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ANTIOXIDANT ACTIVITY OF *BAUHINIA X BLAKEANA LINN.* LEAVES EXTRACT BY USING ISOLATED FROG HEART PREPARATION

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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 Bauhinia X blakeana Linn., leaf 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 desitilization of receptors. It shows negative ionotropic and chronotopic 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 methanolic extract of Bauhinia X blakeana, 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.

KEY WORDS

Frog heart, antioxidant activity, Bauhinia X blakeana Linn., methanolic extract.

INTRODUCTION

Plants play an important role in maintaining human health. *Bauhinia* variety of family Caesalpiniaceae (Fabales) contains 15 species in India. Some of them are bushes or trees while couples are climbers. *Bauhinia x Blackeana* commonly known as Hong Kong orchid tree. It develops around 20 feet tall with a light dark smooth bark and an umbrella-shape propensity [14]. The phytochemical evaluation of Bauhinia blakeana revealed the presence of Alkaloids, Flavonoids, Glycosides, Terpenoids, Anthocyanins, Phytosterols, Tannins, Carbohydrates, Saponins and Phenols [4]. Flavonoids and phenols are strong antioxidants and have an important role in the health care system [3]. According to WHO third world countries depends mainly native medicinal plants for their health purpose. Various part i.e. flowers, buds, stem, roots, bark, seeds, leaves have been used since ancient times for the treatment of a wide range of diseases. The Bauhinia species traditionally used in dysentery, diarrhea, hemorrhoids, piles, edema, laxative, anti-helminthic, astringent, anti-leprotic, wound healing, anti-goitrogenic, anti-tumor, antidote for snake poisoning, dyspepsia, bladder stone, asthma and carminative disease [5,2].

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 which is caused by the reactive oxygen species (ROS) [10]. 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 [7]. Antioxidants were synthesized within the body or taken in the diet which acts as a natural defense against free radical induced damage [10]. 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 [15].

MATERIALS AND METHOD

Plant collection and Authentication:

For the present investigation *Bauhinia X blackeana* leaves were collected in the month of September from Thimmapur village of the Karimnagar district. The plant was identified and authenticated by BSI/DRC/2017-2018/TECH/779. The leaves were dried in shade and stored at 25ºc. It was powdered, passed through sieve no.40 and stored in air tight container.

Preparation of extract:

Methanolic extract of *Bauhinia X blackeana* leaves were prepared by soxhlation method at suitable temperature. 50gms of powdered leaves are prepared as a thimble and placed in the condenser and in the round bottomed flask required amount of methanol was taken. Soxhlation process was carried out for 6-8 hours. The extract obtained was evaporated and dried in desiccator [12].

Materials: Acetyl choline chloride were purchased from Burgoyne laboratories, Mumbai. NaCl, KCl, $Cacl_2$, Dextrose, NaHCo3 were purchased from Finar 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 [8].

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. Frog was stunned by head-blow using a steel rod and pithed. Then frog was placed on frog dissecting board, pin the fore limbs. 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. Heart was then covered with a thin layer of cotton and poured some frog Ringer solution periodically to prevent drying. 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 [8,10].

METHOD:

H_2O_2 induced oxidative stress on isolated frog heart:

- 1mM of 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 10ng, 30ng were showed muscarinic action like negative ionotropic, negative chronotropic and decreased cardiac output. But 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 [9].
- The same dose levels of 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) **[**13].

RESULTS:

Fig1: Effect of 1mM H_2O_2 solution Induced Oxidative Stress on Isolated Frog Heart Preparation

