

**ANTIOXIDANT ACTIVITY OF ASCORBIC ACID ON ISCHEMIC AND REPERFUSED RAT HEART**

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**Running title:** Ascorbic acid protects oxidative stress

**Summary**

Oxidative stress plays a major role in the biochemical and pathological changes associated with myocardial ischemic-reperfusion injury (IRI). The need to identify agents with a potential for preventing such damage has assumed great importance. In the present study, the effect of oral administration of ascorbic acid on oxidative stress induced by ischemic-reperfusion injury in isolated rat heart was investigated. Eighteen Wistar rats were divided into sham-operated control group (I) (n=6), ischemia and reperfusion group (II) (n=6) and ascorbic acid treated group (1mg kg<sup>-1</sup> p.o./day for 7 consecutive days before induced ischemia reperfusion) group (III) (n=6). Hearts from all the groups were then processed for biochemical analysis. 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 increase in myocardial catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in group III as compared to group II. There was depletion of myocardial endogenous antioxidants (SOD, CAT and GPx) was observed in control rats subjected to ischemia-reperfusion group II. The present study demonstrated for the ascorbic acid protected rat heart from oxidative stress associated with ischemic-reperfusion injury.

**Key words:** Ascorbic acid, Vitamin-C, Antioxidants, Oxidative stress, Ischemic reperfusion injury

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## **Introduction**

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 (ROS) play a cardinal role in the pathogenesis of oxidative myocardial damage with consequential cardiac malfunction. This review presents a comprehensive account of the present day knowledge regarding the oxygen free radicals involved in the genesis of ischemic heart disease, the mechanism(s) of oxidative myocardial damage and the endogenous as well as exogenous antioxidant defense systems. Furthermore, the role of ischemic pre-conditioning, some antioxidants and the ability of some cardioprotective drugs in providing protection against the ischemic myocardial damage are also discussed.

In addition to this, Vitamin C acts as the first line of defence against oxidative stress during ischemia reperfusion cycle [1]. It is the only antioxidant in plasma capable of completely inhibiting oxidative modification of low density lipoproteins by aqueous peroxy radicals [2]. Vitamin-C administration exerts a protective role against peroxidative damage of lipids [3]. The damage caused by ROS is generally countered by a synergistic, multilevel defence antioxidant system [4]. A large number of natural or synthetic antioxidant compounds (e.g. Vitamin-E and C) which have the ability to inhibit the oxidative damage by scavenging the highly destructive free radical species [5]. They may be overwhelmed under pathological conditions. In recent times, attention has been given to the concept of reducing myocardial injury at the time of reperfusion by pretreatment with free radical scavenger vitamins [6, 7]. Therefore, the study was designed to evaluate the effects of ascorbic acid on myocardial endogenous oxidative stress associated with ischemic-reperfusion injury in isolated rat heart.

## **Materials and Methods**

### **Chemicals**

All chemicals were of analytical grade and chemicals were obtained from Sigma Chemicals, St Louis, USA.

### **Animal and treatment**

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  $25\pm 2^{\circ}\text{C}$ , relative humidity  $50\pm 15\%$  and normal photo period (12 h dark/12 h light) was used for the study. Animal House (Reg. No.118/ac) IFTM, Moradabad. Eighteen Wister rats were divided into sham-operated control group (I) (n=6), ischemia and reperfusion group (II) (n=6) and Ascorbic acid treated group (1mg/kg body weight daily by oral route for 7 days before induced ischemia reperfusion) group (III) (n=6). Male Wister rats weighing 200-250g were used for induction of ischemia reperfusion injury as described previously. [8].

**Infracts size determination**

At the end of 15min ischemia followed by 45min 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°C for 30minutes. The slices were then fixed in formalin. The cardiac ischemic zone was determined by using computer assisted planimetry [9].

**Biochemical parameters**

Isolation of Mitochondria: 200mg of cardiac tissue was weighed and homogenized with 0.35M sucrose buffer at 4°C and centrifuged at 10,000g for 5min. The resultant mitochondrial pellet 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 [10].

**Assay of enzymes**

Estimation of myocardial antioxidant such as reduced Glutathione [11] Catalase activity [12] and Superoxide dismutase [13] were determined as described previously.

**Statistical analysis**

All the results were statistically interpreted using one-way analysis of variance (ANOVA) followed by Bonferroni test. A value of  $P < 0.05$  was considered statistically significant.

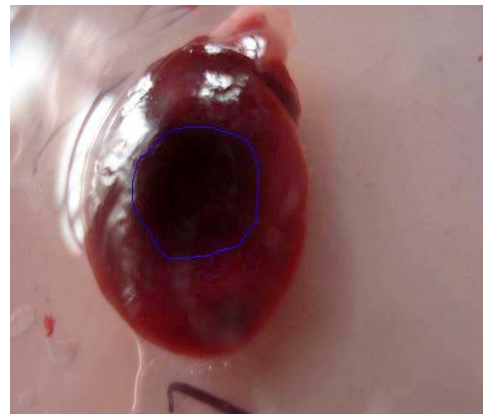
**Results**

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). Ascorbic acid treated group reduce infracts size as compared to IR group which was 46% of the total surface of heart (Fig. 1C).

