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Original article

Cardioprotective properties of natural medicine in isoproterenol induced myocardial damage in the male Albino rats

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ABSTRACT

The main aim of this study is to investigate cardioprotective properties of natural medicine inmyocardial damage induced male Albino rats. The aqueous extractof *Allium sativum*was used for the determination of phenolic compounds and flavonoids. The amount of phenol (1.39 ± 0.37 GAE/g dry weight) and flavonoids (49.1 ± 2.79 QE/g dry weight) were high in aqueous extract. *A. sativum*extract and showed 68.39 ± 3.6% DPPHscavenging activity. Isoproterenol was used to induce myocardial injury in Albino rats *in vivo* by subcutaneous injection (100 mg/kg body weight). To achieve this, experimental animals were categorized into six groups (n = 4), namely, positive, negative control, only isoproterenol administered groups, and garlic extract administered group at 100–300 mg extract/kg body weight. Oxidative stress marker and cardiac markers were assayed to analyze the cardioprotective properties of garlic extract. At 300 mg/kg doseof garlic extract, rat was recovered from various altered factors such as, aspartate aminotransferase, alkaline transminase and alkaline phosphatase. The rats treated with 300 mggarlic extract/kg body weight decreased the level of asparate aminotransferase (126 ± 6.4 IU/L) than other lower doses (100 mg extract/kg and 200 mg extract/kg). Alkaline transaminase level of rat serum level was 81 ± 4.34 IU/L. In the isoproterenol treated rats elevated level was observed (152 ± 4.42 IU/L enzyme activity). Pretreatment of Albino rat with *A. sativum* extract reduced cardiac damage. Isoproterenol exposed animal showed 207.6 ± 1.2 mg/dL triglyceride and the garlic administered rat (300 mgextract/kg) reduced LDL-cholesterol level (61.3 ± 1.3 mg/dL) significantly ($p < 0.05$). Creatinine kinase -MB level was 269.5 ± 12.5 IU/L in the control animal and stress induced animal showed elevated level (572.3 ± 19.4 IU/L). Garlic treated experimental animal (300 μg/kg bw) decreased CK-MB level. To conclude, the aqueous extract of *A. sativum*showed cardio protective properties against myocardial injury.

1. Introduction

Cardiovascular diseasescause severe mortality throughout the world. In haemostasis mechanism platelets contributed in the development of various diseases (Sambrano et al., 2001). Hyperactivity of platelets and a sudden raise of coagulation process are the prominent factors in the formation of atherosclerosis and the development of thrombus (Linden and Jackson, 2010). During the process of haemostasis mechanism, platelets are generally activated. The activated platelets undergo changes in shape and secrete their intracellular granules, adhere to blood vessels, other platelets, and aggregate firmly. The formation of atherosclerosis is mediated by the interactions of the various proteins synthesized by platelets with the blood vessels of animals at the site of injury (Shahriyary and Yazdanparast, 2007). Calcium ion is essential in the release of various granules that leads to aggregation of platelets (Kim et al., 2008). The pathophysiological condition is mainly associated with increased stress, endothelial-, and chronic overactivation of the adrenergic system. These abnormal functions are involved in the progress and the development of heart failure (Russomanno et al., 2017). Generally, development of oxidative stress was linked with heart failure and the level of reactive oxygen species (ROS) has been increased in the myocardium. Generally oxidative stress happens due to variation in the endogenous antioxidant content in the body and the generated ROS involved in oxidative stress (Conti et al., 2017; Shazhni et al., 2018; Parameswari et al., 2019). Antioxidants are useful to delay or slow down the process of oxidation in cells by hindering the sequence of

