ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Comparing acute effect of Preconditioning, Postconditioning, and combined Pre- and Post-conditioning on oxidative stress markers in healthy subjects

Paramjot Kaur Othee[1], Sudhir Varma[2], Kanchan Vohra[3*], Harpreet Singh Kalra[4]

1. Research Fellow, Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala, Punjab (India), Pin-147002.
2. Consultant (D.M., Cardiology), Sadbhavna Medical and Heart Institute, Patiala, Punjab (India), Pin-147001.
3. Assistant Professor (M. Pharm., Pharmacy Practice) Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala, Punjab (India), Pin-147002
4. General Physician (M. D., Physician), Sadbhavna Medical and Heart Institute, Patiala, Punjab (India).

Manuscript Info

Manuscript History:

Received: 15 December 2014
Final Accepted: 26 January 2015
Published Online: February 2015

Key words:

Antioxidants, Forearm Ischemia Reperfusion Injury, Healthy Subjects, Reactive oxygen species

Corresponding Author

Kanchan Vohra

Abstract

INTRODUCTION: In context to the mechanism that potent oxidant radicals are produced within the first few minutes of reflow and play a crucial role in the development of reperfusion injury, the aim of our study was comparative evaluation of preconditioning versus post-conditioning versus combined pre- and post- conditioning on acute level of oxidative stress markers induced by forearm ischemia-reperfusion injury in healthy subjects.

METHODS: A parallel group, open label, pilot study was carried out on healthy subjects. 40 healthy subjects were randomized to four different treatments (n=10/per group). Forearm Ischemia Reperfusion injury (IRI) (group I) was produced by inflation of non-dominant forearm cuff at 200mm Hg for 10 min and then reperfusion for 5 min. Pre-conditioning (group II), Post-conditioning (group III) and, both pre- and post- conditioning (group IV) treatments were also applied to subjects after forearm IRI. Various serum markers of oxidative stress were measured after 10, 30 and 60 min of reperfusion. Results were expressed as mean ± standard error mean and considered significant at $p \leq 0.05$. One-way and Two-way analysis of variance followed by Student-Newman-Keuls test was used.

RESULTS: There was found an increase in thiobarbituric acid reactive substances level during oxidative stress at all time periods after IRI, but decrease in levels of nitric oxide, ascorbic acid, reduced glutathione, α-tocopherol and catalase at 10 min and subsequently further increase in levels at 30 and 60 min of reperfusion.

CONCLUSION: No synergistic effect was seen of combined pre- and post-conditioning in comparison to individual treatment.

INTRODUCTION

Although the reperfusion is beneficial for the survival of the ischemic tissue, yet reperfusion to previously ischemic tissue induces detrimental changes in myocardium resulting in inflammation, oxidative damage and cardiac dysfunction (Rohilla *et al*., 2011). The most common mechanisms and mediators of reperfusion injury may include oxygen free radicals, endothelial and microvascular dysfunction, intracellular calcium overload, and altered myocardial metabolism (Hoffman *et al*., 2004). Bolli and colleagues have shown that potent oxidant radicals are

produced within the first few minutes of reflow and play a crucial role in the development of reperfusion injury, as the structures of both lipids and proteins are affected by them (Bolli *et al*., 1989). Reactive oxygen species (ROS) strongly interact with unsaturated bonds in lipids, leading to a chain reaction and formation of peroxides, hydro-peroxides, aldehydes and conjugated dienes and thus, inactivation of several enzymes (Murin *et al*., 2001). Although pre-clinical (Xing, 2008; Mahfoudh-Boussaid *et al*., 2012; Thomaz *et al*., 2013) and clinical studies (Ambros *et al*., 2007; Zhao *et al*., 2009; Yang *et al*., 2011) have explored role of oxidative stress during pre-conditioning and post-conditioning, none has explored yet acute effects on levels of markers of oxidative stress after pre-, post- and both combined pre- and post- conditioning in healthy subjects. The study is aimed to know the comparative and acute effect of preconditioning versus (vs.) postconditioning vs. combined pre- and post- conditioning on oxidative stress markers induced by forearm ischemia-reperfusion injury (IRI) in healthy subjects.

MATERIALS AND METHODS

A prospective, parallel group, open label study was carried-out on healthy subjects. The subjects were randomized into four groups (n=10/group) and assigned different treatments: subjects with IRI only (group I), with pre-conditioning treatment (group II), post-conditioning treatment (group III), both pre- and post-conditioning treatment (group IV). The study was approved by Institutional clinical ethics committee (ICEC Clearance Number-ICEC/71/2013) of the institute (Punjabi University, Patiala). All healthy subjects had provided a written informed consent to participate after a full explanation of the study. All subjects were assessed for eligibility of inclusion/exclusion (I/E) criteria at baseline. I/E criteria stated inclusion of healthy male volunteers between the age of 18 to 35 years and able to give written informed consent, and exclusion of subjects with smoking habits or any significant medical illness requiring monitoring or treatment, cardiovascular disease, or risk factors for coronary artery disease. Fasting blood samples were obtained from all subjects. Both serum and plasma obtained from blood samples were then stored at -40º C until they were analyzed. Ischemia-reperfusion injury was produced by inflation of non-dominant forearm cuff at 200mm Hg for 10 min and then reperfusion for 5 min (Kilian *et al*., 2005). Preconditioning was achieved by three min cuff inflation at 200mm Hg separated by three min deflation preceding IR at 200 mm Hg by a further five min (Kilian *et al*., 2005). Post-conditioning was produced by three cycles of reperfusion followed by ischemia (each lasting ten or 30 seconds) applied immediately after ten minutes of arm ischemia (Loukogeorgakis *et al*., 2012). Detailed plan of assigning different treatments and procedures are described in table I. Various biomarkers of oxidative stress were estimated using spectrophotometer (DU 640BSpectrophotometer, Beckman Coulter Inc. CA, USA).

