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Freshly Crushed Garlic is a Superior Cardioprotective Agent than Processed Garlic

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Abstract

In this study, we compared the cardioprotective effects of freshly crushed garlic vis-à-vis that of processed garlic. Two groups of rats were gavaged with respective garlic preparations while the control group received vehicle only. After 30 days, all the rats were sacrificed and isolated hearts were subjected to 30 min ischemia followed by 2 h of reperfusion. Both the garlic preparations provided cardioprotection, but superior cardiac performance was noticed for those fed with freshly crushed garlic. Consistent with these results, freshly crushed garlic group displayed significantly greater phosphorylation of anti-apoptotic ERK1/2 proteins, reduced Bax/Bcl-2 ratio and reduced phosphorylation of proapoptotic p-38MAPK and JNK. Moreover, the survival signaling network consisting of Akt-FoxO1 was increased in the freshly crushed garlic treated hearts. Freshly crushed garlic, but not the processed garlic, showed enhanced redox signaling as evident by increased level of p65 subunit of NFκB, Nrf2 and enhanced GLUT 4, PPARα and PPARδ. The results thus show that although both freshly crushed garlic and processed garlic provide cardioprotection; the former has additional cardioprotective properties presumably due to the presence of H₂S.

Keywords

Garlic; Ischemia/reperfusion; Cardioprotection; Hydrogen sulfide; Redox signaling

INTRODUCTION

Garlic has been used for centuries for culinary purposes; while its health benefits have been known since at least 1500 B.C. when ancient Chinese and Indians used it as a blood-thinning agent (1). Hippocrates, the father of modern medicine, used garlic to treat cervical cancer (2). In China, garlic was shown to reduce the risk of esophageal and stomach cancers by ~70% (3). Garlic is also effective against breast and prostate cancers due to the presence of s-allyl mercaptocysteine (4). Documented scientific investigations on garlic were initiated by Louis Pasteur who first reported its antibacterial and antifungal properties (5,6). Albert Schweitzer used this concept and treated dysentery in Africa with garlic (7).

Subsequent studies found efficacy of garlic as a cardioprotectant. Numerous studies documented the hypoglycemic, antiatherogenic and antiatherosclerotic properties of garlic (8-10). Garlic was also found to be beneficial against ischemic heart disease (11). A significant number of clinical trials found garlic to lower total as well as LDL cholesterol (12), although

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negative findings also exist (13). A recent study showed garlic to be useful for lowering high blood pressure (14).

Many of the physiological effects of garlic are attributed to the volatile sulfur compounds like thiosulfates, which are also responsible for its pungent aroma. Many pharmacological properties of garlic are also derived from the organo-sulfur compounds like allicin and diallyl disulfide (9). Most recently, additional cardioprotective ability of garlic was attributed to s-allylcysteine (15). Raw fresh garlic contains alk(en)yl cysteine sulfoxides and γ -glutamyl alk(en)yl cysteine, which upon activation, is converted into s-allylcysteine (deoxyallin) due to the deactivation of the enzyme allinase. Allicin is then formed from S-allyl-L-cysteine, which is readily broken down in volatile sulfur compounds including hydrogen sulfide (H_2S) [Figure 1]. For that reason, when crushed, raw garlic generates H_2S (16). Processed and cooked garlic loses their abilities to generate H_2S , although they still retain their antioxidant actions, thereby suggesting that raw garlic possesses more health benefits than processed garlic. The purpose of the present study was to compare the cardioprotective ability of freshly crushed garlic vis-à-vis processed garlic. We fed a group of rats raw fresh garlic while another group was given the processed one. After 30 days, isolated hearts were subjected to 30 min ischemia followed by 2 hour of reperfusion. Cardiac functional parameters like heart rate, LVDP (Left ventricular Develop Pressure), $LVdp/dt$ (First derivative of LVDP), Coronary flow and Aortic flow were measured. To check the cardioprotective ability of both type of garlic preparations, some related protein (Bax, Bcl2, Akt, ERK1/2, JNK, PPAR, Glut4 etc) levels were also measured. The results of this study clearly demonstrated superior cardioprotective effects of raw garlic compared to its processed counterpart.

