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Article

Cardioprotective effects of native herb-derived cardiac tonic on infarct size in a mouse model of experimental myocardial infarction

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Abstract: Cardiac remodeling is a significant issue involving molecular, cellular, and interstitial changes that affect the size, mass, integrity, and function of the heart following a myocardial infarction (MI). We hypothesized that therapy with mixed herbal extracts has a protective effect in mouse models of MI. The underlying mechanisms are thought to be protective against left ventricular (LV) remodeling through reduced collagen deposition. We therefore studied the effects of mixed herbal extracts commonly used in various ways in the belief that they have beneficial effects on cardiac disease. MI was induced by left anterior descending (LAD) coronary artery ligation in switch albino mice. Ethanol extracts of herbs Arjuna bark, oleander leaves, ginseng root, garlic, cardamom seeds and oregano leaves were prepared by ethanol extraction and rotary evaporation technique of dried herbs and then mixed the extracts to make a therapeutic cocktail. The mixture is then administered orally by lavage for 28 days. At the end of 28 days cardiac tissues were harvested and stored for histopathology analysis and assessment of SOD levels. Survival curve data over a period of 28 days showed significant (P<0.05) differences between groups and mortality was reduced in the herbal extract treated group. Heart mass especially ventricular and atrial space and ratio of infarct to total heart area revealed better improvement in treated group compared to untreated group. Histopathological studies showed that the myocardium of the herbal extract treated group had fewer collagen fibers than the non-treated group. SOD activity levels were up-regulated in animals treated with herbal extracts indicating reduction of oxidative stress by herbal extract mixture. Hence, their effects reduce collagen deposition, reduce LV mass and prevent myocardial tissue damage, reduce structural changes and increase survival by their anti-stress effects. Mixed herbal tonic may provide a new therapeutic approach in ischemic cardiomyopathy and ischemic heart failure.

Keywords: herbal extract; coronary infarction; mortality; animal model; remodeling

1. Introduction

Myocardial infarction (MI) due to coronary occlusion leads to myocardial remodeling and heart failure, leading to cardiovascular-related death due to hypoxia, oxidative stress, and myocardial cell apoptosis (Moe and Marín-

García, 2016; Teringova and Tousek, 2017). The World Health Organization (WHO) reported in 2017 that cardiovascular disease (CVD) is a major concern responsible for 31% of deaths worldwide, while in Bangladesh it may account for 80% of deaths (Laslett *et al*., 2012). This high death rate is very alarming and measures should be taken to reduce it. Most people in our country rely only on generic drugs for CVD but do not take preventive and management measures for CVD. Cardiac tonics can be a good adjunct to medication for people at high risk for CVD. Recently, several plants used in traditional medicine have shown promise in alleviating cardiovascular diseases such as hyperlipidemia and ischemic heart disease. Our hypothesis is that a combination of herbal extracts might enhance their overall effect on CVD. To investigate this, we prepared a mixture of herbal extracts and tested its cardioprotective effects using a left anterior descending (LAD) coronary artery occlusion model in mice. Left ventricular (LV) remodeling after MI is often linked to heart failure, posing a major source of morbidity and mortality and significantly impacting the heart's size and shape. Drug therapy aimed at preventing infarct expansion is crucial to limiting LV remodeling. Future advancements in preventing post-MI heart failure will not rely solely on identifying drugs targeting specific mechanisms and diagnostic techniques. Improved preventive and management practices are needed. Reports indicate that approximately 1.2 million Americans experience a new or recurrent MI each year, making coronary artery disease the leading cause of death in the USA (Brent and Christopher, 2007). Another study reported that approximately 38% of people who have an MI in a given year will eventually die (Rosamond *et al*., 2007). Therefore, various novel therapeutic approaches are being explored to reduce MI size in patients with acute coronary syndromes. Current studies focus on the use of indigenous herbs reputed for their beneficial effects on cardiovascular conditions. Botanical and herbal medicines have increasingly been integrated into modern clinical practice. The selection of plants in this study was based on their documented medicinal properties and toxicology, as reported in various research articles and reviews. Among the herbs included, *Terminalia arjuna* bark stands out as a potent cardio-active agent, known for its ability to improve left ventricular parameters and functional capacity (Dwivedi and Chopra, 2014; Amalraj and Gopi, 2016). They also reported that *T. arjuna* exhibits a range of activities, such as antioxidant, hypotensive, antiatherogenic, and anti-inflammatory effects. The bark extracts of *T. arjuna* have various pharmacological properties, including inotropic, anti-ischemic, antioxidant, blood pressure-lowering, antiplatelet, hypolipidemic, antiatherogenic, and antihypertrophic effects (Maulik and Kumer, 2012). *Narium oleander*, another perennial herb, crude ethanol extract of its dried leaves has been tested on isolated guinea pig hearts and shown to improve contractile force, heart rate and cardiac output by its digoxin like action (Adome *et al*., 2003; Gayathri *et al*., 2011). Previous study of Tang *et al*. (2023) showed that, ginsenoside could inhibit coronary artery dysfunction and increase coronary blood flow and reduce infarct size. *Allium sativum* (garlic) has cardioactive functions such as it prevents atherosclerosis and hyperlipidemia (Sun *et al*., 2018). Reports are also available that cardamom seeds are beneficial for stress-induced myocardial damage (Verma *et al*., 2010). Oregano leaves provide antioxidants (Dabravolski *et al*., 2023), and essential oil (Cheng *et al*., 2018).

