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CARDIOPROTECTIVE EFFECT OF ORTHOSIPHON STAMINEUS ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RAT
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Abstract:
Orthosiphon stamineus benth (OSE) (Family: Lamiaceae) has been widely used in Malaysia for treating kidney problems, gout and diabetes. The present study was designed to evaluate the cardioprotective activity of ethanolic extract of Orthosiphon stamineus on the basis of biochemical parameters in isoproterenol induced myocardial infarction in rats. Male albino rats were pretreated with OSE (100 and 200 mg /kg-1) daily for a period of 14 days. After the treatment period, Isoproterenol (ISO) (200 mg /kg-1) was subcutaneously injected to rats at an interval of 24 hr for two days to induce myocardial injury. After 48 hr, rats were anaesthetized with anesthetic ether, the levels of biochemical and histological observations of the heart tissues were performed. The activities of serum marker enzymes (ALT, AST, LDH and CPK) were increased significantly in ISO-induced rats. OSE at concentration of 200 mg kg-1, when administered orally showed a decrease in serum enzyme levels and brought to the near normal values. The finding results were further confirmed by histopathological observations. The histological sections obtained from ISO alone showed various degrees of focal lesions in many sections, and fragmentation of muscle fibers. Animals treated with OSE showed marked improvement in ISO-induced alterations such as vacuolar changes, edema and leukocyte infiltration compared to ISO-OSE administered group. Our data showed that OSE (200 mg /kg-1, p.o) significantly restores most of the biochemical parameters. The present study concluded that OSE may be therapeutic value in the treatment of myocardial infarction.
Key words: Myocardial infarction, Orthosiphon stamineus, Methanol, Isoproterenol.

Introduction

Cardiovascular Disease are the secondary cause of deaths in many parts of the world, although modern drugs are effective in preventing the disorders, their use is often limited because of their side effects and adverse reactions. A wide variety of plants and its active principles, with minimal side effects, provide an alternate therapy for heart disease. *Orthosiphon stamineus* benth (OSE) (Lamiaceae) better known as poonai meesai by the locals is rich in flavanoids. Most flavanoids are bioactive compounds due to the presence of phenolic group in their molecule. Twenty phenolic compounds were isolated from this plant including nine lipophilic flavones, two flavonol glycosides, nine caffeicacid derivatives. It is widely used in India for treatment of eruptive fever, urinary lithiasis, edema, hepatitis, jaundice and heart diseases.

Isoproterenol (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction. ISO is a β-adrenergic agonist that causes severe stress in myocardium and necrotic lesions in the heart muscles. ISO induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membrane. Myocardial infarction is induced by Isoproterenol in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia and increase in serum creatine phosphokinase, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activities. The mechanism proposed to explain isoproterenol induced cardiac damage involves generation of highly cytotoxic free radicals through autooxidation of catecholamine and has been implicated as one of the causative factor.

Materials and Methods;

Chemical

Isoproterenol hydrochloride (ISO) purchased from Sigma Chemical Company, US. All other chemicals were obtained from local sources and were of analytical grade.
Plant Materials

The leaves of *Orthosiphon stamineus* were collected from Siddha research institute, Arumbakkam, Chennai. The plant was identified and voucher specimen was deposited in the Herbarium of the department of biology, Annamalai University, Chidambaram. The material was dried in shade and powdered leaves 1kg were extracted with methanol in a Soxhlet extractor for 36 hr. Extract was evaporated under low pressure by using Buchi type evaporator.

Animals

Adult male wistar rats weighing 200-250g were obtained from R.V.S.College of Pharmaceutical sciences,sulur,Coimbatore They were maintained at standard housing conditions and fed with commercial diet and provided with water and libitum during the experiment. The institutional animal ethical committee (Reg.no 1012/C/06/CPCSEA) permitted the study.

Acute toxicity study:

Acute toxicity study was carried out using female Albino Rats (150-200 g) by up and down/staircase method as per OECD guidelines. The OSE was orally administered to different groups of rats at the doses of 50, 300, 1000, 2000 and 3000 mg kg-1 body weight respectively. Animals were observed for 48 h to study the general behavior of animals, sign of discomfort and nervous manifestation. The OSE was found devoid of mortality of animals at the dose of 3000 mg kg-1 body weight. Hence (100 mg kg-1, p.o.) and (200 mg kg-1, p.o.) of the dose selected for the screening of cardio-protective activity.

Evaluation of Cardioprotective activity;

Induction of myocardial injury:

Rats were treated with different doses of Methanol extract of Orthosiphon stamineus (OSE) orally using an intra-gastric tube daily for 14 days. On 14th day, myocardial injury was induced in experimental rats by injection of
ISO (200 mg kg⁻¹, s.c.) twice at an interval of 24 h (i.e., on 14th and 15th day of extract treatment) while normal control and ISO treated rats were given an equivalent volume of the vehicle.

**Treatment protocol:**

The experimental rats were divided into four groups of 6 animals each and treated as follows:

**Group 1:**

Normal Control Rats received single daily dose of 5% tween 80 (5 ml/Kg; po)

**Group 2**

Rats treated with 5% tween 80 (5 ml/Kg; po) and ISO (200 mg kg/day for 2 days, s.c.)

**Group 3:**

Rats pretreated with OSE (100 mg/ kg/ day, p.o.) and then ISO (200 mg kg-1day-1; for 2 days, s.c.)

