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Cardioprotective effect of garlic extract in isoproterenol-induced myocardial infarction in a rat model: assessment of pro-apoptotic caspase-3 gene expression

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Abstract

Background: Myocardial Infarction (MI), also known as heart attack, is one of the most common cardiovascular diseases. Although certain drugs or mechanical means are used, day by day natural products such as herbs and spices based MI treatment is getting much popularity over the drugs or mechanical means for their pharmacological effects and have low or no side effects. This study was designed to assess the cardio-protective effect of methanolic extract of Bangladeshi multi clove garlic (*Allium sativum*) cultivar, a highly believed spice having cardioprotective activity, against isoproterenol (ISO) induced MI through cardiac histopathology as well as cardiac apoptotic caspase-3 gene expression study in female Wistar albino rats. Four groups containing 35 rats treated with respective agents like distill water / garlic extract (200 mg/kg-body-weight/day) up to 28 days and normal saline / ISO (100 mg/kg-body-weight/day) on 29th and 30th day were sacrificed (two rats/group/sacrifice) on the day 31, 46 and 61 and collecting the heart, cardiac histology and gene expression analysis were performed.

Results: ISO induced MI rats pretreated with garlic extract revealed up regulated expression of the cardiac apoptotic caspase-3 gene at the initial stage but finally the expressions gradually getting down regulated along with gradual improving the cardiac damage caused by apoptosis. Furthermore, only garlic extract pretreated rats were found undamaged cardioarchitecture and normal expressions of this gene.

Conclusions: These findings suggested that garlic extract confers having significant cardioprotective effect and consuming this spice with regular diet may reduce the risk of MI.

Keywords: Garlic, Myocardial infarction, Histopathology, Gene expression, Caspase-3 gene

Background

Myocardial Infarction (MI) is one of the most lethal metabolic disease of CVDs and has been the object of intense investigation by clinicians and basic medical scientists [1]. The World Health Organization (WHO) estimated that in 2008, out of 17.3 million CVD deaths globally, MI was responsible for 7.3 million deaths [2]. According to the WHO, MI was predicted to be the major cause of death in the world by the year of 2020 [3].

MI, generally due to coronary artery occlusion, results in an inadequate oxygen supply to the downstream myocardium [4]. Due to the influences of gene-environmental interactions, epigenetic mechanisms regulate at least a part of the pathological mechanisms. The specific mechanism involving MI have been proved to be associated with apoptosis [5] along with inflammation [6] and oxidative stress [7]; causes a massive loss of cardiac muscle and the left ventricle, in an attempt to maintain normal pump

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function, undergoes structural and functional adaptations, ultimately leading to heart failure (HF) [8–10].

Over the past few decades, a variety of human genetics approaches have identified apoptotic genes that are involved in the possible pathogenesis of MI through apoptosis. Two major classes of genes, one represented by the Bcl-2 family and the other by the caspase family control the apoptotic activities. Pro- and anti-apoptotic genes of the Bcl-2 family act by stabilizing (Bcl-2-like) or destabilizing (Bax-like) the mitochondrial membrane, thus altering the release of cytochrome c that, in turn, activates Casp9 and Casp3 sequentially [11]. Moreover, membrane-dependent stimuli such as tumor necrosis factor α (TNF-α)/TNF-αR interaction may trigger Casp8 activation, which eventually leads to Casp3 activation [12]. Activation of Casp3 initiates apoptosis of cardiac tissue and causes MI.

Although certain drugs or mechanical means are being used, day by day natural products such as herbs and spices based MI treatment is getting much popularity over the drugs or mechanical means for their pharmacological effects and have low or no side effects. Garlic (*Allium sativum*), a spice belongs to the Alliacae family, and its preparations have been widely recognized as agents for prevention and treatment of CVDs like atherosclerosis, hyperlipidemia, thrombosis, and hypertension [13]. Recent molecular studies have also demonstrated that active ingredients like s-allyl-cysteine sulfoxide (alliin) [14], diallyl trisulfide (DATS) [15], Allicin (2-propene-1-sulfinothioic acid S-2-propenyl ester, diallyl thiosulfinate) [16, 17] of garlic protect cardiac tissue through down regulating the expression of some CVD causing apoptotic genes.

In this study, isoproterenol (ISO) induced rats with MI were employed to investigate whether methanolic extract of the multi clove Bangladeshi garlic cultivar, one of the highly believed spices having cardioprotective activity, had a down regulating potentiality of cardiac apoptotic Casp3 gene expression; in other words the protective effect against MI-induced cardiomyocytes apoptosis and myocardial dysfunction.

