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EFFECT OF *INDIGOFERA TINCTORIA* LINN AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION ON ALBINO WISTAR RATS

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ABSTRACT

The present study was undertaken to evaluate the effect of hydroalcoholic leaf extract of *Indigofera tinctoria* linn (ITE) against isoproterenol (ISO) induced myocardial infarction in albino wistar rats. Rats were pre-treated with ITE (100 mg/Kg & 200 mg/Kg) daily for 28 days. After pre-treatment, Isoproterenol (5.25 mg/Kg and 8.50 mg/Kg, S.C) was injected to rats at an interval of 24 hrs for two consecutive days to induce myocardial infarction. Cardio protective activity was investigated by estimating the levels of Transaminases (Aspartate Transaminase, Alanine Transaminase), Lactate Dehydrogenase (LDH), Creatine Phosphokainase (CPK), Cardiac total protein and antioxidant enzymes such as Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Glutathione Peroxidase (GPx), and Thiobarbituric acid reactive substances (TBARS) in both serum and heart tissue. The levels of serum marker enzymes Transaminases (aspartate transaminase, alanine transaminase), LDH, CPK were significantly ($p < 0.05$) increased and cardiac total protein was decreased in isoproterenol treated group. ITE pre treated groups showed decrease in the levels of serum marker enzymes by increase in cardiac total protein. Antioxidant levels such as SOD, GSH and GPx was decreased significantly in serum of ISO treated group with increase in TBARS levels. ITE pre treated groups showed significantly ($p < 0.05$) increased antioxidant levels such as SOD, GSH and GPx with decreased TBARS levels. The histopathological evidence indicates that ISO alters all the bio-chemical parameters in the body and it can be protected by ITE treatment. This study concluded that hydroalcoholic extract of *Indigofera tinctoria* linn possess protection to ameliorate the myocardial infarction induced by isoproterenol in albino wistar rats.

Key words: *Indigofera tinctoria*, Isoproterenol, Myocardial infarction, Antioxidants and Cardio protective activity.

INTRODUCTION

Cardiovascular diseases including atherosclerosis and cardio tissue injury after myocardial infarction (MI) are the most common cause of death and disability. The world health organization (WHO) estimates 17 million people die of cardiovascular diseases[1]. MI is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demands[2]. It can occur when myocardial ischemia exceeds time period results in irreversible myocardial cell damage or death[3]. The extent of cell death during and after ischemic is the primary determinant of the outcome from the acute MI[4]. Isoproterenol is a synthetic catecholamine, β-adrenergic agonist has been found to be cause severe stress in

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the myocardium, resulting in infarct-like necrosis of heart and it is also well known to generate free radicals, stimulate lipid peroxidation which may be a causative factor for irreversible damage to myocardium membrane in experimental MI[5]. Isoproterenol induced myocardial infarction has been used as model for evaluation of cardioprotective agents[6]. In recent times, numbers of plant researches have been conducted all over the world to treat various diseases. Novel antioxidants may offer an effective and safe means of counteracting some of the defense against free radicals and cardiovascular diseases[7]. The plant *Indigofera tinctoria Linn* (fabaceae) is popularly known as indigo[8]. I*. tinctoria* was traditionally used to treat various kinds of diseases such as constipation, liver diseases, heart palpitation, gout, deobstruent, purgative, antiseptic, astringent, antibacterial, antioxidant, cytotoxic effect, hepato protective activity[9], antidyslipidemic activity[10], anti-hyperglycemic activity[11], anti-proliferative activity[12], anti-inflammatory activity[13] and antihelmenthic activity[14]. Indigo leaves powder mixed with honey was used for enlargement of liver and spleen. Juice is also used in asthma, cough and kidney disorders as in drops. The present study was designed to evaluate the effect of hydroalcoholic extract of I*. tinctoria* leaves against isoproterenol induced myocardial infarction in albino wistar rats.

