Emerging Trend of Herbal Drug Terminalia Belerica Role in Treatment of Ischemic Heart Disease

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A B S T R A C T

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 while no change was noted in heart with both doses 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.B 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

A R T I C L E  I N F O

CONTENTS

1. Introduction ........................ ................................................................. 912
2. Materials and Methods ................................................................. 912
3. Results and discussion ................................................................. 913
4. References ................................................................. 916

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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 myrobolan fruits 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 (coarse16/22). 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 15hours, 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 in to experimental and control groups. They had free assessed 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 was 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, 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 was given a 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 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. The hearts were dissected out immediately, chilled, and perfused with Ice-cold saline. (Suchalatha and Shyamala, 2004)

Gross Examination of Rat Heart
The hearts were dissected out and 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, 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)

3. Results and Discussion

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

<table>
<thead>
<tr>
<th>GP0</th>
<th>Treatment</th>
<th>Serum enzyme parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AST (IU/L)</td>
</tr>
<tr>
<td>Group 1</td>
<td>Normal control (0.9% saline)</td>
<td>67.72±1.89</td>
</tr>
<tr>
<td>Group 2</td>
<td>Iso.control (85mg/kg/day, s.c)</td>
<td>82.95±2.83**</td>
</tr>
<tr>
<td>Group 3</td>
<td>Standard metoprolol (10mg/kg/day, p.o.)</td>
<td>69.59 ±1.44*</td>
</tr>
<tr>
<td>Group 4</td>
<td>Ethanolic extract of T.belerica (150mg/kg/day, p.o.)</td>
<td>74.37±0.41*</td>
</tr>
<tr>
<td>Group 5</td>
<td>Ethanolic extract of T.belerica (300mg/kg/day, p.o.)</td>
<td>70.02±1.00*</td>
</tr>
</tbody>
</table>

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.)

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 CK levels of ISO 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 (70.02±1.00 IU/L) measured. This was significantly lower (P<0.05) when compared to CK 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±1.25IU/L). Iso Rats treated with Ethanolic extract of T.belerica (150,300mg/kg, p.o, once daily) for 28 days had serum level ALT (1241.00±39.22 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± 38.37 IU/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).

**Table 2:** Effect of pretreatment of administration of ethanolic extract of T.belerica (150 and 300mg/kg, p.o, once daily) on isoprenaline (85 mg/kg/s.c.) for two consecutive days induced myocardial necrosis.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Gross examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Necrosis</td>
</tr>
<tr>
<td>Normal control (0.9%saline)</td>
<td>A</td>
</tr>
<tr>
<td>ISO control (85 mg/kg/s.c.)</td>
<td>+++</td>
</tr>
<tr>
<td>Metoprolol (10mg/kg/day,p.o.)</td>
<td>+</td>
</tr>
<tr>
<td>T.belerica (150mg/kg/day p.o.)</td>
<td>A</td>
</tr>
<tr>
<td>T.belerica(300mg/kg/day p.o.)</td>
<td>A</td>
</tr>
</tbody>
</table>

(A) no changes, (+++)severe changes,(++)moderate changes,(+)mild changes
Figure 5 (I) T. belerica (150 mg/kg/day p.o.)

Figure 5 (II) Isoprenaline (85 mg/kg/s.c)

Figure 5 (III) Standard metoprolol (10 mg/kg/day, p.o.)

Figure 5 (IV) Normal control (0.9% saline)

Figure 6 (I) T. belerica (300 mg/kg/day p.o.)

Figure 6 (II) Isoprenaline (85 mg/kg/s.c)
Normal control- No lesion.
ISO control Isoprenaline (85 mg/kg/s.c) - Diffuse heart, Scar formation, and yellowish calour of atrium and ventricle part of heart.
Standard metoprolol (10mg/kg/day,p.o.)
Edema, capillary dilatation, ventricle portion yellowish.Scar formation, yellowish calour of atrium and ventricle part of heart.

Discussion
Medicinal plants have long been valued as sources of new compounds with cardioprotective activity. The present study demonstrated that the *Terminalia belerica* 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 β-adrenergic stimulation. In the present study, pretreatment with the

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