CARDIOPROTECTIVE AND ANTIOXIDANT EFFECTS OF SEEDS OF
SPERMACOE HISPIDA LINN., ON ISOPROTERENOL INDUCED
MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Myocardial infarction (MI) continues to be a major public health problem in the world. Spermacoce hispida exhibit cardio-protective effects by several mechanisms. Spermacoce hispida possesses significant anti-oxidant and cardioprotective activities. The present study aimed to investigate the effects of pretreatment with spermacoce hispida seed extract on isoprenaline-induced 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). Markers chosen to assess cardiac damage included the activity of creatine phospho kinase, LDH, Aspartate Transaminase(AST) and Alanine Transaminase (ALT). Oxidative stress markers such as lipid peroxides and hydroperoxides were assessed. Pre-treatment of ISO administered animals with 200 mg/kg b.wt, of spermacoce hispida was found to exhibit a better cardioprotective and antioxidant effect.

Key Words: Myocardial infarction, isoproterenol, oxidative stress and antioxidant.

INTRODUCTION

Cardio Vascular Diseases (CVD) are the number one cause of death globally: more people die annually from CVDs than from any other cause.[1] Coronary artery disease (CAD) starts...
with the formation of atherosclerotic plaques in the coronary arteries. The arteries harden and narrow due to buildup of a material called plaque on their inner walls. The buildup of plaque is known as atherosclerosis. As the plaque increases in size, the insides of the coronary arteries get narrower and less blood can flow through them. Eventually, blood flow to the heart muscle is reduced, and, because blood carries much-needed oxygen, the heart muscle is not able to receive the amount of oxygen it needs. Reduced or cutoff blood flow and oxygen supply to the heart muscle can result in: unstable angina, acute myocardial infarction and sudden death.\[2\] Most cardiovascular diseases can be prevented by addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids.\[3,4\]

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. To prevent free radical damage the body has a defense system of antioxidants.\[5\] Free radicals have been implicated in cardiac diseases which result due to exposure to chemicals and environmental agents. Isoproterenol induces oxidative stress\[6\] and results in alterations of cardiac function and ultra structure in experimental rats.\[7\] The positive inotropic and positive chronotropic response of isoproterenol augments myocardial oxygen consumption.\[8\] The increase in energy demands\[9\] and the decrease in blood flow induce an energy imbalance by the Ca\(^{2+}\) overload.\[10\] This results in the necrosis of cardiac muscle leading to the irreversible damage to the myocardial membrane.

Millions of people are taking beta-adrenoreceptor blocker drugs for reducing blood pressure and cholesterol which may lead to serious side effects. It is no surprise, then, that the use of alternative medicine, such as botanicals and nutritional supplements, has become popular for curing various diseases.

*Spermacoce hispida* Linn is an important plant belonging to the family of Rubiaceae and has been extensively used in Siddha system of medicine for curing various diseases. All the parts of the plant have an ethnomedical importance.\[11\] It is an effective natural drug for the treatment of hypertension and it has hepatoprotective, antiinflammatory and antioxidant properties. Recent pharmacological studies have shown that seeds of *Spermacoce hispida* posses antidiabetic and antihyperlipidaemic activity in rats. Experimental evidence on
biochemical role of *Spermacoce hispida* on myocardial infarction induced by ISO is lacking. In this context, an attempt has been made to elucidate the maintenance of myocardial integrity in presence of seed extract of *Spermacoce hispida* on ISO induced cardiac damage in rats.

**MATERIALS AND METHODS**

**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.

**Chemicals:** Isoproterenol hydrochloride, a ketoglutaric acid and ATP were purchased from Sigma chemical company, USA. Trisodium citrate and bovine serum albumin were taken from MERCK. Other chemicals used were of analytical grade.

**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. \[^{12,13}\]

**Experimental design:** Group 1: 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 daily at the interval of 24 hours. \[^{14}\]

Group 3: Rats were administered with ISP (100mg/kg b.wt) dissolved in 0.9% saline subcutaneously twice daily at the interval of 24 hours

Group 4: Rats were administered with ISP (200mg/kg b.wt) dissolved in 0.9% saline subcutaneously twice daily at the interval of 24 hours

Group 5: Rats were administered with 100mg/kg body wt. of *Spermacoce hispida* seed extract for 45 days. ISP was injected subcutaneously on 45th day.

Group 6: Rats were administered with 200mg/kg body wt. of *Spermacoce hispida* seed extract for 45 days. ISP was injected subcutaneously on 45th day.

**Collection of blood:** Blood was collected from the retro-orbital sinus without anti-coagulant for isolation of serum. The blood was centrifuged and the serum was used for the biochemical assay. The heart was excised immediately and washed off from blood with ice cold physiological saline.
Preparation of tissue homogenate: 10 % organ homogenate was prepared in 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 assays: The CPK activity was assayed as per the method adopted by Okinaka et al (1961). The Lactate dehydrogenase activity was assayed by the method of King. Activity of transaminases (AST and ALT) in serum and homogenate was assayed by the method of Mohun and Cook, (1957).

