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CARDIO-PROTECTIVE EFFECT OF ALCOHOLIC EXTRACT OF *CYPERUS ROTUNDUS* RHIZOME ON ISOPROTERENOL-INDUCED MYOCARDIAL NECROSIS IN RATS

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ABSTRACT

The study was designed to scientifically evaluate the effect of alchoholic extract of *Cyperus rotundus* rhizomes, a traditional medicinal herb, on isoproterenol-induce myocardial necrosis in rats model. The albino wistar rats were divided into five different groups with six animal in each group. Group I serves as a control group, group II were given isoproterenol (85 mg/kg s.c) for 2 days while Group III was treated with metoprolol (10 mg/kg) for 28 days and isoproterenol on last 2 days. This group serves as standard group and its effect was used for comparison with that of test drug (*Cyperus rotundus* extract). Group IV and V were given *Cyperus rotundus* rhizome extract (300 mg/kg and 150 mg/kg respectively) for 28 days and isoproterenol on last 29th and 30th day. The level of enzymes transaminases (aspartate transaminase and alanine transaminase), Lactate dehydrogenase (LDH), Cretinine kinase (CK) were estimated in serum and the extent of necrosis was studied by grading. Isoproterenol significantly increased the activities of CK, LDH and the transaminases in serum. Pretreatment with the alchoholic extract of *Cyperus rotundus* had a significant effect on the activities of marker enzymes compared to the other groups. The effect was compared with the effect on marker enzymes in the group treated with Metaprolol. The metaprolol treated group shows reduced level of marker enzymes, nearer to the control group. The pretreatment with *Cyperus rotundus* extract at the dose 300 mg/kg shows more significant reduction then at the dose of 150 mg/kg. The study confirms the protective effects of *Cyperus rotundus* rhizome extract against isoproterenol-induced biochemical alterations in rats.

INTRODUCTION: The cardiovascular diseases, today, has become a triple paradox. They are, (i) easy to diagnose often remain undetected, (ii) simple to treat often remain untreated, and (iii) despite availability of potent drugs, the treatment all too often is ineffective. Myocardial infarction is the major cardiovascular disease which is making an increasingly important contribution to mortality statistics, mainly due to changing life styles especially in urban areas in developing countries [1].

Myocardial infarction is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in the necrosis of the myocardium. This is followed by numerous pathophysiological and biochemical changes such as lipid peroxidation, hyperglycemia, hyperlipidemia etc [2]. It is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart [3].

Although modern drugs are effective in preventing cardiovascular disorders, their use is often limited because of their side effects [4]. Now a day a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects [5]. It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life [6].

The need of the hour is to screen a number of medicinal plants for promising biological activity. Considering the aforesaid, *Cyperus rotundus* (Cyperaceae), the traditionally used medicinal plant well known for its diuretic activity beside being a proven anti-oxidant [7], hpolipidemic and hypotensive drug [8], is been screened for cardioprotective activity.

Several methods have been used to study the beneficial effects of many drugs on cardiac function [9]. Isoproterenol (ISO), a synthetic catecholamine and a β-adrenergic agonist, has been documented to produce myocardial infarction in large doses [10]. On auto-oxidation, ISO generates highly cytotoxic free radicals known to stimulate peroxidation of membrane phospholipids and cause severe damage in the myocardial membrane [11] resulting in the infarct-like necrosis of the heart muscle. Isoproterenol-induced MI serves as a well-standardized model because the pathophysiological changes following isoproterenol administration are comparable to those taking place in human MI [12].

The cardioprotective effect of *Cyperus rotundus* is been compared with that of metoprolol, a selective β antagonist widely used in the management of myocardial infarction, to prove its clinical significance.

MATERIAL AND METHOD:

Animals: Adult male albino rats of the Wistar strain weighing 120–150 g were selected for the study. The rats were fed with commercial pellets and water *ad libitum*. The animals were housed in clean polypropylene cages lined with husk, changed every 24 hours under a 12-hour light/ dark cycle at 25°C. The study was carried out after obtaining the necessary approval from the Institutional Animal Ethics Committee. Experimental protocols conform to the Indian National Science Academy Guidelines for the use and Care of Experimental Animals in Research. All animals were handled with humane care.

Drugs and Chemicals: The isoproterenol was generously supplied by Prof. (Dr.) S. K. Maulick, Professor Department of Pharmacology, AIIMS, New Delhi, while metoprolol was purchased from Cipla (Brand Metolar 50) via local chemist. The other chemicals were obtained from the store room of Faculty of Pharmacy, Integral University Lucknow.

Plant Material: The dried rhizomes of *Cyperus rotundus* were purchased from local shop and were authentified by Dr. D.V. Amla, National Botanical Research Institute, Lucknow. Sample 1, Ref. no: NBRI/CIF/198/2011.

Preparation of Extract: The drug powder was dried and crushed into coarse powder and was passed from the sieve no 18. The drug was de-fattied using petroleum ether to remove essential oils. The powdered drug (500 gm) was extracted with ethanol (80%) for 24 hours using Soxhlet extractor. This ethanolic extract was then concentrated to a constant weight under reduce pressure and control temperature (50-60˚c) to yield a solid mass that was completely free from adulterants.

Experimental Procedure: The experimental animals were divided into five different with six animals in each group. Group I serves as control group. Group II was administered isoproterenol (85 mg/kg) on two consecutive days at the interval of 24 hr. Group III was given metoprolol (10 mg/kg) for 28 days followed by isoproterenol (85 mg/kg) on last two days. Group IV and V were given alcoholic extract of *Cyperus rotundus* (300 mg/kg and 150 mg/kg respectively) for 28 days followed by isoproterenol (85 mg/kg) on 29th and 30th day.