In the present investigation, we attempted to develop a mixed herbal cocktail to be used in a rat MI model to investigate the potential cardioprotective effects. The plant ingredients used in this study were arjuna bark, oleander leaves, ginseng root, garlic, cardamom seeds and oregano leaf extract as a therapeutic cocktail. Although, the combined herbal extracts each act by different mechanisms, the cumulative action would more beneficial in preventing infarction. The test and formula were designed to evaluate this hypothesis with the objectives of mixed herbal extract treatment preventing adverse structural remodeling after MI by inhibiting LV dilation, myocardial collagen deposition, and ultimately reducing mortality. The goal of the present investigation aimed to establish a tonic for the heart for adjuvant therapy including allopathic to reduce mortality due to cardiac remodeling after myocardial infarction. The findings would provide a strong rationale for ethnomedicinal use in CVD morbidity.

2. Materials and Methods

2.1. Ethical approval

The research protocol was reviewed and approved by the BSMRAU Animal Research and Ethics Committee (AREC) for the use and care of experimental animals in research. The experiment was conducted at the Department of Physiology and Pharmacology and the Department of Anatomy and Histology of BSMRAU after approval from the Institutional Ethical Committee for the use of animals for experiments. Ethical approval Ref. No. FVMAS/AREC/2023/44.

2.2. Experimental animals

Male Swiss albino mice, aged 10 to 12 weeks and weighing between 35 to 45 grams, were used in this study. The animals were sourced from ICDDRB, Mohakhali, Dhaka, and housed in standard laboratory conditions

within polyacrylic cages. They were kept in an air-conditioned room with a stable temperature of 25 ± 2 °C, following a natural light/dark cycle of approximately 14 hours of light and 10 hours of darkness. The mice had unrestricted access to standard mouse chow and tap water and were allowed to acclimate for one week prior to the experiments. The commercial pellet diet provided contained 24% protein, 5% fat, 4% fiber, 55% carbohydrate, 0.6% calcium, 0.3% phosphorus, 10% moisture, and 9% ash. All experiments were conducted between 9:00 and 16:00 hours.

2.3. Experimental models of myocardial infarction

In the present study, myocardial injury was induced using an open chest left anterior descending (LAD) coronary artery occlusion method. This involved creating a permanent occlusion of the left coronary artery (LCA) to induce myocardial infarction (MI) in the mice, following previously established protocols (Maruyama *et al*., 2021). Briefly, mice were anesthetized with ketamine (90 mg/kg) and xylazine (2 mg/kg) before undergoing a left lateral thoracotomy to perform the ligation. To manage pain from the surgical procedures, flunixin meglumine (Meglunix Vet Inj, Popular Pharmaceutical Ltd) was administered subcutaneously at a dose of 2.5 mg/kg body weight. The mice were sacrificed 28 days after MI induction, and cardiac tissue samples were collected for histological and chemical analysis. Only mice with successful LAD ligation were included in the final analysis.

2.4. Preparation of herbal extracts

Hydro-alcoholic extracts of arjuna bark, oleander leaves, ginseng root, garlic, cardamom seeds and oregano leaves were prepared in the laboratory. Arjuna bark was extracted based on the modified method of Subramaniam *et al*. (2011). After ringing with water the fresh bark was air-dried and then oven-dried at 40°C. Then 750 g of ground bark powder was ground and soaked in 1 L of 80% ethanol at 4°C overnight to make dried bark powder. The liquid part was collected through the mesh connected with funnel and filtered by the whatman filter paper grade-40. Ethanol were evaporated from suspensions by rotary evaporator (RE-100 Pro, DLAB, USA) connected to DLAB water bath and chiller. In this case, a pressure of 175 mbar (to remove ethanol) and 72 mbar (to remove water) was maintained continuously at a rotation speed of 160 rpm, resulting in the extraction of 75 g of the extract. Oleander leaves were extracted using a modified method based on Adome *et al*. (2003) and Abdou *et al*. (2019). The leaves were washed in clean water and dried in hot air at 40°C, and 500 g of dried leaves were extracted several times with 90% ethanol and finally evaporated as described above. The ether extract of ginseng was prepared from Korean red ginseng were extracted according to the procedure stated by Lee *et al.* (1981) with slight modification which was grinded and extracted 500 g of fine powder in 1 L of 90% ethanol for overnight at 4°C on each extraction. Garlic was extracted based on the method described by Rahman and David (2000). Garlic slices were soaked overnight at room temperature in 15–20% aqueous ethanol then filtered and evaporated under reduced pressure and low temperature to obtain the extract. Ethanol extract of cardamom seeds was prepared following the method described by Goyal *et al*. (2015). For each extract, 50 g of dried and crushed powder for each extract was placed in a flask with 300 mL of ethanol. The solvent was evaporated until a crude extract was obtained. Dried oregano leaves were crusted and powdered for ethanol extraction as per the method of Tsai *et al*. (2013). Briefly, 50 g of fine oregano powder was immersed and extracted with 500 mL of ethanol. The ethanol extract was then collected and evaporated. Finally the mixture of all extracts was prepared as Table 1.

Table 1. The extraction preparing mixture and doses.

