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Preventive effect of *Ziziphus nummularia* Burm on Cardiac Markers in Normal and Isoproterenol induced Myocardial Necrosis in Rats

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ResearchArticle

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Keywords: Cardiovascular diseases; Isoproterenol;Ziziphusnummularia

ABSTRACT

Myocardial infarction is making an increasingly important contribution to mortality statistics of Cardiovascular diseases. The present study was aimed to investigate the cardioprotective effect of fruits of *Ziziphus nummularia* in isoproterenol induced myocardial infarcted rats. Subcutaneous injection of isoproterenol (150 mg kg^{-1}) to rats at an interval of 24 h for 2 days showed a significant ($p<0.001$) increase in cardiac marker enzymes (LDH, CKMB, SGOT and SGPT). Pre-treatment with *Ziziphus nummularia* to isoproterenol treated rats for a period of 42 days showed significant ($p<0.001$) cardioprotective effect. Administrations of *Ziziphus nummularia* to normal rats have shown very less significant effect on above parameters. So, the study revealed that the cardioprotective effect of *Ziziphus nummularia* in isoproterenol induced myocardial infarction may be due to decrease in the levels of cardiac markers, which was further confirmed by histopathological studies.

INTRODUCTION

Myocardial infarction is making an increasingly important contribution to mortality statistics of Cardiovascular diseases. The present study was aimed to investigate the cardioprotective effect of fruits of *Ziziphus nummularia* in isoproterenol induced myocardial infarcted rats [1]. Subcutaneous injection of isoproterenol (150 mg kg^{-1}) to rats at an interval of 24 h for 2 days showed a significant ($p<0.001$) increase in cardiac marker enzymes (LDH, CKMB, SGOT and SGPT). Pre-treatment with *Ziziphus nummularia* to isoproterenol treated rats for a period of 42 days showed significant ($p<0.001$) cardioprotective effect.

Administrations of *Ziziphus nummularia* to normal rats have shown very less significant effect on above parameters [2]. So, the study revealed that the cardioprotective effect of *Ziziphus nummularia* in isoproterenol induced myocardial infarction may be due to decrease in the levels of cardiac markers, which was further confirmed by histopathological studies [3].

Therapeutic management of Acute Myocardial Infarction:

1) Oxygentherapy
2) Analgesics
3) Antiplatelet agents
4) Thrombolytics
5) Anticoagulants
6) Nitrates
7) β-blockers
8) Angiotensinconverting enzyme(ACE) inhibitors
9) Calciumchannel blockers
10) Antioxidant

India has a rich flora that is widely distributed throughout the country. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Naturally occurring antioxidants have received a great deal of attention because they are able to ameliorate oxidative damage induced by free radicals and are safer than synthetic antioxidants. The association between the consumption of fruits and vegetables and decreased risk of heart disease and cancer is supported by epidemiological evidence. Hence forth, the interest in the evaluation of antioxidant activity of fruits and vegetables has substantially inc reased and numerous studies have been undertaken.

Ziziphus nummularia Burm. f. (syn. *Ziziphus rotundifolia Lamk, Rhamnusnummularia Burm*. f.) belongs to the buckthorn family *Rhamnaceae* [4]. It is a thorny shrub with pale purplish and velvety stems and branches. Fruits are red, edible drupes, locally known as “Konkanber”, “Badber” or “Jhar Beri” [5]. They are anticancer, anodyne, refrigerant, sedative, pectorial, styptic, stomachic and tonic. They are used to purify the blood and aid digestion. They are also used internally in the treatment in few conditions including loss of appetite, chronic fatigue, diarrhoea, pharyngitis, bronchitis, burns, anaemia, irritability, hysteria [6]. The drug has been scientifically validated for certain pharmacological effects viz. antitumor, anthelmintic, antibacterial, and antifertility effects. Phytochemical reports on *Ziziphus nummularia* have revealed the presence of polysaccharides, a pectin composed of l-rhamnose, d-galacturonic acid, d-galactose, l-arabinose, peptide alkaloids, cyclopeptides, flavonoids, saponins, triterpenoids, fatty acids, Ziziphin N, O, P and Q and dodecacetylprodelphinidin B3 [5-7].

