



Cardioprotective Activity of *Terminalia Belerica* on Isoprenaline Induced Myocardial Necrosis in Rat

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Abstract

The present study was to evaluate the cardioprotective effect of ethanolic extract of *T.belerica* against isoprenaline (ISO) induced myocardial necrosis in rats. Male wistar rats were administered *Terminalia belerica* (150 and 300mg/kg/p.o.) for 28 days and metoprolol (meto, 10 mg/kg) for 28 days orally in their respective groups. Myocardial damage was induced by subcutaneous administration of isoprenaline (85 mg/kg) for two consecutive days. A change in biomarkers levels reflects the influence of treatment. The creatine kinase (CK) activities were fallen in serum and elevated in heart tissue of animals treated with low and high doses of T.B as well as Metoprolol compared to ISO control. The enzymes-lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum activity were significantly reduced in serum with both and high doses of T.belerica while no change was noted in heart with both doses compared to ISO control. Hence it is concluded that *T. belerica* possess potential to ameliorate the myocardial damage induced by isoproterenol in rat.

Keywords: *Terminalia belerica*, Isoprenaline, Cardioprotective Activity

Contents

1. Introduction	127
2. Experimental	128
3. Results and discussion	129
4. References	133

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1. Introduction

Isoprenaline (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction. ISO is a β -adrenergic agonist that causes severe stress in myocardium and necrotic lesions in the heart muscles. ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes. MI induced by ISO in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia and increase in serum creatine phosphokinase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities. The mechanism proposed to explain isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through auto-oxidation of catecholamine and has been implicated as one of the causative factor. Excessive endogenous release or exogenous administration of catecholamines depletes the energy reserve of cardiac muscle cells. This leads to

complex biochemical and structural changes that cause irreversible cellular damage, which is a prelude to necrosis (Rona et al, 1963).

Cardiovascular Diseases (CVD) are the secondary cause of deaths in many parts of the world, although modern drugs are effective in preventing the disorders, their use is often limited because of their side effects and adverse reactions. A wide array of plants and its active principles, with minimal side effects, provide an alternate therapy for heart disease. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds. ***Terminalia belerica* Roxb.** of family **Combretaceae** is distributed throughout. It is found in abundance in Madhya Pradesh, Uttar Pradesh, Punjab, Maharashtra, and also in Ceylon and Malaya. It is used for various ailments in Indian Traditional System of Medicine. The famous myrabolan fruit *Terminalia belerica* is the best single herb for treatment. It is a powerful rejuvenative herb that nourishes the lungs, throat, voice, eyes and hair. It expels stones, and digestive, urinary, and respiratory tracts. Gastric ulcers, hemorrhoids, chronic diarrhea, dysentery, parasites, cholelithiasis, ophthalmia, headache, alopecia and premature graying, edema, rheumatism (topical), wounds (topical).

The present study was designed to evaluate the cardioprotective effect of ethanolic extract of *T. belerica* against isoproterenol (ISO) induced myocardial damage in rats on the basis of biochemical and gross examination of heart observation.

2. Materials and methods

Plant material (*Terminalia belerica*)

The dried ripe fruits of *Terminalia belerica* were purchased from the local market. The plant authenticated by a botanist of National Botanical Research Institute Lucknow (UP). Ref No. NBRI/CIF/194/2011, Specification-NBRI-SOP-202 specimen sample of the same was preserved in the section of the Faculty of Pharmacy, Integral University, Lucknow, with the voucher No. NBRI-SOP-202 for future reference.

Preparation of ethanolic extract

The dried ripe fruits of *Terminalia belerica* (1kg) was powdered and passed through the sieve (coarse 16/22). The powder was used for the preparation of ethanolic extract. An 80% ethanolic extract was prepared by Soxhlet extraction method. Firstly dried powdered fruits of *Terminalia belerica* (50g) were defatted with petroleum ether (40-60) for 2 hours, and then powder drug is dried under shade and extracted with 80% v/v ethanol for 15 hours, using Soxhlet extractor. The extracts were concentrated at 40°C to obtain dark brownish syrupy residue. The yield obtained from the above process was found to be 40% w/w. The extracts were preserved in a refrigerator at 4°C.

