*Available online at* www.scholarsresearchlibrary.com

*Scholars Research Library*

**Archives of Applied Science Research, 2009, 1 (2) 74-80**
***(http://scholarsresearchlibrary.com/archive.html)***

**ISSN 0975-508X**

# Ischemic reperfusion injury alters the rat heart activity

**Najam Ali Khan[1], Pronobesh Chattopathyay[1], Dinesh Kumar[4], Kamal Kishore[2*], Arun Kumar Wahi[3]**

*[1]Department of Pharmacology and Clinical Research, College of Pharmacy, IFTM, Moradabad, Uttar Pradesh, India*
*[2]Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh, India*
*[3]Faculty of Pharmacy, MIT, Moradabad, Uttar Pradesh, India*
*[4]Division of Pharmacology & Toxicology, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh, India*

---

## Abstract

Heart disease is one of the major health problems of advanced as well as developing countries of the world. Extensive research through the last decade has shown beyond doubt that free radicals, particularly, reactive oxygen species play a cardinal role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction. In the present study, the effect on oxidative stress associated with IR injury was investigated in a rat heart model. Twelve rats of 200-250g body weight were divided into sham-operated control group (I) (n=6) and ischemia and reperfusion group (II) (n=6) used for induction of ischemia-reperfusion injury. Hearts from all the groups were then processed for biochemical and histopathological studies. One way ANOVA followed by Bonferroni test was applied to test for significance and values are expressed as mean±SEM ($p<0.05$). There was a significant decrease in myocardial catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) & activities of these enzymes in mitochondria in I/R group. Ischemia and reperfusion induced toxicity reduced remarkable amount of RNA content as compared to sham operated control rat. Histopathology studies showed degree of myocardial damage in ischemia and reperfusion group. The study strongly suggests oxidative stress and associated ultra structural changes induced by myocardial ischemic-reperfusion injury.

**Keywords:** Superoxide dismutase, Reduced glutathione, Ischemic heart disease, Reactive oxygen species.

---

## Introduction

Ischemic heart disease secondary to acute myocardial infarction is among the most prevalent health problems in the world, and is a major cause of morbidity and mortality [1, 2]. It is generally acknowledged that reactive oxygen species (ROS) play an important role in producing lethal cell injury associated with cardiac ischemia and reperfusion (3, 4). Experimental evidence has shown that during the first minutes after cardiac post ischemic reperfusion, a burst of ROS generation occurs [5, 6]. Oxygen radicals can be generated by several mechanisms, including the xanthine/xanthine oxidase reaction [7-10] and the activity of NADPH oxidase [11]. Another potential source of oxygen radicals is thought to be the mitochondrial respiratory chain [12]. Because of the abundance of mitochondria in cardiac myocytes, mitochondrial electron transport might be an important subcellular source of ROS and hence a potential contributor to heart reperfusion injury [13] following ischemia and reperfusion radical formation is greatly increased triggering cellular injury.

Although mammalian cells including cardiomyocytes express endogenous free radical scavenging enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, these antioxidative defenses are overwhelmed after ischemia and reperfusion [14]. The highly reactive radicals causes DNA damage and alter the cellular antioxidant defense system that comprises SOD and catalase hydrogen peroxide to water, which work in a sequential manner in the disposal of super oxide radicals and conversion of changes in Glutathione (GSH) homeostasis have also been implicated in the etiology progression of number of pathological diseases [15]. Mitochondria are the principal targets in the development of ischemia-reperfusion (I/R) induced injury [16]. The effects of free radicals are expressed by the accumulating of oxidative damage to biomolecules: nucleic acids, lipids and proteins [17].

