MedCrave Step into the World of Research
International Journal of Complementary & Alternative Medicine

Research Article

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Synergistic potential of fresh garlic on atenolol mediated attenuation of ischemia-reperfusion induced myocardial damage in rats

Abstract

This research was done to explore the role of fresh garlic homogenate (GH) on attenuation of myocardial damage when administered concurrently with atenolol (ATN) in experimental animals. At the end of treatment of male albino rats, hearts were excised from anesthetized animals and mounted on modified Langendorff setup. After initial perfusion with physiological solution, hearts were subjected to 15 min global no flow ischemia reperfusion injury (IRI) and perfused again. Significant recovery in developed tension and heart rate was exhibited by hearts of animals pretreated with ATN, GH-125 and GH-250. There was significant elevation of LDH and CK-MB in heart tissue homogenate and fall in perfusate of treated groups (ATN, GH-125 and GH-250). Additionally, simultaneous administration of GH-250 with ATN resulted in significant increase in endogenous antioxidant enzymes (SOD and catalase) activities. The augmented antioxidant activities could be due to the possible synergistic potential of ATN in GH mediated scavenging of oxidative free radicals produced during IRI. On the contrary, high dose of GH (500 mg/kg) failed to demonstrate similar potential even in presence of ATN. Hence, only moderate intake of garlic is good for cardioprotection when administered prophylactically and addition of ATN produces synergistic effect.

Keywords: garlic, interaction, ischemia-reperfusion, isolated heart, atenolol

Volume 13 Issue 1 - 2020

Syed Mohammed Basheeruddin Asdaq,[1] Majed Alshammari[2]

[1]Associate Professor of Pharmacology, Al Maarefa University, Saudi Arabia

[2]King Abdulaziz Medical City, Ministry of National Guard, Saudi Arabia

Correspondence: Dr. Syed Mohammed Basheeruddin Asdaq, College of Pharmacy, Al-Maarefa University, Dariyah, Riyadh-11597, Saudi Arabia, Fax 00966-4903501-3399, Email sasdaq@gmail.com

Received: January 24, 2020 | **Published:** February 13, 2020

Abbreviations: GH, garlic homogenate; ATN, atenolol; IRI, ischemia reperfusion injury; LDH, Lactate dehydrogenase; SOD, superoxide dismutase; HTH, heart tissue homogenate; VFITS, volume fraction of interstitial space; H&E, hematoxylin and eosin; SAMC, S-allylmercaptocys-teine

Introduction

Concurrent use of herbs and conventional drugs may either increase or decrease the pharmacological benefits of each other1. Undoubtedly, several herbal therapies carry potential for alleviating diseases and illness, but therapeutic efficiency of most of them does not have any validation through standard procedures. Additionally, use of these therapies are also found along with conventional drugs that may sometime benefit the patient and even sometime it may harm them. Therefore, it is necessary to do in-depth analysis of simultaneous use of the most commonly used herbs with routinely prescribed conventional therapies. One of the common ingredient of house hold and traditional remedy for treatment and prevention of cardiovascular manifestation is garlic.[2] Different types of garlic preparation are reported to have therapeutic benefit in prevention, treatment and progression of diseases such as atherosclerosis, arrhythmia, hyperlipidemia, thrombosis, hypertension and diabetes.[3] Fresh garlic homogenate was recognized as cardioprotective,[4] antioxidant,[5] antineoplastic and antimicrobial agent.[6] Antiarrhythmic potential of garlic was found both in ventricular and supraventricular arrhythmias.[7] Chronic administration of garlic was reported to cause enhancement of endogenous antioxidants activities and fall in oxidants level through either triggering the generation of endogenous antioxidants or obviating the formation of oxidative free radicals.[8] Moreover, garlic was found to produce anti-oxidant effect in isoproterenol-induced myocardial damage in experimental rat.[9] Fresh garlic homogenate was exhibited earlier to produce dose dependent negative inotropic effect in isolated rabbit heart.[10] Interaction studies of garlic showed its synergistic potential on the cardioprotective properties of calcium channel blockers.[11] Augmentation of therapeutic potential of captopril and hydrochlorothiazide by garlic was demonstrated earlier.[12,13] Furthermore, efficacy of garlic in potentiating cardiopropective action of non-selective beta blocker, propranolol.[14] in animal experimental models was also exhibited in published literature. However, no study was found on elucidating the role of garlic in presence of selective beta blocker, atenolol. The use of selective beta blockers is more common in patients with cardiovascular risk than non-selective blockers due to its lower risk of bronchoconstriction. Therefore, it was our interest to explore their role if given along with garlic homogenate in animals with IRI damage.

