Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

PHARMACODYNAMIC INTERACTION OF AQUEOUS EXTRACT OF GARLIC WITH ATORVASTATIN IN DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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Received: 15 Mar 2013, Revised and Accepted: 16 Apr 2013

ABSTRACT

Objective: The purpose of the present study was to determine the Pharmacodynamic interaction of the garlic homogenate with Atorvastatin on Doxorubicin induced myocardial damage in Albino rats.

Methods: Myocardial damage was induced by intraperitoneal Doxorubicin (15mg/kg) administration.Garlic homogenate at different doses of 125 and 250mg/kg orally for 28 days and Atorvastatin (60mg/kg p.o.) was incorporated in the interactive groups during the garlic homogenate treatment was assessed by cardiac marker enzymes such as Lactate Dehydrogenase (LDH) and Creatine Kinase (CK-MB) levels, oxidative stress parameters such as Reduced Glutathione, Thiobarbituric acid reactive substances (TBARS), Superoxide dismutase and Catalase levels were measured. Blood was collected for biochemical estimation.

Results: Doxorubicin administration significantly increased the serum levels of CK-MB and LDH and also increased levels of TBARS and decreased levels of Superoxide dismutase, reduced glutathione and catalase in heart homogenate was observed in Doxorubicin treated group. Further elevation of ST, increased QRS complex and QT interval was also observed. The moderate dose of GH alone or in combination with Atorvastatin (ATO) was found to ameliorate the effect of Doxorubicin and significantly improved biochemical, antioxidant and histological status of myocardium during myocardial damage.

Conclusion: In the present study mild to moderate doses of garlic homogenate (125, 250mg/kg) respectively offers protection from myocardial injury. Incorporation of Atorvastatin augments myocardial protection.

Keywords: Garlic homogenate, Doxorubicin, Atorvastatin, Myocardial infarction, Cardiac marker enzymes.

INTRODUCTION

Myocardial infarction commonly known as heart attack is a disease that occurs when a blood supply to a part of heart is interrupted, causing death of heart tissue. By 2020, heart disease and stroke will become the leading causes of both death and disability worldwide, with the number of fatalities projected to increase to more than 20 million a year, and to more than 24 million a year by 2030 [1]. About 5% of heart attacks occur in young people under the age of 40 years particularly in those with major risk factors to develop atherosclerosis like hypertension, diabetes mellitus, cigarette smoking, familial hypercholesterolemia etc. Males are at higher risk than women probably due to productive influence of estrogen. The use of herbal supplements has become increasingly popular in recent years. The study of mechanism of herbal drug interaction will be of much value in ensuring safety and efficacy of the drugs [2]. The prophylactic and therapeutic effect of herbal extracts such as *Zingiber officinale, Ocimum sanctum* etc., have been reported in reducing cardiovascular disorders. In traditional system *Allium sativum* is used as hypolipidemic and cardiotonic. It was reported that cardioprotective activity of statins is mainly due to the prevention of lipid peroxidation, tissue fibrosis and preservation of anti oxidants as well as scavenging of free radicals. There is a chance of interaction of garlic taken along with diet with that of Atorvastatin in patients undergoing cardioprotective therapy.

Hence the present study was an effort in this direction to evaluate the pharmacodynamic interaction of aqueous extract of garlic with Atorvastatin in Doxorubicin-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Plant collection and Authentication

Garlic bulbs were purchased from local market. The material was authenticated by Prof.P.Jayaraman, Plant Anatomy Research Centre [PARC] Chennai. Voucher specimen (PARC/2011/842) was submitted to Department of Pharmacognosy, SRM College of Pharmacy, Tamil Nadu, India.

Preparation of Garlic Homogenate [GH]

The cloves were peeled, sliced, ground in to paste and homogenate was made in distilled water. Two different concentrations of the extract were prepared 0.05 and 0.1gm/ml, corresponding to 125mg/kg and 250mg/kg body weight of animal. Oral feeding was done within 30 min of the preparation of homogenate [3] [4] [5].

Chemicals and drugs

Atorvastatin was procured from Laila Pharmaceuticals (Chennai) and Doxorubicin was procured from Khandelwal Labs (Mumbai)

Experimental animals

Laboratory bred male Albino Wistar rats weighing between 150-200gm were collected from the animal house of SRM College of Pharmacy. The animals were maintained in well ventilated room with 12:12 hour light/dark cycle in poly propylene cages. Standard feed and tap water was provided *ad libitum* throughout the experimentation period, the study was approved by the institutional animal ethical committee. The proposal number is IAEC/107/2010

Induction of cardiotoxicity in experimental rats

Cardiotoxicity was induced in animals by intraperitoneal injection of Doxorubicin (DOX) in 6 equal doses (2.5mg/kg) over a period of two weeks. After injection, the animal had free access to food and water [6].

Electro cardiograph (ECG)

ECG was recorded at the end of the treatment after the last dosing all rate was fasted over night but had free access to water after the last dose administration. Rats from each group were anesthetized with light ether, needle electrodes was inserted in to skin of the limb at position II. For each ECG tracing ST, QT interval, QRS complex and heart rate was measured

Estimation of cardiac marker enzymes

The cardiac marker enzymes like CK-MB, LDH was estimated by using commercially available kits.