MATERIALS AND MEHODS

Preparation of garlic slurry

Garlic was purchased from local supermarket. Fresh garlic was crushed and made a slurry-like preparation with water [60 gm garlic in 400 ml water]. For preparation of processed garlic, garlic was similarly crushed and then dried in air for two days to allow all the H_2S to escape. Again, this crushed and air dried garlic was made into a slurry-like preparation with water [same concentration].

Animals

All animals used in this study received humane care in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. Sprague Dawley male rats weighing between 250-300 gm were used for the experiment. The rats were randomly assigned to one of the three groups: (1) control (2) fresh garlic (3) processed garlic [Figure 2]. The rats were fed *ad libitum* regular rat chow with free access to water. The fresh garlic/processed garlic treated rats were gavaged with 1 ml of garlic extract (150 mg/kg body wt/day) for thirty days while the control group of rats were gavaged 1 ml of water for the same period of time. The animals were gavaged with fresh garlic within 5 to 10 min of preparation while the processed garlic was gavaged after two days of preparation.

Isolated working heart preparation

At the end of 30 days, the rats were anesthetized with sodium pentobarbital (80 mg/kg, b.w. i.p.), (Abbott Laboratories, North Chicago, IL, USA) and anticoagulated with heparin sodium (500 IU/kg b.w., i.v.) (Elkins-Sinn Inc., Cherry Hill, NJ, USA) injection. After ensuring sufficient depth of anesthesia, thoracotomy was performed, the hearts were perfused in the retrograde Langendorff mode at 37°C at a constant perfusion pressure of 100 cm of water (10

kPa) for a 5 min washout period (17). The perfusion buffer used in this study consisted of a modified Krebs-Henseleit bicarbonate buffer (KHB) (in mM: sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2, and glucose 10). The Langendorff preparation was switched to the working mode following the washout period with a left atrial filling pressure of 17 cm water. At the end of 10 min, after the attainment of steady state cardiac function, baseline functional parameters were recorded. Then the hearts were subjected to 30 min of global ischemia followed by 2 h of reperfusion. The first 10 min of reperfusion was in the retrograde mode to allow for post ischemic stabilization and there after, in the antegrade working mode to allow for assessment of functional parameters, which were recorded at 30-, 60-, and 120- min of reperfusion.

Cardiac function assessment

Aortic pressure was measured using a Gould P23XL pressure transducer (Gould Instrument Systems Inc., Valley View, OH, USA) connected to a side arm of the aortic cannula, the signal was amplified using a Gould 6600 series signal conditioner and monitored on a CORDAT II real-time data acquisition and analysis system (Triton Technologies, San Diego, CA, USA) (17). Heart Rate (HR), Left Ventricular developed pressure (LVDP) (defined as the difference of the maximum systolic and diastolic aortic pressures), and the first derivative of developed pressure (dp/dt) were all derived or calculated from the continuously obtained pressure signal. Aortic flow (AF) was measured using a calibrated flow-meter (Gilmont Instrument Inc., Barrington, IL, USA) and coronary flow (CF) was measured by timed collection of the coronary effluent dripping from the heart.

Infarct size estimation

At the end of reperfusion, a 1% (w/v) solution of triphenyl tetrazolium chloride in phosphate buffer was infused into aortic cannula for 20 min at 37 °C (18). The hearts were excised and the sections (0.8 mm) of the heart were fixed in 2% paraformaldehyde, placed between two cover slips and digitally imaged using a Microtek ScanMaker 600z. To quantitate the areas of interest in pixels, a NIH image 5.1 (a public-domain software package) were used. The infarct size was quantified and expressed in pixels.