Name of the herbs	Amount of the herbs/ amount of the alcohol	Amount of extract used in mixture	Final dose of extract received by the experimental animal (0.5ml/40 gm body weight)
Bark of arjuna	750 g in 1 liter Ethanol	200 mg/25 mL	100 mg/kg-bw/day
Leaves of oleander	Exactly 500 g 1 liter Ethanol	200 mg/25 mL	100 mg/kg-bw/day
Root of ginseng	500 g of fine powder which was exposed 1littre ethanol	80 mg/25 mL	40 mg/kg-bw/day
Garlic	400 gm in 1200 ml ethanol	400 mg/25 mL	200 mg/kg-bw/day
Cardamon seed	50gm 300 ml Ethanol	200 mg/25 mL	100 mg/kg-bw/day
Oregano leaves	50 gm 500 ml Ethanol	400 mg/25 mL	200 mg/kg-bw/day

2.5. Hematoxylin and eosin staining

The heat were excised and preserved in formaldehyde for hematoxylin and eosin (H&E) staining. Heart Section were stained with according to the method described in available literature. Briefly, 10µ tissue section was taken on slide and heated at 37^{o}C using hot plate for 20-30 min then immersed in 4% paraformaldehyde for 10 min. The tissue samples were first immersed in diluted ethanol, followed by staining with Mayer's hematoxylin (Sigma Aldrich, USA) for 30 seconds, subsequent washing, and staining with alcoholic eosin. They were then dehydrated through another ethanol series, followed by three changes of xylene for one minute each. A coverslip was mounted over the sections using xylene-based mounting media. Subsequently, the stained samples were imaged at 4x magnification using a microscope equipped with a Zeiss axiocam 705 camera. The acquired images were then transferred to a computer for further analysis.

2.6. Picrosirius red staining

Some sections were used for picrosirus red (Polyscience, India) staining for analysis of collagen deposition in heart tissue as per the supplied protocol. Stained samples were then imaged at 20x magnification. Collagen accumulation in infarcted and non-infarcted areas was quantified as percentage of stained tissue in random areas of muscle and connective tissue using ImageJ software (NIH). Photographs of four separate regions are considered for representative Sirius red-stained cross-sections of non-infarcted control and 28-day infarcted hearts in tissue.

2.7. Superoxide dismutase (SOD) activity

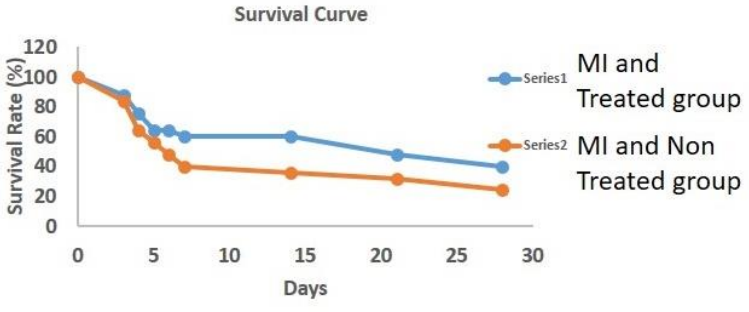
The SOD was determined in the same cardiac tissue homogenate using the SOD assay kit (Dojindo Molecular Technologies Inc. Kumamoto, Japan). The assay was prepared following the company's protocol and measured at 450 nm with an absorbance spectrophotometer (Biotech 800 TS, Agilent, USA).

2.8. Statistical analysis

The data are presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using the SPSS software package. Group differences were assessed using a Bonferroni post hoc test following a non-repeated one-way analysis of variance compared to the control group. For comparisons involving both time and treatment effects, a non-repeated two-way analysis of variance was performed, followed by a Bonferroni post hoc test for survival data over the 4-week period. A significance level of $P<0.05$ was considered statistically significant for all intergroup comparisons.

3. Results

MI mice surviving day 0 were observed and death was recorded up to day 28. The figure showed that the death rate was maximum between 3 and 10 days and gradually the death rate decreased. The MI group that received treatment had longer survival than the non-treated group, and at day 15 survival rates in these groups were 60% and 40%, respectively. Survival rates at the end of 28-days were in the upper range of 40% for those who received the herbal extract treatment. Survival, on the other hand, continued to decline in mice that did not receive treatment, and 24% survival was found at the end of 28 days after MI (Figure 1).



Day	0	3	4	5	6	7	14	21	28
Treated	25	22	19	16	16	15	15	12	10
Non Treated	25	21	16	14	12	10	9	8	6

Figure 1. Survival curves were constructed for treatment efficacy between groups. Herbal extract treatment significantly ($P<0.05$) increased the survival rate compared to the non-treated group. Animal were day matching surgery and treatment for 28 days. Animal mortality and survival were calculated according to date of surgery and treatment given.

Histopathology evaluation of the photograph shows a clear border between the living and infarcted regions in Figure 2. The data indicated that the infarct area was greater in the hearts of untreated mice and the mean ratio of infA/TotalA in the untreated group was measured to be 0.28 ± 0.07 while the values in the treated group were calculated to be 0.20 ± 0.04. These values were significantly reduced in the treated group compared to the non-treated group as shown in Figure 3. Sham surgery showed no evidence of ischemia, while MI groups showed dead and healthy tissue areas on H&E stained images of whole heart sections (Figure 3).

