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Research Article

ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *LEUCAS ZEYLANICA* LINN. USING ISOLATED FROG HEART

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ABSTRACT

Objective: The present study was aimed to evaluate the antioxidant activity of methanolic extract of the leaves of Leucas zeylanica Linn. using isolated frog heart as a model.

Methods: About 1 mM of hydrogen peroxide (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 desensitization of receptors. Cardiac output, heart rate, and cardiac arrest parameters were estimated.

Results: The present study results supports that the frog heart model for induction of oxidative stress by H_2O_2. It shows negative inotropic and chronotropic effects and the cardiac arrest was produced at 20th min. In the presence of a methanolic extract of the leaves of *L. zeylanica*, the cardiac arrest was observed at 38th min, i.e., heart was protected longer period that indicates antioxidant activity which was compared with the standard ascorbic acid.

Conclusion: The results obtained in this work showed that methanolic extract of the leaves of *L. zeylanica* exhibits antioxidant activity against H_2O_2-induced oxidative stress on isolated frog heart model and compared with a standard antioxidant agent (ascorbic acid).

Keywords: Frog heart, Antioxidant activity, *Leucas zeylanica*, Methanolic extract, Oxidative stress.

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 which is caused by the reactive oxygen species (ROS) [13]. ROS are highly reactive substances, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, superoxide anion radical, hydrogen peroxide (H_2O_2), 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 [10]. Antioxidants were synthesized within the body or taken in the diet which acts as a natural defense against free radical-induced damage [13]. The oxidative stress in animals or cell cultures has been successfully induced by H_2O_2 and was chosen for the induction of oxidative stress on isolated frog heart [17].

Herbs and plants play an important role in maintaining human health. *Leucas zeylanica* belongs to the family Lamiaceae commonly called as Ceylon slitwort [9]. Synonyms are *Leucas bancana*, *Phlomis zeylanica* Linn., and *Spermacoce denticulate*. In Telugu, it is commonly known as Thummi [7,8]. It is a small, earthy, non-woody, annual erect plant or sometimes tufted, hispid, and aromatic plant growing to a height of up to 120 cm, stipules absent. Stem is green in color. Leaves are oval in shape and green in color, which occur opposite sides of stems and large in number. These are subsessile leaves which are liner lanceolate or elliptic-lanceolate which is 2.5–7.5 cm long. Roots are mainly taproot and fibrous. In India, leaves and flowers were used for fever, scorpion, snakebites, and jaundice. In Sri Lanka, mostly used as a vermifuge ingredient and also used for anorexia, flatulence, colic pain, malaria, mild fevers associated with indigestion, and intestinal worms infection [5,6]. The phytochemical evaluation of the methanolic extract of the leaves of *L. zeylanica* revealed the presence of alkaloids, flavonoids, glycosides, tannins, carbohydrates, saponins, and phenols [3]. Flavonoids and phenols are strong antioxidants and have an important role in the health-care system [2]. Hence, there were no reports available for the antioxidant activity of methanolic extract of the leaves of *L. zeylanica* using frog heart model.

MATERIALS AND METHODS

Plant collection and authentication

The fresh leaves of *L. zeylanica* were collected from local areas of the Karimnagar district, Telangana, India. The plant was identified and authenticated by BSI/DRC/16-17/Tech.05. The leaves were dried in shade and powdered, passed through sieve no.40. Finally, fine coarse powdered was obtained and stored in air-tight container.

Preparation of extract

Methanolic extract of the leaves of *L. zeylanica* was prepared by soxhlation method at suitable temperature. 50 g of powdered leaves were 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 h. The extract obtained was evaporated and dried in desiccator [15].