EXPERIMENTAL

Plant collection

Indigofera tinctoria Linn leaves was collected from the city of Thanjavur, Tamil Nadu, India. This plant species was identified and authenticated by Dr. G. V. S. Murthy, Head of office, Botanical Survey of India, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India.

Preparation of plant extract

The collected *Indigofera tinctoria* leaves were washed thoroughly in water and allowed for drying under shade, after drying leaves were pulverized and made in to coarse powder. The powdered material was passed through the 40# sieve. A weighed quantity (100 g) of fine powder was extracted in 60% ethanol solution by maceration process for 7 days. Afterwards, the extract was filtered and evaporated to dryness in a rotary evaporator with reduced pressure. The % yield of the extract was approximately (22.6%)[15,16].

Experimental animals

Albino wistar rats weighing approximately 150-200 g were taken from Prist University animal house, Tanjore, Tamil nadu, India. They were acclimatized to standard environmental conditions as $25^{o} \pm 5^{o}C$ in a well-ventilated animal house under 12 : 12 h light/dark cycle. They were fed with pellet diet and given water ad libitum. This study was performed in accordance with the CPCSEA guide lines.

Chemicals

Isoproterenol hydrochloride (ISO) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) purchased from Sigma. All other chemicals used were of analytical grade.

Experimental design

The animals were divided into four groups, each group consists of 6 animals (n = 6). Group I and II were considered as control (Normal saline for 28 days) and Disease control (Normal saline for 28 days). Group III & IV was pre-treated with I.*tinctoria* extract (100 mg/Kg and 200 mg/Kg) for 28 days. Isoproterenol hydrochloride was administered to all the groups except control group on 29^{th} and 30^{th} at two different dose levels (5 mg/Kg and 8.50 mg/Kg) subcutaneously on interval of 24 hr in saline[15].

Determination of *in vitro* total antioxidant activity

In vitro antioxidant activity of I. *tinctoria* extract was determined by using 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay. 50% inhibitory concentrations (IC_{50}) of the extract was calculated versus MeOH as a negative control and vitamin-C was used as a positive control. Briefly, a stock solution of EtOH extract of I. *tinctoria* was prepared in DMSO to achieve the concentration of 1 mg/mL. Serial dilutions were made to obtain concentrations from 100 mg/mL to 1.56 mg./mL. 200 µL of each solution was added to 2 mL of DPPH solution. The absorbance was measured at 517 nm after incubated for 45 min at 25°C. The experiments were performed in triplicate and IC_{50} was calculated for each concentration[15,17,18].

Serum and tissue preparation

After 30 days of experimental period, blood sample were collected from all the animals and centrifuged at 3,000 rpm for 10 min at room temperature. Serum was separated and stored at -40°C to measure serum aspartate transaminase, serum alanine transaminase, serum lactate dehydrogenase, creatine phasphokainase and total protein content. All the animals were sacrificed by cervical decapitation and heart was immediately removed. A known weight of heart tissue was homogenized in 5.0 mL of 0.1 M of tris-HCl buffer (pH 7.4) solution and centrifuged at 3,500 rpm for 10 min in cold homogenizer. The clear supernatant liquid was separated and stored at -40°C for the examination of Antioxidant activity and bio-chemical parameters.

Biological assays

The heart (100 mg) was removed and homogenated, to estimate the antioxidant properties. biochemical parameters i.e., superoxide dismutase, lipid peroxidase, catalase, glutathione S-transferase, GSH, were analyzed according to the reported methods[19,20-27].

Histological studies

Histological evaluation was performed on lower portion of the heart tissue. Fresh heart tissue were excised and then fixed in 10% formalin for 24 hr. The fixative was removed by washing through tap water for overnight. At dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate embedded in paraffin wax. Sections were cut into 5 µm thickness and stained with hematoxylin and eosin. A repeated dehydration and cleaning of sections was taken for mounted and observed under light microscope for histological changes[19].

Statistical analysis

Results obtained in this study were subjected to statistical analysis using ANOVA by comparing each individual group with control. Data are expressed as Mean ± SEM. The value of $p < 0.05$ were considered as statistical significant.

