Effect of Fresh Garlic Homogenate (FGH) on Rat Heart after Exposure to Hot Water Immersion Stress

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ABSTRACT: The present study aims to investigate the possible cardioprotective role of garlic on adult male Sprague-Dawely albino rats after stress exposure. Animals were exposed to stress by immersion in hot water for one day, one week and three weeks of stress. The myocardial activity was evaluated by estimating ECG, heart to body weight ratio, serum creatine phosphokinase (CPK-MB) activity, antioxidant enzymes activities, as well as histopathological alterations in cardiac muscle. Garlic was administered to the animals at a dosage of 250 mg/kg b.w. Exposure for one day stress was of weak effect, while one week and three weeks of stress exposure were nearly of the same effect, causing significant changes in ECG, cardiac hypertrophy, elevation in serum CPK (MB) activity, significant decrease in the antioxidant enzyme activities and increase in MDA content, and significant alterations in histopathological analysis when compared with control. Supplementation of garlic (250 mg/kg b.w.) orally for one day stress exposed and one week stress exposed animals could not produce any significant change when compared to untreated stress exposed animals. However, after 28 days aqueous garlic homogenate supplementation has significantly improved ECG, decreased cardiac hypertrophy, decreased serum CPK (MB) activity, increased all the antioxidant enzyme activities and decreased MDA content as well as improved the myocardial histopathology compared the three weeks stress exposed group. The results indicated that stress exposure affects the myocardial function through direct effect: on the heart muscle, leading to arrhythmia and hypertrophy, and indirect effect: by generating free radicals and oxidative stress.

Key words: Garlic, Stress, Reactive oxygen species (ROS), Antioxidant.

INTRODUCTION:
Stress is considered as an adaptive physiological response to disruption of homeostasis. Moderate stress load causes protection, while stress overload can cause injury or contribute to diseases, such as diabetes, gastric ulcer, obesity, cancer, and Parkinson's disease. There is a relationship between stress and the risk of cardiovascular diseases as high blood pressure, high cholesterol, and other cardiac risk factors (Yun et al., 2007). Most stressful stimuli cause the release of corticotropin-releasing hormone (CRH) from neurons in the brain through activation of the hypothalamic-pituitary adrenal (HPA) axis. The increased release of CRH from the hypothalamic paraventricular nucleus causes the secretion of Adrenocorticotropic (ACTH) from anterior pituitary, which inturn stimulates the secretion of glucocorticoids from the adrenal cortex (Veniaki et al., 1997). Glucocorticoids possess broad spectrum of actions affecting expression and regulation of genes throughout the body (Levine, 2005). Increased glucocorticoids is associated with cardiovascular risk factors such as high blood pressure, blood glucose and triglyceride levels, and low high density lipoprotein cholesterol levels (Fardet et al., 2007) and many forms of lipodystrophy are associated with metabolic disorders and premature atherosclerosis (Guaraldi et al., 2010). Emotional and physical stresses have a negative impact on the heart and the vascular system. Stress can cause increased oxygen demand on the body, spasm of the coronary blood vessels, and electrical instability in the heart's conduction system. Chronic stress has been shown to increase the heart rate and blood pressure, making the heart work harder to produce the blood flow needed for bodily functions (Janet, 2007). Scientists reported that psychosocial stress accounted for approximately 30% of the risk of acute myocardial infarction (Das & O’Keefe, 2008). They indicated that hostility, depression, and anxiety are all related to increased risk of coronary heart disease and cardiovascular death. Since cardiovascular diseases are the leading cause of mortality and morbidity in the modern world (Jialal & Devaraj, 1996), it is necessary to understand the role of stress on the heart and a possible antidote. It has also been proved that antioxidants decrease cellular oxidation, thereby reducing the damaging effects of stress exposure (Quirk et al., 1994).

