

## PHARMACODYNAMIC INTERACTION OF GARLIC WITH HYDROCHLOROTHIAZIDE IN RATS

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**Abstract :** The purpose of current study was to evaluate the influence of hydrochlorothiazide (HCTZ) on protective effects of garlic homogenate (GH) in rats subjected to myocardial damage induced by ischemia-reperfusion (IRI) and isoproterenol (ISO). Female Wistar albino rats were treated with GH at three different doses of 125; 250 and 500 mg/kg orally for 30 days and HCTZ (10 mg/kg, p.o.) was incorporated in the interactive groups during the last seven days of GH treatment. At the end of treatment, animals were subjected to IRI or ISO induced myocardial damage in their respective models. The GH 250 mg/kg was found to dislodge the effect of ISO and IRI on superoxide dismutase & catalase and retained the activities of LDH and CK-MB. Adding HCTZ improved biochemical, antioxidant and histological status of myocardium during myocardial damage. Since no toxic effects were observed, HCTZ may safely be used to improve GH therapy.

**Key words :** garlic interaction isoproterenol  
ischemia-reperfusion hydrochlorothiazide

### INTRODUCTION

The use of herbal supplements has become increasingly popular in recent years. However, concern has been expressed regarding the safety of these products, in particular the potential interaction of these herbs with conventional drugs. It has been documented that as many as 31% of patients use herbal supplements concurrently with the prescribed conventional drugs and 70% of them do not report the use of these products to their healthcare providers (1).

In order to tackle this problem and provide a better service, healthcare professionals should have knowledge of at least commonly occurring or anticipated interactions between herbal supplements and conventional drugs (1).

Garlic (*Allium sativum*, family: *Lilliaceae*) is one of the herbs that are widely believed to hold promise as therapeutically effective medicament for cardiovascular diseases. Epidemiologic studies show an inverse correlation between garlic consumption and

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progression of cardiovascular diseases (2). Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases such as atherosclerosis, arrhythmia, hyperlipidemia, thrombosis, hypertension and diabetes (3).

The cardiovascular effect of hydrochlorothiazide (HCTZ) is not only attributed to its inhibitory effect on carbonic anhydrases in the vascular tissue but also through vasodilatation by activating calcium-activated potassium channels (large conductance) in vascular smooth muscles (4). Eventhough, HCTZ is exploited for various cardiac manifestations; there is dearth of reports on HCTZ role during myocardial stress. Hence efforts were necessary to find out cardiac effects of HCTZ alone and in presence of cardioprotective herb such as garlic at times of myocardial damage. We recently reported improved activity of moderate doses of garlic and propranolol (5)/captopril (6) during myocardial damage when they were administered concurrently. In the present study we investigated the pharmacodynamic interaction of garlic with HCTZ in rats subjected to myocardial damage induced by isoproterenol or ischemia-reperfusion.

#### MATERIALS AND METHODS

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

**Preparation of garlic extract** – Garlic (*Allium sativum*, family: *Lilliaceae*) bulbs were

purchased from the local market. It was identified and authenticated by Dr. Jaiprakash, Department of Pharmacognosy, Krupanidhi College of Pharmacy, Bangalore. The cloves were peeled, sliced and ground into a paste and suspended in distilled water. Three different doses of the garlic homogenate (GH) corresponding to 125, 250 and 500 mg/kg were administered orally (7). GH was administered within 30 min of preparation.

**Experimental animals** – Laboratory bred female Wistar albino rats (200–250 g) were housed at  $25^{\circ}\pm 5^{\circ}\text{C}$  in a well-ventilated animal house under 12:12 h light dark cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, 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. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

