EVALUATION OF CARDIOPROTECTIVE EFFECT OF *CYPERUS TRICEPS*(CYPERACEAE) AGAINST ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS

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A B S T R A C T

This study was conducted to evaluate the cardioprotective effect of *Cyperus triceps* against isoproterenol produced myocardial infarction. The extract of *Cyperus triceps* in the doses of 200 mg/kg and 400 mg/kg was administered orally for 21 days prior to isoproterenol administration to test their cardioprotective effect. Isoproterenol (85 mg/kg) was administered intraperitoneally on 20^{th} and 21^{st} days respectively to induce myocardial infarction. The parameters for evaluation of cardioprotective activity were the Biochemical estimations and Histopathology examination. In biochemical estimations, the cardiac parameters such as CK-MB, SGOT SGPT and Triglycerides were determined. The levels changed by isoproterenol were restored significantly by the administration of the both doses of test extract especially at higher dose. The results of this study shows that *Cyperus triceps* extract has significant cardioprotective effect against isoprenaline induced myocardial infarction.

Key words :

Cardio protective effect,Cyperus triceps, cardiac parameters, myocardial infarction.

INTRODUCTION

Acute necrosis of the myocardium due to an imbalance between coronary blood supply and myocardial demand is known as myocardial infarction (MI). [1] Reduced blood flow to the heart causes ischemia, which can result in a number of metabolic changes that can affect the function of the heart and eventually cause cell death. [2] Free radicals produced in ischemic tissues are known to trigger metabolic stresses that weaken tissue defense mechanisms, resulting in cardiac damage and necrosis. [3]

Isoproterenol (ISO) is a synthetic catecholamine and adrenergic agonist that has been found to trigger myocardial infarction when administered in high doses [4]. One of the mechanisms by which ISO exerts its damaging effects is through autoxidation, leading to the production of potent cytotoxic free radicals. These free radicals, in turn, instigate peroxidation of membrane phospholipids, resulting in considerable harm to the myocardial membrane. Consequently, this oxidative stress can lead to significant damage to the heart tissue, ultimately contributing to the development of myocardial infarction.

Enhanced comprehension of the mechanisms underlying myocardial infarction (MI) has sparked the search for drugs that could mitigate damage to the heart muscle. Such medications that offer myocardial protection from toxic substances hold promise in preventing or delaying the occurrence of myocardial infarction. Among the potential candidates, free radical scavengers, also known as antioxidants, including carotene, vitamin C, vitamin E, and selenium, have garnered significant attention due to their potent antioxidant properties. Given that free radical generation plays a crucial role in causing substantial injury during MI, these nutritional advantages with strong antioxidant effects may offer valuable therapeutic potential.

The therapeutic properties of medicinal plants, plant-based foods, and their constituents have garnered significant interest for their potential in treating different aspects of ischemic heart disease, including myocardial infarction (MI) [5]. As a result, there is an increasing trend in the utilization of herbs in pharmacotherapy. Herbal products are believed to have the capacity to influence the progression of heart disease and offer an integrative approach by providing essential nutrients that aid in restoring and maintaining balance in the body's systems. This growing awareness of the potential benefits of herbal remedies highlights their importance in supporting heart health and promoting overall well-being.[6]

Phytochemicals are potent compounds obtained from natural resources, particularly plants and have therapeutic activities [7] One possible safe and efficient treatment for postprandial hyperglycemia is Kyllinga triceps. [8] Kyllinga triceps also posess diuretic action. [9] It also posses analgesic and anti inflammatory properties[10]

The synonym of Kyllinga triceps is Cyperus triceps. Kyllinga triceps is Glabrous,stems are 5 to 23 cm long, tufted, Leaves are rarely longer but usually shorter than the stem 2 to 4 mm broad, linear,acute spikes, ovoid-oblong or subcylindric usually 3 together(rarely solitary). Kyllinga triceps is used to relieve thirst in fevers, diabetes and also to relieve pruritus of the skin. Kyllinga triceps is used in diseases of the blood. [11] It is used as refrigerant, demulcent tonic and used as antidote to poisons.[12]

However, the literature lacks investigation of Cyperus triceps in cardiac ischemia so the current study investigated the Cyperus triceps extract for its cardioprotective potential.