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However, there are no reports on the cardioprotective activity of the *Ziziphus nummularia* fruits. Hence the present research work is an attempt in this direction to evaluate the Cardioprotective potentials of *Ziziphus nummularia burm* in Isoproterenol induced myocardial necrosis in albino wistar rats B3 [7].

MATERIALS AND METHODOLOGY

Chemicals

Isoproterenol was procured from the Sigma- Aldrich chemicals Ltd, St. Louis, USA. 5, 5'-Dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione was obtained from Himedia Laboratories, Mumbai. All the other chemicals were procured from Merck laboratories, Nice chemicals, Loba chemie, Sd. fine chemicals were of analytical grade.

Induction of myocardial ischemia:

Myocardial ischemia was induced by subcutaneous injection of isoproterenol hydrochloride (ISO, 150 mg kg^{-1} dissolved in saline, once a day for 2 days [8].

Collection of fruits

The dried fruits of *Ziziphus nummularia* burm was purchased from Abhirami botanicals private Ltd, Tuticorin, Chennai. Authentified by V. Chelladurai, Research Officer- Botany (Scientist –C), Central Council for Research in Ayurveda and Siddha, TamilNadu.

Preparation of ethanolic extract

Dried fruits of *Ziziphus nummularia* burm was coarsely powdered and then stored in air tight container. Dried powder of *Ziziphus nummularia* was extracted by using ethanol as a solvent with Soxhlet extractor [6]. The powder was then packed into Soxhlet column and extracted at 65°C-70°C. After completion of extraction, the solvent was removed by vaccum evaporator till the semi-solid mass was obtained. The dried extracts were stored in airtight container.

Phyto-chemical screening of the extract: The dried extract of *Ziziphus nummularia burm* was subjected to qualitative screening for the various phytoconstituents like alkaloids, carbohydrates, glycosides, triterpenoic acids, saponins, tannins, and flavanoids. Tests for common phytochemicals were carried out by standard methods [7].

Dose selection (LD50): Acute toxicity studies were done as per OECD guidelines no: 425 and 1/5 and 1/20 were fixed as doses.

Preparation of drug solutions: Ethanolic extract of *Ziziphus nummularia* was soluble in aqueous solution. Two doses like *Ziziphus nummularia* (100 mg/kg) and *Ziziphus nummularia* (400 mg/kg) were prepared [8].

Experimental animals: Laboratory bred female Wistar albino rats weighing between 150 and 200 g were obtained from Acharya and B M Reddy College of Pharmacy and were housed at 25˚C ± 5˚C in a well-ventilated animal house under 12:12 h light and dark cycle. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard

conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental protocol: Rats were randomly divided into SIX groups, each consisting of eight animals.

Group 1: Administered with 0.2 ml saline orally to each animal for 42 days.

Group 2: Maintained as isoproterenol control without any drug treatment (positive control) given 150 mg/kg subcutaneously once a day for 2 days.

Group 3: Administered (100 mg/kg) *Ziziphus nummularia* orally respectively for 42 days.

Group 4: Administered (400 mg/kg) *Ziziphus nummularia* orally respectively for 42 days.

Group 5: Administered (100 mg/kg) *Ziziphus nummularia* orally respectively for 42 days and then subcutaneously injected with isoproterenol (150 mg/kg dissolved in saline, once a day for 2 days at interval of 24 h).

Group 6: Administered (400 mg/kg) *Ziziphus nummularia* orally respectively for 42 days and then subcutaneously injected with isoproterenol (150 mg/kg dissolved in saline, once a day for 2 days at interval of 24 h).

Twenty-four hours after the second dose of isoproterenol, all the rats were anaesthetized with Thiopental sodium (30 mg/kg, intraperitoneally). Blood samples was collected from carotid artery and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 2500 rpm at 30˚C for 15 min was used for estimation of marker enzymes viz., CKMB, LDH (serum and heart tissue), SGPT, SGOT (serum).