Drugs and Chemicals:

Isoprenaline Hydrochloride Sigma Chemicals, USA, Metoprolol Tartrate (METOLOR- 50) Cipla Ltd Sikkim, Serum ALT, AST and Creatinine (CK) diagnostic kit from Span Diagnostics Ltd, Surat. Serum LDH diagnostic kit from Accurex Biomedical PVT Ltd Mumbai. Other chemicals and reagents were of analytical grade.

Animal

Male Wistar rats (150-200g) were used for the study. They were housed, housed five each in sanitized polypropylene cages containing paddy husk as bedding under standard laboratory conditions at room temperature (23°C ± 2°C) with 12 h light / dark cycle. The animals were randomized into experimental and control groups. They had free access to standard pellets as basal diet and water ad libitum. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) Regd. No. Regd. No. IU/Pharm/M. Pharm/CPCSEA/10/25 Faculty of Pharmacy Integral University, Dasauli, P.O. Bas-ha Kursi Road; Lucknow 226026 (U.P)

Experimental protocol:

Rats were weighed (150-200) g and randomly divided into a group marked to permit individual identification and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Rats were treated with two different doses of 80% ethanolic extract of *Terminalia belerica* (150 and 300 mg/kg/day) by orally using an oral feeding tube daily for 29 days. On 29th day, myocardial injury was induced in experimental rats by injection of ISO (85 mg/kg/day, s.c.) twice at an interval of 24 hours (i.e., on 29th and 30th day of extract treatment) while normal control and ISO treated rats were given an equivalent volume of the vehicle. (Rona, et al., 1961)

Group-I Normal control rats treated with normal saline

Group-II Rats treated with Isoprenaline (85mg/kg/day, subcutaneous)

Group-III Rats pretreated with standard drug metoprolol (metolor -50) (10 mg/kg/day, oral) and ISO (85 mg/kg/day for 2 days, subcutaneous.)

Group-IV Rats pretreated with *T. belerica* extract (150 mg/kg/day, oral) and ISO (85 mg/kg/day; for 2 days, subcutaneous.)

Group-V Rats pretreated with *T. belerica* extract (300 mg/kg/day, oral) and ISO (85 mg/kg/day; for 2 days, subcutaneous.)

At the end of the 28 days of pretreatment with test drug rats were weighed and Isoprenaline Hydrochloride injection was administered at the dose of ISO (85 mg/ kg/day, s.c.) twice at an interval of 24 hours (i.e., on 29th and 30th day of extract treatment). The rats were sacrificed on day 31th blood was collected by cardiac puncture by using a 2ml syringe under light ether anesthesia and allowed to clot for 30minutes 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. The hearts were dissected out immediately, chilled, and perfuse with Ice-cold saline. (Suchalatha and Shyamala, 2004)

Gross Examination of Rat Heart

The hearts were dissected out and wash 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, Chauhan *et al.*, 2010)

Estimation of cardiac marker enzymes

Serum aspartate aminotransferase (AST), alanine transaminase (ALT) and creatinekinase (CK) was determined by using standard kits from Span Diagnostics Ltd, Surat,India. Serum lactate dehydrogenase (LDH) was estimated by using standard kits from Accurex Biomedical Pvt Ltd., Mumbai. All estimation were carried out using UV spectrophotometer (Shimadzu,india). (Vandana S. Panda, Suresh R. Naik,2009)

Table:1 Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on serum enzymes levels in rats against isoprenaline (ISO) induced myocardial necrosis.

GROUP	Treatment	Serum enzyme parameters			
		AST (IU/L)	ALT (IU/L)	LDH (IU/L)	CK (mg/dl)
Group 1	Normal control (0.9% saline)	67.72±1.89	48.93±1.25	1168.55±23.31	1.030±0.024
Group 2	Iso.control (85mg/kg/day, s.c)	82.95±2.83**	63.99±3.06**	1365.56±38.37**	1.246±0.041**
Group 3	Standard metoprolol (10mg/kg/day,p.o.)	69.59 ±1.44*	50.26±1.02*	1183.00±38.54*	1.089±0.032*
Group 4	Ethanolic extract of T.belerica (150mg/kg,day p.o.)	74.37±0.41*	54.37±1.73*	1248.55±33.62*	1.125±0.032*
Group 5	Ethanolic extract of T.belerica (300mg/kg/day p.o.)	70.02±1.00*	51.35±1.05*	1241.00±39.22*	1.124±0.019*

Values expressed as mean ±SEM for six animals

**P<0.05 considered statistically significant as compared to normal control group.

* P<0.05 considered statistically significant as compared to ISO control group.

