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Chronic garlic administration protects rat heart against oxidative stress induced by ischemic reperfusion injury

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Abstract

Background: 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. Chronic oral administration of raw garlic has been previously reported to augment myocardial endogenous antioxidants. In the present study, the effect of chronic oral administration of raw garlic homogenate on oxidative stress induced by ischemic-reperfusion injury in isolated rat heart was investigated.

Results: Raw garlic homogenate (125, 250 and 500 mg/kg once daily for 30 days) was administered orally in Wistar albino rats. Thereafter, hearts were isolated and subjected to IRI (9 min. of global ischemia, followed by 12 min of reperfusion; perfusion with K-H buffer solution; 37°C, 60 mm Hg.). Significant myocyte injury and rise in myocardial TBARS along with reduction in myocardial SOD, catalase, GSH and GPx were observed following IRI. Depletion of myocardial endogenous antioxidants and rise in TBARS were significantly less in the garlic-treated rat hearts. Oxidative stress induced cellular damage as indicated by ultrastructural changes, like disruption of myofilament, Z-band architecture along with mitochondrial changes were significantly less.

Conclusions: The study strongly suggests that chronic garlic administration prevents oxidative stress and associated ultrastructural changes, induced by myocardial ischemic-reperfusion injury.

Background

Oxidative stress is a well established etiopathogenic factor of ischemic heart disease (IHD) and its consequences. Reperfusion of the ischemic myocardium is the only logical approach for the successful management of patients with acute obstruction of coronary arteries. Morphologic observations of the ischemic myocardial tissue undergoing reperfusion suggest that reperfusion injury is a true pathologic phenomenon and a distinct entity from the preceding ischemic injury. 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 [1]. Upon

reperfusion, molecular oxygen undergoes sequential reduction to form reactive oxygen species, including superoxide anion and hydroxyl radical, in addition to hydrogen peroxide. The interaction of oxygen-derived free radicals with cell membrane lipids and essential proteins contribute to myocardial cell damage, leading to depressed cardiac function and irreversible tissue injury with concomitant depletion of certain key endogenous antioxidant compounds, e.g., superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and glutathione peroxidase (GPx) [2].

Although antioxidant administration during reperfusion injury has been shown to reduce the severity of the ischemic reperfusion injury, yet some properties of antioxidants such as cytotoxicity [3], pro-oxidant activity [4] or high molecular weight in case of SOD, limited their therapeutic application. Use of plant extracts [5], food supplement [6] and even drugs [7] which augment major cellular endogenous antioxidants following chronic administration have been identified as a promising therapeutic approach to combat oxidative stress associated with ischemic heart disease. The property of augmenting cellular endogenous antioxidants has been defined as a major constituent of myocardial adaptation against oxidative stress [8].

Both epidemiological and experimental studies have claimed that garlic (*Allium sativum*) has significant beneficial effects in ischemic heart disease (IHD) [9,10]. Garlic reduces a multitude of risk factors which play a decisive role in the genesis and progression of IHD, e.g., hypolipidemic effect, lowering of arterial blood pressure and inhibition of platelet aggregation [11]. Recently, it has been shown that chronic administration of garlic augmented SOD and catalase in rat heart [12]. In view of this observation, the present study was designed to investigate whether this property of garlic could offer protection against oxidative stress arising out of ischemic-reperfusion injury in isolated rat heart.

Results

Biochemical parameters

There was no mortality, change in body weight (table 1) as well as food and water intake pattern of rats in any group. The effects of various doses of raw garlic homogenate on lipid peroxidation and endogenous antioxidants of ischemic-reperfused hearts are shown in table 2.

Myocardial TBARS

There was significant ($p < 0.05$) increase in myocardial TBARS in the group C-IR when compared to the control (C). Significant decrease in the level of myocardial TBARS was observed in groups 1, G-125 ($p < 0.02$), G-250 ($p < 0.001$) and G-500 in comparison to the C-IR group.

Table 1: Effect of chronic administration of raw garlic homogenate on body weight gain in one month

Groups	gm/ 100 gm rat
Control (C)	14.44 ± 1.89
Garlic 125 mg/kg (G-125)	13.75 ± 1.37
Garlic 250 mg/kg (G-250)	16.94 ± 1.48
Garlic 500 mg/kg (G-500)	13.97 ± 1.25

All values are expressed as Mean ± SE (n = 6). There was no significant change among all four groups. One way ANOVA was applied to test for significance. Significance was set at $p < 0.05$.

Myocardial SOD

In C-IR group, there was a significant ($p < 0.05$) reduction in myocardial SOD activity when compared to control (C) group. Significant ($p < 0.001$) increase in myocardial SOD activities was observed in groups G-125 and G-250 but not in group G-500 when compared to C-IR.

