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PROTECTIVE ROLE OF *ICHNOCARPUS FRUTESCENS* AGAINST THE ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN RATS

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ABSTRACT

The cardioprotective effect of phenols and flavonoids from *Ichnocarpus frutescens* (*Apocynaceae*) Leaves were evaluated against Isoproterenol induced myocardial infarction in wistar albino rats. Isoproterenol causes marked decrease in the levels of antioxidant enzymes and increase in the levels of cardiac marker enzymes and lipid peroxidation. The elevated levels of Cardiac marker enzymes such as aspartate aminotransferase, Alanine transaminase, *Lactate dehydrogenase* and creatinine kinase were restored towards normalization significantly by *Ichnocarpus frutescens* in a dose dependent manner. Meanwhile, *Ichnocarpus frutescens* extract also produced a significant and dose-dependent reversal of Isoproterenol diminished activity of the antioxidant enzymes and also reduced elevated level of Malanaldehyde. α- Tocopherol was used as standard drug and *Ichnocarpus frutescens* extract (400mg/kg) produced significant effect compared to Isoproterenol and α-Tocopherol treated Group.

Key words: *Ichnocarpus frutescens*, Isoproterenol, Marker enzymes, Antioxidant enzymes, Myocardial infarction.

INTRODUCTION

Myocardial infarction (MI), commonly known as heart attack, is the interruption of blood supply to the part of the heart causing to die (Mallison T, 2010) Globally MI is one of the leading causes of death for both man and women (Anonymous 1)**.** Isoproterenol (ISO) is a synthetic catecholamine and β adrenergic agonist that causes severe stress in the myocardium resulting in infarct like- necrosis of heart muscle (Rajadurai M *et al*., 2006). Catecholamine's rapidly undergoes autooxidation and it has been suggested that the oxidative products are responsible for changes in myocardium (Yates JC, Dhalla NS, 1975)**.** Higher concentrations of catecholamines are responsible for necrotic lesions in the heart resulting in myocardial infarction in experimental rats (Knufman NM *et al*., 1987)**.** *Ichnocarpus frutescens* (IF) is a large evergreen, woody creeper with red appearance found almost throughout India (Anonymous 2; Ragunathan K, 1999; Kirtikar KR *et al*., 1999) and pharmacological study on *Ichnocarpus frutescens* revealed woundhealing (Pandurangan A *et al*., 2010), hepatoprotective (Deepak KD *et al*., 2007), antioxidant (Deepak KD *et al*., 2007), analgesic, and anti-inflammatory (Mishra A *et al*., 2009) antidiabetic (Rakesh B *et al*., 2008), antiurolithiatic (Anbu J *et al*., 2011), antihyperlipidemic (saravanan *et al*., 2011) and antitumor activity (Kumarappan CT, Subhash, 2007)**.** Aerobic organs such as the heart, liver generate reactive oxygen species that induce oxidative tissue damage. These radicals, which react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer **(**Slater TF, 1984; Vuillaume M, 1987; Meneghini R, 1988). A major defense mechanism is the antioxidant enzymes, which convert active oxygen molecules into non-toxic compounds (Halliwell B, Gutteridge JMC, 1984; Hochstein P, Atallah AS, 1988)**.** This study was designed to ascertain cardio protective

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effect of *Ichnocarpus frutescens (IF)* in Isoproterenol induced myocardial infarcted Wistar albino rats.

MATERIALS AND METHODS

Plant material: The plant material *Ichnocarpus frutescens* (Family: *Apocynaceae)* was collected from chittoor district, Andhrapradesh, India. The plant material was taxonomically identified and authenticated by prof. Madhavashetty, Botanist, S.V.University, Tirupati. The voucher specimen was placed in S.V.University.

Preparation of extract

The roots of the plant *Ichnocarpus frutescens (IF)* were dried in shade, powdered and were extracted using 95% ethanol in a Soxhlet apparatus (final yield was 6.3%). The resultant extract was used for cardio protective activity.

Experimental animals

Study was carried out using wistar albino rats of about 180-200 g. the animals were obtained from Uni-sankyo Ltd., Hyderabad (Reg. No. 93/1999/CPCSEA). The animals were grouped and housed in polyacrylic cages maintained under standard laboratory conditions (Temperature 25±2º c) with 12/12 h dark and light cycle. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee. (Approval No: 1019/SPIPS/Wgl/IAEC/2011 [1126/BC/07/CPCSEA dated 02/01/2008]).

Drugs and Chemicals

Isoproterenol hydrochloride (ISO), α-Tocopherol (Sigma-Aldrich, Mumbai), Riboflavin(Astra IDL, Bangalore), Trichloroacetic acid, Methanol (Qualigens fine chemicals, Mumbai) 1,1,3,3 Tetraethoxy propane (Sigma St. Louis, USA), DTNB reagent, Thiobarbituric acid, (Hi media Laboratories Ltd, Mumbai) and remaining reagents used were of analytical grade obtained from Sisco research laboratories, Mumbai, India.

