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Interaction of Propranolol with Garlic in Isoproterenol Induced Myocardial Infarction in Rat

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Abstract: The current study dealt with the interaction of Garlic Homogenate (GH) with propranolol (PRO) on isoproterenol (ISO)-induced Myocardial Infarction (MI) in rats. Albino rats were treated either with GH at three different doses of 125 mg kg⁻¹, (GH-125), 250 mg kg⁻¹ (GH-250) and 500 mg kg⁻¹ (GH-500) orally for 30 days or different doses of GH along with PRO (10 mg kg⁻¹, p.o.) during the last 7 days of GH treatment. Myocardial damage was induced by administration of ISO (150 mg kg⁻¹ body weight s.c.) for 2 consecutive days. The PRO, moderate dose of GH alone or in combination with PRO was found to ameliorate the effect of ISO on superoxide dismutase (SOD), catalase and retained the activities of the diagnostic marker enzymes such as lactate dehydrogenase (LDH) and creatine phosphokinase isoenzyme (CK-MB). Incorporation of PRO during GH treatment provided further protection to myocardium from injury. However, higher dose of GH alone or in presence of PRO failed to prevent ISO induced myocardial injury. The results of the present study indicate that mild to moderate doses of GH exerts a protective effect, whereas, high dose of GH shows toxic effect against ISO-induced MI either alone or with PRO.

Key words: Garlic, isoproterenol, propranolol, CK-MB, superoxide dismutase, catalase

INTRODUCTION

Several plant products are known to exhibit creditable medicinal properties for the treatment of cardiac ailments. Simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other. Herbal drugs are consumed along with conventional drugs with a perception, that they may produce additive/synergistic activity (Fugh-Berman, 2000).

Garlic (*Allium sativum*, Family: Liliaceae) is one of the herbs that is widely believed to hold promise as therapeutically effective medicament for cardiovascular diseases. Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular diseases (Rahman and Lowe, 2006). Garlic and its products have been recognized globally as agents for prevention and management of atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes (Banerjee *et al.*, 2002a). Garlic is also known to possess cardioprotective, antioxidant, antineoplastic and antimicrobial characteristics (Isensee *et al.*, 1993; Banerjee *et al.*, 2002a; Tattelman, 2005). Furthermore, garlic has remarkable antiarrhythmic effect in both ventricular and supraventricular arrhythmias (Rietz *et al.*, 1993). It is reported that chronic administration of moderate doses of garlic augments the endogenous antioxidants activities and depletes the oxidative damaging effects by either

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increasing the synthesis of endogenous antioxidants or decreasing the generation of oxidants such as oxygen free radicals (Banerjee *et al.*, 2002a). Furthermore, it also exerts anti-oxidant effect in isoprenaline-induced myocardial infarction in rat (Ciplea and Richter, 1988). Garlic juice mimics beta blocking property by inhibiting norepinephrine-induced contractions of rabbit and guinea pig aortic rings. Moreover, it is also reported to inhibit the force of contraction of isolated rabbit heart in a concentration-dependent manner (Aqel *et al.*, 1991).

Earlier reports on the drug interaction studies of garlic indicate that it produces synergistic effect with calcium channel blocker due to its calcium blocking property (Martin *et al.*, 1997). However, no scientific observations are available regarding the interaction of garlic with propranolol (PRO) during conventional cardioprotective therapy. Hence, the present investigation was undertaken to demonstrate the effect of different doses of garlic homogenate and to determine its interaction with PRO in isoproterenol (ISO) induced myocardial damage in rats.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits like lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) were procured from Crest Biosystems (Goa, India).

Preparation of Plant Extract

Garlic bulbs were purchased from the local market. The cloves were peeled, sliced, grounded into a paste and suspended in distilled water. Three different doses of the garlic homogenate corresponding to 125, 250 and 500 mg kg⁻¹ were administered orally (Banerjee *et al.*, 2002b). The Garlic Homogenate (GH) was administered within 30 min of preparation.

