IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2018 Vol.:12, Issue:1

Antioxidant Activity of *Paspalidium flavidum* Grass Extract by Using Isolated Frog Heart

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Swetha Bindu CH.*, Radhika B, Harika S.

Department of Pharmacology,Vaageswari College of Pharmacy, Karimnagar, Telangana.

Submission: 27 March 2018
Accepted: 03 April 2018
Published: 30 April 2018

Keywords: Frog heart, antioxidant activity, *Paspalidium flavidum* grass, aqueous methanolic extract

ABSTRACT

The present study was aimed to develop a model of isolated frog heart for the induction of oxidative stress by using H_2O_2 and evaluate the antioxidant activity of *Paspalidium flavidum* Linn. grass extract. When ringer solution containing 1mM of H_2O_2 perfused to frog heart preparation, which indicating the induction of oxidative stress on frog heart by H_2O_2 solution, this might be due to destabilization of receptors. It shows negative ionotropic and chronotropic effects and the cardiac arrest was produced at 20^{th} minute. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of an aqueous methanolic extract of *Paspalidium flavidum* grass, the cardiac arrest was observed at 39^{th} minutes i.e. heart was protected longer period that indicates antioxidant activity which was compared with the standard ascorbic acid.

INTRODUCTION

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 cells, tissue or organ that is caused by the reactive oxygen species (ROS) [6]. Reactive oxygen species (ROS) is a term that encompasses all 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 [3]. Antioxidants were synthesized within the body or taken in the diet, which acts as a natural defense against free radical-induced damage [6]. 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 [9].

Herbs and plants play an important role in maintaining human health. *Paspalidium flavidum Linn.*, (Poaceae) commonly known as Madhana Ghas has been largely distributed in tropical Asia, India, China and Pakistan [7]. It is a perennial grass, which is slender, smooth, branched, sharply pointed tip with minute projection. An ethnomedicinal survey it is used to treat Skin diseases, eyes, teeth, heart, skin itching, headache, liver diseases, dropsy (inflammation/swelling), prevent abortion, miscarriage and uterine pains after delivery [1]. Hence there were no reports were available for the antioxidant activity of thequeous methanolic extract of *Paspalidium flavidum* grass by using frog heart model.

MATERIALS AND METHODS

Plant collection and authentication:

The fresh grass of *Paspalidium flavidum* was collected from local areas of Tirupathi, Andhra Pradesh, India and authenticated by Prof. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi. The grass was dried in shade and powdered, passed through sieve no.40. Finally, fine coarse powdered was obtained and stored in airtight container.

Preparation of extract:

Aqueous methanolic extract of the plant (30:70) was prepared by using cold maceration process. The fine coarse powder (500gms) was soaked in 2 liters of an aqueous methanolic mixture for 72hrs at room temperature, with occasional shaking for better extraction. After three days, the whole mixture was filtered by Whatman No.1 filter paper and the filtrate was dried at room temperature. The extract was dark brown in color and soluble in distilled water [1].

Materials: Acetylcholine chloride were purchased from Burgoyne laboratories, Mumbai. NaCl, KCL, Cacl2, Dextrose, NaHCo3 were purchased from Final chemicals, Ahmedabad. Ascorbic acid and hydrogen peroxide (H_2O_2) were purchased from Himedia, Laboratories Ltd., Mumbai, India. Kymograph paper, starlings 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[4].

Isolation of frog heart preparation:

Frogs of Rana tagrina species from the animal house of Vaageswari College of pharmacy, Karimnagar were used for the studies. 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. The 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 out put on rotating drum, to which a smoked kymograph paper was affixed [4,6].

Method:

H_2O_2 induced oxidative stress in isolated frog heart:

- 1mM of the H_2O_2 solution in frog Ringer solution was used to induce oxidative stress in isolated frog heart. Cardiac output, heart rate, and cardiac arrest parameters were estimated. Initially acetylcholine at doses of 10ng, 30ng were showed muscarinic action like negative ionotropic, negative chronotropic and decreased cardiac output. But continuous perfusion of- 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 [5].

- The same dose levels of aqueous methanolic 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 were compared with standard drug ascorbic acid (3mM) [8].