The heart was dissected out, washed immediately in ice-chilled physiological saline, blotted and weighed. A known weight of the heart tissue was homogenized in 0.1 M Tris-HCL (pH 7.4) buffer solution to produce 10% w/v homogenate. Similarly, hearts were fixed in 10% buffered neutral formalin for histological studies.

Cardiacmarkers

- Assay of Lactate dehydrogenase (LDH) in serum and heart.
- Assay of Creatinine Kinase MB (CKMB) in serum and heart.
- Assay of Serum glutamic oxaloacetic transaminase (SGOT) in serum.
- Assay of Serum glutamic pyruvic transaminase (SGPT) in serum.

Histopathology procedure: At the end of the experiment, myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. The tissue was carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic moulds and kept under freezing plate to allow paraffin to solidify. Cross section (5 µm thick) of the fixed myocardial tissues was cut. These sections were stained with hemato-xylin and eosin (H and E) and visualized under light microscope to study the light microscopic architecture of the myocardium. The investigators performing the histological evaluation were blind to biochemical estimations[9].

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Statisticalanalysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) by Prism-5 software followed by Dunnett's test.

The results are expressed as mean ± SD from n=6 rats in each group. P<0.001 was considered to be significant.

RESULTS

All the results obtained with various groups regarding serum lactate dehydrogenase, tissue lactate dehydrogenase, serum creatinine kinase MB, tissue creatinine kinase MB, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase is given individually.

Cardiac Markers: LDH, CKMB, SGPT, SGOT

- Results of Serum LDH levels are presented in Table 1; Graph 1.
- Results of tissue LDH levels are presented in Table 1; Graph 2.
- Results of serum CKMB levels are presented in Table 2; Graph 3.
- Results of tissue CKMB levels are presented in Table 2; Graph 4.
- Results of serum SGOT levels are presented in Table 3; Graph 5.
- Results of serum SGPT levels are presented in Table 3; Graph 6.

In the present study, there was significant increase in cardiac marker enzymes in isoproterenol group when compared to normal saline group. In treatment groups, *Ziziphus nummularia* (100, 400 mg/kg) significantly (p<0.001) decreased cardiac marker enzymes in rat hearts subjected to isoproterenol induced myocardial necrosis.

Table 1. Effect of *Ziziphus nummularia* on serum and tissue LDH levels.

Groups	LDH in serum	LDH in tissue
Saline	183.34 ± 6.18	230.54 ± 5.11
ISO	517.53 ± 10.48	563.14 ± 6.78
ZN (100 mg/kg)	156.09 ± 4.03	197.41 ± 3.65
ZN (400 mg/kg)	147.34 ± 3.64	183.57 ± 5.21
ZN (100 mg/kg) +ISO	352.52 ± 5.23***	378.45 ± 3.52***
ZN (400 mg/kg) +ISO	256.66 ± 8.46***	293.96 ± 6.26***
Note: Results are expressed as Mean ± S.D, n=6; ***p<0.001 considered as significant when compared to ISO control group; Values are expressed in IU/L.		