Experimental Animals

Laboratory bred female Wistar albino rats weighing between 200-250 g were housed at 25±5°C in a well-ventilated animal house under 12:12 h light and dark cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal Feed, Maharashtra, India) containing protein 22.10%, oil 4.13%, fibre 3.15%, ash 5.15%, sand (silica) 1.12% w/w) and water *ad libitum*. There was no significant difference in the body weight of the treated rats when compared with control, either at the beginning or at the end of the study period. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The experiment was conducted in the Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, India during the period of April, 2007.

Experimental Protocol

The animals were divided into different treatment groups. The first group and second groups served as normal control and toxic control, respectively. The animals of group 3 received PRO orally at a dose of 10 mg kg⁻¹ (Hashimoto and Ogawa, 1981). The animals of 4, 5 and 6 were treated orally with three different dose of GH at 125, 250 and 500 mg kg⁻¹, respectively, for 30 days. The animals of group 7, 8 and 9 received three different doses of GH for 30 days at 125, 250 and 500 mg kg⁻¹, respectively, along with PRO (10 mg kg⁻¹) during the last seven days of GH treatment.

Experimental Procedure

At the end of treatment period, animals of all the groups excluding normal control were administered ISO (150 mg kg⁻¹ s.c.) for two consecutive days. Blood was drawn from retroorbital vein

48 h after the first dose of isoproterenol under anesthesia and serum was separated by centrifugation for LDH and CK-MB measurement. The heart was isolated from each animal 2 h after the last dose of the drugs under ketamine (70 mg kg⁻¹, i.p.) and xylazine (10 mg kg⁻¹, i.p.) anesthesia and homogenized to prepare Heart Tissue Homogenate (HTH) using sucrose (0.25 M) (Buerke *et al.*, 1998). The activity of LDH, CK-MB, superoxide dismutase (SOD) (Erich and Heupel, 1976) and catalase (Eva, 1988) was determined in HTH. Microscopic slides of myocardium were prepared for histopathological studies. Volume Fraction of Interstitial Space (VFITS) in myocardial tissue was determined from hematoxylin and eosin (H and E) stained transverse sections by using the equation (Zhai *et al.*, 2000).

$$\text{VFITS} = \frac{100\% \times \text{Area of interstitial space}}{\text{Total tissue area}}$$

The myocardial damage was determined by giving scores depending on the intensity as follows (Karthikeyan *et al.*, 2007); no changes-score 0; mild-score 1 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate-score 2 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked-score 3 (necrosis with diffuse inflammatory process).

Statistical Analysis

Results are expressed as mean±SEM. Statistical significance was assessed using One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison tests. p<0.05 was considered significant.

RESULTS

LDH and CK-MB

The LDH and CK-MB activities were significantly increased by ISO treatment when compared to normal group. These enzyme activities increased significantly (p<0.01) in serum and decreased very significantly (p<0.001) in Heart Tissue Homogenate (HTH) after GH-500 treatment when compared to normal group. There was a very significant (p<0.001) reduction in these biological marker enzyme activities in PRO, GH-250, GH-125+PRO and GH-250+PRO groups in serum when compared to ISO control. Further, there was significant (p<0.001) elevation in these biomarker activities in HTH in groups treated with PRO, GH-125, GH-250, GH-125+PRO and GH-250+PRO when compared to ISO control. Furthermore, at high dose (GH-500) either in presence or absence of PRO, there was no significant difference in biomarker activities when compared to ISO toxic group (Table 1).

SOD and Catalase

There was a significant (p<0.001) decline in SOD and catalase activities in HTH after ISO treatment when compared to normal animals. Pretreatment with PRO, GH-125 and GH-250 either alone or with PRO significantly reversed the ISO induced depletion of SOD and catalase in HTH. However, GH-500 alone or with PRO did not affect the antioxidant enzyme activities significantly when compared to ISO group (Table 2).

