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Research Article

CARDIOPROTECTIVE EFFECT OF *INDIGOFERA TINCTORIA* LINN. AGAINST MYOCARDIAL INFARCTION-INDUCED RATS

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ABSTRACT

Objective: The aim and objective of the present study are to study the protective effects of ethanolic extract of *Indica tinctoria* (EEIT) in isoproterenol-induced acute myocardial infarction (AMI) in rats.

Methods: The effect of two doses of isoproterenol (5 mg/kg and 8.5 mg/kg body weight) changes in aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and total protein (TP) and lipid hydroperoxides, superoxide dismutase (SOD), and glutathione (GSH) in blood serum and heart homogenate, for the two consecutive days (29 and 30), were administered subcutaneously in rats in pre-treatment with EEIT for the 28-day study.

Results: There was increased the AST, ALT, LDH, CPK, and lipid peroxides levels and decreased the TP, SOD, GSH with Isoproterenol-induced group. Pre-treatment for 28 days with *Indigofera tinctoria* significantly ($p<0.05$) increased antioxidants (SOD , GSH) , TP levels and decreased the cardiotoxic agents significantly ($p<0.05$) such as LPO, AST, ALT, LDH, and CPK levels in treatment groups.

Conclusion: *I. tinctoria* contains beneficial bioactive photochemicals that have been protective effective against isoproterenol-induced AMI in rats.

Keywords: *Indigofera tinctoria*, Cardiotoxic, Oxidative stress, Isoproterenol, Antioxidants.

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INTRODUCTION

Myocardial infarction (MI) or acute MI (AMI) was commonly known as a heart attack. It occurs when decrease the blood and oxygen supply to the cardiac muscle due to occlusion of a atherosclerotic plaque in coronary artery leads to the death of heart tissues [1]. The various mechanisms propose that the generation of free radicals has been implicated in the pathophysiology of AMI [2]. Isoproterenol is a beta-adrenoceptor agonist that induces MI by causing imbalance between oxidants and antioxidants in the myocardium [3,4]. Several antioxidants have been producing the possible protective actions against AMI [5].

Medicinal plants play an important role in health-care systems as well as in international herbal and pharmaceuticals. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds that produce a definite antioxidant action against free radicals [6,7]. The plant *Indigofera tinctoria* known as Neeli in Tamil and belongs to the family Fabaceae which popularly found throughout India. The plant roots, stems, and leaves of *I. tinctoria* are bitter and used as thermogenic, laxative, trichogenous, expectorant, anthelmintic, gastropathy, splenomegaly, cardiopathy, hepatoprotective, anticancer, epilepsy, neuropathy, chronic bronchitis, and diuretic and also useful for promoting the growth of hair [8,9]. The present investigation of the cardioprotective effect of ethanolic extract of *Indica tinctoria* (EEIT) against isoproterenol-induced AMI in rats as preventive model was conducted.

METHODS

The male albino Wister rats were selected about 150-200 g and taken the approval of the Institutional Animal Ethics Committee of SRR college of Pharmaceutical sciences, Volbhapur, Karimnagar. Animals were divided into four groups; each group consists of 6 animals (n=6) after. Group I animals served as control, received orally normal saline alone for 28 days. The Group II (Disease control) animals were received normal saline for 28 days. Group III and IV animals were pre-treated with EEIT Linn extract (100 mg/kg and 200 mg/kg) for 28 days, and on 29th day and 30th day, all three Groups (II-IV) were administered with isoproterenol hydrochloride at two different dose levels (5 mg/kg and 8.5 mg/kg) subcutaneously except control group. After 30 days of experimental period, the animals of groups were anesthetized with phenobarbitone sodium (35 mg/kg). Blood samples were withdrawn from the retro-orbital route for estimation of serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum lactate dehydrogenase (LDH) [10], creatine phosphokinase (CPK), total protein (TP) [11], antioxidants levels such as superoxide dismutase (SOD) [12], reduced glutathione (GSH) [13], and thiobarbituric acid (TBRS) [14]. Animals were then sacrificed by cervical dislocation under anesthesia, and hearts of each rat were isolated and rinsed in ice-chilled saline. A known weight of heart tissue was homogenized in 5.0 ml of 0.1 M of tris-Hcl buffer (P^H 7.4) solution. The homogenate was centrifuged, and the supernatant was used for the estimation of various biochemical parameters same as serum. Results are expressed as mean±SD. The difference between experimental groups was compared by one-way analysis of variance.

RESULTS

Serum samples and heart homogenates results show significant ($p<0.05$) increase in AST, ALT, LDH, and CPK levels and decrease in TP levels in isoproterenol-treated Group II in comparision with control. Pretreated with *I. tinctoria* showed significantly ($p<0.05$) decrease in levels of serum marker enzymes AST, ALT, LDH, and CPK and increase TP levels in Groups III and IV when comparison with disease control Group II (Tables 1 and 2).