Estimation of thiobarbituric acid reactive substances (TBARS):

Method of Liu *et al*., 1997, was used to measure malondialdehyde (MDA) concentration by estimating TBARS in serum (Liu *et al*., 1997; Narendra *et al*., 2008). The absorbance was noted at 532 nm against blank solution. A standard curve for TBARS was plotted using 1,1,3,3-tetramethoxypropane (Loba Chemie Pvt. Ltd., India).

Estimation of catalase:

Method of Luck, 1974, was used for the estimation of serum catalase enzyme level (Luck *et al*., 1974; Pathak and Pathak, 2012).

Estimation of Ascorbic Acid:

Method of Roe and Keuther, 1943, were used to estimate serum ascorbic acid levels (Roe and Kuether, 1943; Rutkowski and Grzegorczyk, 2007).

Estimation of Alpha-tocopherol:

Method of Rosenberg, 1992 was used for estimation of serum alpha-tocopherol levels (Rosenberg, 1992; Jayachitra *et al*., 2012).

Estimation of Reduced Glutathione:

Method of Moron *et al*., 1979, was employed for estimation of reduced glutathione [Moron *et al*., 1979; Parkash and Sudha, 2012).

Estimation of Ferric Reducing Ability of Plasma (FRAP):

Method of Benzie and Strain, 1999, was used for estimation of plasma FRAP levels [Benzie and Strain, 1999; Jansen and Ruskovska, 2013).

Estimation of Serum Nitrite:

Method of Sastry *et al*., 2003 was employed for estimation of serum nitrite (Sastry *et al*., 2002; Narendra *et al*., 2007). Nitrate was first reduced to nitrite by copper-cadmium alloy. Nitrite thus formed was treated with sulphanilamide, a diazotizing agent, in the acidic medium to form transient diazonium salt. This intermediate was allowed to react with coupling reagent, N-naphthylethylenediamine (NED) to form stable pink colored azo compound. The pink color so developed was measured at 545 nm.

STATISTICAL ANALYSIS

All data were analyzed using SPSS (Statistical Program for Social Sciences, version 17 for windows, 2007, SPSS Inc. Chicago, Illinois, USA) and Sigma Stat 3.2. Continuous variables were expressed as mean ± standard error mean and discrete variables were presented as percentages. One- and Two- Way analysis of variance (ANOVA) followed by Student-Newman-Keuls test were used to assess the difference between various treatments. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Clinical characteristic of subjects at baseline are presented in table II. All healthy young male volunteers (n=40) were in the age range of 18-35 years (mean age, 21 ± 0.36 years). None was obese or having high blood pressure ($p>0.05$) in any group at baseline. Figure 1-7 describes baseline and post treatment levels of oxidative stress markers in various groups. Figure 1, figure 2, figure 3, figure 4, figure 5, figure 6, and figure 7 shows the absolute serum/plasma levels of ascorbic acid, catalase, ferric reducing ability of plasma (FRAP), reduced glutathione, thiobarbituric acid reactive substances, alpha-tocopherol, and nitrite at baseline in healthy subjects, then after inducing IR injury (group I) and, after various conditioning treatments in group II, III and IV, respectively. The *p* values represent statistical significant comparison of levels of markers in all groups viz., II, III and IV versus group I and among themselves.

In comparison to IR group, on an average, levels of ascorbic acid were decreased maximally and levels of FRAP minimally as - 21.01% vs. -20.61% vs. -20.82% and ; -5.64% vs. -5.93% vs. -6.26%, after 10 min of IR injury; similarly, catalase levels were increased maximally to 38.06% (in all groups) and FRAP levels minimally to 1.50% vs. 1.15% vs. 1.78%, after 60 min of IR injury, in pre-conditioned group (group II) vs. post-conditioned group (III) vs. combined pre- and post-conditioned group (IV), respectively.

Table III shows association (Pearson Univariate correlation analysis) of MDA level with anti-oxidants levels at baseline and after 60 min of IR in different groups. Significant negative correlation was observed between MDA and FRAP level at baseline(r= -0.92, $p=0.001$). Similar pattern was observed for MDA and catalase after 60 min of IR injury (r= -0.87, $p=0.001$). All other markers with respect to MDA did not show any significant correlation (all $p \geq 0.05$).

DISCUSSION

Cardiovascular diseases are major contributors to mortality in developed countries (Parasuraman *et al*., 2010). Although few clinical studies have explored role of oxidative stress in IR injury, yet very scarce evidences are available that depicts timely and acute variation in oxidative stress markers after conditionings.

ROS generation leads to lipid peroxidation of cell membranes (Talla and Veerareddy, 2011). The present findings of acute (within 1 hr of IRI) increased levels of TBARS are supported by the results of Janssen *et al*., 1993, who reported increased levels of TBARS in tissue samples of cardioplegic heart after every 5 min upto 30 min in rats (Janssen *et al*., 1993). Another study by Cizova *et al*., 2004, reported increased serum TBARS levels in rat intestinal ischemic model after 30 and 90 min of reperfusion (Cizova *et al*., 2004)]. Present results of pre-conditioning effect are supported by the study of Montalvo-Javé *et al*., 2011, that too lead to decrease in TBARS level in liver biopsy samples after 2, 4, 8, 12 and 24 hour of reperfusion in ischemic rats (Montalvo-Javé *et al*., 2011). Zhang *et al*., 2012, reported that post-conditioning too leads to decrease in tissue TBARS level in ischemic reperfusion induced myocardium in rats after 5, 10, 30, 60 and 120 min of reperfusion (Zhang *et al*., 2012). Lintz *et al*., 2013 had found that both ischemic pre- and post- conditioning decreased muscular levels of TBARS after 60 min of reperfusion in skeletal muscle injury produced by ischemia and reperfusion in rats (Lintz *et al*., 2013). In present study, due to pre-conditioning, post-conditioning & combined pre- and post-conditioning there was decrease in TBARS levels on an average of 19.6% at 10 min, 46.5% at 30 min and 25.8% at 60 min in each group, respectively. These results postulate that due to IR there may be increase in ROS levels which leads to more lipid peroxidation and hence enhanced TBARS levels, but, thereafter a decrease in TBARS levels may be due to protective effect of the treatments which attenuate the production of ROS and lipid peroxidation. Moreover, significant negative correlation between TBARS and FRAP level revealed that as oxidative stress increases antioxidant levels decreases.