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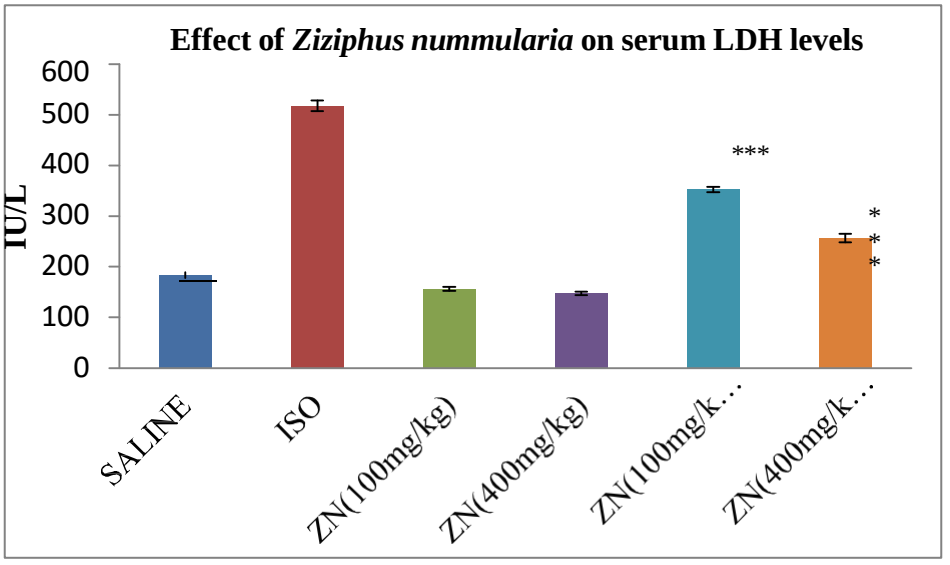
Graph 1. Effect of *Ziziphus nummularia* on serum LDH levels.



Graph 2. Effect of *Ziziphus nummularia* on tissue LDH levels.