Grouping of animals

Groups	Treatment	Dosage, Route and Duration
Group I	VEHICLE	Animals were treated with vehicle(saline) for 4 weeks
Group II	DOX	First 2 weeks vehicle+ cumulative dose of Doxorubicin (15mg/kg, i.p. in 6 equal doses) for next 2 weeks.
Group III	ATO+DOX	Atorvastatin (cumulative dose of 60mg/kg in 12 equal oral doses over a period of 4 weeks) first 2 weeks only ATO+ next 2 weeks along with DOX.
Group IV	GH 1+DOX	GH 1-(125 mg/kg, p.o.) first 2 weeks only GH+ next 2 weeks along with DOX
Group V	GH 2+DOX	GH 2-(250 mg/kg, p.o.) first 2 weeks only GH+ next 2 weeks along with DOX
Group VI	GH1+ ATO+DOX	GH 1-(125 mg/kg, p.o.) first 2 weeks (GH+ ATO) 2 weeks along with DOX
Group VII	GH2+ ATO+DOX	GH 2-(250 mg/kg, p.o.) first 2 weeks (GH+ ATO) 2 weeks along with DOX

Oxidative Parameters

Superoxide Dismutase (SOD) was assayed utilizing the technique of Kakkar *et al*. The activity of SOD was expressed as units/mg protein [7]. Reduced Glutathione (GSH) levels were estimated by Ellman's method. The data was expressed as µmol/g tissue [7]. Lipid Peroxidation (LPO) indicated by levels of TBARS was estimated according to Ohkowa method. TBARS was expressed as nano moles/gm wet weight of tissue [8]. Estimation of Catalase activity was performed by the method described by Aebi. [9]

Histopathological examination

Cardiac muscle samples were taken from rats in different groups, fixed in 10% formal saline for 1 day, then washed with water. Ascending serial dilutions of ethyl alcohol was used for dehydration. samples was cleared in xylene, then embedded in paraffin at 56 degree in hot oven for 24hrs.paraffin blocks were prepared and cut at 4micron thickness. Sections were mounted on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathalogical examinations through the light microscope [7].

Statistical analysis

The results were expressed as the mean ± S.E.M. The results obtained from the present study were analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph pad Prism 5 software.

RESULTS

General Appearance

In the latter half of the study, the Doxorubicin-induced animals developed a pink tinge, and the animals' fur became scruffy. These rats also had red exudates around the eyes and the nose, soft watery faeces and enlargement of the abdomen. These conditions were more severe at the end of study period. There were no deaths in the normal, ATO, GH-125,GH-250,GH-125+ATO,GH-250+ATO group but a mortality rate of 20.0% was observed (i.e,1 out of 5 animals) in DOX group. Rats in DOX-induced groups showed a gradual decrease in their feed and water consumption and subsequent decrease in body weight as compared with the normal group.

Evaluation of ECG alterations

Doxorubicin administration showed significant changes in the repolarization phase of the ECG. Doxorubicin induced a significant prolongation in ST segment, QT interval, with less effect on QRS complex as compared to control group. In addition, a significant increase in heart rate of doxorubicin-induced rats was observed as compared to normal group. Pretreatment with ATO, GH-125 and GH-250 significantly altered these changes in ST AND QT intervals as well as the heart rate as compared to doxorubicin-induced group. There was a very significant alteration in ST and QT intervals and heart rate in GH-250+ATO and GH-125+ATO combination groups when compared to GH-125 and GH-250 alone compared to doxorubicin group.

Table 1: Effect of GH and ATO individually and in combination on doxorubicin induced changes in ECG parameters

Group No.	Treatment	QRS(sec)	Q-T interval (sec)	S-T Segment (sec)	HR(b/min)
I	Normal	0.020±0.223	0.078±0.682	0.009±0.086	379.1±1.904
II	DOX control	0.0166±0.118$^{\#\#\#}$	0.097±0.507$^{\#\#}$	0.012±0.081$^{\#\#\#}$	452±2.066$^{\#}$
III	ATO (60mg/kg)	0.017±0.047***	0.088±0.350***	0.010±0.017***	424±0.796***
IV	GH (125mg/kg)	0.017±0.166**	0.093±0.0225**	0.011±0.031**	438±0.463**
V	GH (250mg/kg)	0.018±0.028***	0.091±0.266***	0.010±0.081***	428.8±0.356***
VI	GH 125+ATO	0.018±0.136***	0.085±0.283***	0.010±0.005***	417.4±0.572***
VII	GH 250+ATO	0.019±0.043***	0.081±0.302***	0.010±0.015***	405.3±1.631***

All values are mean ± SEM; n=6 in each group,$^{\#}$P<0.05,$^{\#\#}$P<0.001 when compared to normal, *p<0.05, **P<0.01, ***P<0.001 when compared to control