VFITS and Histological Scores

There was a significant (p<0.001) rise in VFITS and histological scores in ISO control group (Fig. 1), when compared to normal group (Fig. 2). These parameters were found to diminish significantly (p<0.001) in groups treated with PRO (Fig. 3), GH-125 (Fig. 4) and GH-250 (Fig. 5) in presence (Fig. 7, 8) or absence of PRO (Fig. 9) when compared to ISO control. Histological

Table 1: Effect on LDH and CK-MB activities in serum and heart tissue homogenate of isoproterenol-induced myocardial infarction in rats

Treatments	LDH		CK-MB	
	Serum (U L ⁻¹)	Heart homogenate (U g ⁻¹ wet tissue)	Serum (U L ⁻¹)	Heart homogenate (U g ⁻¹ wet tissue)
Normal	432.38±11.10	716.00±16.80	25.91±2.73	46.08±2.19
ISO Control	556.86±11.90***	286.25±14.18***	39.51±1.82***	19.21±0.36***
PRO	455.33±10.85***	489.61±17.85***	31.43±1.14***	32.52±1.31***
GH-125	533.61±12.41	636.40±10.06***	31.58±1.85***	32.15±0.83***
GH-250	465.28±13.36***	760.61±14.00***	29.33±1.97***	49.35±2.02***
GH-500	603.46±2.57***	270.31±14.28***	39.11±1.76***	20.95±0.54
GH-125+PRO	436.78±06.47***	555.28±8.01***	26.48±1.12 ***	39.90±0.71***
GH-250+PRO	420.30±3.27***	885.03±15.76***	23.96±1.31***	65.21±1.89***
GH-500+PRO	553.15±24.18***	363.01±8.42**	36.71±4.63***	23.95±1.05

Values are expressed as mean±SEM for eight rats in each group. ***Significantly different from ISO control p<0.001. **Significantly different from normal p<0.001. Garlic Homogenate (GH)-125 mg kg⁻¹, 250 mg kg⁻¹ and 500 mg kg⁻¹ (30 days treatment, p.o) propranolol (PRO)-10 mg kg⁻¹ (7 days treatment, p.o)

Table 2: Effect on SOD, catalase, volume fraction of interstitial space (VFITS) and histological scores in isoproterenol-induced myocardial infarction in rats

Treatments	Heart tissue homogenate		Heart tissue	
	SOD (U mg ⁻¹ protein)	Catalase (U mg ⁻¹ protein)	VFITS	Histological scores
Normal	2.51±0.08	3.26±0.05	12.85±0.89	0.50±0.12
ISO Control	1.53±0.03***	1.66±0.03***	37.98±0.81***	3.50±0.34***
PRO	2.61±0.07***	2.65±0.06***	27.18±0.16***	1.83±0.30**
GH-125	2.39±0.03***	2.27±0.07***	25.86±0.16***	1.50±0.22*
GH-250	3.84±0.02***	3.79±0.06***	24.72±0.19***	1.33±0.21***
GH-500	1.43±0.03***	1.44±0.03***	38.32±0.26***	3.66±0.21***
GH-125+PRO	2.93±0.02***	2.79±0.03***	22.83±0.13***	1.33±0.21***
GH-250+PRO	4.14±0.04***	4.15±0.05***	20.14±0.23***	1.16±0.16***
GH-500+PRO	1.63±0.03***	1.65±0.03***	32.15±0.27***	3.00±0.36***

Values are expressed as mean±SEM for eight rats in each group. ***Significantly different from ISO control p<0.001. **Significantly different from normal p<0.001. Garlic Homogenate (GH)-125 mg kg⁻¹, 250 mg kg⁻¹ and 500 mg kg⁻¹ (30 days treatment, p.o) propranolol (PRO)-10 mg kg⁻¹ (7 days treatment, p.o)

