ORIGINAL ARTICLE

Amelioration of Acute Myocardial Infarction by *Tribulus terrestris* (Gokhru) Extract on Isoproterenol-Induced Myocardial Infarction Rats

B. Mahammad Rahmathulla S.[1*], Kodidhela Lakshmi Devi[2], S. Manjunatha[3]

[1,2,3]Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India-515003

ABSTRACT Heart Disease continues to be a major public health complication not only in western countries but also intensify in developing countries and makes symptomatic contribution to the mortality statistics. The current study investigates the defensive effects of Tribulus terrestris extract (TTEt) a medicinal herb following isoproterenol (ISO) induced myocardial Infraction (MI) rats, 2, 3, 5-Triphenyl tetrazolium chloride (TTC) staining on myocardial infarct size, evaluate the preventive effects of TTEt on altered electrocardiogram (ECG). ECG changes such as increase heart rate reduced R-wave amplitude and ST-segment elevation. From the present study it is concluded that TTEt exerts a beneficial effect against ISO-induced MI due to its tissues defense system against cardiac damage.

Keywords: Oxidative stress, Myocardial infarction, ECG, TTC, Histological examination

Address for correspondence: B. Mahammad Rahmathulla S, Department of Biochemistry, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India-515003. E-mail: mdrtl.biochem@gmail.com

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in worldwide and according to the world health organization it will be the major cause of death in the world by the year 2020[1]. Heart and liver disease often co-exist, Cardiac hepatopathy, hepatic injury caused by cardiac dysfunction. Cardiac hepatopathy is incidence of 10-24% in developing countries[2]. Tribulus terrestris (family Zygophyllaceae, known as Ikshugandha in Sanskrit and Gokhru in Hindi) is a common plant, trailing in sandy soils throughout India. Fruits and roots are the medically potent parts of these small caltrops. The fruits contain trace amounts of alkaloids, fixed oils, essential oils, resins and fair amounts of nitrates[3].

MATERIALS AND METHODS

TTEt was procured from Dabur Research Foundation, India. The multiple solvent (methanol: isopropyl alcohol: acetone) extraction procedure was used to prepare the extract by the supplier. The whole plant was used for extraction. Adult male albino rats (Rattus norvegicus), weighing 125-150 g, were procured from the National Centre for Animal Science, National Institute of Nutrition, Hyderabad, India. The study was approved by Animal Ethics Committee of S. K University, Anantapuramu (Regd. 1889/GO/Re/S/16/CPCSEA). The rats were fed with commercial pellet rat chow (M/s. Hindustan Lever Ltd., Bangalore, India) and water ad libitum and maintained under standard laboratory conditions with 12:12 hrs light: dark cycle.

Dosage Fixation

The effective dose of TTEt and duration of treatment was fixed based on the estimation of various biochemical parameters in ISO-induced myocardial infarction in rats, according to previous literature.

Experimental Design

In the present study, a total of twenty four rats were used. The rats were divided into four groups of six rats each.

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Group 1: Control.

Group 2: TTEt [2.5 mg/kg BW]: received TTEt daily mode of administration.

Group 3: ISO [85 mg/kg BW]: received ISO (85 mg/kg) sub-cutaneously on 29th and 30th days with a 24 hr time interval.

Group 4: ISO+TTEt [85 mg/kg BW]: Daily intake of TTEt (2.5 mg/kg) (30 days) and injection of ISO (85 mg/kg BW) on 29th and 30th days (with a gap of 24 hours).

Intubation was accomplished by means of a slightly bend steel intubation needle with ball like thickening terminal, attached to a syringe and extract was infused down the food pipe.

Macroscopic Enzyme Mapping of Myocardium

The rats were divided into four groups of TTC test used for macroscopic enzyme mapping of the ischemic myocardium was a modification of the method described by Sandritter and Jestadt (1958), done without the addition of an exogenous substrate[4]. Freshly prepared 150-200 mL of a solution of TTC (1%) in phosphate buffer was pre-warmed at 37-40 °C for 30 min in a darkened Stender glass dish with a lid. The heart was cut transversely across both the ventricles to obtain slices not more than 1 cm in thickness. Then, the heart slices were washed rapidly in cold water to remove the excess blood; care was taken to avoid the macerate of the tissue. Place the heart slice in the covered, darkened Stender glass dish containing pre-warmed TTC solution, and put the dish in an incubator heated to 37 °C for 35-45 min. Turn the heart slice over once or twice and make certain that it remains immersed and covered by 1 cm of the TTC solution. At the end of the incubation period, place the heart slice in formal saline, which not only fixes the tissue but also enhances the color contrast developed. The expected reactions of the TTC test are as follows: Normal myocardium (enzyme active) will turn bright red, ischemic myocardium (enzyme deficient)-pale gray or grayish yellow and fibrous scars-white.