GP1- Normal control

GP2- ISO control

GP3- Standard metoprolol (10mg/kg/day,oral)

GP4- Ethanolic extract of T.belerica (150mg/kg/day p.o.)

GP5- Ethanolic extract of T.belerica (300mg/kg/day p.o.)

CK=Creatine kinase, LDH= Lactate dehydrogen

3. Results and Discussion

Effect on serum Creatininekinase (CK)

Rats treated with Iso(85mg/kg/s.c.) for two consecutive days had serum CK level of (1.246±0.041mg/dL) measured. This was significantly higher (P<0.05) when compared to serum levels of CK in normal control rats (1.030±0.024mg/dL). Iso Rats treated with Ethanolic extract of T.belerica (150,300mg/kg, p.o, once daily) for 28 days had serum level CK of(1.125±0.032and 1.125±0.019 mg/dL) measured. This was significantly lower (P<0.05) when compared to CK levels in Iso control rats (1.246±0.041mg/dL)

Effect on serum Aspartate transaminase (AST)

Rats treated with Iso (85mg/kg/s.c.) for two consecutive days had serum AST level of (82.95±2.83IU/L)measured. This was significantly higher (P<0.05) when compared to serum levels of AST in normal control rats (67.72±1.89IU/L)

Iso Rats treated with Ethanolic extract of T.belerica (150,300mg/kg, p.o, once daily) for 28 days had serum level AST of(74.37±0.41and 70.02±1.00 IU/L) measured.This was significantly lower(P<0.05) when compared to AST levels in Iso control rats (82.95±2.83IU/L)

Effect on serum Alanine transaminase (ALT)

Rats treated with Iso(85mg/kg,s.c.) for two consecutive days had serum ALT level of (63.99±3.06IU/L) measured. This was significantly higher (P<0.05) when compared to serum levels of ALT in normal control rats (48.93± 1.25I U/L). Iso Rats treated with Ethanolic extract of T.belerica (150,300mg/kg, p.o, once daily) for 28 days had serum level ALT of(54.37±1.73and51.35±1.05 IU/L) measured.This was significantly lower(P<0.05) when compared to ALT levels in Iso control rats (63.99±3.06IU/L)

Effect on Serum Lactate dehydrogenase (LDH)

Rats treated with Iso(85mg/kg,s.c.) for two consecutive days had serum LDH level of (1365.56±38.37IU/L) measured. This was significantly higher (P<0.05) when compared to serum levels of LDH in normal control rats (1168.55±23.31IU/L). Iso Rats treated with Ethanolic extract of T.belerica (150,300mg/kg, p.o, once daily) for 28 days had serum level LDH of (1248.55±33.62 and 1241.00±39.22 IU/L) measured. This was significantly lower (P<0.05) when compared to LDH levels in Iso control rats (1365.56±38.37IU/L)

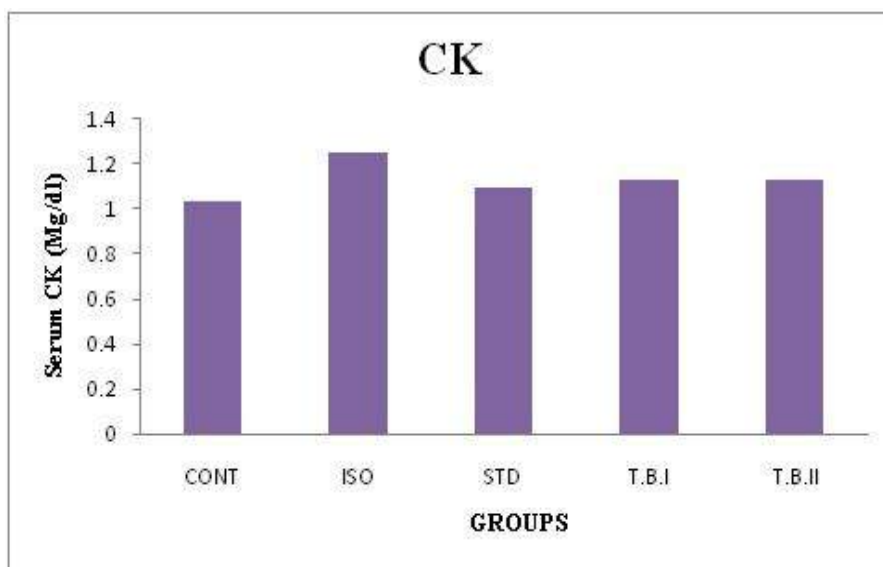


Figure 1: Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on serum CK levels in rats against (ISO) isoprenaline (85 mg/kg/s.c.) for two consecutive days induced myocardial necrosis.