The anion nitrite (NO_2^-) constitutes a biochemical reservoir for nitric oxide (NO) (Gladwin et al., 2005). In this context, nitrite can be considered an "ischemic NO buffer," maintaining hypoxic–ischemic NO homeostasis (Dezfulian and Raat, (2007). A study by Yang *et al*., 2004, has revealed that NO also acts as an intracellular signaling molecule implicated in the cardioprotective effects of pre-conditioning (Yang *et al*., 2004). Pagliaro *et al*., 2004, reported the involvement of NO via the cGMP pathway in post-conditioning (Pagliaro *et al*., 2004). The

present finding of acute increase in levels of NO are supported by the results of Gumustas *et al*., 2007, who reported decreased plasma and brain tissue NO levels after 60 min of reperfusion in rat cerebral IRI models (Gumustas *et al*., 2007) and, Koken *et al*., 1999, who also reported decreased NO levels in rats models with liver IRI (Koken *et al*., 1999). It has been reported that pre-conditioning leads to increase in NO levels in serum of fatty liver after 24 hours of reperfusion in rats (Jiang *et al*., 2013). No published evidence had been found to support the present investigations for effect of post-conditioning and combined pre- and post-conditioning on NO levels. Present results of average decrease of 18.48% NO levels in 10 min, and then increase of 10.59% in 30 and 8.91% in 60 min after reperfusion due to pre-conditioning, post-conditioning and combined pre- and post-conditioning, in each group, respectively, postulate that due to ischemia there was increase in NO as per defensive mechanism in ischemia but during reperfusion there was sudden rise in ROS which caused depletion of NO *i.e.* NO may be used up in scavenging hydroxyl radicals (Chan, 2002). Treatments showed protection by enhancing NO levels as they are important mediators in these treatments hence, decrease in ROS. Oxygen free radicals generated during early reperfusion play major role in maintenance of balanced redox status of body (Mohan *et al*., 2010; Mahmud *et al*., 2012).

Present study reported significantly lowered antioxidant enzymatic and non-enzymatic activities in IR group, possibly resulting from the depletion of antioxidant pool used for scavenging excess ROS due to IR. We found that antioxidant enzyme activities were significantly elevated suggesting that some redox enzymes may be over-expressed in IR and sub-lethal oxidative stress triggers natural protective mechanisms via up-regulation (Ucar *et al*., 2005). The opening of mitochondrial K-ATP channels is considered a pivotal step in the mechanism of pre-conditioning (Kher *et al*., 2005)

The present finding of acute increase in levels of catalase are supported by the results of Rasoulian *et al.,* 2008, who reported decreased tissue catalase levels after 24 hour in rat IRI model (Rasoulian *et al.,* 2008); and, Khan *et al*., 2009, which too reported decreased catalase levels in rat myocardial tissue samples after 45 min of IRI (Khan *et al*., 2009). It has been reported that pre-conditioning leads to increase in tissue catalase level in kidney after 24 hour of reperfusion in rat IRI model (Song *et al*., 2007). Similar to IPC, present study has found increased serum catalase levels after IPostC. These results are supported by studies of Yun *et al*., 2009 and, Zang *et al*., 2012, reported increased catalase levels in renal homogenates after 24 hour of reperfusion (Yun *et al*., 2009); and in myocardium after 15-90 min of reperfusion, respectively (Zang *et al*., 2012).

The present finding of acute increase in levels of ascorbic acid are supported by study of Layton *et al*., 1996, reported decreased levels of ascorbic acid after 90 min of reperfusion in microdialysis perfusates of rat model with hepatic ischemia. It has been reported that pre-conditioning leads to increase in ascorbic acid level in kidney after 15 and 60 min in rabbit IR injury model (Liu *et al*., 2010).

The present finding of acute increase in levels of reduced glutathione (GSH) are supported by the results of Rasoulian *et al*., 2008, which reported decreased tissue GSH levels after 24 hour of reperfusion in rat IRI model and, Khan *et al*., 2009, too reported decreased GSH levels in rat myocardial tissue samples after 45 min of IRI. It has been reported that pre-conditioning leads to increased tissues GSH level in ischemic lungs after 30 min (Soncul *et al*., 1999) and in ischemic pulmonary vascular bed (Kandilci *et al*., 2006) in rats, respectively. Similar to IPC, present study has found increased serum GSH levels after IPostC. These results are supported by the study of Nedvig *et al*., 2011, who reported increased tissue GSH levels after 1, 3 and 6 hour of reperfusion in superior ischemic mesenteric artery in IRI models of rat (Nedvig *et al*., 2011).

The present finding of acute increase in levels of α-tocopherol are supported by the results of Bhaskar *et al.*, 1995, who reported decreased α-tocopherol levels in rat models with colon IRI (Bhaskar *et al.*, 1995); and, Yoshikawa *et al*., 1991, reported decreased serum α-tocopherol levels after 30 and 60 min in rat models of gastric mucosal IRI (Yoshikawa *et al*., 1991). It has been reported that pre-conditioning leads to increase in tissue α-tocopherol level in serum after 24 hours of reperfusion [57] and in plasma after 70 min of reperfusion in right renal artery of rat IRI models (Kadkhodaee *et al*., 2004), respectively.