Separation of Serum LDH Isoenzymes by Agarose Gel Electrophoresis

LDH isoenzyme was separated and quantified by Agarose gel electrophoresis method[5]. Agarose gel (1%) was prepared and applied immediately to the glass slide. After the agar gel sets properly, serum samples were applied into a well. After the run, the gels were removed and stained by the following method. The staining solution contained 1.0 ml of 1.0 M lithium lactate, 1.0 ml of 0.1 M sodium chloride, 1.0 ml of 5.0 mM magnesium chloride, 2.5 ml of 0.1% (w/v) nitro blue tetrazolium (NBT), 0.25 ml of 0.1% phenazine methosulphate, 2.5 ml of 0.5 M phosphate buffer, pH 7.5 and 10 mg of NAD in a total volume of 10 ml. The gels were incubated with the staining solution at 37 °C in the dark for a suitable period. The separated LDH isoenzymes appeared as purple bands. The gels were washed with 7.5% acetic acid, preserved in 5% acetic acid.

Histopathological Studies

At the end of the study, all the rats were sacrificed by cervical decapitation and the hearts were dissected out, washed in ice cold saline. Then myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under light microscope and photographs were taken.

Statistical Analysis

All quantitative measurements were expressed as means±SD for control and experimental rats. The data were analyzed using one way analysis of variance (ANOVA) on SPSS/PC (statistical package for social sciences, personal computer) Ver. 16 and the group means were compared by Duncan's Multiple Range Test (DMRT) (Duncan, 1955). The results were considered statistically significant if the p value is less than 0.05.

RESULTS

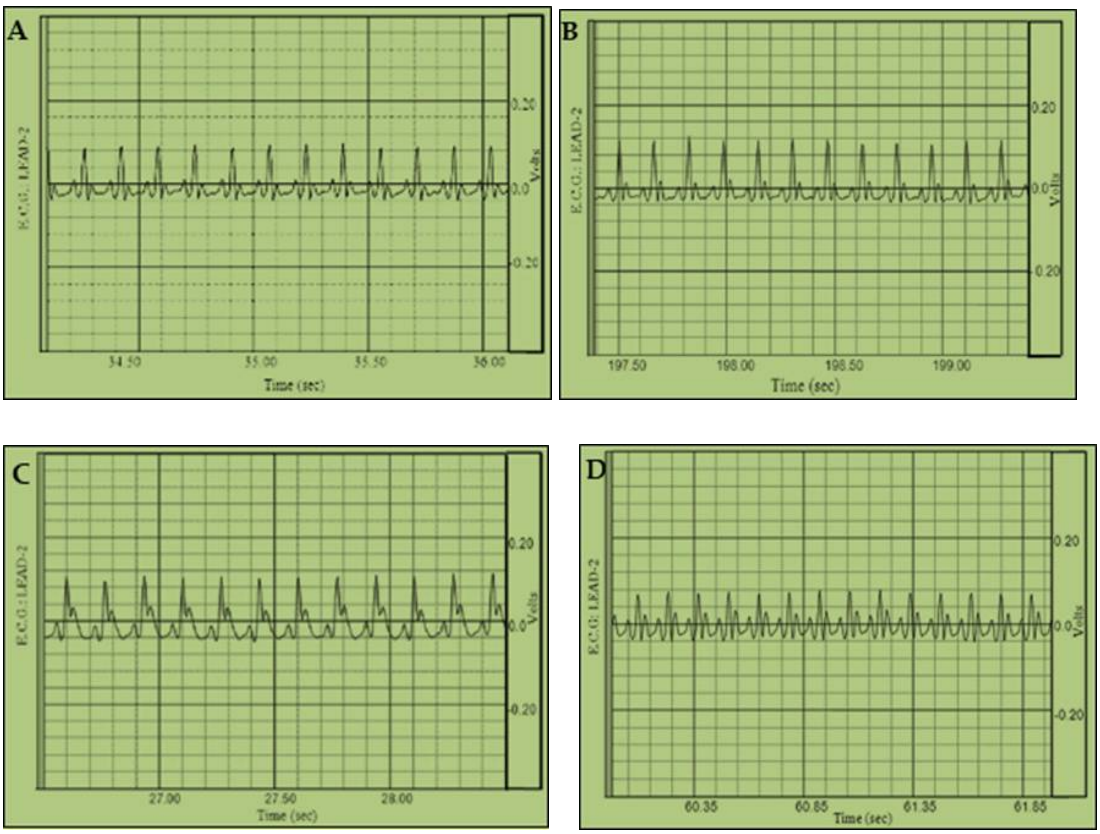
Effect of TTEt on Different ECG Parameters