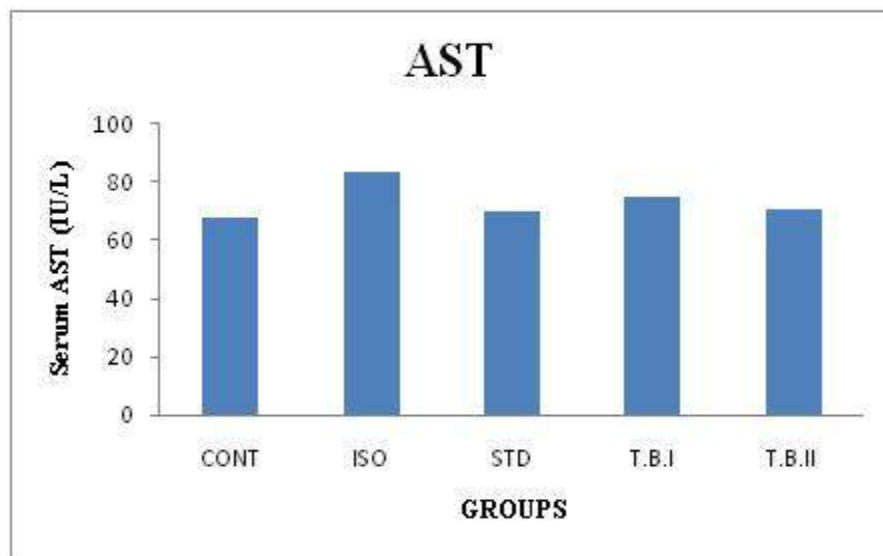


Figure 2: Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on serum AST levels in rats against (ISO) isoprenaline (85 mg/kg/s.c.) for two consecutive days induced myocardial necrosis.

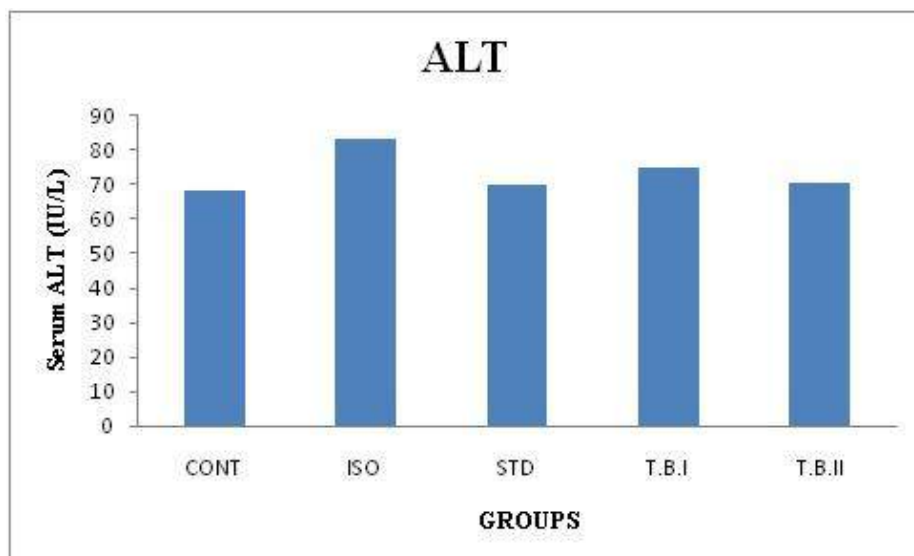


Figure 3: Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on serum ALT levels in rats against (ISO) isoprenaline (85 mg/kg/s.c.) for two consecutive days induced myocardial necrosis.