The present finding of acute increase in levels of FRAP are supported by study of Kadkhodaee *et al*., 2008, which reported decreased plasma FRAP levels in hemodialytic patients (Kadkhodaee *et al*., 2008). No published evidence had been found to support present findings for effect of pre-conditioning, post-conditioning and combined pre- and post-conditioning on FRAP levels.

Present results of initial decrease in all antioxidant levels at ten min, and then increase at 30 and 60 min after reperfusion postulate that due to ischemia there was increase in antioxidants as per defensive mechanism in ischemia but during reperfusion there was sudden rise in ROS which caused depletion of antioxidants as all antioxidants were used up in overcoming the situation.

CONCLUSION

Data from the present study also revealed that all the treatments had approximately similar level of acute effect on modulating markers of oxidative stress within one hour of reperfusion i.e. none was better than other. Present study has concluded that no synergistic effect was seen with combined pre- and post- conditioning as compared to individual treatment alone on markers of oxidative stress.

CONFLICTING INTEREST: NIL

SOURCE(S) OF SUPPORT: Funding from external organizations – NIL (The academic institute, Punjabi University, Patiala, provided all the necessary financial support to the research fellow for completing her dissertation work)

ACKNOWLEDGEMENT: We acknowledge Ms. Jasmeen for assisting the research fellow in completing her dissertation work.

Table I: Detailed Plan of Treatments and Procedures

Healthy Subjects	**Treatment Schedule**
Group I Healthy volunteers with IRI[11] only	Step 1: 2 ml blood was withdrawn before inducing IR to check normal levels of markers of oxidative stress. Step 2: IR was produced by inflation of non-dominant forearm cuff at 200mm Hg for 10 min and was reperfused for 5 min. Step 3: 5 ml blood was withdrawn after 10 min, then after 30 min and subsequently after 60 min of IR.
Group II Healthy volunteers with preconditioning[11]	Step 1: 2 ml blood was withdrawn before inducing IR to check normal levels of markers of oxidative stress. Step 2: Preconditioning was achieved by 3 min cuff inflation at 200mm Hg separated by 3 min deflation preceding IR at 200 mm Hg by a further 5 min. Step 3: IR was produced by inflation of non-dominant forearm cuff at 200mm Hg for 10 min and was reperfused for 5 min. Step 4: 5 ml blood was withdrawn after 10 min, then after 30 min and subsequently after 60 min of IR.
Group III Healthy volunteers with postconditioning[12]	Step 1 and Step 2: As step 1 and 2 in group 1 Step 3: Post-conditioning, was produced by 3 cycles of reperfusion followed by ischemia (each lasting 10 or 30 seconds) applied immediately after 10 minutes of arm ischemia. Step 4: 5 ml blood was withdrawn after 10 min, then after 30 min and subsequently after 60 min of step 3.
Group IV Healthy volunteers with Pre and Post-conditioning	Step 1: 2 ml blood was withdrawn before inducing IR to check normal levels of markers of oxidative stress. Step 2: Preconditioning was achieved by 3 min cuff inflation at 200mm Hg separated by 3 min deflation preceding IR at 200 mm Hg by a further 5 min. Step 3**:** IR was produced by inflation of non-dominant forearm cuff at 200mm Hg for 10 min and was reperfused for 5 min. Step 4: Post-conditioning, was produced by 3 cycles of reperfusion followed by ischemia (each lasting 10 or 30 seconds) applied immediately after 10 minutes of arm ischemia. Step 5: 5 ml blood was withdrawn after 10 min, then after 30 min and subsequently after 60 min of step 4.

IR: ischemia reperfusion, IRI: ischemia reperfusion injury

Table II: Clinical Characteristics of healthy subjects (at baseline)

S. No.	PARAMETERS	Group 1	Group II	Group III	Group IV	*p* values
1	Age (years)	21 ± 0.36	20±.47	21±.24	22±.37	0.98
2	Weight, kg	64.43 ± 1.47	67±1.23	65±1.23	64±1.34	0.96
3	BMI, Kg/m²	22.18 ± 0.38	22.65±.58	21.76±.47	21.87±.69	0.99
4	SBP, mm Hg	122.23 ± 0.69	121.45±.24	122.87±.45	122.23±.55	0.97
5	DBP, mm Hg	79.53 ± 0.24	79.87±.32	78.98±.34	79.45±.47	0.93

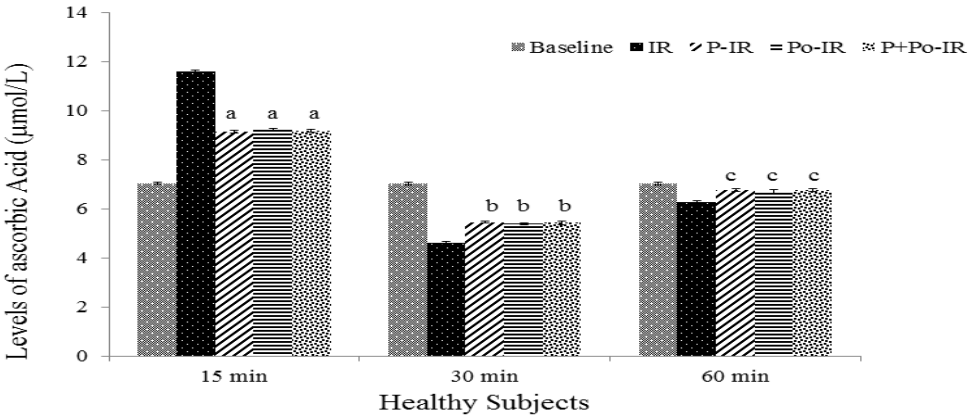
BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

