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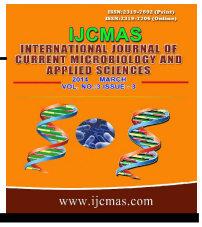

Original Research Article

Evaluation of cardioprotective effect of Tinospora cordifolia against isoprenaline induced myocardial infarction in rats

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Keywords

Cardio-protective effect; *Tinospora cordifolia*; biochemical estimations; myocardial infarction.

A B S T R A C T

The study was conducted to evaluate cardioprotective effect of *Tinospora cordifolia* on isoprenaline-induced myocardial infarction. This study deals with the cocoons, which is called Abresham in the Unani system of medicine. It is one of the 64 drugs which Avicenna has mentioned in Avicenna's tract on cardiac drugs and used in the treatment of cardiovascular diseases. The methanolic extract of *Tinospora cordifolia* in the dose of 350 mg/kg and 650 mg/kg body weight was administered orally for 28 days before isoprenaline administration to test their cardioprotective effect. Isoprenaline (85 mg/kg) was administered subcutaneously on days 29th and 30th, respectively in order to induce myocardial infarction. The parameters for evaluation of cardioprotective activity were the physical parameters and the biochemical estimations. The physical parameters were gross examination of heart, heart weight/body weight ratio and histopathology examination. In biochemical estimations, the activity of various cardiac enzymes such as aspartate transaminase, alanine transaminase, creatinine kinase, lactate dehydrogenase, and the gold marker troponin-I were determined. The levels altered by isoproterenol were restored significantly by the administration of the both doses of test extract especially at higher dose. The result of this study shows that methanolic extract *T.Cordifolia* has significant cardioprotective activity against isoprenaline-induced myocardial infarction.

Introduction

Myocardial infarction (MI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Dhalla, N.S.). Ischemia caused due to reduced blood supply to heart causes several biochemical alterations which may lead to cardiac dysfunction and ultimately cell death (Ferrari, R.). It is well recognized that free radicals generated in ischemic tissues cause metabolic stresses which results in degradation of tissue defence system, leading to myocardial damage and

necrosis (Hearse, D.J.). The development of myocardial ischemia and infarction is a dynamic process with the widespread occurrence of coronary atherosclerosis and involvement of oxidative stress in the humans. Among several pharmacological interventions to protect heart against oxidative stress, the use of antioxidants is most promising. Epidemiological, clinical and experimental studies have provided compelling evidence that MI is largely preventable by antioxidant intervention via suppression of free radical generation and/or augment endogenous antioxidant (Cooper R, Hertog MGL) Medicinal plants, plants based foods and their constituents have received great attention for their salutary effects and potential to treat many aspects of ischemic heart disease or MI (Ojha, S.K., Goyal, S.N., Mohanty I). In parallel, the use of herbs in pharmacotherapy is also rising along with a realization that herbal products can influence the course of heart diseases and may provide an integrated approach of nutritional substances, which helps in restoring and maintaining, the balanced body systems (Hertog et al., 1993).

Isoproterenol (ISO), a synthetic catecholamine and β-adrenergic agonist, has been documented to produce myocardial infarction in large doses (Rona G,) .On auto oxidation, ISO generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids and cause severe damage to the myocardial membrane.

Tinospora cordifolia (Eilld) Miers ex Hook. F. & Thomas (Family-Menispermaceae) *(Guduchi)* is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial (Sinha Kirti *et al*. 2004). A dose dependent reduction in infarct size and in lipid peroxide levels of serum and heart tissue has been reported with ethanol extracts of TC at various doses (Stanley M. et al. 2001). The cardioprotective activity of an herbal formulation "Caps HT2", which contains methanol extract of TC as a component, has antioxidant, anticoagulant, platelet antiaggregatory, lipoprotein lipase releasing, anti-inflammatory and hypolipidaemic activity in rats (Singh N et al. 2005). Administration of 2.5 and 5.0 g/kg body weight of aqueous root extract for 6 weeks results in a significant reduction of serum and tissue cholesterol, phospholipids and free fatty acids in alloxan induced diabetic rats (Sen P et al. 1992). Dichloromethane extracts of TC shows concentration dependent decline in the clonogenicity, glutathione-S-transferase activity as well as increase in lipid peroxidation with a peak at 4 h and a lactate dehydrogenase release with a peak at 2 h (Pahadiya S et al. 2003). In Ehrlich ascites carcinoma bearing mice, the highest number of survivors were observed at an optimal dose of 50 mg/kg in dichloromethane extract of TC (Singh RP et al. 2006). The hydroalcoholic extract of aerial parts has potent chemopreventive effect against cancer, in which oxidative stress plays an important causative role (Rawal A et al. 2004). It is suggested that consumption of hepatoprotective herbs like TC with the above said drugs can minimize the liver toxicity (Jana U et al. 1999). The alcoholic root extract significantly reduces the blood and urine glucose, and lipids in serum and tissues in alloxan induced diabetic rats. The extract also prevents decrease in body weight (ICMR; 2003). In a prospective double blind randomized controlled study lasting for over 18 months in 50 patients, produced significantly better outcome with improvement in wound healing, indicating