**Isoproterenol (ISO) induced myocardial damage** – Female albino rats were divided into several groups as follows: Group I, normal vehicle treatment for 30 days, *p.o.*; Group II, ISO control vehicle treatment for 30 days, *p.o.*; Group III, Hydrochlorothiazide (HCTZ) 10 mg/kg (8) for seven days, *p.o.*; Group IV, V and VI, garlic homogenate (GH) 125, 250 and 500 mg/kg respectively for 30

days, p.o.; Group VII, GH-125 for 30 days and HCTZ for last seven days p.o.; Group VIII, GH-250 for 30 days and HCTZ for last seven days p.o. and Group IX, GH-500 for 30 days and HCTZ for last seven days p.o. In interactive groups, GH was simultaneously administered with HCTZ during last seven days of 30 days GH treatment. At the end of treatment period, animals of all groups excluding Group I was administered ISO (150 mg/kg s.c) for two consecutive days. Blood was withdrawn from retroorbital vein 48 hrs after the first dose of ISO under anesthesia and serum was separated by centrifugation for LDH and CK-MB measurement. The heart was isolated from each animal under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia and homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) (9). The activity of LDH, CK-MB, superoxide dismutase (SOD) (10) and catalase (11) was determined in HTH. Microscopic slides of myocardium were prepared for histopathological studies. The myocardial damage was determined by giving scores depending on the intensity as follows (12); no changes – score 00; mild - score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate score 02 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked score 03 (necrosis with diffuse inflammatory process).

*Ischemia-reperfusion (IRI) induced myocardial damage* – The animals were divided into eight treatment groups. The first group served as control and the animals of group II received HCTZ orally at a dose of 10 mg/kg. The animals of III, IV and V were treated orally for 30 days with three different

doses of GH at 125, 250 and 500 mg/kg respectively. The animals of group VI, VII and VIII received three different doses of GH for 30 days at 125, 250 and 500 mg/kg respectively along with HCTZ during the last seven days of GH treatment. In interactive groups, GH was simultaneously administered with HCTZ during last seven days of 30 days GH treatment. A modified Langendorff apparatus for the isolated perfused heart was set up as mentioned elsewhere (13). The heart was isolated from each animal 2 hrs after the last dose of the drug(s) under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia. The isolated heart was perfused with Krebs-Henseleit (K-H) solution gassed with carbogen (95% O<sub>2</sub> and 5% CO<sub>2</sub>) at 37°C at a constant flow rate of 5 ml/min. The composition of K-H solution was (mM) NaCl 118, KCl 4.7, NaHCO<sub>3</sub> 25, NaHPO<sub>4</sub> 1.0, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.57, CaCl<sub>2</sub> 2.5 and glucose 11). The pH of K-H solution was adjusted to 7.4 to avoid K-H buffer acidosis that may occur after prolonged gassing with carbogen. The heart was allowed to equilibrate for 10 min and then regular recordings were taken for a perfusion period of 15 min. Measurement of contractile force was done using force displacement transducer and recorded on a Student Physiograph (INCO, Mumbai, India). After the initial preischemic perfusion, heart was subjected to 15 min of global no-flow ischemia (14) by blocking the flow of K-H solution and carbogen supply followed by 15 min of reperfusion. The heart rate and developed tension were measured during pre-ischemic and post-ischemic period and recovery (%) was calculated. Lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activity were measured in the perfusate during pre-ischemic and post-ischemic period. The heart was then

homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) (9) and the activity of LDH, CK-MB, superoxide dismutase (SOD) (9) and catalase (11) was determined. Microscopic slides of myocardium were prepared for histopathological studies after post-ischemia (12). The measurements of histological scores were done as discussed in ISO procedure.

**Statistical analysis** – The mean values $\pm$ SEM are calculated for each parameter. For determining the significant inter-group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out and the individual comparisons of the group mean values were done using Tukey multiple comparison tests.  $P<0.05$  was considered significant.

## RESULTS

*Isoproterenol (ISO) induced myocardial damage (Table 1)* – The LDH, CK-MB, SOD and catalase activities were significantly decreased in heart tissue homogenate (HTH) by ISO treatment when compared to normal group. Further, prior treatment of animals with HCTZ ( $P<0.05$ ), GH-125 ( $P<0.01$ ) and GH 250 mg/kg ( $P<0.001$ ) demonstrated significant increase in these parameters when compared to ISO control. Addition of HCTZ during the last seven days treatment of GH 125 mg/kg provided significant ( $P<0.05$ ) rise in LDH and CK-MB activities in HTH when compared to GH 125 mg/kg alone. Similarly, histological examinations (Fig. 1–3) of slides prepared from myocardium of experimental animals treated with HCTZ, GH-125, GH-250, GH-125+HCTZ and GH-250+HCTZ indicated a decrease in scores when compared to ISO control.

