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# Cardio Protective Efficacy of Seeds of *Spermacoce Hispida* Linn on Lysosomal Enzymes and Membrane Bound ATPases in Cardiotoxic Rats

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## ABSTRACT

*Spermacoce hispida* possesses significant anti-oxidant and cardioprotective activities. This manuscript reveals the preventive potential of seed extract on lysosomal damage in isoproterenol induced myocardial infarction (MI) in rats. Two different doses of the plant extract such as 100 and 200 mg/kg body weight was used to prove the cardioprotective effect against 100mg/kg body weight of isoproterenol (ISO). The activities of lysosomal enzymes in the heart of ISO- induced MI rats were increased significantly. Pretreatment with hydro alcoholic extract of seeds of *Spermacoce hispida* prevented the changes in the levels of lysosomal enzymes in isoproterenol induced MI rats. The activity of enzymes like β –D- Galactosidase, β –D- Glucuronidase, N-Acetyl β-D –glucoseaminidase, Cathepsin D and ATP ases are found to be increased in heart homogenate in diseased rats against normal animals.

**Keywords:** anti-oxidant, cardioprotective, isoproterenol, lysosome

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## INTRODUCTION

Isoproterenol is a b-adrenoceptor agonist and produces myocardial infarction in large doses. Upon auto-oxidation, it generates highly cytotoxic free radicals known to stimulate the peroxidation of membrane phospholipids causing severe damage to the myocardial membrane. Hence, it is widely used as a model to produce MI in rats. Isoprenaline has positive inotropic and chronotropic effects on the heart[1,2].

Myocardial infarction is the irreversible damage of myocardial tissue caused by prolonged ischemia and hypoxia. This is a condition which most commonly occurs when the coronary artery is occluded following the rupture of an atherosclerotic plaque, which then, leads to the formation of a blood clot. The occluded block decreases the flow of blood to the myocardium and results in chronic ischemia and necrosis[3]. Autopsy study reveals that the commonest cause of myocardial infarction is atherosclerosis.

Oxidation of low density lipoprotein (Ox-LDL) is mainly responsible for the genesis of atherosclerosis[4]. Ox-LDL induces pro-atherosclerotic NADPH oxidase expression and superoxide anion formation in human vascular endothelial cells[5] and this may be one of the mechanisms by which ox-LDL stimulates ROS generation.

Now a days herb are used to prevent and treat diseases and ailments or to support health and healing[6]. These are drugs or preparations made from plants and used for any of such purposes. Herbal drugs are the oldest form of health care known to mankind[7]. The use of herbal drugs due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers.

In this study cardioprotective effect of seeds of *Spermacoce hispida* was analysed with its lysosomal enzyme activity. *Spermacoce hispida* is known to have lots of therapeutic activity in Siddha medicine. It has hepatoprotective, anti-inflammatory and antioxidant activities. In our previous communications, we have reported that the seed extract of *Spermacoce hispida* possess cardio protective effect by the activities of various biochemical parameters and also maintaining cardiac marker enzymes in ISO induced myocardial infracted rats[8]. Seed extract of SH also had an impact on mitochondrial lipid peroxides, TCA cycle and antioxidant enzymes. Hence this study was undertaken to assess the efficacy of seeds of *Spermacoce hispida* in the treatment of myocardial infarction.

# MATERIALS AND METHOD

## Animals

Adult male albino wistar rats weighing 150-250g were obtained from Sri Venkateshwara Enterprises, Bangalore-560021, India. The animals were housed in polypropylene cages. They were fed with standard diet and water *ad libitum* and housed under standard environmental conditions.

## Experimental design

Group1: The rats of group 1 serve as control and they did not receive any treatment

Group 2: Rats were administered with ISP (100mg/kg b.wt) dissolved in 0.9% saline subcutaneously twice at the interval of 24 hours[9].

Group 3: Rats were administered with 100mg/kg body wt. of hydro alcoholic extract (HAE) of seeds of *Spermacoce hispida* for 45 days. ISP was injected subcutaneously on 45th day.

Group 4: Rats were administered with 200mg/kg body wt. of *Spermacoce hispida* seed extract for 45 days. ISP was injected subcutaneously on 45th day

Group 5: Rats were administered with 100mg/kg body wt. of *Spermacoce hispida* seed extract alone for 45 days.

Group 6: Rats were administered with 200mg/kg body wt. of *Spermacoce hispida* seed extract alone for 45 days.

Group 7: Rats were administered with Vitamin E at 100 mg/ kg b.wt. for 45 days. ISP was injected subcutaneously on 45th day.