TUNEL assay for assessment of apoptotic cell death—Immunohistochemical detection of apoptotic cells was carried out using TUNEL method [18] (Promega, Madison, WI). The heart tissues were immediately put in 10% formalin and fixed in an automatic tissue-fixing machine. The tissues were carefully embedded in the molten paraffin in metallic blocks, covered with flexible plastic moulds and kept under freezing plates to allow the paraffin to solidify. The metallic containers were removed and tissues became embedded in paraffin on the plastic moulds. Prior to analyzing tissues for apoptosis, tissue sections were deparaffinized with xylene and washed in succession with different concentrations of ethanol (absolute, 95%, 85%, 70%, 50%). Then the TUNNEL staining was performed according to the manufacturer's instructions. The fluorescence staining was viewed with a fluorescence microscope (AXIOPLAN2 IMAGING) (Carl Zeiss Microimaging, Inc. NY) at 520 nm for green fluorescence of fluorescein and at 620 nm for red fluorescence of propidium iodide. The number of apoptotic cells was counted throughout the slides and expressed as a percent of total myocyte population.

Preparation of Subcellular Fractions

Tissues (frozen in liquid nitrogen and stored at -80°C) were homogenized in 1 ml buffer A (25 mM Tris-HCl, pH 8, 25 mM NaCl, 1mM Na-Orthovanadate, 10 mM NaF, 10 mM Na-Pyrophosphate, 10 nM Okadaic acid, 0.5 mM EDTA, 1 mM PMSF, and 1x Protease inhibitor

cocktail) in a Polytron-homogenizer. Homogenates were centrifuged at 2000 rpm at 4°C for 10 min and the nuclear pellet was resuspended in 500 µl of Buffer A with 0.1% Triton X-100 and 500 mM NaCl. Supernatant from the above centrifugation was further centrifuged at 10,000 rpm at 40°C for 20 min, and the resultant supernatant was used as cytosolic extract. The nuclear pellets were lysed by incubation for one hour on ice with intermittent tapping. Homogenates were then centrifuged at 14,000 rpm at 4°C for 10 min, and the supernatant was used as nuclear lysate. Cytosolic, nuclear extracts were aliquoted, snap frozen and stored at -80°C till use. Total protein concentration in cytosolic and nuclear extract was determined using BCA Protein Assay Kit (Pierce, Rockford, IL).

Western blot Analysis

Either cytosolic or nuclear proteins were separated in SDS-PAGE and transferred to nitrocellulose filters. Filters were blocked in 5% nonfat dry milk, and probed with primary antibody for overnight (19). Primary antibodies such as Nrf2, Akt, Phospho-Akt, Glut-4, p-65, histone, glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology, Santa Cruz, CA; whereas Bax, Bcl-2, FoxO1, Phospho-FoxO1, Jnk, Phospho-Jnk, ERK, Phospho-ERK were obtained from Cell Signaling Technology, Beverly, MA. PPAR α , PPAR δ were obtained from Abcam Inc. Cambridge, MA. All primary antibodies were used at the dilution of 1:1000. Protein bands were identified with horseradish peroxidase conjugated secondary antibody (1:2000 dilution) and Western blotting Luminol Reagent (Santa Cruz Biotechnology, Santa Cruz, CA). GAPDH and histone were used as cytosolic and nuclear loading control respectively. The resulting blots were digitized, subjected to densitometric scanning using a standard NIH image program, and normalized against loading control.

Statistical Analysis

The values for myocardial functional parameters, total and infarct volumes and infarct sizes and cardiomyocyte apoptosis are all expressed as the mean $\pm$ standard error of mean (SEM). Analysis of variance test followed by Bonferroni's correction was first carried out to test for any differences between the mean values of all groups. If differences between groups were established, the values of the treated groups were compared with those of the control group by a modified t-test. The results were considered significant if $p < 0.05$.