Myocardial Catalase

In the C-IR group there was a significant ($p < 0.001$) decrease in myocardial catalase activity compared to control (C). In the groups G-125 and G-500, there was no significant increase in the level of myocardial catalase activities compared to C-IR. However, significant ($p < 0.001$) increase in myocardial catalase activity was seen only in group G-250.

Myocardial GSH

Significant ($p < 0.001$) decrease in myocardial GSH level was observed in the group C-IR in comparison to the control (C). In the groups G-125, G-250 and G-500, there was an significant ($p < 0.02$) increase of myocardial GSH levels when compared to C-IR.

Myocardial GPx

Significant ($p < 0.05$) reduction in GPx activity was observed in C-IR group. No change of GPx activity was observed in any of the garlic fed groups when compared to C-IR group.

Light microscopic changes

In C IR group, there was patchy necrosis of muscle fibers with infiltration of acute and chronic inflammatory cells. Marked interstitial edema was also noted (Fig 1A). The degree of myocardial damage in G-125 group was similar to C IR in regard to morphological changes (Fig 1B). In G-500 group there was focal acute and chronic inflammation with interstitial edema but no myonecrosis was seen (Fig 1D). In G-250 group, the interstitial edema as well as inflammation was less than G-500 group (Fig 1C). The myonecrosis was also not remarkable in this group.