Garlic (Allium sativum) is used in herbal medicine for thousands of years (Ramaa et al., 2006). The Egyptians cited 3500 years before, the usage of garlic for the treatment of heart diseases, tumors, bites and worm infections (Rahman, 2001). Garlic contains 0.1-0.36% of volatile oil consisting of sulfur-containing compounds: allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), etc. These volatile compounds are
generally considered to be responsible for most of the pharmacological properties of garlic. The biological effects of additional constituents of intact garlic are due to lectins, proteolipidins, fructan, pectin, adenosine, vitamins B₁, B₂, B₆, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids which have been studied for over several decades (Fenwick & Hanley, 1985). There are several reports supporting the pharmacological importance of garlic for hypoglycemic, hypolipidemic, antimiobacterial, antitumor, antioxidant and anti-atherosclerotic effects (Banerjee & Maulik, 2002, Alder et al., 2003 and Elkayam et al., 2003). Most studies on garlic during the past 15 years have been primarily in the cardiovascular field and have been mainly related to atherosclerosis, where effects of different garlic preparations have been examined on serum cholesterol and oxidative stress (Zhang et al., 2001 and Ramaa et al., 2006). The present study investigates whether exposure for one day, one week and three weeks of stress exposure deteriorates the heart function, and whether garlic supplementation could ameliorate such harmful effect the heart function.

MATERIALS AND METHODS:

Animals: Adult male Sprague-Dawley rats (150-180g) were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. Animals were kept under standard laboratory animal housing conditions and allowed free access to food and tap water. All animals were treated daily at fixed time as described in the experimental groups.

Preparation of the Fresh Garlic Homogenate (FGH): Garlic was purchased from local market. The bulbs were peeled and cut into small pieces and homogenized with double distilled water in a motor driven Teflon-glass homogenizer on ice. A concentration of 250 mg/kg was administered orally to the rats (Sumanta et al., 2003) within 30 min of its preparation. Garlic was given for 7 days before stress induction (Stress), 4 days before one week and 3 weeks hot stress. The 4th gp animals were exposed to stress one day, one week, 3 weeks along with FGH treatment for 14, 28 consecutive days.

Stress Procedure: Rats were immersed in a tube that contained 4 to 5 inches of hot water (50 °C). The 4 to 5 inch water level allows the rats to just touch the bottom and keep their noses above the water. At the first sign of fatigue (inability to maintain head out of water), the rats were promptly removed from the tube. (Jacob & Kazem, 1973).

At the last day of stress according to each duration, the Electrocardiography (ECG) was measured according to the method describe by Papich & Riviere (2009) using Multimedia biofeedback Biograph infinity SA7900PD Version 1.1.2 apparatus (Thought Technology Ltd., Canada). Blood samples were withdrawn from the retro-orbital plexus of each rat (Cocchette & Bjoransson, 1983) for estimation of creatine-phospho kinase (CPKMB). Animals were weighed and killed by dislocation. Hearts were rapidly excised, washed in ice-cold saline, blotted dry in filter paper, weighed and divided into two halves; the first half was fixed in 10% neutral buffered formalin and processed for histological analysis, while the other half was homogenized in 10% (w/v) 0.1 M tris buffer (pH 7.4) and centrifuged at 10,000 xg for 30 min at 4°C, the clear supernatant was kept at -80°C for the determination of: Serum CPK (MB) was analyzed according to Bais & Edwards (1982) its absorbance was read at 340 nm. Myocardial lipid peroxidation (MDA) was carried out according to Uchiyama & Mihara (1978), its colour absorbance was measured at 535 nm and 520 nm against a reagent blank. Myocardial glutathione content (GSH) was assayed according to Ellman (1959), its optical density was measured at 412 nm against a reagent blank. Myocardial glutathione-S-transferase (GST) activity, was assayed by using the method of Habig et al. (1974), its color absorbance was observed by continuous recording at 340 nm at 1 min intervals for 3 min, the data were expressed as nmol/min/mg protein. Myocardial catalase (CAT) was carried out according to the method of Clairborne (1985), the changes in absorbance were followed for 2 min. at 240 nm at an interval of 1 min. Myocardial superoxide dismutase (SOD) was carried out using Pyrogallol method according to Marklund (1985), changes in the absorbance at 420 nm were recorded at 1 min interval for 3 min. Protein concentration was measured by Lowry method (Lowry et al., 1951). The mean ratio between heart and total body weight in each group of animals was then calculated. Heart samples were fixed in 10% formalin, processed and embedded in paraffin according to the standard histological techniques. 3 µm thick paraffin sections were cut through the heart (n=4). Sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS) reagent and examined by light microscopy (Broun, 1969).