Materials and Methodology

Plant authentication

The plant Cyperus triceps was collected after the authentication by Dr.K. Madhava Chetty, Plant Taxonomist (IAAT: 357), Professor of the Department of Botany, SV University, A.P, India.Voucher number is 2385.

Preparation of extract

The fresh whole plant was carefully dried in the shade and subsequently ground into a moderately coarse powder. The powdered material was then subjected to consecutive extractions using a soxhlet extractor. For the extraction of dry Kyllinga triceps Rottb powder, 80% ethanol was utilized, and the process was carried out for 24 hours through the cold percolation method. The resulting alcoholic extract was filtered using an 80 mesh nylon cloth to ensure the removal of any solid residues. To prevent contamination, a raw material to solvent ratio of 1:8 was maintained, and the entire extraction procedure was repeated five

times. Throughout the extraction process, strict adherence to clean and sterile conditions was maintained.

Afterward, all the filterates obtained from the extraction procedure were combined, and the resultant mixture was subjected to further filtration using a nylon cloth to refine the extract. Finally, the extract was concentrated under reduced pressure to obtain the desired product.

ACUTE TOXICITY STUDIES

An acute oral toxicity study for Cyperus triceps was performed according to the acute toxic classic method described by OECD-423 guidelines. Twelve Albino mice weighing from 15-25gms were utilized for the acute toxicity study. The animals were kept fasting total night giving only water. The test animals were categorized into four groups of three rats each and were given doses of Cyperus triceps (i.e.,50, 300, 1000, 2000 mg /kg). The animals are kept under close observation for 24 hrs. for any visible signs of toxicity, aggressive behavior (i.e., biting and scratching behavior, and diarrhea and observed for up to 14 days for mortality).

Experimental animals

In the present study, male and female Wistar rats weighing between 180-200 g were used as experimental subjects. Prior to conducting the study, ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC/CESCOP/2023-08). The rats were housed in cages with access to natural light and dark cycles and were allowed to acclimate to the laboratory conditions, including the environmental temperature, for a period of 7 days before commencing the experiments. Throughout the study, the animals were provided with a standard rodent diet and had free access to water.

The experimental protocol followed in this study was in full compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (1305/Po/Re/S/09/CPCSEA), and was duly approved by the Institutional Animal Ethical Committee. Proper care and ethical practices were strictly adhered to in the treatment and handling of the animals throughout the study.

Experimental Procedure

Grouping

Total of 30 rats were taken for the experiment. After acclimatization, the animals were allocated into 5 groups containing 6 animals each.

- ✓ Group I animals received the normal saline and were termed as control group (1ml/kg/day p.o) for 21 days.
- ✓ Group II animals served as isoproterenol treated at 85mg/kg/i.p daily for the last two consequent days.
- ✓ Group III animals were treated with standard propranolol (10 mg/kg, p.o) for one week after two weeks of saline treatment.
- ✓ Group IV and Group V animals were pre-treated with Cyperus triceps at 200mg/kg and 400mg/kg BW orally for 21 days respectively.
- ✓ Animals from Group II to Group V were administered isoproterenol (85mg/kg), intraperitonially on the 20^{th} and 21^{st} days at an interval of 24 hours

Induction of Myocardial infarction

To induce myocardial infarction, Isoproterenol hydrochloride at a dose of 85mg/kg body weight was dissolved in normal saline and administered via intraperitoneal injection for two consecutive days (20^{th} and 21^{st}). After 24 hours from the final intraperitoneal injection of ISO, the rats were weighed and euthanized [13].

Blood collection was conducted following Good Laboratory Practices. Blood samples were obtained through the retro-orbital plexus from the inner canthus of the eye using capillary tubes, and through cardiac puncture under light ether anesthesia. The collected blood was allowed to clot for 30 minutes at room temperature, and then the serum was separated through centrifugation at 3000 rpm and 30°C for 15 minutes. The obtained serum was used for assessing parameters such as SGOT, SGPT, CK-MB, and Triglycerides. Cervical decapitation was performed to humanely sacrifice all animals. The hearts were promptly dissected out, weighed, and fixed in a 10% buffered neutral formalin solution.