Methods

Preparation of garlic extract (GE)

Multi clove Bangladeshi garlic cultivar was collected from local municipal market in Dhaka city and confirmed by the researchers of Biomedical and Toxicological Research Institute, Bangladesh Council of Scientific and Industrial Research, Dhanmondi, Dhaka, Bangladesh. The garlic was peeled off, separated cloves and chopped into pieces with knife and dried under sun at 32 ± 3 °C till the complete dehydration. Dehydrated chopped cloves were crushed into fine pure powder by an electric blender machine (YT-4677A-S Miyako, Japan). After complete dehydration of 1.50 kg raw garlic we got 400.167 g crude powder. Garlic powder was defatted by n-hexane application with sample to solvent ratio of 1:3 [18]. Methanolic extract was prepared through using aqueous methanol (80%) with sample to solvent ratio of 1:5 [19] by soxhlation method. During this method continuous agitation on rotary shaker at 150 rpm and ultrasonic vibration in sonicator machine at 37 kHz ultrasonic frequency and 40 °C temperature [20] was performed in 40/20-min shaking/sonication cycle. Obtained extract was condensed at 50 °C under reduced pressure by rotary evaporator and concentrated extract was collected in amber colored bottle, then covered and sealed properly and stored at 4 °C temperature. The total yield of extract was 201.09 g (50.25% of dry weight).

Maintenance of experimental animals

Laboratory bred healthy thirty five Wistar albino female rats weighing between 145 to 210 g and aged of 12–14 weeks were provided for this experiment by Biomedical and Toxicological Research Institute, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh. Selected animals were allowed to acclimatize to laboratory condition for two weeks then housed in rectangular polypropylene cages (50 × 35 × 20 cm) with free access to food and water during the course of experiment; maintained under standard laboratory conditions at 80 ± 5 °F and a relative humidity of 60 ± 10% with 12/12 h light/dark photoperiodic cycle. Rats were provided with a standard laboratory pellet diet. The maintenance of the animals was carried out in accordance with the OECD guidelines for use of animals [21] and all the experimental protocol was approved by the institutional ethical committee on animal care and use in experiment.

Experimental dose and MI induction

GE dose was determined following the existing studies of Nwanjo and Oze 2008 [22], Budoff et al. 2009 [23], Ebrahimi et al. 2015 [24], Vibha et al., 2011 [25] as suspending GE (200 mg/kg-bw/day) in 1.00 ml distilled water for each rat of two treatment groups. Experimental MI was induced in rats by a single subcutaneous injection of ISO hydrochloride dissolving 100 mg/kg-bw/day [26, 27] powder in 0.5 ml 0.9% normal saline for each rat of disease control group with 24 h interval for successive 2 days.

Experimental design

The female rats were randomly divided into four groups. Five rats in normal control group and ten rats per group in the rest three other groups of similar average body weight were taken; housed them separately in different rectangular polypropylene cages under same standard laboratory conditions.

Group 1 (Normal control): Standard laboratory diet and tap water ad libitum for successive 28 days and injected normal saline on successive 29th and 30th day.
Group 2 (ISO/Disease control): Lab diet for successive 28 days and administrated with ISO (100 mg/kg-bw/day) on 29th and 30th day.
Group 3 (GE): Lab diet+GE dose (200 mg/kg-bw/day) fed orally for successive 28 days and injected normal saline on successive 29th and 30th day.
Group 4 (GE + ISO): Lab diet+GE dose (200 mg/kg-bw/day) fed orally for successive 28 days and injected ISO (100 mg/kg-bw/day) on 29th and 30th day.

Within 24 h, after 15 days and after 30 days of last ISO administration, i.e. on the day 31, 46 and 61, rats were anaesthetized and sacrificed for histopathology and gene expression study.