beneficial effects of immunomodulation for ulcer healing (Tripathi I. et al.2003). Diabetics are prone to the development of cataract; alcohol extract of TC has preventive effect on the development of cataract and produces a significant reduction of plasma glucose levels in alloxan induced diabetic rats (Rathi SS et al. 2002).

Materials and Methods

Plant material

The Tinospora cordifolia, were purchased from the local market of Lucknow. The drug was authenticated by Division of Taxonomy, National Botanical Research Institute Lucknow (UP) (Ref No. NBRI/CIF/270/2011 and Specification NBRI-SOP-202).

Preparation of extract

The clean fresh roots of the *Tinospora cordifolia* were cut in to small pieces convenient for the purpose of extraction. The drug was then messed up by petroleum ether (40-60) in order to remove its volatile oil component. The drug was extracted with 80% methanol in a soxlet apparatus. The excess of methanol was recovered. Then conc. extract is subjected to evaporation on water bath. When extract was concentrated by evaporation to half of the volume, a waxy material separate out. The concentrated extract was weighed according to body weight, and accurately dissolves in saline for dose treatment daily.

Animals

Albino Wistar rats of 125-150 g of either sex were used for the study. The inbred species of rats were obtained from animal house of Central drug research institute (CDRI), Lucknow for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2ºC) before the study. The animals were randomized into experimental, normal and control groups, housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Faculty of Pharmacy, Integral University, Lucknow (Registration No. **IU/Pharm/M.pharm/CPCSEA/12/10**), India.

Chemicals

Normal saline (0.9%) (Albert David Ltd, Ghaziabad), Isoprenaline Hydrochloride, Metoprolol Tartrate (Pure drug) (Sigma Chemicals, USA), Petroleum Ether (40-60) (S.D.Fine Chemicals, Mumbai), Methanol (Jiangsu Huaxi Ltd, China), Formaldehyde (Fisher scientific Ltd, Navi Mumbai), Serum ALAT diagnostic kit, Serum ASAT diagnostic kit, Serum Creatinine diagnostic kit (Span Diagnostics Ltd, Surat), Serum LDH diagnostic kit (Accurex Biomedical Pvt Ltd Thane, Mumbai), all the chemicals used was of analytical grade.

Experimental Procedure

Grouping*

Group I – Termed as normal control group (NC group), received distilled water (0.5ml, po) daily
for 30 days.

Group II – Termed as Isoprenaline group (ISO group), received distilled water (0.5ml, po) daily for
30 days, in addition received injections of ISO (85 mg/kg, sc) at an interval of 24 hours
on 29th and 30th day.
Group III – Termed as standard group (STD group), receiving metoprolol (pure) (10 mg/kg/day, p.o.)
daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at
an interval of 24 hours.
Group IV–Termed as Test group 1(TG1), *Tinospora cordifolia* extract (350 mg/kg/day, p.o.) administered
daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at
an interval of 24 hours.
Group V – Termed as Test group 2 (TG2), *Tinospora cordifolia* extract (650 mg/kg/day, p.o.)
administered daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th
and 30th days at an interval of 24 hours.

ISO = Isoproterenol
*Each group contains 5 animals (either sex)

Induction of myocardial infarction

Myocardial infarction was induced by Isoproterenol hydrochloride 85mg/kg body weight, dissolved in normal saline given through subcutaneous injection for two consecutive days (29th and 30th). (Murugesan M,).

Rats were weighed and put down 24 hours after the final subcutaneous injection of ISO. Blood collection was done by adhering to Good Laboratory Practices. Blood was collected through retro-orbital plexus from the inner canthus of the eye using capillary tubes and cardiac puncture under light ether anesthesia and allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes and used for the estimation of marker enzymes, including aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK). All animal were sacrificed by cervical decapitation. The hearts were dissected out immediately, weighed, and then the heart was fixed in 10% buffered neutral formalin solution. (Panda VS)

Estimation parameters

Gross examination of rat heart

The dissected hearts was washed with Ice-cold saline. The visually examined the Inflammation, redness, capillary dilatation, Scar formation, colour, in all part of heart and grading was performed. (Rona, *et al.*, 1963).