RESULTS

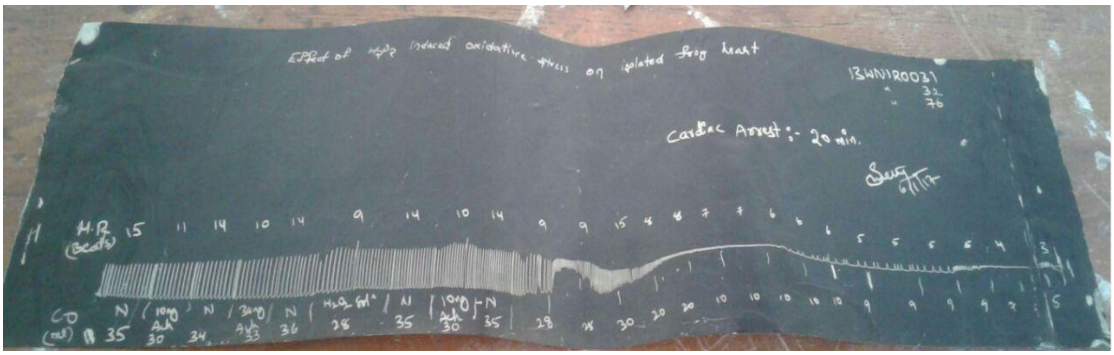

Fig 1: Effect of 1mM H_2O_2 solution Induced Oxidative Stress on Isolated Frog Heart Preparation