Statistical Analysis: The data were expressed as mean ± SEM and were statistically analyzed at P<0.05 using ordinary one way Analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons. A computer program Graphpad Prism (version 5) was used to test the significance of the difference between groups.

RESULTS:

Electrocardiography (ECG): Figure (1) showed that exposure to one day stress caused no significant change in heart wave (QRS) interval, significant increase in the distance between two waves (R-R) interval and
significant decrease in the peak of heart wave (QRS) amplitude as compared to control. After treatment with FGH, QRS interval decreased, R-R interval increase and no change in QRS amplitude. One week stress exposure caused significant decrease in QRS interval and QRS amplitude and significant increase in R-R interval. On using FGH, only QRS amplitude was significantly increased when compared to stress exposure group. Three weeks stress exposure showed significant increase in QRS interval and R-R interval and significant decrease in QRS amplitude as compared to control group. Treatment with FGH significantly decreased QRS interval and increased QRS amplitude as compared to stress exposure group.

**Effect of FGH on biochemical parameters in rats exposed to different stress levels:** Figure (2) showed that, one day stress exposure didn’t cause significant change in CPK (MB) activity as compared to control group. One week stress exposure caused significant increase in serum CPK (MB) activity by 360.5% as compared to control group. Pretreatment with FGH caused significant decrease in CPK (MB) activity by 24.6% as compared to stress exposure group. Three weeks stress exposure caused significant increase in serum CPK (MB) activity by 504.6% as compared to control group. Pretreatment with FGH caused significant decrease by 60.4% as compared to stress exposure group. 

**Myocardial lipid peroxidation:** One day stress exposure didn’t cause significant change in MDA as compared to control group. One week stress exposure caused significant increase in MDA content by 43.0% as compared to control group. Upon pretreatment with FGH, there was no significant change as compared to stress exposure group. Three weeks stress exposure caused significant increase in MDA content by 59.7% as compared to control group. Pretreatment with FGH caused significant decrease by 36.0% as compared to stress exposure group.

**Myocardial GSH:** One day stress exposure caused significant decrease in GSH content by 14.0% as compared to control group. Pretreatment with FGH; it caused significant increase by 29.0% as compared to stress exposure group. One week stress caused significant increase in GSH content by 73.4% as compared to control group. Pretreatment with FGH caused significant increase by 48.0% as compared to stress exposure group. Three weeks stress exposure caused significant decrease in GSH content by 73.0% as compared to control group. Upon pretreatment with garlic, it caused significant increase by 142.9% as compared to stress exposure group. 

**Myocardial GST:** One day stress exposure didn’t cause significant change as compared to control group. One week stress exposure caused significant decrease in GST enzyme activity by 65.0% as compared to control group. Pretreatment with FGH caused significant increase in GST by 30.0% as compared to stress exposure group. Three weeks stress exposure caused significant decrease in GST enzyme activity by 72.8% as compared to control group. Upon pretreatment with FGH caused significant increase by 101.5% as compared to stress group. 

**Myocardial CAT:** One day stress exposure didn’t cause significant CAT change as compared to control group. One week stress exposure caused significant decrease in CAT activity by 78.5% as compared to control group. Whereas pretreatment with FGH, there was no significant change as compared to stress exposure group. Three weeks stress exposure caused significant decrease in CAT activity by 82.8% as compared to control group. Whereas pretreatment with FGH caused significant increase by 301.0% as compared to stress exposure group.

**Myocardial SOD:** One day stress exposure caused significant decrease in SOD activity by 17.5% as compared to control group. Upon pretreatment with FGH caused significant increase by 15.5% as compared to stress exposure group. One week stress caused significant decrease in SOD activity by 46.2% as compared to control group. Three weeks stress exposure caused significant decrease in SOD activity by 47.4% as compared to control group. Pretreatment with FGH caused significant increase by 82.9% as compared to stress exposure group.