Table III: Pearson correlation of TBARS (oxidant) with various antioxidants levels (at baseline and after 60 min of IR injury in various groups

S. No.	Parameters		Baseline	Group I (IRI)	Group II (Pre-conditioning)	Group III (Post-conditioning)	Group IV (Pre and Post conditioning)
1.	Ascorbic acid	*r*	-0.57	0.29	0.60	0.63	-0.71
		p	0.32	0.40	0.65	0.48	0.20
2.	Reduced Glutathione	*r*	0.32	0.69	0.31	0.74	-0.12
		p	0.60	0.25	0.37	0.12	0.78
3.	Alpha-Tocopherol	*r*	0.81	0.67	0.63	0.63	0.28
		p	0.95	0.31	0.47	0.47	0.47
4.	Catalase	*r*	0.27	**-0.87**	0.19	0.38	0.19
		p	0.67	0.01*	0.59	0.26	0.59
5.	FRAP	*r*	**-0.92**	-0.18	0.76	0.78	0.42
		p	0.01*	0.61	0.83	0.63	0.21

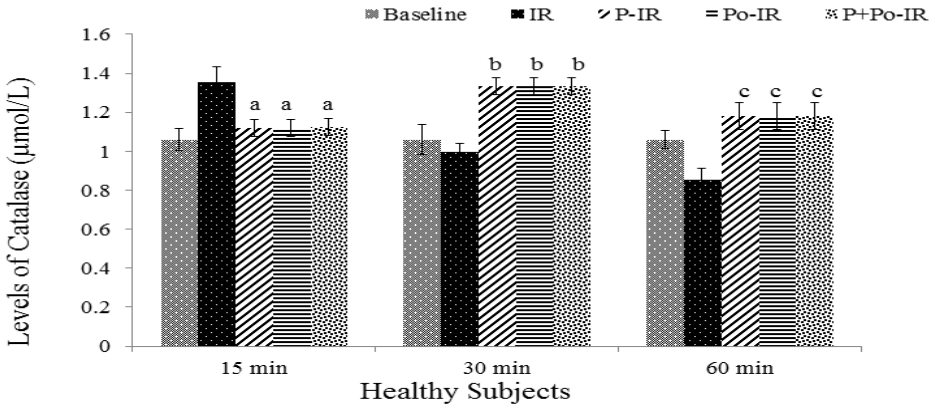
* Represents statistical significant p values (all $p \leq 0.05$); r: correlation value; FRAP: Ferric reducing ability of plasma; IRI: ischemia reperfusion injury

Figure 1: Effect on Ascorbic acid level



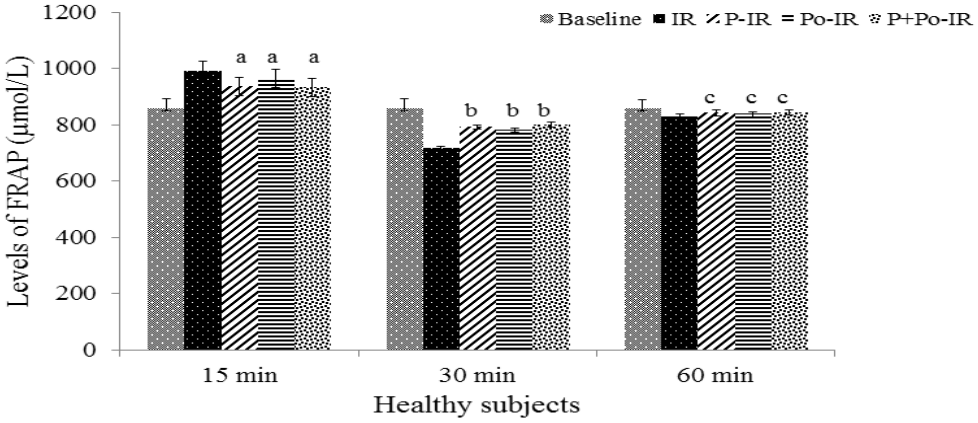
Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 2: Effect on Catalase level



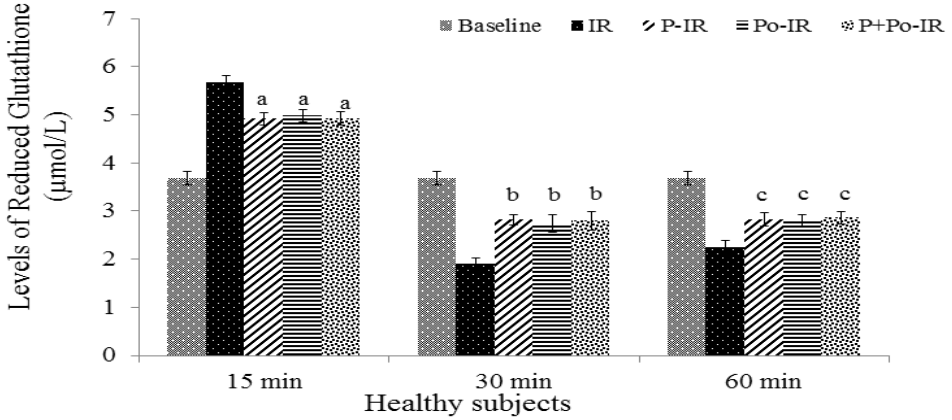
Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 3: Effect on Ferric Reducing Ability of Plasma (FRAP) level