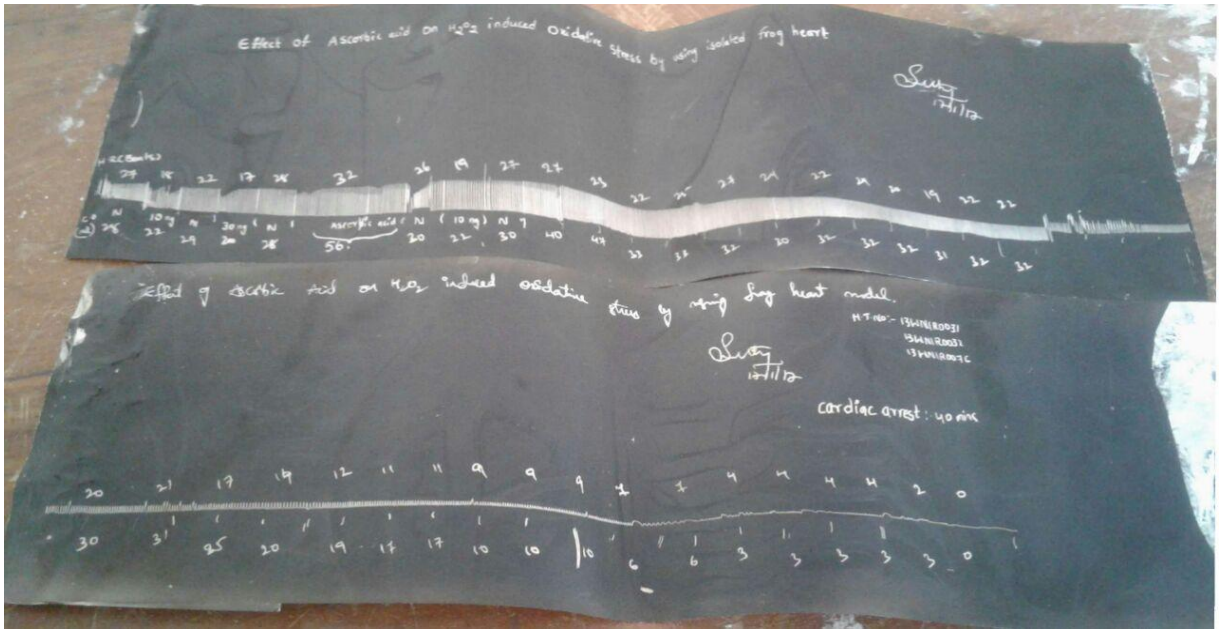

Fig 2: Effect of 3mM Ascorbic Acid solution on Isolated Frog Heart Preparation

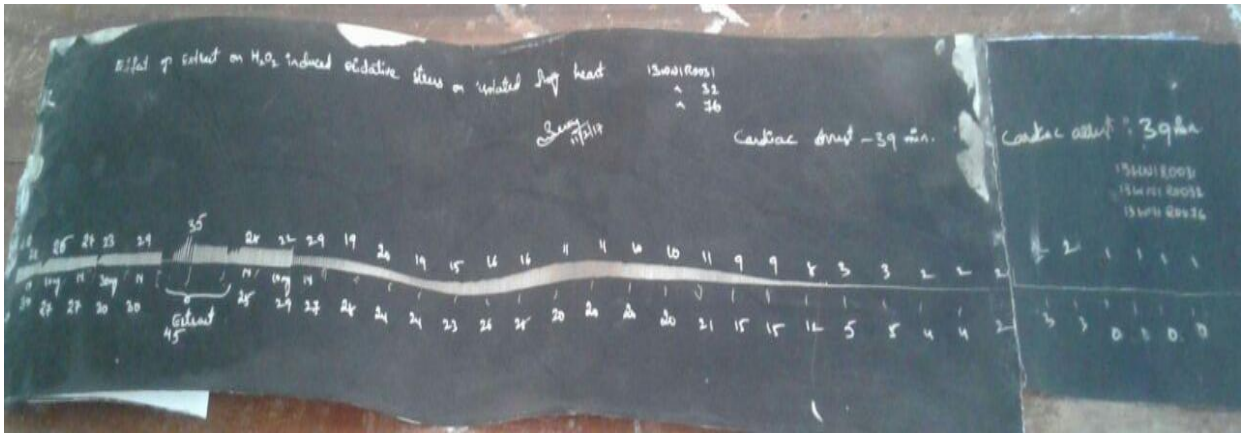

Fig 3: Effect of aqueous methanolic extract of *Paspalidium flavidum* grass on Isolated Frog Heart Preparation

Table 1: Effect of Hydrogen peroxide, Ascorbic acid, and extract on Isolated Frog Heart Preparation

	Heart Rate (Beats/min)	Cardiac Output(ml)	Cardiac Arrest(min)
Hydrogen peroxide	21	28	20
Ascorbic acid	32	56	40
Leaf extract	35	45	39

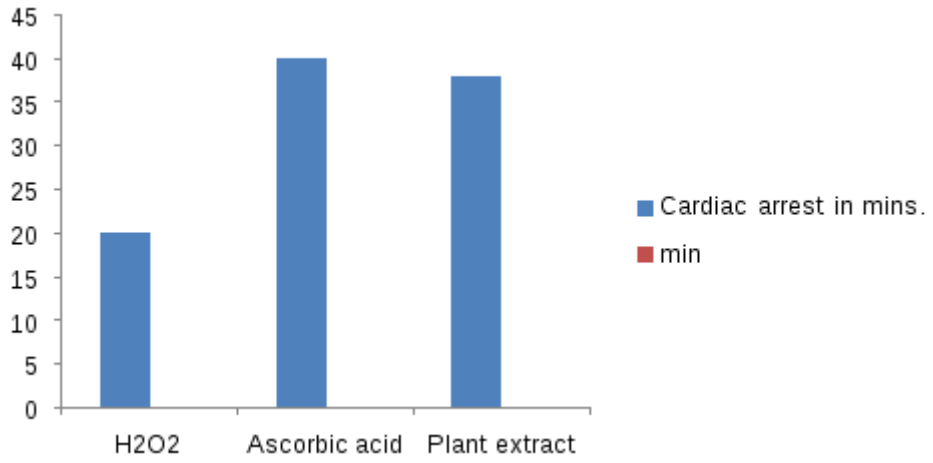

Figure 4: Graphical Representation of Hydrogen peroxide, Ascorbic acid, and extract on cardiac arrest (min)

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 [6]. Earlier reports suggest that oxidative stress or cell damage was induced to the human colon carcinoma cells, Caco-2, cells by exposing hydrogen peroxide at concentrations varying from 0 to 250 µM [2,9]. By the present results, it was observed that induction of oxidative stress by H_2O_2 solution, the cardiac arrest was observed at **20th** minutes.In the presence of an aqueous methanolic extract of *Paspalidium flavidum* grass, the cardiac arrest was observed at **39th** 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 aqueous methanolic extract of *Paspalidium flavidum* grass 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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