Article

A REVIEW ON CELASTRUS PANICULATUS WILLD.

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ABSTRACT

Celastrus paniculatus linn. belongs to the family Celastraceae commonly known as Malkanguni. It is found as a large climbing shrub with yellow fruits in the sub himalayan tract & all over hilly parts. It contains carboxylic acids, fatty acids, sterols, triterpenoids, etc. According to the ayurvedic system of medicine it is used to remove 'Vata' and 'Kapla'. Malkanguni oil is reported to be toxic to rats. LD50 i.p. of the malkanguni oil is 1.75 gm/kg. It enriches blood and cures abdominal complaint. It is also used as appetizer, laxative, emetic, aphrodisiac, powerful brain tonic, in diarrhoea & dysentery. Seeds supposed to have good stimulating properties, for sharpening the memory & used in rheumatism, gout and leprosy. The bark is abortifacient, depurative and brain tonic, leaves are emmenagogue, and leaf juice is used in dysentery. It is reported to be a good antidote for opium poisoning.

Key words: *Celastrus paniculatus*, Malkanguni, brain tonic, emmenagogue, abortifacient.

Introduction:

Ischemic heart disease is the leading cause of mortality and morbidity worldwide (Bernard *et al*; 2010). Myocardial ischemia occurs due to inadequate blood flow to the heart (micheal *et al*; 2006). Reperfusion after a prolonged period of ischemia damages the myocardium rather than restoration of normal cardiac function and it is known as ischemia reperfusion injury (kloner *et al*; 1993). Ischemic preconditioning originally described by Murry and coworkers as the protection

conferred to ischemic myocardium by preceding brief periods of sublethal ischemia separated by periods of reperfusion (Murry *et al*; 1986). The IPC mediated cardioprotection is biphasic. The early phase (classical IPC) is immediated in onset and lasts 2-3hr (Murry *et al*; 1991,Van winkle *et al*; 1991, Burckhartt *et al;* 1995). The cardioprotective responses , reappears after 12-24h, lasts for 3-4 days (Kuzuya *et al*; 1993) and is known as late phase of IPC. Early phase of IPC is mediated through different existing cellular kinases .i.e. protein kinase C(PKC) , Glycogen synthase kinase 3β (GSK3β) (Yadav *et al* ; 2010) . Late phase of cardioprotection by synthesizing new proteins viz. inducible nitric oxide synthatase (NO), (Guo *et al*; 1999) aldose reductase (Shinmura *et al*; 2002), superoxide dismutase (yamashita *et al*; 1994) and cyclooxygenase-2 (COX-2) (Guo *et al*; 2000). The signaling steps in IPC involves activation of nitric oxide synthase (NOS) by phosphorylation, with the production of NO and subsequent activation of guanylyl cyclase, production of cGMP, activation of protein kinase G, opening of mitochondrial K_{ATP} channels and generation of reactive oxygen species (Cohen *et al*; 2006).

Ischemic preconditioning is attenuated in hyperlipidaemic rat heart (yadav *et al;* 2010). Moreover, the impaired activation of mito K_{ATP} channels (Katakam *et al ;*2007) and a decrease in ecto-5′-nucleotidase activity may be responsible for attenuation of the cardioprotective effect of preconditioning (Ueda *et al;* 1999). Ecto-5′-nucleotidase is a key enzyme for synthesizing adenosine and plays an important role in ischemic preconditioning (Taichi Sakaguchi *et al*;2000).

HO-1 is localized in the inner leaflet of the plasma membrane. Activation of .haemoxygenase (HO-1) produces cardioprotective during IPC (Wang *et al*; 2010). During hyperlipidemia, activity of HO-1 is reduced (William Durante *et a;* 2011). It has been reported that adenosine is a upstream pathways of HO-1 and it enhance the activity of haemoxygenase-1 (Glia *et al*; 2008). The activity of Adenosin is diminished in hyperlipidemia (Schwemmer *et al;* 2000). It has been noted that adenosine exerts its cardioprotective effects of IPC through its binding with A1 receptors in rats (Singh *et al* ; 2012).

Therefore, the present study has been design to investigate the involvement of adenosine and heme-oxygenase-1 in attenuated

cardioprotective effect of ischemic preconditioning in hyperlipdemic rar heart.

Materials and Methods

The experimental protocol used in the present study was approved by Institutional Animal Ethical Committee.

Reagents and Chemicals

Serum total cholesterol and triglycerides estimation kits was purchased from Span diagnostics ltd., Surat, India. N6-(2-phenylethyl)adenosine (10µg/kg/i.p) and zinc protophorphyrin (50µg/kg/i.p) (Sigma Aldrich, Ltd., Banglore, India) was dissolved in DMSO and injected 24 hr before isolation of heart. L-NAME (30µM/L) (Sigma Aldrich, Ltd., Banglore, India) was dissolved in K-H buffer and perfused to isolated rat heart. The 1% w/v solution of TTC Stain (CDH Pvt. Ltd., New Delhi) was prepared in Tris-choloride buffer (CDH Pvt. Ltd., New delhi) was used to measure infarct size. The LDH enzymatic estimation kit was purchased from Span diagnostics Ltd., Surat, India and CK-MB enzymatic estimation kit was purchased from Coral Clinical Systems, Goa, India. For the estimation of nitrite level, sulphanilamide (CDH Pvt. Ltd., New Delhi), phosphoric acid (CDH Pvt. Ltd., New Delhi), N-(1-Naphthyl) ethylene ediamine dihydrochloride (Himedia Laboratories Pvt. Ltd., Mumbai) and standard solution nitrite (CDH Pvt. Ltd., New Delhi) were used. All other reagents used in this study were of analytical grade and always freshly prepared before use.