Materials and methods

Chemicals

Chemicals used in this study were of analytical grades and procured from standard companies. All biochemical kits for estimating LDH and CK-MB were obtained from Crest Biosystems, Coral Clinical Systems, Goa- 403 722, India).

Preparation of extract

The garlic (Allium sativum) bulbs were bought from local vendors. After peeling the cloves, they were sliced and grinded to form a paste and suspended in distilled water. Three different concentrations of the garlic homogenate were prepared corresponding to 125mg/kg, 250mg/kg and 500mg/kg body weight.[3] The homogenate of garlic was administered to animals within 30minute of its preparation.

Experimental animals

All animal experiments were carried out in accordance and approval of institutional ethical committee of the college of Pharmacy. Laboratory bred male Wistar albino rats weighing between 200-250g

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 and water ad libitum. No significant variation was noted in the body weight of rats who received GH or ATN treatment when compared with control, both at the beginning or at the end of the study period.

Experimental protocol

The animals of this study were categorized into eight groups (eight animals in each group). Group I received distilled water oral treatment for 30days; group II were given GH (125mg/kg) for 30days; group III were administered GH (250mg/kg) for 30 days; Group IV animals were treated with GH (500mg/kg) for 30days; Group V animals received GH (125mg/kg) for 30days and atenolol (6mg/kg)15 for last seven days of treatment; Group VI rats were given GH (250mg/kg) for 30days and atenolol (6mg/kg) for last seven days; Group VII animals were administered GH (500mg/kg) for 30days and atenolol (6mg/kg) for the last seven days and finally Group VIII rats received atenolol (6mg/kg) for last seven days and distilled water treatment for all 30days.

Experimental procedure

Modified Langendorff apparatus was developed and used in this study as per the specification outlines in earlier research16. The heart was excised from anesthetized [ketamine (70mg/kg, i.p) and xylazine (10mg/kg, i.p)] rats two hours after the last day treatment. The isolated heart was perfused with Kreb-Henseleit solution gassed with carbogen (95% O2 and 5% CO2) at 37°C at a constant flow rate of 5ml/min. The composition of K-H solution was (mM) NaCl 118, KCl 4.7, NaHCO3 25, NaHPO4 1.0, MgSO4.7H2O 0.57, CaCl2 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 mounted hearts were allowed to stabilize for around 10min and developed tension and heart rate was recorded under normal perfusion for a period of 15minutes. Measurement of contractile force was done using force displacement transducer and recorded on a Student Physiograph (INCO, Mumbai, India). Further, at the end of preischemic perfusion of 1minutes, heart was subjected to 15min of global no-flow ischemia.[15–17] by blocking the flow of K-H solution & carbogen supply followed by 15min 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)[18] and the activity of LDH, CK-MB, superoxide dismutase (SOD)[19] and catalase[20] was determined. Microscopic slides of myocardium were prepared for studying volume fraction of interstitial space (VFITS) in myocardial tissue after staining with hematoxylin and eosin (H&E) stain by using the equation.[21]

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

Statistical analysis

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

Results

Effect on LDH & CK-MB levels

There was a significant increase in LDH and CK-MB enzymes activities during pre-ischemia in hearts of animals pretreated with GH 500mg/kg. However, significant fall in LDH and CK-MB activities were noted in perfusate of animals pretreated with GH 125mg/kg, GH 250mg/kg, GH 125mg/kg + ATN, GH 250mg/kg + ATN and ATN. In post-ischemic perfusate, significant decline in enzyme activity was observed with GH 250mg/kg group as well as in groups which received GH-125, GH-250 along with ATN when compared to control (Table 1). There was no significant impact of GH (500mg/kg) was observed on activities of these enzymes in heart tissue homogenate (HTH), whereas, both GH-125 and GH-250 treatment produced significantly increased activities of enzymes both in presence and absence of ATN, when compared to control (Table 2).