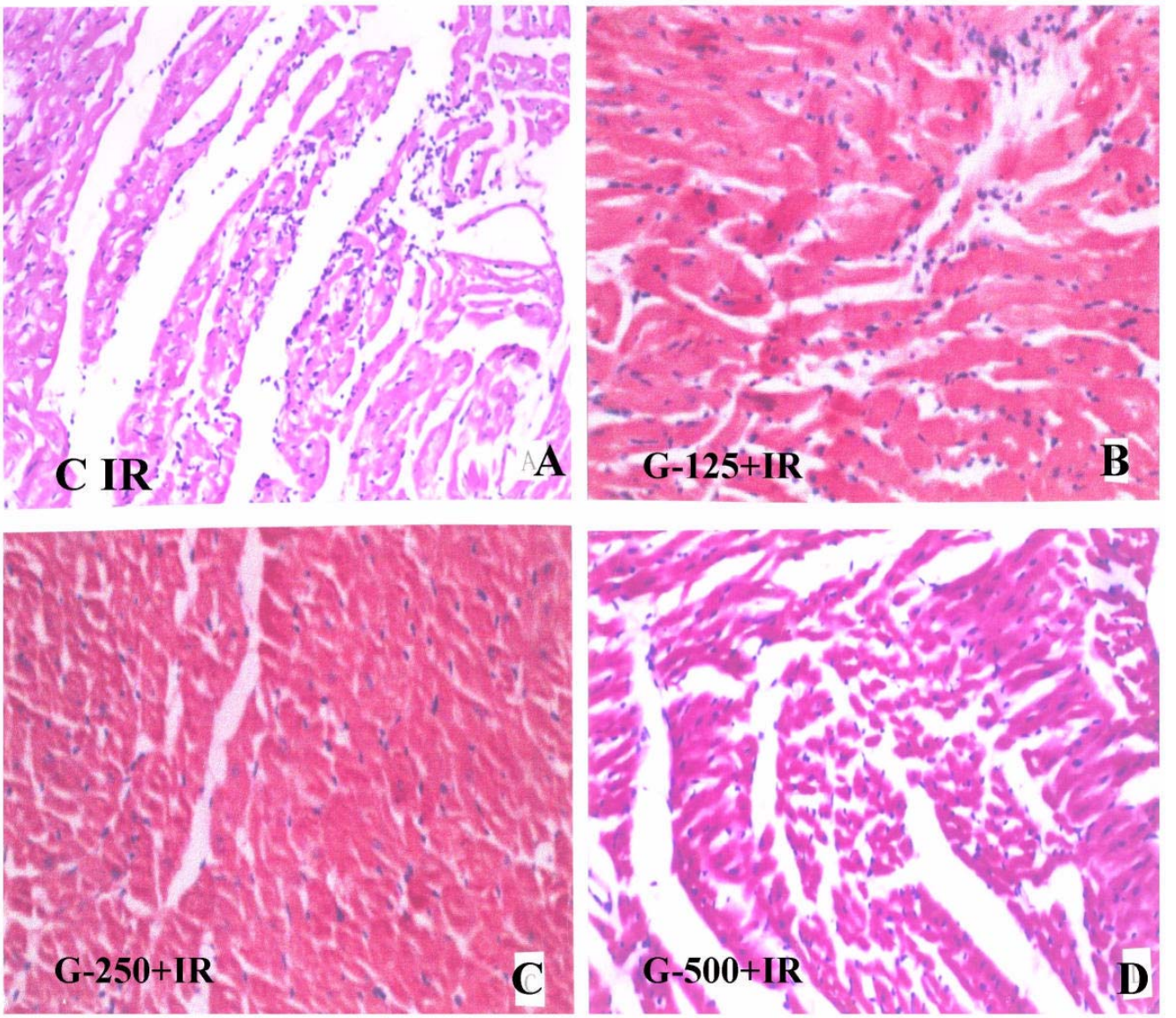

Figure 1
Light micrograph of **A]** control rat heart subjected to ischemic reperfusion injury showing focal loss of muscle fibre with inflammation (H & E × 10). **B]** 125 mg/kg/day garlic treated rat heart subjected to ischemic reperfusion injury showing occasional loss of muscle fiber with mild inflammation (H & E × 10). **C]** 250 mg/kg/day garlic treated rat heart subjected to ischemic reperfusion injury showing normal structure of myocyte with slight edema (H & E × 10). **D]** 500 mg/kg/day garlic treated rat heart subjected to ischemic reperfusion injury showing occasional loss of myofibre with mild edema (H & E × 10).

Ultrastructural changes (transmission electron microscopy)

Characteristic changes were seen in the rat heart subjected to IRI (group C-IR). There were significant disruption of myofilament and Z-band architecture in C-IR group (Fig. 2A &2B). Other ultrastructural changes were manifested by loss of cell membrane integrity, interstitial edema, the appearance of vacuoles within the cell and changes in the mitochondrial architecture (Fig. 2D). Extensive loss of crystae and double membrane and presence of vacuoles in mitochondria were prominent (Fig. 2D). However, myocardial ultrastructure was found to be well preserved and less evidence of myocyte injury was observed in both 250 and 500 mg/kg/day dose of chronic garlic fed groups (G-250 and G-500) and not in the G-125 group (Fig. 3). Only occasional disruption of myofilament, mild interstitial edema and less accumulation of electron dense material in mitochondria were noticed in G-250 and G-500 groups (Fig. 4 &5).

Table 2: Effect of chronic administration of raw garlic homogenate on TBA-RS, GSH, catalase, SOD and GPx in rat heart after ischemic-reperfusion injury

Group	TBA-RS (n mole/gm wet wt.)	GSH (μg/gm wet wt.)	CATALASE (U/mg protein)	SOD (U/mg protein)	GPx (mU/mg protein)
C	203.03 ± 11.52	479.17 ± 40.76	49.49 ± 2.91	6.88 ± 0.79	357.41 ± 38.41
C-IR	259.96 ± 22.48^{ac}	284.57 ± 24.33^{cc}	14.41 ± 1.94^{cc}	4.61 ± 0.65^{ac}	272.30 ± 24.02^{ac}
G-125	161.43 ± 26.05^{b}	366.93 ± 18.44^{b}	21.01 ± 5.56	16.29 ± 3.16^{c}	287.83 ± 37.29
G-250	130.78 ± 11.36^{c}	398.19 ± 13.90^{b}	45.35 ± 5.69^{c}	17.99 ± 2.93^{c}	316.20 ± 51.26
G-500	197.77 ± 25.79^{a}	371.18 ± 16.53^{b}	12.35 ± 1.70	4.23 ± 0.59	288.31 ± 28.92

All values are expressed as Mean ± SE (n = 6). p values: ac < 0.05, cc < 0.001 vs Control a < 0.05, b < 0.02, c < 0.001 vs C-IR (One way ANOVA)

Discussion

In the present study, ischemic reperfusion injury (IRI) was associated with increased oxidative stress, as evidenced by increase in myocardial TBARS and depletion of myocardial endogenous antioxidants such as SOD, catalase, GSH and GPx. Similar observations were made earlier by different other studies, using similar models [13,19–21]. Ultrastructural changes, like disruption of myofilaments and Z-band architecture along with mitochondrial swelling also occurred in the myocytes following ischemia reperfusion. Increased oxidative stress might be responsible for such myocyte injuries. Chronic garlic administration prevented the oxidative stress and the ultrastructural changes associated with IRI. The mechanism of such protection of chronic garlic administration may be postulated in the light of the phenomenon of myocardial adaptation.