Induction of Experimental Hyperlipidaemia

Wistar rats (180-300 g) of either sex were employed in present study. Experimental hyperlipidaemia was produced by feeding cholesterol 1 g, cholic acid 0.5 g) for 8 weeks (Lorkowska, et al., 2006; Reeves, 1997). Hyperlipidaemia was documented by estimating the level of total cholesterol (TC) and triglycerides (TG) in serum using commercially available kits (Vital Diagnostics (P) Ltd., Mumbai, India).

high fat diet (corn starch 44.74 g, casein 14 g, sucrose 10 g, butter 20 g, fiber 5 g, mineral mix 3.5 g, vitamin mix 1 g, choline 0.25 g, terbutylhydroquinone 0.0008 g,

Isolated Rat Heart Preparation

Heparin (500 U; i.p.) was administered about 20 min before sacrificing the animal by cervical dislocation. Heart was rapidly excised and immediately mounted on Langendorff's apparatus (Langendorff, 1895). The heart was enclosed by double-walled jacket, the temperature of which was

maintained by circulating water heated to 37ºC. The isolated heart was retrogradely perfused at a constant perfusion pressure of 80 mm Hg with Kreb's Henseleit (K-H) solution (NaCl, 118mM; KCl, 4.7 mM; $CaCl_2$ 2.5 mM; $MgSO_4.7H_2O$, 1.2 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM and $C_6H_{12}O_6$, 11 mM), pH 7.4, maintained at 37°C and bubbled with carbogen (95% oxygen and 5% carbondioxide). Coronary flow was maintained at 7-9 ml/min using Hoffman's screw. Global ischemia was produced for 30 min by blocking the inflow of Kreb's Henseleit solution and was followed by reperfusion for 120 min. Coronary effulent was collected before ischemia; immediately, 5 min and 30 min of reperfusion for estimation of lactate dehydrogenase (LDH) and Creatine kinase (CK-MB) (Singh and Sharma *et al*).

Assessment of Myocardial Injury

NITRITE ESTIMATION

Nitrite is stable nitrogen intermediate formed from the spontaneous degradation of production (Marletta et al., 1988). Nitrite release in coronary effluents was measured (Szabo et al., 1993a, Szabo et al., 1993b). Griess reagent 0.5 ml (1:1 solution of 1% sulphnilamide in 5% phosphoric acid and 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride in water) was added to 0.5 ml of coronary effluent.The optical density at 550nm was measured using spectrophotometer (UV-1700 spectrophotometer, Shimadzu, Japan). Nitrite concentration was calculated by comparison with spectrophotometer reading

To determine the extent of myocardial injury, the release of LDH and CK-MB in coronary effluents was measured using commercially available kit (Span diagnostics Ltd., Surat, India). Values were expressed in international units IU per liter.

Myocardial infarct size

The heart was removed from the Langendorff apparatus. Both the atria and root of aorta were excised and ventricles were kept overnight at -40C. Frozen ventricles were sliced into uniform sections of 1-2 mm thickness. The slices were incubated in 1% TTC at 37ºC in 0.2 MTr buffer pH 7.4 for 30 min (Fishbein et al., 1981). The normal myocardium was stained brick red while the infarcted portion remained unstained. Infarct size was measured by the volume method (Chopra et al., 1992).

NO. Unlike NO , nitrite can be measured easily and nitrite concentration can be used to infer levels of NO

of standard solution nitrite prepared in K-H buffer (Parikh and singh, 1999b).

EXPERIMENTAI PROTOCOL

A diagrammatic representation of experimental protocol is shown in Fig. 1. In all groups, the isolated rat heart was perfused with K-H solution and allowed for 10 min of stabilization.

Group 1: (Sham Control; n=6): Isolated rat heart preparation was stabilized for 10 min. and then perfused continuously with K-H buffer solution for 190 min. without subjecting them to global ischemia and reperfusion.

Group 2: (Ischemia-Reperfusion Control; n=6): Isolated rat heart preparation was allowed to stabilize for 10 min. and then heart preparation from hyperlipidemic rat was allowed to stabilize for 10 min, and subjected to four cycles of ischemic preconditioning, each cycle comprised of 5 min. ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected to 30 min. global ischemia followed by 120 min. of pretreated hyperlipidemic rat was allowed to stabilize for 10 min. and then subjected to four cycles of ischemic preconditioning, each cycle comprised of 5 min. ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected perfused continuously with K-H buffer solution for 40 min. Then it was subjected to 30 min. global ischemia followed by 120 min. of reperfusion.

Group 3: (Ischemic Preconditioning Control; n=6): Isolated rat heart preparation was allowed to stabilize for 10 min. and subjected to four cycles of ischemic preconditioning each cycle comprised of 5 min. global ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected to 30 min. global ischemia followed by 120 min. of reperfusion.

Group 4: (Ischemic Preconditioning in Hyperlipidemic Rat Heart; n=6): Isolated

reperfusion. Group 5: (Ischemic Preconditioning in N6-(2- phenylethyl) adenosine (10µg/kg/i.p) Pretreated Hyperlipidemic Rat Heart; n=6): N6-(2-phenylethyl)adenosine dissolved in DMSO and injected 24 hours before isolation of heart. Isolated rat heart preparation from N6-(2-phenylethyl)adenosine (10µg/kg/i.p) to 30 min. global ischemia followed by 120 min. of reperfusion.

Group 6: (Ischemic Preconditioning in Zinc protoporphyrin (50µg/kg/i.p) and N6-(2-phenylethyl) adenosine (10µg/kg/i.p) Pretreated Hyperlipidemic Rat Heart; n=6):

Zn protophorphyrin and N6-(2-phenylethyl) adenosine was dissolved in DMSO and injected 24 hours before isolation of rat heart. Isolated rat heart preparation from Zn protophorphyrin and N6-(2 phenylethyl) adenosine pretreated hyperlipidemic rat was allowed to stabilize for 10 min. and then subjected to four cycles of ischemic preconditioning, each cycle comprised of 5 min. ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected to 30 min. global ischemia followed by 120 min. of reperfusion.

Group 7: (Ischemic Preconditioning in N6-(2-phenylethyl)adenosine (10µg/kg/i.p) Pretreated and L-NAME (30µM/L) Perfused Hyperlipidemic Rat Heart; n=6): L-NAME, a nitric oxide synthase inhibitor (30µM/L) was perfused for 30 min in N6-(2-phenylethyl) adenosine pre-treated hyperlipidemic rat heart significantly attenuated restoration of N6-(2-phenylethyl)adenosine pretreatment induced cardioprotection. Hyperlipidemic rat was allowed to stabilize for 10 min. and then subjected to four cycles of ischemic preconditioning, each cycle comprised of 5 min. ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected to 30 min. global ischemia followed by 120 min. of reperfusion.

Group 8: (Ischemic Preconditioning in N6-(2-phenylethyl)adenosine (10µg/kg/i.p) and Zinc protoporphyrin (50µg/kg/i.p) Pretreated and L-NAME (30µM/L) Perfused Hyperlipidemic Rat Heart; n=6): Hyperlipidemic rat heart treated by Znpp (50µg/kg/i.p) and N6-(2-phenylethyl) adenosine (30µM/L) 24h before the isolation of heart. Heart was perfused with L-NAME, a nitric oxide synthase inhibitor (30µM/L) and was subjected to four cycles of ischemic preconditioning, each cycle comprised of 5 min. ischemia followed by 5 min. reperfusion with K-H solution. Then the preparation was subjected to 30 min. global ischemia followed by 120 min. of reperfusion.