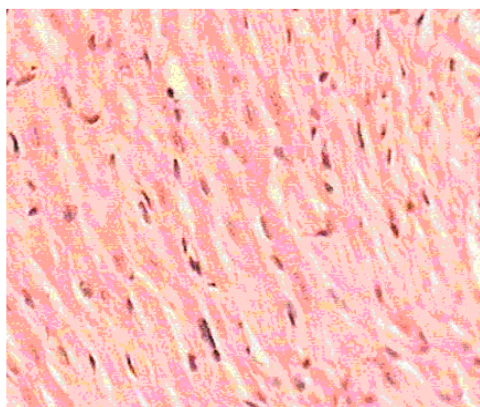


Fig. 1: Normal-Clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils

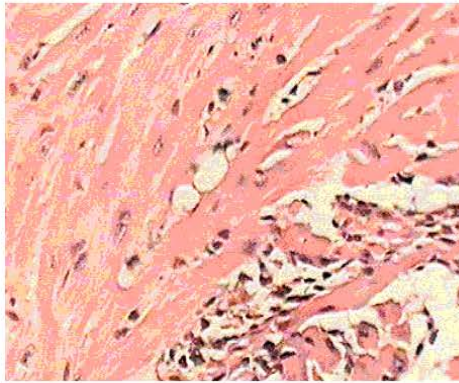


Fig. 2: ISO control-patchy areas of necrosis, hyalinization of muscle fibers with focal cellular infiltrations. The muscle fibers showed vacuolar changes with fragmentation suggestive of necrosis

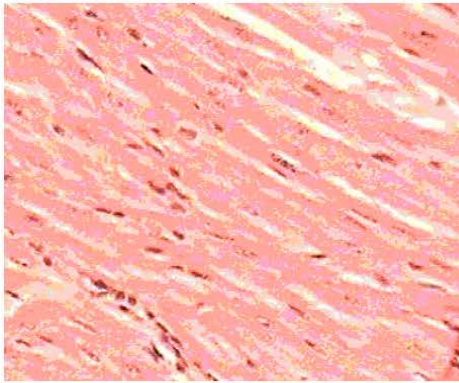


Fig. 3: PRO-normal cellular architecture with decrease interstitial space and minor infiltration

