Evaluation of Cardioprotective Activity of Ethanolic Extract of Leaves of the *Cyanthillium Cinereum* against Isoprenaline Induced Cardiotoxicity in Rats

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**A B S T R A C T**

The aim of the study was to evaluate the cardioprotective activity of ethanolic extract of leaves of the *Cyanthillium cinereum* against isoprenaline induced cardiotoxicity in rats. Cardiac damage was induced by administration of isoprenaline (85mg/kg) subcutaneously on 29th and 30th days. Rats were pretreated with *Cyanthillium cinereum* leaf extract at the dose of 250mg/kg and 500 mg/kg body weight for 28 days. Propranolol 10mg/kg was administered as a standard drug for 28 days per orally. In this study analysis of biochemical parameters like LDH, SGOT, Troponin I, CK-MB, and tissue antioxidant markers like Catalase, Glutathione. *Cyanthillium cinereum* was showed the cardioprotective activity by lowering isoprenaline induced elevation of SGOT, LDH, Troponin, CK-MB and decreased levels of Catalase, Glutathione. Cardiac protection was confirmed by the histopathological examination of cardiac tissues treated with plant extract. This result concluding *Cyanthillium cinereum* leaves extract showed cardioprotective activity.

**Keywords:** Cardioprotective activity, *Cyanthillium cinereum*, Isoprenaline, myocardial infarction.
flowers are many pinkish violet colour with small heads.1) Cyanthilliumcinereumcontaining various phytoconstituents like alkaloids, Cardiac glycosides, , phenols, glycosides, flavonoids, steroids, tannins, phlobatannins, saponins, luteolin- 7 mono-beta D-glucopyranoside along with triterpene compounds like beta-amyrin acetate, lupeol acetate.2) This phytoconstituents are involved in treatment of different diseases. This plant has great medicinal values in diverse traditional usage in different nations. Whole plant used in ayurvedic preparation for the treatment of kidney disorders, it’s also used in the medication of diuretic, and menstrual pains. It given in form of decoction for swellings, stomach pain and diarrhea.3) Seeds are used in the preparation of anthelmintic agents. Leaves are used for the treatment of various diseases such as analgesic, antimicrobial, antipyretic and anti-inflammatory.4) Cyanthilliumcinereum (Vernoniacinerea) has antioxidant, anti-chemotherapeutic anti-hyperglycemic and sedative activity.5) Hepatoprotective activity.6) Anti-smoking agent.2)

2. Materials and Methods

Chemicals: Isoprenaline(Samarth pharma Pvt. Ltd, Mumbai, India), Propranolol (Abbott healthcaro Pvt. Ltd. India), Normal saline (Claris Life Sciences, Ahmadabad, India), Ethanol (Avantor performance materials India Ltd Maharashtra, India), Formaldehyde (Finar chemicals limited, Ahmadabad, India), Sodium citrate (Virat Labs, Hyd, India), Lactate dehydrogenase kit (Cornal clinical system, Verna, Goa, India), Aspartate transaminase kit(Excel diagnostics Pvt. Ltd, India) and Troponin I kit (Oscar Medicare Pvt. Ltd. New Delhi, India). CK-MB kit (Cornal clinical system, Verna, Goa, India).

Experimental animals: Albino Wistar rats of 125-150 g of female were used for the study. The animals were maintained under standard condition in animal house of Vaageswari College of Pharmacy. The animals were maintained under controlled conditions of temperature (23 ± 2°C)Humidity (35-60%), 12:12 light and dark ratio. The animals were randomized into different groups, accommodated in polypropylene cages containing sterilized paddy husk as bedding. They had standard pellets as basal diet and water ad libitum. All the studies conducted and approved by the Institutional Animal Ethical Committee (IAEC). (Registration No. VCP/Cology/005/11/2017)

Acute toxicity study:

Acute toxicity study was carried using rats as per OECD guideline. The aim of this experiment was to determine the LD50 of the crude extract. In this study using 20 rats of both sexes were randomly divided into 4 groups. Each group containing five rats and dose of 100, 500, 1000 and 3500 mg/Kg of the plant extract per orally by gastric gavage. After 48 hours there was observed signs of mortality and toxicity. This study suggested that Cyanthilliumcinereum does not cause any apparent acute toxicity.

Experimental procedure

Inducing procedure for cardio toxicity: Myocardial infarction was induced by the isoprenaline hydrochloride at the dose of 85mg/kg body weight dissolved in normal saline, it given by subcutaneously for two consecutive days.

Study design for cardioprotective activity:

Experimental Animal: Female albino wistar rats (150-200 gm).Animals were grouped into 4 groups, each group contains 5 rats. Group I: Normal control group-administration of distilled water orally (0.5ml) daily for 30 days. Group II: Cardiac control group - administration of distilled water orally for 30 days. In addition received injections of Isoprenaline (85mg/kg, S.C) at an interval of 24 hours on the 29th and 30th day. Group III: Standard control group - receiving of Propranolol (10mg/kg/day) orally for 28days. Group IV: Test group 1 - Pre-treated with Cyanthilliumcinereum ethanolic leaf extract of 250mg/kg orally for 28days.Group V: Test group 2 - Pre-treated with Cyanthilliumcinereum ethanolic leaf extract of 500mg/kg orally for 28days.Group III, IV and V groups are received the treatment of ISO at two consecutive days (29th and 30th). At the end of the treatment blood samples were collected and assayed for the levels of LDH, Troponin, SGOT and antioxidant biomarker Catalase, Glutathione.
End of the experiment the blood samples were collected by retro orbital puncture using capillary tubes. Serum were separated from blood sample and used for the estimations of different cardiac biomarkers, like Troponin-I, serum glutamic oxalacetic transaminase, creatine kinase myoglobin and Lactate dehydrogenase along with antioxidant biomarker such as Catalase and glutathione. These estimations are performed by using commercially available Diagnostic kits.