RESULTS AND DISCUSSION

In vitro antioxidant activity of *I. tinctoria* extract

The ethanolic extract of I. *tinctoria* was tested for its free radical scavenging effect on DPPH. The inhibition of DPPH concentration dependently by the extract. The free radical scavenging potency of the I. *tinctoria* extract was found to be as IC_{50} = 30.23 µg/mL and standard vitamin-C as 2.62 µg/mL.

Effect of *Indigofera tinctoria* Linn on Serum Biochemical parameters

Group II (Isoproterenol treated) animals having increase in serum AST, ALT, LDH, CPK levels with decrease in total protein levels in comparison with Groups I, III & IV. Animals pre-treated with *I. tinctoria* and treated with ISO showed significant ($p < 0.05$) decrease in levels of serum marker enzymes AST, ALT, LDH, CPK with increasing in total protein levels and comparison with disease control (**Table 1**).

Table 1: Effect of *Indigofera tinctoria* Linn on Serum Biochemical parameters (AST, ALT, LDH, CPK and total protein content)

Groups	I	II	III	IV
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	38.00 ± 1.807	44.67 ± 2.092	41.67 ± 1.926	41.00 ± 1.862
CPK	49.00 ± 2.352	60.83 ± 2.688*	54.83 ± 2.845	51.17 ± 2.664
Total protein (g/dl)	10.85 ± 0.2790	4.767 ± 0.2124*	6.433 ± 0.2824#	10.38 ± 0.3420*#

Values are expressed as mean ± S. E. M. (n = 6), *P < 0.05, When compared with the control group; #P < 0.05, When compared with the disease control group; (One way ANOVA followed by Bonferroni's Multiple Comparison Test)

Effect of *indigofera tinctoria* Linn on Heart Biochemical parameters

Isoproterenol treated animals having increase in heart AST, ALT, LDH, CPK levels with decrease in total protein levels in comparision with groups I, III and IV. Animals pretreated with *I. tinctoria* and ISO showed significant ($p < 0.05$) decrease in levels of heart AST, ALT, LDH, CPK and total protein levels showed significant ($p < 0.05$) increased when comparison with disease control (Table 2).

Table 2: Effect of *Indigofera tinctoria* Linn on heart Biochemical parameters (AST, ALT, LDH, CPK and total protein content)

Groups	I	II	III	IV
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	35.00 ± 1.826	45.00 ± 1.291*	40.83 ± 1.276*#	38.17 ± 0.7923#
CPK	50.00 ± 1.291	58.67 ± 1.406*	53.33 ± 0.8433#	51.00 ± 0.8563*
Total protein(g/dl)	9.800 ± 0.6202	9.283 ± 0.5173*	9.6 ± 0.5053#	9.750 ± 0.5632#

Values are expressed as Mean ± S. E. M. (n = 6), *P < 0.05, When compared with the control group, #P < 0.05, When compared with the disease control group, (One way ANOVA followed by Bonferroni's Multiple Comparison Test)

Effect of *Indigofera tinctoria* Linn on Heart Antioxidant activity

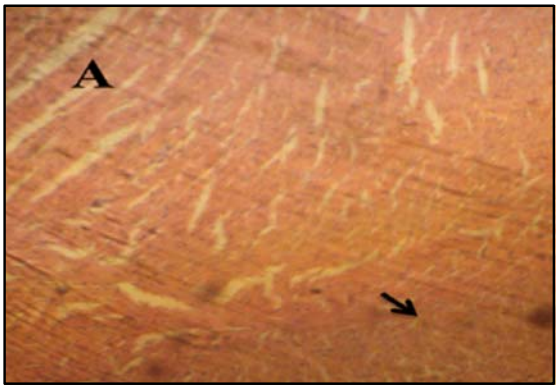
In Isoproterenol treated animals, we noticed that the antioxidant levels such as SOD, GHS and GPX were significantly decreased with increased levels of TBRS when compared to control. Group III & IV were pre treated with I. *tinctoria* and follows treated with ISO indicates increased levels of SOD, GPX, GSH and decreased levels of TBARS (Table 3).