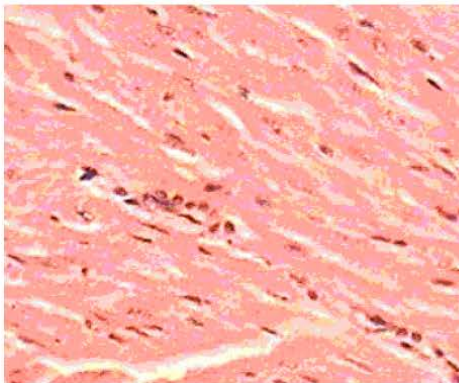


Fig. 4: GH-125-normal architecture restored with least interstitial space

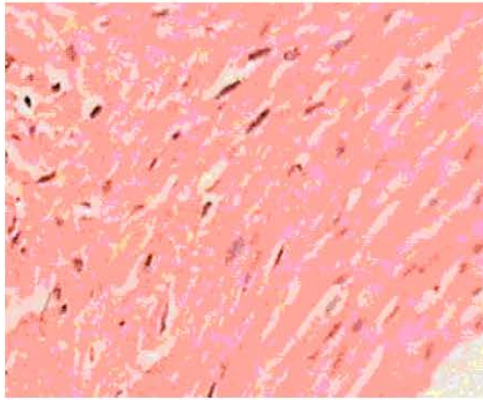


Fig. 5: GH-250-normal architecture restored with very less interstitial space

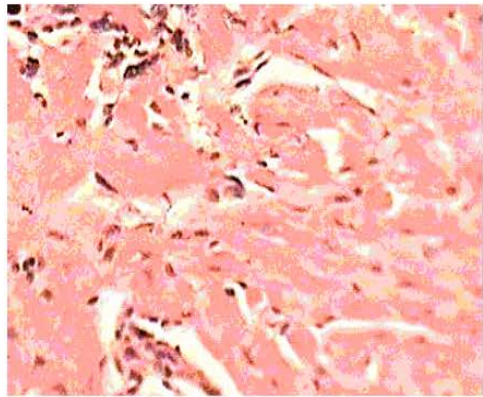


Fig. 6: GH-500-focal infiltration with increased interstitial space

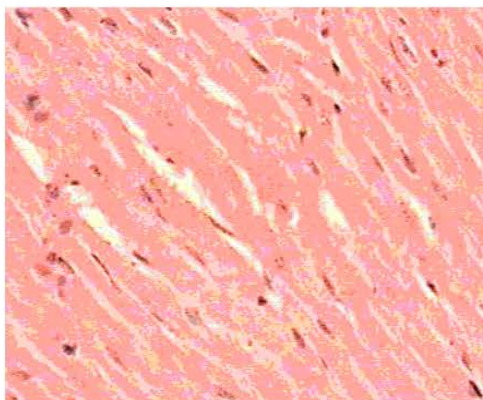


Fig. 7: GH-125+PRO-normal architecture restored with least interstitial space

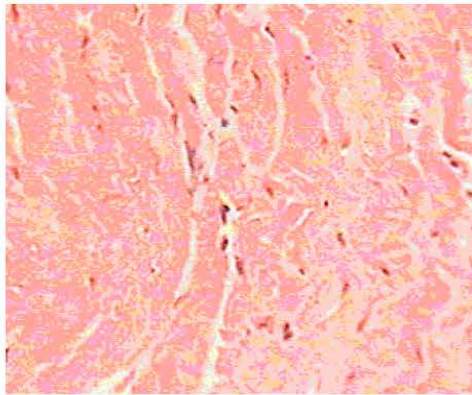


Fig. 8: GH-250+PRO-normal architecture restored

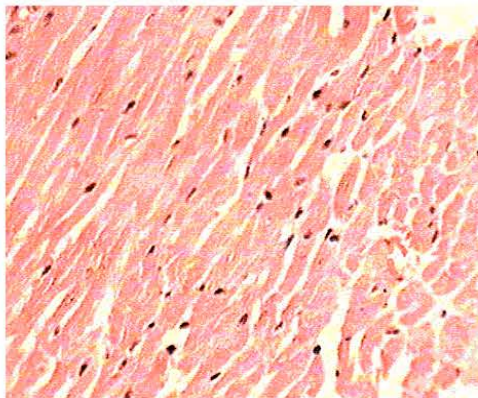


Fig. 9: GH-500+PRO-loss of cellular structure with focal cellular infiltrations

examination of myocardial tissue of the normal group (Fig. 1) depicted clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils. Heart tissues of animals treated with ISO showed patchy areas of necrosis, hyalinization of muscle fibers with focal cellular infiltrations (Fig. 6). The muscle fibers showed vacuolar changes with fragmentation suggestive of necrosis (Fig. 2). In animals pretreated with GH-250+PRO (Fig. 5), the morphology of the myocardium was almost similar to that observed in normal animals (Table 2).

DISCUSSION

Herbs are often administered in combination with therapeutic drugs, raising the potential of herb-drug interactions (Hu *et al.*, 2005). Currently, there is very little information published on herb-drug interactions whilst the use of herbs is progressively growing across the world. Certain herbal supplements can cause potentially dangerous side effects when taken with prescription drugs and the number of cases reported for the emerging herb-drug interactions are already on the rise

(Gohil and Patel, 2007). Hence it is widely accepted that in-depth and appropriate studies on drug-herb interactions should be carried out to confirm the efficacy of combined drug-herb treatments. The present study was carried out to determine the interaction of GH with PRO. It is demonstrated by epidemiologic studies that there is an inverse correlation between garlic consumption and progression of cardiovascular disease.

GH was administered at three different doses that were reported to be safe (125, 250 and 500 mg kg⁻¹). Earlier studies on the effect of GH on cardiovascular system suggests that GH induced cardioprotection is due to its active organosulfur metabolites; S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), which have potent antioxidant activity (Ide and Lau, 1997; Imai *et al.*, 1994; Wei and Lau, 1998). Allicin (allyl 2-propenethiosulfinate) was earlier thought to be the principle bioactive compound responsible for the cardioprotective effect. However, recent studies suggest that allicin is an unstable and transient compound with oxidant activity (Freeman and Kodera, 1995), that is virtually undetectable in blood circulation after garlic ingestion and decomposes to form the SAC and SAMC (Lawson *et al.*, 1992).