Overall, it is thought that the combined effects of ROS and elevated $Ca^{2+}$ play a critical role in the transition from reversible to irreversible reperfusion injury. In particular, they lead to the opening of the mitochondrial permeability transition pore that is now widely accepted to play a critical role in reperfusion injury [18-20]. Generation of reactive oxygen species immediately upon reperfusion has been documented in experimental conditions, as well as in patients with acute myocardial infarction undergoing thrombolysis, coronary angioplasty or open heart surgery [21]. Free radicals are generated by one electron reduction or oxidation of molecules creating an unpaired electron. In normal mitochondrial oxidative phosphorylation, $O_2$ is reduced by four electrons to form $H_2O$. The energy derived from this reduction of $O_2$ serves to meet the energy demands of the cell. Paradoxically, it is also the actual process of oxygen reduction that leads to the formation of oxygen free radicals. Incomplete reduction of $O_2$ leads to the generation of superoxide anion ($O_2$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH). $O_2$ is unstable with a lifetime of milliseconds at neutral pH, and in aqueous solution it spontaneously reacts or dismutates to yield $H_2O_2$ and $O_2$. The OH is an extremely reactive and short-lived free radical produced in biological systems. In the Haber-Weiss reaction, $O_2$ and two OH are formed when $O_2$ reacts spontaneously with $H_2O_2$. In the Fenton reaction (also known as the iron-catalyzed Haber-Weiss reaction), reduction or oxidation of a trace metal in the presence $O_2$ and $H_2O_2$ gives rise to OH. The chemical mechanisms of free radical reactions in biological systems have been previously described [22]. We have also evaluated other changes induced by heart ischemia/reperfusion related to oxidative stress associated mitochondrial function.

## Materials and Methods

***Chemicals***

$CoQ_{10}$ was obtained as a gift sample from Tishcon Corporation, New York, USA. Other chemicals were obtained from Sigma (Sigma, St louis, Mo, USA) were of analytical grade.

***Animal care***

Twelve Wister rats were divided into sham-operated control group (I) (n=6) and ischemia and reperfusion group (II) (n=6) Male Wister rats weighing 200-250g were used for induction of ischemia reperfusion injury as described previously [23]. The study was approved by Institute Animal Ethics Committee and all animal care and experimental protocols were in compliance with the NIH guidelines for the care and use of the Laboratory Animals (NIH Publication #85-23, 1985). Wistar rats (200-250g) of either sex were maintained under standard laboratory conditions at temperature 25±2°C, relative humidity 50±15% and normal photo period (12 h dark/12 h light) was used for the study. Animal House (Reg. No.118/ac) IFTM, Moradabad.

***Infracts size determination***

At the end of 15 min ischemia followed by 45 min reperfusion, the heart were removed and cut into thin cross sectional slices and then incubated in a 0.08% solution of 2,3,5-triphenyltetrazolium chloride dissolved in Kerbs-Henseleit buffer at 37$^{o}$C for 30 minutes. The slices were then fixed in formalin. The cardiac ischemic zone was determined by using computer assisted plainimetry [24].

***Estimation on DNA and RNA***

Extraction of myocardial nucleic acid [25] & estimation of myocardial DNA and RNA [26].

***Biochemical parameters***

Isolation of Mitochondria: 200mg of cardiac tissue was weighed and homogenized with 0.35M sucrose buffer at 4$^{o}$C and centrifuged at 10,000 g for 5min. The resultant mitochondrial pallet was then resuspended in 0.25M sucrose solution containing 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA and made up to a final volume of 2 ml with the same [27]. Estimation of myocardial antioxidant such as reduced Glutathione [28], Catalase activity [29] and Super oxide dismutase [30].

***Histopathological Examination***

Myocardial tissue was fixed in 10% formalin, routinely processed and embedded in paraffin. Paraffin sections (3µm) were cut and stained with hematoxylin and eosin (H&E), examined under a microscope [31].