TABLE I: Effect of GH and HCTZ individually and in combination on LDH, CK-MB, SOD & catalase activities and histological scores in rats during myocardial damage induced by isoproterenol (ISO).

Group No.	Treatment	LDH activity		CK-MB activity		Heart tissue homogenate		Histological scores
		Serum (U/l)	Heart tissue homogenate (U/g)	Serum (U/l)	Heart tissue homogenate (U/g)	SOD (Unit/mg protein)	Catalase (Unit/mg protein)	
I	Normal	432.38 $\pm$ 11.10	716 $\pm$ 16.80	25.91 $\pm$ 2.73	46.08 $\pm$ 2.19	2.51 $\pm$ 0.08	3.26 $\pm$ 0.05	0.5 $\pm$ 0.22
II	ISO control	556.86 $\pm$ 11.90 <sup>***</sup>	286.25 $\pm$ 14.18 <sup>***</sup>	39.51 $\pm$ 1.82 <sup>***</sup>	21.21 $\pm$ 0.36 <sup>***</sup>	1.53 $\pm$ 0.03 <sup>**</sup>	1.66 $\pm$ 0.03 <sup>***</sup>	2.66 $\pm$ 0.21 <sup>***</sup>
III	HCTZ 10 mg/kg	503.21 $\pm$ 7.65 <sup>*</sup>	333.09 $\pm$ 12.43 <sup>*</sup>	35.28 $\pm$ 1.32 <sup>*</sup>	26.22 $\pm$ 2.11 <sup>***</sup>	1.91 $\pm$ 0.17 <sup>***</sup>	1.91 $\pm$ 0.27 <sup>***</sup>	1.5 $\pm$ 0.34 <sup>***</sup>
IV	GH-125 mg/kg	497.61 $\pm$ 12.41 <sup>**</sup>	446.4 $\pm$ 10.06 <sup>***</sup>	33.58 $\pm$ 1.85 <sup>***</sup>	32.12 $\pm$ 0.83 <sup>***</sup>	2.10 $\pm$ 0.05 <sup>***</sup>	2.07 $\pm$ 0.13 <sup>***</sup>	2.0 $\pm$ 0.25 <sup>***</sup>
V	GH-250 mg/kg	412.28 $\pm$ 13.36 <sup>***</sup>	522.61 $\pm$ 14.00 <sup>***</sup>	29.33 $\pm$ 1.97 <sup>***</sup>	42.22 $\pm$ 2.21 <sup>***</sup>	2.24 $\pm$ 0.10 <sup>***</sup>	3.16 $\pm$ 0.04 <sup>***</sup>	0.5 $\pm$ 0.22 <sup>***</sup>
VI	GH-500 mg/kg	603.46 $\pm$ 2.57 <sup>***</sup>	270.31 $\pm$ 14.28 <sup>***</sup>	35.11 $\pm$ 1.76 <sup>***</sup>	20.95 $\pm$ 2.32 <sup>***</sup>	1.43 $\pm$ 0.03 <sup>***</sup>	1.54 $\pm$ 0.03 <sup>***</sup>	2.5 $\pm$ 0.22 <sup>***</sup>
VII	GH-125+HCTZ	452.21 $\pm$ 9.21 <sup>**a</sup>	488.11 $\pm$ 4.32 <sup>**a</sup>	29.29 $\pm$ 2.11 <sup>**a</sup>	38.66 $\pm$ 0.96 <sup>**a</sup>	2.28 $\pm$ 0.05 <sup>**a</sup>	2.29 $\pm$ 0.04 <sup>***</sup>	1.5 $\pm$ 0.22 <sup>**a</sup>
VIII	GH-250+HCTZ	429.20 $\pm$ 5.79 <sup>**</sup>	569.09 $\pm$ 22.43 <sup>**a</sup>	25.87 $\pm$ 1.32 <sup>**a</sup>	46.87 $\pm$ 0.98 <sup>**</sup>	2.55 $\pm$ 0.06 <sup>**</sup>	3.38 $\pm$ 0.67 <sup>**</sup>	0.5 $\pm$ 0.22 <sup>**</sup>
IX	GH-500+HCTZ	588.20 $\pm$ 10.01 <sup>***</sup>	302.12 $\pm$ 9.22 <sup>***a</sup>	34.21 $\pm$ 3.77 <sup>***</sup>	23.99 $\pm$ 2.21 <sup>***</sup>	1.51 $\pm$ 0.13 <sup>***</sup>	1.65 $\pm$ 0.43 <sup>***</sup>	2.33 $\pm$ 0.33 <sup>***</sup>