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events. Medicinal plants have various antioxidant compounds that protect against several diseases, thus emerging as alternative therapeutic substances. Herbal medicines have rich of traditional value; also have good medical and public interest throughout the world as potential sources of new lead molecules for drug discovery (Dianat et al., 2014; Surendra et al., 2016). The medicinal plants such as, *Cichorium intybus*, *Mucuna pruriens*, *Hydrocotyle asiatica* Linn, *Tinospora cordifolia*, *Terminalia arjuna*, *Ginkgo biloba*, *Amaranthus viridis*, *Nerium oleander* (NO) Linn, and *Daucus carota* Linn showed cardioprotective activity. Digoxin is one of the phytochemical reported in *Digitalis lanata*which has lot of potential to treat cardiac diseases (Dec 2003). Atropineis one of the useful medicines to treat the symptoms of low heart rate which was characterized from the medicinal plant, *Atropa belladonna*(Vukajlovic et al., 2006). In recent years, research has been focussed to search low-cost natural antioxidants as alternative to various synthetic substances including, *tert*-butyl hydroquinone, and propyl gallate. These synthetic chemical substances are highly toxic to the animals (Baydar et al., 2007; Valsalam et al., 2019; Al-Ansari et al., 2019). In recent years various natural sources, nanoparticles and materials have been proposed for the treatment of cardiovascular diseases, energy and environmental applications (Al-Dhabi and Valan Arasu, 2018; Lee et al., 2021; Theerthagiri et al., 2021; Theerthagiri et al., 2020; Ramu et al., 2020; Malar et al., 2020).

Allium sativum L. is one of the important medicinal plants and has been widely used as alternative medicine. Medicinal plants from the genus Alliumreduced the risk of diabetes, various cardiovascular problems, enhance immunity to the organisms, anti-aging, anti-cancer, antifungal and antibacterial properties (Rahman, 2001). Aged garlic extract has been widely used to protect heart disorders, protect the development of cancer cells in various organs, and activate immunity to the organism (Mathew and Biju, 2008). Allicin is the one of the antioxidant substancesextracted from A. sativumthat showed an antioxidant property at very low doses. Diallyl trisulfide and diallyl disulfide are the allicin derived compounds also showed antioxidant and antimicrobial properties (Amagase et al., 2001). In this study the antioxidant properties of *A. sativum*was studied and to assess its cardioprotective role in experimental animal.

2. Materials and methods

2.1. Sample

The experimental sample (bulb from A. sativum) was obtained from the market. Sample was blended and homogenized with aqueous (100 mL) using a glass homogenizer. After complete homogenization, the extract was stirred for 20 min at 4 °C and filtered using four layered cheesecloth. The residue was again homogenized and repeated this process three times. It was further filtered using a filter paper (No.1) by vacuum filtration. The filtrate was collected and dried under vacuum (65 °C). The dried residue was re-suspended in aqueous (5 mL) and used as the sample for the determination of phytochemical analysis and antioxidant properties.

2.2. Analysis of total phenol

The extract was diluted with Millipore water (five-fold) and used for the determination of phenols. To the sample (0.1 mL), sodium carbonate (2.0 mL, 2%,w/v) and Folin–Ciocalteu's solution (0.6 mL) was added and incubated for 10 min at 30 ± 2 °C. Then the sample was read at 630 nm against reagent blank.

2.3. Analysis of total flavonoid

Aluminium - chloride method has been used for the determination of flavonoid. In this method, 0.2 mL diluted sample was mixed with potassium acetate (0.1 mL, 1 M), aluminium chloride (0.1 mL, 10%, w/v), anddiluted appropriately with Millipore water. The reaction was carried out for 10 min at room temperature and the OD of the reaction mixture was tested at 415 nm using a UV– Visible spectrophotometer. Quercetin has been used at various concentrations and standard curve was plottedat 10 – 100 μg/mL.

2.4. Antioxidant activity of A. Sativum extract

1,1-diphenyl-2-picryl-hydrazil (DPPH) method was applied for the analysis of antioxidant power of the extract. Rutin was used as the positive control. Briefly, the solvent extract was diluted appropriately (50, 100, 150, 200 and 250 μg/mL) and DPPH (2 mL) was incorporated. It was kept for 20 min at 30 ± 2 °C. Then the absorbance of the sample was measured using a UV– Visible spectrophotometer at 517 nm (Nahar et al., 2005).

2.5. Experimental animal

Adult male Albino rat (200 ± 25 gm) was used for thisexperiment. A total of 24 animals were maintained in the aluminium cages (1 × 0.5 × 0.5 m) in an animal house with well-ventilation at 30 ± 2 °C with 12 h light/dark condition. The humidity 60 ± 5% was maintained under standard laboratory condition. The laboratory animals were maintained according to use of laboratory animals. This work has been approved by the ethical committee.

