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Evaluation of Antioxidant Activity of Ethanolic Extract of Leaves of *Cyanthillium cinereum* (L) H. Rob. by Using Isolated Frog Heart

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Keywords: Frog heart, antioxidant activity, *Cyanthillium cinereum*, ethanolic extract, oxidative stress.

ABSTRACT

Objective: The present study was aimed to evaluate the *in vitro* antioxidant activity of ethanolic extract of leaves of *Cyanthillium cinereum Linn*. by using isolated frog heart as a model. **Method:** 1mM of an H_2O_2 solution in frog Ringer solution was used to induce oxidative stress on isolated frog heart. When ringer solution containing H_2O_2 perfused to frog heart preparation, which indicating the induction of oxidative stress on frog heart, this might be due to destabilization of receptors. Cardiac output, heart rate, and cardiac arrest parameters were estimated. **Results:** The present study results support that the frog heart model for induction of oxidative stress by H_2O_2. It shows negative ionotropic, negative chronotropic effects and cardiac arrest was produced in the 16th minute. In the presence of the ethanolic extract of leaves of *Cyanthillium cinereum*, cardiac arrest was observed at 31st minutes i.e. heart was protected longer period which indicates antioxidant activity when compared with the standard ascorbic acid. **Conclusion:** The results obtained in this work showed that ethanolic extract of leaves of *Cyanthillium cinereum* exhibits anti-oxidant activity against H_2O_2 induced oxidative stress on isolated frog heart as a model and compared with the standard antioxidant agent (Ascorbic acid).

INTRODUCTION

Cyanthillium cinereum (L) H.Rob. also called as *Vernonia cinerea* belongs to the family of Asteraceae. The species is native to tropical Africa, tropical Asia, India, Indochina, tropical South America, West India and US state of Florida. *Cyanthillium cinereum* is an annual herb grows up to 120cm tall and produces flat-topped arrays of numerous flower head. It is used in Ayurvedic herbal medicine [1]. *Cyanthillium cinereum* containing various chemical constituents like luteolin 7 mono beta D glucopyranoside along with triterpene compounds like beta amyrin acetate, lupeol acetate [2].

Figure no 1: Leaves and Flowers of *Cyanthilium cinereum* (L) H. Rob.

Phytochemical screening of this plant showed a presence of cardiac glycosides, alkaloids, phenols, flavonoids, steroids, tannins, phlobatannins and saponins [12]. Flavonoids and phenols are strong antioxidants and have an important role in the health care system [3]. These constituents were involved in the treatment of various diseases and show antioxidant activity. Whole plant used in ayurvedic preparation for the treatment of kidney disorders, in the form of decotion used for swellings, stomach pain, and diarrhea. It is also used as the diuretic and in menstrual pains [2]. Seeds are also used as the anthelmintic agent. Leaves are used for the treatment of various diseases such as analgesic, antimicrobial, antipyretic and anti-inflammatory [12].

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants. Free radicals are the unstable molecules that react with other substances to damage cell, tissue or organ, which is caused by the reactive oxygen species (ROS) [8]. Reactive oxygen species (ROS) are highly reactive substances, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. The free radicals have capable of reacting with membrane nucleic acids, lipids, proteins, enzymes and other small molecules [5]. Antioxidants were synthesized within the body or taken in the diet, which acts as a natural defense against free radical-induced damage [8]. The oxidative stress in animals or cell cultures has been successfully induced by hydrogen peroxide and was chosen for induction of oxidative stress on isolated frog heart [10].

MATERIALS AND METHODS

Plant collection and authentication:

The healthy leaves of *Cyanthillium cinereum* (L) H. Rob. Belongs to the family Asteraceae were collected from the roadsides of Thimmapur village, Karimnagar district, Telangana, India and authenticated by the Botanical Survey of India. Reg no: BSI/DRC/2017-18/Tech./699.