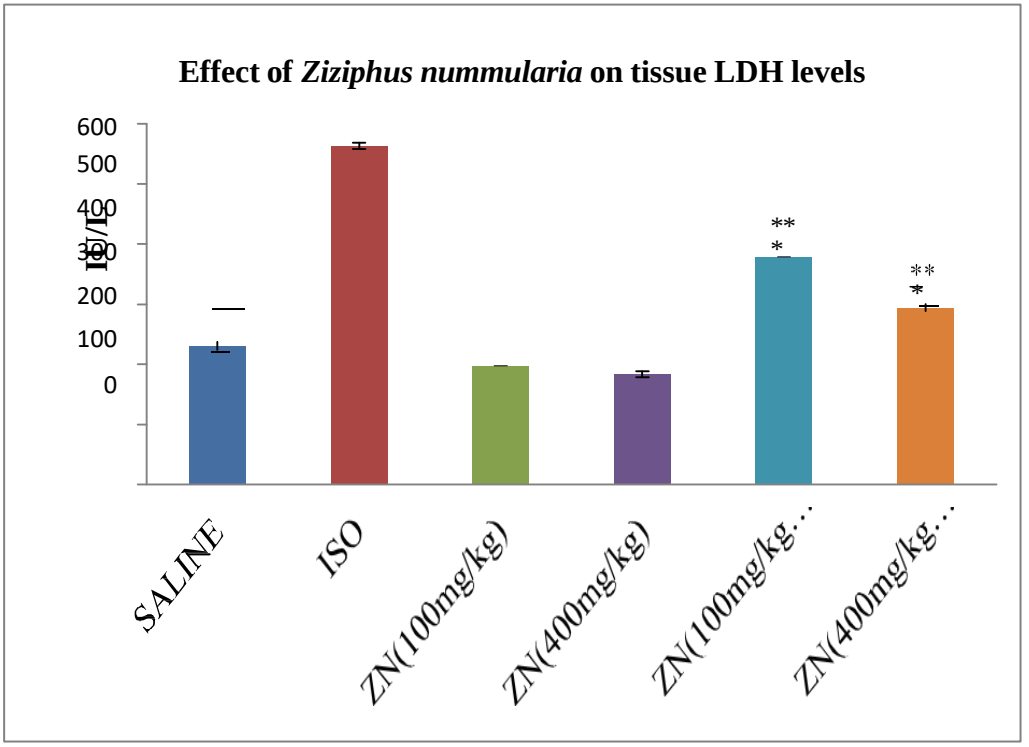


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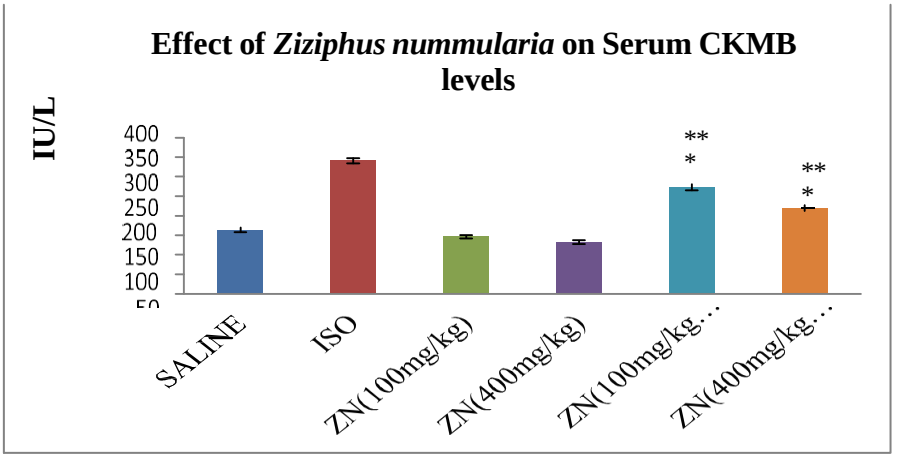
Table 2. Effect of *Ziziphus nummularia* on serum and tissue CKMB levels.

Groups	CKMB in Serum	CKMB in Tissue
Saline	163.65 ± 6.01	197.73 ± 3.95
ISO	340.52 ± 6.65	404.08 ± 7.37
ZN (100 mg/kg)	146.26 ± 4.21	180.1 ± 4.74
ZN (400 mg/kg)	132.51 ± 4.78	158.74 ± 4.74
ZN (100 mg/kg) +ISO	272.98 ± 7.37***	320.54 ± 3.87***
ZN (400 mg/kg) +ISO	219.71 ± 7.33***	283.79 ± 4.41***
Note: Results are expressed as Mean± S.D, n=6; ***p<0.001 considered as significant when compared to ISO control group; Values are expressed in IU/L..		

Graph 3. Effect of *Ziziphus nummularia* on serum CKMB levels.



Graph 4. Effect of *Ziziphus nummularia* on Tissue CKMB levels.

