

# PHARMACODYNAMIC INTERACTION OF GARLIC WITH PROPRANOLOL IN ISCHEMIA-REPERFUSION INDUCED MYOCARDIAL DAMAGE

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## ABSTRACT

The present study was undertaken to explore the interaction of garlic homogenate (GH) with propranolol (PRO) on ischemia-reperfusion injury (IRI) in isolated rat heart preparation. Albino rats were treated with GH at three different doses of 125 mg/kg, (GH-125), 250 mg/kg (GH-250) and 500 mg/kg (GH-500) for 30 days orally. The hearts were excised and mounted on modified Langendorff setup and subjected to 15 min global no flow ischemia and reperfused for 15 min. Pretreatment of animals with PRO, GH-125 and GH-250 (either alone or in combination) provided significant protection to myocardium from IRI damage as indicated by significant decrease in LDH and CK-MB activities in perfusate and an increase in activities of these enzymes in heart tissue homogenate. Similarly, the recovery (%) in developed tension and heart rate were significantly more in treated groups during post-ischemia when compared to control. Moreover, GH-250 either alone or with PRO showed significant increase in activities of antioxidant enzymes such as superoxide dismutase and catalase during IRI damage. However, GH-500 failed to show cardioprotective effect when given alone or along with PRO. These biochemical findings were supported by changes in histopathological studies.

**Keywords:** Garlic; interaction; ischemia-reperfusion; isolated heart; propranolol.

## INTRODUCTION

Simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other (Fugh-Berman, 2000). It is widely believed that although herbs hold promise as therapeutically effective medicaments, in-depth and appropriate studies should be carried out to confirm their efficacy in the presence of modern medicines.

Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular diseases (Rahman and Lowe, 2006). 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 (Banerjee and Moulik, 2002). Garlic is also reported to possess cardioprotective (Isensee *et al.*, 1993), antioxidant (Banerjee *et al.*, 2002), antineoplastic and antimicrobial properties (Tattelman, 2005). Further, garlic has significant antiarrhythmic effect in both ventricular and supraventricular arrhythmias (Rietz, 1993). It is reported that garlic in moderate doses for long period augments the endogenous antioxidants activities and depletes the oxidants damaging effects by either increasing the synthesis of endogenous antioxidants or decreasing the generation of oxidants like oxygen free radicals (Banerjee, 2002). Furthermore, it also exerts antioxidant effect in isoprenaline-induced myocardial

infarction in rat (Ciplea and Richter, 1988). Garlic juice inhibits norepinephrine-induced contractions of rabbit and guinea pig aortic rings. It is also reported to inhibit the force of contraction of isolated rabbit heart in a concentration-dependent manner (Aqel, 1991).

Earlier reports on the drug interaction studies of garlic with calcium channel blockers indicate that it produces concentration dependent synergistic effect due to its own calcium channel blocking effect (Martin, 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 protective effect of different doses of garlic and to determine its interaction with PRO, during IRI damage to myocardium using isolated perfused rat heart preparation.

## MATERIALS AND METHODS

### Chemicals

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 Plant extract

Garlic (*Allium sativum*) bulbs were purchased from the local market. The cloves were peeled, sliced, ground into a paste and suspended in distilled water. Three different

doses of the garlic homogenate corresponding to 125 mg/kg, 250 mg/kg and 500 mg/kg were used (Banerjee *et al.*, 2002). 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  $25^{\circ} \pm 5^{\circ}\text{C}$  in a well-ventilated animal house under 12:12 hour 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).

### Experimental Protocol

The animals were divided into different treatment groups. The first group served as control and the animals of group II received propranolol orally at a dose of 10 mg/kg (Hashimoto and Ogawa, 1981). The animals of III, IV and V were treated orally for 30 days with three different dose of GH at 125 mg/kg, 250 mg/kg and 500 mg/kg respectively. The animals of group VI, VII and VIII received three different doses of GH for 30 days at 125 mg/kg, 250 mg/kg and 500 mg/kg respectively along with PRO (10 mg/kg) during the last seven days of GH treatment.

### Experimental Procedure

A modified Langendorff apparatus for the isolated perfused heart was set up as mentioned elsewhere (Inamdar *et al.*, 1994). 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 (Mouhieddine *et al.*, 1993) by blocking the flow of K-H solution & 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) (Buerke *et al.*, 1998) and the activity of LDH, CK-MB, superoxide dismutase (SOD) (Erich and Elastner, 1976) and catalase (Eva, 1988) was determined. Microscopic slides of myocardium were prepared for histopathological studies after post-ischemia (Karthikeyan *et al.*, 2007). Volume fraction of interstitial space (VFITS) in myocardial tissue was determined from hematoxylin and eosin (H &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 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).