RESULTS

Freshly crushed garlic has better cardioprotective effects

We compared the efficacy of freshly crushed garlic vs. processed garlic as a cardioprotectant. Although rats fed with either freshly crushed garlic or processed garlic had superior ventricular performance compared to control during the reperfusion phase (Figure 3), the freshly crushed garlic group displayed significantly greater recovery of aortic flow [AF], left ventricular developed pressure [LVDP] and the maximum first derivative of developed pressure [LV_{max} dp/dt] compared to that achieved from processed garlic. The heart rate and coronary flow did not vary between the groups.

Myocardial infarct size determined by TTC staining was about $37 \pm 1.2\%$ normalized to area of risk (Figure 4A). There was no infarction when the hearts were perfused with the KHB buffer without subjecting to ischemia and reperfusion protocol (control). While both the experimental groups showed lower infarct size compared to ischemia/reperfusion control, the freshly crushed garlic group displayed smaller infarct size ($20 \pm 1.7\%$) as compared to that from processed garlic ($23 \pm 2.01\%$).

The hearts subjected to ischemia/reperfusion showed about 33 % apoptotic cardio-myocytes compared to the control (5 %) (Figure 4B). Garlic treated hearts demonstrated substantial

reduction in the number of apoptotic cells and it was more prevalent in freshly crushed garlic group ($15 \pm 1.75\%$) than that in processed garlic group ($21 \pm 1.82\%$).

Garlic treatment generates survival signal

It is well known that ischemia/reperfusion causes cellular injury by generating a death signal (20). Such death signal is produced from defective MAP kinase/tyrosine kinase signaling and increased proapoptotic Bax/Bcl-2 ratio (21). We, therefore, determined these pro- and anti-apoptotic parameters in the hearts with and without garlic treatment. As shown in Figure 5A, both fresh and processed garlic had the ability to enhance the phosphorylation of ERK1/2 several-fold, but freshly crushed garlic had higher potential than processed garlic. An opposite phosphorylation patterns for proapoptotic JNK and p38MAPK were obtained with garlic treatment, wherein fresh garlic being superior over processed garlic.

Ischemia/reperfusion increases proapoptotic protein Bax and reduces anti-apoptotic protein Bcl-2, as expected (Figure 5B). While garlic increased the Bcl-2/Bax ratio, the greater degree of enhancement was noticed from freshly crushed garlic group. Taken together, these results indicate that although garlic generates an overall survival signal, freshly crushed garlic has better efficacy than processed garlic in suppressing proapoptotic factors and boosting the antiapoptotic ones.

Garlic induced survival signal includes Akt-FoxO axis

Akt promotes cell survival by phosphorylating its downstream target FoxO, which then exit the nucleus, resulting in the suppression of proapoptotic genes (22). We thus examined if garlic could activate the Akt-FoxO axis. As shown in Figure 6, garlic treatment caused extensive phosphorylation of Akt and FoxO1 even at the baseline level. Further, while processed garlic only moderately enhanced the phosphorylation of FoxO1, freshly crushed garlic was more potent and ischemia/reperfusion (I/R) showed no effect. The results thus indicate that while both garlic preparations activate Akt, only the freshly crushed garlic has the ability to stimulate its downstream target i.e., FoxO1.

Fresh garlic boosts redox signaling

Since antioxidants present in garlic have been attributed to its cardioprotective effects, we compared the effects of freshly crushed and processed garlic on induction Nrf2 and NFkB, two known mediators of redox signaling [Figure 7]. We presumed that both garlic preparations would contain comparable amounts of antioxidants while H₂S would be absent from the processed garlic. However, to our surprise, only freshly crushed garlic could activate Nrf2 and the p65 subunit of NFkB. In fact, in processed garlic group p65 reduced after ischemia/reperfusion.