Values are mean $\pm$ SEM of 8 animals, significant at – \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  when compared to control group; <sup>a</sup> $P<0.05$ , <sup>aa</sup> $P<0.01$ , <sup>aaa</sup> $P<0.001$  when compared to corresponding dose of GH alone and <sup>b</sup> $P<0.05$ , <sup>bb</sup> $P<0.01$ , <sup>bbb</sup> $P<0.001$  when compared to Group I.

However, there was significant ( $P < 0.001$ ) incline and decline in activities of LDH and CK-MB enzymes in serum and HTH respectively in animals pretreated with GH 500 mg/kg when compared to normal group. The high dose of GH 500 mg/kg does not show any significant ( $P > 0.05$ ) change in LDH, CK-MB, SOD, catalase and histological scores when compared to ISO control. Moreover, administration of HCTZ to GH 500 mg/kg treated rats unable to show any significant change when compared to ISO control and GH 500 mg/kg alone.

*Ischemia-reperfusion injury (IRI) induced myocardial damage (Table IIa & IIb)* – There was a significant ( $P < 0.001$ ) increase in LDH, CK-MB, SOD and catalase activities in HCTZ, GH-125, GH-250, GH-125+HCTZ and GH-250+HCTZ groups in HTH when compared to IRI control. Further, prophylactic treatment of HCTZ to GH 125 mg/kg treated animals shows significant ( $P < 0.05$ ) increase in LDH and CK-MB activities in HTH when compared to GH-125 alone. Moreover, GH-250 alone and in presence of HCTZ

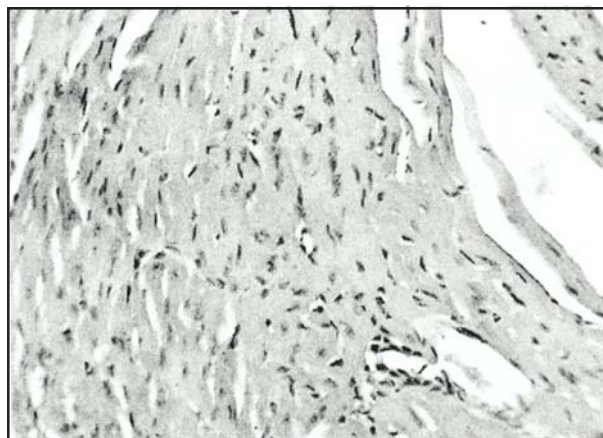


Fig. 1: H&E ( $\times 400$ ) stained microscopic section of isoproterenol (ISO) control. There is loss of cellular architecture with prominent hyperchromasia.

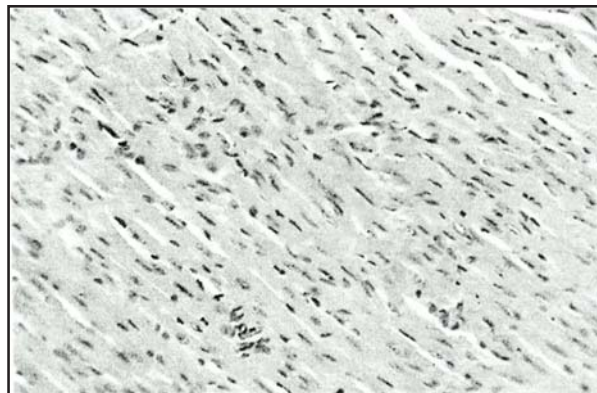


Fig. 2: H&E ( $\times 400$ ) stained microscopic section of heart tissue of animals pretreated with GH 250 mg/kg for 30 days orally and subsequently two doses of isoproterenol (ISO) 150 mg/kg, s.c. Normal architecture restored with focal infiltration as evident from the figure.