Heart weight: body weight ratio

The mouse was euthanized, weighed and recorded total body weight. Mouse was placed on its back and pinned onto board with extended extremities (inner side of hands and foot). The mouse was wiped or wetted with 70% ethanol to control hair and dander. Removal of the heart was performed by dissecting the aortic root immediately above the aortic valves and the superior vena cava above the atria. Adjacent mediastinal fat pads were removed from the excised heart carefully with forceps. Heart blood was removed from heart by tapping the heart on a kim wipe (absorbent pad) or surgical compress.

This process was repeated until the heart was totally dry. The dry heart was weighed and recorded. Then the heart was place in fixative. (Firoz M).

Biochemical estimations

At the end of experimental period the blood samples were taken and serum was separated for analysis of different enzymes related to myocardial infarction such as lactate dehydrogenase (LDH), creatine kinase-MB fraction (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT). All the analyses were performed with commercially available kits purchased from Span Diagnostics Ltd, Surat, Accurex Biomedical Pvt Ltd Thane, Mumbai.and measured spectrophotometrically, Shimadzu. Release of TROPONIN-I was estimated by Troponin –I Rapid test kit commercially purchased from Reckon diagnostic Pvt Ltd. (Panda VS, 2009).

Statistical analysis

Statistical analysis was performed using one way ANOVA followed by Dunnett t test (GraphPad Instat, USA).

Histopathological study

At the end of study, the heart was isolated, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and five micrometer thick sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope and photomicrograph was taken.

Results and Discussion

Gross examination of heart

The visually examination of the heart showing inflammation, redness, capillary dilatation, Scar formation, colour, was performed and grading of the heart has been done.

Grading of heart parameter

Grade 0 = No Lesion

Grade 1= Inflammation, redness, capillary dilations.

Grade 2 = Edema, yellowish ventricle portion

Grade 3 = Atrium & ventricle turns yellow, scar formation

Grade 4 = Diffuse heart, absolute scar formation, increased necrosis portion.

The isoprenaline (ISO) group showed marked inflammation, scar formation, diffused heart when compared with Normal control (NC) group. The standard (STD) group showed marked reduction in edema, capillary dilation, and scar formation, with little redness when compared with Isoprenaline (ISO) group. The test group 2 (TG2) (100mg/kg) showed remarkable decrease in inflammation, redness, capillary dilatation and scar formation as compared to test group 1 (TG1) (350mg/kg) when both of the extract have been compared with isoprenaline (ISO) group(Table 1).

Heart weight/ body weight ratio

The heart weight and heart weight/body weight ratio was analysed in various treatment group. The isoprenaline (ISO) group showed marked increase in heart weight due to hypertrophy, when compared with Normal control (NC) group. The standard (STD) group showed reduction in heart weight and heart/body weight ratio when compared with Isoprenaline (ISO) group. Test group 2 (TG 2) (100 mg/kg) demonstrated significant decrease in heart weight and decrease in heart/body weight ratio when compared with Isoprenaline group When both test groups (350mg/kg, 100mg/kg) was compared with Isoprenaline (ISO) group, the highest dose (TG2) (650mg/kg) showed remarkable reduction in heart weight and heart/body weight ratio as compared to lowest dose (TG1) (350mg/kg) (Table.2).

Biochemical estimations

The effects of ethanolic extract of Coleus forskohlii oral treatments on serum marker enzymes AST, ALT, LDH, and CPK for 30 days are outlined in (Table 3). Rats treated with ISO showed a highly significant increase ($p<0.001$) in activities of serum marker enzymes compared with the normal rat (NC) group. Rats pretreated with metoprolol (STD group) when compared with Isoprenaline (ISO) group showed a highly significant ($p<0.001$) reduction in cardiac marker enzyme. Pretreatment with *Coleus forskohlii* high dose (TG2) (100 mg/kg) to rats for 30 days, followed by ISO subcutaneous injection on the 29th and 30^{th} days, elicited a highly significant ($p<0.001$) reduction in the ISO-induced increased activities of AST,ALT, LDH, and CPK. The low dose of coleus forskohlii (TG1) (50 mg/kg) when compared with Isoprenaline (ISO) group was significant ($p<0.01$) in lowering ISO elevated serum enzyme activities (Table 3).

The release of troponin-I was estimated by Rapid test kit after 4 hours of infarction. When Isoprenaline (ISO) group compared with normal control (NC) group, all animal of ISO group showed presence of troponin in serum. When Standard group was compared with Isoprenaline group (ISO) more than half animal showed absence of troponin in serum while in rest of them troponin was found to be present. When both test groups (TG1, TG2) (350mg/kg, 650mg/kg) was compared with (ISO) group, the highest dose (TG2) (650mg/kg) showed remarkable reduction in release of troponin as compared to lowest dose (TG1) (350mg/kg) (Table 4).

