ANTIOXIDANT ACTIVITY OF RED KINO TREE USING FROG HEART MODEL

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Summary

The present study was aimed at evaluation of antioxidant activity of methanolic extract of medicinally important red kino tree bark using 1mM H_2O_2 on isolated frog heart preparation. Induction of cardiac arrest was used as a end point in this method, which was at 15th and 29th minutes respectively when perfusing frog Ringer solution containing hydrogen peroxide and methanol extract to isolated frog heart. In the presence of animal bile, the cardiac muscle was protected up to another 14 minutes than control, indicated its antioxidant activity which was comparable with standard ascorbic acid.

Keywords: red kino tree, antioxidant activity, frog heart model

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Introduction

Pterocarpus marsupium Roxb., popularly known, as "red kino tree" a member of the Papilionaceae family, is a widely used medicinal species useful in the treatment of diabeties, dysentery, stomachache, elephantiasis, leucoderma, cholera, urinary complaints and cough [1]. The gum obtained from the stem is used as astringent, in diarrhea and for toothache and the leaves are useful as external applications for boils, sores and skin diseases [2]. Heart wood of *Pterocarpus marsupium* has been used in ayurvedic medicine for centuries for its anti-heperglycemic activity. Earlier studies reported that the phenolic constituents of *Pterocarpus marsupium* significantly lowered the blood glucose level in diabetic rats [3]. The flowers are used in fever, and the gum is locally applied in leucorrhoea and passive hemorrhage [4]. Oxidative Stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species (ROS). ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them [5]. The effects of free radicals are expressed by the accumulation of oxidative damage to biomolecules: nucleic acids, lipids and proteins [6]. So far, no reports are available for the antioxidant activity of methanolic extract of *P. marsupium* using frog heart model.

Methods

Materials: Acetylcholine bromide, CaCl2 and dextrose purified were purchased from Loba chemicals Pvt. Ltd. Mumbai, India. NaCl, KCl and $NaHCO_3$ acids were purchased from S.D. Fine Chemicals, Mumbai, India. Ascorbic acid and hydrogen peroxide (H_2O_2) were purchased from Himedia, Laboratories Ltd., Mumbai.

Kymograph: Starlings heart lever and kymograph (Inco, Ambala, India) were used to record the responses of acetylcholine, hydrogen peroxide on smoked paper.

Physiological solution: Frogs Ringer solution was used as a perfusion solution to maintain the rhythm of the isolated tissue. The composition of Ringer solution includes NaCl (9 g L), KCl (0.42 g/L), $CaCl_2$ (0.24 g/L), dextrose (1.0 g/L) and $NaHCO_3$ (0.5 g/L). 1 litre of Ringer solution was diluted to 1.4 liter with distilled water forms frog's Ringer solution [7].

Plant material collection: The bark of *Pterocarpus marsupium* was collected from forest office, Warangal, Andhra Pradesh, India.

Extract preparation: The material was air dried under shade and cut into small pieces and macerated with methanol for one week. The extracts were vacum dried and stored in refrigerator until further use.

Isolated frog heart preparation using symes technique:

Isolation of frog heart was done by standard procedure [7]. Briefly an Indian frog (*Rana tigrina*) was stunned and abdomen were cut and opened. The pectoral girdle was cut using a bone cutter and removed the pericardium carefully. Syme's cannula was connected to the reservoir containing frog Ringers solution and introduced 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 connected to the Starling 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. The heart was allowed to stabilize and when the heart rate and cardiac out put were taken, the recordings were made on a slow rotating drum, to which a sooted kymograph paper was affixed. The study protocol was approved by Institutional Animal Ethical Committee, UCPSc, Kakatiya University, Warangal, India.

Effect of plant extraction on H_2O_2 induced oxidative stress:

To induce oxidative stress on isolated frog heart, 1mM of H_2O_2 in Ringer solution was used **[**8]**.** Influence of plant methanol extract on oxidative stress was studied by perfusing frog Ringer solution containing plant extract and H_2O_2 solution to the isolated frog heart preparation. The parameters studied include force of contraction, heart rate and cardiac output (n=6). Then time taken for induction of cardiac arrest was noted by continuous perfusion of frog Ringer solution containing plant extract and is compared with that of control (H_2O_2) and standard ascorbic acid (3mM).