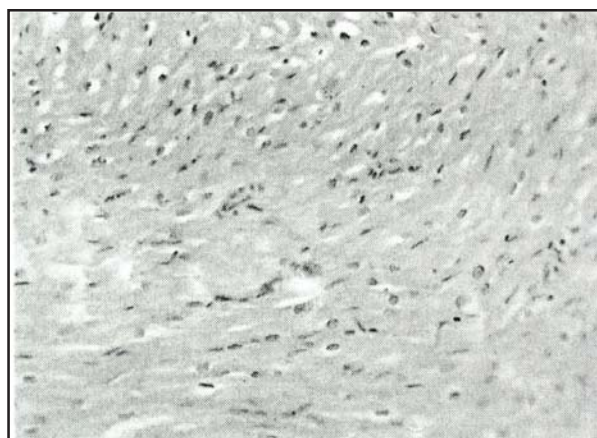


Fig. 3: H&E ( $\times 400$ ) stained microscopic section of heart tissue of animals pretreated with GH 250 mg/kg for 30 days and HCTZ 10 mg/kg for 7 days orally and subsequently two doses of isoproterenol (ISO) 150 mg/kg, s.c. There is protection from myocardial damage as revealed by restoration of normal architecture with reduced interstitial space.

provided significant recovery in developed tension and heart rate (Table IIb) when compared to ISO control. On the contrary, treatment of animals with high dose of GH (500 mg/kg) did not show any significant change that were remained unchanged even

after incorporation of HCTZ during last seven days of GH treatment. Histological examination of myocardial tissue of animals subjected to IRI showed vacuolar changes with fragmentation suggestive of necrosis (Fig. 4). In animals pretreated with GH-250 mg/kg (Fig. 5), the morphology of the myocardium was almost similar to that observed in normal animals. The histological examination of tissues of animals pretreated with both GH 250 mg/kg and HCTZ 10 mg/kg (Fig. 6) depicted clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils (Table II).

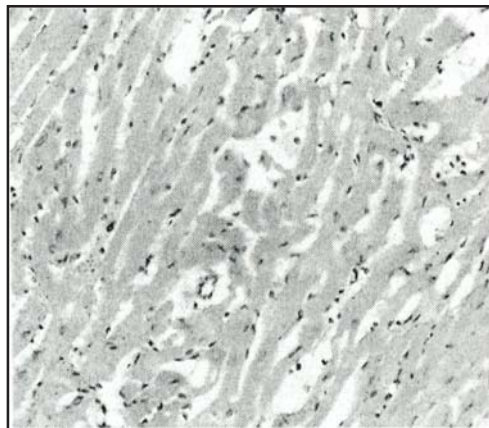


Fig. 4: H&E ( $\times 400$ ) stained microscopic section of IRI control. Heart subjected to global no flow 15 min ischemia and subsequent reperfusion. Heart section shows vacuolar changes with fragmentation suggestive of necrosis. Nuclear duplication, increased interstitial space and loss of cellular architecture with mild interstitial edema are also evident.

TABLE IIa: Effect of GH and HCTZ individually and in combination on LDH, CK-MB, SOD & catalase activities in rats during myocardial damage induced by ischemia-reperfusion injury (IRI).