Histopathological examination

The 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 eosin18. The slides were examined under light microscope and microphotographs were taken.

Photomicrograph of rat heart of normal control group shows, the endocardium, myocardium, and epicardium as well as papillary muscles and vasculature were all normal and healthy in both the groups. There was no muscular hypertrophy or evidences of myositis (necrosis and/or round cell infiltrates), clearly visible in 10x (prominently) (Fig 1. a). Isoprenaline treated (ISO) group shows focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium vacuolar changes and

prominent oedema along with chronic inflammatory cells are clearly visible in 10x (prominently) **(**Fig 1.b). Rat heart pretreated with the Standard (STD) Metoprolol (10mg/kg) showing very lesser degree of myonecrosis, myophagocytosis and lymphocytic infiltration, oedema and very little infiltration of inflammatory cells are clearly visible in 10x (prominently) (Fig 1.c). Photomicrograph of rat heart pretreated with *Tinospora cordifolia* (TG1) (350mg/kg) group showing decreased degree of myonecrosis and lesser infiltration of inflammatory cells but myophagophytosis and subendocardium vacuolar changes are present & clearly visible in 10x (prominently) (Fig 1.d). Pretreated rat heart with *Tinospora cordifolia* (TG 2) (650 mg/kg) treated group showing little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagophytosis and subendocardium vacuolar changes are present & clearly visible in 10x (prominently) (Fig 1.e).

Cardiovascular disease is major global health problem reaching epidemic proportion in Indian subcontinent. (Banerjee A) and low and middle income countries accounting for 78% of all death.(WHO) myocardial cell protection and prevention of cell ischemia or necrosis have been therapeutics targets for a long time. New therapies are needed to treat ischemia because current treatment has only a limited impact on survival and annual cost (Milano CA).

The present investigation is aimed to explore and evaluate the cardioprotective effect of Tinospora cordifolia root and stem on isoprenaline induced myocardial infarction in rats. Myocardium contains an abundant concentration of diagnostic marker enzyme of myocardial infarction viz., AST, ALT, LDH, CK-MB and TROPONIN once myocardium is damaged, releases of its content into the extra cellular fluid serves as the diagnostic enzyme marker of myocardial damage tissue (Suchalatha S).

In our study we observed significant increase in the level on marker enzyme (AST, ALT, LDH, and TROPONIN) in serum of isoprenaline treated rats. It is noticeable that isoprenaline induced rat showed increased level of AST, ALT and LDH,CK-MB when compared to control rats. This finding could be a consequence of a reduction in the number of viable myocytes due to enhanced cell death in heart as these animals showed the highest level of AST, ALT, LDH and CK-MB (Mair J). Among both dose of *Tinospora cordifolia*, treatment with the highest dose (TG2) (650mg/kg) decreased the level of these marker enzyme quite significantly when compared to low dose (TG1) (350mg/kg) pointing clearly that *Tinospora cordifolia* could be a cardioprotective against the MI.

The isoprenaline (ISO) group showed marked increase in heart weight due to hypertrophy, when compared with Normal control (NC) group. The standard (STD) group showed reduction in heart weight and heart/body weight ratio when compared with Isoprenaline (ISO) group showing significantly less hypertrophy. Test group (TG2) (650 mg/kg) demonstrated more significant decrease in heart weight and decrease in heart/body weight ratio than Test extract (TG1) (350mg/kg) when compared with isoprenaline group, showing marked reduction in hypertrophy, change in shape of heart.

The visually examination of the heart showing inflammation, redness, capillary

Table 1. Observation table for grading of heart	
Groups	**Grading of cardiac damage**
Normal control (NC)	Grade 0
Isoprenaline (ISO)	Grade 4
Standard (STD)	Grade 1
Test group 1(TG 1) (350mg/kg)	Grade 3
Test group 2(TG 2) (650mg/kg)	Grade 2

Table 2. body weight, heart weight, heart: body weight ratio			
Groups	**Body weight (g)**	**Heart weight (g)**	**Heart/body weight ratio (x $10^{3)}$**
Normal control (NC)	208	0.81	3.8
Isoprenaline (ISO)	191	1.08	5.6
Standard (STD)	216	0.88	4.0
Test group 1(TG 1) (350mg/kg)	220	1.01	4.5
Test group 2(TG 2) (650mg/kg)	225	0.98	4.3

Table.3 Effect of methanolic extract of *Tinospora cordifolia* on CK–MB, LDH, AST and ALT levels in rats by Isoprenaline -induced