Lipid peroxidation in cardiac tissues was estimated by the determination of thiobarbituric acid reactive substances content that was evaluated as malondialdehyde (MDA) in heart homogenate by the method of Nichans and Samuelson, (1968). The tissue and serum hydroperoxydes were estimated by the method of Jiang et al., (1992).

Statistical analysis: The results were statistically evaluated by one way Analysis of Variance (ANOVA). They were further evaluated by Duncan Multiple Range test (DMRT) and the results were expressed as Mean ± Standard deviation (SD) for six rats in each group. A value of $P<0.05$ was considered statistically significant. All the statistical analysis was computed using SPSS software version 12.0.

RESULT

Table 1: Effect of HAE on Cardiac markers in normal and ISO induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>CPK Heart</th>
<th>CPK Serum</th>
<th>LDH Heart</th>
<th>LDH Serum</th>
<th>AST Heart</th>
<th>AST Serum</th>
<th>ALT Heart</th>
<th>ALT Serum</th>
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</thead>
<tbody>
<tr>
<td>(Group I) Control</td>
<td>909.3 ± 60.2 e</td>
<td>90.1 ± 21.3 a</td>
<td>75.7 ± 12.3 b</td>
<td>52.5 ± 6.9 a</td>
<td>459.8 ± 31.8 d</td>
<td>21.3 ± 2.9 a</td>
<td>422.5 ± 19.8 d</td>
<td>13.9 ± 1.9 a</td>
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<tr>
<td>(Group II) ISO</td>
<td>596.3 ± 39.8 a</td>
<td>152.2 ± 20.1 c</td>
<td>52.2 ± 9.0 a</td>
<td>204.3 ± 54.9 c</td>
<td>263.6 ± 17.4 a</td>
<td>43.5 ± 4.6 c</td>
<td>220.9 ± 19.3 a</td>
<td>25.8 ± 2.2 d</td>
</tr>
<tr>
<td>(Group III) 100 mg/kg b.wt. HAE + ISO</td>
<td>694.1 ± 30.2 b</td>
<td>124.7 ± 14.3 b</td>
<td>54.2 ± 19.7 a</td>
<td>171.9 ± 10.1 bc</td>
<td>414.9 ± 27.6 bc</td>
<td>40.2 ± 4.5 bc</td>
<td>300.2 ± 23.4 b</td>
<td>22.7 ± 2.3 c</td>
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<tr>
<td>(Group IV) 200 mg/kg b.wt. HAE + ISO</td>
<td>815.5 ± 67.1 d</td>
<td>94.8 ± 19.7 a</td>
<td>71.2 ± 11.5 ab</td>
<td>129.1 ± 56.1 b</td>
<td>453.5 ± 33.1 d</td>
<td>35.2 ± 9.8 b</td>
<td>391.9 ± 39.1 d</td>
<td>17.9 ± 2.6 b</td>
</tr>
<tr>
<td>(Group V) 100 mg/kg</td>
<td>902.2 ± 71.3 e</td>
<td>89.1 ± 19.7 a</td>
<td>77.8 ± 15.4 b</td>
<td>49.9 ± 4.9 a</td>
<td>454.4 ± 22.5 d</td>
<td>20.5 ± 4.1 a</td>
<td>421.8 ± 19.8 d</td>
<td>13.9 ± 1.2 a</td>
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<tr>
<td>Groups</td>
<td>TBARS Heart (nM of MDA/mg of protein)</td>
<td>Serum (nM of MDA/mg of protein)</td>
<td>Hydroperoxide Heart (µM/100 mg of tissue)</td>
<td>Serum (µM/dl)</td>
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<tr>
<td>Normal</td>
<td>29.2 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.1 ± 11.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.07 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>ISO</td>
<td>54.3 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99.8 ± 37.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.31 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>100 mg/kg b.wt. HAE + ISO</td>
<td>47.2 ± 6.2&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>94.9 ± 53.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.22 ± 0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.61 ± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>200 mg/kg b.wt. HAE + ISO</td>
<td>31.9 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.1 ± 11.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.31 ± 0.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.43 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>100 mg/kg b.wt. HAE</td>
<td>24.2 ± 6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.2 ± 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.02 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.49 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>200 mg/kg b.wt. HAE</td>
<td>20.2 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.2 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.01 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.43 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

Note: Activity of CPK, LDH, AST, ALT are represented as nM of Phosphate liberated/min/mg of protein, nM of pyruvate liberated/min/mg of protein, µM of pyruvate formed/min/mg of protein, (µM of pyruvate formed/min/mg of protein. 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,cd,d of same column are differ significantly (P<0.05).