Animal treatment

Wistar albino rats (180-200 g) were used in the study. Animals were divided into 6 groups of 6 each. *IF* root extract was dissolved in 5% gum acacia gum acacia was used as a solubilising agent, α- Tocopherol was used as standard drug.

Group 1: Received vehicle (5% Gum acacia)
Group 2: Received ISO
Group 3: Received I.F extract (400mg/kg)
Group 4: Received ISO+I.F extract (200mg/kg)
Group 5: Received ISO+IF (400mg/kg)
Group 6: ISO+ α- Tocopherol (10mg/kg)

The animals were treated with vehicle (5% gum acacia) or α-Tocopherol (10 mg/kg) or *IF* extract (200 and 400 mg/kg) orally for a period of 10 days. Normal and ISO control rats received 5% gum acacia only and all the rats, except normal control received ISO (85 mg/kg) twice at an interval of 24 h on 11^{th} and 12^{th} day. At the end of the experimental period after 12 h of second ISO injection, all rats were sacrificed, blood was collected and serum was separated for the determination of marker enzymes. The heart tissues were excised and homogenized in 0.1M Tris–Hcl (pH 7.4) buffer solution for biochemical estimations.

Estimation of cardiac marker enzymes

a. Alanine amino transferase (ALT) and aspartate amino transferase (AST) in plasma were determined (Mohur A, Cook IJY, 1957) at 540 nm and expressed as µmol of pyruvate liberated /hr/l.
b. The creatinine phosphokinase (CPK) was determined as µmol creatinine liberated /hr/l, at 520 nm (Okinaka S *et al*., 1961).
c. The activity levels of LDH were measured at 505 nm in DU-2 Beckmann's spectrophotometer (King J, 1965).

Estimation of antioxidant enzymes and Lipid Peroxidation (LPO)

a. Superoxide dismutase (SOD) activity was determined by the method of misra and fridovich at 480 nm for 2 min (Aebi H, 1974).
b. Catalase (CAT) activity was measured by method of Aebi at 240 nm for 3 min as nmol of hydrogen peroxide decomposed /min/mg protein (Maehly AC and Chance B).
c. Glutathione peroxidase activity was measured at 340 nm for 5 min as nmol GSH oxidized /min/mg protein (Wood JL, 1970).
d. Glutathione-s-transferase (GST) was measured at 340 nm for 5 min as µmol 1-chloro-2, 4-di nitro benzene (CDNB) conjugate formed /min/mg protein (Jocelyn PC, 1972).
e. Lipid peroxidation was assayed by method of Brogan in which malonaldehyde (MDA) released served as the index of LPO. The absorbance was measured at 532 nm (Buege JA and Aust SD, 1978).

Statistical Analysis

Results were expressed as mean ± SEM and were analyzed by one way ANOVA followed by Dunnette's multiple comparison tests. $P<0.01$ was found to be significant.

RESULTS AND DISCUSSION

In the present investigation, there was a significant rise in the levels of cardiac marker enzymes (AST, ALT, LDH and CK) in the serum of ISO treated

Fig 1. Effect of *Ichnocarpus frutescens* on the levels of AST, ALT, LDH and CK in the serum of normal and ISO-induced myocardial-infarcted rats

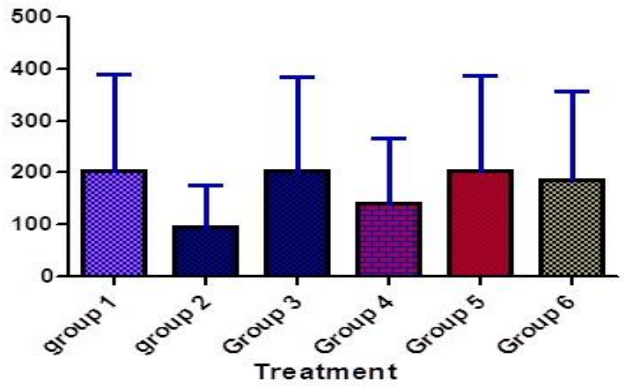

Fig 2. Effect of *Ichnocarpus frutescens* on the activities of SOD, CAT, GPx, GST and LPO in the heart of normal and ISO-induced myocardial-infarcted rats

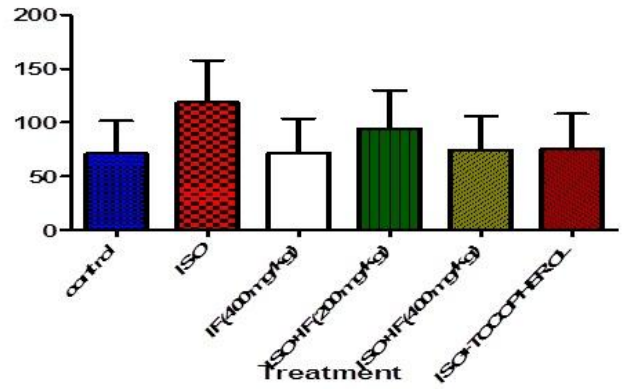

Table 1. Effect of *Ichnocarpus frutescens* on the levels of AST, ALT, LDH and CK in the serum of normal and ISO-induced myocardial-infarcted rats

