ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

ANTIOXIDANT ACTIVITY OF TRADITIONALLY USED BACKYARD INDIAN MEDICINAL PLANTS USING FROG HEART AS A MODEL

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ABSTRACT

The present study was aimed at evaluation of oxidative stress and antioxidant activity of different juices of medicinal plants on isolated frog heart; the plants were collected from Botanical garden. Perfusion of frog Ringer solution containg hydrogen peroxide to isolated frog heart, results cardiac arrest at ±17th minute, which indicated the induction of oxidative stress. In the presence of juices of medicinal plants, cardiac arrest was prolonged above ±17minutes indicated their antioxidant activity which was comparable with standard ascorbic acid.

Key Words: Frog heart, antioxidant activity

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ISSN: 0975-8585

INTRODUCTION

Oxidative Stress (OS) 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) as a result of one of three factors: a) an increase in oxidant generation, b) a decrease in antioxidant protection, or c) a failure to repair oxidative damage. 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(1). Examples are hydroxyl radical, superoxide, hydrogen peroxide, and peroxynitrite. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, cancer, and aging [1,2]. The effects of free radicals are expressed by the accumulation of oxidative damage to biomolecules: nucleic acids, lipids and proteins [2]. Antioxidants synthesized within the body or taken in the diet that form a natural defense against free radical induced damage [3]. The oxidative stress in animals or cell cultures has been successfully induced by hydrogen peroxide [4], and was chosen for induction of oxidative stress on isolated frog heart.

MATERIALS AND METHODS

The plant materials were collected from the herbal garden of Shri Vishnu college of pharmacy, Bhimavaram. The authentication was done by M.C.Prabhakara, Department of pharmacology, Shri Vishnu College of pharmacy. The fresh leaves 35gm of *Ocimum tenuiflorum* (syn. O. sanctum, Holy Basil,Sri tulsi), 35gm of *Ocimum sancium* (Krishna Tulsi) ,70gm of *Trachyspermum ammi* leaves and 50gms of fresh stems of *Cissus quadrangularis* collected from herbal garden. 30gm of *Neem flowers* were collected in the month of March (2009). After authentification fresh plant material was collected in bulk, washed, dried under shade and made into juice with frog Ringers solution with the help of mechanical grinder. Finally 25ml of *Ocimum tenuiflorum,* 25ml of Ocimum sancium, 60 ml of Trachyspermum ammi, 25 ml of *Cissus quadrangularis* and 20ml of Neem flower juice were collected.

Materials: Acetyl choline bromide, $CaCl_2$ and dextrose purified were purchased from Loba chemicals Pvt. Ltd. Mumbai, India. NaCl, KCl and $NaHCO_3$ 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.1 litre of Ringer solution was diluted to 1.4 liter with distilled water forms frogs Ringer solution.

ISSN: 0975-8585

Isolated frog heart preparation using Symes cannula

An Indian frog (*Rana tigrina*) was stunned by head-blow using a steel rod and pithed. The skin 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 then covered with a thin layer of cotton wool 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. 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 [5]. The study protocol was approved by Institutional Animal Ethical Committee, Shri Vishnu College of pharmacy, Andhra University.

H_2O_2 induced oxidative stress on isolated frog heart [6]

230 µl of H_2O_2 in 400ml of frog Ringer solution was used to induce oxidative stress on isolated frog heart. The parameters studied include cardiac output, force of contraction, heart rate and cardiac arrest. Acetylcholine at 10ng, 20ng dose levels elicited its muscarinic action like negative ionotropic, negative chronotropic and decreased cardiac output. The same dose levels were repeated in continuous perfusion of frog Ringer solution containing H_2O_2 to the heart preparation and observed the parameters.The time taken to induce cardiac arrest was compared with those of treated heart.

Effect of juice of medicinal plants on oxidative stress

Influence of diluted juice of medicinal plants on oxidative stress was studied by perfusing frog Ringer solution containing juice of plants and H_2O_2 solution the isolated frog heart preparation. The parameters studied include force of contraction, heart rate and cardiac output (n=6). The time taken for the cardiac arrest was noted by continuously perfusing frog Ringer solution containing juices of medicinal plants and H_2O_2 solution.