Table 2: Effect of HAE on oxidative stress markers in normal and iso induced cardiotoxic rats

DISCUSSION

Spermacoce hispida Linn., is popularly known as ‘Nattaisuri’ in Tamil and ‘Shaggy button weed’ in English is widely distributed in western Ghats of Kerala and Tamil Nadu of India. The seed extract of the plant has been used as a remedy for curing various diseases. In the current study, MI was induced in rats by subcutaneous administration of isoprenaline in a dose of 100 mg/kg for two successive days.

Table 1 shows the effect of hydroalcoholic extract of seeds of Spermacoce hispida in cardiotoxicity induced rats. Isoprenaline-induced cardiac damage are varied and include generation of highly cytotoxic free radicals, increased calcium overload, and mitochondrial injury or
dysfunction.\textsuperscript{20,21} Myocardium contains enormous concentration of proteins and marker enzymes like CPK, LDH and transaminases and once they are metabolically damaged releases its content into the extracellular fluid (ECF). Administration of ISO leads to a cascade of metabolic events in the myocardial tissues beginning from anaerobic glycolysis, inhibition of ATP dependent transport process in cell membrane, electrolyte shift, and cellular edema and finally loss of cell membrane integrity. This leads to release of those cardio specific marker enzymes from heart to serum.

Serum levels of creatine kinase, lactate dehydrogenase and transaminases are the diagnostic indicators of myocardial infarction.\textsuperscript{22} In the current study, HAE of \textit{S.Hispida} has prevented the acute release of diagnostic marker enzymes in the serum as compared with ISO treated rats. Activity of CPK, LDH, AST and ALT is observed to be decreased significantly in heart tissue and increased in serum of diseased rats (Group II) against normal animals (P<0.05). The amount of enzymes present in the serum is directly proportional to the number of necrotic cells present in the damaged cardiac tissue.\textsuperscript{23} Pre treating animals with HAE of \textit{S.Hispida} has diminished the activity of these enzymes in serum and elevated in heart tissue and also found to be dose dependent. Significant difference has not been observed in cardiac markers of Group V and VI against Group I animals.

Sheela and Shyamaladevi, (2000)\textsuperscript{24} have stated that the damage caused by ISO is probably due to action on the sarcolemmal membrane, stimulation of adenylate cyclase, activation of Na\textsuperscript{+} and Ca\textsuperscript{2+} channels, exaggerated Ca\textsuperscript{2+} inflow and energy consumption leading to cellular death and these effects may be responsible for the increased level of marker enzymes in serum of diseased rats (Group II). The acute elevation of diagnostic markers in ISO treated rats has also been reported by Sathish \textit{et al}., (2003).\textsuperscript{25} Ischemic condition formed by ISO administration, causes anaerobic glycolysis. The ischemic damage is caused mainly by the accumulation of lactate and this might be due to the fall in LDH level in heart tissue of ISO administered (Group II) rats. The cardiac damage caused by ISO administration resulted in the release of various enzymes and their concentration is observed to be increased in serum. But the animals pretreated with \textit{S.Hispida} seed extract significantly reduced the release of the cardiac marker enzymes in serum and possess protective action against myocardial ischemia. Isoprenaline-generated free radicals are known to initiate peroxidation of membrane-bound polyunsaturated fatty acids leading to damage of the structural and functional integrity of the myocardium with consequent changes in membrane permeability.\textsuperscript{20} In the present study
increased level of oxidative stress markers like TBARS and HP is observed in heart and serum of diseased rats (Group II) against the normal animals ($P<0.05$, Table 2). Pretreatment of ISO administered rats with HAE (Group III and IV) is observed to decrease the level of these markers significantly ($P<0.05$) against diseased rats (Group II).

Seed extract of *Spermacoce hispida* exhibits satisfactory antioxidant effect against oxidative stress induced tissues. It may react with free radical to neutralize their effect. Fractions rich in flavonoids of *S.Hispida* seeds has antioxidant activity both *invitro* and *invivo*. The decreased level of TBARS and hydroperoxides of *S.Hispida* pretreated animals may be due to its inhibitory activity on the lipoxygease enzyme.

The plant has a large number of phytoconstituents such as Borreline, β-sitosterol, Ursolic acid and isorhamntin. Structure of β-sitosterol is similar to that of cholesterol and is sometimes used for the treatment of hypercholesterolemia. Hence various phytoconstituents present in the plant material might be responsible for the antioxidant and cardioprotective activity of *S.Hispida*.

**CONCLUSION**

The findings of the present study reveal that HAE of *S.Hispida* is a more potent antioxidant and cardioprotective agent. Seed extract of *S.Hispida* has shown a significant reduction in the release of cardiac markers from heart and also registered a significant reduction in lipid peroxidation. It is also concluded that the dose 200mg/kg bw of the extract has shown good effect. The results of this study are encouraging enough for further studies aimed at targeting its mechanism involved in cardioprotection.

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**REFERENCES**