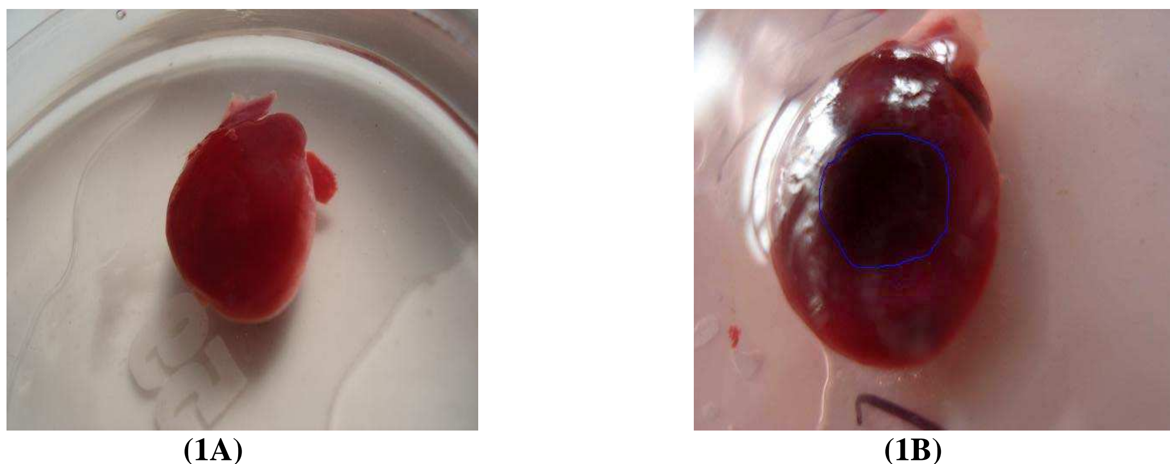
***Statistical analysis***

All values were expressed as mean±SD. Differences in mean values were compared using SPSS 11.0 by one-way ANOVA and Student-Newman-Keul (SNK) test. A value of $P<0.05$ was considered statistically significant.

## Results

The infract size shown in (Fig. 1 and table 1). Sham-operated Group infract size was 5.71% of the total surface of heart (Fig. 1A). After 15 min ischemia and 45 min reperfusion infracts size was 65% of total surface of the heart in IR group (Fig. 1B).

**Fig.1. Showing ischemic zone in (1a): Sham operated rat heart and (1b): Ischemia and reperfused rat heart after 2, 3, 5 triphenylterazolium chloride staining. Ischemic zone marked with blue lining**



**(1A)** **(1B)**

**Tab 1. Effect on rat heart after ischemia reperfusion injury**

| Groups | Percentage Infract Size |
|---|---|
| Sham-operated (Group I) | 5.71 ± 0.81 |
| IR Injury (Group II) | 65.60 ± 7.34[a] |

Results are expressed as mean ±SD (n=6). [a]Significantly different (P<0.01) from sham operated rats

Tables 2, represent the activity of myocardial antioxidant enzymes (GSH, SOD and CAT) after ischemic-reperfusion injury. There was a significant decrease in myocardial GSH, SOD and CAT activity in group (II) (4.53±0.60, 6.87±0.97 and 0.81±0.20 units/mg protein; p<0.05) as compared to sham operated group (7.15±0.73, 12.38±1.32 and 1.54±0.49 units/mg protein).

**Tab 2. Effect of $Q_{10}$ on mitochondrial antioxidant level of GSH, SOD, and CAT of rat suffered from acute heart injury induced by ischemic and reperfusion**

| Groups | GSH † | SOD † | CAT † |
|---|---|---|---|
| Sham operated (Group1) | 7.15±0.73 | 12.38±1.32 | 1.54±0.49 |
| IR (Group II) | 4.53±0.60[a] | 6.87±0.97[a] | 0.81±0.20[a] |

Results are expressed as mean ±SD (n=6). [a]Significant different (P<0.05), from sham operated rats. †Activity is expressed as: nmol per 100mg protein for GSH; unit per mg per 100 mg protein for SOD; nmol of $H_2O_2$ decomposed per min per mg protein for CAT.

Table 3, depicts the effect of $CoQ_{10}$ on DNA and RNA of rat suffered from acute heart injury induced by ischemic and reperfusion in control and experimental groups. DNA and RNA were 2.96±1.49, and 14.54±2.17 mg/g of heart tissues respectively in the sham operated rats decreased to 2.32±1.01, 8.29±0.73 mg/g of heart tissues after ischemic followed by reperfusion

**Tab 3. Effect on DNA and RNA of rat suffered from acute heart injury induced by ischemic and reperfusion**

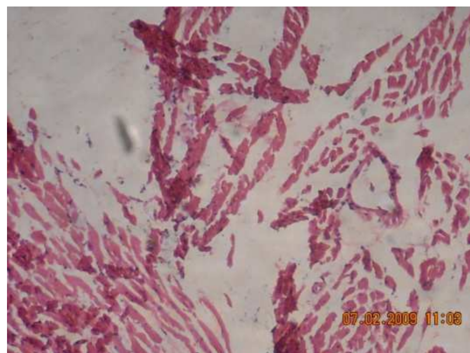
| Groups | DNA[‡] | RNA[‡] |
|---|---|---|
| Sham operated(Group1) | 2.96±0.79 | 14.54±2.17 |
| IR (Group II) | 2.32±0.45[a] | 8.29±0.73[a] |

Results are expressed as mean ±SD (n=6). [a] Significant different (P<0.05), from sham operated rats. [‡] Expressed as mg/g of heart tissues.