High doses of isoproterenol hydrochloride lead to increase the oxidative stress in the heart and serum which causes decreases in the antioxidant

Table 1: Serum levels of AST, ALT, LDH, CPK and TP content in normal and treated groups

Groups/parameters	I (normal)	II (isoproterenol)	III (EEIT 100 mg/kg)	IV (EEIT 200 mg/kg))
AST(IU/L)	26.83±1.778	54.83±1.869*	51.17±1.400	34±1.506**
ALT(IU/L)	21.17±1.922	62.00±2.477*	58.17±2.613*	30.33±1.764**
LDH(IU/L)	38.00±1.807	44.67±2.092*	40.67±1.926	39.00±1.862*
CPK(IU/L)	49.00±2.352	60.83±2.688*	54.83±2.845	51.17±2.664*
TP(g/dL)	10.85±0.2790	4.767±0.2124**	6.433±0.2824*	10.38±0.3420**

Data results were (mean ± SD) compared significant (*p<0.05, **p<0.01) between control and treated groups, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase, TP: Total protein

Table 2: Heart homogenate levels of AST, ALT, LDH, CPK, and TP content in normal and treated groups

Groups/parameters	I (normal)	II (isoproterenol)	III (EEIT 100 mg/kg)	IV (EEIT 200 mg/kg)
AST(IU/L)	29.00±1.366	47.00±0.7303**	42.00±0.894	34.67±1.282**
ALT(IU/L)	25±1.366	55.00±08944**	40.50±3.585*	33.83±1.833*
LDH(IU/L)	35.00 ±1.826	45.00±1.291*	40.83±1.276*	38.17±0.7923**
CPK(IU/L)	50.00± 1.291	58.67±1.406	53.33±0.8433	51.00±0.8563*
TP(g/dL)	9.800± 0.6202	9.283±0.5173	9.6±0.5053*	9.750±0.5632**

Data results were (mean±SD) compared significant (*p<0.05, **p<0.01) between control and treated groups. AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CPK: Creatine phosphokinase, TP: Total protein

Table 3: Heart homogenate content of SOD, GSH, and TBRS in normal and treated groups

Groups/parameters	I (normal)	II (isoproterenol)	III (EEIT 100 mg/kg)	IV (EEIT 200 mg/kg)
SOD(units/mg)	2.767±0.0954	1.850±0.1176*	1.700±0.1751*	2.350±0.2045**
GSH(nmoles/100 g tissue)	3.033±0.1174	1.133±0.1282*	2.083±0.1195*	2.117±0.1195**
TBRS(nmoles/100 g tissue)	0.9500±0.099116	1.517±0.1424*	1.017±0.1108	0.8000±0.1065**

Data results were (mean±SD) compared significant (*p<0.05, **p<0.01) between control and treated groups. SOD: Superoxide dismutase, GSH: Glutathione

levels. Pre-treatment with *I. tinctoria* leads to the increase in the antioxidant levels in the heart compared with Group II. Isoproterenol showed decreased levels of antioxidants such as SOD and GSH and increased levels of TBRS when compared with Groups control, III, and IV which were pretreated with *I. tinctoria* and decreased levels of TBRS. The antioxidant property of *I. tinctoria* was shown in Table 3.

DISCUSSION

Isoproterenol (ISO) is a synthetic catecholamine, β-adrenoreceptor agonist and has been producing MI in large doses [15]. The formation of free radicals as well as lipid peroxides, which causes to damage the myocardial muscles [16,17]. The myocardium contains the LDH and transaminases as enzymatic markers of MI, which were releases from damaged tissues into extracellular fluid [18]. The results of phytochemical screening of EEIT contain the flavonoids, saponins, tannins, and phenols. The phenols and flavonoids were possess the potent antioxidant properties [19].

In the present study, the transaminases (ALT and AST), LDH, CPK, and TP serve as sensitive indicators in the severity of MI [20]. Isoproterenol-induced group increased the cardiac markers such as AST, ALT, LDH, and CPK, and it may be due to the myocardium necrosis and decrease in the level of TP in serum as compared with control group. The alteration levels will be more in serum when compared to the heart homogenate.

Pre-treatment with Ethanolic extract of *Indica tinctoria* (EEIT) significantly ($p<0.05$) decreased the elevated cardiotoxic effects of large doses of Isoproterenol in rats. The contents of EEIT scavenging the free radicals, lipid peroxides, and increases the antioxidant enzymes such as SOD and GSH are the first line of cellular defenses of cell functionality and viability in cardioprotection. Depletion of GSH results in increased lipid peroxidation which can cause increased GSH consumption develops the number of chronic diseases such as cancer, neurodegenerative, and cardiovascular diseases [21,22]. Pre-treatment with EEIT significantly increased the antioxidant levels when compared with isoproterenol-treated groups. These results may indicate the *I. tinctoria* Linn which is a beneficial as protective agent against MI induced by isoproterenol in albino Wistar rats due to its antioxidant and free radical scavenger properties.

CONCLUSION

The results of the present study conclude that EEIT elicited cardioprotective effect when given against isoproterenol-induced myocardial necrosis in albino Wistar rats. EEIT contains the beneficial phytochemicals such as flavonoids, saponins, and tannins. Phenols have cardioprotective property by decreasing the levels of serum aminotransferase, aspartate transferase, LDH, CPK, and thiobarbituric acid and increasing the antioxidant enzymes such as SOD, reduced GSH, and GSH peroxidase. Further studies are recommended to elucidate the mechanisms of the cardioprotective action of these plant active agents.

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