Myocardial adaptation against oxidative stress is mediated through augmentation of a number of cellular antioxidants, such as SOD, catalase, glutathione peroxidase, glutathione [8,22–24]. As IRI is a common sequel of ischemic heart disease (IHD) 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 obnoxious stimuli, like ischemia [25], certain endotoxins [26], reactive oxygen species [27], etc. and protects heart from subsequent exposure to injuries of similar or more severe nature [28,29]. Although protective in nature, the basic mechanisms of such induction of adaptation are harmful in themselves and, therefore, cannot be relied upon as acceptable therapeutic methods. Therefore, pharmacological [30,31], as well as transgenic approaches [32,33] of myocardial adaptation has recently become the focus of recent scientific interest.

It is interesting to note that different plants and plant extracts can also stimulate the synthesis of cellular antioxidants [5,34–36]. It was reported earlier that aged garlic extract increases the level of SOD, GSH and GPx in vascular endothelial cell [37,38] and inhibits oxidative-stress mediated ischemic-reperfusion damage in rat brain [39]. We have also reported that chronic oral administration of raw garlic homogenate caused a significant increase in basal SOD and catalase activities of rat heart, which was associated with a concomitant decrease in basal lipid peroxidation [12]. Simultaneous increase in SOD and catalase activities, as observed with chronic administration of garlic homogenate has special importance of being more beneficial than increase in SOD activity alone, because without a concomitant increase in catalase activity, increased SOD activity may lead to intracellular accumulation of H_2O_2[40] with detrimental effects.

Protection against ischemia reperfusion induced oxidative stress in garlic treated rat hearts was evidenced by preservation of endogenous antioxidants and prevention in rise of TBARS. Among the three garlic treated groups, only 250 mg/kg group showed the best protection in terms of biochemical and histopathological evidences. Garlic in 125 mg/kg group did not show better histopathological improvement compared to garlic 500 mg/kg group. This can be explained by our previous study [12] where only 250 and 500 mg/kg doses of chronic garlic administration augmented both SOD and catalase activities, while 125 mg/kg garlic administration augment only SOD activity.

Conclusions

The observations made in the present study showed for the first time that chronic garlic administration prevents oxidative stress and associated ultrastructural changes induced by myocardial ischemic reperfusion injury.

Methods

Plant extract

Garlic (*Allium sativum*) bulbs were purchased from a local market. The cloves were peeled, sliced, ground into a paste and a homogenate was made in distilled water. Three different concentrations of the extract were prepared, 0.05,