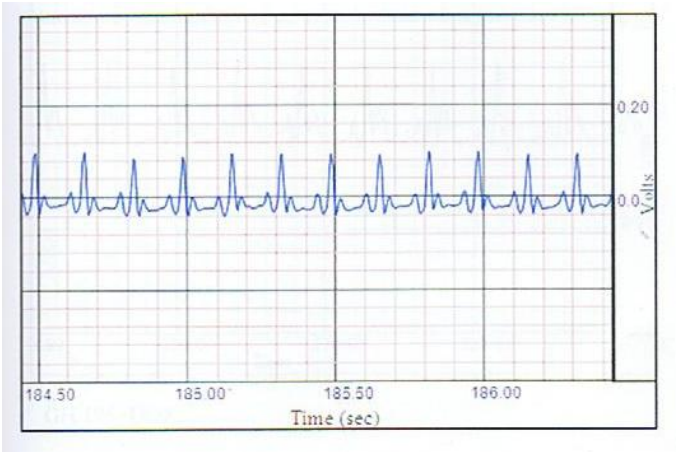

Fig. 1: Normal group

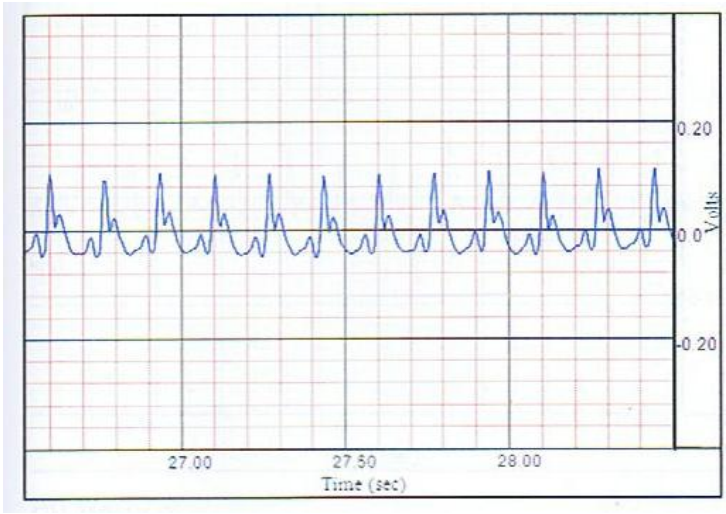

Fig. 2: DOX treated

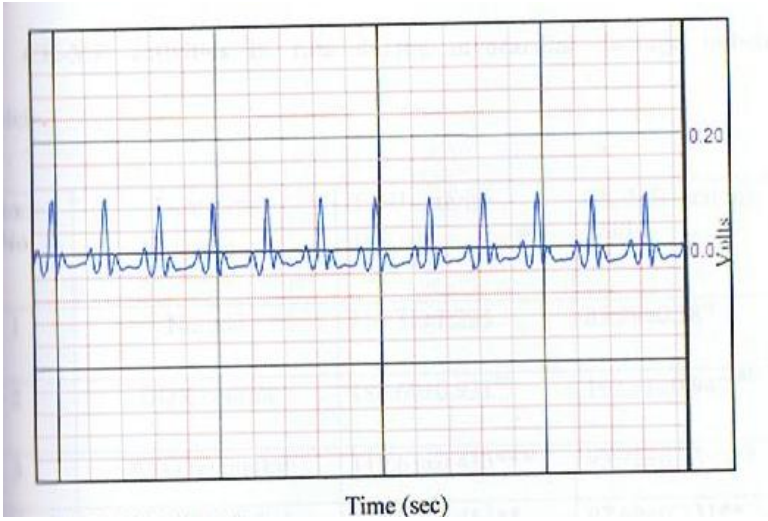

Fig. 3: ATO treated

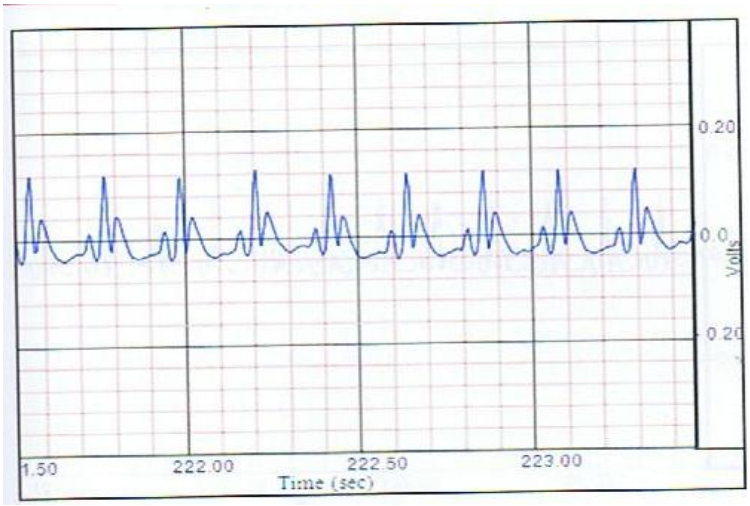

Fig. 4: GH-125 mg/kg + DOX treated

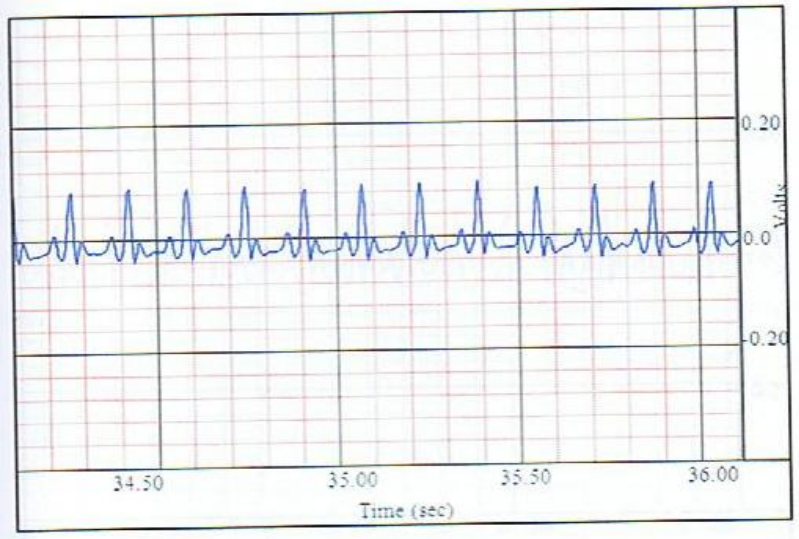

Fig. 5: GH-250 mg/kg + DOX treated

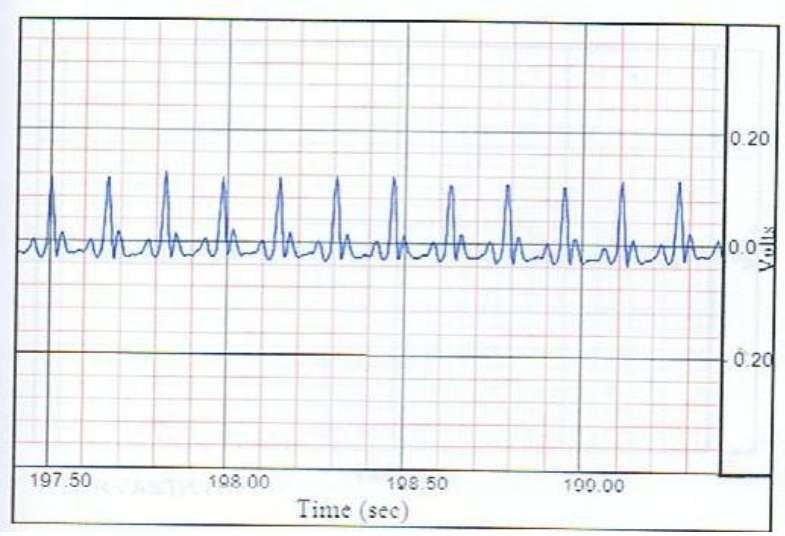

Fig. 6: GH-125 mg/kg + ATO + DOX treated

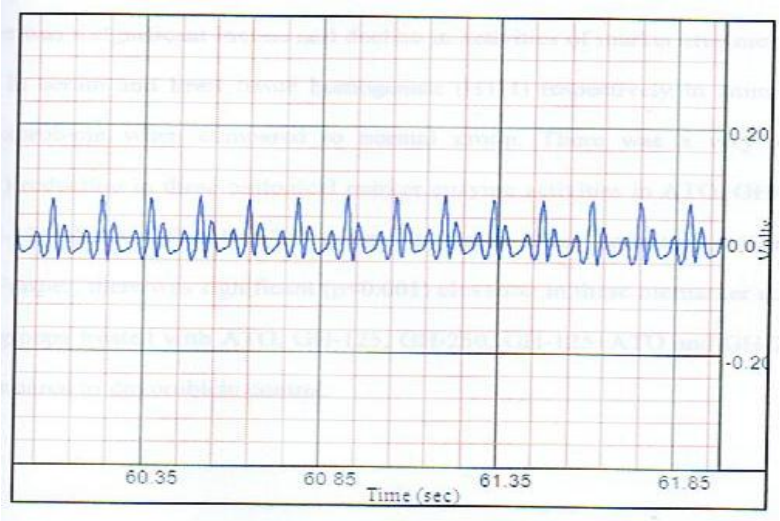

Fig. 7: GH-250 mg + ATO + DOX treated

Results of cardiac marker enzymes

There was a significant incline and decline in activities of marker enzymes LDH and CK-MB in serum and heart tissue homogenate (HTH) respectively in animals treated with Doxorubicin when compared to normal group. There was a very significant (P<0.001) reduction in these biological marker enzyme activities in ATO, GH-125,GH-250,GH-125+ATO,GH-250+ATO groups in serum when compared to Doxorubicin control. Further, there was significant (P<0.001) elevation in these biomarker activities in HTH in groups treated with ATO, GH-125, GH-250, GH-125+ATO, GH-250+ATO when compared to Doxorubicin control.

Table 2: Effect of GH and ATO individually and in combination on serum LDH and CK-MB activities in rats during myocardial damage induced by Doxorubicin

Group No.	Treatment	LDH activity(IU/L)	CK-MB activity
I	Normal	114.31±1.293	85.99±0.387
II	DOX control	188.60±0.931$^{\#\#}$	192.31±0.942$^{\#\#\#}$
III	ATO 60mg/kg	119.61±0.413***	93.71±0.339***
IV	GH(125mg/kg)	124.01±0.464**	97.69±0.331**
V	GH(250mg/kg)	121.91±0.479***	95.75±0.329***
VI	GH-125+ATO	117.63±0.413***	91.72±0.333***
VII	GH-250+ATO	115.52±0.478***	89.73±0.337***

All values are mean ± SEM; n=5 in each group,$^{\#}$ P<0.05, $^{\#\#}$P<0.01, $^{\#\#}$P<0.001 when compared to normal. *P<0.05, **P<0.01, ***P<0.001 when compared to control