### STATISTICAL ANALYSIS

Results are expressed as mean  $\pm$  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

#### Effect on LDH & CK-MB activities

The biological activities of endogenous enzymes like LDH and CK-MB were evaluated in coronary effluent (perfusate) during pre-ischemic and post-ischemic period as well as in heart tissue homogenate (HTH). There was significant ( $p < 0.001$ ) elevation in these enzyme activities in heart perfusate of animals pretreated with GH-500 and depletion in enzyme activities were observed after treatment with GH-125 and GH-250 alone or in combination with PRO when compared to control. During post-ischemia, there was significant ( $p < 0.001$ ) decline in enzyme activities in the perfusate of GH treated groups and GH with PRO treated groups when compared to control (Table 1). Further, high dose of GH-500 was found to significantly ( $p < 0.001$ ) reduce the activities of these enzymes in HTH when compared to control and addition of PRO in GH-500 regimen failed to protect the myocardium. Furthermore, there was significant elevation

( $p < 0.001$ ) in activities of enzymes in HTH of animals pretreated with GH 250 and GH-250 + PRO when compared to control (Table 2). Moreover, it was also noted that the pretreatment of animals with PRO significantly ( $p < 0.001$ ) imparted protection to myocardium by declining the endogenous enzyme activities in perfusate both during pre and post-ischemia and increasing the activities in HTH (tables 1 and 2).

#### Effect on SOD and catalase activity

The SOD and catalase activity in the HTH were significantly ( $p < 0.001$ ) increased after treatment with GH-250 and GH-125 alone or along with PRO. However, the GH-500 did not produce any significant increase in activities of these enzymes when given alone but produced significant increase in enzyme activities when given along with PRO (table 2).

#### Developed Tension and Heart rate

Pretreatment of PRO significantly ( $p < 0.001$ ) imparts the recovery to ischemic heart in terms of developed tension

and heart rate. There was also significant ( $p < 0.001$ ) recovery from global ischemia in groups treated with different doses of GH either alone or with PRO (table 3).

#### VFITS and histological scores (figs. 1, 3)

IRI induced elevation in VFITS values and histological scores. These damages were reversed significantly ( $p < 0.001$ ) by administration of GH-250 alone or with PRO. The GH-500 alone or along with PRO did not produce any significant change in these microscopic parameters (table 3).

## DISCUSSION

The research envisaged was carried out to determine the effect of different doses of GH and its interaction with PRO during IRI induced myocardial infarction (MI) in isolated rat heart preparation. The results show that high dose of GH (500 mg/kg) aggravates the IRI whereas moderate dose of GH (250 mg/kg) protect the myocardium against IRI damage. It was also

**Table 1:** Effect on LDH and CK-MB activities in rat heart perfusate

| Treatment    | LDH Activity (U/L) |                  | CK-MB (U/L)   |                 |
|--------------|--------------------|------------------|---------------|-----------------|
|              | Pre-ischemia       | Post- ischemia   | Pre-ischemia  | Post- ischemia  |
| IRI CONTROL  | 201.27±4.42        | 546.75±11.89     | 21.21±0.59    | 41.16±1.05      |
| PRO          | 168.12±1.69***     | 393.24±7.15 ***  | 13.61±0.24*** | 26.40±0.78 ***  |
| GH-125       | 192.99±2.15        | 485.66±5.68***   | 19.10±0.61**  | 34.81±0.35***   |
| GH-250       | 162.51±1.63***     | 388.49 ± 3.32*** | 13.85±0.42*** | 26.58 ± 0.83*** |
| GH-500       | 406.81±9.55***     | 576.70±9.57*     | 29.06±0.55*** | 46.48±0.80***   |
| GH-125 + PRO | 173.52±1.27***     | 380.75±10.86***  | 14.64±0.20*** | 31.40±0.52***   |
| GH-250 + PRO | 152.46±1.35***     | 275.33±9.13***   | 12.48±0.18*** | 21.64±0.25***   |
| GH-500 + PRO | 323.56±3.91***     | 439.16 ± 3.60*** | 24.69±0.29*** | 35.93 ± 0.62*** |

Values are expressed as mean ± SEM for eight rats in each group.