2.6. Experimental procedure

The experimental animals were grouped into six having 4 animals each (n = 4). Experimental rats were acclimatized for 14 daysbefore to perform the experiments.Aspirinwas injected at 1.0 mg/kgbw and various doses of *A. sativum*(100, 200, 300 mg/kg body) were administered orally to the experimental groups. To the control group, double distilled water was provided. Then, isoproterenol was administered intraperitoneally to the rat (100 mg/kg bw). To the first group, physiological saline was administered and only isoproterenol was provided to the second group. To the experimental groups III, IV and V, 100, 200, 300 mg/kg body weight of extract was administered. To the group VI, asprin was administered (1.0 mg/kg bw) before intraperitoneal injection of isoproterenol.After the experiment, blood was withdrawn from the experimental and control animals without anticoagulant.

2.7. Biomarker analysis from the serum sample

Cardiacmarkers were analyzed from the serum sample. After 48 h of the final treatment, blood was collected from the control and experimental animals. Serum samples were subjected for the determinationof HDL-C, LDL-C, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, cholesterol and triglyceride. HDL-cholesterol was estimated by the method of Kostener (1977) and LDL cholesterol was assayed as suggested by Glatter (1984). Total cholesterol content was analyzed as described previously (Allain et al., 1974). Alanine transaminase and aspartate aminotransferase were analyzedas described previously (Moussa and Bashandy, 2008).

2.8. Biomarker analysis from the heart tissues

The experimental animals were sacrificed and heart muscle was separated. The muscle was repeatedly washed with sodium

phosphate buffer (pH 7.0). Then it was centrifuged at 10000 rpm for 10 min and clear extract was obtained. It was used for the analyses of CPK and LDH. Analyses were performed using clinical diagnostic kit (Merck, Germany). Creatine kinase–MB of heart muscle was assayed from the tissue homogenate using Urdal and Landaas (1979) method.

2.9. Data analysis

One - way analysis of variance (ANOVA) was used to evaluate the significant changes of biomarker level between control and treatment groups.

3. Results and discussion

3.1. Total flavonoid and phenolic content of aqueous extract

Total flavonoid and phenol contents were determined using aqueous extract. Aqueous extract showed the presence of flavonoid (49.1 ± 2.19 QE/g dry weight) and phenolic compound (1.38 ± 0.37 GAE/g dry weight). The amount of phenolic content was generally described as gallic acid equivalent (GAE), which described to the average value of the all available phenolic substances detected in fruits and vegetables (George et al., 2005). Generally, antioxidant properties of medicinal plants are associated with the availability of these phenol containing molecules (Cai et al., 2004). Flavonoids are highly complex structures have various function in plants and these have protective roles against various pathogens, insects and are considered as natural antioxidants (Franco et al., 2007). In plants, phenolic-type compounds involved in antioxidant properties, however no positive correlation has been reported (Chandrasekara and Shahidi, 2011).

3.2. Antioxidant properties

To determine antioxidant potential of phytochemicals, 2,20-diphenyl-1-picrylhydrazyl (DPPH) assay was performed. The aqueous extract of *A. sativum* showed 68.39 ± 3.6% DPPH inhibitory activitiesat 250 μg/mL concentration of the extract (Fig. 1). The antioxidant properties of plants mainly based on the existence of organosulfur and phenolic compounds (Boivin et al., 2009; Miller et al., 2008). *A. sativum* contains flavonoids, phenols and various sulphur containing compounds including, disulfide and S-ally-(l)-cysteine. These compounds have potential radical scavenging activities (Colin-Gonzalez et al., 2012). The number of flavonoids and phenolic compounds were positively correlated with DPPH scavenging activity due to the donation of hydrogen ion from hydroxyl groups of these phytochemicals (Rice-Evans et al., 1996). In *A. sativum*, allicin is the major compound that showed antibacterial, antifungal and antioxidant activities (Martins et al., 2015). In a study, the enhanced antioxidant property of garlic has been reported after heat treatment, and maximum activity was observed after three weeks (Zhang et al., 2015). Recently, Jang et al. (2018) analyzed antioxidant property of aged and fresh garlic in various solvents.