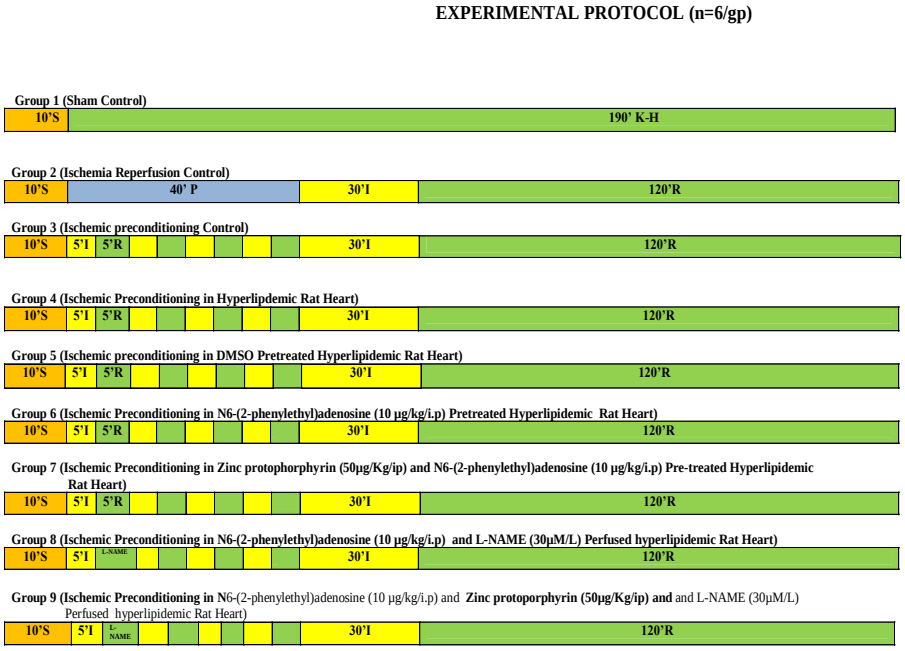

Fig.1. Diagrammatic representation of experimental protocol.

2.7: Statistical Analysis

All the values were expressed as mean ± standard deviation (S.D.). Statical analysis was performed by using Sigmastat and Graphpad software. Data obtained from the various groups were significantly analysed using one way analysis of variance (ANOVA) $P < 0.05$ was considered to be significantly static.

3: Results

Effect of high fat diet on body weight:

By feeding high fat diet for 8 weeks to the rats, there is significant increase in body weight as compared to basal value (fig 4.1).

Effect of high fat diet on serum cholesterol and triglyceride level

By feeding high fat diet for 8 weeks to the rats, there is significant increase in serum cholesterol and serum triglyceride level as compared to basal value (fig. 4.2).

Effect of Ischemia and Reperfusion on Myocardial Injury

Global ischemia of 30 min. followed by reperfusion of 120 min. significantly increased the myocardial infarct size, release of LDH and CK-MB as compared to sham control group (fig. 4.3,4.4,4.5).

Effect of Ischemic Preconditioning in N6-(2-phenylethyl)adenosine (10µg/kg/i.p) pretreated hyperlipidemic rat heart

Administration of N6-(2-phenylethyl)adenosine (10µg/kg/i.p) 24 hr before isolation of heart, followed by four Moreover, N6-(2-phenylethyl)adenosine pre-treatment significantly increased the

Effect of ischemic preconditioning on normal and hyperlipidemic rat heart

Four episodes of IPC significantly reduced ischemia-reperfusion induced increase in myocardial infarct size and release of LDH and CK-MB in the normal rat heart. However, ischemic preconditioning mediated cardioprotection measured in terms of myocardial infarct size and release of LDH and CK-MB was significantly attenuated in hearts obtained from hyperlipidemic rats (fig 4.3,4.4,4.5). IPC significantly attenuate the ischemia-reperfusion induced decrease in release of nitric oxide measured in terms of nitric oxide measured in terms of nitrite in coronary effluent of the normal rat heart. However, ischemic preconditioning did not significantly attenuate the ischemia-reperfusion induced decrease in nitrite release in coronary effluent of hyperlipidemic rat heart (fig 4.9).

episode of IPC significantly restored the attenuated cardioprotection noted in terms of decrease in myocardial infarct size and the release of LDH and CK-MB levels in coronary effluent (fig. 4.6,4.7,4.8).

release of nitrite in coronary effluent as compared to untreated hyperlipidemic rat heart (fig.4.10).

Effect of Ischemic Preconditioning in Zinc protoporphyrin(50µg/kg/i.p) and N6-(2-phenylethyl)adenosine(10µg/kg/i.p) pretreated hyperlipidemic rat heart

Administration of Zinc protoporphyrin(50µg/kg/i.p) and N6-(2-phenylethyl) adenosine (10µg/kg/i.p) 24 hr before isolation of heart, followed by four episodes of IPC, Znpp almost completely attenuated ischemic preconditioning induced increase in myocardial infarct size and the release of LDH and CK-MB (fig. 4.6,4.7,4.8) and decrease in nitrite release in coronary effluent obtained from N6-(2-phenylethyl)adenosine pretreated hyperlipidemic rat heart (fig. 4.10).

Effect of perfusion of L-NAME (30µM/L) in N6-(2-phenylethyl)adenosine (10µg/kg / i.p) pretreated hyperlipidemic rat heart

30 min perfusion of L-NAME, a nitric oxide synthase inhibitor (30µM/L) in N6-(2-phenylethyl)adenosine pre-treated hyperlipidemic rat heart significantly attenuated restoration of N6-(2-phenylethyl)adenosine pretreatment induced cardioprotection noted in terms of increase in myocardial infarct size and LDH and CK-MB levels in coronary effluent (fig 4.6,4.7,4.8). Moreover, N6-(2-phenylethyl)adenosine induced restoration of the release of nitrite in coronary effluent of hyperlipidemic rat heart was significantly suppressed by perfusion of L-NAME (fig 4.10).

Effect of perfusion of L-NAME (30µM/L) in N6-(2-phenylethyl)adenosine (10µg/kg/ i.p) and Zinc protoporphyrin(50µg/kg/i.p) pretreated hyperlipidemic rat heart

30 min perfusion of L-NAME, a nitric oxide synthase inhibitor (30µM/L) in N6-(2-phenylethyl)adenosine and Zinc protophorphyrin pre-treated hyperlipidemic rat heart significantly attenuated restoration of N6-(2-phenylethyl)adenosine pretreatme-nt induced cardioprotection noted in terms of increase in myocardial infarct size and LDH and CK-MB levels in coronary effluent (fig 4.6,4.7,4.8). Moreover, N6-(2-phenylethyl)adenosine induced restoration of the release of nitrite in coronary effluent of hyperlipidemic rat heart was significantly suppressed by perfusion of L-NAME (fig 4.10).

