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Original Article

Interaction of Propranolol with Garlic on Biochemical and Histological Changes in Rat

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Abstract

The current study dealt with the interaction of garlic homogenate (GH) with propranolol (PRO) in rat, and to determine whether this effect is associated with biochemical and histological changes.

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) orally for 30 days and PRO was incorporated in the interactive groups during the last seven days of GH treatment. Blood was withdrawn under ether anesthesia from the retroorbital route and serum was separated. Heart tissue homogenate (HTH) of the excised heart was also prepared. Both serum and HTH were used for biochemical estimation. Histopathological studies were carried out subsequently for confirmation of biochemical findings.

GH-125 and GH-250 were found to significantly augment the endogenous antioxidant synthesis whereas, GH-500 was found to significantly diminish the synthesis of superoxide dismutase (SOD) and catalase. Toxicity of GH-500 cannot be reversed by the addition of PRO in the therapy. However, incorporation of PRO in GH-125 or GH-250 treatment showed significant synergistic effect in terms of increasing antioxidant synthesis. Mild and moderate doses of GH were also shown to keep the integrity of myocardium intact, whereas GH-500 damages the myocardium.

The findings of the present study indicate that it is safe to administer garlic in low to moderate doses in cardiac patients receiving propranolol. However, high doses of GH are found to be toxic to myocardium and hence care should be taken for proper selection of doses.

Keywords: Garlic; Propranolol; CK-MB; SOD; Catalase.

Introduction

The use of complementary and alternative medicines is burgeoning globally, especially in developed countries including US (1). However, over 80% of the population in developing countries depend on traditional healing modalities, including herbal remedies, for health maintenance and therapeutic management of the disease (2). Although many studies have identified the increasing prevalence of herbal remdies throughout the world, only a few have reported on how patients perceived the efficacy of this healthcare modality in specific diseases (3). It is interesting to note that herbs are often administered in combination with therapeutic drugs, raising the potential of

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herb-drug interactions (4). Herbal medicines are ubiquitous. However the dearth of reports of adverse events and interactions probably reflects a combination of under-reporting and the benign nature of most herbs used. Experimental data in the field of herb-drug interactions are limited, case report scarce, and case series rare. 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 (5). 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.

In traditional medicine, garlic *(Allium sativum)* and its preparations have been widely recognized as agents for the prevention and treatment of cardiovascular and other metabolic diseases, such as atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes (6). Previous studies have demonstrated that garlic has a significant anti-arrhythmic effect in both ventricular and supraventricular arrhythmias (7). These beneficial effects have been proposed to be due to its alteration on cardiac electrophysiology, including effective refractory period (ERP) prolongation, Ca^{2+} influx suppression, as well as it's free radical scavenging activity (7). It is also demonstrated by epidemiologic studies that there is an inverse correlation between garlic consumption and progression of cardiovascular disease (8). Garlic juice mimics beta-blocking property by inhibiting norepinephrine-induced contractions of rabbit and guinea pig aortic rings (9).

Earlier reports on the drug interaction studies of garlic with calcium channel blocker indicate that it produces a concentration dependent synergistic effect via its calcium blocking property (10). As mentioned above, garlic is known to mimic beta-blocking property. However, no scientific observations are available regarding the interaction of garlic with propranolol (PRO), when they are used together during conventional cardioprotective therapy. Hence, the present investigation was undertaken to demonstrate the protective effect of different doses of garlic homogenate and to determine its interaction with PRO, in rats.

Experimental

Materials

All the 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 mg/kg, 250 mg/kg and 500 mg/kg were administered orally (11). 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 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 the "Purpose of Control and Supervision on Experiments on Animals Committee".

Experimental protocol

The animals were divided into different treatment groups. The first group served as control and the animals in group II received propranolol orally at a dose of 10 mg/kg (12). The animals in III, IV and V were treated orally for 30 days with three different GH doses of 125 mg/kg, 250 mg/kg and 500 mg/kg, respectively. The animals in groups VI, VII and VIII received