Estimation of parameters

Biochemical parameters

At the conclusion of the experimental period, blood samples were collected, and the serum was separated to analyze various parameters associated with myocardial infarction. These parameters included Triglycerides, Creatine Kinase-MB fraction (CK-MB), Aspartate transaminase (AST), and Alanine transaminase (ALT).

Statistical analysis

The values were presented as mean ± standard deviation (SD) for six rats in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. Graph Pad Software version 5.3 was utilized for the statistical analysis, and a p-value of less than 0.001 was considered statistically significant.

Histopathological study

At the conclusion of the study, the heart was isolated and rinsed with ice-cold saline. The cardiac tissue was then fixed in a 10% buffered neutral formalin solution. After the fixation process, the tissues were embedded in paraffin-wax, and sections of five micrometers thickness were cut. These sections were subsequently stained with hematoxylin and eosin. The stained slides were observed under a light microscope, and photomicrographs were captured for analysis and documentation.

Results and Discussion

Acute Toxicity study

An Acute Oral Toxicity study was conducted on the extract of Cyperus triceps following the acute toxic classic method described by OECD-423 guidelines. The test animals were divided into four groups and administered different doses of Cyperus triceps extract, namely 50, 300, 1000, and 2000 mg/kg. These animals were closely monitored for any signs of mortality or toxic manifestations over a period of 72 hours.

The results of the study indicated that the extract of Cyperus triceps did not cause any mortality or exhibit toxic effects at the highest dose tested, which was 2000 mg/kg body weight. Based on these findings, doses of 200 mg/kg (considered low dose) and 400 mg/kg

(considered high dose) were selected for further investigation of the extract's biological activity.

Effect of EECT on serum biochemical parameters

In the isoproterenol-treated group, the activities of CK-MB, SGOT, and SGPT enzymes were significantly elevated compared to the control group. However, the Standard group demonstrated notable improvements in SGOT and SGPT levels when compared to the EECT-treated groups. In all the treatment groups, there was a significant reduction in the enzyme levels compared to the isoproterenol-treated group.

A marked increase in the levels of biochemical enzymes in the serum is used as a sensitive indicator of cardiac injury. The increase of these biomarkers in the serum suggests leakage of the enzymes from the mitochondria due to myocardial injury. Thus, it indicates that the extent of cardiac myocyte injury in pre-treated groups was significantly lesser. This proves the cardio protective activity of the trial drugs

Histopathology Examination

The myocardial tissue was promptly immersed in a 10% buffered neutral formalin solution for fixation. Following fixation, the tissues were embedded in paraffin, and consecutive sections were cut. Each section was then stained with hematoxylin and eosin. Subsequently, the slides were carefully examined under a light microscope, and microphotographs were captured for further analysis and documentation

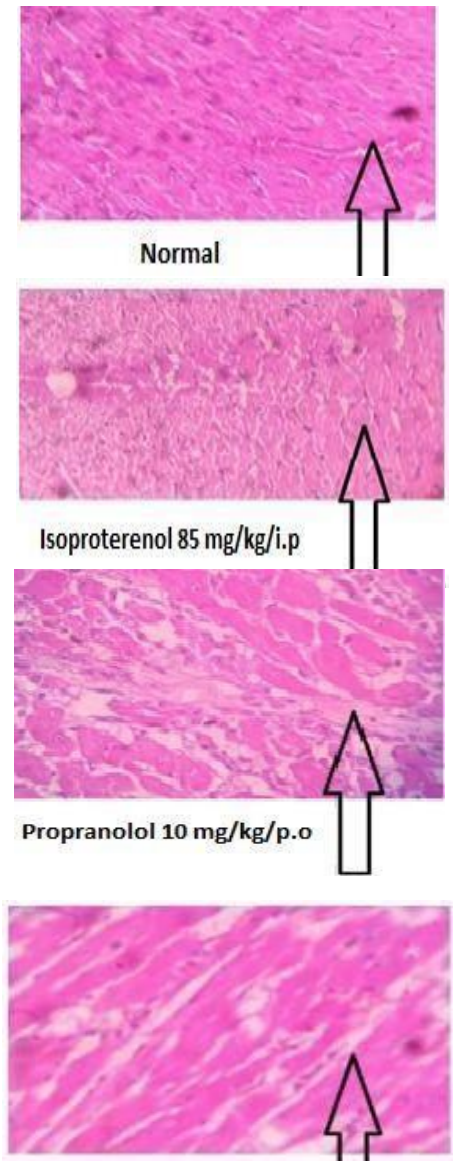
In the normal group, the myocardial cell membrane exhibited intact integrity. The myofibrillar structure displayed clear striations and maintained continuity with adjacent myofibrils. Additionally, the interstitial space appeared to be intact without any signs of damage..