Discussion

Wistar rats of either sex were employed in the present study because of their small size, low cost and easy availability. The isolated rat heart preparation perfused retrogradely on langerdroff's apparatus was employed because changes in systemic circulation do not affect working of this preparation (Verdouw *et al*., 1998). Langerdroff's preparation and working heart preparation are hemodynamically comparable to investigate the effects of pharmacological interventions on ischemia and reperfusion-induced myocardial injury (Neely and Rovetto *et al.,* 1975).

Myocardial ischemia is due to inadequate blood flow to the heart (Michael *et al*., 2006). Moreover, early reperfusion is necessary to restore the normal physiology of heart (Collard and Gelman *et al*., 2001). The ischemic preconditioning induced by four episodes of 5 min. global ischemia followed by 5 min. reperfusion is reported to produce cadioprotective effect in isolated rat heart preparation (Fralix *et al*., 1993; Parikh and singh, 1998,1999a; Sharma and Singh,2001; Yadav *et al*., 2010). Therefore same protocol is employed in the present study. It has been reported that maximum release of LDH occurred immediately after reperfusion and peak release of CK-MB occurred 5 min. after reperfusion (Parikh and singh, 1998,1999; Sharma and Singh *et al*.,2001). Therefore, samples of coronary effluent were collected at these time intervals to estimate the amount of LDH and CK-MB release which was measured spectrophotometrically using commercial kits. In present study infarct size has been measured macroscopically using triphenyltetrazolium chloride (TTC) staining (Banka *et al*., 1981). The NADH and dehydrogenase enzymes present in viable myocardium convert triphenyltetrazolium chloride (TTC) staining it deep red colour (Nachalas and Schnitka *et al* ., 1963). However, infarcted cells lose dehydrogenase enzyme and cofactor NADH and thus remained unstained or dull yellow (Fishbein *et al*., 1981). The increase in infarct size and release of LDH and CK-MB are documented on be index of ischemia-reperfusion induced myocardial injury (Sharma and Singh *et al.*, 2000). Nitrite is stable nitrogen intermediate formed from the spontaneous degradation of NO. Therefore, samples of coronary effluents were collected at particular time intervals to estimate the nitrite release. Nitrite concentration can be used to infer the concentration of NO (Marletta *et al*., 1988; Parikh and Singh *et al*., 1999b).

Hyperlipidemia was produced by feeding high diet (corn starch 44.74g, casein 14g, sucrose 10g, butter 20g, fiber 5g, mineral mix 3.5g, vitamin mix 1g, choline 0.25g, terbutylhydroquinone 0.0008g. cholic acid 0.5g) for 8 weeks (Lorkowska, et al.,2006; Reeves, 1997). Rats with blood serum cholesterol level in between 800-1000mg/dl and serum triglyceride level 200-300 mg/dl after 8 weeks of administration of high fat diet were considerd to be hyperlipidemic.

Nitric oxide (NO) plays an important role in the regulation of redox signaling and cellular function (Hare *et al*., 2005). NO can be generated by nitric oxide synthase (NOS) or by the breakdown of nitrite or other compounds to NO (Duranski *et al*., 2005). Both endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively expressed in distinct subcellular locations within cardiomyocytes. Endothelial nitric oxide synthase (eNOS) is generally thought to be predominantly localized to invaginations of the sarcolemma called caveolae (Harrison *et al*., 2002) whereas nNOS is mostly found in the sarcoplasmic reticulum (SR) (Barouch *et al*., 2002). Caveolae are flask-like investigations of the plasma membrane enriched with cholesterol, sphingolipids, and the marker protein, caveolin which play an important role in cellular signaling (Patel *et al*., 2008). Increased expression of caveolin lead to the, decreased phosphorylation of endothelial nitric oxide synthase and consequent decreased generation of nitric oxide (Feron *et al*., 1996). Further, it has been reported that expression of caveolin is upregulated in hyperlipidemia (Bucci *et al*., 2004; Penumathsa *et al*., 2008a). Thus it may facilitate the binding with eNOS which decreases the availability of nitric oxide (Feron and Balligand *et al*., 2006). Moreover, the level of NO gets diminished into hyperlipidemic heart (Feron *et al*., 1999). It has been reported that NO is responsible for cardioprotection effect of ischemic preconditioning (Prendes et al., 2007).

In the present study, cardioprotective effect of ischemic preconditioning was attenuated in hyperlipidemic rat heart. This is in agreement with earlier published studies (Kersten *et al*., 2000; Del Valle *et al*., 2002; Tsang *et al*., 2005; Wynne *et al.*, 2007; Gross *et al*., 2007; Yadav *et al*., 2010,2012) that release of nitric oxide during the ischemic preconditioning has been reported to produce cardioprotection against ischemia-reperfusion induced injury (Prendes *et al*., 2007; Sun and Murphy., 2010). In our result four episodes of ischemia and reperfusion, significantly

increased the release of NO (measured in coronary effluent) as compared to ischemia reperfusion control group. However, IPC mediated increase in release of nitric oxide got significantly impaired in hyperlipdemic rat heart. It may be probable to say that increased caveolin expression and subsequently decrease the level of nitric oxide is responsible for attenuation of cardioprotective effect of IPC in hyperlipidemic rat heart. This is in accordance with our previous studies (Ajmani *et al*., 2011).

Heme-oxygenase is the rate limiting enzyme in the biochemical pathway responsible for catabolism of heme into ferrous (Fe^{+2}) ion, carbon monoxide, and biliverdin, the later being subsequently converted into bilirubin by biliverdin reductase (Ryter *et al*., 2006). Heme-oxygenase-1 is localized in the membrane caveolae of the plasma membrane where it is interacts with caveolin (Kim *et al*., 2004). It has been reported that, in the transgenic mice, the overexpression of heme-oxygenase-1 shows decreased expression of caveolin (Penumathsa *et al*., 2008b). It has been reported that a decrease the expression of HO-1 exacerbates (Liu *et al*., 2005) and upregulation of HO-1 produces cardioprotection against ischemia-reperfusion injury (Thirunavukkarasu *et al.,* 2007; Penumathsa *et al*., 2008b). It has been documented that HO-1 facilitates release of NO by disrupting complex of caveolin and eNOS (Penumathsa *et al*., 2008). The expression of HO-1 is diminished into hyperlipidemic myocardium (Csonka *et al*., 1999). It has been reported that adenosine activate HO-1 by acting on adenosine-1 (A-1) receptor (Glia *et al* ,2008). Administration of N6-(2-phenylethyl)adenosine (adenosine agonist) in hyperlipidemic rat, 24 hr before the ischemia restore attenuated cardioprotective effect of IPC in hyperlipidemic rat and increase the release of NO. Moreover, administration of Znpp, inhibitor of HO-1 significantly attenuated the observed cardioprotection and increase in release of NO in heart of N6-(2-phenylethyl)adenosine pretreated hyperlipidemic rat. It may be suggest that the pretreatment of N6-(2-phenylethyl)adenosine an A1 agonist restore the attenuated cardioprotection effect of IPC in hyperlipidemic rat heart by activation of HO-1 which was attenuated by administration of Znpp (HO-1 inhibitor).