\*\*\*Significantly different from IRI control  $P < 0.001$ . Garlic Homogenate (GH)- 125 mg/kg, 250 mg/kg & 500 mg/kg (30 days treatment,  $p.o$ ), Propranolol (PRO)-10 mg/kg (7 days treatment,  $p.o$ )

**Table 2:** Effect on LDH, CK-MB, SOD and Catalase activities in heart tissue homogenate of isolated rat heart preparation

| Treatment    | LDH (U/g wet tissue) | CK-MB (U/g wet tissue) | SOD (Units/mg protein) | Catalase (Units/mg protein) |
|--------------|----------------------|------------------------|------------------------|-----------------------------|
| IRI CONTROL  | 761.95±35.18         | 49.76±0.97             | 1.44±0.00              | 2.08±0.05                   |
| PRO          | 947.80±24.03***      | 74.06±0.52***          | 4.49±0.03***           | 7.14±0.18***                |
| GH-125       | 739.46±26.13         | 51.82±0.44             | 2.72±0.04***           | 3.32±0.10***                |
| GH-250       | 979.35±4.62***       | 63.83±0.85***          | 5.31±0.04***           | 6.69±0.17***                |
| GH-500       | 431.30±14.19***      | 46.55±1.11***          | 1.95±0.00              | 2.35±0.06                   |
| GH-125 + PRO | 845.55±8.51*         | 55.96±1.29***          | 5.10±0.05***           | 5.87±0.12***                |
| GH-250 + PRO | 1095.83±26.85***     | 87.25±0.97***          | 7.96±0.12***           | 9.65±0.18***                |
| GH-500 + PRO | 786.40±12.64         | 53.77±0.20             | 3.01±0.01***           | 4.27±0.09***                |

Values are expressed as mean ± SEM for eight rats in each group.

\*\*\*Significantly different from IRI group  $P < 0.001$ . Garlic Homogenate (GH)- 125 mg/kg, 250 mg/kg & 500 mg/kg (30 days treatment,  $p.o$ ), Propranolol (PRO)-10 mg/kg (7 days treatment,  $p.o$ )

SOD Units: One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 10 % heart tissue required to inhibit the reduction of 24 mM NBT by 50%.

Catalase Units: One international unit of catalase is the amount, which catalyzes the decomposition of 1 mM hydrogen peroxide per minute at 37°C.

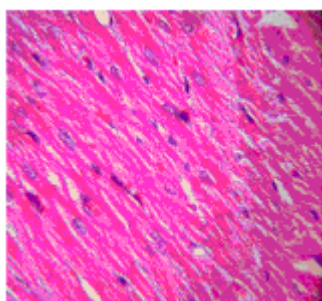
**Table 3:** Effect on percentage recovery of developed tension & heart rate, volume fraction of interstitial space (VFITS) and histological scores

| Treatment    | Percentage Recovery |               | VFITS <sup>1</sup> | Histological scores <sup>2</sup> |
|--------------|---------------------|---------------|--------------------|----------------------------------|
|              | Developed Tension   | Heart Rate    |                    |                                  |
| Vehicle      | 22.27±4.17          | 33.72±2.23    | 39.03±1.71         | 3.33±0.33                        |
| PRO          | 65.98±6.70***       | 74.66±1.63*** | 28.79±1.63***      | 1.66±0.21*                       |
| GH-125       | 51.06±8.87          | 57.29±2.16*** | 28.09±0.69***      | 2.52±0.42                        |
| GH-250       | 75.02±9.23***       | 79.04±3.14*** | 25.24±0.93***      | 1.5±0.22**                       |
| GH-500       | 13.68±6.22          | 32.04±1.71    | 35.49±0.54         | 3.66±0.33                        |
| GH-125 + PRO | 56.44±5.94*         | 76.46±1.37*** | 27.39±0.60***      | 1.83±0.30                        |
| GH-250 + PRO | 91.44±1.25***       | 90.42±1.32*** | 23.44±1.30***      | 1.16±0.16**                      |
| GH-500 + PRO | 57.72±4.78**        | 60.88±1.92*** | 30.49±0.50***      | 3.00±0.51                        |

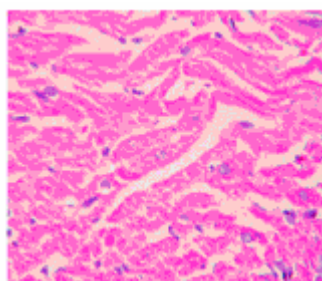
Values are expressed as mean ± SEM for eight rats in each group. \*\*\*Significantly different from IRI group  $P < 0.001$ .