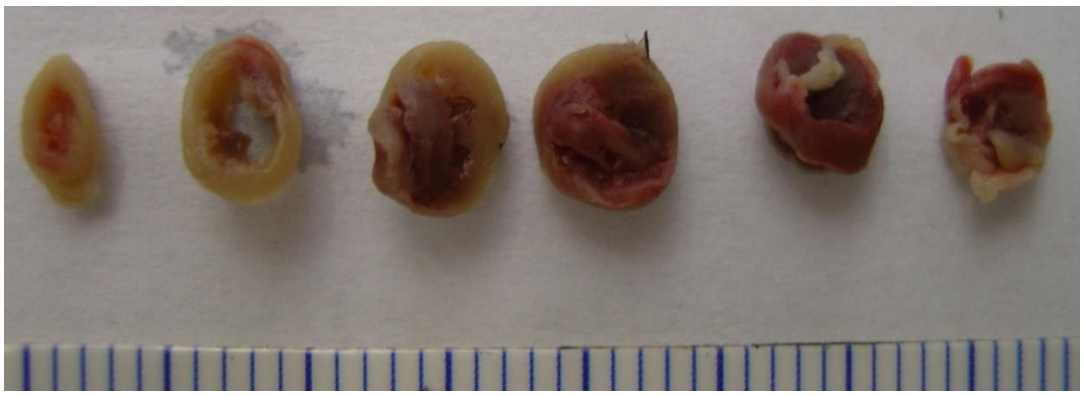


Figure 2. Images of heart slices from successfully induced MI to confirm infarction. At the end of 28 days after MI surgery, the heart was excised and approximately 3 mm slices were cut into 5-6 transverse sections with a surgical blade. The photograph was taken by an ordinary camera and transferred to the computer for observation and quantification using ImageJ software.

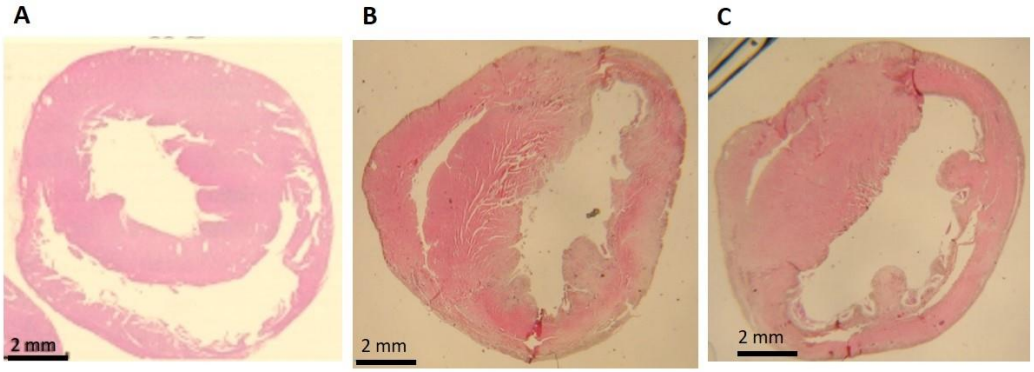


Figure 3. Coronary ligation in rat hearts showing extensive myocardial damage. Representative images show whole hearts at 4X magnification after 28 days. A. The chest cavity was opened in sham-operated rats, without LAD ligation. B and C from treated and non-treated groups, respectively, 28 days after LAD ligation. Histologic sections stained with H&E. Data from sham-operated mice are shown as controls. Images are representative of 5 MI mice.

Ventricular and atrial space of both groups were calculated and analyzed. Ventricular space was found to be significantly (P<0.05) decreased in the treated group compared with untreated MI. Differences in arterial space were not significant and a slight elevation was found in the treatment group. This structural change may indicate that the mixed herbal extract attempted to reveal the size and shape of the heart (Figure 4).

The observation under the microscope focused on the stained sections of the left ventricle and indicated that the myocardial tissue architecture and degree of necrosis, tissue mass and texture were found irregular in the non-treated group, on the other hand, all these contexts were found to be improved in the treated group. For detection of collagen tissue deposition picrosirius red staining was performed at 4 weeks after MI to visualize collagen deposition after MI (Figure 5). The microscopic observation revealed that collagen deposition in LV cross-sections was minimal in the control tissue. A high collagen deposition was observed in the non-treated (5B) and comparatively decreased in the treated (5C) group. Observational study conclude with a higher density of collagen deposition and a thicker fibrotic wall at the border-zone area (5B) compared with the Herbal extract treated group (5C).