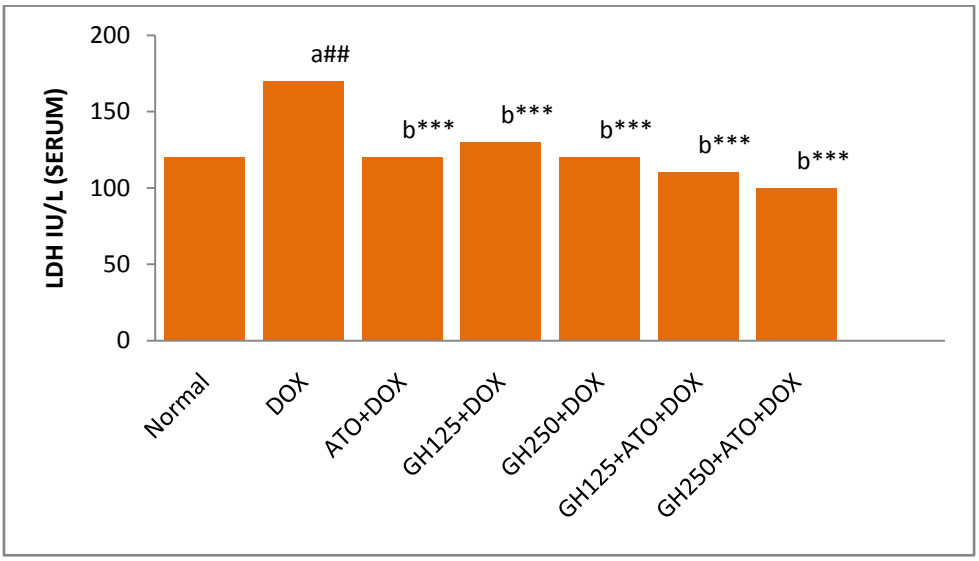

Fig. 8: Serum LDH activity in rats

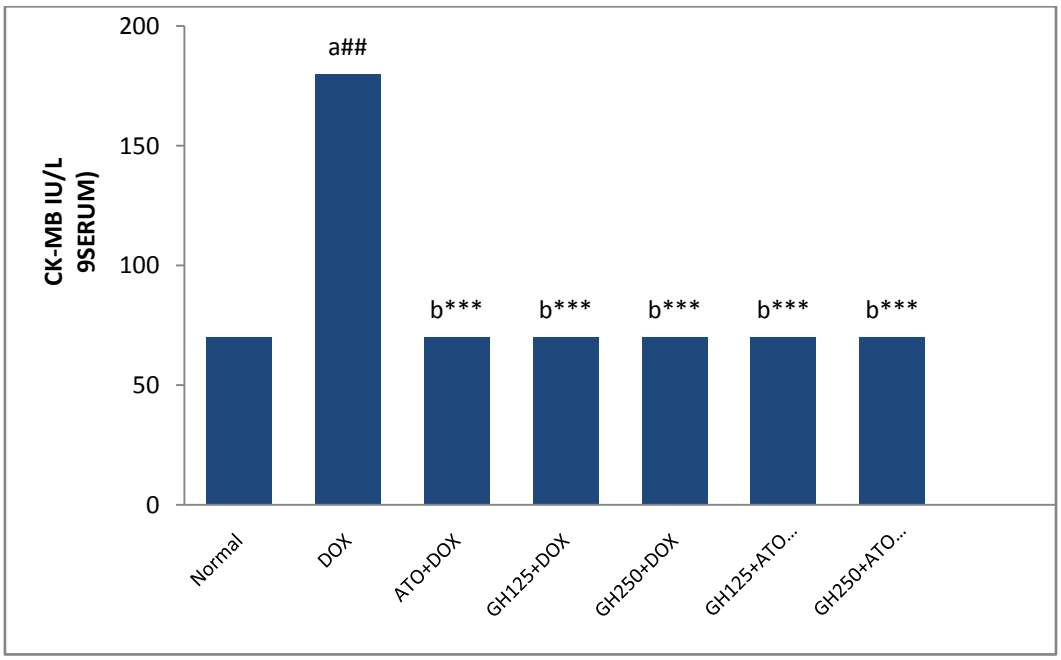

Fig. 9: Serum CK-MB activity in rats

Table 3: Effect of GH and ATO individually and in combination on heart tissue homogenate LDH and CH-MB activities in rats during myocardial damage induced by DOX

Group No.	Treatment	LDH activity(IU/L)	CK-MB activity
I	Normal	96.9±0.757	97.04±0.619
II	DOX control	47.48±0.379$^{\#\#}$	48.31±0.305$^{\#\#\#}$
III	ATO(60mg/kg)	88.78±0.228***	92.01±0.392***
IV	GH(125mg/kg)	84.79±0.233**	87.94±0.388**
V	GH(250mg/kg)	86.78±0.235***	89.83±0.412***
VI	GH-125+ATO	90.75±0.219***	93.01±0.391***
VII	GH-250+ATO	93.03±0.072***	96.17±0.253***

All values are mean ± SEM; n=5 in each group,$^{\#}$ P<0.05, $^{\#\#}$P<0.01, $^{\#\#}$P<0.001 when compared to normal. *P<0.05, **P<0.01, ***P<0.001 when compared to control.