## Induction of Myocardial infarction

MI was induced in rats by subcutaneous injection of 100 mg/kg isoprenaline hydrochloride dissolved in saline once daily for two successive days[10,11].

## Preparation of sample for total lysosomal hydrolases and membrane bound phosphatases

About 200 mg of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH 7.4) solution. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used for the various biochemical parameters.

## Biochemical parameters

The activity of β-Glucuronidase was determined by the method of Kawai and Anno, (1971)[12]. The activity of β-N-Acetyl glucosaminidase was determined by the procedure of Moore and Morris, (1982)[13]. The activity of β-D-Galactosidase was assayed by the method of Conchie *et.al*. (1967)[14]. The enzyme activity was expressed as µM of p- nitrophenol liberated /h/100 mg of protein.

Cathepsin D activity was determined by the method of Sapolsky *et al*., (1973)[15] and was expressed as µmol of tyrosine liberated/h/100mg protein**.** $Na^+/K^+$- ATPase was estimated by the method of Bonting, (1970)[16]. The activity of $Ca^{2+}$- ATPase was assayed according to the method of Hjerten and Pan, (1983)[17]. The activity of $Mg^{2+}$ - ATPase was assayed according to the method of Ohnishi, *et al.* (1982)[18]. The enzyme activity was expressed as µM of phosphorus liberated/hr /mg protein under incubation conditions.

**Statistical analysis**

The data were analysed by using One way ANOVA followed by DMRT. The results from experimental groups were compared with respective control and *p* values < 0.05 were considered statistically significant.

## RESULTSAND DISCUSSION

The activities of the lysosomal enzymes in the control and experimental groups are displayed in Table I and II. The activity of the β-Glucuronidase, β-N-Acetyl glucosaminidase, β-D-Galactosidase, Cathepsin D, $Na^+/K^+$- ATPase, $Ca^{2+}$- ATPase and $Mg^{2+}$ - ATPase were found to be significantly increased in the heart homogenate of Group II rats subjected to ISO exposure as compared with those of control (Group I). Rats pretreated with Spermacode hispida seed extract (Group III&IV) significantly decreased the alterations induced by ISO when compared with Group II animals. Prior oral treatment with SH alone (Group V&VI) registered no significant changes when compared to control. Rats treated with Vitamin E and ISO (Group VII) also shows similar activity of SH treated rats.

**Table: 1: Effect of seeds of SH on tissue (heart) Lysosomal enzymes in normal and ISO induced cardiotoxic rats**

| **Groups** | **β–D Galactosidase** | **β–D-glucuronidase** | **N-Acetyl β-D glucoseaminidase** | **Cathepsin D** |
|---|---|---|---|---|
| | **(µM of p- nitrophenol liberated /h/100 mg of protein)** | | | **µmol of tyrosine liberated/h/100mg protein)** |
| Normal | 33.0 ± 10.5 [a] | 48.5 ± 11.6 [a] | 123.7 ± 27.8 [a] | 31.4 ± 8.5 [a] |
| ISO | 171.5 ± 16.0 [d] | 120.2 ± 13.3 [d] | 369.4 ± 45.2 [c] | 78 ± 15.3 [c] |
| 100 mg/kg b.wt. HAE + ISO | 72.5± 17.2 [b] | 70.3 ± 25.2 [ab] | 217.2 ± 25.2 [b] | 56 ± 12.8 [b] |
| 200 mg/kg b.wt. HAE + ISO | 51.8 ± 21.2 [b] | 51.2 ± 35.1 [a] | 127.1 ± 28.3 [a] | 42 ± 15.9 [a] |
| 100 mg/kg b.wt. HAE | 35.5 ± 7.6 [a] | 47.5 ± 12.5 [a] | 128.5 ± 26.5 [a] | 32.1 ± 12.4 [a] |
| 200 mg/kg b.wt. HAE | 31.6 ± 4.8 [a] | 45.1 ± 5.9 [a] | 132.6 ± 20.5 [a] | 30.8 ± 17.5 [a] |
| Vitamin E + ISO | 67.3 ± 21.2 [b] | 68.9 ± 15.7 [ab] | 192.6 ± 24.6 [b] | 51.5 ± 15.4 [b] |

Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c,d of same column are differ significantly ($P$<0.05).