Isoprotenerol [1 - (3, 4 - dihydroxyphenyl) -2- isopropylamino- ethanolhydrochloride] (Chagoya *et al.*, 1997) is a synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to development of MI. The MI is produced due to its action on the cardiac β_1 -receptors (Brouria *et al.*, 2002; Witteveen *et al.*, 1975). ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membrane. A number of studies are available that suggest the crucial role of free radicals in pathogenesis of ISO-induced myocardial damage. The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial alterations (Karthikeyan *et al.*, 2007). Hence ISO-induced myocardial infarction model was used in this study (Ithayarasi and Devi, 1997). Animals were pretreated with varying doses of GH alone or along with PRO, a known β -adrenergic receptor blocker that is found to have good prophylactic effect (Satoskar *et al.*, 1995).

The diagnostic marker enzymes of Myocardial Infarction (MI) are CK-MB and LDH (Witteveen *et al.*, 1975; Fontes *et al.*, 1999). Presence of these biomarkers in heart tissue homogenate (HTH) is indicative of myocardial integrity and their release in serum signifies myocardial injury. The release of cellular enzymes reflects a non-specific alteration in the plasma membrane integrity (Hearse, 1975; Licka *et al.*, 2002). In the present study, there was decrease in the activities of these marker enzymes in HTH and increase in the activities in serum in ISO induced MI. Oral pretreatment with GH-125 and GH-250 with or without PRO restored the activities of these enzymes to near normal in both the heart and the serum. Similar protective effect was also seen upon pretreatment with PRO before subjecting to MI. This indicates that both GH (in low and moderate doses) as well as PRO possesses protective effect individually and their combined treatment augments cardioprotection. However, high dose of GH (GH-500) failed to provide similar protection to myocardium suggesting that GH produces cardioprotective effect only in mild and moderate doses. Furthermore, addition of PRO to GH-500 treatment failed to produce cardioprotection (Fig. 9). The exact reason for this can not be explained with the present data but it is speculated that high dose of GH on its own may aggravate the ISO induced MI as it showed a non-significant changes in serum LDH, SOD and catalase. The earlier reports by Banerjee *et al.* (2002b) that high doses of garlic do not possess cardioprotective effect support the present results.

Among the different oxygen free radicals (OFRs), that cause destruction of myocardial membrane and leakage of bioenzymes in serum (McCord, 1988; Murphy and Steenbergen, 2008), contribution of superoxide to myocardial damage is believed to be the highest and this radical is combated by elevated activities of endogenous antioxidant enzyme-the superoxide dismutase (SOD) (Guarnieri *et al.*, 1980; Bafna and Balaraman, 2006). In addition to this, measurement of catalase

activity was carried out as elevation in SOD dismutates superoxide but results in accumulation of hydrogen peroxide (H₂O₂), which could further precipitate the MI (Yim *et al.*, 1990; Liu *et al.*, 2008). Pretreatment of animals with GH (125 and 250 mg kg⁻¹) alone or along with PRO produced remarkable elevation in SOD and catalase activities when compared to control indicating cardioprotective effect. However, pretreatment of GH (500 mg kg⁻¹) produced a significant decrease in the antioxidant enzyme activities and PRO failed to reverse the GH (500 mg kg⁻¹) induced aggravation of myocardial damage. The result clearly demonstrates that GH in moderate and low doses reduces oxidative damage and in high doses aggravates oxidative stress.

The ISO induced damage to cardiac musculature and its prevention by different treatments was also demonstrated and confirmed by histopathological parameters such as VFITS and histological scores. Any increase in these parameters is indicative of myocardial damage. Pretreatment of GH-125 and GH-250 with or without PRO substantially decreased the VFITS and histological scores and showed normal musculature under microscope indicating protection offered by mild and moderate doses of GH alone as well as along with PRO.

The findings of the present study suggest garlic in low to moderate doses possess cardioprotective effect and this effect may be enhanced if garlic is taken along with β -blockers such as PRO. Further, administration of high doses of garlic may not produce any beneficial effects on its own and on the contrary, it may attenuate the cardioprotective action of PRO.

CONCLUSION

In conclusion, pretreatment of GH was found to offer dose dependent protection from myocardial injury in ISO-induced myocardial injury model at doses of 125 and 250 mg kg⁻¹. Incorporation of PRO in the treatment further facilitates the cardioprotection offered by GH. However, GH-500 mg kg⁻¹ was failed to prevent ISO-induced myocardial injury either on its own or when administered along with PRO.

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