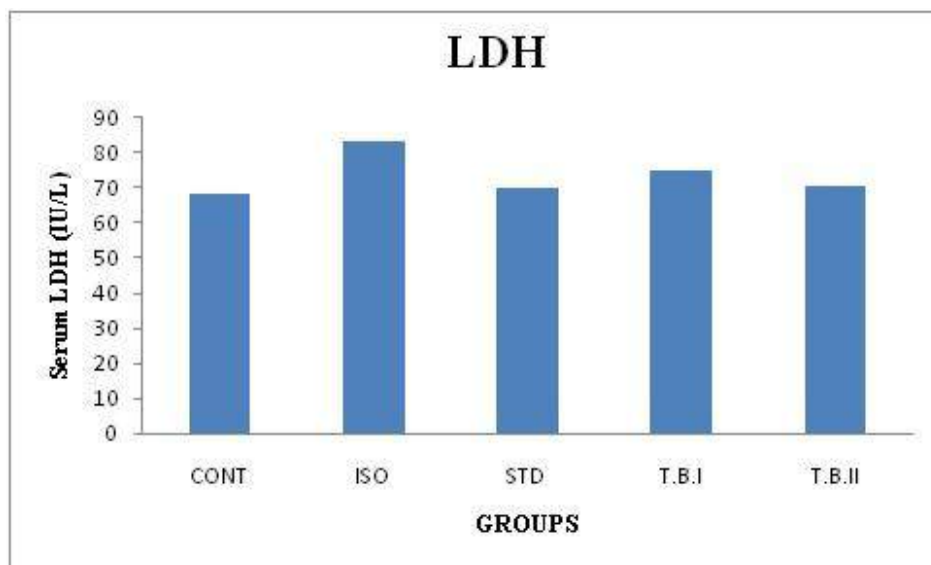


Figure 4: Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on serum LDH levels in rats against (ISO) isoprenaline (85 mg/kg/s.c.) for two consecutive days induced myocardial necrosis.

CONT - Normal control

ISO - ISO control

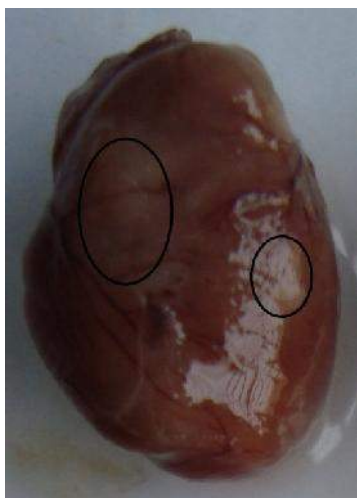
STD - Standard metoprolol (10mg/kg/day,oral)

T.B.I - Ethanolic extract of T.belerica (150mg/kg/day p.o.)

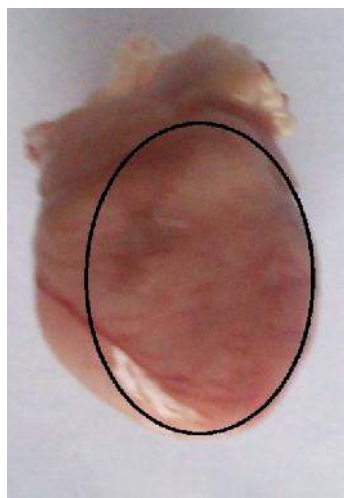
T.B.II- Ethanolic extract of T.belerica (300mg/kg/day p.o.)

Table 2: Gross examination (grading) of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) against isoprenaline (ISO) induced myocardial necrosis in rat.

Group	Grading number
Normal control (0.9% saline)	0
ISO control (85 mg/kg/s.c.)	4
Metoprolol (10mg/kg/day,p.o.)	3,4
T.belerica (150mg/kg/day p.o.)	2,1
T.belerica(300mg/kg/day p.o.)	1,2



(I) T.belerica (150 mg/kg/day p.o.)



(II) Isoprenaline (85 mg/kg/s.c)



(III) Standard metoprolol (10mg/kg/day,p.o.)

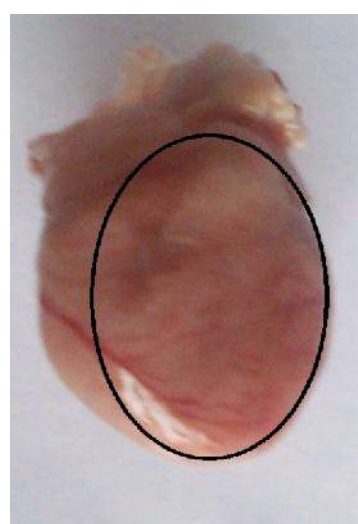


(IV) Normal control (0.9%saline)