Group No.	Treatment	LDH activity		CK-MB activity		Heart tissue homogenate	
		Serum (U/I)	Heart tissue homogenate (U/g)	Serum (U/I)	Heart tissue homogenate (U/g)	SOD (Unit/mg protein)	Catalase (Unit/mg protein)
I	IRI control	546.75 $\pm$ 11.89	361.95 $\pm$ 15.10	39.16 $\pm$ 1.21	29.76 $\pm$ 0.97	1.44 $\pm$ 0.07	1.81 $\pm$ 0.15
II	HCTZ 10 mg/kg	428.32 $\pm$ 6.11 <sup>***</sup>	419.30 $\pm$ 13.04 <sup>**</sup>	32.21 $\pm$ 1.21 <sup>***</sup>	34.32 $\pm$ 1.31 <sup>***</sup>	1.79 $\pm$ 0.23 <sup>*</sup>	2.24 $\pm$ 0.21 <sup>*</sup>
III	GH-125 mg/kg	485.66 $\pm$ 5.68 <sup>*</sup>	439.21 $\pm$ 16.09 <sup>**</sup>	34.81 $\pm$ 0.35 <sup>*</sup>	41.72 $\pm$ 1.24 <sup>***</sup>	1.86 $\pm$ 0.14 <sup>**</sup>	2.38 $\pm$ 0.13 <sup>***</sup>
IV	GH-250 mg/kg	388.49 $\pm$ 3.32 <sup>***</sup>	621.15 $\pm$ 14.12 <sup>***</sup>	26.58 $\pm$ 0.83 <sup>***</sup>	47.83 $\pm$ 0.25 <sup>***</sup>	2.36 $\pm$ 0.11 <sup>***</sup>	2.89 $\pm$ 0.12 <sup>***</sup>
V	GH-500 mg/kg	576.70 $\pm$ 9.57	421.30 $\pm$ 11.10 <sup>***</sup>	40.38 $\pm$ 0.42	30.25 $\pm$ 1.13	1.54 $\pm$ 0.06	1.90 $\pm$ 0.10
VI	GH-125+HCTZ	420.75 $\pm$ 7.86 <sup>**a</sup>	515.21 $\pm$ 10.34 <sup>a</sup>	29.27 $\pm$ 0.33 <sup>***a</sup>	45.34 $\pm$ 2.54 <sup>***a</sup>	1.98 $\pm$ 0.10 <sup>**</sup>	2.43 $\pm$ 0.23 <sup>*</sup>
VII	GH-250+HCTZ	355.21 $\pm$ 3.13 <sup>***</sup>	678.21 $\pm$ 14.54 <sup>***a</sup>	24.32 $\pm$ 0.65 <sup>***</sup>	51.25 $\pm$ 0.82 <sup>***</sup>	2.46 $\pm$ 0.24 <sup>***</sup>	2.98 $\pm$ 0.13 <sup>***</sup>
VIII	GH-500+HCTZ	532.16 $\pm$ 3.60 <sup>a</sup>	446.32 $\pm$ 11.11 <sup>a</sup>	39.93 $\pm$ 0.78	31.65 $\pm$ 0.41	1.62 $\pm$ 0.03	1.98 $\pm$ 0.19

Values are mean $\pm$ SEM of 8 animals, significant at - <sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01, <sup>\*\*\*</sup>P<0.001 when compared to control group; <sup>a</sup>P<0.05, <sup>aa</sup>P<0.01, <sup>aaa</sup>P<0.001 when compared to corresponding dose of GH alone.

TABLE IIb: Effect of GH and HCTZ individually and in combination on histological scores, developed tension and heart rate in rats during myocardial damage induced by ischemia-reperfusion injury (IRI).

Group No.	Treatment	Histological scores	% age recovery in developed tension	% age recovery in heart rate
I	IRI control	2.33 $\pm$ 0.33	20.21 $\pm$ 3.11	23.72 $\pm$ 2.23
II	HCTZ 10 mg/kg	2.00 $\pm$ 0.25 <sup>*</sup>	48.68 $\pm$ 3.70 <sup>***</sup>	44.31 $\pm$ 2.21 <sup>***</sup>
III	GH-125 mg/kg	2.0 $\pm$ 0.25 <sup>*</sup>	49.06 $\pm$ 5.77 <sup>***</sup>	47.31 $\pm$ 2.26 <sup>***</sup>
IV	GH-250 mg/kg	0.5 $\pm$ 0.22 <sup>***</sup>	65.21 $\pm$ 5.22 <sup>***</sup>	71.31 $\pm$ 2.14 <sup>***</sup>
V	GH-500 mg/kg	2.5 $\pm$ 0.22	18.38 $\pm$ 5.22	22.14 $\pm$ 1.33
VI	GH-125+HCTZ	1.5 $\pm$ 0.22 <sup>**a</sup>	56.34 $\pm$ 4.94 <sup>***a</sup>	59.32 $\pm$ 1.31 <sup>***a</sup>
VII	GH-250+HCTZ	0.5 $\pm$ 0.22 <sup>***</sup>	78.31 $\pm$ 2.21 <sup>***a</sup>	83.41 $\pm$ 1.22 <sup>***a</sup>
VIII	GH-500+HCTZ	2.33 $\pm$ 0.33	22.61 $\pm$ 3.78	26.21 $\pm$ 1.31

Values are mean $\pm$ SEM of 8 animals, significant at - <sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01, <sup>\*\*\*</sup>P<0.001 when compared to control group; <sup>a</sup>P<0.05, <sup>aa</sup>P<0.01, <sup>aaa</sup>P<0.001 when compared to corresponding dose of GH alone.