Sacrifice of experimental animals

Within 24 h, after 15 days and after 30 days of last ISO administration, i.e. on the day 31, 46 and 61, rats (two rats/group/sacrifice) from Group 2, 3 and 4 were anaesthetized by 0.5 ml/rat intra peritoneal injection of drug Ketamine Hydrochloride (conc. 50 mg/ml) (Popular Pharmaceuticals Ltd.) as it has less effect on the rate and blood pressure. But from the Group 1 (control group) only two rats were anaesthetized only on the day 31 as this group was neither GE nor ISO administrated. The anaesthesia was confirmed by monitoring the zero movements of the rats and the anaesthetized rats were placed on a wax tray gradually in operation theatre room of the laboratory. Lying in dorsal position, their legs were extended as per the needs and fixed gently with supporting pins. Then, dorsal incision was made from neck to abdomen under clean and sterilized condition. Using small pins, gently the incised skin and tissue was kept stretched to make the heart clearly visible. Finally, the hearts were incised and collected and rinsed with normal saline. In each group the heart weight/body weight ratio was measured on the day of sacrifice. Heart weight was measured after keeping the heart in ice cold saline and squeezing out the blood. One portion of heart tissue was excised and soaked into buffered formalin (10%) for a week at room temperature for histopathology study and rest of the myocardial tissue was drenched into 0.9% NaCl and stored at − 80 °C temperature for molecular study.

Assessment of histopathological studies

Formalin fixed myocardial tissue was processed through consecutive soaking into 50, 60, 70, 80, 90 and 100% ethanol, pure acetone, pure xylene and then embedded in paraffin wax to prepare formalin fixed paraffin embedded (FFPE) (tissue embedding system). Myocardium was sectioned at 3 μm thin slices in automated microtome and fixed onto histological slides then deparaffinized through incubation at 60 °C. Histological slides were stained through Hematoxylene and Eosin (H&E) staining and examined through fluorescence microscope over normal spectra (Olympus BX 43, Tokyo, Japan) at × 200 magnification. Photomicrographs were taken using an attached digital camera.

Relative gene expression study

Total RNA was extracted from heart sample following SV Total RNA Isolation system protocol (Promega Corporation, USA) and quantified by measuring the absorbance at 260 nm by qubit 3.0 fluorometer (applied biosystem) and then isolated pure total RNA was stored at − 80 °C for next use, especially for subsequent gene expression study using primers Casp3F 5′-GAGCTTGGAACGCGAAGAAA-3′, Casp3R 5′-GCCCATTTCAGGGTAATCCA-3′ and B2MF 5′-CGGTGACCGTGATCTTTCTG-3′, B2MR 5′-GTGGAACTGAGACACGTAGC-3′. Relative gene expression was evaluated through one-Step RT-qPCR method using GoTaq® one-Step RT-qPCR system (Promega, USA). Each 10 μl reaction mix contains 5.00 μl GoTaq® qPCR master Mix (2X), GoScript™ RT Mix for 1-Step RT-qPCR (50X) 0.20 μl, $MgCl_2$ (25 mM)- 0.8 μl, 0.30 μl forward primer (10 μM), 0.30 μl reverse primer (10 μM), RNA Template (500 fg–100 ng) 2.00 μl and 1.40 μl nuclease free water. Gene expression study was conducted in Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System. The applied reaction conditions were as shown in Table 1. The relative gene expression level was calculated by $2^{-\Delta\Delta CT}$ method [28].

Data analysis

Regulation of Casp3 gene was analyzed by $2^{-\Delta\Delta CT}$ method using Microsoft Office Excel 2010. All the required statistical analyses for this study were performed by Statistical Package for Social Science program (SPSS 22.0 version). One-way ANOVA (analysis of variance) along with LSD post hoc test was used to analyze the $2^{-\Delta\Delta CT}$ values between different groups. For ANOVA the

Table 1 One Step RT-qPCR Reaction Conditions

Stages	Cycles	Program in Standard / Fast Mode
1. Reverse Transcription	1	≥37 °C for 15 min
2. RT inactivation/Hot-start activation	1	95 °C for 10 min
3. 3-Step qPCR		
i. Denature	40	95 °C for 10 s
ii. Anneal/ Collect Data		56 °C for 30 s
iii. Extend		72 °C for 30 s
4. Dissociation	1	95 °C

fold difference data were used. The findings were considered as statistically significant, if $p < 0.5$.

Results

Among the four experimental animal groups, rats from the control group (Group 1) were found normal. Rats of other two GE and GE + ISO treated groups (Group 3 and Group 4) were seemed very healthy and spontaneous up to 3 weeks but at the last week some of the rats became a bit unhealthy and less spontaneous than before and a decrease in food consumption was also found and gradually lose the body weight. As a consequence one rat from both of the treatment groups was expelled due to the mild sickness. Two rats from the ISO induced group (Group 2) found dead on 32th day after three days of ISO treatment. That is why; only two mice were sacrificed at each time point and analyzed.

200x magnified photomicrographs of transverse section of myocardium were analyzed to assess histological condition of heart tissue under different treatment and diseased condition.