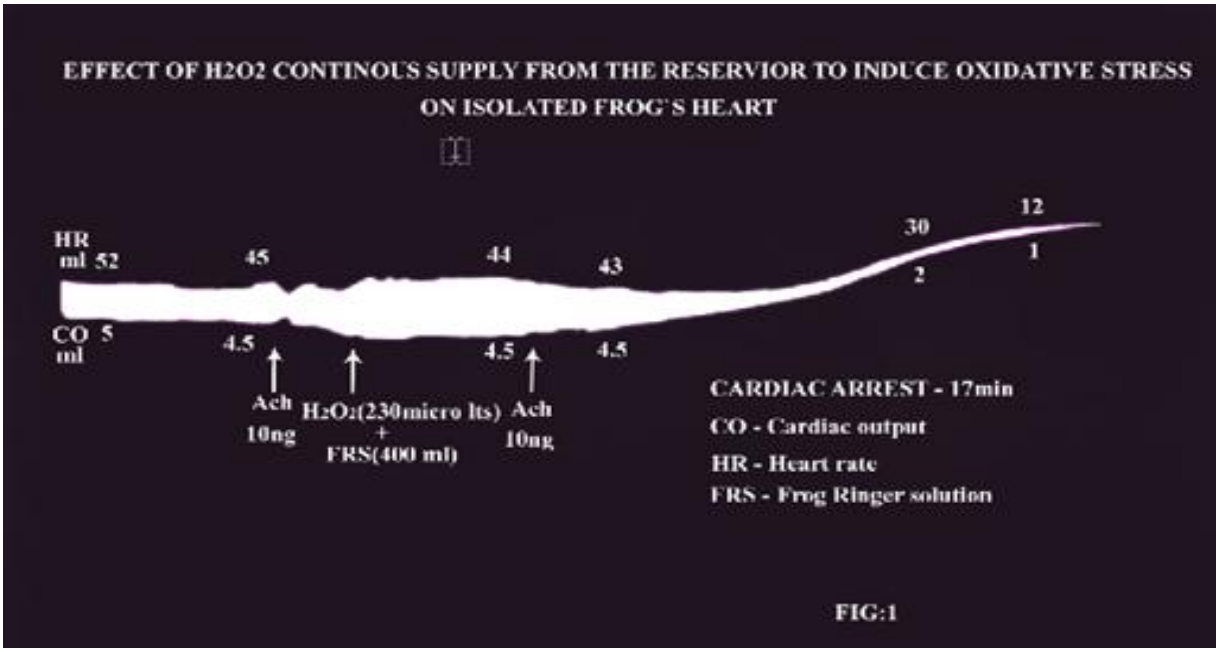
When isolated frog heart was perfused with normal frog Ringer solution, acetylcholine elicited muscarinic action i.e. negative ionotropic, negative chronotropic and decreased cardiac output at 10 ng, 20ng dose levels. But, in continuous perfusion of frog Ringer solution containing H2O2 to the heart preparation, the muscarinic actions were not observed, indicating that the damage of muscarinic receptors by H2O2 continuous exposure and this might be non specific damage to the receptor due to oxidative stress induced by H_2O_2. Finally it produced cardiac arrest at 17th minute and was taken as a control (n=6). This investigation supported the model of induction of