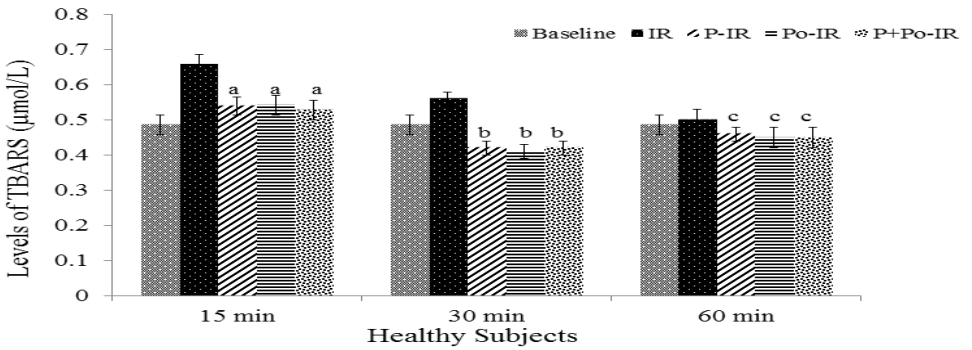
Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 4: Effect on Glutathione level



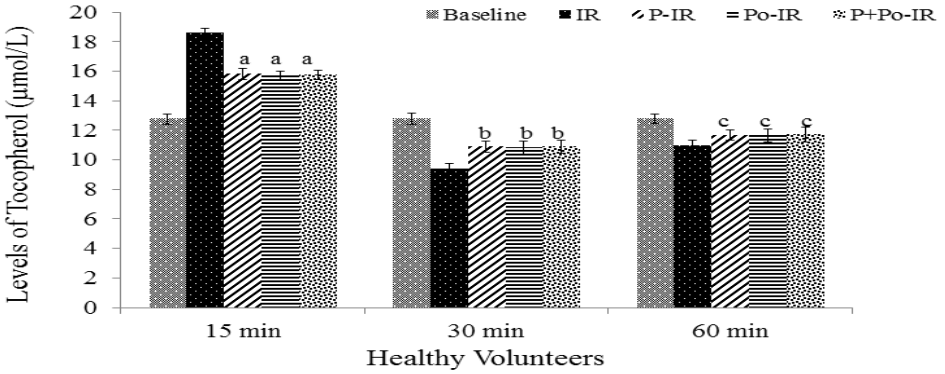
Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 5: Effect on Thiobarbituric Acid Reactive Substances (TBARS) level



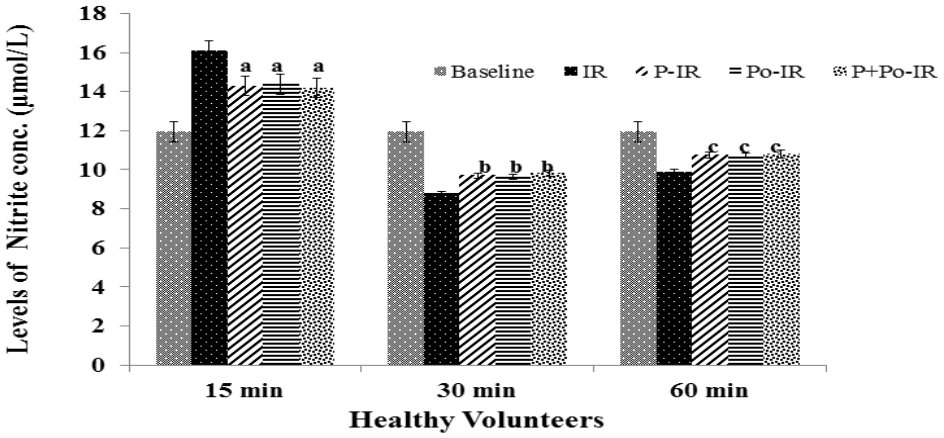
Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 6: Effect on Tocopherol level



Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

Figure 7: Effect on Nitrite Concentration level



Values are expressed as mean ± SEM. significant levels considered at $p<0.05$ a: as compared with IR in 10 min; b: as compared with IR in 30 min; c: as compared with IR in 60 min; IR: Ischemia Reperfusion episode; P-IR: Preconditioning; Po-IR: Postconditioning; P+Po-IR: Combined Preconditioning and Postconditioning.

REFERENCES:

Ambros JT, Herrero-Fresneda I, Borau OG, Boira J.M. (2007): Ischemic preconditioning in solid organ transplantation: from experimental to clinic. Transpl Int., 20: 219-29.

Aryamanesh S, Ebrahimi SM , Abotaleb N, Nobakht M, Rahimi-Moghaddam P. (2012): Role of Endogenous Vitamin E in Renal Ischemic Preconditioning Process: Differences between Male and Female Rats. Iran Biomed J., 16: 44-51.

Benzie I.F, Strain JJ. (1999): Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol., 299: 15–27.

Bhaskar L, Mathan MM, Balasubramanian KA. (1995): Oxygen free radical-induced damage during colonic ischemia/reperfusion in rats. Mol Cell Biochem., 151: 9-14.

Chan KL. (2002): Role of Nitric Oxide in Ischemia and Reperfusion Injury. Curr Med Chem - Anti-Inflammatory & Anti-Allergy Agents, 1: 1-13.

Chen H, Xing B, Liu X, Zhan B, Zhou J, Zhu H, *et al*. (2008): Ischemic postconditioning inhibits apoptosis after renal ischemia/reperfusion injury in rat. Transpl Int., 21: 364-71.

Cízová H, Lojek A, Kubala L, Cíz M. (2004): The Effect of Intestinal Ischemia Duration on Changes in Plasma Antioxidant Defense Status in Rats. Physiol Res., 53: 523-31.

Dezfulian C, Raat N, Shiva S, Gladwin MT. (2007): Role of the anion nitrite in ischemia-reperfusion cytoprotection and therapeutics. Cardiovasc Res., 7: 327-38.

Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, *et al*. (2005): The emerging biology of the nitrite anion. Nat Chem Biol., 1: 308–14.