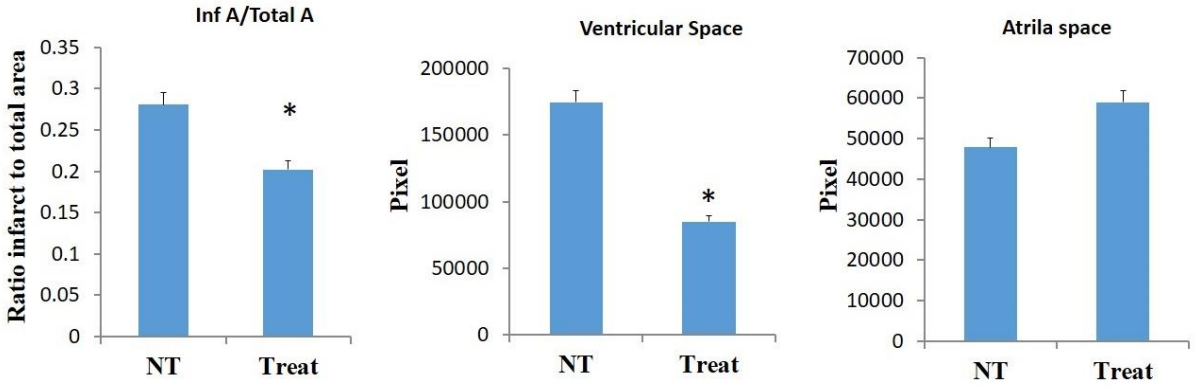


Figure 4. Cardiac mass was assessed after 28 days to measure quality. Whole heart slices and normal photographs were used to quantify healthy and infarct areas in hearts collected at day 28 after LAD ligation. The ratio of infarct area to total heart area, ventricular space, and atrial space was quantified in histological images stained with H&E using. Ratios and pixel values were calculated using ImageJ software and ANOVA statistical analysis was performed to express the mean value and level of significance. *P <0.05 considered statistical significant in compared with NT control vs. Treated group. Values= mean ± SEM; Inf A= infarct area, Total A= total area, NT = non-treated, Treat = treatment with herbal extract.

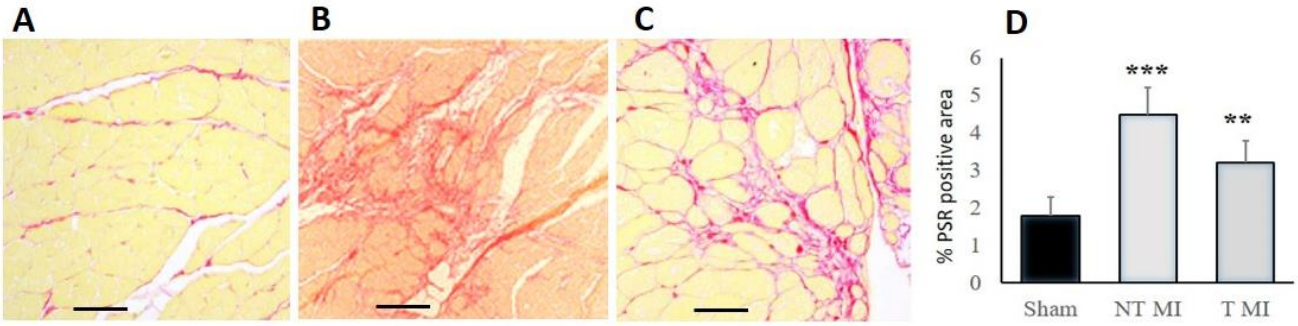


Figure 5. Heart section for quantification of fibrous connective tissue. The histological images here are representative picrosirius red-stained slides at 20X magnification 28 days after MI surgery. A. Sham control MI group; B. Non-treated MI group; C. Treated MI group. D. Measurement of cardiac fibrosis. **P<0.01 vs sham-operated animals. Values= mean ± SEM; SE= Standard error mean; ***P<0.001 compared with group. PSR= Picrosirius red, MI= myocardial infarction, NT= untreated, T= treated.

When collagen tissue was measured it gave a quantitative value in all groups and showed an average collagen count of 1.8 ± 0.5 percent in the control group whereas values for untreated and treated groups were found to be 4.5 ± 0.7 and 3.2 ± 0.6 percent, respectively (5D). There found a significantly difference of P<0.001 for non-treated vs. control and P<0.01 for treated vs. control. The herbal extract showed a greater than 30% reduction in collagen deposition in the treated group compared to the non-treated group. Superoxide dismutase (SOD) activity plays an important role in cardiac tissue when it is challenged by ischemic conditions. SOD then tries to protect it by the underlying oxidant-antioxidant balance mechanism. For this reason the SOD values of three groups of cardiac tissues were determined. In control hearts the value was found to be 2.35 ± 0.06 U/mL. Whereas the values recorded were 1.15 ± 0.07 U/mL and 1.62 ± 0.08 U/mL in non-treated and treated groups respectively. The mean SOD activity was significantly decreased (P<0.01) in the non-treated group that survived compared to the control and treated groups. Mean SOD activity was significantly (P<0.05) higher in patients receiving herbal extract treatment than non-treat group at the end of 28 days of MI.

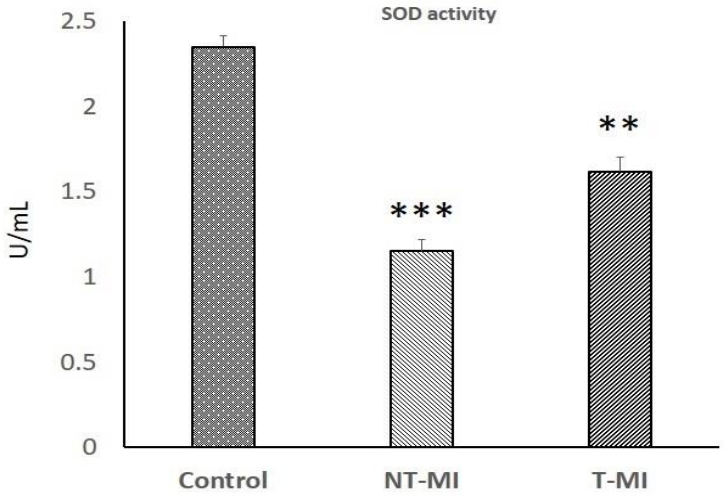


Figure 6. Superoxide dismutase (SOD) activity (U/mL) in cardiac tissue of control, NT-MI and T-MI groups was measured by colorimetric assay. Values are expressed as U/mL superoxide anion. Value= mean ± SEM; NT-MI= untreated MI; T-MI= treated MI; MI= myocardial infarction; ******P*<0.001, *****P*<0.01 compared to control.