Table 3: Effect of *Indigofera tinctoria* Linn on heart Antioxidant activity (SOD, GSH, GPX, TBARS)

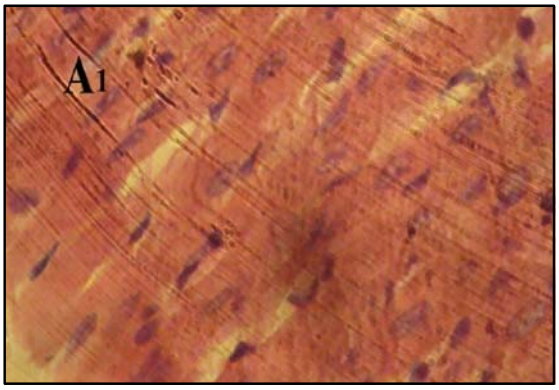
Groups	I	II	III	IV
SOD (units/mg)	2.767 ± 0.0954	1.850 ± 0.1176*	1.700 ± 0.1751	2.350 ± 0.2045
GSH (n moles/100 g tissue)	3.033 ± 0.1174	1.133 ± 0.1282*	2.083 ± 0.1195*#	2.117 ± 0.1195#
GPX (n moles/100 g tissue)	2.050 ± 0.1478	1.067 ± 0.08028*	1.233 ± 0.08819*	1.367 ± 0.09189*
TBARS (n moles/100 g tissue)	0.9500 ± 0.099116	1.517 ± 0.1424*	1.017 ± 0.1108#	0.8000 ± 0.1065#

Values are expressed as Mean ± S. E. M. (n = 6), $^{*}P < 0.05$, When compared with the control group; $^{\#}P < 0.05$, When compared with the disease control group, (One way ANOVA followed by Bonferroni's Multiple Comparison Test)

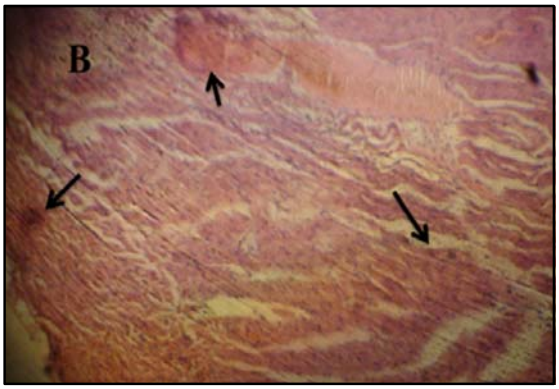
Histopathological study



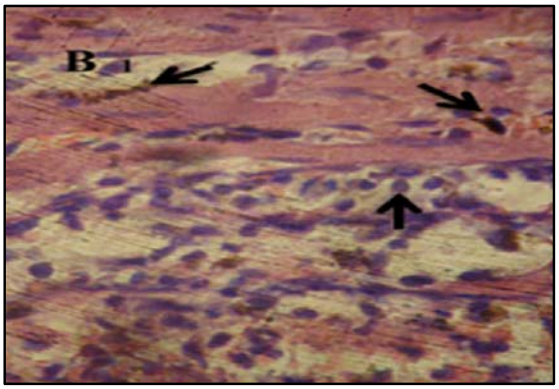
[A]. A photogram of a section in rat heart of control group, showing normal architecture of heart (10x)



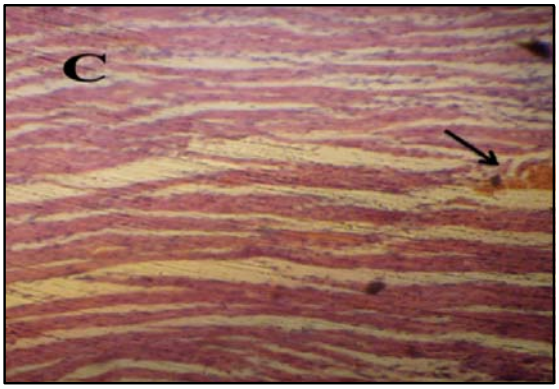
[A1]. Hematoxylin and eosin (100x) stained microscopic section in rat heart of control group showing normal cardiac muscle fibers