Treatment	**AST (IU/L)**	**ALT (IU/L)**	**LDH (IU/L)**	**CK (mg/dl)**
NC	155.137±24.5	103.630±2.41	107.780±2.11	1.034±0.020
ISO	285.911±24.9[a]	255.144±16.9[a]	307.743±22.44[a]	1.260±0.044[a]
STD	161.277±23.4[b]	105.226±22.8[b]	111.106±4.27[b]	1.068±0.029[b]
TG1	191.65± 18.80[c]	210.67± 8.17	243.22± 12.06	1.24± 0.065
TG2	171.81±24.77[d]	168.09± 17.11	218.91± 15.18	1.11± 0.025

Values are mean ± SEM for five animals in each group

[a]**P < 0.001** When ISO group is compare with NC group

[b]**P < 0.001** When standard group is compare with ISO group

[c]**P < 0.01** When experimental group is compare with ISO group

[d]**P < 0.001** When experimental group is compare with ISO group

AST- Aspartate amino transferaces; ALT- Alanine transaminase; LDH - Lactate dehydrogenase

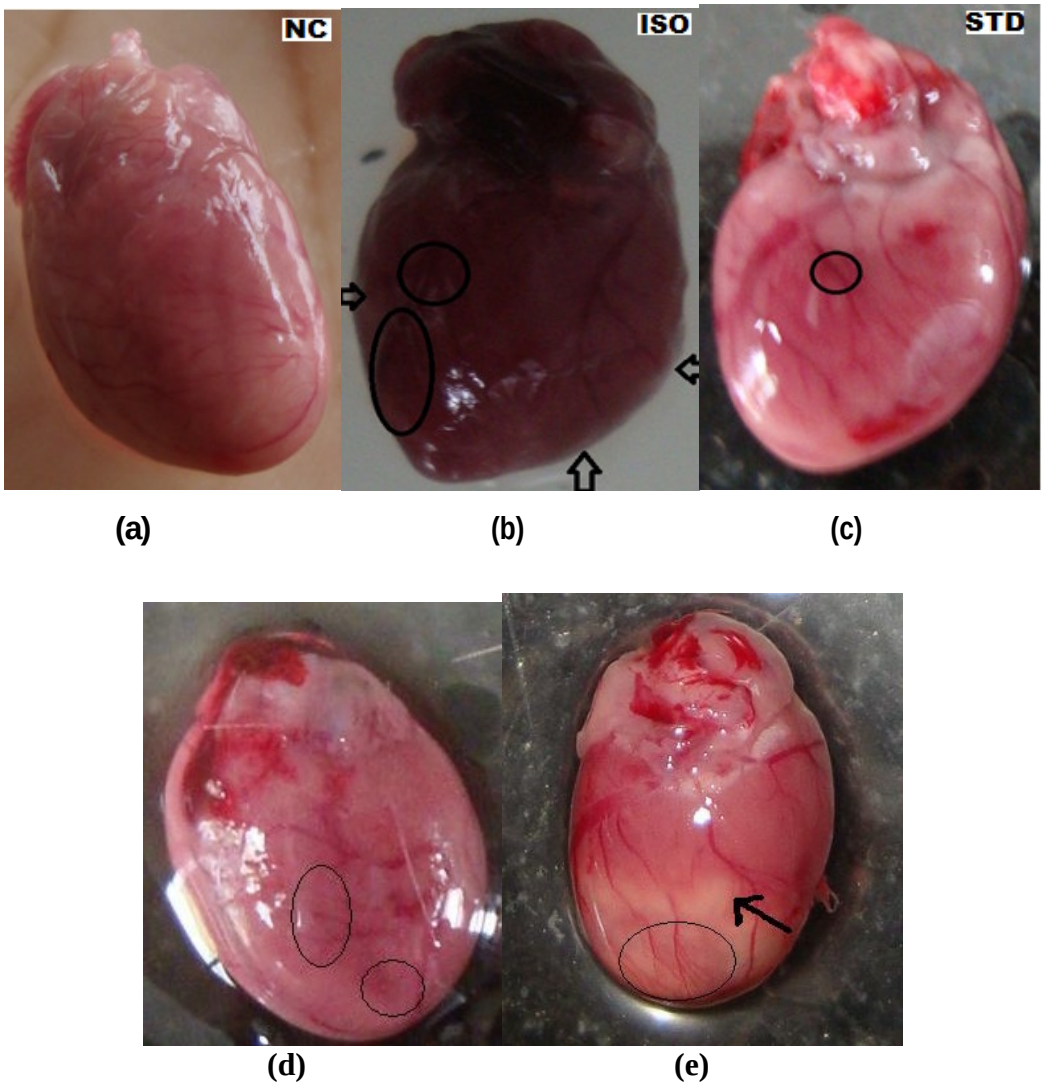
CK-MB- Creatine kinase; NC- Normal control; ISO-Isoprenaline; STD- Standard; TG1- Test group 1; TG2- Test group 2