ISSN: 0975-8585

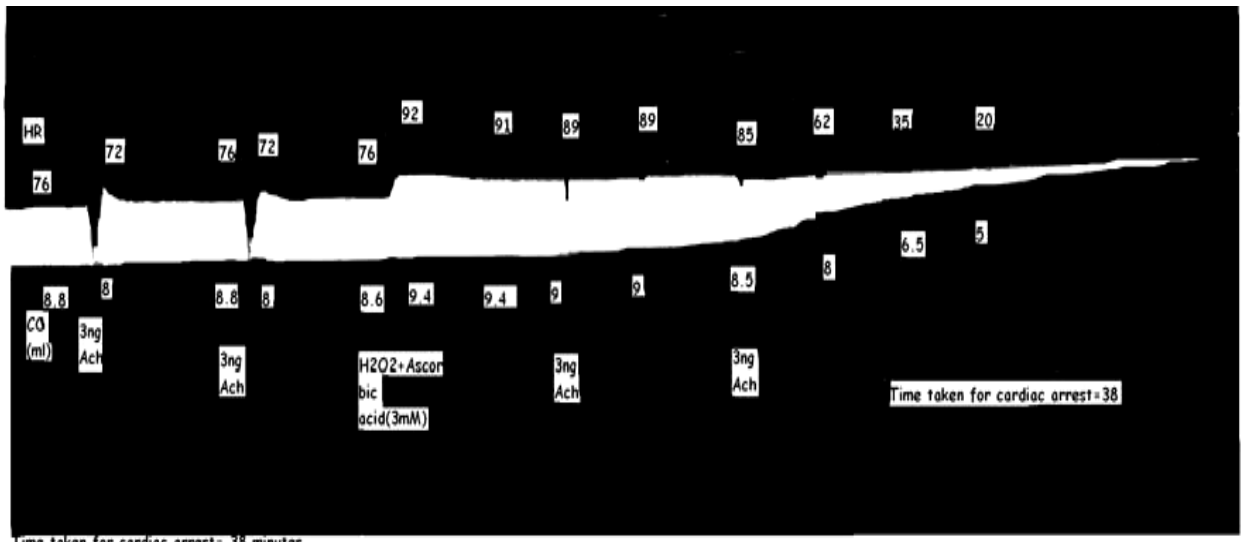
oxidative stress on isolated frog heart. The influence of juice of medicinal plants on H2O2 induced oxidative stress is show in figures 2, 3, 4, 5 and 6 respectively.

The effect of 230µl of H_2O_2 in 400 ml of frog Ringers solution on isolated heart was showed in

FIG: 1



Study of the antioxidant activity of Ascorbic acid on isolated frog's heart .