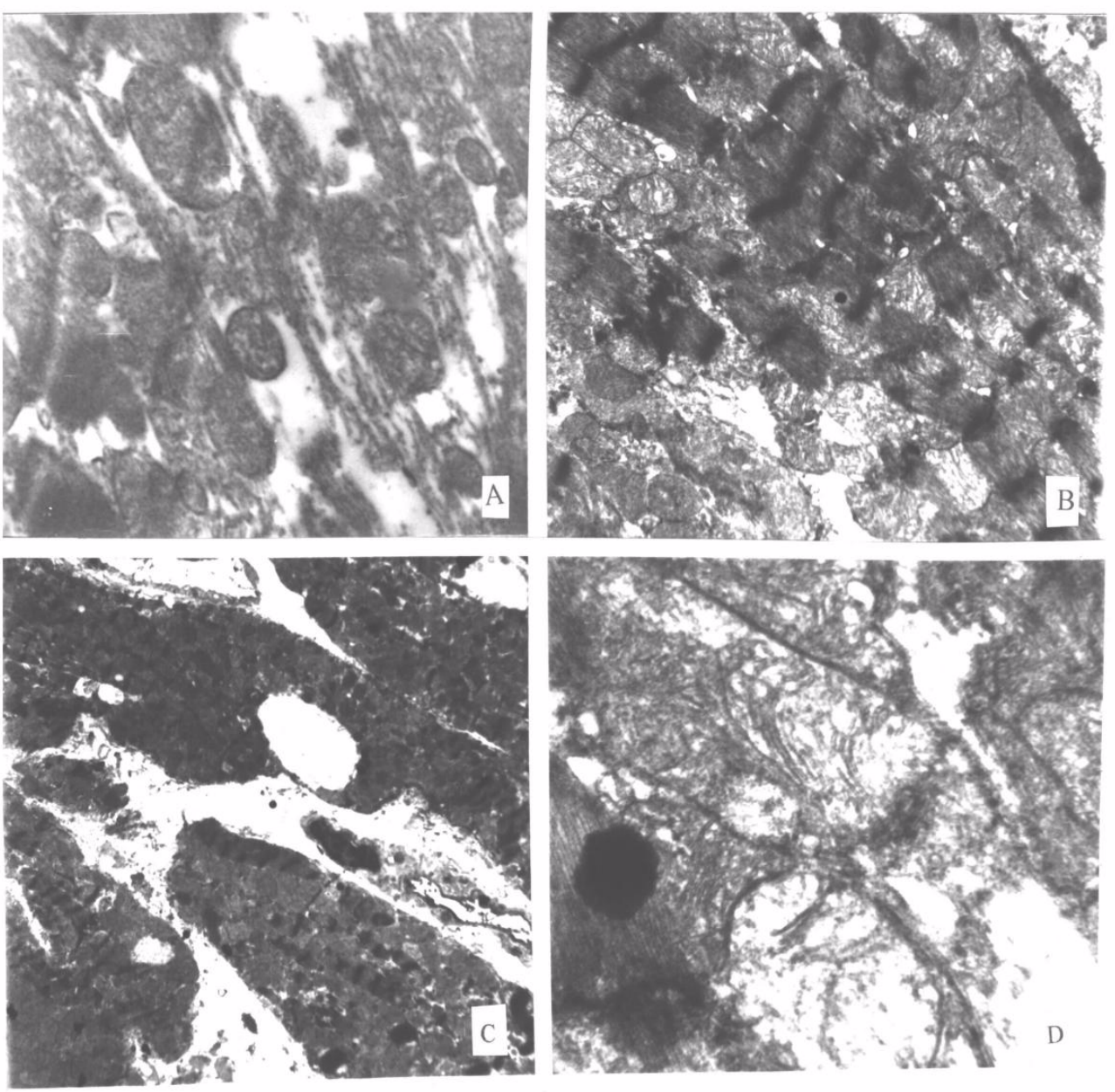

Figure 2
Electron micrograph of rat heart subjected to ischemic-reperfusion injury showing **A]** extensive muscle necrosis with disruption of architecture (× 4600). **B]** focal viable muscle fiber (× 2650) **C]** marked damage of mitochondria (× 870). **D]** extensive loss of crystae, double membrane and vaculisation in mitochondria (× 8400).

0.1 and 0.2 gm/ml, corresponding to 125 mg, 250 mg and 500 mg/kg body weight of animal. Oral feeding was done within 30 minutes of the preparation of homogenate.

Animals

The study was approved by the Institute *Animal Care Ethics Committee*. Laboratory bred Wistar albino rats of both sexes weighing between 150 and 200 gm, maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photo period (12 hr dark/12 hr light) were used for the experiment. Commercial pellet diet and water were provided ad libitum. Commercial pellet diet (Ashirwad, India) contains protein: 24%, fat: 5%, fiber: 4%, carbohydrate: 55%, calcium: 0.6%, phosphorous: 0.3%, moisture: 10% and ash: 9% w/w.