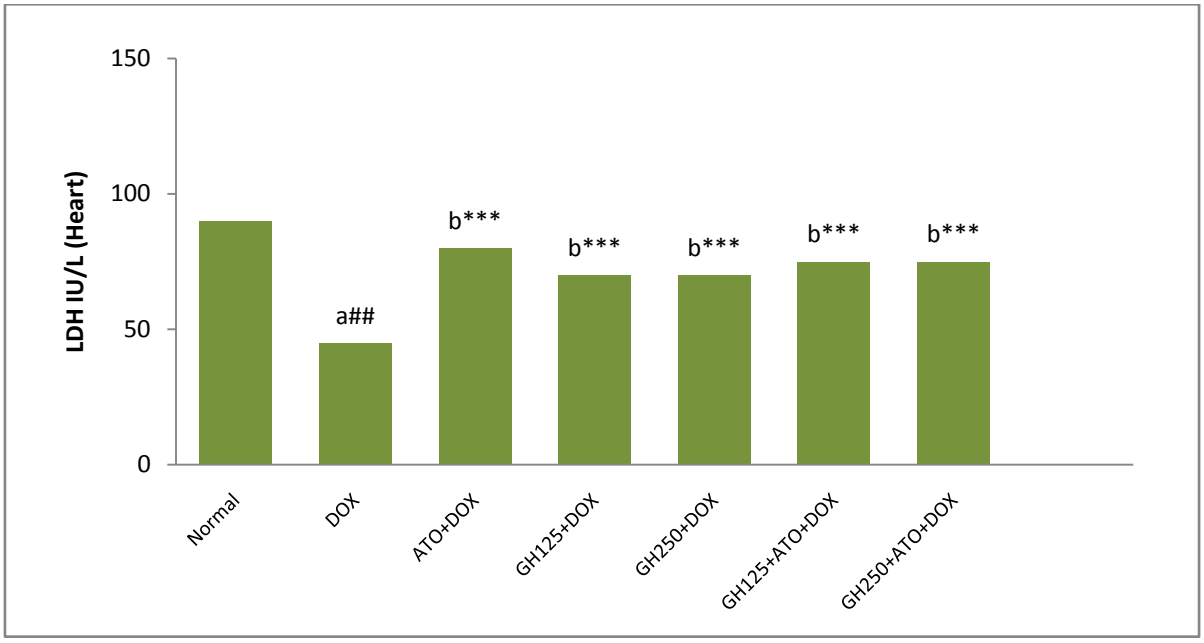

Fig. 10: LDH from heart homogenate activity in rats

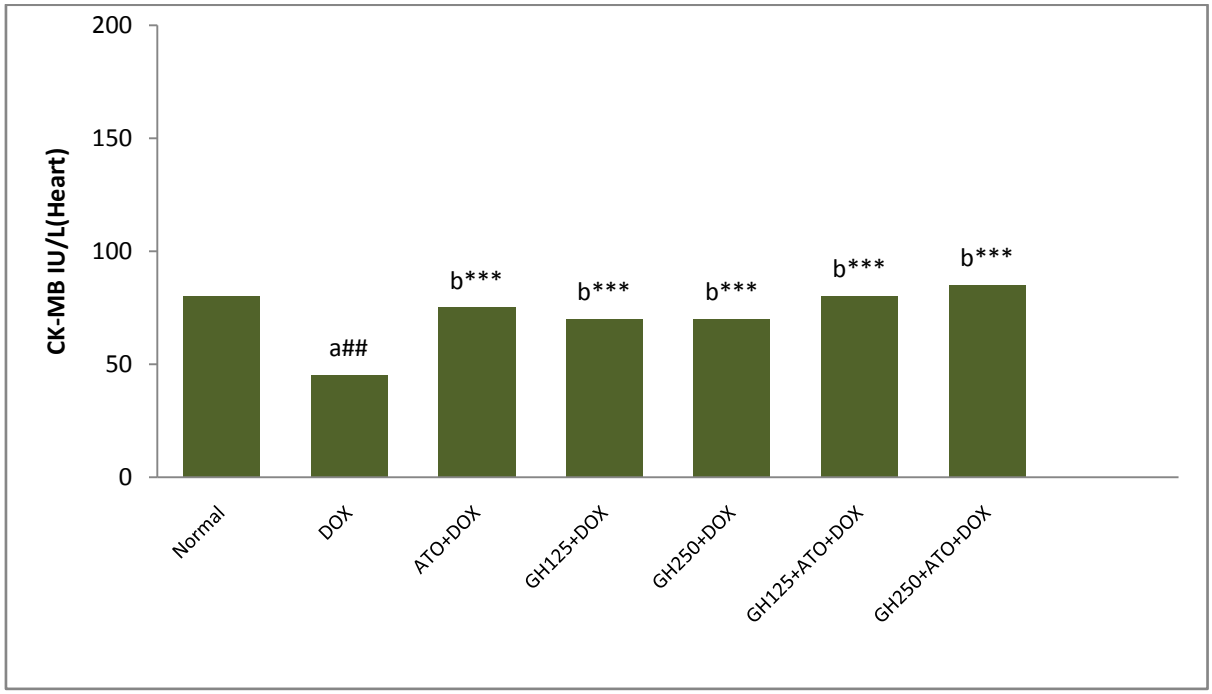

Fig. 11: CK-MB from heart homogenate activity in rats

RESULTS

Doxorubicin administration causes a significant increase in myocardial TBARS level,(P<0.001) when compared to that of normal group. The pre-treatment and concurrent treatment with ATO, GH-125,GH-250,GH-125+ATO,GH-250+ATO significantly reduced this effect (P<0.001) compared to DOX treated group. The level of endogenous antioxidant (GSH, SOD, CAT) was decreased significantly in DOX treated group compared with normal group. Moreover pre and concurrent treatment of GH 125+ATO, GH-250+ATO significantly reduced this effect than ATO,GH-125,GH-250 when treated alone compared to DOX group.

Table 4: Effect of GH and ATO individually and in combination on heart tissue homogenate SOD,TBARS, GSH and CAT activities in rats during myocardial damage induced by DOX

Group No.	Treatment	SOD activity (u/mg)	TBARS activity (moles/g)	GSH(µmol/g wet tissue)	CAT(U/mg protein)
1	Normal	7.27±0.088	25.07±0.085	973.2±0.744	59.79±0.172
2	DOX control	2.51±0.172$^{\#\#}$	48.92±0.217$^{\#\#\#}$	552.3±0.412$^{\#\#\#}$	927.4±0.546***
3	ATO(60mg/kg)	6.73±0.128***	30.12±0.207***	927.4±0.546***	51.14±0.156***
4	GH(125mg/kg)	4.67±0.178**	33.42±0.089**	909.4±0.361***	46.37±0.149***
5	GH(250mg/kg)	5.89±0.314***	32.88±0.097***	919.2±0.368***	50.12±0.132***
6	GH-125+ATO	6.41±0.133***	29.68±0.172***	936.8±0.208***	52.73±0.126***
7	GH-250+ATO	8.76±0.143***	26.28±0.181***	955.4±0.698***	56.98±0.097***

All values are mean ± SEM; n=5 in each group,$^{\#}$ P<0.05, $^{\#\#}$P<0.01, $^{\#\#}$P<0.001 when compared to normal. *P<0.05, **P<0.05, **P<0.01, ***P<0.001 when compare to control.