Results

The effect of 1 mM H_2O_2 solution on isolated heart was showed in figure 1.

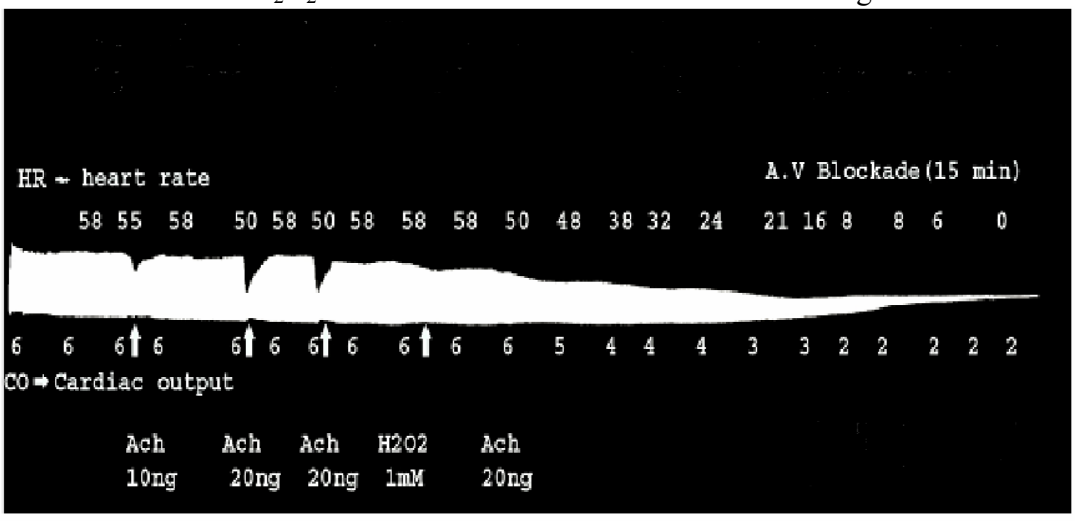

Figure 1: Effect of 1 mM H_2O_2 solution on isolated heart preparation

The effects of bark extract and ascorbic acid on isolated heart were showed in figure 2 and 3.