**Group 4:**

Rats pretreated with OSE (200 mg kg/ day, p.o.) and then ISO (200 mg kg/ day; for 2 days, s.c.)

**Biochemical analysis:**

At the end of experimental period (after 24hr of second ISO injection or 16th day of extract/vehicle treatment) the rats were anaesthetized with light anaesthetic ether. Blood was collected from retro-orbital puncture, serum was separated and used for estimation of marker enzymes. The activities of aspartate Aminotransferase (AST) and alanine Aminotransferase (ALT) in serum were determined spectrophotometrically by the method of Mohur and Cook and the absorbance was measured at 520 nm and enzyme activity was expressed as U L⁻¹. The Lactate Dehydrogenase (LDH) activity in serum was assayed according to the method of King and the absorbance was measured 520 nm and the enzyme activity was expressed as U L⁻¹. The Creatine Phosphokinase (CPK) activity in serum was determined by the method of Okinaka et al. and the absorbance was measured at 640 nm and the enzyme activity was expressed as IU L⁻¹.
Histopathological studies:

At the end of the study, all the rats were sacrificed by cervical dislocation and the hearts were dissected out, washed in ice cold saline. Then myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under light microscope and photographs were taken.

Statistical Analysis

The results were expressed as mean ± SEM of six animals from each group. The statistical analysis were carried out by one way analysis of variance (ANOVA) P values < 0.05 were considered significant.

Results

Effect of Orthosiphon Stamineus on serum marker enzymes:

ISO treated rats exhibited significantly (p<0.001) higher levels of serum myocardial injury marker enzymes such as AST ALT, LDH and CPK compared to normal control rats (Table 1). The pretreatment of OSE (200 mg/kg) for 14 days and ISO (200 mg/kg, for 2 days) administration showed highly significant (p<0.001) reduction in all the tested diagnostic markers. Whereas, lower dose of OSE (100 mg/kg) and ISO (200 mg/kg) showed significant (p<0.01) reduction in all diagnostic markers.

Histopathological findings:

Histopathological examination of myocardial tissue obtained from normal control animals and animals treated with OSE showed clear integrity of myocardial membrane and an infiltration of inflammatory cells were not seen in experimental group 1 (Fig.1). The histological sec ISO alone (Fig. 2) shows various degrees of focal lesions in many sections consisting of fragmentation of muscle fibers, vacuolar changes along with hyaline necrosis were observed. Pretreatment with OSE (200 mg kg-1, respectively) (Fig3) Showed marked improvement in ISO-induced alterations such as vacuolar changes, edema, and leukocyte infiltration compared to ISO administered group.
Fig-1: Normal control showing normal myocardium.

Fig-2: Isoproterenol (200 mg kg⁻¹) group showing fragmentation of myocardial fibers and greater focal interstitial inflammatory response.

Fig-3: ISO + OS (200 mg kg⁻¹) showing reduced focal interstitial inflammatory response.

Discussion and conclusion

Isoproterenol is well known cardiotoxic agent due to its ability it will destruct myocardial cells. As a result of this, cytosolic enzymes such as Lactate Dehydrogenase (LDH), transaminases (ALT, AST) and Creatine Phosphokinase
(CPK) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in blood reflects the alterations in plasma membrane integrity and/or permeability. In the present study, ISO treated rats showed significant elevation in the levels of these diagnostic marker enzymes (AST, ALT, LDH and CPK). Moreover, elevated levels of these enzymes are an indicator of the severity of ISO-induced myocardial membrane necrosis, which is in line with an earlier report. The prior administration of OSE (100 and 200 mg /kg) showed significant reduction in ISO induced elevated serum marker enzymes. At a dose of 100 mg/kg the effect is only marginal whereas at higher dose 200 mg/kg effectively prevented isoproterenol induced cardiac damage. This reduction in enzyme levels could be due to its action on maintaining membrane integrity thereby restricting the leakage of these enzymes. In the present study, we found that Orthosiphon stamineus leaves methanol extract protected myocardium from isoproterenol-induced myocardial functional injury via normalization levels of diagnostic marker enzymes, suggesting the beneficial action of OSE as a cardioprotective agent.

Table-1: Effect of Methanol extract of Orthosiphon stamineus on different Biochemical parameters in ISO-induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspartate amino transferase (AST)</th>
<th>Alanine amino transferase (ALT)</th>
<th>Lactate dehydrogenase (LDH)</th>
<th>Creatine phosphokinase(CPK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>100.33±3.16</td>
<td>65.42±2.16</td>
<td>324±16</td>
<td>260.07±2.45</td>
</tr>
<tr>
<td>II ISO(200mg/kg)</td>
<td>207±4.05</td>
<td>180±3.03</td>
<td>643±3.1</td>
<td>324.57±2.08</td>
</tr>
<tr>
<td>III ISO+OSE(100mg/kg)</td>
<td>174.5±3.13</td>
<td>155.67±3.84</td>
<td>416±18</td>
<td>281.4±3.13</td>
</tr>
<tr>
<td>IV ISO+OSE(200mg/Kg)</td>
<td>115.17±3.63</td>
<td>70.83±3.32</td>
<td>396±40</td>
<td>274.5±2.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM for 6 animals in each group Group II compared with Group I (P<0.001). Group III and IV compared with Group II (P<0.001)
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