4. Discussion

Many studies have demonstrated the efficacy of natural medicines in treating myocardial infarction (MI). In recent years, several herbs have also been identified for their potential to enhance MI treatment through various inherent qualities. Since we used a mixed herbal extract instead of one, it is difficult to say whether an action is due to a specific herb. Therefore we will discuss the chemical properties and cardio potentiating action of all the plants used in this investigation. The results of the present study showed that herbal mixture treatment following MI in rats prevented ventricular size expansion, reduced collagen tissue deposition, increased antioxidant activity in MI tissue and improved the survival rate of mice. The most notable feature of this report is that the herbal extract mixture reduces mortality, which is expected to have a strong effect on LV lesion healing and remodeling. It is particularly useful for discovering new chemical constituents of cardiopotent medicinal plants and their pharmacological actions. *T. arjuna* is an anti-ischemic, anti-atherogenic and potent antioxidant agent that has exploded in various experimental and clinical reports. The results of the present study showed that the mixture has anti-ischemic effect which can significantly (P<0.05) reduce the ratio of infarct to total area (Inf A/Total A) in the treatment group compared to the untreated group (Figure 4). This results is suggested by previous study showed anti-ischemic action in subjects treated with 500 mg of *T. arjuna* extract along with other drug for 1 to 3 months. MI was significantly reduced, E/A ratio, a markers of left ventricular function, improved and anginal frequency decreased (Dwivedi *et al*., 2005).

While *N. oleander* is known for its cardiac glycoside content, which can be highly toxic to the heart, the lethal dose of oleander leaves varies significantly among different species. For instance, the lethal dose is approximately 250 mg/kg body weight in sheep, whereas rats can tolerate much higher doses, around 4,000 mg/kg body weight (Aslani *et al*., 2004). Another study by Shridhar (2022) reported that 100 and 200 mg/kg orally administration for 14 and 30 days is toxic for ruminant but not for rodents. Another study by Galey *et al*. (1996) suggested that ingestion of small amounts of oleander leaves, even as little as 0.005% of the animal's body weight, can be fatal to cattle and horses. All of the available data suggest that oleander is not recommended for human consumption, but there are no limits to its use in experimental animals. In the present investigation sub-acute toxic dose of oleander leaves extract is used and the mortality due to the effect of *N. oleander* is not been considered rather it makes benefit for the cardiac arrest after MI is proved and several previous research also reported the same beneficial effects. A report from Mwafy and Yassin (2011) showed that rats administered orally 250 mg/kg body weight of *N. oleander* plant extract for 28 days improved insulin and glucose levels, also improved alkaline phosphatase and liver enzyme activities. Gayathri *et al*. (2011) flowers of *N. oleander* is cardioprotective in rat model of oxidative stress by stimulating SOD activity and scavenging the free radicals. It is stated that force of contraction, cardiac output and heart rate all are increase in isolated rodents heart (Adome *et al*., 2003) which provide indications for use in CVD.

Another herb, ginseng, has beneficial effects on the heart in many ways. It can limit myocardial infarct size by increasing the endothelial nitric oxide synthase (eNOS) pathway and increasing coronary circulation (Tang *et al*., 2023). In the context of CVD, ginseng exhibits hypotensive effects due to its effect on increasing arterial function and thus ginsenosides facilitate vasorelaxation action (Kim *et al.*, 1999).

Allium sativum (garlic) is a well-established example of an herb used in the management of cardiovascular disease (CVD). It is renowned for its diverse properties that combat CVD-related conditions such as hypertension, oxidative stress, inflammation, and hyperlipidemia (Ashraf *et al.*, 2013). Garlic has been used traditionally for a long time to lower total cholesterol and serum LDL levels. It reduces lipid content in arterial cells and inhibits the proliferation of vascular smooth muscle cells and thus garlic can manage atherosclerosis and hyperlipidemia (Sun *et al*., 2018). Several research suggests that regular consumption of cardamom fruit powder can reduce high blood pressure which is a frequent cause of heart failure and stroke. The protective effect of cardamom against stress-induced myocardial damage has been studied in animals and cardio-adaptogenic properties have been found by Verma *et al.* (2010). *Origanum vulgare* is another herb that contains the chemicals carbachol, thymol, terpinene, and cymene, which show anti-inflammatory effects of its essential oil by inhibiting interleukin and TNF alpha expression in injured tissues (Cheng *et al*., 2018; Dabravolski *et al*., 2023).