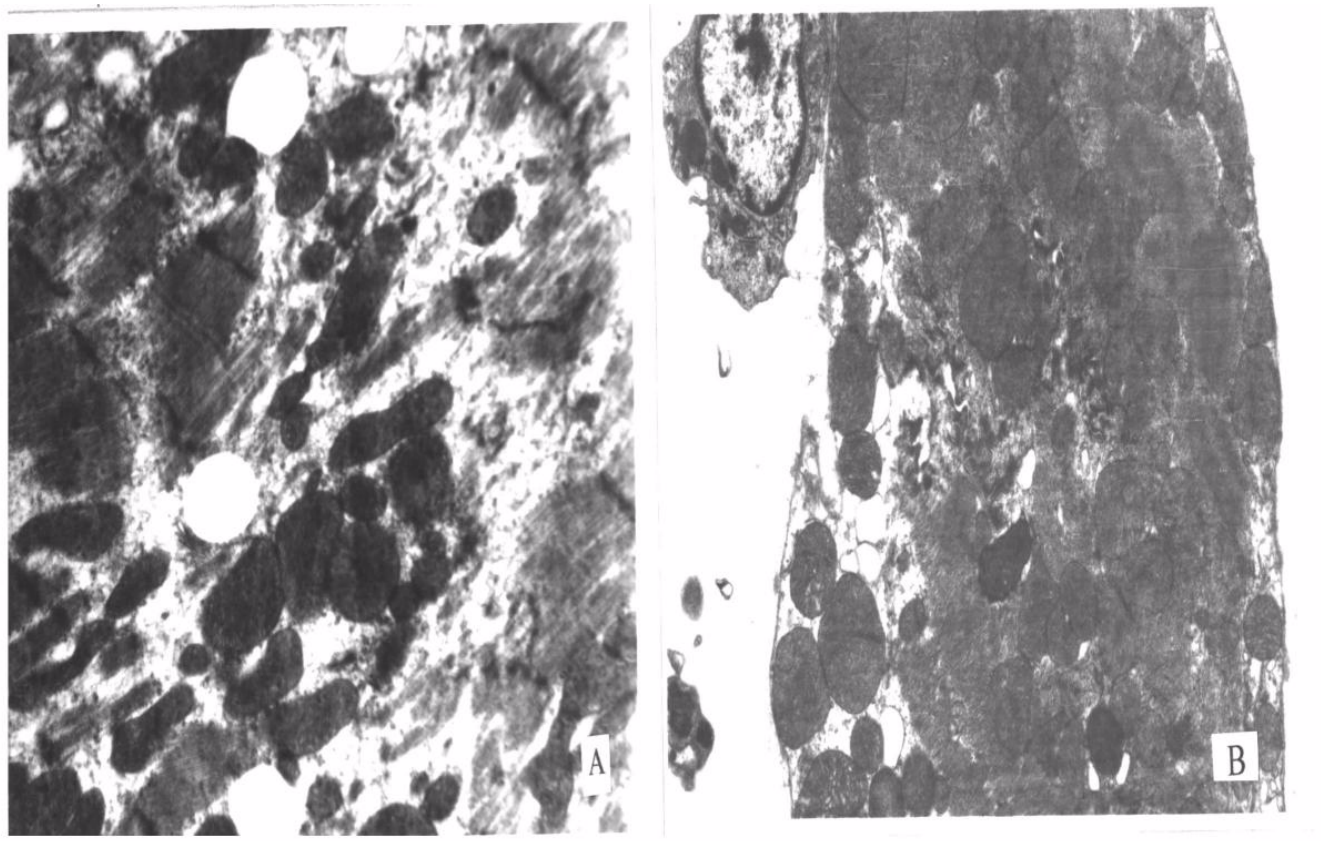

Figure 3
Electron micrograph of rat heart treated with garlic 125 mg/kg for 30 days and subjected to ischemic-reperfusion injury showing **A]** loss of myofilament along with mitochondrial damage. Disruption of crystae and double membrane in mitochondria is prominent (× 3400). **B]** disruption of mitochondria and vaculisation (× 2050).

Chemicals

All chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Sigma Chemicals, St Louis, USA. Double distilled water was used in all biochemical assays.

Experimental protocol

Rats were randomly divided into four groups, each group having 6 rats. Aqueous homogenate of garlic bulb was fed by oral gavage everyday at a fixed time (10.00 a.m) for 30 days in three different doses. Control rats were fed normal saline daily for 30 days. Changes in body weight, food and water intake patterns of rats in all groups were noted throughout the experimental period. After 48 hours of the last dose, rats were heparinised [375 units/200 gms i.p], anaesthetized after 30 min. with sodium pentobarbitone (60 mg/kg i.p) and subjected to the following protocol.

Production of ischemic-reperfusion injury in isolated rat heart

Hearts were rapidly excised and washed in ice-cold saline, and then perfused with the non-recirculating Langendorff's technique (Hufesco, Hungary), in constant pressure mode with modified Kreb's Henseleits buffer [13] containing [in mM]: glucose 11.1; NaCl 118.5; $NaHCO_3$ 25; KCl 2.8; KH_2PO4 1.2; $CaCl_2$ 1.2; $MgSO_4$ 0.6, with a pH of 7.4. The buffer solution equilibrated with 95% O_2 + 5% CO_2 was delivered to the aortic cannula at 37°C under 60 mm Hg pressure. Following 5 min-equilibration period, hearts were subjected to 9 min. of zero-flow [ischemia] and 12 min of re-flow [reperfusion] (IR injury).

Group C : Normal saline-fed rat hearts subjected to 26-min. perfusion.

Group C-IR : Normal saline-fed rat hearts subjected to IR injury.

Group G-125: 125 mg/kg/day for 30 days of garlic homogenate fed rat hearts subjected to IR injury.

Group G-250: 250 mg/kg/day for 30 days of garlic homogenate fed rat hearts subjected to IR injury.