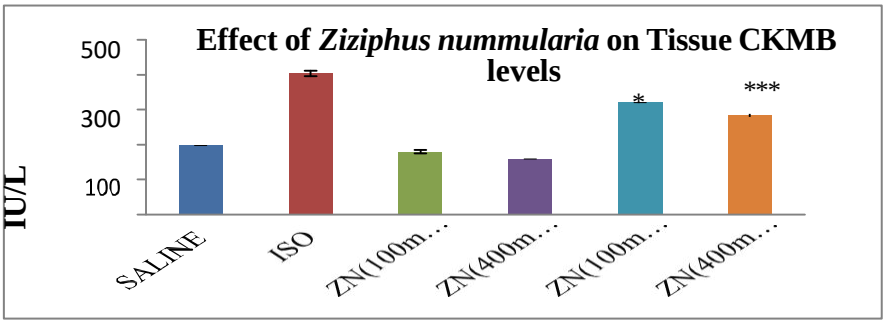


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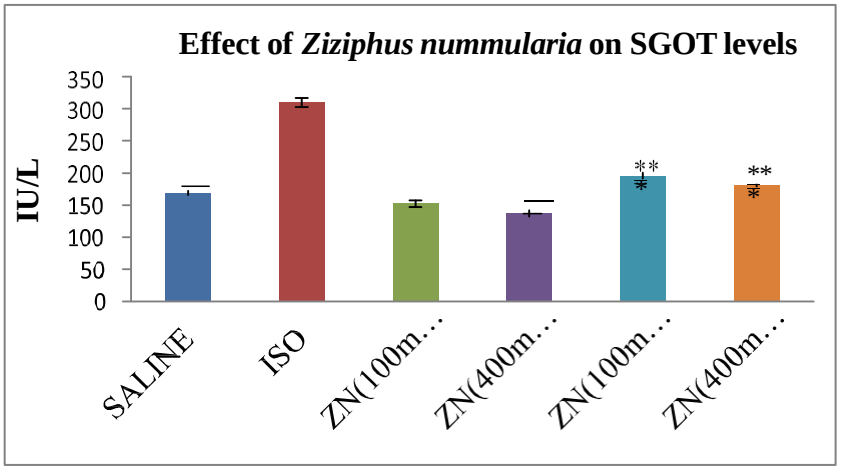
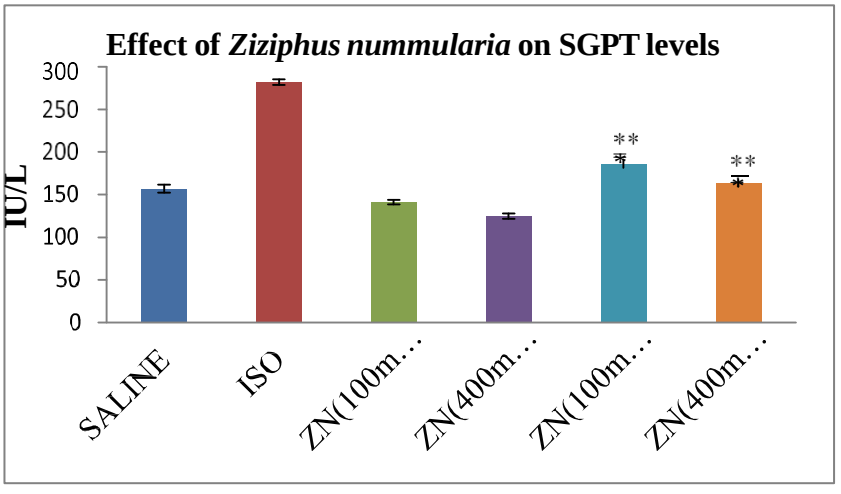
Table 3. Effect of *Ziziphus nummularia* on SGOT and SGPT levels.

Groups	SGOT	SGPT
Saline	168.91 ± 3.54	157.24 ± 4.74
ISO	309.89 ± 7.28	282.1 ± 3.32
ZN (100 mg/kg)	152.27 ± 5.17	141.08 ± 2.69
ZN (400 mg/kg)	137.22 ± 5	124.77 ± 3.19
ZN (100 mg/kg) +ISO	195.94 ± 4.48***	186.18 ± 4.87***
ZN (400 mg/kg) +ISO	180.77 ± 2.63***	163.33 ± 4.13***
Note: Results are expressed as Mean± S.D, n=6; *** p<0.001 considered as significant when compared to ISO control group; Values are expressed in IU/L.		

Graph 5. Effect of *Ziziphus nummularia* on SGOT levels



Graph 6. Effect of *Ziziphus nummularia* on SGPT levels



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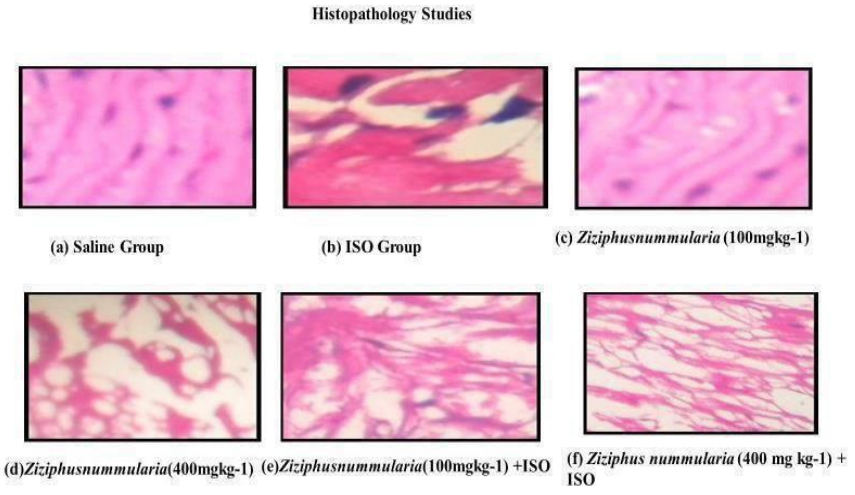
Histopathology examination