Cardiac histology of the normal control rats (Group 1) as shown on Fig. 1 revealed a normal structure with centrally arranged nuclei and undamaged myofibrils with clear striations.

Myocardial sections of stressed rats with ISO represented in Fig. 2(a), 2(b) and 2(c) exhibited myocardial necrosis, edema with inflammation, distorted myofibrils with damaged striation and nuclei aggregation but these myocardial aberrations gradually became worsen on day 46 and 61 compared to the day 31.

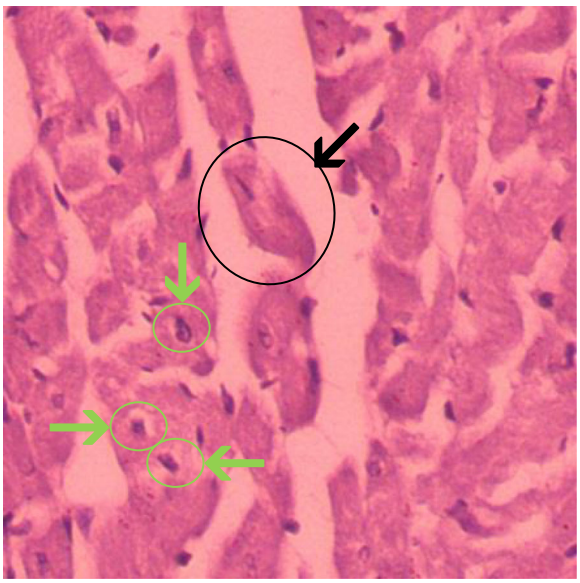

Fig. 1 Cardiac histology of normal control rat (Group 1) revealed regular arrangements of myofibrils with clear striation (pointed with black arrow) and normal and central distribution of nuclei (pointed with green arrows) (black dots on myofibrils); no disruption or inflammation in myofibrils

Photomicrographs of Fig. 3(a), 3(b) and 3(c) represented the cardiac histology of GE treated group (Group 3) pretreated with GE (200 mg/kg-bw) depicted a normal alike well-structured myocardial histo-architecture without any distortion.

In case of 200 mg GE + ISO treated group (Group 4) cardiac histology of 2 days stressed rats with MI sacrificed on 31st day represented in Fig. 4(a) revealed highly pronounced cardio-architecture aberration with confluent focal necrosis of myofibrils, edema with inflammation and nuclei aggregation but gradually these cardiac distortions became lessened and replenished to the near normal condition on day 46 and 61as shown in Fig. 4(b) and 4(c) respectively.

The relative expressions of 1st, 2nd and 3rd sacrifice on the day 31, 46 and 61 respectively of cardiac apoptotic gene is represented in Fig. 5. In this current study the mRNA expression levels or fold changes of Casp3 gene were approximately similar to the normal control group in GE (200 mg/kg-bw) treated rat (Group 3) ($p < 0.5$). In ISO treated group (Group 2), it was observed that on day 31 though the expression of Casp3 gene was similar to the control group (Group 1), the expressions were gradually and significantly up regulated on day 46 and 61 ($p < 0.5$). But in case of GE + ISO treated group (Group 4) the expressions were gradually became down regulated on day 46 and 61 compared to the day 31 and the expression value of 3rd sacrifice was not significantly different form the control group ($p < 0.5$). Gene expressions on day 31, 46 and 61 within the rats of ISO treated group (Group 2) were significantly different with gradual increasing ($p < 0.5$). Comparing the gene expression of ISO stressed (Group 2) rats sacrificed on day 31 with GE treated group (Group 3), no significant difference was observed in rats on day 31, 46 and 61 but in sacrificed GE + ISO treated (Group 4) rats gene expressions were gradually decreased with significant differences on day 31 and 46 and no significant difference on day 61 ($p < 0.5$). The gene expressions in GE treated (Group 3) rats were down regulated compared to the ISO stressed rats (Group 2) on day 46 and except the day 31, the values of day 46 and day 61 were significantly different ($p < 0.5$). On the other hand, in GE + ISO treated (Group 4) rats the gene expression on day 31 was significantly up regulated but down regulated on day 46 and 61 with no significant difference ($p < 0.5$). In GE treated (Group 3) rats the gene expressions on day 31, 46 and 61 were significantly down regulated with fluctuation from the rats of day 61 of ISO stressed group (Group 2) but in GE + ISO treated (Group 4) rats the gene expressions gradually down regulated with no significant difference