Gümüştaş K, Meta Güzeyli FM, Atükeren P, Sanus GZ, Kemerdere R, Tanriverdi T, *et al*. (2007): The effects of vitamin E on lipid peroxidation, nitric oxide production and superoxide dismutase expression in hyperglycemic rats with cerebral ischemia-reperfusion injury. Turk Neurosurg., 17: 78-82.

Hoffman JW, Gilbert TB, Poston RS, Silldorff E.P. (2004): Myocardial reperfusion injury: etiology, mechanisms, and therapies. J Extra Corpor Technol., 36: 391-411.

Jancso G, Arató E, Sinay L. (2012): Ischemic Postconditioning Reduces Reperfusion Injury After Aortic Revascularization Surgery. In: Dai Yamanouchi, (eds) Vascular Surgery, 1st ed. China: Intech; pp. 237-26.

Jansen HJM E, Ruskovska T. (2013): Comparative Analysis of Serum (Anti) oxidative Status Parameters in Healthy Persons. Int J Mol Sci., 14: 6106-15.

Janssen M, Koster JF, Bos E, de Jong, J.W. (1993): Malondialdehyde and glutathione production in isolated perfused human and rat hearts. Circ Res., 73: 681-8.

Jayachitra A, Shreelatha S, Padma PR. (2012): Antioxidant and hepato protective effects of clitoria ternatea leaf extract by using in-vivo model. Int J Med Arom Plants., 2: 232-332.

Jiang Y, Tang JJ, Wu BQ, Yuan B, Qu Z. (2013): The Protective Effects of Different-Time-Ischemic Preconditioning on the Reperfusion Injury in Fatty Livers in Rats. PLoS One., 8: e58086. doi:10.1371/journal.pone.0058086

Kadkhodaee M, Aryamanesh S, Faghihi M, Zahmatkesh M. (2004): Protection of rat renal vitamin E levels by ischemic-preconditioning. BMC Nephrol., 5: 6.

Kadkhodaee M, Hemmati M, Zahmatkesh M, Ghaznavi R, Mirershadi F, Mahdavi-Mazde M, et al. (2008): Assessment of plasma antioxidant status in hemodialysis patients. Ther Apher Dial., 12: 47-51.

Kandilci HB, Gumusel B, Topaloglu E, Ucar G, Korkusuz P, Ugur Y, *et al*. (2006): Effects of ischemic preconditioning on rat lung: role of nitric oxide. Exp Lung Res., 32: 287-03.

Khan NA, Chattopadhyay P, Rai A, Kishore K, Wahi A.K. (2009): Antioxidant activity of ascorbic acid on ischemic and reperfused rat heart. Pharmacology Online, 3: 1-8.

Kher A, Meldrum KK, Wang M, Tsai BM, Pitcher JM, Meldrum DR. (2005): Cellular and molecular mechanisms of sex differences in renal ischemia–reperfusion injury. Cardiovasc Res., 67: 594-03.

Kilian JG, Nakhla S, Griffith K, Harmer J, Skilton M, Celermajer D.S. (2005): Reperfusion injury in the human forearm is mild and not attenuated by short-term ischemic preconditioning. Clin Exp Pharmacol Physiol., 32: 86-90.

Köken T, Inal M. (1999): The effect of nitric oxide on ischemia-reperfusion injury in rat liver. Clin Chim Acta., 288: 55-62.

Layton ME, Wood JG, Yan ZY, Forster J. (1996): Ischemia/reperfusion alters uric acid and ascorbic acid levels in liver. J Surg Res., 64: 1-5.

Lintz JA, Dalio MB, Joviliano EE, Piccinato CE. (2013): Ischemic pre and postconditioning in skeletal muscle injury produced by ischemia and reperfusion in rats. Acta Cir Bras., 28: 441-6.

Liu J, Yeo HC, Doniger SJ, Ames BN. (1997): Assay aldehydes from lipid peroxidation: gas chromatograph mass spectrometry compared to thiobarbituric acid. Anal Biochem., 245: 161-66.

Liu L, Lin YQ, Yan LT, Hong K, Hou XF, Mao LQ, *et al*. (2010): Extracellular ascorbic acid fluctuation during the protective process of ischemic preconditioning in rabbit renal ischemia-reperfusion model measured. Chin Med J (Engl)., 123: 1441-6.

Loukogeorgakis SP, Panagiotidou AT, Yellon DM, Deanfield JE and MacAllister R.J. (2006): Postconditioning Protects Against Endothelial Ischemia-Reperfusion Injury in the Human Forearm. Circulation, 113: 1015-9.

Luck H. (1974): Catalase. In: Bergmeyer HU, (eds). Methods in Enzymatic Analysis, 2nd ed., New York Academic Press; pp. 885-94.

Mahfoudh-Boussaid A, Zaouali MA, Hadj-Ayed K, Miled AH, Saidane-Mosbahi D, Rosello-Catafau J, *et al*. (2012): Ischemic preconditioning reduces endoplasmic reticulum stress and and upregulates hypoxia inducible factor-1α in ischemic kidney: the role of nitric oxide. J Biomed Sci., 19: 7.

Mahmud ZA, Bachar SC, Qais N. (2012): Antioxidant and hepatoprotective activities of ethanolic extracts of leaves of *Premna esculenta R*oxb against carbon tetrachloride-induced liver damage in rats. J Young Pharma., 4: 228-34.

Mohan M, Patankar P, Ghadi P, Kasture S. (2010): Cardioprotective potential of *Punica granatum* extract in isoproterenol-induced myocardial infarction in Wistar rats. J Pharmacol Pharmacother., 1(1): 32-7.

Montalvo-Javé EE, García-Puig MA, Escalante-Tattersfield T, Peña-Sánchez J, Vázquez-Meza H, Ortega-Salgado JA. (2011): Biochemical analysis and lipid peroxidation in liver ischemic preconditioning. Cir Cir., 79: 132-40.