Adenosine is one of the upstream triggers and mediators involved in Ischemic Preconditioning (IPC) induced cardioprotection (Giacometti and Lishmanov *et al*., 2010). It has been noted

that adenosine exerts its cardioprotective effects of IPC through its binding with A1 and A3 receptors in rats (De Jonge *et al*., 2002). Activation of adenosine A1 receptors activates phospholipase (PLC), (Rogel *et al*., 2005) which in turn activates protein kinase C (PKC) (Yellon *et al*., 2000). Activated PKC phosphorylates and opens the mitochondrial KATP channels (Mito-KATP) and block mitochondrial permeability transition pore (MPTP) to afford IPC mediated cardioprotection (Haunsenloy *et al*., 2002). Administration of N6-(2-phenylethyl)adenosine (adenosine agonist) in hyperlipidemic rat, 24 hr before the isolation of heart, produces cadioprotection by decreasing the infarct size and reducing of LDH, CK-MB and it also increase the release of nitrite/ nitrate in coronary effluent. Moreover, perfusion of L-NAME, inhibitor of NO significantly attenuated the observed cardioprotection.

On the basis of above discussion, it may be concluded that attenuation of cardioprotective effect of ischemic preconditioning is due to impairment of HO-1 induced release of nitric oxide in the hyperlipidemic rat heart. Activation of adenosine-1 receptor by a pretreatment of specific agonist N6-(2-phenylethyl)adenosine, restored the attenuated cardioprotective effect of ischemic preconditioning in hyperlipidemic rat heart by the activation of HO-1 and enhanced the release of NO.