Sham operated rats showed normal structure similar to normal heart. (Fig. 2A) IR group showed severe necrosis, marked edema and focal destruction of myocardial in fibers (Fig. 2B)

**Fig 2. Histopathology of rat heart. (2a): Showing intact microfibrils and myocytes are embedded in tissue in sham operated group (H&E; x 200.). (2b): showing severe necrosis, marked edema and focal destruction of myocardial in fibers in IR group (H&E; x 200)**

**(2A)**




**(2B)**

## Discussion

Reactive oxygen ROS have been implicated in the cardiac tissue injury that follows ischemia and reperfusion. The mechanisms for the enhanced ROS generation and the cellular and subcellular targets of ROS attack are not well established. Mitochondria consume >90% of the oxygen used by the cell, and the mitochondrial respiratory chain generates a continuous flux of oxygen radicals. It has been estimated that 2% of the oxygen reacting with the respiratory chain leads to formation of superoxide radical. Subsequent dismutation of superoxide anion generates $H_2O_2$, which in turn can lead to production of OH·. Ischemia may cause mitochondrial alterations that would favor oxygen radical production when the oxygen concentration is reestablished by reperfusion and oxidative phosphorylation resumes because oxygen concentration increases and the level of reduced one-electron donors in the respiratory chain is concomitantly increased due to a decreased availability of ADP [32]. A similar situation is likely to be encountered during ischemia. When ischemic tissue is reoxygenated, electron transport through the respiratory chain is impaired because of depletion of ADP during ischemia, and this leads to a burst of ROS

generation during the first minutes of reoxygenation [33-35]. Cardiac myocytes are the likely targets of reactive oxygen species (ROS) attack in the failing heart. It is conceivable that free radicals cause damage at or near the site of their formation. Therefore, as a major source of ROS production, mitochondria could also be the major targets susceptible to ROS attack [36]. Histopathology showed that I/R induced injury damaged the degree of cardiocellular structure. Earlier study showed that defects in mitochondrial architecture would lead to the alteration of the mitochondrial metabolism, resulting in decreased activities of mitochondrial enzymes, in the heart, injury induced by IR, thus become a key contributor to intrinsic cell dysfunction [37]. Also it was demonstrated in previous study that DNA content decreases in I/R injury, presumably as a result of increase activity of TNF by toxic effect of ischemia and reperfusion. TNF may mediated direct toxicity to mitochondria and induce apoptosis or cell death [25]. Cellular antioxidant enzymes such as SOD, CAT, and free radical scavengers like GSH protect cells and tissues against noxious radicals. An imbalance between cellular pro-oxidant and antioxidant levels results in the oxidative stress that leads to tissue damage. The antioxidant enzymes react directly with ROS to yield non-radical products. SOD, a mitochondrial as well as cytosolic enzyme, $O_2^{\cdot-}$ is converted to $H_2O_2$ by dismutation, which is decomposed by CAT to $H_2O$ [38].

## Conclusion

In the present study, the profile of oxidative/antioxidative status in heart after acute injury induced by ischemic and reperfusion revealed marked alterations in antioxidant enzyme activities. Low activities of antioxidant enzymes such as SOD, CAT, and GSH might be due to the overwhelming effects of free radicals. Further experimentation is necessary to development of therapeutics in the treatment of ischemic myocardial complication.

## Acknowledgments

The authors are grateful to the Dr. R. M. Dubey, Managing Director, IFTM, Moradabad for providing all help and facility to carry out the research work and Division of pathology, Indian Veterinary Research Institute Izatnagar-243122, UP, India for histopathology study.

## References

[1] Hansen PR. *Eur Heart J*, **1995,** 16, 734-740.
[2] Ferrari R. *Am J Cardiol*, **1995,** 76, 17B-24B.
[3] Hess ML; Manson NH. *J Mol Cell Cardiol*, **1984**, 16, 969-985.
[4] Das DK. *Ann N Y Acad Sci*, **1994**, 723, 116-127.
[5] Zweier JL; Flaherty JT; Weisfeldt ML. *Proc Natl Acad Sci USA*, **1987**, 84, 1404-1407.
[6] Vanden Hoek TL; Shao Z; Li C; Zak R; Schumaker PT; Becker LB. *Am J Physiol*, **1996**, 270, H1334-H1341.
[7] Chambers DE; Parks DA; Patterson G; Roy R; McCord JM; Yoshida S; Parmley LF; Downey JM. *J Mol Cell Cardiol*, **1985**, 17, 145-152.
[8] Thompson-Gorman SL; Zweier JL. *J Biol Chem*, **1990**, 265, 6656-6663.
[9] Xia Y; Zweier JL. *J Biol Chem*, **1995**, 270, 18797-18803.