Effects of garlic on PPAR activities

Since several reports link garlic with diabetes and obesity, the two well known cardiovascular risk factors; we examined the effects of garlic on the expression of Glut-4 and PPARs, markers for diabetes/obesity. As shown in Figure 8, at the baseline level, the amount of Glut-4 did not vary between the groups. However, upon ischemia/reperfusion, significant reduction in Glut-4 and PPAR were observed. In contrast, at the baseline level, PPAR α and PPAR δ were higher for the garlic group. Further, in ischemia/reperfusion group, Glut-4 increased significantly in case of freshly crushed garlic group while it was reduced for the processed garlic group. PPAR δ also followed a similar pattern. In contrast, PPAR α significantly increased for both the freshly crushed garlic and processed garlic group.

DISCUSSION

The results of the present study showed for the first time that freshly crushed garlic possesses superior and diverse cardioprotective abilities compared to processed garlic as evidenced by its ability i) to produce greater post-ischemic ventricular recovery, lower myocardial infarction and reduced cardiomyocyte apoptosis compared to processed garlic; ii) to generate greater degree of survival signal by boosting anti-apoptotic ERK1/2 and Bcl-2/Bax ratio and by suppressing the death signal by decreasing the phosphorylation proapoptotic JNK and p38MAPK; iii) to stimulate Akt-FoxO survival network signaling; iv) to generate redox signaling by activating Nrf2 and p65 subunit of NFκB and v) to reduce cardiovascular risk factors associated with diabetes and obesity through the upregulation of GLUT-4, PPARα and PPARδ. The superiority of freshly crushed garlic over processed garlic might be due to H₂S, which is absent in processed garlic.

The long-held belief that most of the health benefits of garlic are derived from its antioxidant constituents has recently been challenged by Chuah et al. providing evidence that S-allylcysteine provide protection from myocardial infarction via an H₂S-dependent pathway (20). Almost simultaneously, Benavides et al. also demonstrated that the vasoactivity of garlic is derived from H₂S (21). Taken together, these two studies thus provided novel evidences that the cardioprotective function of garlic is in part is through the generation of H₂S.

To understand why freshly crushed garlic can produce H₂S while processed garlic is devoid of this key gaseous molecule, it is necessary to know the chemistry of H₂S generation by garlic. Raw garlic contains alk(en)yl cysteine sulfoxides and γ-glutamyl alk(en)yl cysteines, which upon crushing yields s-allylcysteine (deoxyallin) due to the activation of the enzyme allinase. The final product allicin is then formed from s-allyl-L-cysteine, which is readily broken down into several volatile sulfur compounds including H₂S (Figure 1). H₂S being volatile then escapes. Processed garlic contains only the oxidized products of allicin, presumably over 75 sulfur-containing compounds including s-allyl-cysteine and ajoene methyl allyl sulfide, with cholesterol lowering properties (22). In the heart, cystathione-γ-lyase appears to be involved in the endogenous generation of H₂S (23).

As mentioned above, two classes of organosulfur compounds are present in garlic; gamma glutamylcysteines and cysteine sulfoxide, about 80% of this later compound being allylcysteine sulfoxide or allin. The enzyme allinase catalyzes the formation of sulfenic acids, which spontaneously react with each other to form thiosulfinate including allicin, which possesses cardioprotective properties (24). Allicin and allicin-derived compounds are metabolized to allyl methyl sulfide. Gamma glutamylcysteines, on the other hand, are hydrolyzed to S-allylcysteine and S-1-propenylcysteine. Most of these organosulfur compounds are present in the processed garlic, and some of them might contribute to cardioprotection.

H₂S has long been considered as a harmful chemical, responsible for diverse health problems including neurotoxicity (25). Only recently, H₂S was shown to protect ischemic myocardium through preconditioning effect (26). It has thus been proposed that H₂S is the “third” endogenous signaling gasotransmitter (after NO and CO) capable of providing cardioprotection (27). Several recent studies demonstrated the ability of H₂S to preserve myocardial contractility, activate ATP-dependent potassium channel and exert vasodilation, all in tune with its potential preconditioning function (28-30).