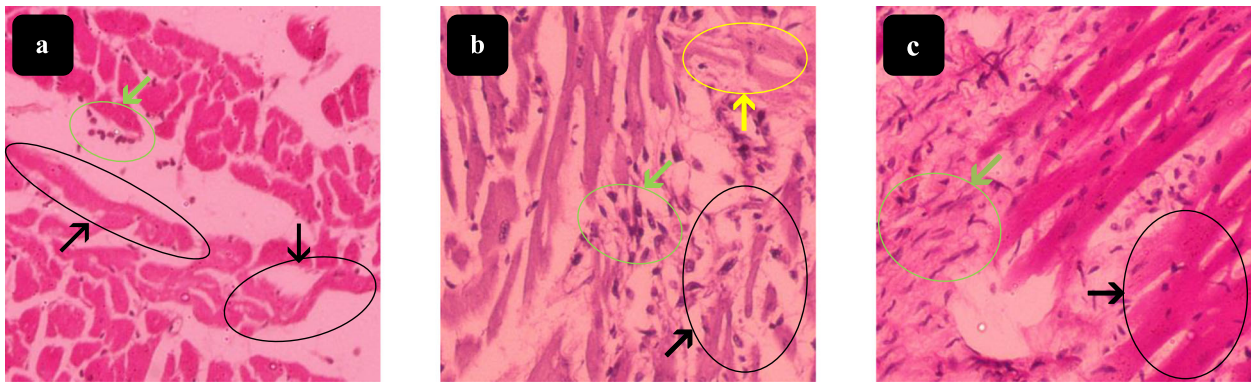

Fig. 2 Cardiac histology of ISO administered rat with MI (Group 2): (**a**) sacrificed on 31st day exerted distorted myofibrils (pointed with black arrow) and nuclei aggregation (pointed with green arrow), (**b**) sacrificed on 46th day also showed pronounced inflammation (pointed with yellow arrow) in cardiomyocytes, damaged myofibrils (pointed with black arrow) and nuclei aggregation (pointed with green arrow), **(c)** sacrificed on 61st day depicted less pronounced myocardial damage with deformed myofibrils (pointed with black arrow) and nuclei aggregation (pointed with green arrow)

on day 31 and significant difference on day 46 and 61 ($p < 0.5$).

Discussion

The history of pharmacology of the East dating back thousand years proves that herbs and spices was a growing and major interest as a complementary and alternative medicine [29]. With the growing awareness of self-care and concern on the foreseeable adverse effects of conventional medicine, CVD patients accept herbal medicines more confidently for their unique characteristics in preventing and curing diseases, rehabilitation, and health care [30].

In this research we have selected the Bangladeshi multi clove garlic cultivar which exert highly commendable cardio-protective effect and commonly consumed in Bangladesh culinary. The yield of garlic powder is depends on moisture content in raw garlic and blending system. Moisture content in garlic is approximately 60–70% i.e. the yield of dried/powdered garlic is about 40–30% [31–33]. June, 2014 also found approximately 185 g i.e. 37% of garlic powder from 500 g of raw garlic [34]. In our study the yield of powder from raw garlic is about 26.68% which is closely relevant with the above mentioned studies. For GE preparation, here we used ultrasonic vibration for complete extraction from the sample because Altemimi et al., 2015 [20] and Anaya-Esparza et al., 2018 [35] studies have revealed that ultrasonic vibration increase the mean yield of plant extract and in our study we also obtained 50.25% total extract from crude powder that was a commendable yield of extraction.

To study the effect of GE against MI induced by ISO through histology and gene expression study, Wistar