Moron MS, Depierre JW, Mannervik B. (1979): Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta., 582: 67-78.

Narendra M, Bhatracharyulu NC, Padmavathi P, Varadacharyulu N.C. (2007): Prallethrin induced biochemical changes in erythrocyte membrane and red cell osmotic haemolysis in human volunteers. Chemosphere, 67: 1065-71.

Narendra M, Kavitha G, Padmavathi P, Helah Kiranmai A, Varadacharyulu NC. (2008): Allethrin-induced biochemical changes and properties of human erythrocyte membrane. Afr J Biochem Res., 2: 24-9.

Nedvig K, Völgyi E, Wéber G, Röth E, Ferencz A. (2011): Effect of ischemic postconditioning on oxidative stress and structural tissue changes in intestinal warm ischemic and auto transplantation model. Magy Seb., 64: 294-300.

Pagliaro PR, Rastaldo R, Penna C, Mancardi D, Cappello S, Losano GA. (2004): Nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway is involved in ischemic postconditioning in the isolated rat heart. Circulation, 110: 111-36.

Parasuraman S, Kumar EP, Kumar A, Emerson SF. (2010): Free radical scavenging property and diuretic effect of triglize, a polyherbal formulation in experimental models. J Pharmacol Pharmacother., 1: 38-41.

Parkash S, Sudha S. (2012): Lipid peroxidation and antioxidant status in south Indian patients with Type 2 Diabetes Mellitus. IRJP, 30: 132-4.

Pathak R and Pathak A. (2012): Study of life style habits on risk of type 2 diabetes. Int J Appl Basic Med Res., 2: 92–6.

Rasoulian B, Jafari M, Noroozzadeh M, Mehrani H, Wahhab-Aghai H, Hashemi-Madani SMH, et al. (2008): Effects of ischemia-reperfusion on rat renal tissue antioxidant systems and lipid peroxidation. Acta Medica Iranica., 46: 353-60.

Roe JH, Kuether CA. (1943): Determination of ascorbic acid whole blood and urine through the 2,4-dinitrodiphenylhydrazine derivate of ascorbic acid. J Biol Chem., 143: 399-406.

Rohilla A, Singh G, Khan MU, Khana R. (2011): Amelioration of myocardial ischemic reperfusion injury by simvastatin in rats. IRJP, 2: 51-55.

Rosenberg HR. (1942): Chemistry and Physiology of the vitamins. New York: Intersceince publishers; pp. 674.

Rutkowski M, Grzegorczyk K. (2007): Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Sci Pol Technol Aliment, 6: 17-28.

Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao G.S. (2002): Spectrophotometeric determination of serum nitrite and nitrate by copper cadmium alloy. Anal Biochem., 306:79-82.

Soncul H, Oz E, Kalaycioglu S. (1999): Role of ischemic pre-conditioning on ischemia-reperfusion injury of the lung. Chest, 11: 1672-77.

Song T, Peng YF, Guo SY, Liu YH, Liul LY. (2007): Brief small intestinal ischemia lessens renal ischemia-reperfusion injury in rats. Comp Med., 57: 200-5.

Talla V, Veerareddy PR. (2011): Oxidative stress induced by fluoroquinolones on treatment for complicated urinary tract infections in Indian patients. J Young Pharm., 3: 304-9.

ThomazNeto FJ, Koike MK, AbrahãoMde S, CarilloNeto F, Pereira RK, Machado JL, *et al*. (2013): Ischemic preconditioning attenuates remote pulmonary inflammatory infilteration of diabetic rats with an intestinal and hepatic ischemia-reperfusion injury. Acta Cir Bras., 28: 174-8.

Ucar G, Topaloglu E, Kandilci HB, Gümüsel B. (2005): Effect of ischemic preconditioning on reactive oxygen species-mediated ischemia-reperfusion injury in the isolated perfused rat lung. Clin Biochem., 38: 681-4.

Xing B, Chen H, Zhang M, Zhao D, Jiang R, Liu X, *et al*. (2008): Ischemic postconditioning inhibits apoptosis after focal cerebral ischemia/reperfusion injury in the rat. Stroke., 39: 2362-69.

Yang G, Cui SY, Huang LB. (2011): Protective Effects of the Preconditioning with Different Doses of Sodium Aescinate on Tourniquet-induced Ischemic Reperfusion. Chin J Integr Med., 17: 542-45.

Yang XM, Proctor JB, Cui L, Krieg T, Downey JM, Cohen M.V. (2004): Multiple, brief coronary occlusions during early reperfusion protect rabbit hearts by targeting cell signaling pathways. J Am Coll Cardiol., 44: 1103-10.

Yoshikawa T, Yasuda M, Ueda S, Naito Y, Tanigawa T, Oyamada H, *et al*. (1991): Vitamin E in gastric mucosal injury induced by ischemia-reperfusion. Am J Clin Nutr., 53: 210S-4S.

Yun Y, Duan WG, Chen P, Wu HX, Shen ZQ, Qian ZY, et al. (2009): Ischemic postconditioning modified renal oxidative stress and lipid peroxidation caused by ischemic reperfusion injury in rats. Transplant Proc., 41: 3597-02.

Zhang L, Ma J, Liu H. (2012): Protective Effect of Ischemic Postconditioning against Ischemia Reperfusion-Induced Myocardium Oxidative Injury in IR Rats. Molecules, 17: 3805-17.

Zhao WS, Xu L, Wang LF, Zhang L, Zhang ZY, Liu Y, et al. (2009). A 60s postconditioning protocol by percutaneous coronary intervention inhibits myocardial apoptosis in patients with acute myocardial infarction. Apoptosis, 14: 1204-11.