Table 4 Release of TROPONIN –I in various treatment groups

Animal no.	Normal Control	Isoprenaline (85mg/kg)	Standard (10mg/kg)	Test extract 50mg/kg	Test extract 100mg/kg
1	-ve	+ve	-ve	+ve	-ve
2	-ve	+ve	-ve	+ve	-ve
3	-ve	+ve	+ve	-ve	+ve
4	-ve	+ve	-ve	+ve	-ve
5	-ve	+ve	+ve	-ve	+ve

+ve - Presence of troponin in serum; -ve - Absence of troponin in serum

Figure 1- Visual photographs of dissected rat heart:



a- (NC) group showing no scar, oedema, capillary dilation; b- (ISO) group showing hypertrophy, oedema, and change in colour (circle and arrow); c- (STD) group showing with no oedema, capillary dilation but showing inflammation and redness (circle); d- (TG1) group showing redness, inflammation near ventricle (circle); e- (TG2) group showing no redness, but shows minor inflammation (arrow)

Figure.2 Photomicrograph of heart section (10x, 10x10):

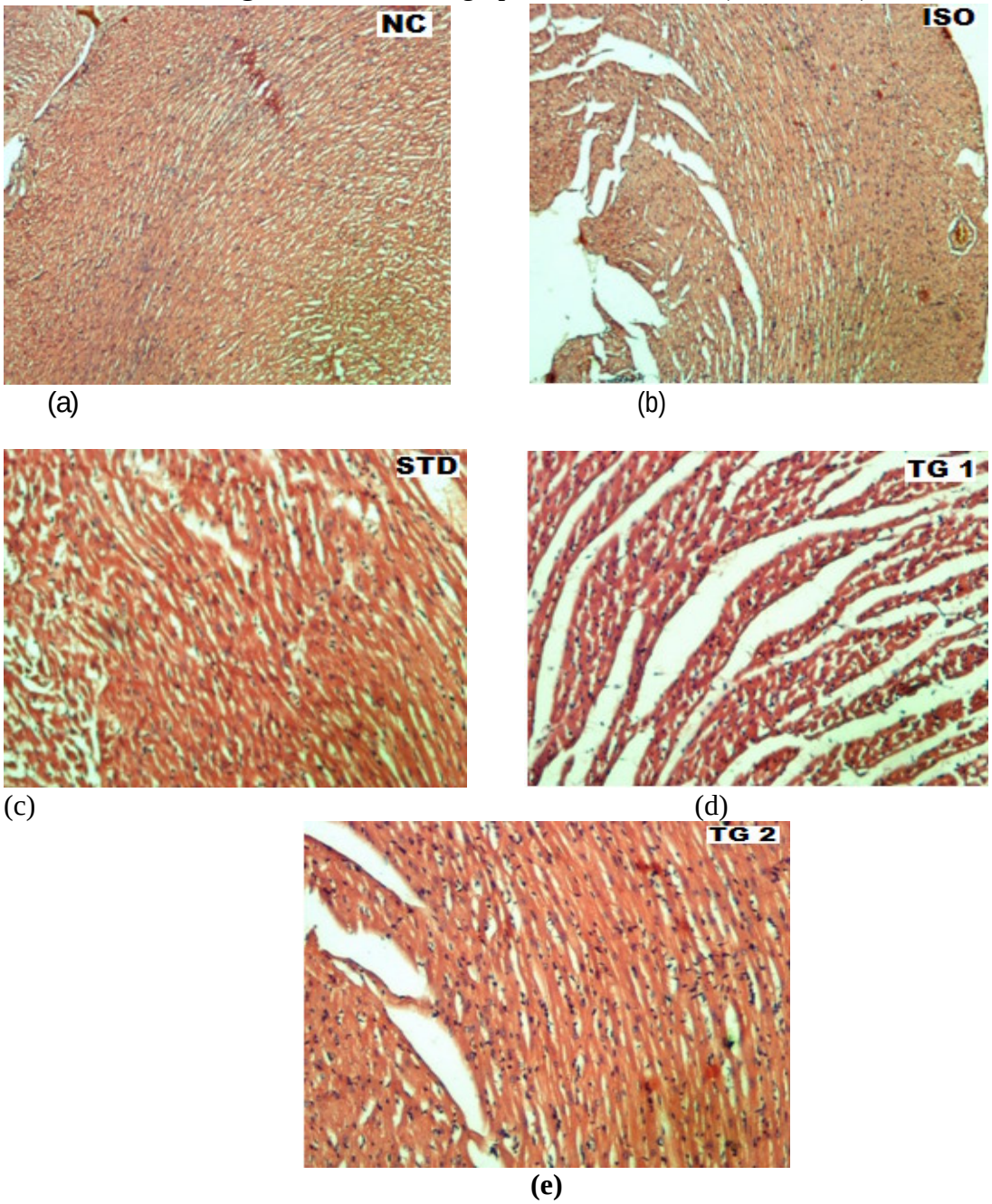


Figure 2- photomicrograph of heart section (10x, 10x10): a- Normal group (NC) showing normal cytoarchitecture; b- Isoprenaline (ISO) group shows shows focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium vacuolar changes and prominent oedema along with chronic inflammatory cells are present; c- Standard (STD) group showing very lesser degree of myonecrosis, myophagocytosis and lymphocytic infiltration, oedema and very little infiltration of inflammatory cells; d- Test group (TG 1) showing decreased degree of myonecrosis and lesser infiltration of inflammatory cells but myophagophytosis and subendocardium vacuolar changes are present; e- Rat heart Pretreated with Test group 2 (TG 2) (650 mg/kg) treated group showing little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagophytosis and subendocardium vacuolar changes are present

dilatation, Scar formation, colour, in all part of heart gives the information regarding degree of necrosis and according to above observation grading was performed. The isoprenaline (ISO) group showed marked inflammation, scar formation, showing grade 4 type of cardiac damage. The standard (STD) group showed marked reduction in edema, capillary dilation, and scar formation showing grade 1 cardiac damage. The test group 2 (TG2) (650mg/kg) showed remarkable decrease in inflammation, redness, capillary dilatation, scar formation owing grade 2 type of cardiac damage as compared to test group 1 (TG1) (350mg/kg) which shows cardiac damage of grade 3 when both of the extract have been compared with isoprenaline group.