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
Control	200.21±7.42	454.75±11.89	22.21±0.49	48.16±1.02
GH-125	198.99±4.15	324.66±5.68***	19.11±0.65**	37.81±0.35***
GH-250	158.51±3.63***	310.49 ± 3.32***	14.65±0.45***	28.58 ± 0.85***
GH-500	385.81±8.55***	488.70±19.59	26.06±0.56***	41.48±0.85***
GH-125+ ATN	164.34±1.27***	312.75±9.86***	11.64±0.26***	31.40±0.55***
GH-250+ ATN	155.46±1.35***	261.33±8.13***	09.48±0.19***	23.64±0.24***
GH-500+ ATN	320.33±3.91***	412.16 ± 6.60	23.69±0.27***	36.93 ± 0.64***
ATN	154.22±4.69***	284.24±7.15***	12.23±0.23***	24.40±0.74***

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

Statistical analysis

One-way ANOVA followed by Tukey multiple comparisons test

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

Effect on SOD and catalase activity

The activities of SOD and catalase enzymes were significantly increased in heart tissue homogenate of animals treated with GH 250mg/kg and GH 125mg/kg when administered individually or concurrently with ATN. However, no significant change was noticed in groups administered GH (500mg/kg) in these parameters. Moreover, addition of ATN to GH 500mg/kg group did not produce any significant increase in these antioxidant activities (Table 2).

Developed tension and heart rate

Prior treatment of animals with GH 250mg/kg and ATN resulted in significant recovery in developed tension and heart rate of ischemic heart during reperfusion. Concurrent administration of GH 250mg and ATN has caused further elevation in percentage recovery in the above parameters. Further, when compared to ATN, GH 250mg/kg produced better recovery from IRI damage. Also, GH 500mg/kg alone did not produce any significant change in both the above parameters, but significantly improved recovery from ischemic damage was noticed when GH 500 mg/kg was given together with ATN (Table 3).

Percentage total tissue space

Pretreatment of animals with GH 125mg/kg and 250mg/kg showed significantly decreased VFITS score. Further, microscopic evaluation of slides of heart tissues of animals treated with GH 125mg/kg and 250mg/kg demonstrated further fall in the VFITS score when compared to control. Moreover, administration of ATN alone has shown modest recovery which further increased when ATN was given along with GH 125mg/kg and GH 250mg/kg. However, high dose of GH (500mg/kg) did not produce any significant change in this value but a significant reduction was observed in groups treated with both GH 500mg/kg and ATN together (Table 3).

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

Treatment	LDH (U/L)	CK-MB (U/L)	SOD (Units/mg protein)	Catalase (Units/mg protein)
Control	656.92±35.12	48.76±0.97	1.67±0.00	2.10±0.05
GH-125	782.42±26.12	62.82±0.44	3.22±0.04***	4.32±0.10***
GH-250	822.32±4.42***	65.83±0.85***	4.43±0.04***	5.44±0.17***
GH-500	587.33±14.39	52.55±1.11*	1.86±0.00	2.25±0.06
GH-125+ATN	887.54±8.51*	61.96±1.29***	4.98±0.05***	6.77±0.12***
GH-250+ATN	998.82±21.35***	84.25±0.97***	6.76±0.12***	8.69±0.18***
GH-500+ATN	686.41±12.34	49.77±0.20	2.11±0.01	2.21±0.09
ATN	843.22±22.11***	67.06±0.52***	3.55±0.03***	6.44±0.18***

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

Statistical analysis

One-way ANOVA followed by Tukey multiple comparisons test

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

SOD Units

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

Catalase units

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

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

Treatment	Percentage Recovery		VFITS[1]
	Developed Tension	Heart Rate	
Control	24.27±4.17	35.72±2.23	38.03±1.71
GH-125	53.068.87	49.29±2.16*	26.23±0.69***
GH-250	63.029.23***	69.04±3.14***	20.24±0.93***
GH-500	31.686.22	36.04±1.71	32.49±0.54
GH-125+ATN	58.44±5.94*	69.46±1.37***	23.39±0.60***
GH-250+ATN	78.44±1.25***	87.42±1.32***	22.44±1.30***
GH-500+ATN	37.72±4.78	46.88±1.92	29.49±0.50*
ATN	54.986.70***	48.66±1.63*	25.79±1.63***

