

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

Swetha Bindu CH.*

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

Article Received on
23 May 2018,
Revised on 13 June 2018,
Accepted on 03 July 2018
DOI: 10.20959/wjpr201814-12844

*Corresponding Author

Swetha Bindu CH.

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

ABSTRACT

The present study was aimed for the induction of oxidative stress by using H₂O₂ on isolated frog heart and evaluates the antioxidant activity of methanolic extract of leaves of *Leucas zeylanica* Linn. When frog ringer solution containing 1mM of H₂O₂ perfused to frog heart preparation, which indicating the induction of oxidative stress on frog heart by H₂O₂ solution, this might be due to desensitization of receptors. By using H₂O₂, shows negative inotropic and chronotropic effects and the cardiac arrest was produced at 20th minute. This result supports the frog heart model for induction of oxidative stress by H₂O₂. In the presence of methanolic extract of leaves of *Leucas zeylanica*, the cardiac arrest was observed at 38th minutes i.e. heart was protected

longer period that indicates antioxidant activity which was compared with the standard ascorbic acid. In the presence of ascorbic acid cardiac arrest was observed at 40th minute.

KEYWORDS: Frog heart, antioxidant activity, oxidative stress *Leucas zeylanica*, methanolic extract.

INTRODUCTION

Herbs and plants play an important role in maintaining human health. *Leucas zeylanica* belongs to the family Lamiaceae commonly called as ceylon slitwort.^[7] Synonyms are *Leucas bancana*, *Phlomis zeylanica* Linn, *Spermacoce denticulate*. In telugu it is commonly known as thummi.^[5,6] 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 sub sessile leaves which are linear lanceolate or elliptic lanceolate

which is 2.5 to 7.5 cm long. Roots are mainly tap root and fibrous. In India leaves and flowers were used for fever, scorpion, snake bites 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, intestinal worms infection.^[3,4] The phytochemical evaluation of methanolic extract of leaves of *Leucas zeylanica* revealed the presence of Alkaloids, Flavonoids, Glycosides, Tannins, Carbohydrates, Saponins and Phenols.^[2] Flavonoids and phenols are strong antioxidants and have an important role in the health care system.^[1]

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).^[12] 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.^[9] Antioxidants were synthesized within the body or taken in the diet which acts as a natural defense against free radical induced damage.^[12] 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

The fresh leaves of *Leucas zeylanica* was 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 leaves of *Leucas zeylanica* were prepared by soxhlation method at suitable temperature. 50gms 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 hours. The extract obtained was evaporated and dried in desiccators.^[13]

Materials

Acetylcholine chloride was purchased from Burgoyne laboratories, Mumbai. NaCl, KCl, CaCl₂, Dextrose, NaHCO₃ were purchased from Finar chemicals, Ahmedabad. Ascorbic acid and hydrogen peroxide (H₂O₂) 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.^[10]

Isolation of frog heart preparation

Frogs of *Rana tagrina* species from the animal house of Vaageswari College of pharmacy, Karimnagar were used for this study. Approval no. is 1720/PO/a/13/CPCSEA. 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 venous 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.^[10,12]

METHOD

H₂O₂ induced oxidative stress on isolated frog heart

- 1mM of H₂O₂ 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 inotropic, negative chronotropic and decreased cardiac output. But continuous perfusions of frog Ringer solution containing H₂O₂, the muscarinic actions

were not observed which indicates the damage of muscarinic receptors due to oxidative stress induced by H_2O_2 .^[11]

- 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).^[14]

RESULTS

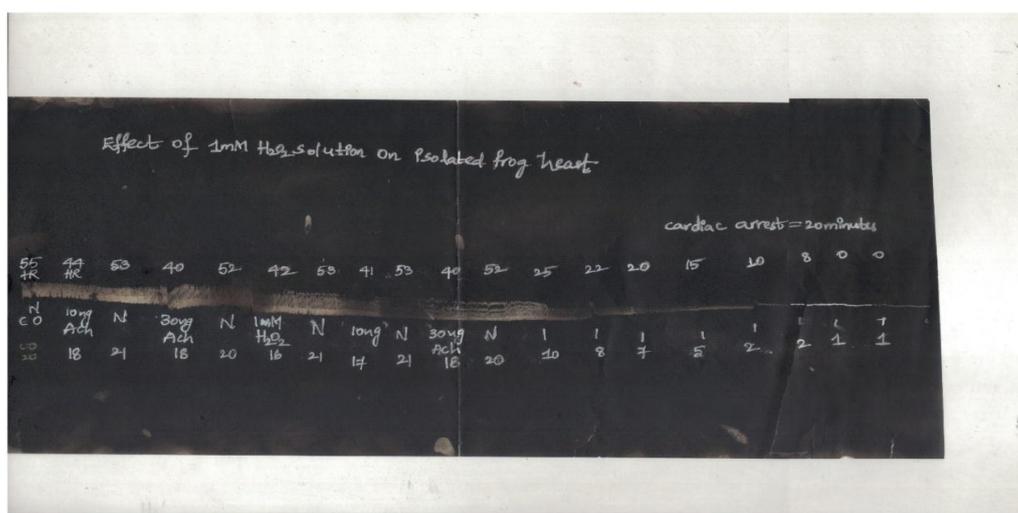


Fig. 1: Effect of 1mM H_2O_2 solution Induced Oxidative Stress on Isolated Frog Heart Preparation.