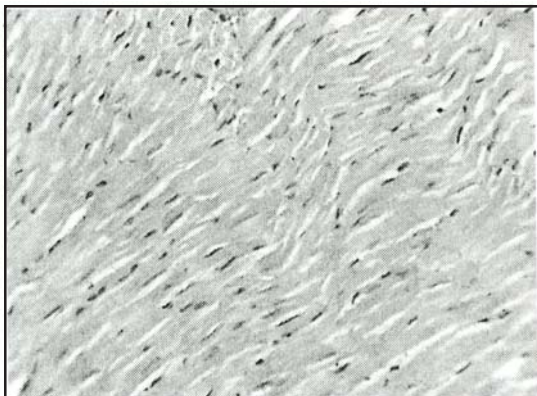


Fig. 5 : H&E ( $\times 400$ ) stained microscopic section of heart of animals pretreated with GH 250 mg/kg orally for 30 days and subsequently heart subjected to global no flow 15 min ischemia and reperfusion, depicted normal architecture, without visible infiltration and decreased interstitial space indicating protective effect of GH 250 mg/kg.

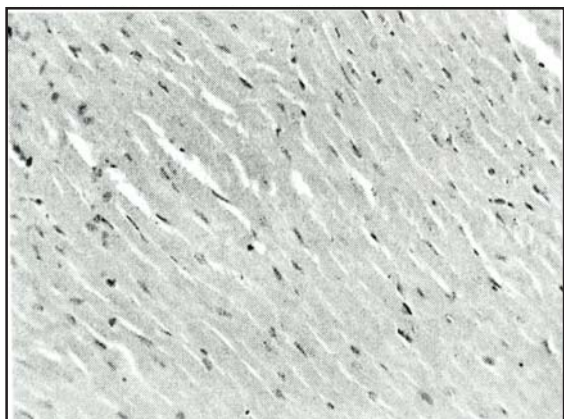


Fig. 6 : H&E ( $\times 400$ ) stained microscopic section of heart pretreated with GH 250 mg/kg for 30 days and HCTZ 10 mg/kg for 7 days by oral route and subsequently heart subjected to global no flow 15 min ischemia and reperfusion. There is clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils indicating protective effect of the combination.

## DISCUSSION

The present research was undertaken to evaluate the effect of different doses of GH

and its possible interaction with HCTZ during and after myocardial damage induced by ISO and IRI in rats. Main observation of the current study was that moderate dose of GH (250 mg/kg) provides protection to myocardium at times of stress such as ISO and IRI which remained stable in presence of HCTZ.

GH was administered at three different doses, which were reported to be safe (125 mg/kg, 250 mg/kg and 500 mg/kg) (7). An earlier study 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 (15–17). 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 (18) that is virtually undetectable in blood circulation after garlic ingestion and decomposes to form the SAC and SAMC (19). GH was administered orally for thirty days to avail the bioactivity of SAC and SAMC at highest possible level. This study was carried out in female rats. Female rats were chosen to keep uniformity among all groups as well as to avail protection against myocardial ailments. It is well known that the females are protected from coronary heart disease due to hormonal influence, whereas males are prone to develop coronary manifestations.

The myocardial damage was produced by administration of Isoproterenol [1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanolhydrochloride], which (20) is a

synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to development of myocardial necrosis. Isoproterenol (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 (12).

To check the possible effect of GH alone and in presence of HCTZ during myocardial ischemia, we also induced myocardial damage by ischemia-reperfusion injury (IRI) in rats. The IRI was induced following no-flow global ischemia, where sudden occlusion of physiological salt solution (PSS) results in immediate biochemical alterations (21, 22). The increase in intracellular  $\text{Na}^+$  serves to drive  $\text{Ca}^{2+}$  intracellularly via  $\text{Na}^+/\text{Ca}^{2+}$  exchange that results in irreversible damage to myocardium at the end of 15 min global ischemia (23).