3.3. Protective role of A. Sativum extract on isoproterenol induced hypercholesterolemia and hypertriglyceridemia

Cardioprotective properties were confirmed by the decreased cardiac marker in the serum sample of the experimental animal. Pre-treatment of Albino rat with *A. sativum* extract reduced cardiac risk in various ways. Elevated level of LDL (92 ± 1.2 mg/dL), total cholesterol (207.6 ± 1.3 mg/dL) and triglyceride was observed in the isoproterenol treated Albino rat. Pre-treatment with *A. sativum* at three different doses revealed considerable reduction in lipid levels. Triglyceride content was 69.2 ± 1.6 mg/dL in the control and it increased as 164.8 ± 2.2 mg/dL in the stress induced animal. Likewise, isoproterenol exposed animal showed 207.6 ± 1.2 mg/L triglyceride in experimental animal and the garlic extract administered rat (300 mgextract/kg) reduced LDL-cholesterol level (61.3 ± 1.3 mg/dL) ($p < 0.05$) (Fig. 2).

The lipid lowering effect was mainly due to the increased bile acid secretion, increase in HDL cholesterol, and increased uptake of cholesterol from the body by the liver. Elevated level of lipids (hyperlipidemia) induced the development of cardiovascular diseases and arthrosclerosis (Hassarajani et al., 2007). High concentration of low-density lipoprotein,total cholesterol, triglycerides and reduced level of high-density lipoprotein was found in the sodium fluoride induced experimental rats (Abdel-Wahab, 2013). The elevated level of cholesterol in isoproterenol induced rats causing interference in lipid metabolism and results elevated level of lipid profile. The experimental animal treated with isoproterenol caused hyperlipidemia. Grucka-Mamczar et al. (2004) tested oxidative stress in animals after the administration of sodium fluoride to experimental animal and found the inhibitory effect of pyro-phosphatase, unspecific esterases, lipases and phospholipases. Parikh et al. (2015) observed a positive relationship between increased serum LDL-cholesterol level and developing ischemic heart disease. Garlic supplemented diet involved in the alterations in lipid content has been previously described. It involved in deac-

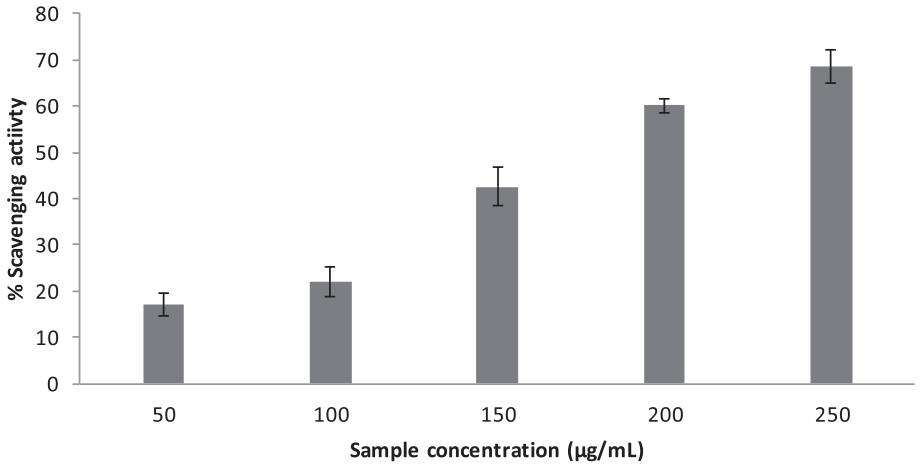

Fig. 1. DPPH scavenging activity of garlic extract *in vitro*. Garlic sample was used at various concentrations and % scavenging activity was determined.