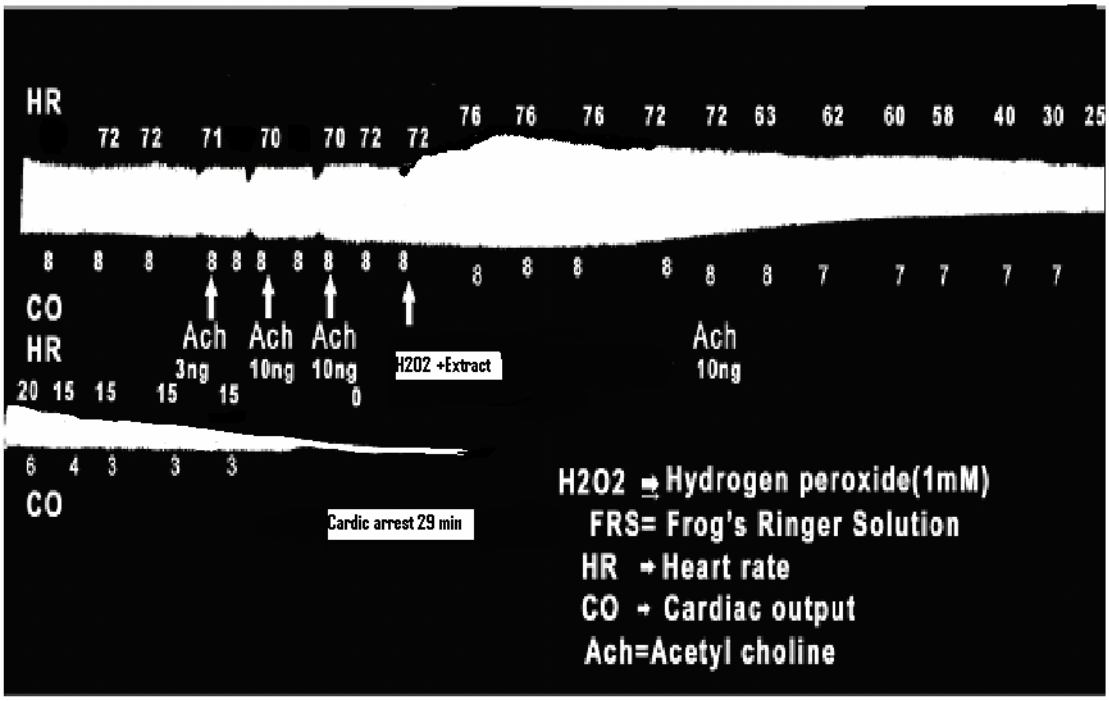

Figure 2: Influence of *P. marsupium bark* extract on H_2O_2 induced oxidative stress

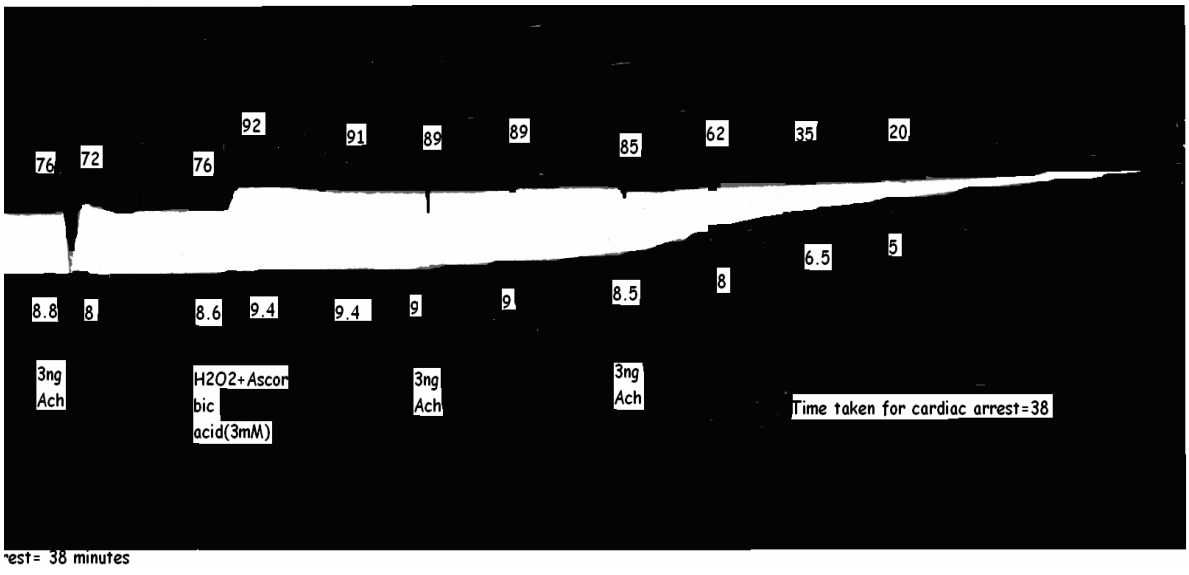

Figure 3: Influence of ascorbic acid on H_2O_2 induced oxidative stress

Upon perfusion of frogs Ringer solution containing H_2O_2 to the heart preparation, the cardiac arrest was observed at 15th minute and was taken as a control (n=6). The influence of plant extract on H_2O_2 induced oxidative stress was showed in figures 2. Initially there was a slight increase in force of contraction and heart rate, but no significant change in cardiac output was observed with plant extract. Upon continuous perfusion of extract and ascorbic acid, the cardiac arrest was observed at 29 and 38 minute respectively (n=6).