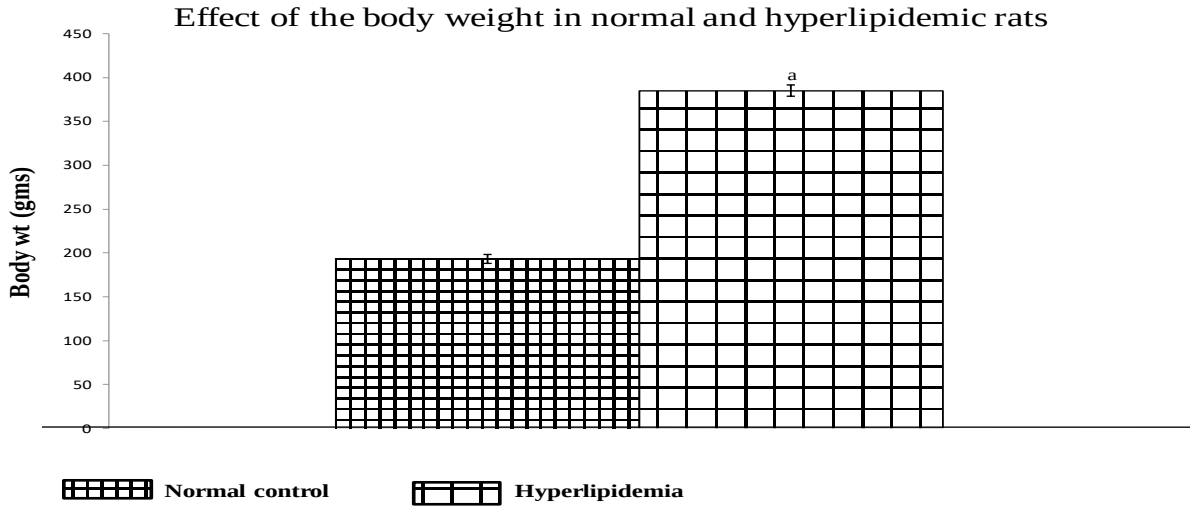

Fig. 4.1: a = p< 0.05 vs. Normal control

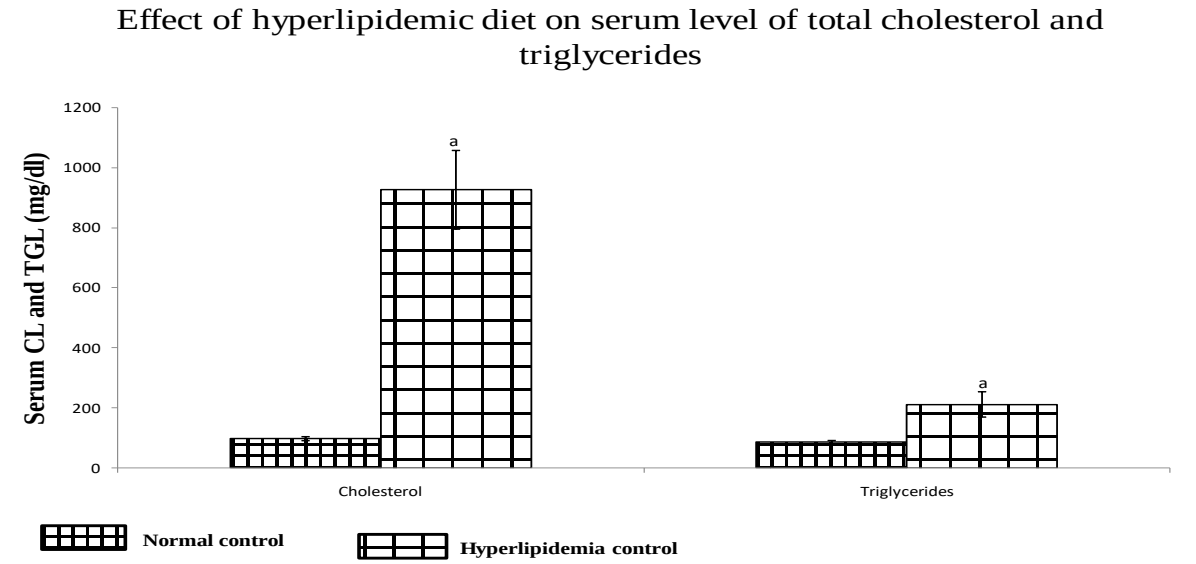

Fig.4. 2: a = p< 0.05 vs. Normal control

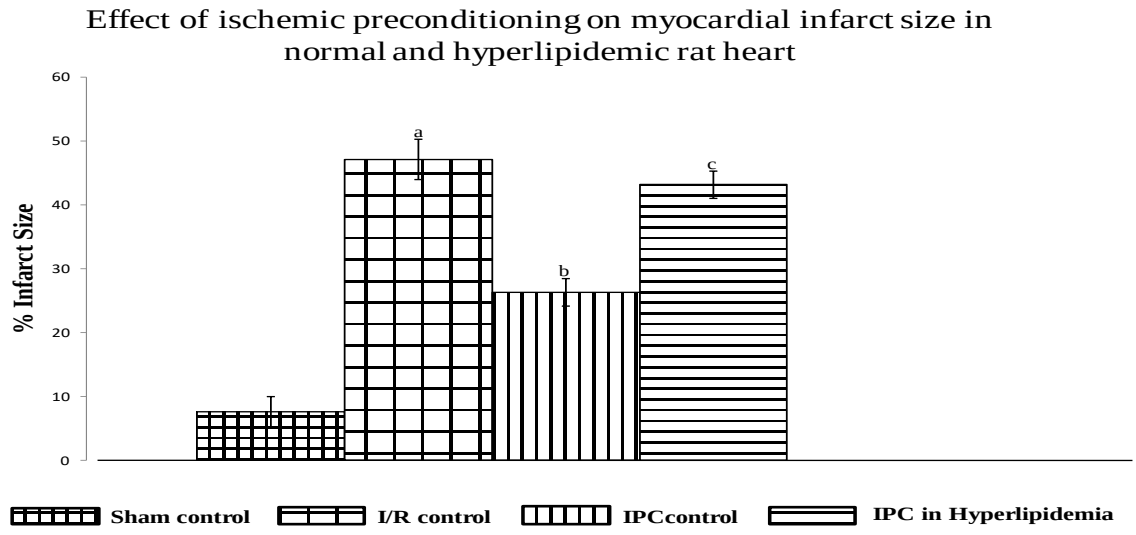

Fig .4.3: I/R denotes ischemic reperfusion; IPC denotes ischemic preconditioning.
Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control ; b = p< 0.05 vs. I/R Control ; c = p< 0.05 vs. IPC Control.

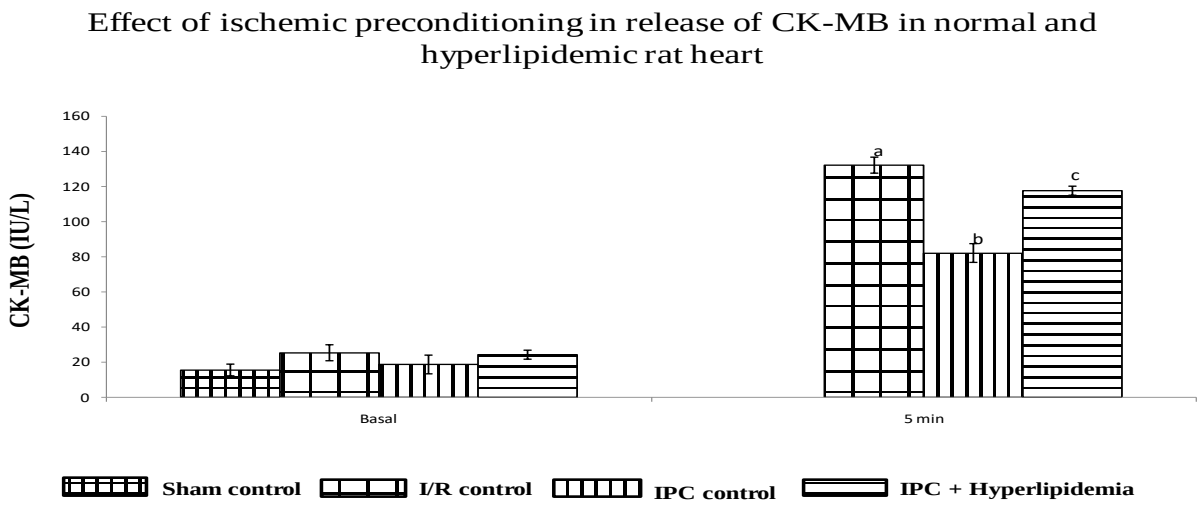

Fig. 4.4: Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R Control; c= p< 0.05 vs. IPC in normal Rat Heart.

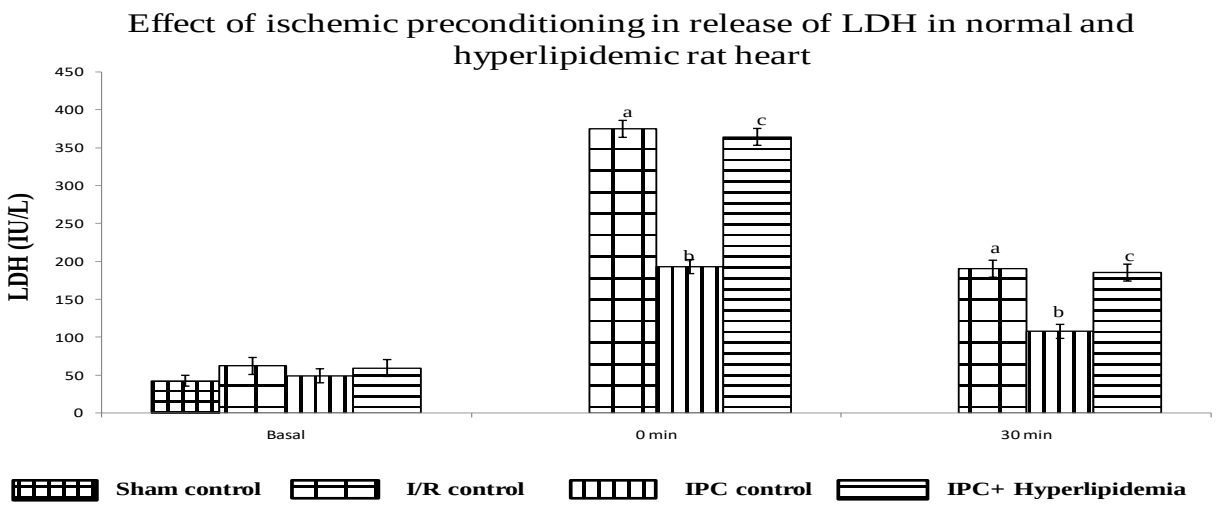

Fig.4.5: Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R Control; c = p< 0.05 vs. IPC in normal rat heart.