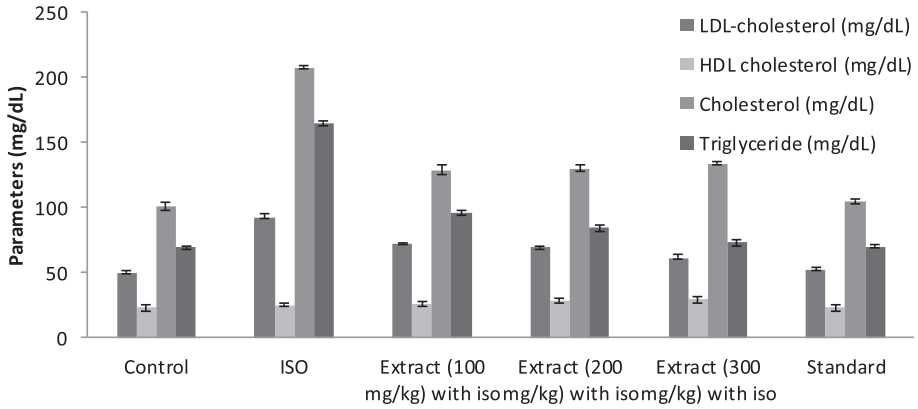

Fig. 2. Isoproterenol induced hypercholesterolemia and hypertriglyceridemia and cardioprotective properties of *A. sativum* extract.

tivation cholesterol synthesis and decreasing fatty acid synthase, glucose-6 phosphate dehydrogenase, and malic acid (Yeh and Liu, 2001). Gebhardt and Beck (1996) reported cholesterol-lowering ability of garlic and the inhibitory role of garlic on hydroxyl methyl glutaryl CoA. Garlic suppressed the LDL oxidation and reduced the level of LDL (Lau, 2001).

3.4. Cardiac markers and analysis of cardioprotective properties in experimental rat

Isoproterenol induced significant increase in Aspartate aminotransferase, alkaline transminase and alkaline phosphatase in experimental animals. Moreover, the garlic extract administered animal reduced these enzymeactivities ($p < 0.05$) in the blood. In the control animal, aspartate aminotransferase was 120 ± 3.21 IU/L and this increased as 146 ± 3.31 IU/L in isoproterenol exposed animal. In the experimental animals treated with 100, 200 and 300 mg/kg body weight garlic extract decreased the level of aspartate aminotransferase (138 ± 12.4, 129 ± 11.3, 126 ± 6.4 IU/L) (Fig. 3). Alkaline transaminase level of animal serum level was 81 ± 4.34 IU/L. In the isoproterenol treated rats showed 152 ± 4.4 2 IU/L. Alkaline phosphatase level was 49.2 ± 2.22 IU/L and it elevated in isoproterenol administered rats.

The damaged myocardium results elevated levels of various diagnostic biomarkers involved in myocardial infarction. This condition revealed heavy discharge of biomarkers (Upaganlawar et al., 2009). Monitoring the levels of creatinine kinase-MB, creatinine kinase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase are very important for the determination of myocardium damage (Al-Dhabi et al., 2018). Creatinine kinase is an important enzyme present in urinary bladder, colon, brain and heart muscle (Atif et al., 2020; Al-Dhabi et al., 2020). Elevated level of creatinine kinase-MB and creatinine kinase level were useful for the determination of myocardial damage. Creatinine kinase-MB was highly specific for heart muscle damage (Jaffe et al., 2000). Isoproterenol induced elevated level of creatinine kinase and lactate dehydrogenasein rats. CK-MB level was 269.5 ± 12.5 IU in the control animal and stress induced animal showed 572.3 ± 19. 4 IU. Garlic treated experimental animal (300 μg/kg bw) decreased CK-MB level considerably ($p < 0.05$) (Fig. 4). Aspirate aminotransferase, alkaline transaminase and alkaline phosphatase level in experimental animals caused significant depletion of various biomarkers than control groups. In sodium fluoride-treated rats, elevated levels of cardiac biomarkers such as, ALT, AST and LDH were reported (Yıldırım et al., 2018). Recenty, Avula et al. (2014) reported isoproterenol induced toxicity in experimental animal and reported cardiac protective role of garlic extract. Severe stress to the heart muscle will induced elevated levels of cardiac enzymes in serum in experimental animals (Badole et al., 2015). The marked reduction of various markers in the animal blood indicated the pre-