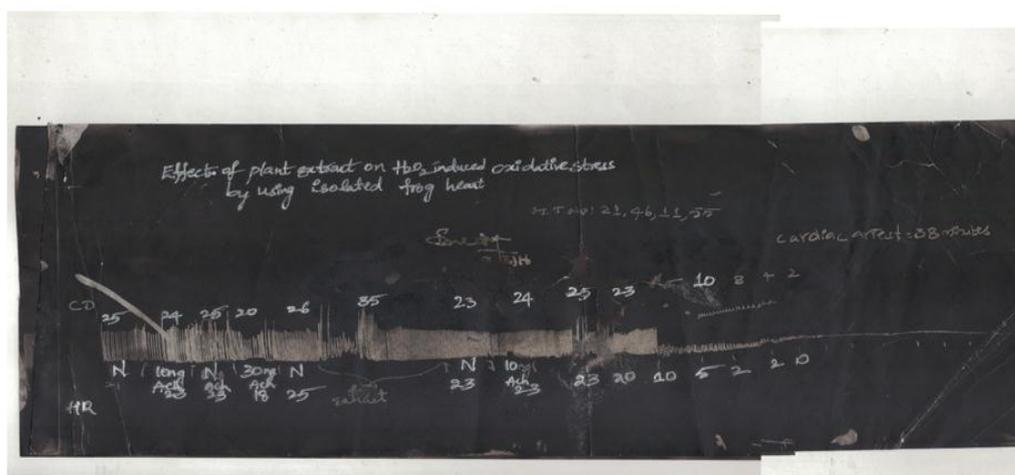


Fig. 2: Effect of methanolic extract of leaves of *Leucas zeylanica* on Isolated Frog Heart Preparation.

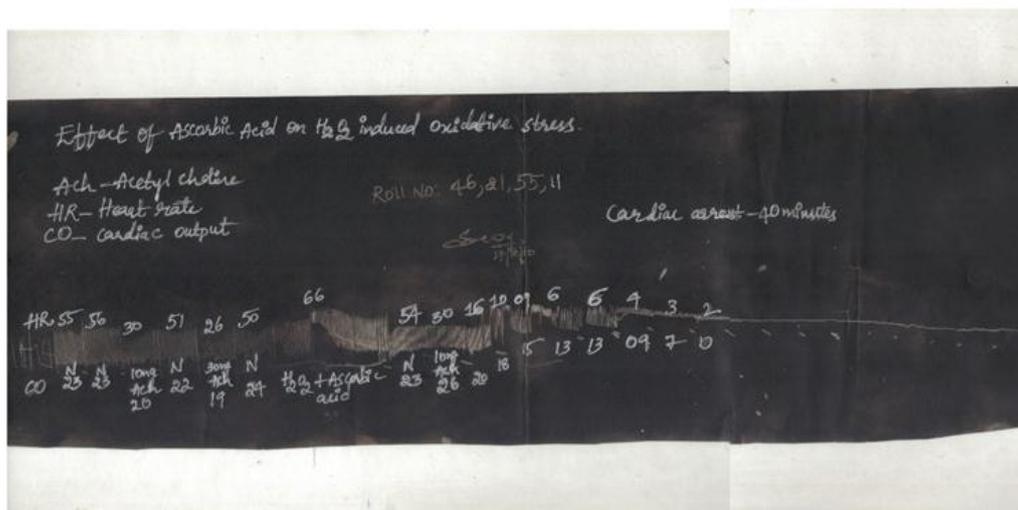


Fig. 3: Effect of 3mM Ascorbic Acid solution on Isolated Frog Heart Preparation.