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

Statistical analysis

One-way ANOVA followed by Tukey multiple comparisons test

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

[1]VFITS =100X Area of Interstitial space/Total tissue area

Citation: Asdaq SMB, Alshammari M. Synergistic potential of fresh garlic on atenolol mediated attenuation of ischemia-reperfusion induced myocardial damage in rats. *Int J Complement Alt Med.* 2020;13(1):36–40. DOI: 10.15406/ijcam.2020.13.00490

Discussion

This research was done to explore the role of different doses of garlic homogenate (GH) in presence and absence of atenolol (ATN). The outcome of the research demonstrate that the high dose of GH (500mg/kg) aggravates the ischemia reperfusion injury (IRI) as exhibited by increase in levels of biological markers of myocardial infarction, the LDH and CK-MB activities in the perfusate and a decrease in the activities of the antioxidant enzymes; SOD and catalase along with a decrease in activities of biological markers in the heart tissue homogenate. The moderate dose of GH (250mg/kg) and the low dose of GH (125mg/kg) reduced the IRI as shown by changes in the activities of biological markers and antioxidant enzymes. Further, administration of moderate dose of GH (250mg/kg) and low dose of GH (125mg/kg) along with atenolol, a known cardioprotective agent, produced synergistic effect. The doses of garlic homogenate selected for this study were reported by published literature as safe and effective in animal models.[3] The cardioprotective potential of garlic homogenate is attributed to the presence of its active organosulfur antioxidant metabolites, S-allylcysteine (SAC) and S-allylmercaptocys-teine (SAMC).[22–24] Allicin (allyl 2-propenethiosulfinate) was initially referred as a bioactive molecule of garlic attributed for its cardioprotective potential of garlic. But, recent published data claimed the unstable and transient nature of allicin[25] and its rapid decomposition into active organosulfur compounds.[26] Since bioactive organosulfur compounds (SAC and SAMC) are virtually responsible for therapeutic potential of garlic and it is formed from conversion of allicin in presence of biological catalyst, garlic homogenate was administered to the live animals instead of its direct injection to the isolated heart or adding it to the perfusing fluid.

The biomarkers for myocardial damage estimated in this study were CK-MB and LDH27. The enzyme markers are suggested to be better markers than others as they are more specific.[28] Fall in activities of these enzymes in heart tissue homogenate indicates myocardial damage due to leakage from injured myocardial cells. Administration of GH in moderate dose of GH (250mg/kg) has caused remarkable increase in the enzyme activities in HTH indicating myocardial protection from IRI damage. In groups treated with ATN and GH (250mg/kg), the integrity of myocardium was higher as evident from further increase in these markers in HTH.

The oxygen free radicals such as superoxide and hydrogen peroxide are usually generated due to IRI damage. The antioxidant (SOD and catalase) activities are also bound to get decreased during IRI damage.[29] Animals treated with GH (125mg/kg & 250mg/kg) individually or in combination with ATN produced remarkable elevation in SOD and catalase level that could be one of the reasons for therapeutic efficacy of GH. Similar results were not found with high dose of GH (500mg/kg). The antioxidant potential of moderate dose of GH (250mg/kg) was augmented in presence of ATN. However, no significant impact of ATN was found in groups with high dose of GH (500mg/kg).

The recovery from myocardial damage caused by IRI was also measured by inotropic (developed tension) and chronotropic (heart rate) recordings of heart during reperfusion.[14] The beta blockers are known to reduce the myocardial oxygen requirement and hence increase the stress tolerance during post myocardial infarction phase.[30] The ability of ATN in tolerating the IRI induced stress was evident in our study with an increase recovery in this parameters in all groups treated with ATN, even in a group that was treated with high dose of GH (500mg/kg).

Conclusion

The outcome of this research emphasized the role of garlic in alleviation damage to myocardium when administered in low to moderate dose. Addition of beta blocker, such as ATN, has significant synergistic potential in augmenting the myocardial protection caused by GH. However, oxidative damage was observed in animals treated with high dose of GH (500mg/kg). Hence, only moderate intake of garlic is good for cardioprotection when administered prophylactically and addition of ATN produces synergistic effect.

Acknowledgments

None.

Conflicts of interest

Author declares that there are no conflicts of interest towards publication.

Funding

None.

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