Groups	AST (µmol/h/l)	ALT (µmol/h/l)	LDH (µmol/h/l)	CK (/min/mg)
Group 1 (Vehicle control)	34.64±6.62	16.52±2.73	78.46±9.32	154.28±12.63
Group 2 (ISO)	76.43±8.46*	31.43±5.48*	162.43±13.46*	204.12±17.58*
Group 3 (*IF* 400 mg/kg)	32.48±6.73	17.42±3.12	74.54±8.62	161.36±13.46
Group 4 (ISO+*IF* 200 mg/kg)	56.57±7.35♦	23.16±4.52♦	113.64±11.43♦	184.75±15.58♦
Group 5 (ISO+*IF* 400 mg/kg)	37.42±6.24♦	18.43±3.58♦	83.42±7.28♦	159.63±13.12♦
Group 6 (ISO+ α-tocopherol 10 mg/kg)	38.63±5.65♦	17.46±3.02♦	84.49±8.53♦	162.46±14.63♦

Values were expressed as mean ± SEM, n=6; * $p<0.01$ when compared to normal control; ♦ $p<0.01$ when compared to ISO control.

Table 2. Effect of *Ichnocarpus frutescens* on the activities of SOD, CAT, GPx, GST and LPO in the heart of normal and ISO-induced myocardial-infarcted rats

Groups	SOD (U/mg protein)	CAT (µmol/ mg protein)	GPx (µg/ mg protein)	GST (nmoles/mg protein)	LPO (nmoles/mg protein)
Group 1 (Vehicle control)	16.36±0.57	47.42±4.62	9.43±0.87	943.62±14.62	6.47±0.18
Group 2 (ISO)	8.84±0.036*	23.36±1.84*	2.64±0.034*	417.52±12.36*	26.58±0.22*
Group 3 (*IF* 400 mg/kg)	18.54±1.23	48.46±5.13	8.42±0.78	932.15±16.54	6.11±0.09
Group 4 (ISO+*IF* 200 mg/kg)	12.38±0.92♦	34.84±4.02♦	4.24±0.34♦	637.16±15.54♦	17.13±0.14♦
Group 5 (ISO+*IF* 400 mg/kg)	15.65±0.64♦	46.54±4.35♦	7.26±0.83♦	938.26±17.38♦	8.36±0.13♦
Group 6 (ISO+α-tocopherol 10 mg/kg)	15.78±0.74♦	42.12±3.72♦	6.33±0.56♦	862.43±17.74♦	7.52±0.17♦

Values were expressed as mean ± SEM, n=6 animals in each group. * $p<0.01$ when compared to normal control.
♦ $p<0.01$ when compared to ISO control.

group of rats. Pretreatment with *IF* extract (200, 400 mg/kg) for 10 days significantly decreased the levels of these marker enzymes as compared with ISO group. The reference standard, α-tocopherol, also showed similar effect. Furthermore, antioxidant enzymes (SOD, CAT, GPx, GST) in the cardiac tissue were significantly lowered with concomitant significant raise in TBARS levels compared to control. Pretreatment with *IF* and α-

tocopherol significantly elevated the levels of these antioxidant enzymes with simultaneous decrease in LPO compared with ISO group. Significant alterations in the levels of marker enzymes and antioxidant enzymes indicate the severity of ISO induced necrotic damage of myocardial membrane. Prior administration of *IF* was found to attenuate the ISO induced changes in the level of above enzymes with concomitant decrease in LPO significantly, indicating the cardiac protective effect of *Ichnocarpus frutescens*. Isoproterenol produces free radicals and stimulates lipid per oxidation, which may be a consecutive factor for irreversible damage to the myocardial membrane. IF (400mg/kg) group pro Earlier study revealed that the roots of *IF* consist of several constituents including phenolic compounds, sterols and flavonoids which were found to be potential antioxidant principles.

CONCLUSION

The present investigation indicates that IF extract exert significant protection against Isoproterenol-induced myocardial infarction by its ability to ameliorate the lipid peroxidation through the free radicals scavenging activity, and also cause decrease in the levels of cardiac marker enzymes and increase in anti-oxidant activity.

Ichnocarpus frutescens at a dose of 400mg/kg produced significant decrease in cardiac marker enzymes and increase in antioxidant enzyme levels when compared to control group. The cardioprotective effect of *IF* could be due to the free radical scavenging property of phenolic compounds and flavonoids present in *IF*.

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