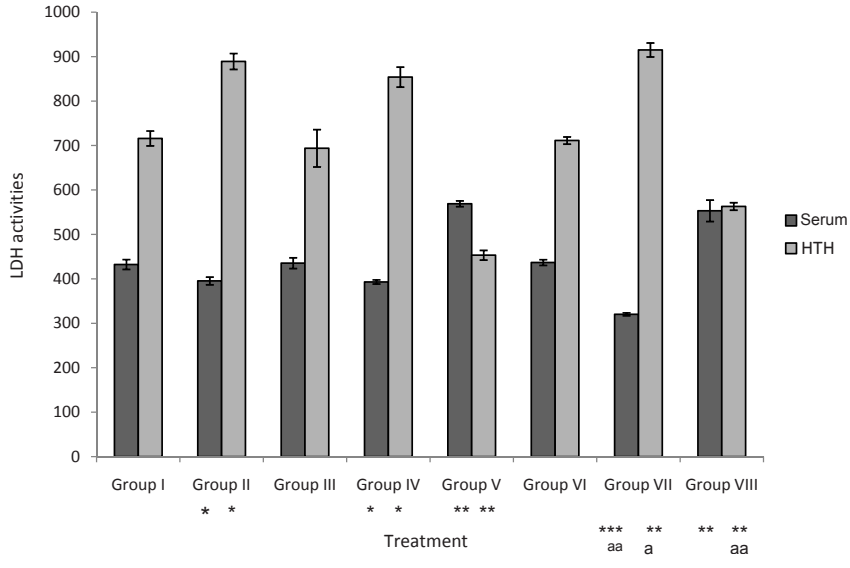

Figure 1. Effect on LDH activities in serum (U/L) and heart tissue homogenate (HTH-U/G).
Values are mean ± SEM , n=8.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ when compared to Group I; $^{a}P < 0.05$, $^{aa}P < 0.01$, $^{aaa}P < 0.001$ when compared to the corresponding dose of GH alone.
Group I, normal vehicle; Group II, PRO; Group III, GH-125 mg/kg; Group IV, GH-250 mg/kg; Group V, GH-500 mg/kg; Group VI, GH-125+PRO; Group VII, GH-250+PRO; Group VIII, GH-500+PRO. PRO (10 mg/kg) treatment was for 7 days, whereas GH treatment was for 30 days, *p.o*.

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

At the end of treatment period, blood was drawn from retroorbital vein of the rat under ether anesthesia and serum was separated by centrifugation for lactate dehydrogenase (LDH) and creatine phosphokinase isoenzyme (CK-MB) measurement. The heart was isolated from each animal 2 h after administration of the last dose of the drugs, under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia and homogenized to prepare heart tissue homogenate (HTH) using 0.25 M sucrose (13). The activity of LDH, CK-MB, superoxide dismutase (SOD) (14) and catalase (15) 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 & E) stained transverse sections by using the following equation (16).

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

The myocardial damage was determined by giving scores depending on the intensity, as follows (17): 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), and marked-score 03 (necrosis with diffuse inflammatory process).

Statistical analysis

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

Results

LDH and CK-MB

In the present study, LDH and CK-MB

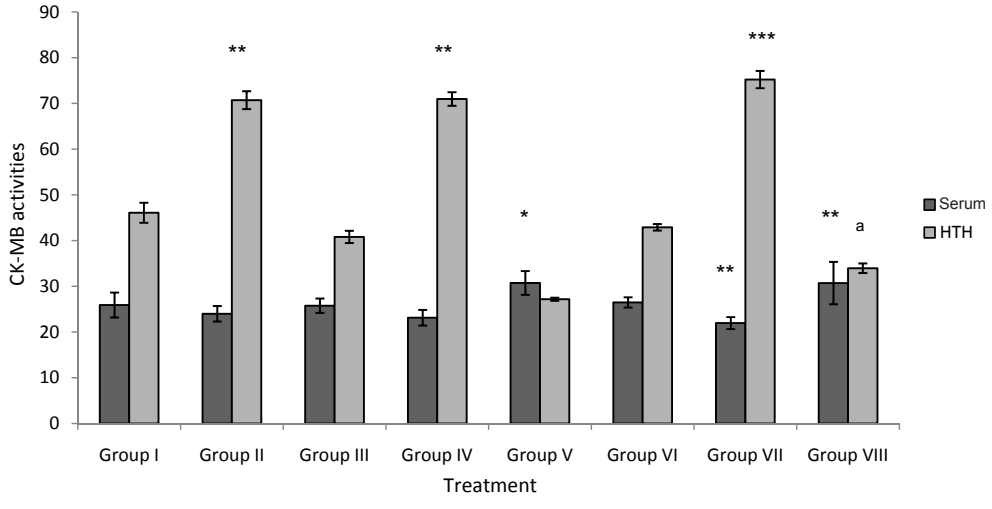

Figure 2. Effect on CK-MB activities in serum (U/L) and heart tissue homogenate (HTH-U/G).
Values are mean ± SEM , n=8.
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P< 0.001$ when compared to Group I; $^{a}P<0.05$, $^{aa}P<0.01$, $^{aaa}P< 0.001$ when compared to the corresponding dose of GH alone. Group I, normal vehicle; Group II, PRO; Group III, GH-125 mg/kg;
Group IV, GH-250 mg/kg; Group V, GH-500 mg/kg; Group VI, GH-125+PRO; Group VII, GH-250+PRO; Group VIII, GH-500+PRO.
PRO (10 mg/kg) treatment was for 7 days, whereas GH treatment was for 30 days, *p.o*.