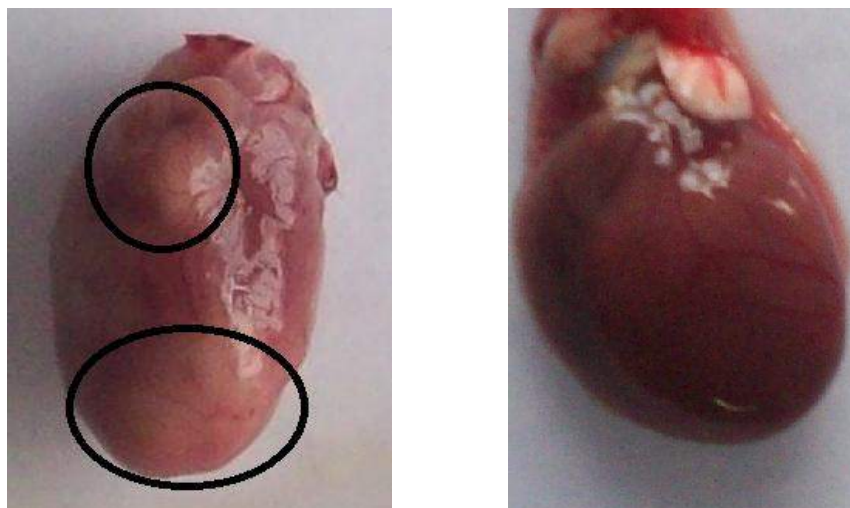
Figure 5



(I) T.belerica (300 mg/kg/day p.o.)



(II) Isoprenaline (85 mg/kg/s.c)



(III) Standard metoprolol (10mg/kg/day,p.o.) (IV) Normal control (0.9%saline)

Figure 6

Normal control- No lesion.

ISO control Isoprenaline (85 mg/kg/s.c) - Diffuse heart, Scar formation, and yellowish colour of atrium and ventricle part of heart.

Standard metoprolol (10mg/kg/day,p.o.)

Edema, capillary dilatation, ventricle portion yellowish.Scar formation, yellowish colour of atrium and ventricle part of heart.

T.B.I - Ethanolic extract of T.belerica (150mg/kg/day p.o.)

Inflammation and redness, Edema, capillary dilatation, ventricle portion yellowish.capillary dilatation.

T.B.II- Ethanolic extract of T.belerica (300mg/kg/day p.o.)

Inflammation and redness, capillary dilatation near to normal control heart

Discussion

Medicinal plants have long been valued as sources of new compounds with cardioprotective activity. The present study demonstrated that the *Terminalia bellerica* extract has efficiently protected the myocardium against isoprenaline-induced MI. Isoprenaline administration brought about a significant decrease in the activities of cardiac marker enzymes such as AST, ALT, CK and LDH in the myocardial tissue (Table I), with a subsequent increase in the activities of these enzymes in the serum. This might be due to the damage in the heart muscle, rendering the leakage of enzymes into the serum.(Ebenezar., *et al* 2003) The significant rise observed in the levels of diagnostic marker enzymes in the serum of Group II isoprenaline administered rats as compared to that of Group I control rats (Table I) is an indication of the severity of the necrotic damage to the myocardial membrane. Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue.[19]

The release of cellular enzymes reflects non-specific alterations in the membrane integrity and permeability as a response to , -adrenergic stimulation. In the present study, pretreatment with the *Terminalia bellerica* extract significantly prevented isoproterenol-induced elevation in the levels of the diagnostic marker enzymes of Groups IV, V and VI animals compared to Group II rats, probably due to the protective effect of the *Terminalia bellerica* extract on the myocardium; this reduced the extent of myocardial damage and thereby restricted the leakage of these enzymes from the myocardium.(Manjula *et al.*,1992) The significant elevation observed in the level of serum uric acid in the isoproterenol-injected groups could be due to the excessive degradation of purine nucleotides and proteolysis. In conclusion, the results of the present study indicate that the prior administration of the *Terminalia bellerica* extract attenuates isoproterenol-induced MI. The cardioprotective effect of the *Terminalia bellerica* extract is probably related to its ability to strengthen the myocardial membrane by its membrane-stabilising action. *Terminalia bellerica* extract at the dose level of 300 mg/kg was found to be more effective dose. In this study, the cardioprotective potential of *Terminalia bellerica* extract is evident.

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