Preparation of extract:

The plant material (leaves of the *Cyanthillium cinereum*) was shade dried and powdered by a mechanical grinder. The dried powder was extracted with ethanol as a solvent by using soxhlet apparatus. The powder (100gm) was taken and placed in thimble made up of filter paper and inserted into the wide central tube of an extractor. Ethanol is placed in the round bottom flask and brought to its boiling point up to 78^{o}c for 6-7 hours. Its vapors passed through the larger right-hand tube into the upper part of an extractor and then to the condenser. During this period, the active constituents were extracted, when the level of the extract reaches the top of the syphon tube. The process was continued until the drug was completely extracted, then the extract processed for evaporation. After the evaporation, the semi-solid jelly is formed. The plant extract was dark green in color and soluble in distilled water [11].

Materials:

Acetylcholine chloride was purchased from Burgoyne laboratories, Mumbai. NaCl, KCL, Cacl2, Dextrose, $NaHCO_3$ were purchased from Final chemicals, Ahmedabad. Ascorbic acid and hydrogen peroxide (H_2O_2) were purchased from Himedia, Laboratories Ltd., Mumbai, India. Kymograph paper, Starling's heart lever and Sherrington rotating drum were purchased from Inco, Ambala, India.

Physiological solution:

The composition of frog ringers solution is NaCl- 6grms, KCl- 0.14grms, Cacl2 – 0.12grms, NaHco3 – 0.2grms, glucose- 2grms made with 1000ml distilled water[6**].**

Isolation of frog heart preparation:

Frogs of Rana tigrina species from the animal house of Vaageswari College of pharmacy, Karimnagar were used for these studies. Approval no. is 1720/PO/a/13/CPCSEA. The frog was stunned by head-blow using a steel rod and pithed. The frog was placed on frog dissecting board, pin the forelimbs. The skin and abdomen were cut and opened. The pectoral girdle was cut by using a bone cutter and removed the pericardium carefully. Introduce the Syme's cannula, connected to the reservoir of frog Ringers solution. Immediately into the Sinus venosus of the heart. The connecting blood vessels were cut and heart was isolated from the animal and mounted on to a stand. The heart was then covered with a thin layer of cotton and poured some frog Ringer solution periodically to prevent drying. A heart was connected to the Starlings heart lever and adjusted for recording the responses of the heart. The level of frog Ringer solution in the Syme's cannula was maintained by fixing a glass tube into the cork fixed to the reservoir (Marriott's bottle) tightly. It helps to maintain a constant pressure head over the heart. Then the heart was allowed to stabilize and record heart rate and cardiac output on rotating drum, to which a smoked kymograph paper was affixed [6,8].

METHOD:

H_2O_2 induced oxidative stress on isolated frog heart:

- 1mM of the H_2O_2 solution in frog Ringer solution was used to induce oxidative stress on isolated frog heart. Cardiac output, heart rate, and cardiac arrest parameters were estimated. Initially acetylcholine at doses of 1ng/ml, 3ng/ml was showed muscarinic action like negative

ionotropic, negative chronotropic and decreased cardiac output. Nevertheless, continuous perfusion of frog Ringer solution containing H_2O_2, the muscarinic actions were not observed which indicates the damage of muscarinic receptors due to oxidative stress induced by H_2O_2 [7].

➢ The same dose levels of ethanolic extract were repeated in continuous perfusion of frog Ringer solution containing H_2O_2 and observed the parameters. The time taken to induce cardiac arrest was compared with the standard drug ascorbic acid (3mM) [9].