**Histopathological examination:**
At the end of the study, the abdomen was cut open to isolate heart from each animal. Isolated hearts were washed with cold saline and cleaned off extraneous tissue and kept in 10% neutral formalin solution. And tissues were embedded in paraffin wax and 5µm thick sections was cut and stained with eosin and hematoxylin. Then the sections were observed under light microscope and photo micrographs were taken.\(^{(9)}\)

**Statistical analysis:**
The data are expressed as Mean ± SEM and Statistical analysis was performed using one way ANOVA followed by Dunnett t-test for cardioprotective activity. \((P<0.05)\) was regarded as statistically significant.

3. Results and Discussion
Myocardial infarction is the most among ischemic heart diseases and is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperlipidermia etc. and leading to qualitative & quantitative alterations of myocardium. Oxygen free radicals are implicated as mediators of tissue injury in cardiovascular pathology thus leading to myocardial damage. On induction of cardiotoxicity with Isoprenaline.

The elevated levels of Troponin, SGOT, LDH and CK-MB and reduced levels of glutathione and catalase in toxic control group than normal in all groups. Plant extract \((Cyanthillium cinereum)\) was administered to group-4 (250mg/kg) and group-5 (500mg/kg). On pretreatment with extract the SGOT, CK-MB and LDH levels were reduced significantly. 250mg/kg showed significant reduction in SGOT \((p<0.01)\), LDH \((p<0.05)\), and CK-MB \((p<0.01)\). 500mg/kg showed significant reduction in SGOT \((p<0.001)\), LDH \((p<0.01)\), CK-MB \((p<0.001)\). Administration of propranolol (10mg/kg) showed significant decreased levels of cardiac parameters and increased levels of catalase and glutathione.

Pretreated extract with 500mg/kg and 250mg/kg have showed elevated levels of catalase \((p<0.01)\) and glutathione \((p<0.01)\) (Table no). In this study isoprenaline was used to induce cardiotoxicity in rats. Isoprenaline causes myocardial infarction which results in increased levels of CK-MB LDH, Troponin I, SGOT in serum and decreased levels of Catalase and glutathione. Pretreatment with leaves of the \(Cyanthillium cinereum\) extract produced significant decrease levels of CK-MB, LDH, SGOT, Troponin I and increased levels of catalase and glutathione which indicating the protective effect of cardiac tissue.

On histopathological examination, normal control group heart section (fig-4.a) shows the cardiocyte cellular and architectural pattern maintained. There is no evidence of fibrosis or inflammation or degenerative changes. ISO group (85mg/kg) heart section (fig-4.b) shows marked myocardial necrosis with lymphocytic infiltration. There is focal cardiocyte degeneration. It indicates cardiac tissue damage. In treated rats with extract (250mg/kg) (fig-4.c) very few cardiocytes shows degenerative changes and mild myonecrosis with mild interstitial fibrosis and sparse inflammation. In treated rats with extract (500mg/kg) (fig-4.d) shows normal cellular and architectural pattern with on degeneration, very lesser degree of myocardial necrosis. In treated rats with standard drug propranolol (10mg/kg) (fig-4.e) shows normal cellular and architectural pattern with no degeneration. Myofibrils and mononuclear cells appeared normal. Thus histopathological results indicates that the pretreatment with extract at doses 250mg/kg and 500mg/kg has less degeneration showing protective effect over isoprenaline control group.
Table 1: Release of Troponin – I in various treatment groups

<table>
<thead>
<tr>
<th>No of animals</th>
<th>Normal control group</th>
<th>ISO induced group (85mg/kg)</th>
<th>Std group (10mg/kg)</th>
<th>Plant extract (250mg/kg)</th>
<th>Plant extract (500mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
<td>+Ve</td>
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<tr>
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<td>-Ve</td>
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<td>-Ve</td>
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<td>-Ve</td>
<td>+Ve</td>
<td>-Ve</td>
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</tbody>
</table>

+Ve – Presence of Troponin – I in serum; -Ve – Presence of Troponin – I in serum

Table 2: Effects of ethanolic extract of leaves of the Cyanthillium cinereum on SGOT, LDH, CK-MB, CAT, & GSH levels in rats treated with Isoprenaline

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Cardiac biomarkers</th>
<th>Antioxidant biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT(IU/L)</td>
<td>LDH(IU/L)</td>
</tr>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>46.74±3.94</td>
<td>98.75±14.9</td>
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<tr>
<td>2</td>
<td>ISO Control</td>
<td>130.09±6.56</td>
<td>179.70±14.5</td>
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<td>3</td>
<td>STD</td>
<td>61.84±3.69</td>
<td>108.47±5.88</td>
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<tr>
<td>4</td>
<td>Extract(250mg/kg)+ISO</td>
<td>99.68±3.22</td>
<td>153.80±5.11</td>
</tr>
<tr>
<td>5</td>
<td>Extract(500mg/kg)+ISO</td>
<td>74.96±3.48</td>
<td>123.04±2.42</td>
</tr>
</tbody>
</table>

4. Conclusion
From the experimental studies carried out on ethanolic extract of leaves of the *Cyanthillium cinereum* at two doses (250mg/kg and 500mg/kg) showed dose dependent cardioprotective activity against ISO induced cardiotoxicity. The higher dose 500mg/kg showed significant protective activity compared to low dose 250mg/kg.

Conflict of Interest: We declare no conflict of interest.

5. References