activities increased and decreased significantly ($P < 0.01$) in serum and heart tissue homogenate (HTH), respectively, at the high dose of GH-500 when compared to the control group. There was a significant ($P < 0.001$) serum reduction in terms of these biological marker enzyme activities in groups II, IV and VII when compared to the control group. Further, there was a significant ($P < 0.001$) elevation in these biomarker activities in HTH in groups treated with PRO, GH-250, and GH-250 + PRO, when compared to the control. However, incorporation of PRO during GH-500 treatment failed to keep the biomarker activities intact within the myocardium (Figures 1 and 2). Moreover, there was a significant fall and rise in LDH activities in serum and HTH, respectively, of animals pretreated with GH-250 in presence of PRO, when compared to the corresponding GH-250 treated group**.**

SOD and catalase

There was a significant ($P < 0.001$) decline in SOD and catalase activities in HTH at the high dose of GH-500, when compared to the control group. Further more, it was a found that there was a significant ($P < 0.001$) rise in these antioxidant activities in groups treated with PRO and GH-250 either alone in combination with PRO in HTH, when compared to the control. However, there was a significant decline in antioxidant activities at the high dose of GH-500 in presence of PRO, when compared to the control group (Table 1).

VFITS and histological scores

Biochemical findings on the effective dose of GH were further confirmed by histopathological studies. There was a significant ($P < 0.05$) rise in VFITS and histological scores at a high dose (GH-500), when compared to the control group. Further more, these parameters were found to remain unchanged upon treatment with GH-125 and GH-250, with or without PRO, when compared to the control group. Examination of myocardial tissue of the control group (Figure 3) depicted clear integrity of myocardial cell membrane. Light microscopy showed normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils. GH-500 administrated heart tissue (Figure 4) showed patchy areas of necrosis. In animals pretreated with GH-250+PRO (Figure 5) the morphology of the myocardium

Table 1. Effect on SOD, catalase, volume fraction of interstitial space (VFITS) and histological scores in rats.

Treatment	Heart tissue homogenate		Heart tissue	
	SOD (Units/mg protein)	Catalase (Units/mg protein)	VFITS	Histological scores
Group I	2.51±0.08	3.26±0.05	23.85±0.89	1.0±0.02
Group II	4.93±0.04*	4.96±0.08*	21.06±0.25	0.5±0.02
Group III	2.56±0.03	3.41±0.06	22.80±0.20	1.33±0.22
Group IV	4.25±0.05*	4.35±0.03*	20.43±0.19	0.5±0.16
Group V	1.68±0.03*	2.17±0.06*	32.84±0.31**	2.5±0.30*
Group VI	2.93±0.02	2.79±0.03^{a}	22.83±0.13	1.0±0.21
Group VII	5.14±0.04^{**a}	5.15±0.05^{**a}	20.14±0.23	0.5±0.16
Group VIII	1.93±0.03^{*a}	2.65±0.03^{*a}	28.15±0.27*	3.0±0.36*

Values are mean ± SEM , n=8.

*P<0.05, **P<0.01, ***P<0.001 when compared to Group I; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P<0.001 when compared to the corresponding dose of GH alone.

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PRO (10 mg/kg) treatment was for 7 days, whereas GH treatment was for 30 days, *p.o*.

Superoxide dismutase 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 that amount which catalyzes the decomposition of 1 mM hydrogen peroxide per minute at 37 °C.

was essentially within normal limits. No area of necrosis and cellular infiltration was observed seen.

Discussion

The research envisaged was carried out to determine the effect of different doses of GH and it's interaction with PRO in rat. The results showed that the high dose of GH (500 mg/kg) induced damage to myocardium which was not prevented by the addition of PRO during GH therapy. It was also demonstrated in the present study that the incorporation of PRO during GH-250 administration produces a synergistic cardioprotective effect.

GH was administered at three different doses, (125 mg/kg, 250 mg/kg and 500 mg/kg) which were reported to be safe. Earlier studies on the effect of GH on cardiovascular system suggest that GH induced cardioprotection is due to it's active organosulfur metabolites [S-allylcysteine (SAC) and S-allylmercaptocys teine (SAMC)], which have potent antioxidant activities (18). Allicin (allyl-2-propene thiosulfinate) 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 (19) that is virtually undetectable in blood circulation after garlic ingestion and decomposes to form SAC and SAMC.