**Effect of FGH on heart to body weight ratio in rats exposed to different stress levels:** As shown in figure (3); one day stress exposure didn’t cause significant change in heart/body weight ration. One week stress caused significant increase in heart to body weight ratio by 30.8% as compared to control group. Pretreatment with FGH; didn’t cause significant change in the heart/body weight ratio as compared to stress exposure group. Three weeks stress exposure caused significant increase in heart to body weight ratio by 40.9% as compared to control group. Pretreatment with garlic there was significant decrease in heart to body weight ratio by 24.5% as compared to stress exposure group.

**Histopathological examination:** As shown in Figure (4); one day stress exposure didn’t cause any histopathological changes in the rat myocardium. One week stress exposure caused myocardial necrosis, vacuoles and edema with inflammatory cells, while pretreatment with FGH there was lessened necrosis and edema. Three weeks stress exposure caused confluent focal necrosis of myofibrils, edema with infiltration of inflammatory cells and extravasations of red blood cells, while pretreatment with FGH, there was near normal myocardial histarchitecture with minimal edema and inflammation.

**DISCUSSION:**

In the present study, stress exposure caused myocardial injury manifested by marked alteration in ECG pattern. This injury was clear by histopathological examination of the heart, and elevation of serum CPK (MB). This comes in accordance with the work of Olga et al. (2002), Zhao et al. (2007) and Kumar et al. (2010). Also stress caused cardiac hypertrophy which is supported by Thiago et al. (2012), and caused oxidative stress as evidenced by increase in myocardial MDA and depletion of myocardial endogenous antioxidants such as SOD, CAT, GSH and GST. Similar observations were observed earlier by different other studies, using similar models (Mercanoglu et al., 2010).
The present study investigated the effect of changing duration of stress on rat myocardium to determine whether the responses could be related to time. It was observed that one day stress exposure is of weak detrimental effect on the heart. While one week and three weeks stress exposure have strong negative effect on the heart. This is supported by the study of Soldani et al. (1997) revealing that long duration of stress exposure causes more cardiac injury than short one. It was also observed in this study that, with the exception of CPKMB, the effects of both one week and three weeks stress exposure were nearly the same. This may be due to the development of adaptation of the hypothalamo-pituitary adrenal (HPA) axis on long term exposure. When the same stressor is repeated, the HPA response undergoes desensitization or become stable (Gadek-Michalska & Bugajski, 2003). The prophylactic treatment with garlic homogenate (250 mg/kg/day) caused marked protection against stress induced cardiac injury. It was noticed that garlic administration for 28 days was the most effective regimen. While garlic pretreatment for short time (7 days or 14 days) was of weak effect. This comes in accordance with the study done by Sathya et al. (2012) where chronic garlic administration was the most effective in comparison with short durations. In the present study, garlic did not alter R-R interval, however it decreased QRS interval and increased its amplitude. The earlier work of Rietz et al. (1993) mentioned that the incidence of ventricular fibrillation (VF) during 20 min occlusion of the descending branch of the left coronary artery (LAD) was significantly reduced in the garlic treated group as compared to untreated controls. Garlic pretreatment in this work prevented the occurrence of cardiac hypertrophy, as manifested by normalizing heart to body weight ratio. A previous study indicated that allicin, protected cardiac function and prevented the development of cardiac hypertrophy through ROS-dependent mechanism involving multiple intracellular signaling (Chen et al., 2010). In the present study, administration of FGH reduced creatine kinase enzyme activity in stress exposed rats. This reduction is in line with Vibha et al. (2011), where pretreatment with garlic decreased the isoprenaline-induced elevation of serum CKMB. Garlic has scavenging properties against ROS, this was supported by the present study where long-term garlic pretreatment nearly normalized the MDA and SOD, and significantly increased GSH, GST and CAT. Earlier study reported that aged garlic extract increases the level of SOD, GSH and GST in vascular endothelial cell (Geng & Lau, 1997) and (Wei & Lau, 1998) and inhibits oxidative-stress mediated ischemic-reperfusion damage in rat brain (Numagami et al., 1996). It was also reported that chronic oral administration of raw garlic homogenate caused a significant increase in basal SOD and CAT activities of rat heart, which was associated with a concomitant decrease in basal lipid peroxidation (Banerjee et al., 2002b). The histopathological evaluation in the present work showed that long term garlic pretreatment decreased the edema and the infiltration with inflammatory cells, nearly normalizing the myocardium histoarchitecture. This is in agreement with Banerjee et al. (2002a), who realized that garlic treated rat heart subjected to ischemic reperfusion injury showed normal structure of myocyte with slight edema.