Materials

Acetyl choline chloride was purchased from Burgoyne Laboratories, Mumbai. Nacl, Kcl, $Cacl_2$, dextrose, and NaHCo3 were purchased from Finar Chemicals, Ahmedabad. Ascorbic acid and H_2O_2 were purchased from Hi-Media, 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 Ringer's solution is Nacl - 6 g, Kcl - 0.14 g, Cacl2 - 0.12 g, NaHco3 - 0.2 g, and glucose - 2 g made with 1000 ml distilled water [11].

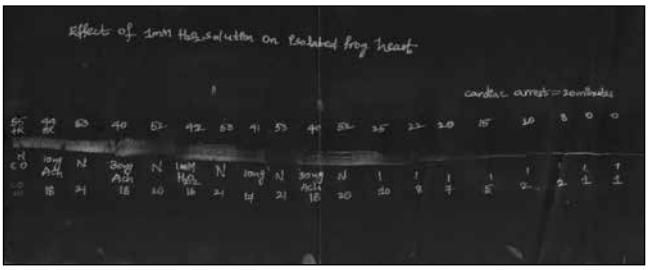

Fig. 1: Effect of 1 mM hydrogen peroxide solution-induced oxidative stress on isolated frog heart preparation

Fig. 2: Effect of 3 mM ascorbic acid solution on isolated frog heart preparation

Isolation of frog heart preparation

Frogs of *Rana tigrina* 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 forelimbs. The skin and abdomen were cut and opened. The pectoral girdle was cut using a bone cutter and removed the pericardium carefully. Introduce the Symes 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 Symes 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 [11,13].

Methods

H_2O_2-induced oxidative stress on isolated frog heart

- About 1 mM 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 10 ng and 30 ng was showed muscarinic action such as negative ionotropic, negative chronotropic, and decreased cardiac output. However, continuous perfusion of frog Ringer solution containing H_2O_2, the muscarinic actions were not observed which indicate the damage of muscarinic receptors due to oxidative stress induced by H_2O_2 [12].
- 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 was compared with standard drug ascorbic acid (3 mM) [16].

DISCUSSION

Oxidative stress was induced by H_2O_2 solution which shows the ischemic reperfusion injury in the heart and overload of H_2O_2 may exhibit post-ischemic myocardial damage [13]. Earlier reports suggest that oxidative stress or cell damage was induced to the human colon carcinoma cells, Caco-2, cells by exposing H_2O_2 at concentrations varying from 0 to 250 µM [4,17]. By the present results, it was observed that induction of oxidative stress by H_2O_2 solution, the cardiac arrest was observed at 20th min. In the presence of a methanolic extract of the leaves of *L. zeylanica*, the cardiac arrest was observed at 38th min, i.e., heart was protected longer period that indicates extract showed antioxidant activity which was compared with the standard ascorbic acid.

Fig. 3: Effect of methanolic extract of the leaves of *Leucas zeylanica* on isolated frog heart preparation

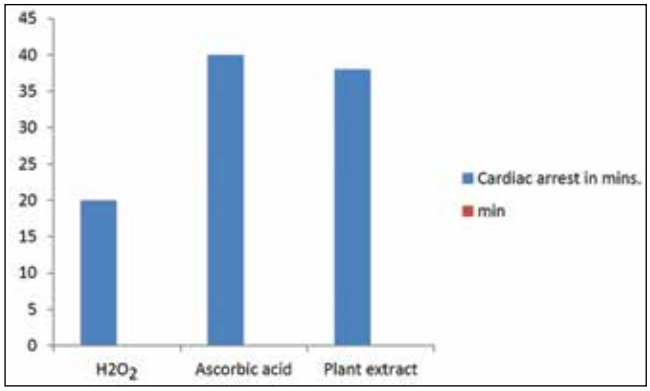

Fig. 4: Graphical representation of hydrogen peroxide, ascorbic acid, and extract on cardiac arrest (min)

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	42	16	20
Ascorbic acid	66	30	40
Leaf extract	60	35	38

CONCLUSION

From the above results, the present study was concluded that methanolic extract of the leaves of *L. zeylanica* exhibits antioxidant 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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