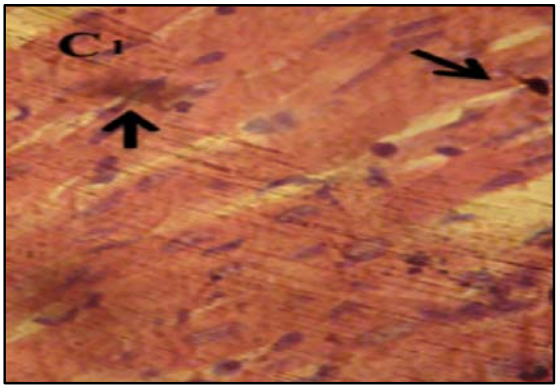
B. A photogram of a section in rat heart of ISO treated group showing focal myonecrosis with myophagocytosis and lymphatic infiltration, vacuolar changes and oedema are prominent with chronoic inflammatory cells visible (Arrows) (10x)



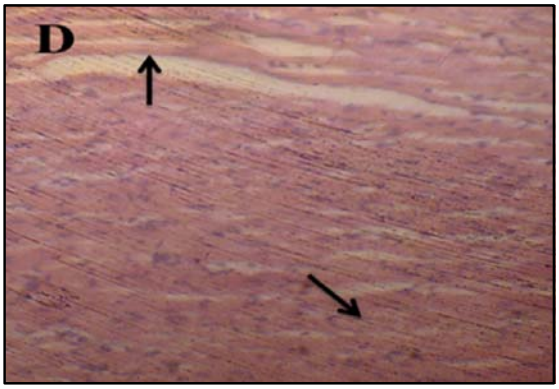
[B1]. Hematoxylin and eosin (100x) stained microscopic section of ISO treated heart showing cardiac necrosis edema with mononuclear collections in the endomyocardial junction



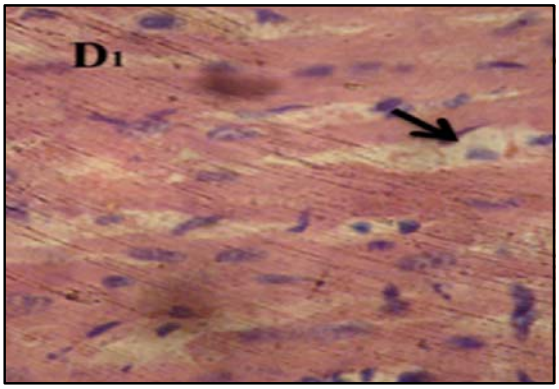
C. A photogram of a section in rat heart of *I. tinctoria* (100 mg/kg) supplemented and ISO-treated groups showing lesser degree of myonecrosis and infiltration of inflammatory cells (Arrows) (10x)



C1. Hematoxylin and eosin (100x) stained microscopic section of group III. *I. tinctoria* (100 mg/Kg) pre- treated + ISO treared heart showing normal cardiac muscle fibres with minor necrosis, edema and inflammatory infiltrate



D. A photogram of a section in rat heart of group IV. I. *tinctoria* (200 mg/kg) supplemented and ISO treated group IV showing lesser degree of myonecrosis and inflammatory cells (Arrows) (10x)



D1. Hematoxylin and eosin (x100) stained microscopic section of group IV. *I. tinctoria* (200 mg/Kg) pretreated + ISO treated heart showing normal cardiac muscle fibres without necrosis, edema and inflammatory infiltrate

The present study demonstrated the effect of hydroalcoholic extract of *Indigofera tinctoria linn* against isoproterenol induced myocardial infarction in albino wistar rats. It ameliorated the myocardial infarction induced by isoproterenol in albino wistar rats. Isoproterenol is a synthetic catecholamine, β-adrenergic agonist causes severe stress in myocardium, result in infarct like necrosis of the heart muscle. The pathophysiological changes following isoproterenol administration are comparable to human myocardial infarction[28].

Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction, such as aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine phasphokainase and total proteins[29].

AST & ALT levels were increased markedly in conditions of extensive damage to muscle especially cardiac muscle. Estimation of this enzyme is widely sought for to the diagnosis of myocardial infarction. LDH has gained much clinical interest recently and measurement of its activity in serum is useful in the

diagnosis of certain cardiovascular disease conditions. CPK is found to be high concentration in skeletal muscle, brain but not found in lungs, RB cells and its level is not affected by heamolysis. It appears to be a sensitive measure of myocardial infarction[30]. CPK and LDH enriched in cardiomyocytes are the important indicators that reflect the determination of myocardial damage. In pathological conditions, the enzymes such as CPK, LDH, AST, ALT leak from the necrotic heart cells to serum which are important measure of cardiac injury.

These are not specific myocardial injury in individually, however evaluation of these enzymes together may be an indicator of myocardial injury. In present study, significant increase in serum AST, ALT, LDH, CPK levels with decrease in total protein levels were noticed in isoproterenol treated group in comparison with control and groups III & IV. Rats pretreated with *I. tinctoria* and treated with ISO showed significant decrease in levels of serum marker enzymes AST, ALT, LDH, CPK and total protein levels showed significant increased when comparison with disease control.

Free radical scavenging enzymes such as SOD, GPx are the first line of cellular defense against oxidative injury decomposing superoxide and hydrogen peroxide before interacting to form the more reactive hydroxyl radical. The balance of these enzymes is an important process for the effective deletion of oxygen free radicals. The second line of defense consists of non-enzymatic scavenger is GSH scavenge residual free radicals escaping decomposition by the antioxidant enzymes. ISP administration was connected with reduced GSH content as well as antioxidant enzyme levels SOD and GPx in cardiac tissue. The fall in the activity of GPx in ISP treated group might be associated to decrease availability of its substrate, reduced GSH. Moreover, due to the destruction of both enzymatic and non-enzymatic antioxidant defense mechanism, it is quite likely that the free radicals are not effectively neutralized and hence myocardium shows enhanced susceptibility to lipid peroxidation. In present study, ISO treated rats, showed decreased levels of antioxidants such as SOD, GHS, GPX, and increased levels of TBRS when compared with control group. Group III & IV rats pre-treated with *I. tinctoria* and treated with ISO showed increased levels of SOD, GPX, GSH and decreased levels of TBARS. The therapeutic efficacy of the extract may due to its antioxidant, anti-lipid peroxidative, free radical scavenging activity that could have prevented the isoproterenol-induced myocardial infarction. Thus, it could be concluded that extract protects the experimentally induced myocardial infarction by using isoproterenol. Further studies have to perform to elucidate the mechanism(S) of protection against myocardial infarction.

CONCLUSION

The results of present study concluded that hydroalcoholic extract of *Indigofera tinctoria* at doses of (100 mg/Kg and 200 mg/Kg) showed significant ($p < 0.05$) cardioprotective effect in isoproterenol induced myocardial necrosis in albino wistar rats. Cardio protective activity was confirmed by estimation of the levels of serum aminotransferase, aspartate transferase, lactate dehydrogenase, creatine phasphokainase, Total protein and antioxidant enzymes (superoxide dismutase, reduced glutathione, glutathione peroxidase, thiobatbituric acid reactive substances). Isoproterenol is a synthetic compound which alters biochemical parameters and it can be prevented significantly ($P < 0.05$) by administration of hydroalcoholic extract of *Indigofera tinctoria*. The results of histopathological studies also confirmed the protective effect of I.*tinctoria*. Further studies are recommended to elucidate the mechanism of the cardioprotective action of this plant and identification of its active agents.

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