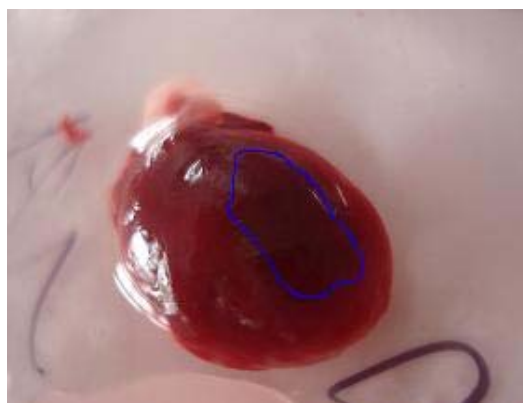
**Fig 1: Showing ischemic zone in (1A): Sham operated rat heart and (1B): Ischemia and reperfused rat heart (1C): Ascorbic acid treated rat heart after 2, 3, 5 triphenylterazolium chloride staining. Ischemic zone marked with blue lining**



(1A)



(1B)



(1C)

**Table 1: Effect of ascorbic acid after ischemia reperfusion of rat heart**

Groups	Percentage Infarct Size
Sham-operated (Group I)	5.71 ± 0.81
IR Injury (Group II)	65.60 ± 7.34**
Ascorbic acid treated (Group III)	46.19± 4.11*##

Results are expressed as mean± SD (n=6). Significantly different (\*P<0.05, \*\*P<0.01) from sham operated rats and significantly different (##P<0.01) from vehicle-treated ischemia and reperfusion were recorded.

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) ( $4.53 \pm 0.60$ ,  $6.87 \pm 0.97$  and  $0.81 \pm 0.20$  units/mg protein;  $p < 0.05$ ) as compared to sham operated group ( $7.15 \pm 0.73$ ,  $12.38 \pm 1.32$  and  $1.54 \pm 0.49$  units/mg protein). Activities of these enzymes in mitochondria were improved ( $p < 0.05$ ) level in Ascorbic acid treated groups with significant change in group (III) ( $6.95 \pm 0.86$ ,  $10.37 \pm 1.39$  and  $1.34 \pm 0.67$  units/mg protein) group when compared to group (II).

**Table 2: Effect of ascorbic acid 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)	$7.15 \pm 0.73$	$12.38 \pm 1.32$	$1.54 \pm 0.49$
IR (Group II)	$4.53 \pm 0.60^*$	$6.87 \pm 0.97^*$	$0.81 \pm 0.20^*$
Ascorbic acid treated (Group III)	$6.95 \pm 0.86^\#$	$10.37 \pm 1.39^\#$	$1.34 \pm 0.67^\#$

Results are expressed as mean $\pm$ SD (n=6). Significant different (\* $P < 0.05$ ), from sham operated rats. Significant different ( $^\#P < 0.05$ ), from vehicle treated ischemia and reperfusion. †Activity is expressed as: nmol per 100mg protein for GSH; unit per mg per 100 mg protein for SOD; nmol of H<sub>2</sub>O<sub>2</sub> decomposed per min per mg protein for CAT.

### Discussion

In the present study, ischemic reperfusion injury (IRI) was associated with increased oxidative stress, as evidenced by depletion of myocardial endogenous antioxidants such as SOD, catalase, and GSH. Similar observations were made earlier by different other studies, using similar models [14, 15, 16]. Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase, glutathione peroxidase, glutathione [17, 18, 19]. As IRI is a common sequel of ischemic heart disease and oxidative stress plays a central role in its etiopathogenesis, protection against oxidative stress through a novel mechanism like myocardial adaptation holds promise as an effective therapeutic approach. Myocardial adaptation occurs in response to various kinds of noxious stimuli, like ischemia [20], certain endotoxins [21], reactive oxygen species [22], etc. and protects heart from subsequent exposure to injuries of similar or more severe nature [23, 24].

The 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 [25]. 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, 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,  $O_2^-$  is converted to  $H_2O_2$  by dismutation, which is decomposed by CAT to  $H_2O$  [26]. Our results concord with the earlier work in ischemic heart, reduced glutathione is only one among many potential antioxidant defenses involved in the protection of various organs against oxidant-induced injury in inflammation [27]. Major antioxidants like SOD, CAT, and GSH are important for cellular protection due to their ability to detoxify free radicals such as reactive oxygen species. A number of studies have reported diverse results for the changes of these antioxidant enzyme activities in animal heart, injury induced by ischemic and reperfusion. It has been recently demonstrated that during reperfusion of the ischemic heart, there is increased generation of oxygen free radicals [28, 29] and depletion of endogenous antioxidants [30]. Evidence of protective effects of vitamin-C in reperfusion injury has been provided by many investigators [31, 32]. The rise in the activities of mitochondrial antioxidant enzyme in group III rats, pre-treated with ascorbic acid highlights the protection rendered by the ascorbic acid in combating the oxidative stress.

Experimental as well as clinical studies with exogenous antioxidants supplementation have been shown to have protective effect in ischemic heart disease [33, 34]. In this regard, the most commonly used exogenous antioxidants are vitamin C. The observations made in the present study have important nutritional significance in relation to ischemic heart disease. Thus, we have evaluated the antioxidant potential of ascorbic acid in prevention on IR injury in our present studies it could be concluded that the ascorbic acid shows antioxidative effect. Further study is needed to establish molecular basis of cardio protective effects of ascorbic acid in ischemic-reperfusion injury.

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