Except for H₂S, most of the bioactive compounds including antioxidants like tocopherols, vitamin A, vitamin C and riboflavin are preserved in processed garlic (31). A recent study also showed the presence of cycloalliin, a stable organosulfur compound, in garlic (32). Thus, it is not surprising that garlic retains many of its cardioprotective functions even after processing. Nevertheless, in spite of the presence of numerous chemicals including antioxidants,

differences in the protective abilities between raw and processed garlic have also been documented. As an example, while raw garlic is highly effective in reducing serum cholesterol and triglyceride, boiled garlic has minimal effects (33). According to a recent study, oven-heating of garlic at 200°C or immersing in boiling water for 3 min or less does not affect its ability to inhibit platelet aggregation, whereas heating crushed garlic for 6 min completely suppressed IVAA (34). Increasing concentration of crushed garlic extract has a dose dependent positive IVAA in the aggregation reaction while uncrushed, microwaved garlic samples are ineffective. It has also been shown that processed garlic loses its antiplatelet activities due to the loss of allicin and thiosulfinates (35). Fresh garlic also has higher antioxidant activities as compared to processed garlic (36).

The present study was specifically designed to determine cardioprotective abilities of freshly crushed garlic vs. processed garlic using ischemic/reperfused rat heart model. As mentioned earlier, both the garlic preparations reduced ischemia reperfusion injury, but freshly crushed garlic revealed superior cardioprotective ability. For example, compared to control ischemia/reperfusion, freshly crushed garlic increased the ventricular function including left ventricular developed pressure and aortic flow from 66.5 ± 1.8 to 99.3 ± 2.6 and from 9.3 ± 1.1 to 26.8 ± 1.3 , respectively, while the processed garlic enhanced these functions from 66.5 ± 1.8 to 88.7 ± 2.1 and from 9.3 ± 1.1 to 17 ± 1.5 , respectively. The differences in the improvement of ventricular function with freshly crushed garlic and processed garlic were significant ($p < 0.05$). Consistent with the results of functional recovery, freshly crushed garlic revealed smaller infarct size ($20 \pm 1.7\%$) compared to processed garlic ($23 \pm 2.01\%$) and both of these values were significantly lower than control ($37 \pm 1.2\%$). In concert, the number of apoptotic cardiomyocytes was significantly lower in the freshly crushed garlic group and processed garlic group. In case of control I/R group, the amount of apoptotic cardiomyocytes was 33%, the fresh garlic reduced it to $15 \pm 1.75\%$ whereas in case of processed garlic the amount were higher than fresh garlic but lower than control I/R group ($21 \pm 1.82\%$).

Our results demonstrated that both the garlic preparations increased ERK1/2 phosphorylation and decreased p38MAPK and JNK activations several-fold compared to control. Similar to the results of cardiac function and infarct size, freshly crushed garlic consistently showed superior abilities (vs. processed garlic) to activate ERK1/2 and/or to down-regulate p38MAPK and JNK. P38MAPK and JNK are known pro-apoptotic kinases while ERK1/2 is a well-known anti-apoptotic factor, which contributes to survival signals (37). Consistent with these results, freshly crushed garlic reduced Bax/Bcl-2 ratio more than processed garlic while both the garlic preparations reduced Bax/Bcl-2 ratio significantly than control. Our results also showed that both the garlic preparations could phosphorylate Akt while only freshly crushed garlic, and not the processed garlic could up-regulate FoxO1. PI-3-kinase-Akt signaling is known to regulate the survival signal while FoxO1, a downstream target of Akt also regulate cell survival (38). There are also reports indicating that FoxO1 could trigger activation of Akt suggesting a feedback regulation of Akt by FoxO1 (39). In concert, freshly crushed garlic, and not the processed garlic, potentiated redox signaling by activating NFκB and Nrf2. It is well known that PI3K-Akt pathway plays a crucial role in regulating Nrf2 mediated redox signaling, which in turn regulates the survival signaling (40).