In the ISO-treated group, there was evident loss of intact integrity of the myocardial cell membrane. The myofibrillar structure displayed disruptions in striations and continuity with adjacent myofibrils. Furthermore, at some areas, there was an increase in the interstitial space.

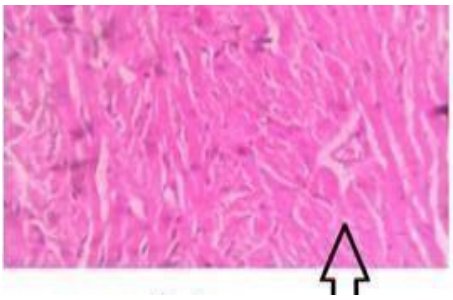
In contrast, the Standard group demonstrated preserved intact integrity of the myocardial cell membrane. The myofibrillar structure exhibited clear striations and maintained continuity with adjacent myofibrils. Additionally, scattered inflammatory infiltration was observed in this group.

At the low dose of EECT (200mg/kg), there was a noticeable loss of intact integrity in the myocardial cell membrane. The myofibrillar structure displayed disruptions in striations and continuity with adjacent myofibrils. Additionally, an increase in the interstitial space was observed at focal areas.

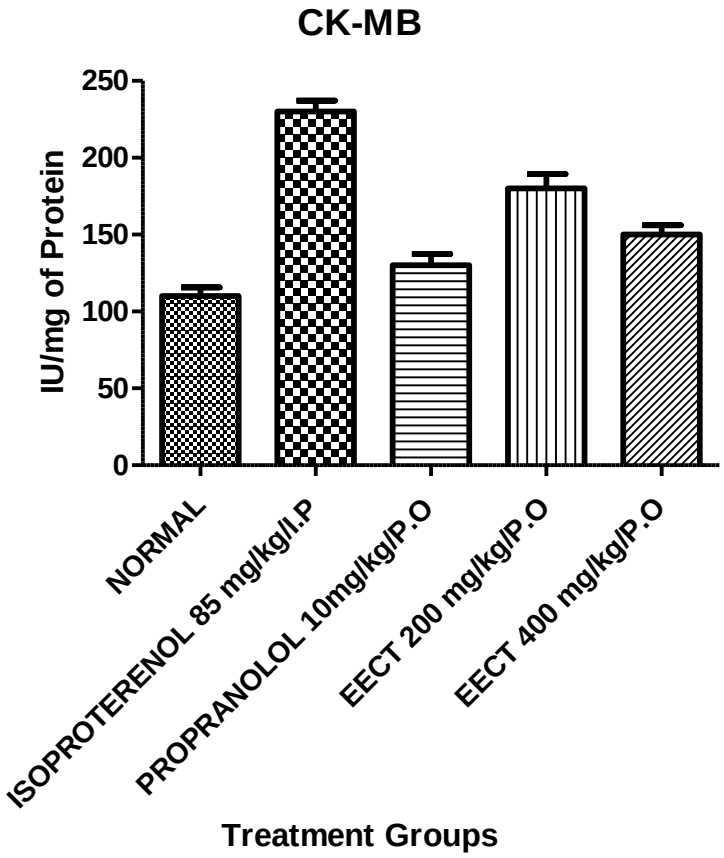
Similarly, at the high dose of EECT (400mg/kg), there was evident loss of intact integrity in the myocardial cell membrane, and disruptions in the myofibrillar structure with striations and continuity with adjacent myofibrils were observed. However, in this group, the interstitial space appeared to be mostly intact with some alterations amidst these areas.