Garlic Homogenate (GH)- 125 mg/kg, 250 mg/kg & 500 mg/kg (30 days treatment, *p.o*)

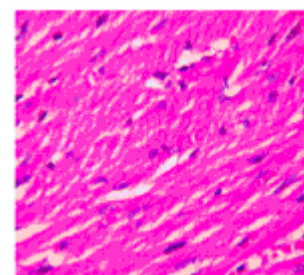
Propranolol (PRO)-10 mg/kg (7 days treatment, *p.o*)



**Fig. 1:** H & E (× 200) stained microscopic section of normal control heart.



**Fig. 2:** H & E (× 200) stained microscopic section of heart subjected to ischemia-reperfusion injury (IRI) induced myocardial infarction (MI).



**Fig. 3:** H & E (× 200) stained microscopic section of heart of animal pretreated with GH-250 mg/kg (30 days *p.o*) and PRO 10 mg/kg -10 (7 days *p.o*) subjected to IRI induced MI.

demonstrated in the present study that the incorporation of PRO during IRI in presence of GH-250 produces synergistic cardioprotective effect.

GH was administered at three different doses, which were reported to be safe (125 mg/kg, 250 mg/kg & 500 mg/kg) (Banerjee *et al.*, 2002). 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-propenethiosulfonate) 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). GH was administered orally instead of introducing it into the

perfusion fluid to avail the activity of SAC and SAMC, which are effective metabolites of garlic formed only in circulation from allicin.

Myocardial damage was induced using ischemia-reperfusion injury (IRI) model. Ischemia is an acute or chronic form of cardiac disability arising due to the imbalance between the myocardial supply and demand for oxygenated blood. The IRI was induced following no-flow global ischemia (Mouhieddine *et al.*, 1993), where sudden occlusion of physiological salt solution (PSS) results in immediate biochemical alterations (James and Keyes, 1963; Jennings *et al.*, 1990). 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 (Jennings *et al.*, 1985).

It is well established that the biological markers like endogenous enzyme are organ specific and leak from the damaged organ during necrosis (Hearse *et al.*, 1979).

Damaged to the cardiac musculature due to IRI results in leakage of cardiac biomarkers such as LDH and CK-MB into the perfusate with resultant decrease in their activities in HTH (Dumoulin *et al.*, 2005; Hearse *et al.*, 1979; Lee *et al.*, 2005). Prophylactic administration of GH-250 (30 days *p.o*) and PRO, a good cardioprotective agent (Satoskar *et al.*, 1995), either alone or together restored the enzyme activity to normal in heart tissue homogenate (HTH) and perfusate to substantial extent.

The IRI damage to myocardium is also due to release of oxygen free radicals (OFRs), that causes destruction of myocardial membrane and leakage of bioenzymes in perfusate. Among number of OFRs associated with myocardial contractile and rhythmic disturbances (Mc Cord, 1988), 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). In addition to this, measurement of catalase activity was carried out as elevation in SOD dismutates superoxide but results in accumulation of H<sub>2</sub>O<sub>2</sub> which could further precipitate the MI (Yim *et al.*, 1990). Pretreatment of animals with GH (125 mg/kg & 250 mg/kg) 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) produced a significant decrease in the antioxidant enzyme activities and PRO failed to reverse the GH (500 mg/kg) 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 increase in functional parameters like developed tension and heart rate at the end of 15 min reperfusion is an indication of good recovery from global ischemia (Mouhieddine *et al.*, 1993). PRO is known to reduce the myocardial oxygen requirement and improve the stress tolerance in patients with MI (Satoskar, 1995). This PRO tolerating ability was demonstrated in this study, which was evident from good recovery in functional parameters in PRO incorporated groups. Maximum recovery was seen in groups with GH (250 mg/kg) alone or along with PRO and good recovery was seen even at low dose GH (125mg/kg) alone or along with PRO. GH (500 mg/kg) showed toxic effect, as indicated by poor recovery from IRI.

Histopathological studies were carried out for confirmation of biochemical findings. The parameters - VFITS and histological scores were used to determine the myocardial damage. Pretreatment with GH at doses of 125 mg/kg and 250 mg/kg alone or with PRO substantially decreased the interstitial cavity and kept the myocardial integrity during IRI damage. This effect might be due to augmentation of endogenous antioxidant

enzyme synthesis. There was also remarkable reduction in the pathological scores with GH (250 mg/kg) in presence of PRO. These results suggest the enhancement of PRO mediated protection to myocardium by moderate dose of GH during MI.

## CONCLUSION

In conclusion, pretreatment of GH (250 mg/kg) offers protection from myocardial injury in IRI myocardial damage. Incorporation of PRO augments myocardial protection. However, high dose of GH (GH-500) was found to increase the oxidative stress that could aggravate the pathological complications. Therefore, diet containing moderate doses of garlic could prove beneficial to the heart and administration of garlic with PRO produces additive effect.

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