Figure 1 shows the electrocardiographic pattern of control and experimental rats. Control and TTEt alone treated rats showed a normal ECG pattern, whereas rats treated with ISO alone showed significant elevation in ST segment, reduction in P wave, QRS complex and R-R interval. In addition there was an increase in heart rate, prolongation of QT interval and cardiac cycles compared to normal control rats. Pretreatment of TTEt rats exhibited normal ECG pattern with a slight elevation in ST segment. Furthermore, treatment also resulted in significant ($p<0.001$) increase in P wave, QRS complex and R-R interval, whereas heart rate, QT interval and cardiac cycle were maintained near to normal values. The data of the experimental rats such as P wave, QRS complex, QT interval, R-R interval, heart rate and cardiac cycle are shown in Table 1.

Triphenyl Tetrazolium Chloride (TTC) Macroscopic Enzyme-Mapping Assay

Figure 2 (A-D) shows the staining of heart tissue slices by 2, 3, 5-phenyl tetrazolium chloride. The 2, 3, 5-triphenyl

Figure 1: (A-D): ECG of Rats



Note: (A) ECG of control rat's heart revealing normal ST-segments. (B) ECG of TTEt treated rat's heart showing normal ST-segments. (C) ECG of ISO induced myocardial infarcted rat's heart revealing elevated ST-segments. (D) ECG of TTEt+ISO pretreated isoproterenol induced myocardial infarcted rat's heart showing minimized ST-segment elevation.

Table 1: Effect of TTEt on ISO-Induced ECG Alterations in Rats

Parameters	Control	Control + TTEt	ISO	TTEt + ISO
P wave (s)	0.017 ± 0.0008[a]	0.016 ± 0.0008[a]	0.005 ± 0.0002[b]	0.015 ± 0.0007[a]
QRS complex (s)	0.045 ± 0.0051[a]	0.050 ± 0.0001[a]	0.030 ± 0.0063[b]	0.040 ± 0.0001[c]
QT interval (s)	0.048 ± 0.0051[a]	0.055 ± 0.0051[a]	0.085 ± 0.0051[b]	0.050 ± 0.0025[a]
R-R interval (s)	0.220 ± 0.011[a]	0.200 ± 0.01[a]	0.145 ± 0.007[b]	0.210 ± 0.0105[a]
Heart Rate (bpm)	300.72 ± 15.036[a]	285.71 ± 14.2[a]	413.79 ± 20.68[b]	352.94 ± 17.64[c]