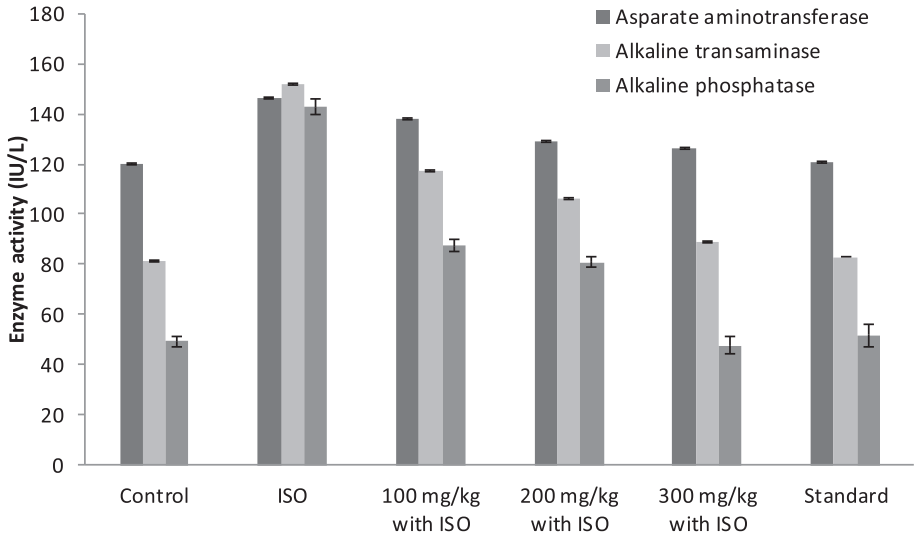

Fig. 3. Effect of garlic extract on elevated level of cardiac markers in experimental animal.

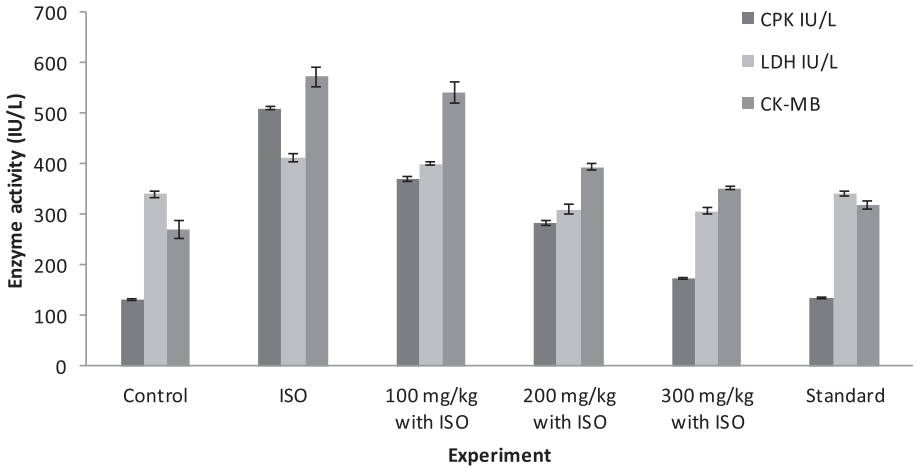

Fig. 4. Influence of garlic extract on CPK, LDH and CK-MB levels in experimental animals.

ventive role of extract to protect the leak of the important biomarkers and maintain membrane integrity and help to protect the cell membranes from damage (Rahman and Lowe, 2006). In garlic extract the phytochemicals such as, Sallylmercaptocysteine, sulfur metabolites and S-allylcysteine have significant antioxidant properties. These phytochemicals and or derivatives have cardiac protective activity (Asdaq et al., 2010). The compound diallyl polysulfide isolated from garlic extract was effectively protected the animal from cardiac damage (Bradley et al., 2016).