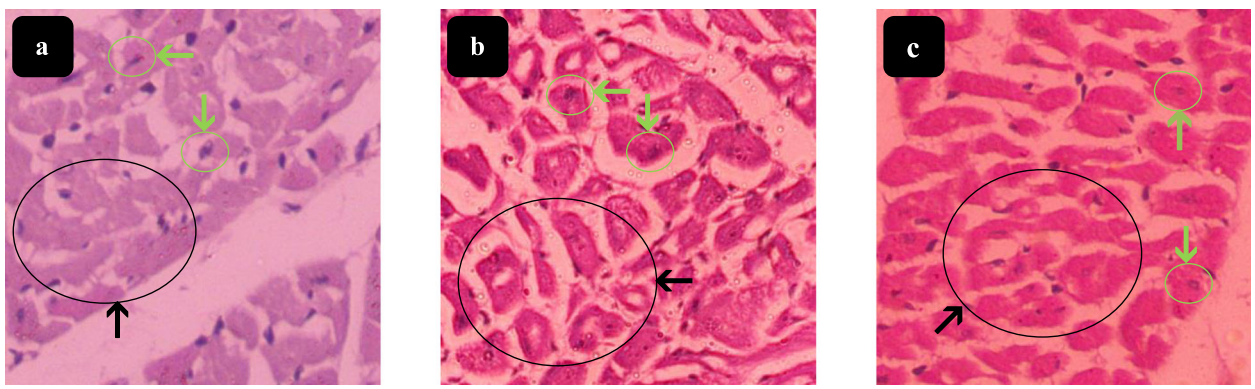

Fig. 3 Cardiac histology GE (200 mg/kg-bw/day) treated rat (Group 3): (**a**) sacrificed on 31st day revealed intact undamaged myofibrils (pointed with black arrow) and normal distribution of nuclei (pointed with green arrow), (**b**) sacrificed on 46th day depicted more pronounced normal and undamaged myofibrils (pointed with black arrow) and regular centered distribution of nuclei (pointed with green arrow), (**c**) sacrificed on 61st day exhibited the most evident normal cardioarchitecture with intact myofibrils without inflammation (pointed with black arrow) and disaggregated central distribution of nuclei (pointed with green arrow)

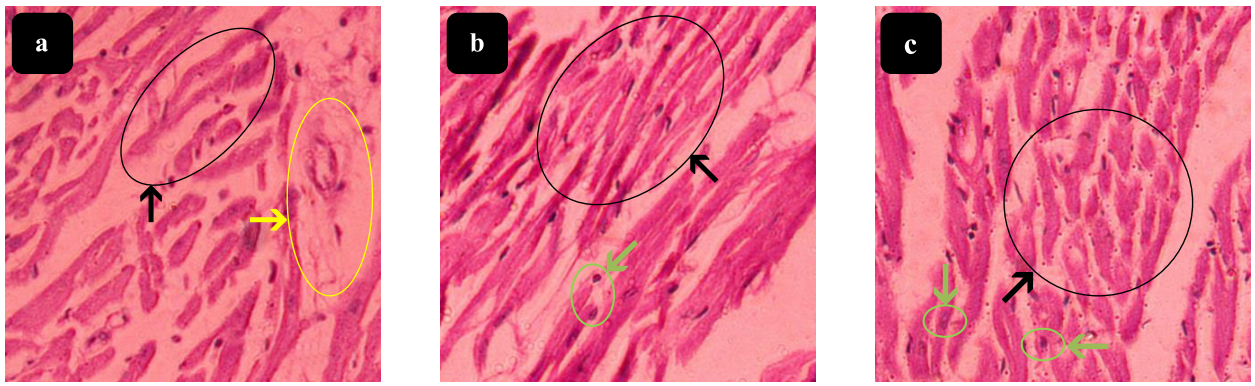

Fig. 4 Cardiac histology of (200 mg GE + ISO) treated rat (Group 4): (**a**) sacrificed on 31st day revealed highly pronounced edema with inflammation and nuclei aggregation (pointed with yellow arrow) in cardiomyocytes and distorted myofibrils (pointed with black arrow), (**b**) sacrificed on 46th day shows less prominent cardiac necrosis with less pronounced distortion of myofibril structure (pointed with black arrow) and less aggregation of nuclei (pointed with green arrow), **(c)** sacrificed on 61st day showed approximately similar looking cardiac histoarchitecture as normal control rats with normal structure of myofibrils (pointed with black arrow) and regular and central distribution of nuclei (pointed with green arrow) but some portions were still damaged

albino female rats were grouped and GE dose concentration at 200 mg/kg-bw/day was applied. Studies by Nwanjo and Oze 2008 [22], Budoff et al. 2009 [23], Ebrahimi et al. 2015 [24], Vibha et al., 2011 [25] have revealed that approximately similar dose was commendably effective against ISO induced MI. In this current study the selected GE dose from the existing studies was experimented to observe how much the apoptotic Casp3 gene regulates histological changes of cardiac tissue at different time intervals.