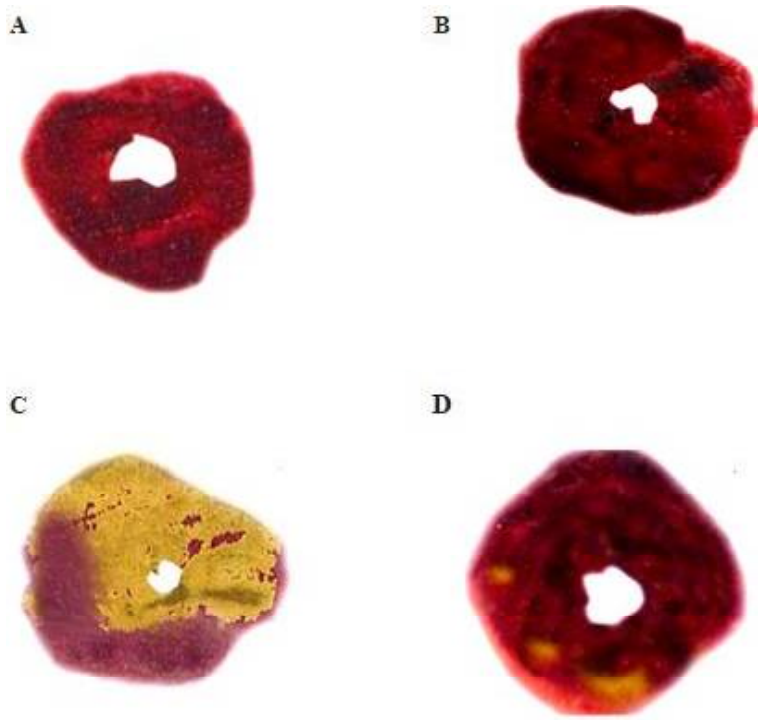
Note: Values are expressed as means ± S.D for six rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT).

tetrazolium chloride stained heart slices of the normal control and TTEt treated rats revealed completely viable tissue (with red color), indicating the presence of LDH and intact myocardial tissue (Figures 2A and 2B). In the isoproterenol induced myocardial infarcted rats the infarcted regions of heart tissue is clearly visible as a bright spot (white) and indicates the absence of LDH (Figure 2C). Pretreatment with TTEt showed signiûcant reduction in infarct size in isoproterenol induced myocardial infarcted rats (Figure 2D). A major portion of the heart tissue as stained positively with 2, 3, 5-triphenyl tetrazolium chloride and revealed greatly reduced infarct size, compared to the heart slices of rats induced with isoproterenol alone.

Effect of TTEt on Serum Cardiac Marker LDH Isoenzyme

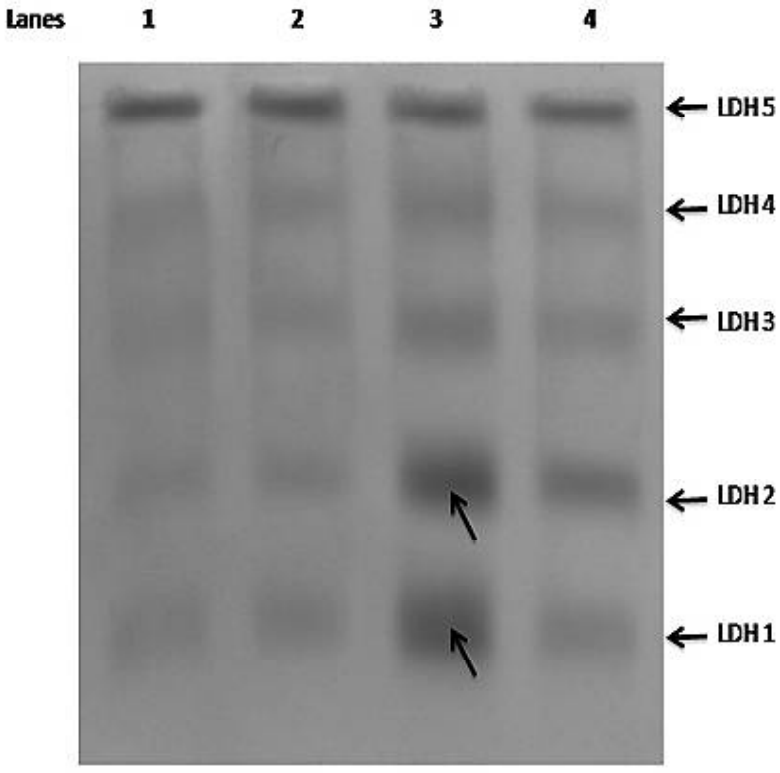
Agarose gel electrophoretic separation of serum LDH isoenzyme patterns of control and experimental group of rats is depicted in Figure 3. ISO induction caused an increased

Figure 2: Effect of TTEt on Macroscopic Enzyme Mapping of Heart in Control and ISO-Induced Rats