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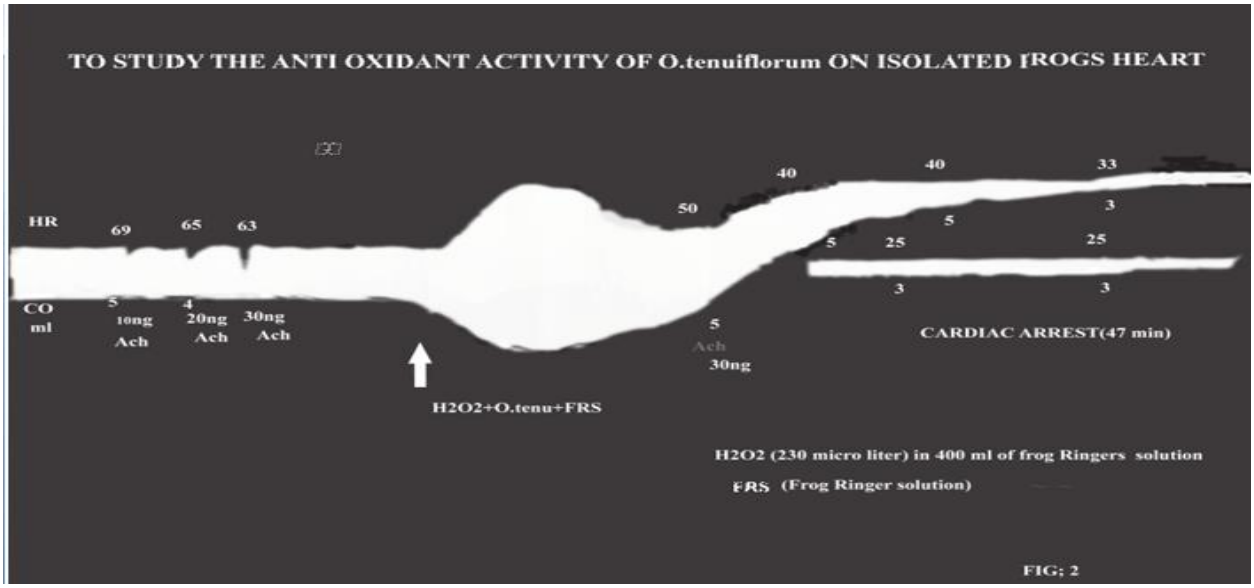
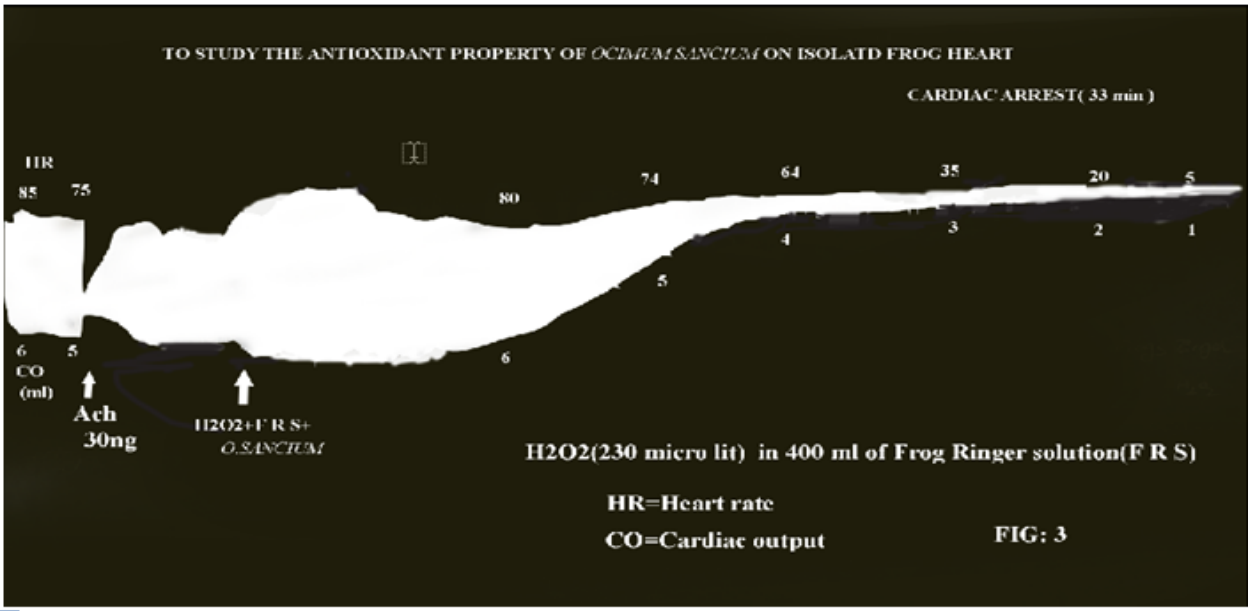
Study of the antioxidant activity of juice of *Ocimum tenuiflorum* on isolated frog's heart.

FIG: 2



Study of the Antioxidant property of juice of ocimum sancium on isolated frog's heart.