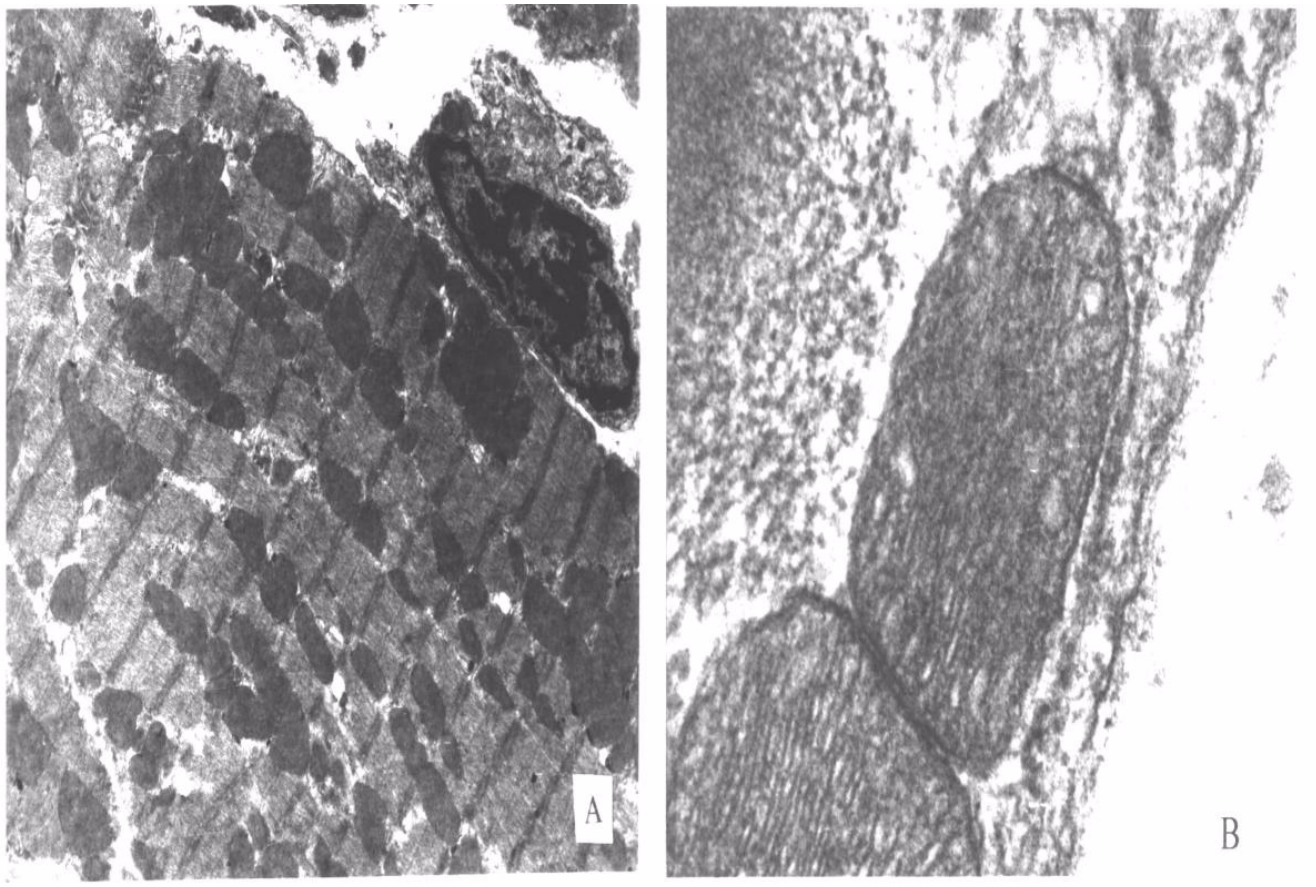

Figure 4
Electron micrograph of rat heart treated with garlic 250 mg/kg for 30 days and subjected to ischemic-reperfusion injury showing **A]** intact Z-band with normal mitochondria (× 1950) **B]** high power of mitochondria showing normal architecture (× 21500).

Group G-500: 500 mg/kg/day for 30 days of garlic homogenate fed rat hearts subjected to IR injury.

At the end of each experiment, myocardial tissue was stored in liquid nitrogen for biochemical estimations, 10% buffered formalin for light microscopic studies and Karnovsky fixative for electron microscopic studies.

Biochemical parameters

Thiobarbituric acid reactive substances (TBARS) [14] was measured as a marker of lipid peroxidation and endogenous antioxidants, e.g., superoxide dismutase (SOD) [15], catalase [16], reduced glutathione (GSH) [17] & glutathione peroxidase (GPx) [18] were estimated.

Histopathology

a) Light microscopic study

Myocardial tissue was fixed in 10% formalin, routinely processed and embedded in paraffin. Paraffin sections (3 μm) were cut on glass slides and stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS) reagent and examined under a light microscope.

b) Ultrastructural study (transmission electron microscopy)

Small pieces of myocardial tissue were washed in cold 0.1 M phosphate buffer after fixation (Karnovsky fixative) and post-fixed for two hours in 1% osmium tetraoxide in the same buffer at 4°C. After several washes in 0.1 M phosphate buffer, the specimens were dehydrated using graded acetone solutions and embedded in CY 212 araldite. Ultrathin sectioning (80–100 nm) of the tissue blocks was carried out using an ultracut E (Reichert, Austria) microtome. The sections were stained in alcohol uranyl acetate and lead citrate and viewed under a transmission electron microscope (Philips CM 10) operated at 60 KV.