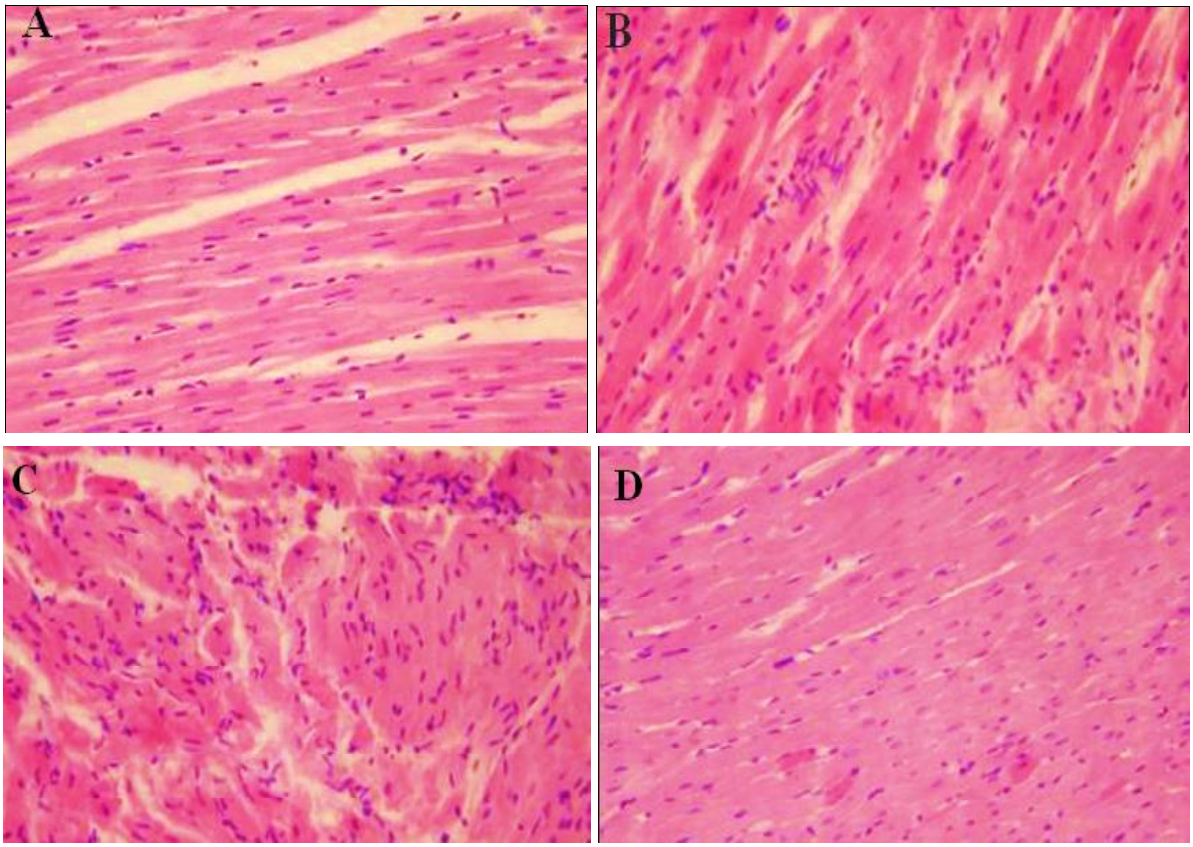
Note: (A) Control rat showing completely viable myocardial tissue. (B) Control+TTEt administered rat showing completely viable myocardial tissue. (C) ISO rat heart showing soaring proportion of infarct size with reduced stain. (D) ISO+TTEt pretreated administered rat showing heart tissue with highly reduced infarct size.

Figure 3: Electrophoretogram Showing the Effect of TTEt on LDH-Isoenzymes in the Serum of Control and ISO-Induced Rats



Note: Lanes 1: Control; 2: Control+TTEt; 3: ISO; 4: TTEt+ISO.

Figure 4: (A-D) Histopathology of Heart Tissue (Hematoxylin & Eosin X 200)



Note: (A) Control untreated rats showing normal cardiac muscle fibers. (B) Control rats with TTEt alone administered rats showing the normal cardiac fibers without any notable changes. (C) ISO showing degeneration and disruption of cardiac myofibers and marked necrosis and also infiltration indicated by arrow marks. (D) ISO+TTEt pretreated rats show reduced the rupture of muscle fibers and mononuclear infiltration.

expression of LDH isoenzyme bands, predominantly LDH1, when compared to control rats. The LDH1 concentration in TTEt-pretreated rats was found to be lower than in ISO administered rats, which indicates the protective effect of TTEt. TTEt alone treated rats also showed similar concentration of LDH isoenzymes as compared with control rats.