In this experiment, MI was induced into the experimental rats by injection of ISO subcutaneously at100 mg/kg-bw/day for two days. ISO, a β-adrenergic agonist, has been shown to cause infarction, such as heart muscle lesions in experimental rats. In ISO administrated rats with MI, the observed pathological and morphological

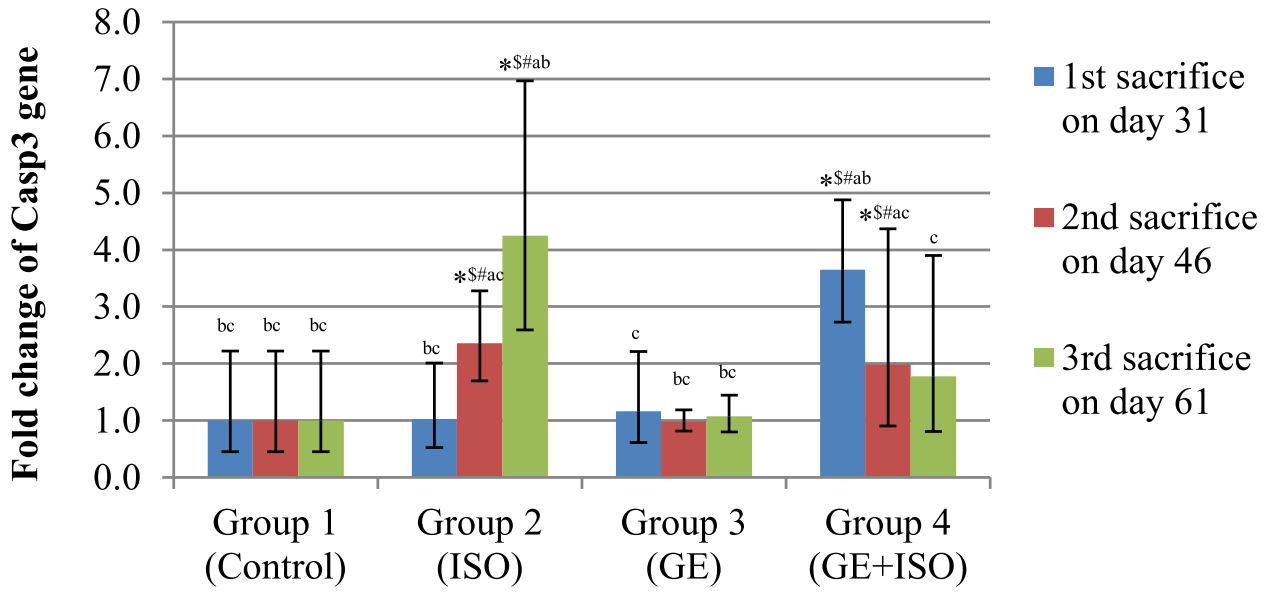

Fig. 5 Relative expression ($2^{-\Delta\Delta CT}$) of Casp3 gene. In Group 1 the same data of the day 31 was used for day 46 and 61 as this group was neither GE nor ISO administrated. Data are presented as fold change with fold difference from the control group. The error bars indicate fold change differences as a range of Casp3 gene. One-way ANOVA (analysis of variance) along with LSD post hoc test was used to analyze the $2^{-\Delta\Delta CT}$ values between different groups. For ANOVA the data of fold change differences were used. The findings were considered as statistically significant, if $p < 0.5$. *****, **$** and **#** indicate the fold change of CASP3 gene significantly different from Group 1 (control group) on the day of rat sacrifice i.e. day 31, 46 and 61 respectively and similarly **a**, **b** and **c** indicate fold change of CASP3 gene significantly different from Group 2 on the day of rat sacrifice i.e. day 31, 46 and 61 respectively

changes such as inflammation, lipid peroxidation, necrosis, hyperlipidemia, myocyte loss, increased calcium overload, changes in membrane permeability etc. are almost like to those observed in human MI [36, 37].

From the histopathological study (Figs. 1, 2, 3 and 4) it was found that the administration of ISO causes moderate to severe myocardial ultrastructure changes. ISO-induced rats with MI till the duration of this study were found infiltration of inflammatory cells, necrosis and disruption of cardiac myofibrils and large aggregation of nuclei that evident the severe myocardial necrosis and morphological aberration. Whereas GE pretreated MI rats exerted significant improving the regular arrangements of myofibrils and normal and central distribution of nuclei, even approximately similar to the normal individuals at the end of this study that was relevant to the findings of Khatua et al., 2016 [38].

Modern molecular genetic studies revealed that the histological changes may be occurred due to the up regulated expression of typical cardiac apoptotic and inflammatory genes [38–42]. Understanding the genetic basis of MI will not only provide insight regarding the pathogenesis of the disease but also a basis for the development of preventive and therapeutic strategies. One-Step RT-qPCR System has been used to assess changes in Casp3 gene expression that result from MI to gain insight into the underlying molecular basis of the disease.