Histopathology studies in the present investigation revealed a complex collagen network in the untreated myocardial infarction (MI) heart. This network plays crucial roles in controlling apoptosis, restoring pathological deformations, and maintaining structural alignment. Additionally, it regulates heart muscle dilatation and facilitates energy transmission during fiber shortening through the release of cytokines and growth factors (Zannad *et al*., 2010). Therefore, collagen plays a crucial role in maintaining cardiac architecture and function. However, in the remodeling process, the balance between collagen synthesis and degradation can be influenced by various adverse factors. Traditional medicine has historically relied on natural resources and their extracts as medicines. Examples supporting this include *Salix alba* as the source of aspirin, digoxin from *Digitalis purpurea*, ephedrine from *Ephedra sinica*, *Monascus purpureus* give lovastatin, taxol from *Taxus brevifolia*, reserpine from *Rauwolfia serpentina* and many others (Harvey, 2000). A dense collagen deposit has now been found in the infarcted area of the myocardium (Figure 5), which can lead to heart failure and death after MI. The combined herbal treatment reduced infarct size and collagen deposition due to its antioxidant activity and anti-inflammatory activity. Our results indicated that SOD activity increased in the treated group. Experimental evidence indicates that inflammatory mediators can induce the re-expression of fetal genes, promote cell proliferation, activate metalloproteinases, stimulate fibroblast proliferation, and contribute to the progressive loss of myocytes through apoptosis. Moreover, modulation of the adaptive response of inflammatory cells may lead to more favorable remodeling, particularly in models of myocardial ischemia (Mann, 2015).

Sequential changes during left ventricular (LV) remodeling after myocardial infarction (MI) encompass the phases of acute MI, healing, and repair, which unfold over weeks to months. Structural deformation leads to decreased tone and increased wall stress, promoting progressive LV dilation and stimulating fibrosis where early and prolong anti-ischemic therapy is advantageous (Prabhu and Frangogiannis, 2016). Oxidative species are produced in ischemic tissue through cellular processes involving enzymes such as cyclooxygenase, cytochrome P450, glucose oxidase, the NADPH oxidase system, lipoxygenase, and xanthine oxidase. The antioxidant properties of the current herbal extract mixture may potentially mitigate the progression of heart failure and prevent death following ischemic injury by neutralizing the effects of free radicals and reactive oxygen species (ROS).

5. Conclusions

This experimental trial of a mixed herbal tonic from different herbs showed significant efficacy against cardiac remodeling associated with MI-induced cardiac wall thinning, antioxidative insufficiency and inadequate cardiac repair. Ventricular myocardial death and collagen formation are greater after MI. Good survival from MI requires a supportive therapeutic management to limit it. In this study we found that the herbal extract mixture showed better defense against oxidative stress but the research gap remains involving unexplained cellular mechanisms.

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Data availability
All relevant data are within the manuscript.

Conflict of interest
None to declare.

Authors' contribution
All authors contributed by active participation in this research. Md. Mizanur Rahman: principal investigator of this work, Md. Shahidul Islam: alternative investigator who critically thinks about the problem raised and solved, Md. Tasmir Rayan Labib: performed the surgery to establish the MI model, Md. Sodrul Islam: read the manuscript and contributed to editorial work, Kazi Khalid Ibne Khalil: established the animal facility for the research, Apurbo Kumar Mondal: biochemical analysis of the samples and Md. Abdullah Al Mahmud: gave us the histopathology facility. He also collaborated giving support of staining protocol and chemicals. All authors have read and approved the final manuscript.

References
Abdou RH, WA Basha and WF Khalil, 2019. Subacute toxicity of *Nerium oleander* ethanolic extract in mice. Toxicol. Res., 35: 233-239.

Adome RO, JW Gachihi, B Onegi, J Tamale and SO Apio, 2003. The cardiotonic effect of the crude ethanolic extract of *Nerium oleander* in the isolated guinea pig hearts. African Heal. Sci., 3: 77-82.

Ashraf R, RA Khan, I Ashraf and AA Qureshi, 2013. Effects of *Allium sativum* (garlic) on systolic and diastolic blood pressure in patients with essential hypertension. Pak. J. Pharm. Sci., 26: 859-863.

Amalraj A and S Gopi, 2016. Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: A review. J. Tradit. Complement. Med., 7: 65-78.

Aslani MR, AR Movassaghi, M Mohri, A Abbasian and M Zarehpour, 2004. Clinical and pathological aspect of experiment oleander (*Nerium oleander*) toxicosis in sheep. Vet. Res. Commun., 28: 609-616.

Brent AF and MK Christopher, 2007. Mechanisms of post-infarct left ventricular remodeling. Drug Discov. Today Dis. Mech., 4: 185-196.

Cheng C, Y Zou and J Peng, 2018. Oregano essential oil attenuates RAW264.7 cells from lipopolysaccharide-induced inflammatory response through regulating NADPH oxidase activation-driven oxidative stress. Molecules, 23: 1857.

Dabravolski SA, VN Sukhorukov, AA Melnichenko, VA Khotina and AN Orekhov, 2023. Potential application of the plant-derived essential oils for atherosclerosis treatment: Molecular mechanisms and therapeutic potential. Molecules, 28: 5673.

Dwivedi S and D Chopra, 2014. Revisiting *Terminalia arjuna* - An ancient cardiovascular drug. J. Tradit. Complement. Med., 4: 224-231.