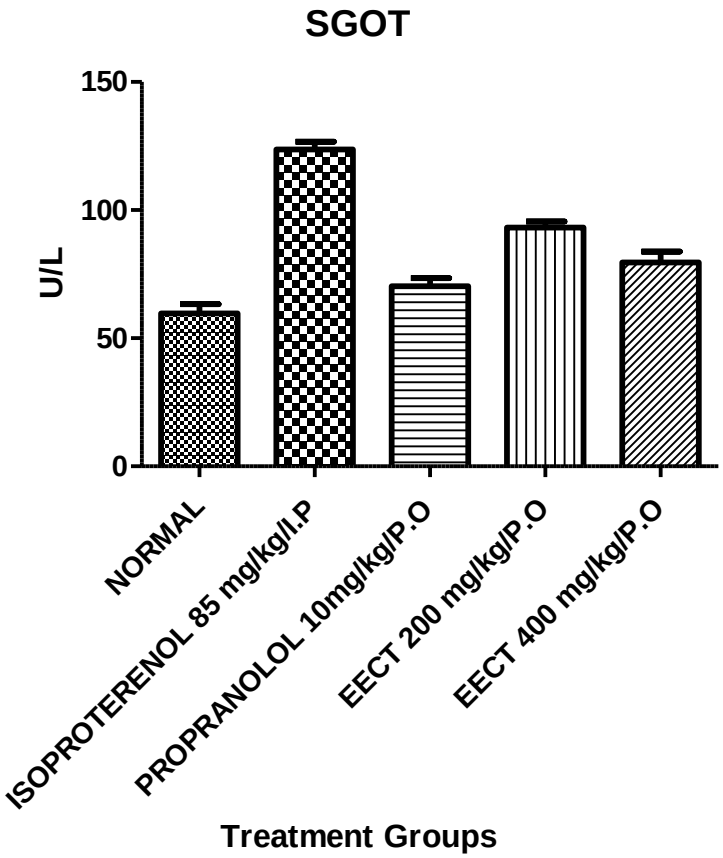


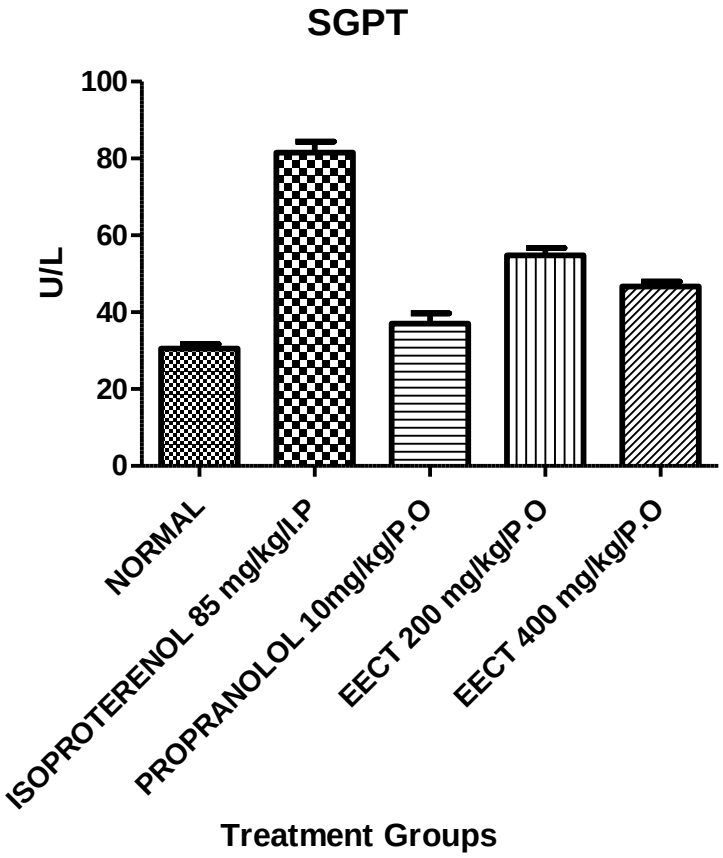
EECT 200mg/kg



EECT 400mg/kg




SGOT
150
100
50
0
U/L
NORMAL
ISOPROTERENOL 85 mg/kg/I.P
PROPRANOLOL 10mg/kg/P.O
EECT 200 mg/kg/P.O
EECT 400 mg/kg/P.O
Treatment Groups