It is well known that LDH and CK-MB are diagnostic marker enzymes of myocardial damage (24). Presence of these biomarkers in heart tissue homogenate (HTH) is indicative of myocardial integrity and their release in serum or perfusate signifies myocardial injury. The release of cellular enzymes reflects a non-specific alteration in the plasma membrane integrity. In the present study, there was decrease in the activities of these marker enzymes in HTH and increase in the activities in serum/perfusate in ISO/IRI induced myocardial damage. Oral pretreatment with GH-125 and

GH-250 with or without HCTZ restored the activities of these enzymes to near normal in both the heart and the serum/perfusate. Eventhough, HCTZ prior administration prevented damage to myocardium, but it is not substantial to prove its protective behavior. However, it has kept the integrity of myocardium when given along with moderate dose of GH 250 mg/kg. Earlier studies demonstrated the stable role of HCTZ during myocardial damage due to its ability to increase GFR. As it is well established that decrease GFR itself may be a risk factor triggering development of heart failure. Accordingly cardiomyocyte apoptosis, and reduced capillary/cardiomyocyte ratios have been observed in rats with mild renal impairment (25, 26). The role of HCTZ during cardiac ailment is not only mediated by its diuretic action but also it causes vasodilatation by activating calcium-activated potassium channels (large conductance) in vascular smooth muscles and inhibiting various carbonic anhydrases in vascular tissue. It is possible that by this vasodilatation, HCTZ may have promoted the healing and prevented the ischemic damage to myocardium, however, higher dose of GH 500 mg/kg may have caused severe damage that HCTZ could not able to revert back to normal condition.

There is substantial evidence that the associated contractile and rhythmic disturbances involve contributions from oxygen free radicals (OFRs) (27). During myocardial damage, OFRs such as superoxide and hydrogen peroxide are produced in enormous amount that contribute to myocardial tissue injury (28). IRI/ISO induced myocardial damage is associated with decreased endogenous antioxidants such as superoxide dismutase (SOD) and catalase in perfusate/serum which are structurally and

functionally impaired by free radicals resulting in damage to myocardium. Inclination in endogenous antioxidant activities in HTH is indication for structural integrity and protection to the myocardium by prior administration of GH. However, low dose of GH 125 mg/kg does not show the similar rise in SOD and catalase when compared to moderate dose (GH 250 mg/kg). It is interesting to note the alteration in SOD is with concomitant fluctuation in catalase after prior treatment of animals with GH. Elevated activity of catalase in HTH is more beneficial than increase in SOD activity alone because without a simultaneous increase in catalase activity, increased SOD activity may lead to intracellular accumulation of  $H_2O_2$  with detrimental effects (29). However, high dose of GH 500 mg/kg failed to show the beneficial effect probably because of excessive release of allicin, which is a proven oxidant (18). There was no interference of HCTZ on the exhibition of antioxidant and oxidant properties of moderate doses and high doses of GH respectively.

Damage to cardiac musculature was also demonstrated and confirmed by histopathological scores. An increase in score is indicative of myocardial damage (30). Pretreatment with GH at doses of 250 mg/kg alone or with HCTZ substantially decreased the pathological scores and kept the myocardial integrity during IRI/ISO

damage. This effect might be due to augmentation of endogenous antioxidant enzyme synthesis. These results suggest the stabilization of GH mediated protection during HCTZ administration. It is difficult to predict specific mechanism for interaction between garlic and hydrochlorothiazide with the present result. However, one of the major complications of hydrochlorothiazide monotherapy for hypertension in patients with ischemic heart diseases is development of hypokalaemia. Hypokalaemic increases the likelihood of cardiac arrhythmias. Since HCTZ did not interfere with garlic mediated protection during myocardial damage, it is speculated that HCTZ induced hypokalemia might have been attenuated by prior administration of garlic. Hence, further studies should be carried out to elucidate the possible role of GH in pharmacokinetic and pharmacodynamic profile of HCTZ's diuretic activity. We hope that this type of study will open new areas of research for interaction and counteraction between herb and conventional drugs when they are taken concurrently.

In conclusion, pretreatment of GH (250 mg/kg) offers protection from myocardial injury in IRI/ISO myocardial damage. Incorporation of HCTZ does not interfere with GH protective activity. However, high dose of GH (GH-500) was found to increase the oxidative stress that could aggravate the pathological complications.

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