Fig. 12: SOD activity

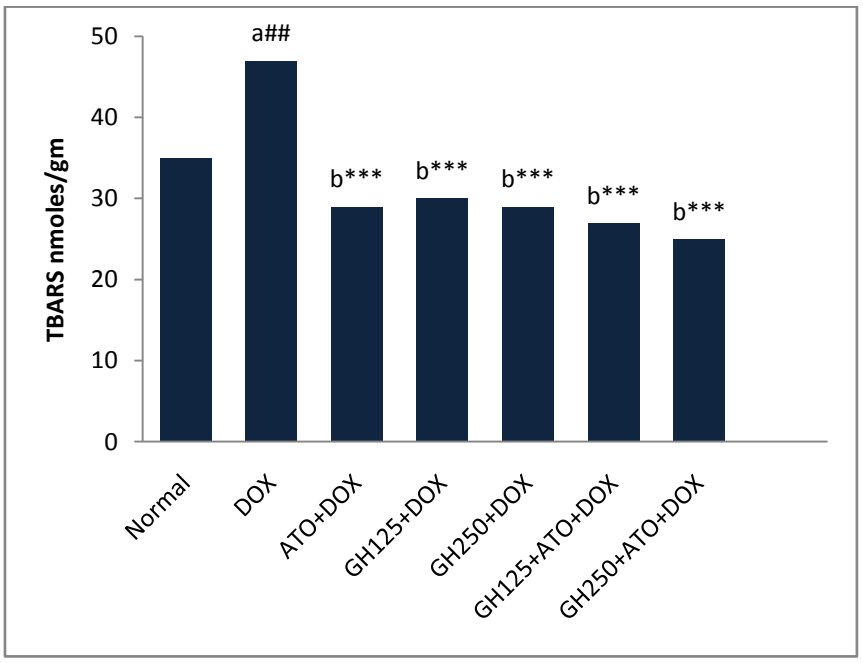

Fig. 13: TBARS level

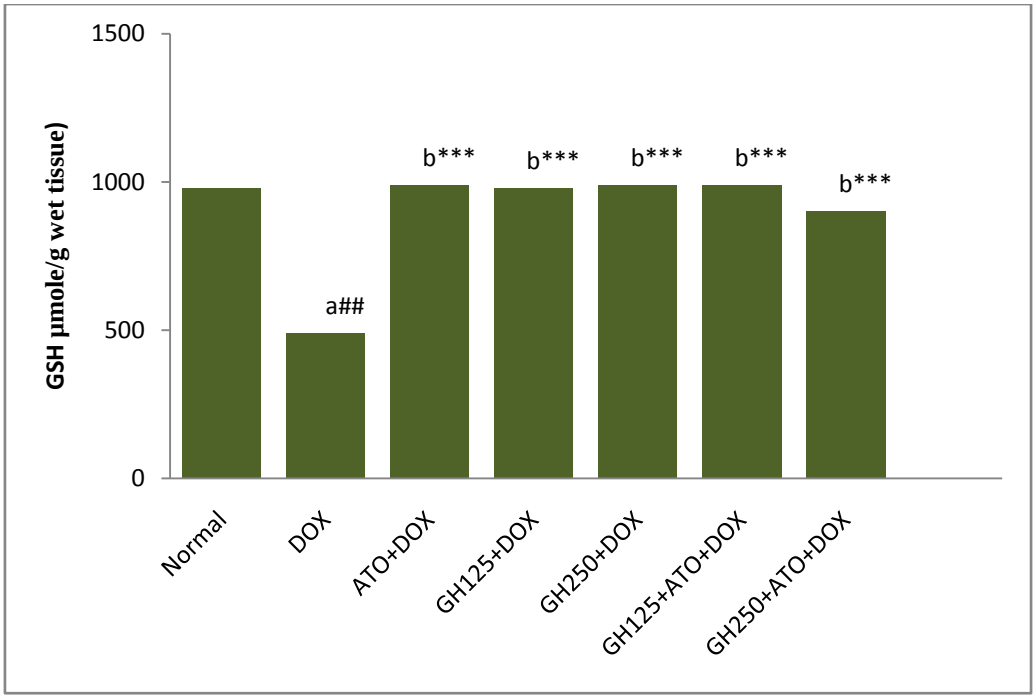

Fig. 14: GSH level

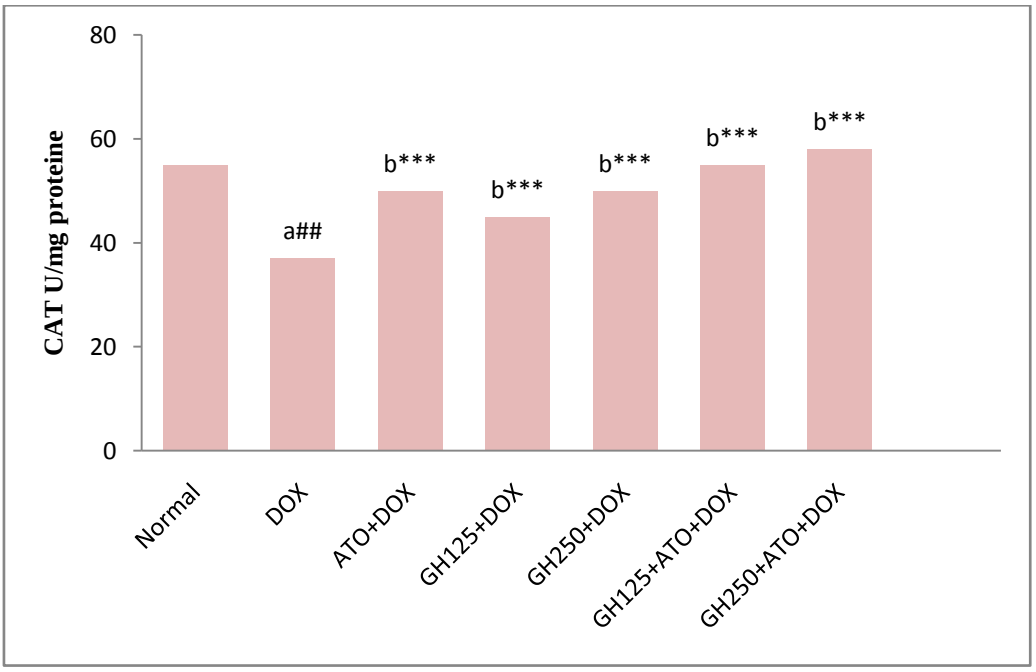

Fig. 15: CAT level

Histopathological examination of cardiac tissue

Normal control cardiac muscle showed normal characteristic features of myocardium without cellular infilteration and normal vasculature. It is depicted clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils.

Rats administered DOX showed typical myocardial toxicity in a form of myocardial muscle coagulative necrosis with focal areas of fibrosis, vascular dilation and congestion, valves edema and massive mononuclear cellular infiltration. The nuclei of myofibril revealed pyknotic nucleus. Interstitial edema was present in the connective tissue spaces.

In animals pre-treated with GH-125mg/kg alone and along with ATO, histological examination of tissue depicted restoration of normal structure with less interstitial space and less integrity of cardiac muscles. In animals pre-treated with GH 250mg/kg alone and along with ATO, the morphology of myocardium was almost similar to that observed in normal animals. The histological examination of tissues of animals pre-treated with both GH-250mg/kg and ATO 60mg/kg depicted clear integrity of myocardial

cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils.