Histopathological examination of myocardial tissue obtained from normal control (NC) group exhibited clear integrity of myocardial membrane. Normal control (NC) rats showed cardiac fibers without any infarction. The heart sections obtained from Isoprenaline (ISO) treated rats showed disruption of several subcellular elements including myonecrosis, myophagocytosis and lymphocytic infiltration, oedema, loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm, formation of lysosomal bodies and dilation of the sarcotubule and dilation of the sarcotubular system, (Olson and Young,1974). Treatment with test extract 2 (TG2- 650 mg/kg, restores the architecture of the heart near to normal as compared to test extract 1 (TG1-350mg/kg) when both group are compared with the Isoprenaline (ISO) group.

The lesions produced by ISO in rat heart are similar to those found in myofibrillar degeneration in human ischemic heart disease (IHD) (Milei J). Hence, the study of ISO-induced myocardial necrosis and its underlying mechanisms might provide better insight and new leads on the pathogenesis of IHD.

In conclusion the result of present study indicated that the prior administration of methanolic extract of *Tinospora cordifolia* attenuates isoprenaline induced MI. The cardioprotective activity of *Tinospora cordifoli*a probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action.

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References

Dhalla, N.S., A.B. Elmoselhi, T. Hata and N. Makino: Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovas. Res., 47, 446-456 (2000).

Ferrari, R., O. Alûeri, S. Curello, S. Ceconi, A. Cargnoni, P. Marzollo, A. Pardini, E. Caradonna and O. Visioli: Occurrence of oxidative stress during reperfusion of the human heart. Circulation, 81, 201- 211 (1990)

Hearse, D.J.: Prospects for antioxidant therapy in cardiovascular medicine. Am. J. Med., 91, 118S-121S (1991).

Cooper, R., J. Cutler, P.D. Nickens, S.P. Fortmann, L. Friedman, R. Havlik, G. Hogelin, J. Marler, P. McGovern, G. Morosco, L. Mosca, T. Pearson, J. Stamler, D. Strye and T. Thom: Trends and disparities in coronary heart

disease, stroke, and other cardiovascular disease in the United States, findings of the national conference on cardiovascular disease prevention. Circulation, 102, 3137-3147 (2000)

Hertog, M.G.L., E.J.M. Feskens, P.C.H. Hollam, M.B. Katan and D. Kromhout: Dietary antioxidant flavonoids and risk of coronary heart diseases. The Zutphen elderly study. Lancet, 342, 1007-1020 (1993)

Ojha, S.K., M. Nandave, S. Arora, R. Narang, A.K. Dinda and D.S. Arya: Chronic administration of Tribulus terrestris Linn extract improves cardiac function and attenuates myocardial infarction in rats. Int. J. Pharmacol., 4, 1-10 (2008)

Goyal, S.N., S. Arora, A.K. Sharma, S. Joshi, R. Ray, J. Bhatia, S. Kumari and D.S. Arya: Preventive effect of crocin of Crocus sativus on hemodynamic, biochemical, histopathological and ultrastuctural alterations in isoproterenol-induced cardiotoxicity in rats. Phytomedicine, 17, 227-232 (2010)

Mohanty, I., D.S. Arya, A. Dinda, K.K. Talwar, S. Joshi and S.K. Gupta: Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction. Basic Clin. Pharmacol. Toxicol., 94, 184-189 (2004).