RESULTS

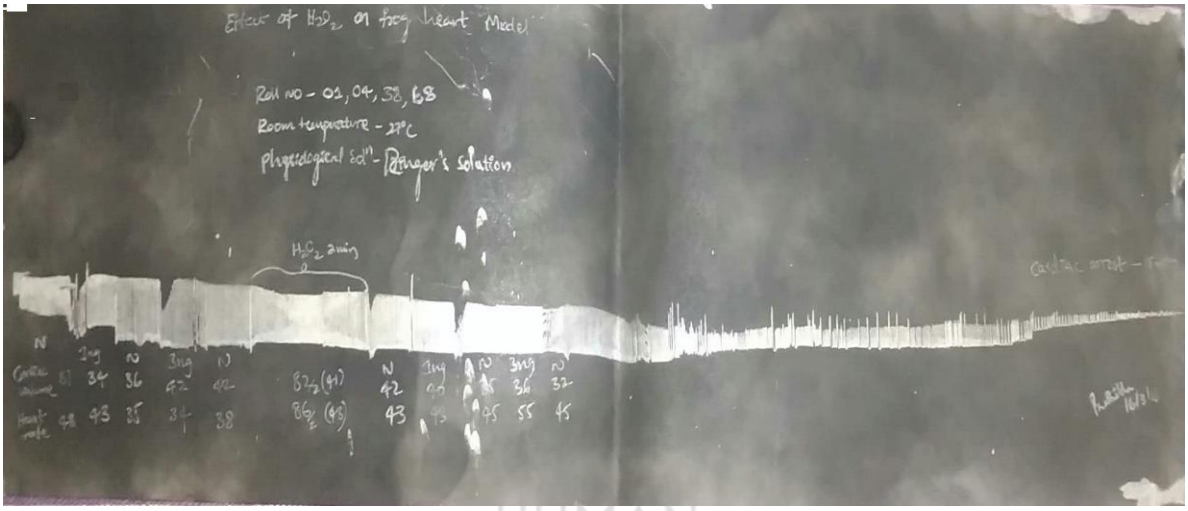

Fig 2: Effect of 1mM H_2O_2 solution Induced Oxidative Stress on Isolated Frog Heart Preparation