Cysteine-dependent aspartate-directed protease, the caspases specifically play the role in the initiation and execution phases of apoptosis by splitting their target substrates at specific peptide sequences. During apoptosis, nuclear, metabolic or externally active stimuli activate the caspases in a cascade fashion which leads to nuclear engulfment and cell death [43]. In the myocardium of experimental animals with heart failure and apoptosis [44], in end-stage heart failure patients [45] and in patients with right ventricular dysplasia [46] elevated expression of Casp3 is found that refers to the association of Casp3 with human heart disease. However, anti-apoptotic interferences can delay ischemic myocardial damage in experiments. Plant based caspase inhibitors have been reported to be effective in reducing myocardial reperfusion injury, an action that was partially attributed to attenuation of cardiomyocyte apoptosis [47].

As in Fig. 5 according to Group 3 and Group 4, GE suppresses the Casp3 up regulation but when any stimulus like ISO is induced GE cannot withstand the up regulation tendency of Casp3 gene at the initial stage of MI. In a 14 days study by Khatua et al. 2016 [38] it was also observed that aqueous garlic homogenate and its one of active compounds diallyl disulfide up regulated the Casp3 gene expression when ISO was induced and cause cardiac hypertrophy. In this current study the expression levels of Casp3 gene at the initial stage i.e. on day 31 was significantly up regulated but gradually were down regulated in MI rats by GE and on day 61 no significant difference was observed compared to the control group ($p < 0.5$). Again as ISO, a β-adrenergic agonist, causes infarction in heart muscles, Casp3 is supposed to be up regulated instantly in contrast to the control group. But practically the Casp3 gene may not be stimulated by ISO at the early stage of induction; even the expression may be lower than the control group [38]. Similarly though the expression in the rats with MI initially were approximately similar to the control group, gradually the Casp3 gene expression was up regulated up to the last stage of this experiment with significant differences ($p < 0.5$). This confirms that GE has the potentiality of Casp3 gene down regulation. It has also been reported that active ingredients like s-allyl-cysteine sulfoxide (alliin) [14], diallyl trisulfide (DATS) [15] decreased Casp3 and protects the heart against ischemia/reperfusion injury in rats by inhibiting apoptosis. So inhibiting the expression of apoptotic Casp3 gene by GE treatment may reduce the risk of myocardial infarction.

The overall findings of this study were that the myocardial infarcted rats pretreated with GE not only improved the cardiac histo-architecture but also down regulated the apoptotic Casp3 gene expression gradually. This suggests that GE administration may gradually reduce the risk the of MI occurrence specially caused by apoptosis.

Conclusions

In conclusion, the present study demonstrated that GE reduced the ISO induced damage in rats with MI, by improving the anti-apoptotic capacity of the body through gradually down regulating the expressions apoptotic gene. Therefore, garlic preparations may be served with regular diets to minimize the occurrence of MI. Our findings suggest that garlic extract (GE) may provide a potential novel therapeutic approach for the treatment of MI.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40816-020-00199-4.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.

Abbreviations

CVDs: Cardiovascular Diseases; B2M: Beta2-microglobulin; Casp3: Caspase-3; Casp8: Caspase-8; Casp9: Caspase-9; BAX: B-cell lymphoma 2-associated X; TNF-α: Tumor necrosis factor α; MI: Myocardium Infraction; HF: Heart Failure; ISO: Isoproterenol; NaCl: Sodium Chloride; GE: Garlic extract; kg-bw: Kilogram body weight; fg: Femtogram; ng: Nanogram; mg: Milligram; gm: Gram; ml: Milliliter; μl: Microliter; μM: Micromolar; qPCR: Quantitative Polymerase Chain Reaction

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Authors' contributions
DI and DCR made an idea or hypothesis for research and/or manuscript and planned methodology to reach the conclusion; MBS, CL, SA and DCR took the responsibility in execution of the experiments, data management and reporting; DI, CL, LCM, EPL, MH and DCR took the accountability in logical interpretation and presentation of the results; MBS, MRI and DCR wrote of the whole or body of the manuscript; DI, MRI and DCR reviewed the article before submission not only for spelling and grammar but also for its intellectual content. All authors have read and approved the manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate
All rats were from the Biomedical and Toxicological Research Institute (BTRI) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. All procedures performed in studies involving animals were in accordance with the ethical standards of the 'Ethical Committee of BCSIR for Animal Research (ECAR)'. Approval no: 39.309.006.00.00.163.2014/403(2019–2).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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