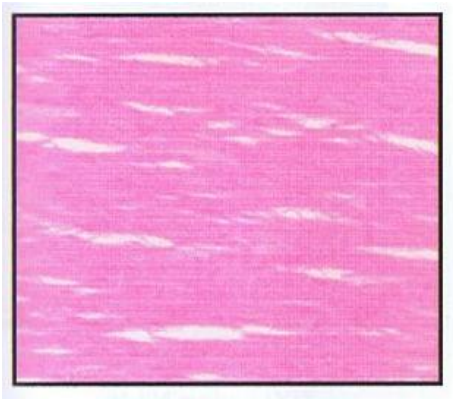

Fig. 16: Normal

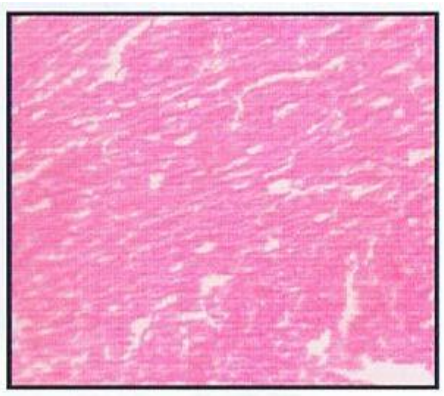

Fig. 17: DOX(control)

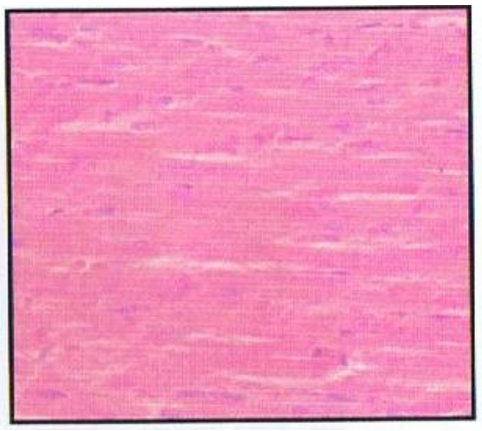

Fig. 18: Atorvastatin

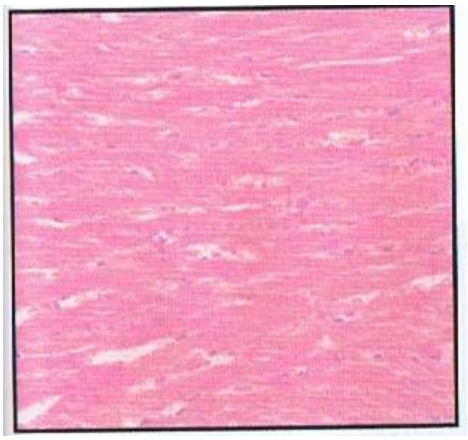

Fig. 19: GH-125mg/kg + DOX

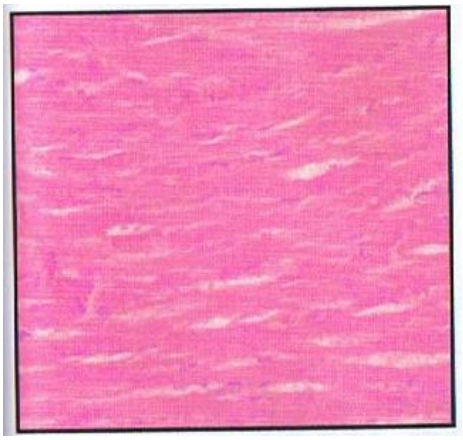

Fig. 20: GH 250mg/kg + DOX

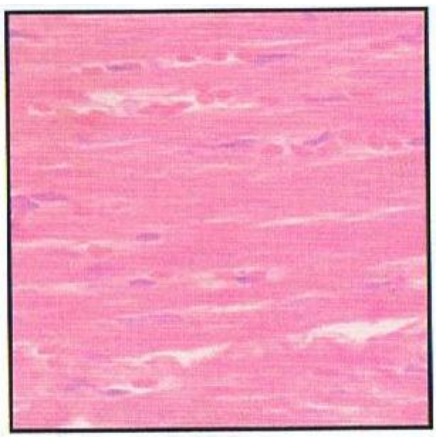

Fig. 21: GH 125mg/kg + ATO + DOX

DISCUSSION

Doxorubicin was converted in to its semiquinone form, which is a toxic, short lived metabolite that reacts with molecular oxygen initiating a cascade of reaction leading to ROS(reactive oxygen species) generation [10]. ROS reacts with lipids, proteins and other cellular constituents causing damage to mitochondria and cell membranes of the heart muscle cells [11][12]. 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 and S-allylmercaptocysteine, which have potent antioxidant activity[13]

In the present study there was a significant alteration in ST and QT intervals and heart rate in GH-250+ATO and GH-125+ATO combination groups when compared to GH-250 alone when compared to DOX control group. It is well known that LDH and CK-MB are diagnostic marker enzymes of myocardial damage. 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 these marker enzymes in HTH and increase in activities in serum in doxorubicin induced myocardial damage. Oral pre-treatment with GH-125 and GH-250

with or without ATO restored the activities of these enzymes to near normal in both heart and the serum

The antracycline drug reduced significantly the cardiac GSH content, besides it notably lowered the cardiac enzymatic activities of SOD and CAT associated with a marked increase in cardiac lipid peroxiadtion as manifested by increased TBARS level. Moreover pre and concurrent treatment of GH-125, GH-250 when treated alone compared to the DOX group

CONCLUSION

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 HMGCoA inhibitors such as Atorvastatin. This effect might be due to augmentation of endogenous antioxidant enzyme synthesis. Therefore diet containing moderate doses of garlic could provide beneficial effect to the heart and administration of garlic with atorvastatin produced additive effect at moderate doses

ACKNOWLEDGMENT

We are very thankful to Dr. K. S. Lakshmi, Dean, SRM College of Pharmacy, SRM University for her valuable support and encouragement during the project work.

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