Statistical Analysis

All values are expressed as mean ± SE. One way ANOVA was applied to test for significance of biochemical data of the different groups. Significance is set at $p < 0.05$.

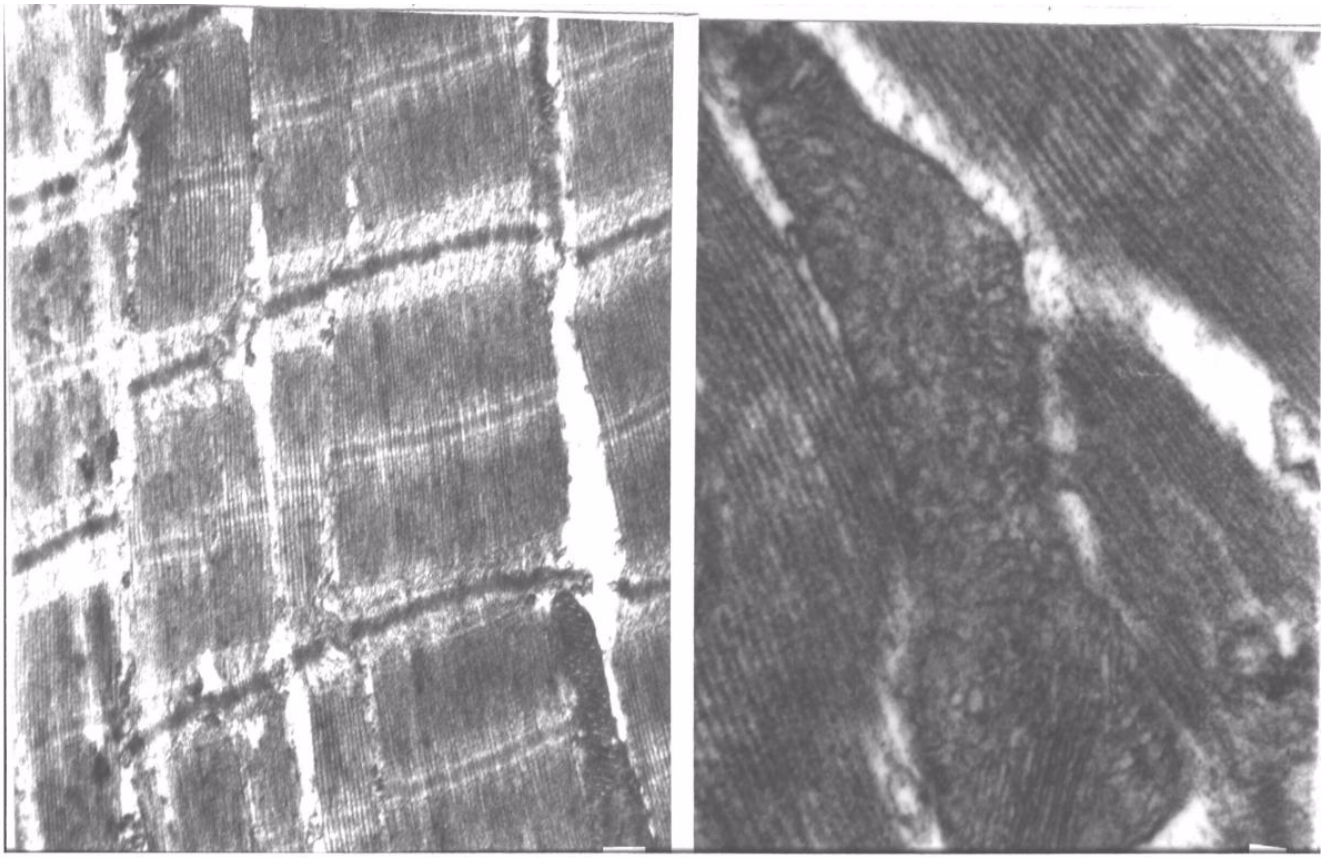

Figure 5
Electron micrograph of rat heart treated with garlic 500 mg/kg for 30 days and subjected to ischemic-reperfusion injury showing **A]** mostly intact Z-band with focal disruption of myofilament (× 6300) **B]** normal structure of mitochondria (× 8400).

Authors' contributions

SKB carried out the animal experimentation, biochemical estimation and statistical analysis of results. AKD carried out the light microscopic and electron microscopic study. SCM participated in the design of the study. SKM conceived the study, and participated in its design, coordination and drafted the manuscript.

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