Histopathological Findings

Histopathological examination of myocardial tissue obtained from normal control rats and rats treated with TTEt depicted clear integrity of myocardial membrane and an infiltration of inflammatory cells were not seen in these experimental groups (Figures 4A and 4B). The histological sections obtained from the hearts of rats receiving ISO alone (Figure 4C) shows various degrees of focal lesions in many sections consisting of molten staining, fragmentation of muscle fibers with confluent retrogressive lesions were observed. In addition marked sequestering mucoid edema and vacuolar changes along with hyaline necrosis were clearly visible in ISO treated rats. Pretreatment with TTEt demonstrated (Figure 4D) marked improvement in ISO-induced alterations such as vacuolar changes, edema, capillary dilatation and leukocyte infiltration compared to ISO administered group.

DISCUSSION

Electrocardiograph-abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. ST-segment elevation was observed either in patient with acute myocardial ischemia[6] or in isoproterenol-induced myocardial infarction in rat[7]. The study shows significant alterations of ECG patterns were observed in ISO administered rats as compared to normal control rats. The characteristic findings were reductions in the P wave intensity, QRS complex, R-R intervals, QT interval and prolongation of cardiac cycle. We also observed a significant elevation in the ST segment and increase in heart rate. These alterations could be due to the consecutive loss of cell membrane in injured myocardium[8]. In the present study, we observed an elevation of ST-segments in isoproterenol-induced rat and pretreatment with TTEt markedly inhibited isoproterenol-induced ST-segment elevation suggestive of its cell membrane protecting effects.

The appearance of Q wave and ST segment elevation are some of the indicative signs of ischemia. In the present study we did not observe pathological Q wave due to conditions of ischemia. The prominent Q wave were seen only on severe ischemia, infarction and in patients with severe heart diseases. The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation[9]. TTEt administration showed a protective effect against ISO-induced altered ECG pattern and eliminated the acute fatal complications by protecting the cell membrane damage.

TTC acts as a proton acceptor for many pyridine nucleotide linked-dehydrogenases along with cytochromes which form an integral part of the inner mitochondrial membrane and make up the electron transport chain[10]. The tetrazolium salt is reduced by the enzymes into a red, lipid soluble formazan. Viable tissues therefore stains deep red while the ischemic zone is known to progress to a dense fibrous scar with no viable muscle fibers, remains unstained. TTEt administration markedly reduced the myocardial ischemic zone in ISO-induced rats. The antioxidant and membrane stabilizing activities of TTEt might be responsible for the reduction in the infarct size, and by prevention of myocardial necrosis[11]. ISO-induced MI has been reported to alter membrane permeability[12] cardiac damage during ISO-induction. Measurement of LDH isoenzymes is necessary for greater specificity of cardiac injury, since a nonspecific increase of total LDH in serum will lead in tissue damage. An increase in the expressions of LDH_1 and LDH_2 bands were observed in ISO-induced rats which are in agreement with the previous findings of[13] have reported that the release of cardiac specific isoenzymes LDH_1 and LDH_2 into the circulation which might be due to the necrosis induced by ISO. TTEt administration to ISO-induced rats showed decrease in the intensity of LDH_1 and LDH_2-bands and activities of cardiac markers.

Electrocardiographic and macroscopic enzyme mapping findings were further confirmed by histopathological studies. Histopathological examination of myocardial tissue in control depicted clear integrity of the myocardial cell membrane. No inflammatory cells infiltration was seen in the rat heart of control group. In ISO administered group, focal lesions in many sections consisting of molted staining and fragmentation of muscle fibers with confluent retrogressive lesions, hyaline necrosis, and sequestering mucoid edema were observed.

Pretreatment with TTEt demonstrated reversal of focal lesions, fragmentation of muscle fibers and retrogressive lesions with hyaline necrosis seen with ISO treated group. Inflammatory cells were seen with reduced density in TTEt treated group confirming further the cardioprotective activity exerted by TTEt. TTEt treated normal rats had no toxic effects on cardiac architecture. These data further confirmed the cardioprotective action of oral administration of TTEt. Higher dose of isoproterenol induce sub-endocardial ischemia, hypoxia, necrosis and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction[13]. In the present study, we found that TTEt protected myocardium from isoproterenol-induced myocardial functional and structural injury. The data of the present study clearly showed TTEt modulated most of the electrophysiological, macroscopic enzyme mapping and histopathological parameters were maintained to normal status in isoproterenol rats, suggesting the beneficial action of TTEt as a cardioprotective agent.

