

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

SWETHA BINDU CH\*

Department of Pharmacology, Vaageswari College of Pharmacy, Karimnagar, Telangana, India. Email: swethabindu225@gmail.com

Received: 8 December 2018, Revised and Accepted: 12 February 2019

### 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 20<sup>th</sup> min. In the presence of a methanolic extract of the leaves of *L. zeylanica*, the cardiac arrest was observed at 38<sup>th</sup> 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 sessile leaves which are linear 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  $NaHCO_3$  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,  $CaCl_2$  - 0.12 g,  $NaHCO_3$  - 0.2 g, and glucose - 2 g made with 1000 ml distilled water [11].



- 1 normal intestinal epithelial cells. J Inflamm 2003;27:123-8.
- 2 **AQ1** 5. *Leucas zeylanica* (L) R.Br.-Lamiaceae-Dicotyledon. Available from: 1
- 3 <http://www.Oswaldasia.org>. 2
- 4 **AQ1** 6. *Leucas zeylanica* Flora of China. Available from: <http://www.Efloras.org>. 3
- 5 7. *Leucas zeylanica* List. Available from: <http://www.Tropicos.org>. 4
- 6 **AQ1** 8. *Leucas zeylanica* List. Available from: <http://www.Ipni.org>. 5
- 7 9. *Leucas zeylanica*. Available from: <http://www.Virboga.de>. 6
- 8 10. Mohammed MT, Kadhim SM, Jassimand AM, Abbas SI. Free radicals and human health. Int J Innov Sci Res 2015;4:218-23. 7
- 9 11. Prabhakar MC. Experiments on frogs. In: Experimental Pharmacology 8
- 10 for Undergraduates. 1<sup>st</sup> ed. India: Orient Longman; 2007. p. 52-8. 9
- 11 12. Prasad N, Reddy AR, Venkatesham A, Krishna DR, Prabhakara MC. 10
- 12 Antioxidant activity of animal bile using frog heart as a model. 11
- 13 Pharmacologyonline 2008;2:390-6. 12
- 14 13. Kumar PP, Satyavathi K, Prabhakar MC. Antioxidant activity of 13
- 15 traditionally used backyard Indian medicinal plants using frog heart as 14
- 16 a model. Res J Pharm Biol Chem Sci 2010;1:131. 15
- 17 14. Rabia N, Sabila B, Azra N, Rahmatullah Q, Rabia A, Khaliq C, *et al*. 16
- 18 Wee flora of pir mehr ali shah arid agriculture university rawalpindi: 17
- 19 Winter aspect. Pak J Weed Sci Res 2008;14:255-72. 18
- 20 15. Radhika B. Comparitive study of soxhlation and maceration extracts of 19
- 21 *Tabernaemontana divaricta* Leaves for antibacterial activity. J Nat Prod 20
- 22 Plant Resour 2017;7:34-9. 21
- 23 16. Rajyalakshmi G, Radhika T, Prasad N. Antioxidant activity of red kino 22
- 24 tree using frog heart model. Pharmacologyonline 2008;3:26-31. 23
- 25 17. Wijeratne KS, Cuppett SL, Schlegel V. Hydrogen peroxide induced 24
- 26 oxidative stress damage and antioxidant enzyme response in caco-2 25
- 27 human colon cells. J Agric Food Chem 2005;53:8768-75. 26
- 28 27 27
- 29 28 28
- 30 29 29
- 31 30 30
- 32 31 31
- 33 32 32
- 34 33 33
- 35 34 34
- 36 35 35
- 37 36 36
- 38 37 37
- 39 38 38
- 40 39 39
- 41 40 40
- 42 41 41
- 43 42 42
- 44 43 43
- 45 44 44
- 46 45 45
- 47 46 46
- 48 47 47
- 49 48 48
- 50 49 49
- 51 50 50
- 52 51 51
- 53 52 52
- 54 53 53
- 55 54 54
- 56 55 55
- 57 56 56
- 58 57 57
- 59 58 58
- 60 59 59
- 61 60 60
- 62 61 61
- 63 62 62
- 64 63 63
- 65 64 64
- 66 65 65
- 67 66 66
- 68 67 67
- 69 68 68
- 69 69 69

#### Author Queries???

AQ1:Kindly provide last accessed details

AQ2:Kindly review the sentence.

AQ3:Kindly cite table 1 in the text part

AQ4:Kindly provide column head

AQ5:Kindly cite figures 1-4 in the text part

AQ6:Kindly cite reference 1 and 14 in the text part and also cite all references in chronological order