3.5. Effect of garlic extract oncardiac markers in heart muscle

CPK and LDH level in the heart muscle of experimental animals were described in Fig. 5. The amount of myocardial serum marker in the isoproterenol group was higher than control animals ($p < 0.05$), however in experimental animal treated with 100–300 mg extract showed lower amount of cardiac markers indicated reduced cardiac damage. In this study cardio protection of aqueous extract of A. sativum was confirmed the presence of lower level of various serum markers of heart and increased level of CAT and SOD (Fig. 6). The amount of CAT and SOD level increased more than 30% in the experimental animal treated with 200 and 300 mg extract. Pre-treatment of A. sativum extract significantly reduced isoproterenol induced oxidative stress in various ways. Elevated level of LDL, total cholesterol and triglyceride indicated the interfering role of isoproterenol in experimental animal (Wang et al., 2020). Heart muscle injury in experimental Albino rats was evidenced by increased level of various markers. The elevated level of LDH in serum shows damage of mitochondria and leakage of mitochondrial enzymes (Othman et al., 2008; Chen et al., 2020; Seshadri et al., 2020). Treatment with isoproterenol increased elevated level of various cardiac markers. The animals pre-treated with A. sativum extract and standard were more protective role against cardiac muscle damage.

4. Conclusions

Garlic extract has cardioprotective properties induced by isoproterenol in male Albino rats. Alkaloids and phenol containing compounds antagonize the stress induced by isoproterenol in experimental rat. The administered aqueous garlic extract protectedthe animal from cardiac damage. Garlic extract at highest concentration reduced myocardial damage and reduced elevated level of cardiac markers in heart muscle.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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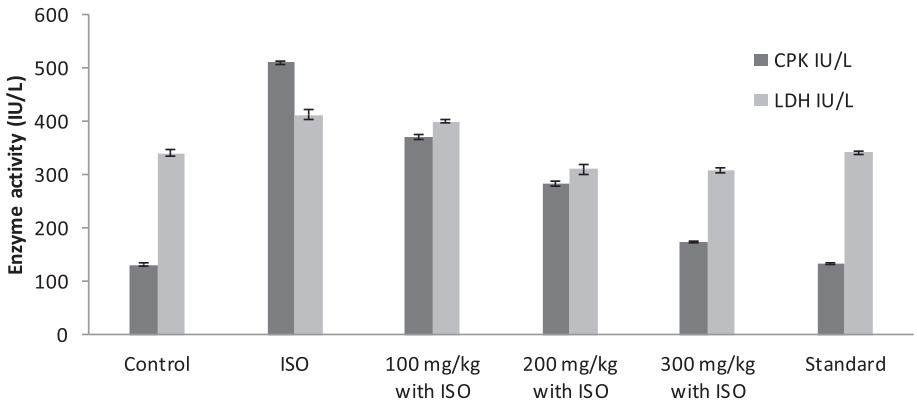

Fig. 5. CPK and LDH level in the heart muscle of experimental animals.

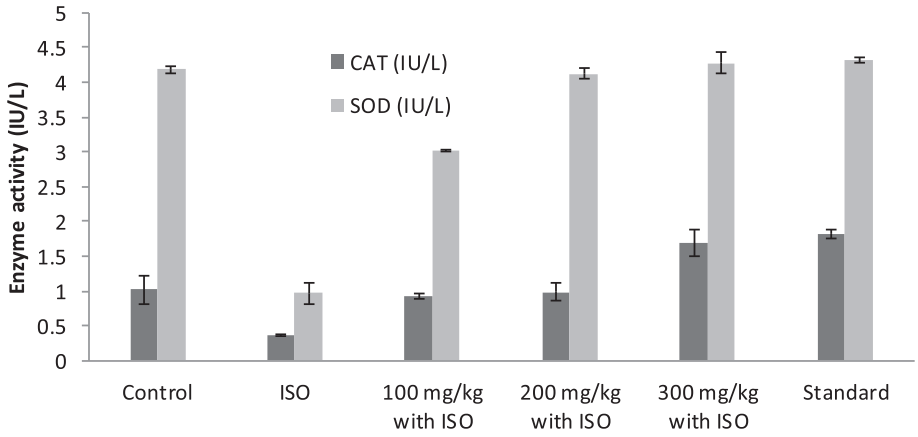

Fig. 6. CAT and SOD activity in the heart muscle of the experimental animals.

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