Dwivedi S, A Aggarwal, MP Agarwal and S Rajpal, 2005. Role of *Terminalia arjuna* in ischaemic mitral regurgitation. Int. J. Cardiol., 100: 507-508.

Galey FD, DM Holstege, KH Pulmee, E Tor, B Johnson, ML Anderson, PC Blanchard and F Brown, 1996. Diagnosis of oleander poisoning in livestock. J. Vet. Diagn. Investig., 8: 358-364.

Gayathri V, S Ananthi, C Chandronitha, G Ramakrishnan, R Lakshmisundaram, RL Sundaram and HR Vasanthi, 2011. Cardioprotective effect of *Nerium oleander* flower against isoproterenol-induced myocardial oxidative stress in experimental rats. J. Cardiovasc. Pharmacol. Ther., 16: 96-104.

Goyal SN, C Sharma, UB Mahajan, CR Patil, YO Agrawal, S Kumari, DS Arya and S Ojha, 2015. Protective effects of cardamom in isoproterenol-induced myocardial infarction in rats. Int. J. Mol. Sci., 16: 27457-27469.

Harvey A, 2000. Strategies for discovering drugs from previously unexplored natural products. Drug Disc. Today, 7: 294-300.

Kim ND, SY Kang, MJ Kim, JH Park and VB Schini-Kerth, 1999. The ginsenoside Rg3 evokes endothelium-independent relaxation in rat aortic rings: role of K+ channels. Eur. J. Pharmacol., 367: 51-57.

Laslett LJ, P Alagona, BA Clark 3rd, JP Drozda Jr, F Saldivar, SR Wilson, C Poe and M Hart, 2012. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American college of cardiology. J. Am. Coll. Cardiol., 60: S1-S49.

Lee DC, MO Lee, CY Kim and DH Clifford, 1981. Effect of ether, ethanol and aqueous extracts of ginseng on cardiovascular function in dogs. Can. J. Comp. Med., 45: 182-187.

Mann DL, 2015. Innate immunity and the failing heart: the cytokine hypothesis revisited. Circ. Res., 116: 1254-1268.

Maruyama K, K Naemura, K Yoshihara, K Imanaka-Yoshida, H Kurihara and S Miyagawa-Tomita, 2021. Surgical protocol for permanent ligation of the left anterior descending coronary artery in mice to generate a model of myocardial infarction. STAR Protoc., 26: 100775.

Maulik SK and S Kumar, 2012. Oxidative stress and cardiac hypertrophy: a review. Toxicol. Mech. Methods, 22: 359-366.

Moe GW and J Marín-García, 2016. Role of cell death in the progression of heart failure. Heart Fail. Rev., 21: 157-167.

Mwafy SN and MM Yassin, 2011. Antidiabetic activity evaluation of glimepiride and *Nerium oleander* extract on insulin, glucose levels and some liver enzymes activities in experimental diabetic rat model. Pak. J. Biol. Sci., 14: 984-990.

Prabhu SD and NG Frangogiannis, 2016. The biological basis for cardiac repair after myocardial infarction: From inflammation to fibrosis. Circ. Res., 24: 91-112.

Rahman K and B David, 2000. Dietary supplementation with aged garlic extract inhibits adp-induced platelet aggregation in humans. J. Nutri., 130: 2662-2665.

Rosamond W, K Flegal, G Friday, K Furie, A Go, K Greenlund, N Haase, M Ho, V Howard, B Kissela, S Kittner, D Lloyd-Jones, M McDermott, J Meigs, C Moy, G Nichol, CJ O'Donnell, V Roger, J Rumsfeld, P Sorlie, J Steinberger, T Thom, S Wasserthiel-Smoller and Y Hong, 2007. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation, 6: e69-171.

Shridhar NB, 2022. *Nerium oleander* toxicity: A review. Int. J. Adv. Aca. Stud., 4: 23-32.

Subramaniam S, R Subramaniam, S Rajapandian, S Uthrapathi, VR Gnanamanickam and GP Dubey, 2011. Anti-atherogenic activity of ethanolic fraction of *Terminalia arjuna* bark on hypercholesterolemic rabbits. Evid. Based Complement. Alternat. Med., 2011: 487916.

Sun YE, W Wang and J Qin, 2018. Anti-hyperlipidemia of garlic by reducing the level of total cholesterol and low-density lipoprotein: A meta-analysis. Medicine, 97: e0255.

Tang MM, ST Zhao, RQ Li and W Hou, 2023. Therapeutic mechanisms of ginseng in coronary heart disease. Front. Pharmacol., 3: 1271029.

Teringova E and P Tousek, 2017. Apoptosis in ischemic heart disease. J. Transl. Med., 15: 87.

Tsai TH, LT Chuang, TJ Lien, YR Liing, WY Chen and PJ Tsai, 2013. *Rosmarinus officinalis* extract suppresses *Propionibacterium acnes*-induced inflammatory responses. J. Med. Food., 16: 324-333.

Verma S, A Bordia and V Jain, 2010. Greater cardamom (*Amomum subulatum* Roxb.) –A cardio-adaptogen against physical stress. J. Herb. Med. Toxicol., 4: 55-58.

Zannad F, P Rossignol and W Iraqi, 2010. Extracellular matrix fibrotic markers in heart failure. Heart Fail. Rev., 15: 319-329.