**Conclusion:** From the above results, it is clear that stress affects the cardiac performance and that long-term consumption of garlic is beneficial. As it is under human consumption, based on the current study reports; dietary supplementation of garlic for prolonged period may be beneficial as one could not avoid stress in the modern way of living. It is concluded that garlic homogenate may provide a nutritional benefit for people exposed to stressful life conditions.

**Fig. (1):** Effect of FGH on ECG parameters in rats exposed to stress for one day, one week and 3 weeks.
- a,b,c indicates a significant difference from control, FGH and stress groups, respectively, at p<0.05
Fig. (2): Effect of FGH on biochemical parameters in rats exposed to stress for one day, one week and 3 weeks. 
- a,b,c: Significant difference from control group, FGH group and stress group, respectively, at p<0.05

Fig. (3): Effect of FGH on heart to body weight ratio in rats exposed to stress for one day, one week and 3 weeks. 
a,b,c indicates a significant difference from control, FGH and stress groups, respectively at p<0.05.
Figure (4): Effect of FGH on myocardial histoarchitecture in rats exposed to different stress levels: (A): Normal cardiac muscle fibres and nuclei (H & E x 200), (B): FGH showing normal cardiac muscle, (C) One day stress rat showing normal architecture of heart muscle, (D): One day stress rat +FGH showing normal cardioarchitecture of the cell, (E): One week stress rat showing myocardial necrosis, vacuoles, edema with inflammatory cells, (F): One week stress rat+ FGH showing near normal myocardium with lessened necrosis and edema, (G): Three weeks stress rat showing confluent focal necrosis of myofibrils, edema with infiltration of inflammatory cells and extravasations of RBC’s, (H): Three weeks stress rat +FGH showing almost normal myocardial histoarchitecture.

Acknowledgement: My deepest thanks to Prof. Dr. Adel B. Khelosy, Prof. of path. Faculty of veterinary medicine, Cairo university for his professional aid and guidance in the histopathological section

REFERENCES:


Tأثير الثوم الطازج على قلب الفئران بعد تعرضهم للأجهاد بوضعهم فى ماء ساخن

يعتبر الأجهاد واحد من العوامل المؤثرة على أمراض القلب. كما يعتبر الثوم من أقدم العلاجات التى تستخدم منذ الأف السنين. تهدف الدراسة الحالية لمعرفة مدى التأثير الواثق للثوم بعد تعرض ذكور الفئران من فصيلة الأسبراجو دوللى للأجهاد لمدة مختلفة (يوم واحد – أسبوع واحد – ثلاثة أسابيع) عن طريق غمرهم في ماء ساخن ثم تقييم نشاط عضلات القلب بعمل رسم تخطيطى لقلب الفئران وحساب وزن القلب بالنسبة لوزن الجسم وقياس نشاط إنزيم كرياتين فوسفوكينيز (CPK-MB) وكذلك مستوي مضادات الأكسدة (MDA) وكانت النتائج كالآتي: الأجهاد لمدة يوم واحد كان له تأثير ضعيف على القياسات المستخدمة بينما الأجهاد لمدة اطول كان له تأثير أدى إلى تغيرات في قياسات عضلة القلب وتقليل مستويات الأكسدة في عضلة القلب. التأثير الواثق للثوم كان الأكبر تأثيرا عند تناوله لمدة 28 يوما : تنظيم إقراض ضروب القلب وإخفاء تضخم عضلة القلب كما قلل من مستوي إنزيم الكريبتين في المصل ومستوى الدهون المؤكسدة كذلك ادى الى زيادة في الأنزيمات المضادة للأكسدة كما حسن من التغيرات الهستولوجية التي حدثت نتيجة التعرض للأجهاد.