SGPT
100
80
60
40
20
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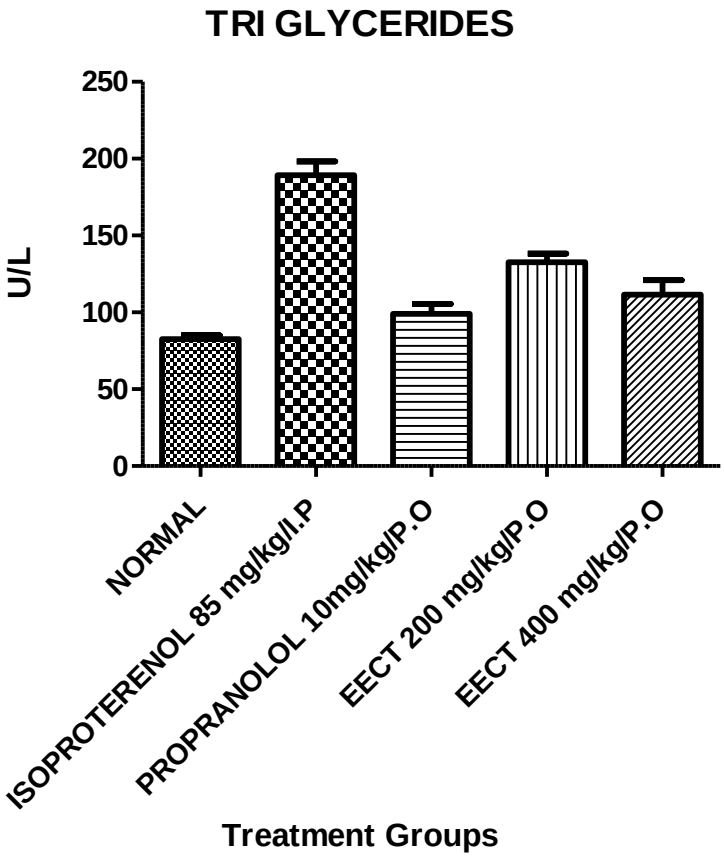
U/L
NORMAL
ISOPROTERENOL 85 mg/kg/I.P
PROPRANOLOL 10mg/kg/P.O
EECT 200 mg/kg/P.O
EECT 400 mg/kg/P.O
Treatment Groups


TRI GLYCERIDES
250
200
150
100
50
0
U/L
NORMAL
ISOPROTERENOL 85 mg/kg/I.P
PROPRANOLOL 10mg/kg/P.O
EECT 200 mg/kg/P.O
EECT 400 mg/kg/P.O
Treatment Groups

Conclusion

Cardiovascular disease is a significant global health concern, and its prevalence is increasing rapidly in the Indian subcontinent [14]. The present study revealed that the ethanolic extract of Cyperus triceps (EECT) possesses cardioprotective properties against isoproterenol-induced myocardial infarction in albino rats. These findings are consistent with the idea that free radicals generated from isoproterenol play a vital role in inducing myocardial infarction. EECT effectively restored the levels of cardiac enzymes (CK-MB, SGOT, SGPT) to normal levels. Notably, the higher dosage of 400 mg/kg exhibited more potent cardioprotective activity compared to 200 mg/kg, possibly attributed to the presence of active constituents like flavonoids in the ethanolic extract of Cyperus triceps. These constituents may contribute to mitigating oxidative stress caused by myocytes. Histopathological studies further corroborated the protective effects of Cyperus triceps. However, additional research is necessary to fully comprehend the precise mechanism underlying the cardioprotective effect of this ethanolic extract and to identify its active compounds.

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CONFLICT OF INTEREST :

We declare that we have no conflict of interest.

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