1. Saline Group: Section of the cardiac muscle showing some congested vascular spaces.
2. ISO Group: Section of the heart showing some areas of coagulative necrosis of cardiocytes, mixed inflammatory infiltration and damaged vascular spaces.
3. *Ziziphus nummularia* (100 mgkg^{-1}): Section of the cardiac muscle showing few congested vascular spaces.
4. *Ziziphus nummularia* (400 mgkg^{-1}): Section of the cardiac muscle showing some proliferating vascular spaces.
5. *Ziziphus nummularia* (100 mgkg^{-1}) +ISO: Section of the cardiac muscle showing focal areas of necrosis of cardiocytes, mild inflammation, few congested vascular spaces.
6. *Ziziphus nummularia* (400 mg kg^{-1}) + ISO: Section of the cardiac muscle showing proliferating and congested vascular spaces.

Histopathology is the study of the microscopic examination and the signs of the disease by biopsy or surgical specimen that is processed and fixed onto glass slides. To visualize and understand different components of the tissue under a microscope, the sections are dyed with one or more stains. The aim of staining is to reveal cellular components. Histopathological examination of the myocardium of normal rat showed clear integrity of myocardial cell membrane. Endocardium and pericardium were within normal limits. No inflammatory cell infiltration was observed. The group of ISO Group treated rats showed moderate to marked myocytic necrosis with moderate infiltration of lymphocytes and macrophages. The changes were more prominent along the endocardium and in papillary muscles. Minimal-to-mild focal myocytic necrosis and minimal diffuse lymphocytic infiltration along the endocardium was seen in the heart sections of the standard drug verapamil treated group. Minimal-to-mild multifocal myocytic necrosis with removal of sarcoplasm and mild diffuse inflammatory cell infiltration along the endocardium was observed in the *Ziziphus nummularia* (400 mg kg^{-1}) + ISO group (Figure 1).

Figure 1. Histopathology studies.



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DISCUSSION

The present investigation is aimed to explore the cardioprotective potentials of *Ziziphus nummularia* burm in Isoproterenol induced myocardial necrosis in albino wistar rats [9]. During myocardial infarction, reactive oxygen species like superoxide, hydrogen Peroxide and hydroxyl radicals are produced in enormous amount which contribute to myocardial tissue injury. Cardiovascular actions of isoproterenol may also lead to cardiac necrosis. The imbalance between oxidants and antioxidant defence is one of the major causes of many human diseases. Potential antioxidant therapy should, therefore, include either exogenous supplementation of natural antioxidants or augmentation of endogenous anti-oxidants. The cardiac marker enzymes viz LDH, CKMB, SGPT and SGOT serve as sensitive index to assess the severity of MI.

When myocardial membrane gets ruptured due to deficient oxygen supply or glucose it leads to leakage of enzymes (LDH, CKMB, SGOT and SGPT) from heart tissue to serum. This accounts for the increased activity of these enzymes in serum of rats with myocardial infarction induced by isoproterenol.

In the present study, a significant increase in cardiac markers LDH, CKMB, SGPT and SGOT activity was observed in both normal and isoproterenol induced myocardial necrosis in rats. Treatment with *Ziziphus nummularia* burm significantly ($p<0.001$) reduced the levels of cardiac markers LDH, CKMB, SGPT and SGOT. This effect may be due to the free radical scavenging properties of *Ziziphus nummularia* burm. Histopathological findings of pretreated groups with *Ziziphus nummularia* burm (100 and 400 mg kg^{-1}) showed reversal of myonecrosis. Doses of *Ziziphus nummularia* burm per se treated rats had no significant reversal of toxic effects on cardiac architecture.

CONCLUSION

Thus, the study concludes that the administration of *Ziziphus nummularia* burm is more effective in reducing the extent of myocardial damage and significantly counteracted the oxidative stress during isoproterenol-induced myocardial infarction in rats.

Declaration of conflicting interests: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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