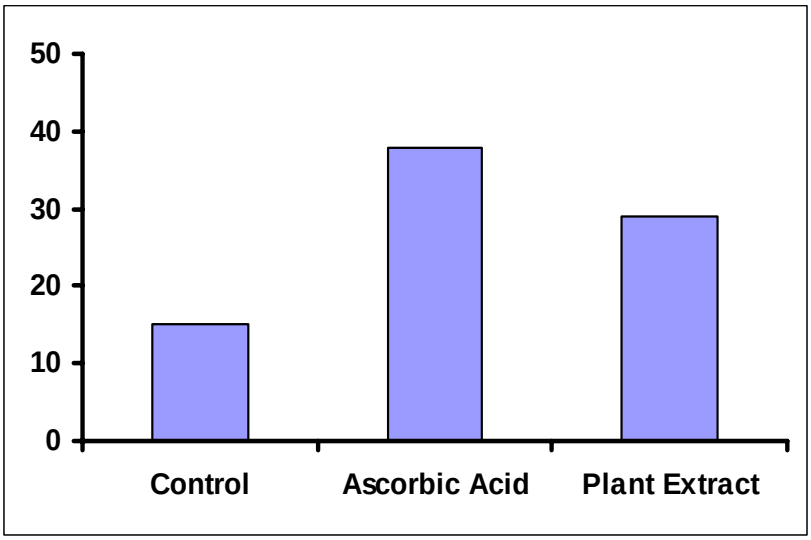

Figure 4: Comparison of antioxidant activity of animal bile with ascorbic acid.

Discussion

Antioxidant activity of animal bile was estimated by induction of oxidative stress using 1mM H_2O_2 on isolated frog heart preparation [8]. Earlier reports suggests 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 [9,10]. In the present study we estimated antioxidant activity of bark tree extract using H_2O_2 model on frog heart. The cardiac arrest time was prolonged by 14 minutes in presence of extract, i.e. heart was protected longer period with plant extract against H_2O_2 induced oxidative stress when compared with the control. This indicates the antioxidant activity of *P. marsupium bark* extract which was comparable with ascorbic acid.

In conclusion, the present study investigation supported the antioxidant activity *Pterocarpus marsupium* extract against H_2O_2 model on isolated frog heart which was comparable with ascorbic acid.

References

1. Tiwari, S., Pankaj, S. and Kanchan, S**.** *In vitro* propagation of *Pterocarpus marsupium* Roxb.An endangered medicinal tree. Indian J. Biotech, 2004; 3: 422-425.
2. Mankani, K.L., Krishna, V., Manjunatha, B.K., Vidya, S.M., Jagadeesh singh, S.D., Manohara, Y.N., Raheem, A.U. and Avinash, K.R. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. Indian J Pharmacol,2005; 37(3): 65-168.
3. Manikam, M., Ramanathan, M., Jahromi, M.A., Chansouria, J.P. and Ray, A,B Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat Prod ,1997; 60: 609-610.
4. Pullaiah, T. Medicinal plants of Andhra Pradesh (India). New Delhi: Regency Publications, 1999.
5. Hayes, J.D. & McLellan LI, Glutathione and glutathione-dependent enzymes represent a co-coordinately regulated defense against oxidative stress. *Free Radic. Res.,* 1999; 31: 273-278.
6. M. C. Polidori, H. R. Griffiths, E. Mariani and P. Mecocci, Hallmarks of protein oxidative damage in neurodegenerative diseases: focus on Alzheimer's disease, *Journal of amino acids*, 2007; 32: 553-560.
7. Prabhakar, M.C., Experiments on frogs in Experimental pharmacology for undergraduates 1st ed., Chennai, India 2007; 52-58.
8. N. prasad, A. Rama Narsimha Reddy, A.Venkatesham, D.R Krishna and M.C. Prabhakara. Antioxidant activity of animal bile using frog heart as a model, Pharmacologyonline, 2008; 2: 390-396
9. Subhashinee S. K, Wijeratne, Susan L. Cuppett, and Vicky Schlegel, Hydrogen peroxide induced oxidative stress damage and antioxidant enzyme response in Caco-2 human colon cells. J. Agric. Food Chem.,2005**;** 53 (22): 8768-8774.
10. Kazuo Yamamoto, Ryoji Kushima, Osamu Kisaki, 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. J of inflammation, 2003; 27:123-128.