It is well established that the biological markers, such as endogenous enzyme, are organ specific and leak from the damaged organ during necrosis (20). Chronic administration of mild and moderate doses of GH keeps the myocardium intact, preventing the damage to cardiac cells, whereas, high doses of GH leak the enzymes from myocardium, indicating damage. Oxygen free radicals (OFRs) are involved in the pathophysiology of a wide range of disease conditions including ischemic heart diseases, resulting usually from deficient endogenous antioxidant defenses. It can be emphasized that in most cases ischemic heart disease occurs due to the imbalance between oxidants and antioxidant defenses. Potential antioxidant therapy should include the supplementation of endogenous antioxidants with natural antioxidants or augmentation of endogenous antioxidant synthesis such as superoxide dismutase, catalase, etc (21).

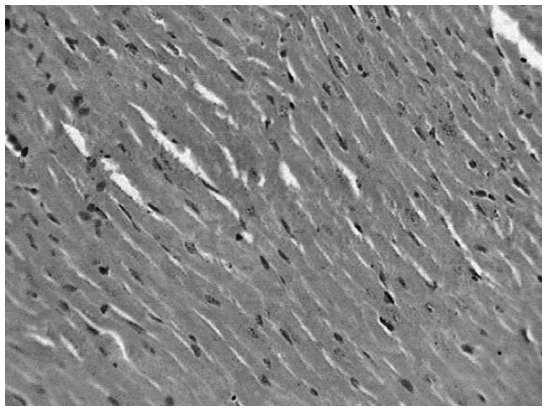

Figure 3. H&E (×200) stained microscopic section of normal control heart.

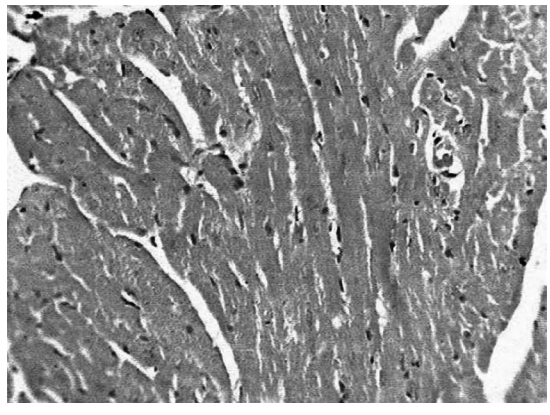

Figure 4. H&E (×200) stained microscopic section of GH-500 heart.

Among number of OFRs associated with myocardial contractile and rhythmic disturbances, contribution of superoxide to myocardial damage is believed to be the highest and this radical is combated by elevated activities of the endogenous antioxidant enzyme, superoxide dismutase (22). In addition to this, measurement of catalase activity was carried out as elevation in SOD dismutes superoxide but results in accumulation of H_2O_2, which could further precipitate MI (23). Chronic administration of GH-125 and GH-250 alone or along with PRO produced remarkable elevation in SOD and catalase activities, when compared to control, indicating augmented synthesis of endogenous antioxidants during garlic treatment. However, administration 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 results obtained clearly demonstrate that GH in moderate and low doses reduces oxidative damage and in high doses aggravates oxidative stress.

It can be speculated that the antioxidant ability of garlic homogenate could be due to the presence of active organosulfur metabolites; S-allylcysteine and S-allylmercaptocys teine, which possess a potent antioxidant activity. Incorporation of conventional cardioprotective agents, such as PRO, further accelerate the protective ability of mild and moderate doses of garlic, however, fails to reverse the damage due to high doses of garlic. It is still difficult to speculate the reason behind the toxic effect of high dose of GH. A number of studies on garlic juice and garlic homogenate have shown that the injurious effect of high doses of garlic on various tissues like intestinal lining and stomach (24) could be attributed to oxidizing ability of allicin.

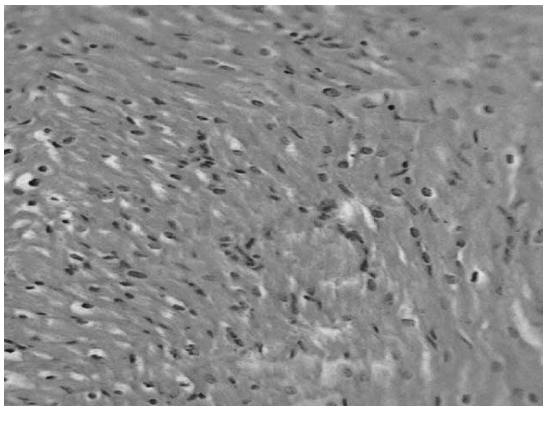

Figure 5. H&E (×200) stained microscopic section of GH-250 + PRO heart.

Conclusion

In conclusion, chronic garlic intake of low to moderate doses augmented endogenous antioxidants, which might have important direct cytoprotective effects on the heart, especially in the event of an oxidant stress induced injury. However, high doses of GH are found to be toxic to myocardium and hence care should be taken for proper selection of doses.

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