[10] Xia Y; Khatchikian G; Zweier JL. *J Biol Chem*, **1996**, 271, 10096-10102.
[11] Shandelya SM; Kuppusamy P; Weisfeldt ML; Zweier JL. *Circulation*, **1993**, 87, 536-546.
[12] Chance B; Seis H; Boveris A. *Physiol Rev*, **1979,** 59, 527-605.
[13] Ambrosio G; Zweier JL; Duilio C; Kuppusamy P; Santoro G; Elia PP; Tritto I; Cirillo P; Condorelli M; Chiariello M; Flaherty JT. *J Biol Chem*, **1993**, 268, 18532-18541.
[14] Dhalla NS; Elmoselhi AB; Hata T; Makino N. *Cardiovasc Res*, **2000,** 47, 446-456.
[15] Avti PK; Kumar S; Pathak CM; Vaiphei K; Khanduja KL. *Toxicological Sci*, **2006,** 89(2), 547-553.
[16] Elimadi A; Sapena R; Settaf A; LeLouet H; Tillement J; Morin D. *Biochem Pharmacol*, **2001,** 62, 509-516.
[17] Polidori MC; Griffiths HR; Mariani E; Meccocci P. *J amino acids*, **2007**, 32, 553-560.
[18] Halestrap AP; Clarke SJ; Javadov SA. *Cardiovasc Res*, **2004**, 61, 372-385.
[19] DiLisa F; Bernardi P. *Cardiovasc Res*, **2006**, 70, 191-199.
[20] Garcia Dorado D; Rodniguez Sinovas A; Ruiz Meana M; Inserte J; Agullo L; Cabestrero A. *Cardiovasc Res*, **2006**, 70, 274-285.
[21] Bolli R. *Prog Cardiovas Dis*, **1998**, 40, 477-516.
[22] Hammond B; Hess ML. *J Am Col Cardiol*, **1985**, 6, 215-220.
[23] Kato B; Nozawa T; Igarashi N; Nonomura M; Fujii N; Igawa A. *J Nucl Med*, **2004**, 45, 1074-1080.
[24] Joshi CN; Jain SK; Murthy PSR. *Brain Res Protocol*, **2004**, 13, 11-17.
[25] Hibbs JB; Taintor RR; Vavrin Z; Rachlin EM. *Biochem Biophys Res Commun*, **1988**, 157, 87-94.
[26] Burton K. *Biochem J*, **1956**, 62, 615-616.
[27] Johnson D; Lardy H. *Methods in Enzymology*, **1947**, 10, 95-96.
[28] Elman GL. *Arch Biochem Biophys*, **1969**, 82, 70-77.
[29] Aebi H. *Methods in Enzymolog*, **1954**, 105, 121-126.
[30] Beatecheemp C; Frindrorich JX. *J Biological Chem*, **1969**, 244, 6049-6055.
[31] Shamatsu K; Kanles IR. *Hepatology*, **1997**, 266, 343-349.
[32] Chance B; Seis H; Boveris A. *Physiol Rev*, **1979**, 59, 527-605.
[33] Zweier JL; Flaherty JT; Weisfeldt ML. *Proc Natl Acad Sci USA*, **1987,** 84, 1404-1407.
[34] Vanden Hoek TL; Shao Z; Li C; Zak R; Schumaker PT; Becker LB. *Am J Physiol*, **1996**, 270, H1334-H1341.
[35] Ambrosio G; Zweier JL; Duilio C; Kuppusamy P; Santoro G; Elia PP; Tritto I; Cirillo P; Condorelli M; Chiariello M; Flaherty JT. *J Biol Chem*, **1993**, 268, 18532-18541.
[36] Statman ER. *Science*, **1992**, 257, 1220-1224.
[37] Mimnaugh EG; Trush MA; Mohit B; Gram FE. *Biochem Pharmacol*, **1985**, 34, 847-856.
[38] Hassan HM; Fridovich I. *J Biol Chem*, **1978;** 253, 8143-8148.