**Table 2: Effect of seeds of SH on tissue (heart) membrane bound ATP ases in normal and ISO induced cardiotoxic rats**

| Groups | $Mg^{2+}$ ATPase (µM of Phosphorous liberated/hr/mg of protein) | $Ca^{2+}$ ATPase (µM of Phosphorous liberated/hr/mg of protein) | $Na^{+}K^{+}$ ATPase (µM of Phosphorous liberated/hr/mg of protein) |
|---|---|---|---|
| Normal | 9.33 ± 1.1 [a] | 13.1 ± 2.2 [a] | 16.2 ± 2.4 [a] |
| ISO | 16.72 ± 2.5 [c] | 17.6 ± 3.1 [b] | 24.1 ± 3.5 [b] |
| 100 mg/kg b.wt. HAE + ISO | 10.54 ± 2.3 [ab] | 12.5 ± 2.4 [ab] | 18.1 ± 2.4 [ab] |
| 200 mg/kg b.wt. HAE + ISO | 9.23 ± 1.6 [a] | 11.4 ± 2.8 [a] | 15.9 ± 4.8 [a] |
| 100 mg/kg b.wt. HAE | 9.83 ± 2.6 [a] | 12.6 ± 3.2 [a] | 16.5 ± 3.8 [a] |
| 200 mg/kg b.wt. HAE | 9.52 ± 3.5 [a] | 11.5 ± 3.4 [a] | 16.1 ± 2.7 [a] |
| Vitamin E + ISO | 13.39 ± 4.9 [bc] | 14.1 ± 4.5 [b] | 19.4 ± 7.8 [b] |

Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab of same column are differ significantly ($P$<0.05).

Oxidative stress, in the form of hydrogen peroxide, formed from auto-oxidation of ISO, rapidly ruptures lysosomes. Rupture of lysosome is followed by caspase dependent apoptosis or caspase independent apoptosis like cell death or necrosis[19]. The lysosomal membrane permeabilisation may occur due to lipid peroxidation of the membrane PUFA and the generated apparent ultra structural changes results in release of a large number of acid hydrolases. Moreover, the lysosomal content of redox-active iron may induce intra-lysosomal Fenton-type reactions at oxidative stress situations with ensuing peroxidation of lysosomal limiting membranes and releases the damaging contents, such as cathepsins and redox-active iron to the cytosol[20]. Consequences of decreased oxygen supply to myocardium or increased anaerobic glycolysis, and increased lactic acid associated with decrease in pH. In the acidic pH most of the acid hydrolases are activated resulting in further damage of the cell[21]. To evaluate the membrane stabilizing effect of HAE various lysosomal markers and membrane bound enzymes are assayed.

The activity of enzymes like β –D- Galactosidase, β –D- Glucuronidase, N-Acetyl β-D – glucoseaminidase, Cathepsin D and ATP ases are found to be increased in heart homogenate in diseased rats against normal animals. Pretreating animals with extract of Spermacoce hispida for 45 days has exhibited a decrease in the activity of lysosomal enzymes significantly at a dose of 200 mg/kg b.wt. However, the membrane stabilizing potential of the extract is observed to be progressive dose dependently against diseased rats. It is interesting to note that HAE at the dose of

200 mg/kg b.wt. decreases ATP ases activity better than that of Vitamin E pretreated rats. Hence, it is considered that SH is a potent membrane stabilizing agent.

The increased activity of lysosomal enzymes in heart homogenate might be due to the damage of membrane. Niebes and Ponard, (1975)[22] have explained that elevated lysosomal enzymes in the extra cellular fluid occur as a result of decreased lysosomal membrane stability. ISO administration may be involved in membrane damage and thereby causing the release of lysosomal enzymes. The phospholipid rich lysosomal membrane is a potential site for free radical attack subsequently causing loss of membrane stability[23]. Hence the damage of lysosomal membrane might be due to lipid peroxidation. Hence, any drug with potent antioxidant activity can protect the lysosome from damage.

ATP ase activities may be altered by increase in cholesterol levels[24]. Sudhahar *et al.*, (2007)[25] have found that rats fed with high-cholesterol diet have indicated significant decrease in the activity of the myocardial $Na^+/K^+$ ATP ase and they have explained the reason for the same as oxidation of membrane lipids and proteins. The level of ATP ase after the SH treatment observed after the SH treatment in the present analysis confirmed that the seeds extract of SH might be the reason for protecting the membrane from damage.

Altogether the results of the present study reveal that HAE exhibits membrane stabilizing activity and protect the membrane including lysosome from damage caused by lipid peroxidation through its free radical quenching activity and antioxidant potential.

## CONCLUSION

The observed effects are due to the membrane stabilizing property of seed extract of SH and this property might be due to decreased lipid peroxidation. Thus SH protects heart from isoproterenol induced myocardial damage by its antioxidant and free radical scavenging activity. It could be concluded that intake of herbs like *Spermacoce hispida* offers protection to the heart. It could be developed as a drug for coronary heart disease after proper clinical trials.

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