The animals were anesthetized 24hr after the second dose of isoproterenol and blood was withdrawn by cardiac puncture. The serum was separated by centrifugation and was tested for the presence of serum marker enzymes creatinine kinase (CK), lactate

dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT). After the experimental period, the rats were sacrificed and the heart was dissected and immediately washed in ice cold saline. A portion of heart was stored in 10% formaline solution for histopathological analysis. Student's *t*-test was used for statistical analysis. Values are expressed as the mean ± standard error of the mean (SEM) for the six animals in each group. A value of $p < 0.001$ was considered statistically significant.

RESULT: There was a significant elevation observed in the activities of transaminases (ALT, AST), CK and LDH in isoproterenol treated group II compared to the control group (**table 1**). However, in the group IV and V, pretreated with *C. rotundus* extract, there was a significant reduction in the activities of marker enzymes compared to the isoproterenol administered rats (group II).

Rats in group II were given isoproterenol through subcutaneous route whereas group IV and V were given extract of *Cyperus rotundus*, per orally for 28 days and then subcutaneous dose of isoproterenol on 29^{th} and 30^{th} days. Rats in group III that was pretreated with metoprolol for 28 days followed by dose of isoproterenol at the end of treatment also showed significant reduction in the activities of serum marker enzymes. This group was serves as standard group and its effect was compared with that of *Cyperus rotundus* extract.

It was found that, group V (150 mg/kg of *Cyperus rotundus* extract) shows minor reduction in serum marker enzyme concentration while group IV (300 mg/kg of *Cyperus rotundus* extract) shows more significant reduction. The activities of serum marker enzyme in group IV was in accordance with the activities of that in group III (standard group treated with metoprolol).

TABLE 1: EFFECT OF *C. ROTUNDUS* PRETREATMENT ON ISOPROTERENOL-INDUCED CHANGES IN THE ACTIVITIES OF SERUM AST, ALT, LDH AND CK

Groups	AST	ALT	LDH	Creatinine ***
Control	67.72 ± 1.8**	48.93 ± 1.2	1168.56 ± 23.3	91.05 ± 0.02
ISO	82.59 ± 2.8	63.99 ± 3.0	1365.56 ± 38.3	110.14 ± 0.04
Standard	69.58 ±1.4	50.26 ± 1.0	1183.40 ± 38.5	96.26 ± 0.03
CRE 1*	71.43 ± 0.71	51.32 ± 0.7	1187.84 ± 26.0	91.05 ± 0.02
CRE 2*	76.14 ± 0.46	56.05 ± 1.8	1243.81 ± 30.9	99.43 ± 0.03

*CRE 1 = *Cyperus rotundus* extract (300mg), CRE 2 = *Cyperus rotundus* extract (150 mg). ** All readings are expressed as mean ± SE.M for 6 animals in each group. ***Concentrations of Creatinine are expressed in µmol/L. All concentrations are expressed in Iu/L. $P < 0.001$, between Control & ISO. $P < 0.001$, between Std. & ISO. $P < 0.001$, between CRE 1& ISO. $P < 0.05$, between CRE 2 & ISO.

DISCUSSION: The present study demonstrated that the C*yperus rotundus* extract has efficiently protected the myocardium against isoproterenol-induced MI. This was evident from the activities of marker enzymes in the serum. Enzymes are the best markers of tissue damage because of their specificity and catalytic activity to the tissue [13].

The biochemical markers that are used widely in detection of myocardial necrosis are Creatine Kinase (CK), LDH, and transaminases (SGOT & SGPT). CK has greater than 95% sensitivity and specificity for myocardial injury when measured between 24-36 hrs [14]. Estimations of elevated serum enzymes are a useful guide for death of heart muscles [15]. The elevated level of marker enzymes in serum might be due to the damage in the heart muscle, rendering the leakage of enzymes into the serum [16].

The significant rise observed in the levels of diagnostic marker enzymes in the serum of Group II isoproterenol administered rats as compared to that of Group I control rats (Table 1) is an indication of the severity of the necrotic damage to the myocardial membrane [17].

The pretreatment with the *Cyperus rotundus* extract significantly prevented isoproterenol- induced elevation in the levels of the diagnostic marker enzymes of Groups IV and V animals compared to Group II rats, probably due to the protective effect of the *C. rotundus* extract on the myocardium; this reduced the extent of myocardial damage and thereby restricted the leakage of these enzymes from the myocardium.

The cardioprotective effect shown by the *Cyperus rotundus* extract was compared with the cardioprotective effect shown by metoprolol, a β antagonist which is a potent cardioprotective drug and is widely used clinically, in the management of myocardial necrosis. The level of serum marker enzymes, in the group pretreated with metoprolol, was significantly reduced and was found closer to the control group level. The pretreatment with *Cyperus rotundus* extract at the dose 300 mg/kg shows more significant reduction then at the dose of 150 mg/kg.

CONCLUSION: To conclude, the results of the present study indicate that the prior administration of the *C. rotundus* rhizome extract attenuates isoproterenol-induced MI. The cardioprotective effect of the *C. rotundus* rhizome extract is probably related to its ability to strengthen the myocardial membrane by its membrane-stabilizing action. *C. rotundus* rhizome extract at the dose level of 300 mg/kg was found to be more effective than the other dose level of 150 mg/kg. In this study, the cardioprotective potential of *C. rotundus* rhizome extract is evident.

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