Finally, to our surprise, garlic could modulate the activities of Glut-4 and PPARs, which are linked with diabetes and obesity related cardiac disorders. To the best of our knowledge, there are only very limited observations on the role of garlic in obesity. One such report showed that garlic could reduce body weight via decrease in serum lipid and increase in UCP-1 and UCP-2 mRNA expressions. Interestingly enough, while PPARα and PPARδ were higher for both the garlic preparations, PPARδ was reduced after I/R only for processed garlic group while it was increased for freshly prepared garlic. PPARδ was increased for both the garlic preparations. GLUT-4 followed the same pattern as PPARδ. The reason for differential regulation of PPARs

and GLUT4 is not clear, and further study is necessary to unveil the mechanisms of PPARs and GLUT4 regulation with garlic.

In summary, the results of the present study clearly demonstrated for the first time that fresh garlic can provide superior cardioprotection as compared to processed garlic. Fresh garlic appears to generate a potent survival signal leading to the activation of antiapoptotic and anti death proteins that's presumably due to the presence of H₂S. These results are potentially important, as there is a growing interest among the heart patients to use natural and complementary medicine. Current evidence indicates that one in three American adults use some form of alternative medicine. The results of the present study strongly suggest that using fresh garlic would provide maximal and added benefits to the cardiovascular patients.

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Figure 1.
Chemical reactions of formation of allicin and H₂S from raw garlic

Figure 2.
Schematic representation of experimental perfusion protocol of different group of heart.
(Abbreviations used: I/R —Ischemia / Reperfusion)

Figure 3.

Effect of fresh and processed garlic on post ischemic left ventricular function, heart rate, coronary flow, left ventricular developed pressure (LVEDP), Maximum first derivative of developed pressure and aortic flow. Results are shown as mean $\pm$ SEM * $p < 0.05$ vs. control and # $p < 0.05$ vs. fresh garlic, $n = 6$ in each group. (Abbreviations used: BL- baseline; RE - reperfusion)

Figure 4. Effect of fresh and processed garlic on myocardial infarct size (A), and cardiomyocytes apoptosis (B). Results are shown as mean $\pm$ SEM * $p < 0.05$ vs. I/R, $n = 3$ in each group. (Abbreviations used: I/R —Ischemia / Reperfusion)

Figure 5.

Western blot analysis and densitometric bardiagram of p-ERK, ERK, p-JNK, JNK, p-p38MAPK, p38MAPK (A), and Bax/Bcl-2 (B) from the control, fresh and processed garlic treated heart samples. GAPDH was used as the loading control. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are shown as mean $\pm$ SEM * $p < 0.05$ vs. control and # $p < 0.05$ vs. fresh garlic. (Abbreviations used: BL- baseline; I/R — Ischemia / Reperfusion)

Figure 6.

Western blot analysis of p-Akt, Akt, from the cytosolic fraction and p-FoxO1, FoxO1 from the nuclear fraction of control, fresh and processed garlic treated heart samples. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are shown as mean $\pm$ SEM * $p < 0.05$ vs. control and # $p < 0.05$ vs. fresh garlic. . (Abbreviations used: BL- baseline; I/R —Ischemia / Reperfusion)

Figure 7.

Western blot analysis of Nrf2 and p65 from the nuclear fraction of control, fresh and processed garlic treated heart samples. Histone was used as the loading control. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are shown as mean $\pm$ SEM. * $p < 0.05$ vs. control and # $p < 0.05$ vs. fresh garlic. (Abbreviations used: BL- baseline; I/R —Ischemia / Reperfusion)

Figure 8.

Western blot analysis of PPAR α and PPAR δ from nuclear fraction (A) and Glut-4 from cytosolic fraction (B) of control, fresh and processed garlic treated heart samples. Histone and GAPDH were used as the loading control for nuclear and cytosolic fraction respectively. Figures are representative images of three different groups, and each experiment was repeated at least thrice. Results are shown as mean $\pm$ SEM * $p < 0.05$ vs. control and # $p < 0.05$ vs. fresh garlic. . (Abbreviations used: BL- baseline; I/R —Ischemia / Reperfusion)