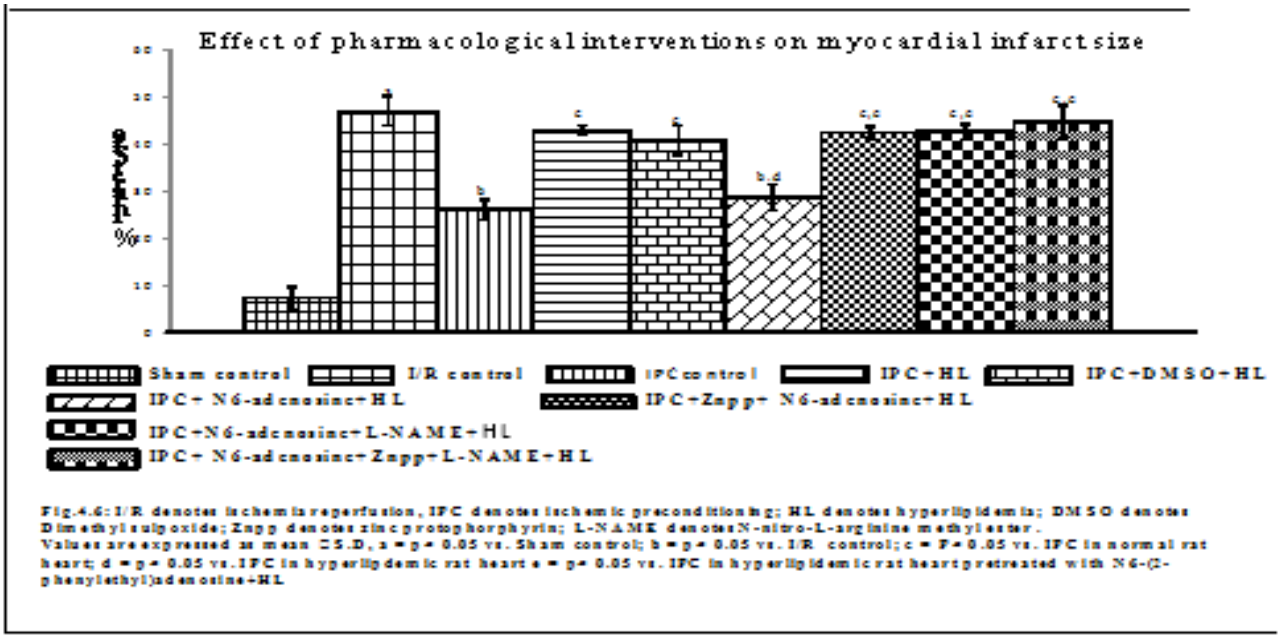

Fig.4.6: I/R denotes ischemia reperfusion, IPC denotes ischemic preconditioning; HL denotes hyperlipidemia; DMSO denotes Dimethyl sulphoxide; Znpp denotes zinc protophorphyrin; L-NAME denotes N-nitro-L-arginine methyl ester. Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R control; c = P< 0.05 vs. IPC in normal rat heart; d = p< 0.05 vs. IPC in hyperlipidemic rat heart e = p< 0.05 vs. IPC in hyperlipidemic rat heart pretreated with N6-(2-phenylethyl)adenosine+HL

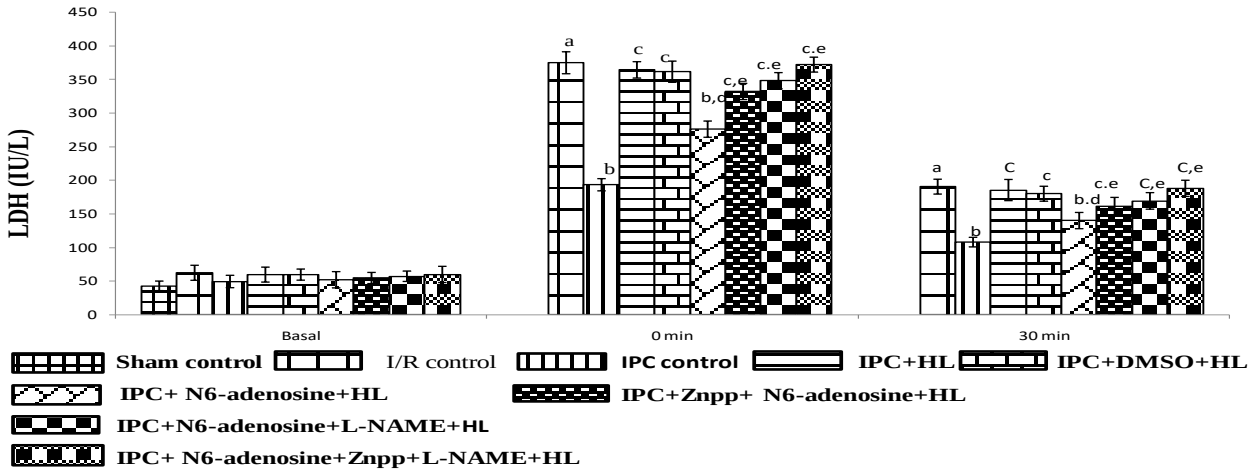

Fig.4.7: I/R denotes ischemia reperfusion, IPC denotes ischemic preconditioning; HL denotes hyperlipidemia; DMSO denotes Dimethyl sulpoxide; Znpp denotes zinc protophorphyrin; L-NAME denotes N-nitro-L-arginine methyl ester .

Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R control; c = P< 0.05 vs. IPC in normal rat heart; d = p< 0.05 vs. IPC in hyperlipdemic rat heart e = p< 0.05 vs. IPC in hyperlipidemic rat heart pretreated with N6-(2-phenylethyl)adenosine+HL .