In conclusion, the present results indicate that the protective effect of TTEt in ISO-induced myocardial infarcted rats could be related to its antioxidant defense system. Our results show that TTEt was safe and highly effective in preventing cardiovascular dysfunction in rats, possibly due to both antioxidative and cardiotonic properties[14,15]. These observations highlight that TTEt is one of the promising herbal drug for improving defense mechanisms in the physiological systems against oxidative stress caused by myocardial infarction.

REFERENCES

1. Murrau, 1998. The global burden disease, 1990-2020. Nat. Med., 4: 124-123. http://www.ncbi.nlm.nih.gov/pubmed/9809543
2. Sherlock, S. & Dooley, J. Diseases of the Liver and Biliary System. 11th ed.Malden, Blackwell Science, 2002. pp. 423-5.
3. Nadkarni, A. K. (1954). Tribulus ferrestris. Indian Material Medica 1, 3rd edn. 1229-1232. Popular Book Depot, Bornbay.
4. Lie, J.T., Pariolero, P.C., Holley, K.E., Titus, J.L., 1975. Macroscopic enzyme-mapping verification of large, homogeneous, experimental myocardial infarcts of predictable size and location in dogs. J. Thorac. Cardiovasc. Surg. 69 (4), 599-605.
5. McKenzie, D., Henderson, A.R., 1983. Electrophoresis of lactate dehydrogenase isoenzymes. Clin. Chem. 29 (1), 189-195.
6. Peacock, W.F., J.E. Hollander, R.W. Smalling and M.J. Bresler, 2007. Reperfusion strategies in the emergency treatment of ST-segment elevation myocardial infarction. Am. J. Emerg. Med.,25: 353-366. DOI: 10.1016/J.AJEM.2006.07.013

7. Rajadurai, M. and S.M.P. Prince, 2007a.Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology, 230: 178-188. DOI: 10.1016/J.TOX.2006.11.053
8. Joshi, C.N., Jain, S.K., Murthy, P.S., 2004. An optimized triphenyltetrazolium chloride method for identification of cerebral infarcts. Brain Res. Protoc. 13 (1), 11-17.
9. Balanehru, S., Nagarajan, B., 1991. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. Biochem. Int. 24 (5), 981-990
10. Han, S.K., Ko, Y.I., Park, S.J., Jin, I.J., Kim, J.M., 1997. Oleanolic acid and ursolic acid stabilize liposomal membranes. Lipids 32 (7), 769-773.
11. S. B Mahammad Rahmathulla, *et al.* Tribulus Terrestris (l) Protects Heart and Liver from Beta Adrenergic-Stimulated Cardiotoxicity: Biochemical and Histological study in Wistar Rats International Journal of Drug Development & Research, January-March 2013, Vol. 5, Issue 1, ISSN 0975-9344.
12. Zhou, R., Xu, Q., Zheng, P., Yan, L., Zheng, J., Dai, G., 2008. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. Eur. J. Pharmacol. 586 (1-3), 244-250.
13. Khalid, M.A., Ashraf, M., 1993. Direct detection of endogenous hydroxyl radical production in cultured adult cardiomyocytes during anoxia and reoxygenation. Is the hydroxyl radical really the most damaging radical species? Circ. Res. 72 (4), 725-736.
14. K.V. Sailaja, V. Leela Shivaranjani, H. Poornima, S.B.Md. Rahamathulla, 2013, PROTECTIVE effect of Tribulus terrestrisl. Fruit aqueous extract on lipid profile and oxidative stress in isoproterenol Induced myocardial necrosis in Male albino wistar rats. EXCLI Journal 2013;12:373-383 – ISSN 1611-2156.
15. Karthick, M. and S.M.P. Prince, 2006. Preventive effect of Rutin, a bioflavonoid, on lipid peroxides and antioxidants in ISO-induced myocardial infarction in rats. J. Pharm. Pharmacol., 58: 701-707. DOI: 10.1211/JPP.58.5.0016.