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

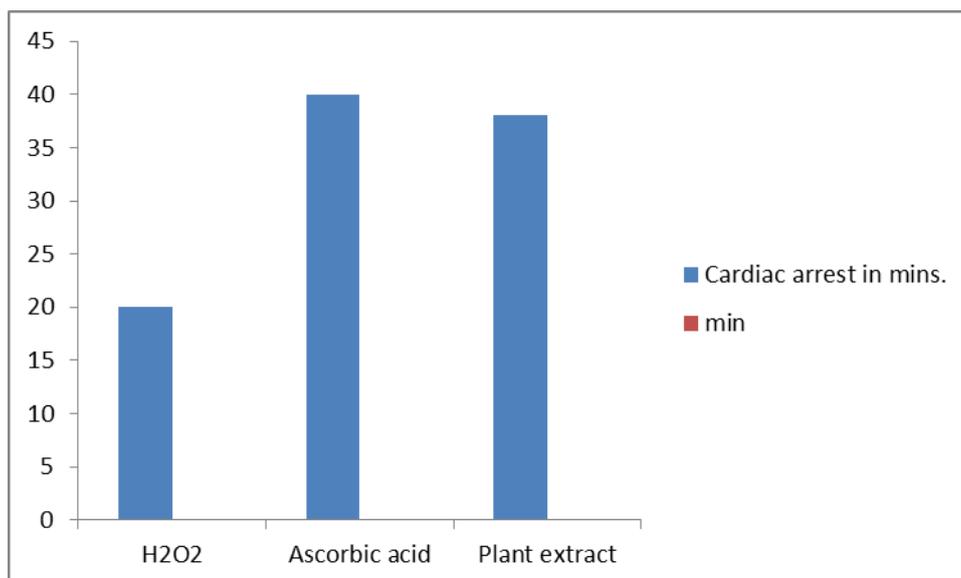


Figure 4: Graphical Representation of Hydrogen peroxide, Ascorbic acid and extract on cardiac arrest (min).

DISCUSSION

Oxidative stress was induced by hydrogen peroxide (H₂O₂) solution which shows the ischemic reperfusion injury in the heart and overload of hydrogen peroxide may exhibits post-ischemic myocardial damage.^[12] Earlier reports suggests 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.^[8,15] By the present results it was observed that induction of oxidative stress by H₂O₂ solution, the cardiac arrest was observed at 20th minutes. In the presence of methanolic extract of leaves of *Leucas zeylanica*, the cardiac arrest was observed at 38th 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 *Leucas zeylanica* exhibits anti-oxidant activity against H₂O₂ induced oxidative stress on isolated frog heart model and compared with a standard antioxidant agent (Ascorbic acid).

ACKNOWLEDGMENTS

The author has grateful to the authorities of Vaageswari College of pharmacy, Thimmapur, Karimnagar for the facilities. The author has no conflict of interest.

REFERENCES

1. Dhan Prakash, Samikasha Suri, Garima Upadhyay and Singh BN. Total phenol, antioxidant and free radical Scavenging activities of some medicinal plants. International Journal of food Science and Nutrition, 2007; 58(1): 18-28.
2. Geethika K, Sunoj Kumar P. Preliminary Phytochemical Screening of 6 Members of *Leucas* (Lamiaceae). International Journal of Pharmaceutical Sciences Review and Research, 2017; 47(1): 60-4.
3. <http://www.Oswaldasias.org>. *Leucas zeylanica* (L) R.Br.- Lamiaceae- Dicotyledon.
4. <http://www.Efloras.org>. *Leucas zeylanica* flora of china.
5. <http://www.Tropicos.org> *Leucas zeylanica* list.
6. <http://www.Ipni.org> *Leucas zeylanica* list.
7. <http://www.Virboga.de> *Leucas zeylanica*.
8. Kazuo Yamamoto, Ryoji Kushima, Osamu Kasaki, Yoshihide Fujiyama and Hidetoshi Okabe. Combined effect of hydrogen peroxide induced oxidative stress and IL-1α on IL-8

- production in CaCo-2 cells (a Human colon carcinoma cell line) and normal intestinal epithelial cells. *Journal of inflammation*, 2003; 27: 123-28.
9. Mustafa Taha Mohammed, Sura Mohammed Kadhim, Abdulkadir Mohammed Noori Jassimand and Sarah Isam Abbas. Free Radicals and Human Health. *International Journal of Innovation Sciences and research*, 2015; 4(6): 218-23.
 10. Prabhakar MC. Experiments on frogs in *Experimental pharmacology for undergraduates* 1st ed., India, 2007; 52-8.
 11. Prasad N, Rama Narsimha Reddy A, Venkatesham A, Krishna DR and Prabhakara MC. Antioxidant Activity of Animal Bile Using Frog Heart As A Model. *Pharmacology online*, 2008; 2: 390-96.
 12. Praveen Kumar P, Satyavathi K, Prabhakar Mc. Antioxidant Activity of Traditionally Used *Backyard* Indian Medicinal Plants Using Frog Heart As A Model. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2010; 1(3): 131-40.
 13. Radhika B. Comparitive Study of Soxhlation and Maceration Extracts of *Tabernaemontana Divaricta* Leaves for Antibacterial Activity. *Journal of natural products and plant resources*, 2017; 7(2): 34-9.
 14. Rajyalakshmi G, Radhika T and Prasad N. Antioxidant Activity Of *Red Kino* Tree Using Frog Heart Model. *Pharmacology online*, 2008; 3: 26-31.
 15. Subhashinee S. Wijeratne K, Susan L. Cuppett, and Vicky Schlegel. Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells. *Journal of Agriculture Food Chemistry*, 2005; 53(22): 8768-75.