Rona G, Chappel CI, Balazs T, Gaudry R. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. *AMA Arch Pathol* 1959;67:443- 455.

De Souza NJ, Dohadwalla AN, Reden J. Forskolin: a labdane diterpenoid with antihypertensive, positive inotropic, platelet aggregation inhibitory, and adenylate cyclase activating properties. *Med. Res. Rev*, 1983; 3: 201-219.

Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Ind. J. Pharmacol. 2011; 43: 516-519.

Panda VS, Naik SR (2009). Evaluation of cardioprotective activity of Ginkgo biloba and Ocimum sanctum in rodents. Altern Med Rev. 14(2):161-71.

Firoz M, Bharatesh K, Patole N, Godse V, Shikalgar T, Nikwade N.Cardioprotective activity of ethanolic extract of *Callistemon lanceolats*leaves on doxorubicin induced cardiomyopathy in rats.Bangladesh J Pharmacol. 2011;6(1):24-27.

Banerjee A. Coronary artery disease and its problem in management. J Indian med assoc 2001; 99: 474-5.

WHO: The world health report 1999 making a difference. Geneva: world health organisation.

Milano CA, Allen LF, Rockman HA, Dolber PC, Mcminn TR, Chien KR, et al. Enhanced myocardial function in transgenic mice overexpressing beta-2 adrenergic receptor. Science 1994; 264:582-86.

Suchalatha S, shyamaladevi CS. Protective effect of terminalia chebula against experimental myocardial injury induced by isoproterenol. Indian J Exp Biol 2004; 42:174-8.

Mair J, Wagner I, Jakob G, Lechleitner P, Drenstl F, Paschenctort B, Michel G. Different time couse of cardiac contractile protein after acute myocardial infarction. Clin Chin Acta 1994;231:47-60.

Olson HM, Young DM. Electrolyte and morphologic alterations of myocardium in adriamycin treated rabbits. Am J Pathol. 1974; 77: 439-54.

Young RC, Myers CE. Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. Science 1977; 19:165-67.

Milei J, Nunez RG, Rapaport M. Pathogenesis of isoproterenol-induced myocardial lesions: its relation to human coagulative myocytolysis. *Cardiology* 1978; 63:139-151.

Stanely Mainzen Prince P, Menon VP. Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. Phytother Res. 2001;15:213–8.

Tripathi I. "Raj Nighantu". Varanasi: Chaukhambha Krishnadas Academy; 2003. p. 31.

Sinha K, Mishra NP, Singh J, Khanuja SP. *Tinospora cordifolia* (Guduchi): A reservoir plant for therapeutic applications: A review. Indian J Trad Know. 2004;3:257–70.

Quality standards of Indian medicinal plants. 1st ed. Vol. I. New Delhi: ICMR; 2003. Anonymous; pp. 212–8.

Sen P, Maiti PC, Puri S, Ray A, Audulov NA, Valdman AV. Mechanism of anti-stress activity of Ocimum sanctum Linn, eugenol and *Tinospora malabarica* in experimental animals. Indian J Exp Biol. 1992;30:592–6.

Jana U, Chattopadhyay RN, Shw BP. Preliminary studies on anti-inflammatory activity of *Zingiber officinale Rosc.*, *Vitex negundo* Linn. and Tinospora cordifolia (Willid) Miers in albino rats. Indian J Pharmacol. 1999;31:232–3.

Pahadiya S, Sharma J. Alteration of lethal effects of gamma rays in Swiss albino mice by *Tinospora cordifolia*. Phytother Res. 2003;17:552–4.

Singh N, Singh SM, Shrivastava P. Immunomodulatory and antitumor actions of medicinal plant *Tinospora cordifolia* are mediated through activation of tumor-associated macrophages. Immunopharmacol Immunotoxicol. 2005;26:145–62.

Rawal A, Muddeshwar M, Biswas S. Effect of Rubia cordifolia, Fagonia cretica linn, and *Tinospora cordifolia* on free radical generation and lipid peroxidation during oxygen-glucose deprivation in rat hippocampal slices. Biochem Biophys Res Commun. 2004;324:588–96.

Rathi SS, Grover JK, Vikrant V, Bisis NR. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. Phytother Res. 2002; 16(8): 774-7.