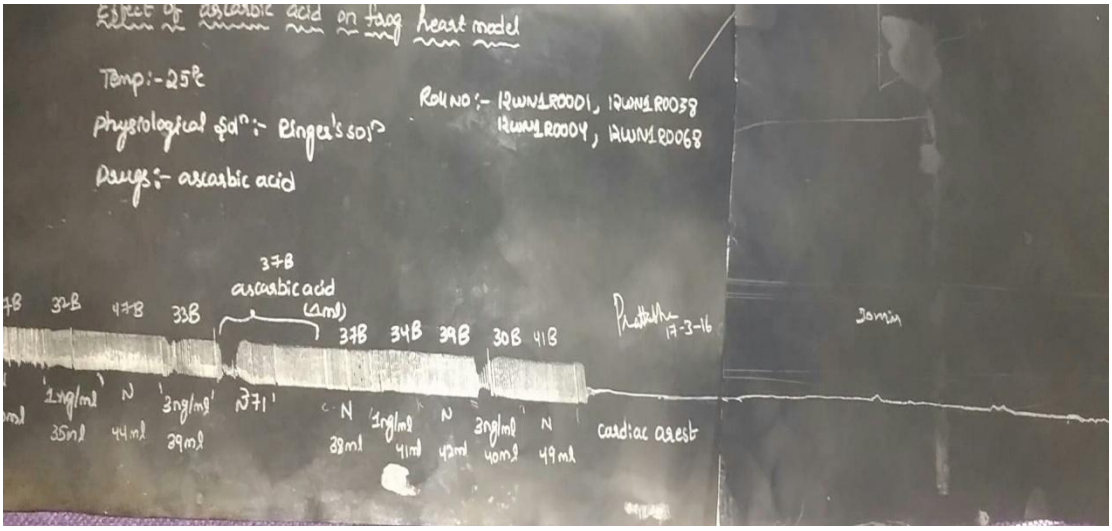

Fig 3: Effect of 3mM Ascorbic Acid solution on Isolated Frog Heart Preparation

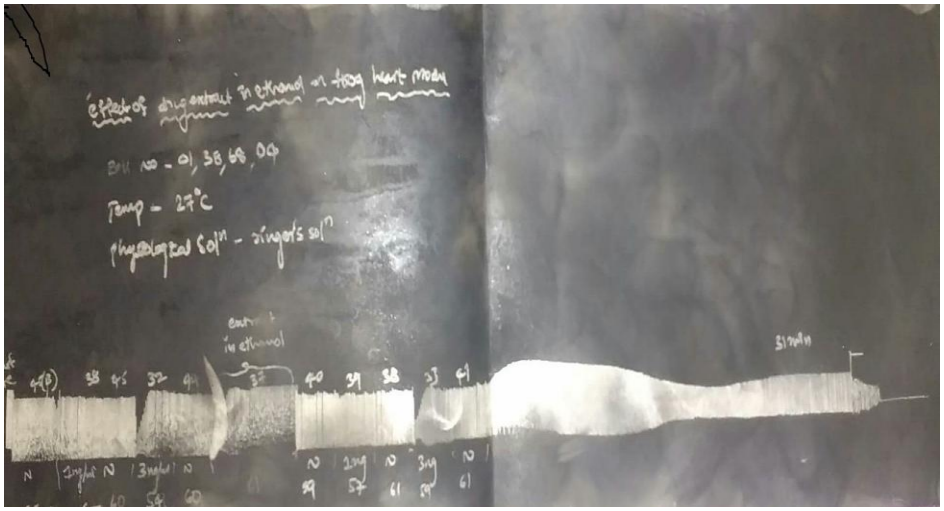

Fig 4: Effect of ethanolic extract of leaves of *Cyanthillium cinereum* on Isolated Frog Heart Preparation

Table 1: Efect of Hydrogen peroxide, Ascorbic acid, and extract on Isolated

Frog Heart Preparation

	Heart Rate (Beats/min)	Cardiac Output(ml)	Cardiac Arrest(min)
Hydrogen peroxide	43	41	16
Ascorbic acid	67	71	30
Leaf extract	67	61	31

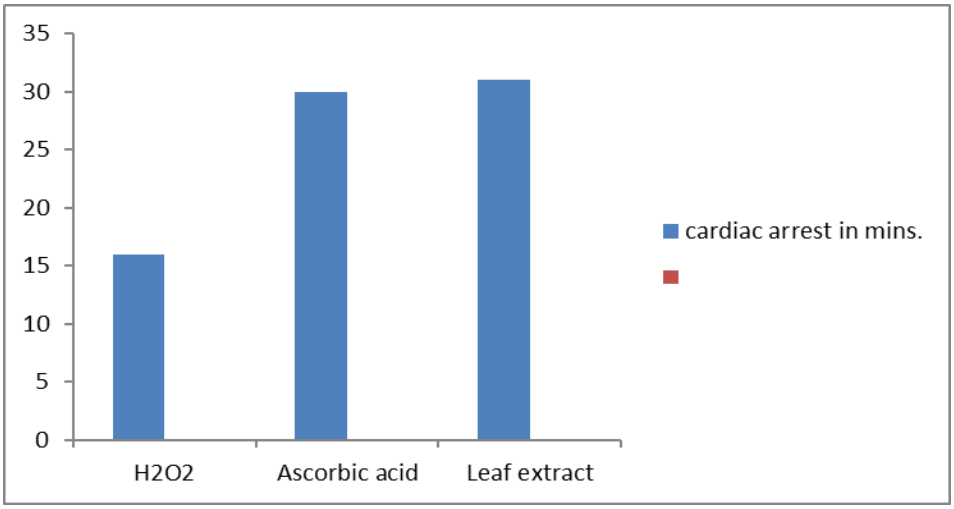

Figure 5: Graphical Representation of Hydrogen peroxide, Ascorbic acid, and extract on cardiac arrest (min)

DISCUSSION

Oxidative stress was induced by hydrogen peroxide (H_2O_2) solution, which shows the ischemic reperfusion injury in the heart, and overload of hydrogen peroxide may exhibit post-ischemic myocardial damage [8]. Earlier reports suggest that oxidative stress or cell damage was induced to the human colon carcinoma cells by exposing hydrogen peroxide at concentrations varying from 0 to 250 µM [4, 10]. By the present results, it was observed that induction of oxidative stress by H_2O_2 solution, the cardiac arrest was observed at the 16^{th} minute. In the presence of the ethanolic extract of leaves of *Cyanthillium cinereum*, the cardiac arrest was observed at 31^{st} minutes i.e. heart was protected longer period that indicates extract showed antioxidant activity which was compared with the standard ascorbic acid.

CONCLUSION

From the above results, the present study was concluded that ethanolic extract of leaves of *Cyanthillium cinereum* exhibits anti-oxidant activity against H_2O_2 induced oxidative stress on isolated frog heart model and compared with a standard antioxidant agent (Ascorbic acid).

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