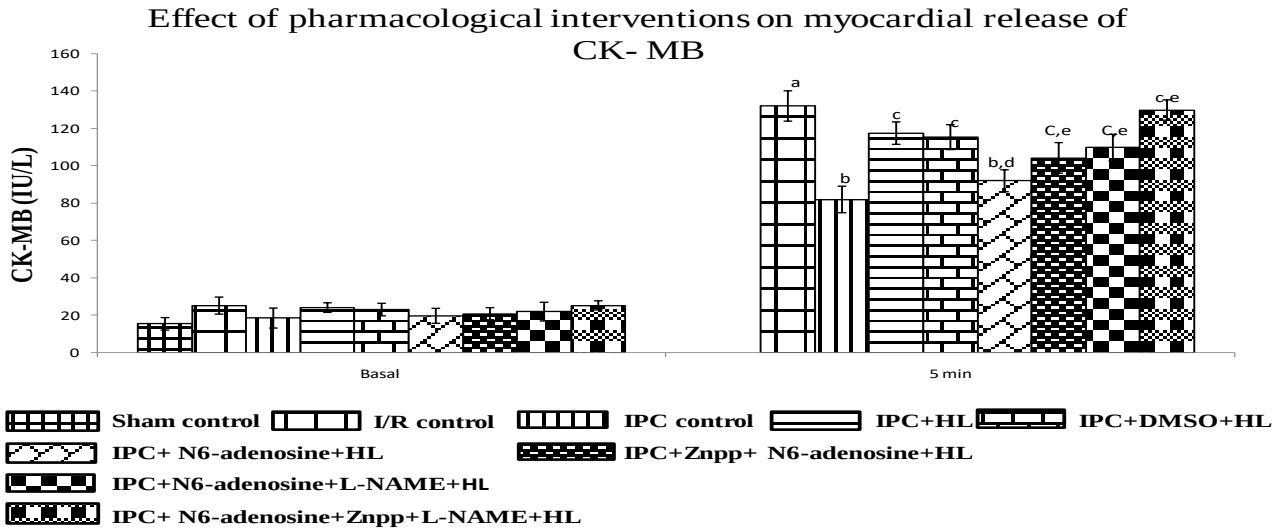

Fig.4.8: I/R denotes ischemia reperfusion, IPC denotes ischemic preconditioning; HL denotes hyperlipidemia; DMSO denotes Dimethyl sulpoxide; Znpp denotes zinc protophorphyrin; L-NAME denotes N-nitro-L-arginine methyl ester .

Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R control; c = P< 0.05 vs. IPC in normal rat heart; d = p< 0.05 vs. IPC in hyperlipdemic rat heart e = p< 0.05 vs. IPC in hyperlipidemic rat heart pretreated with N6-(2-phenylethyl)adenosine+HL

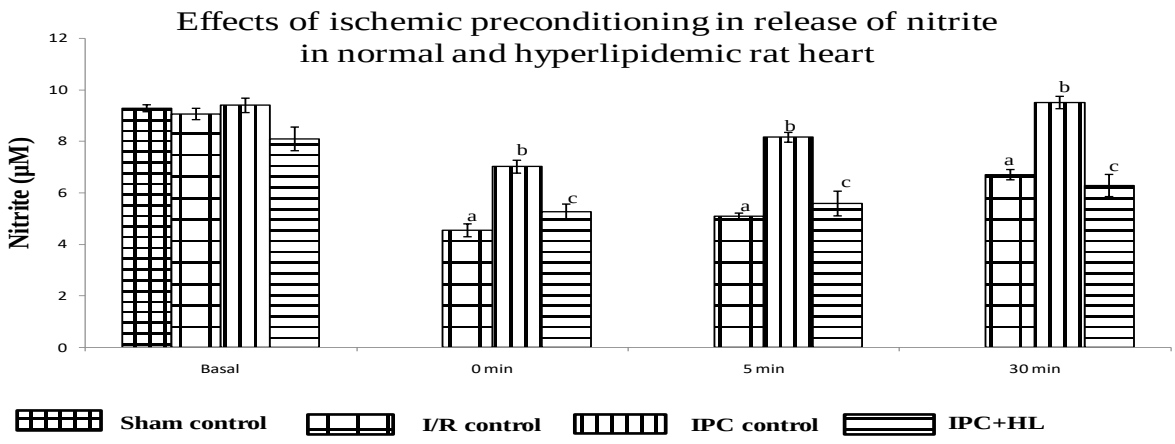

Fig.4.9: Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b= p< 0.05 vs. I/R control; c = p< 0.05 vs. IPC control.

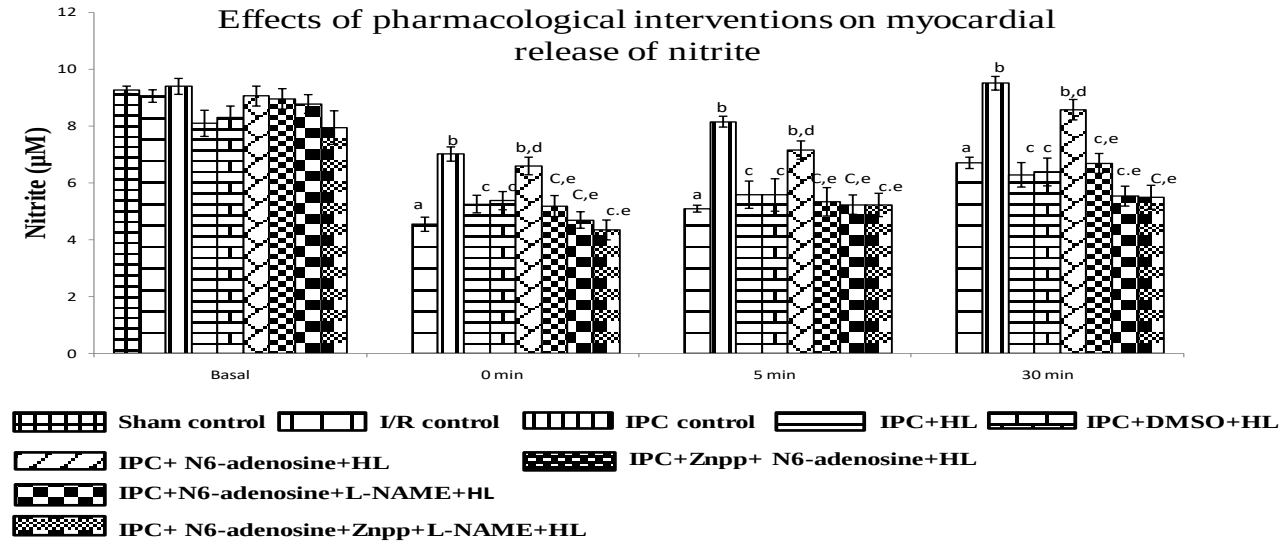

Fig.4.10: I/R denotes ischemia reperfusion, IPC denotes ischemic preconditioning; HL denotes hyperlipidemia; DMSO denotes Dimethyl sulpoxide; Znpp denotes zinc protophorphyrin; L-NAME denotes N-nitro-L-arginine methyl ester .

Values are expressed as mean ± S.D, a = p< 0.05 vs. Sham control; b = p< 0.05 vs. I/R control; c = P< 0.05 vs. IPC in normal rat heart; d = p< 0.05 vs. IPC in hyperlipdemic rat heart e = p< 0.05 vs. IPC in hyperlipidemic rat heart pretreated with N6-(2-phenylethyl)adenosine+HL

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