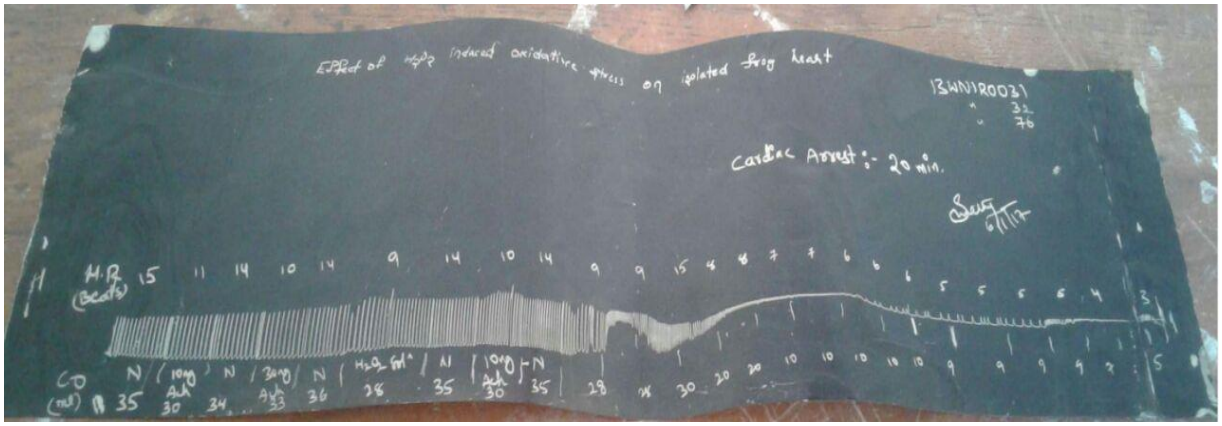


Fig2: Effect of 3mM Ascorbic Acid solution on Isolated Frog Heart Preparation

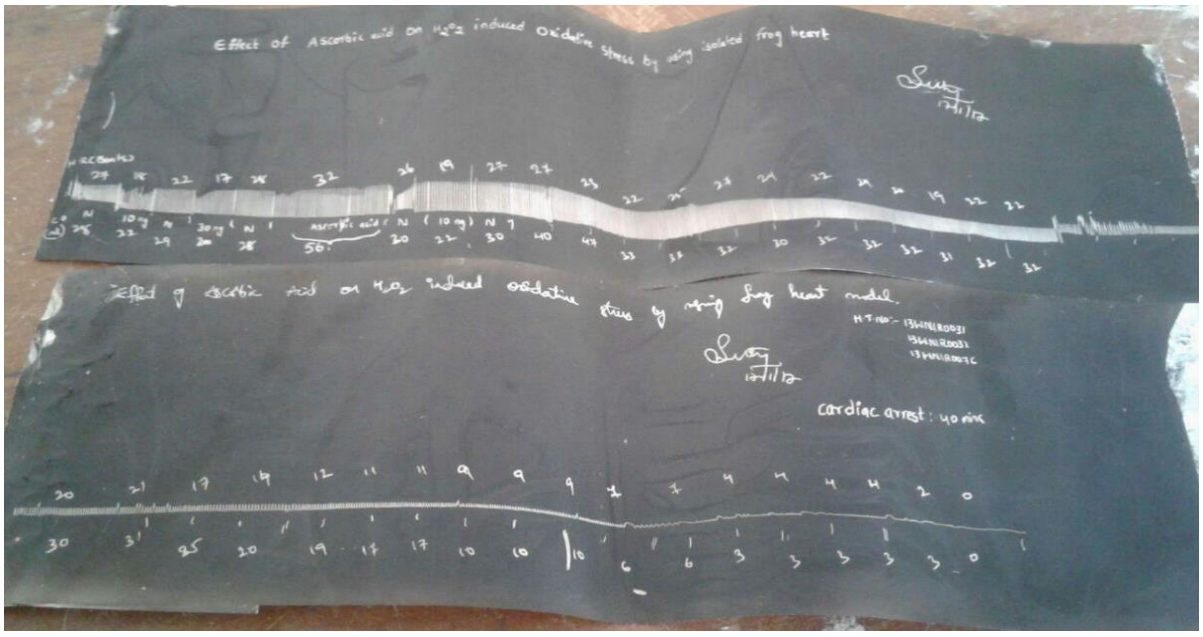
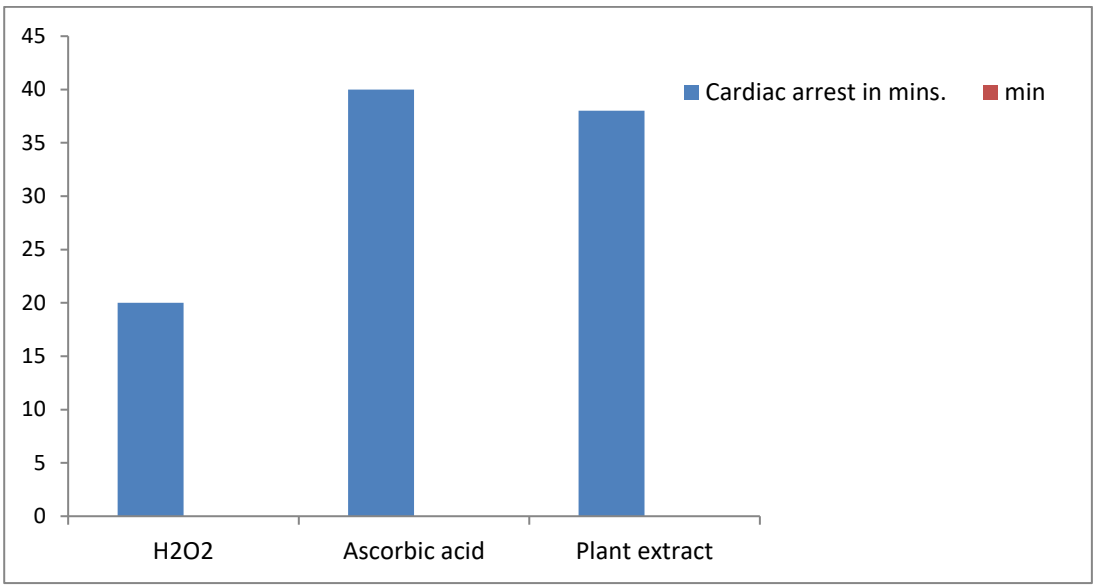


Fig3: Effect of methanolic extract of leaves of *Bauhinia X blackeana* on Isolated Frog Heart Preparation

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	36	49	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 exhibits post-ischemic myocardial damage [10]. 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 [6,15]. By the present results it was observed that induction of oxidative stress by H_2O_2 solution, the cardiac arrest was observed at 20^{th} minutes. In the presence of methanolic extract of *Bauhinia X blackeana*, the cardiac arrest was observed at 39^{th} 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 methanolic extract of leaves of *Bauhinia X blackeana* 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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