FIG: 3



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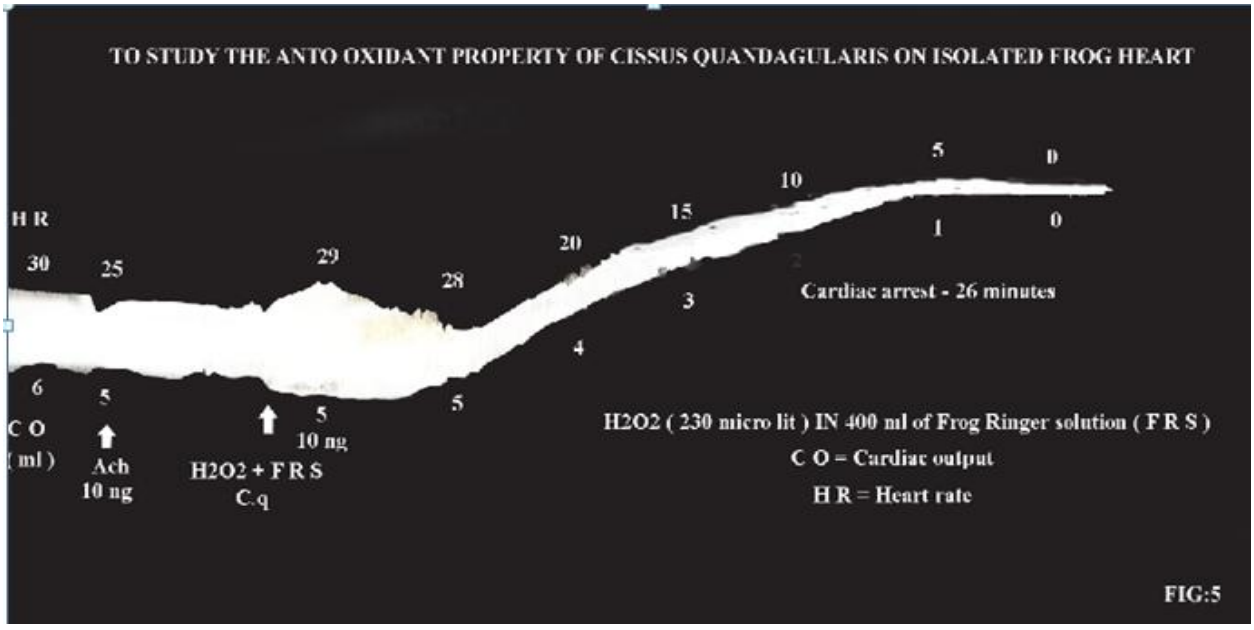
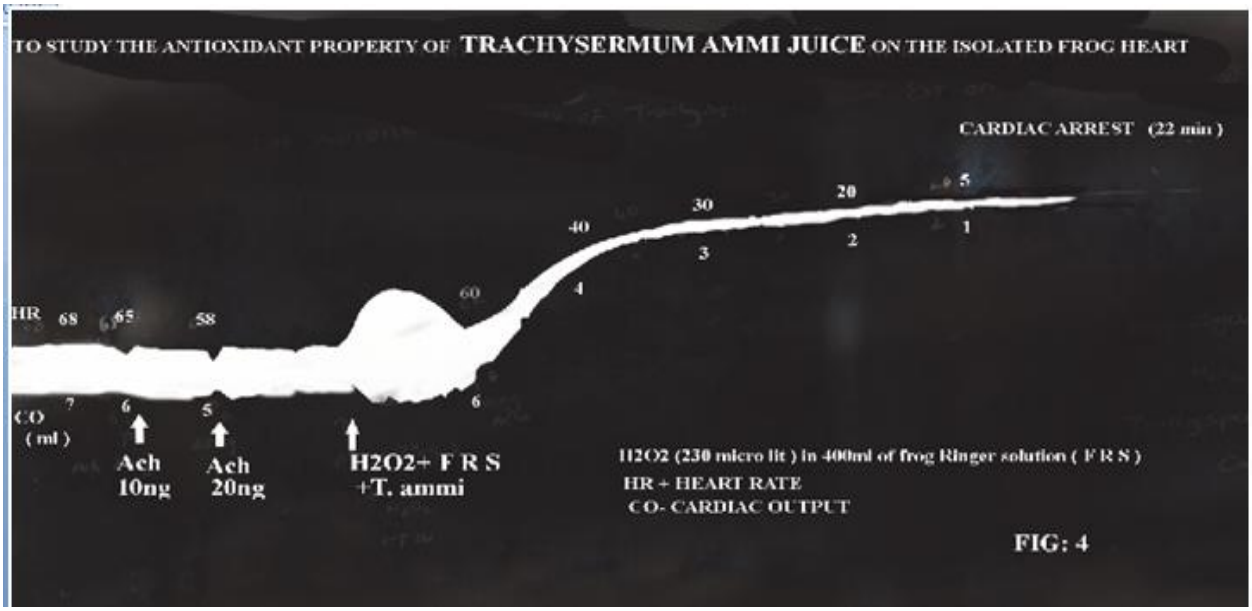
Study of the Antioxidant property of juice of *Cissus quadrangularis* on isolated frog heart.

FIG: 4



Study of the Antioxidant property of juice of *Trachyspermum ammi* on isolated frog heart.

FIG: 5



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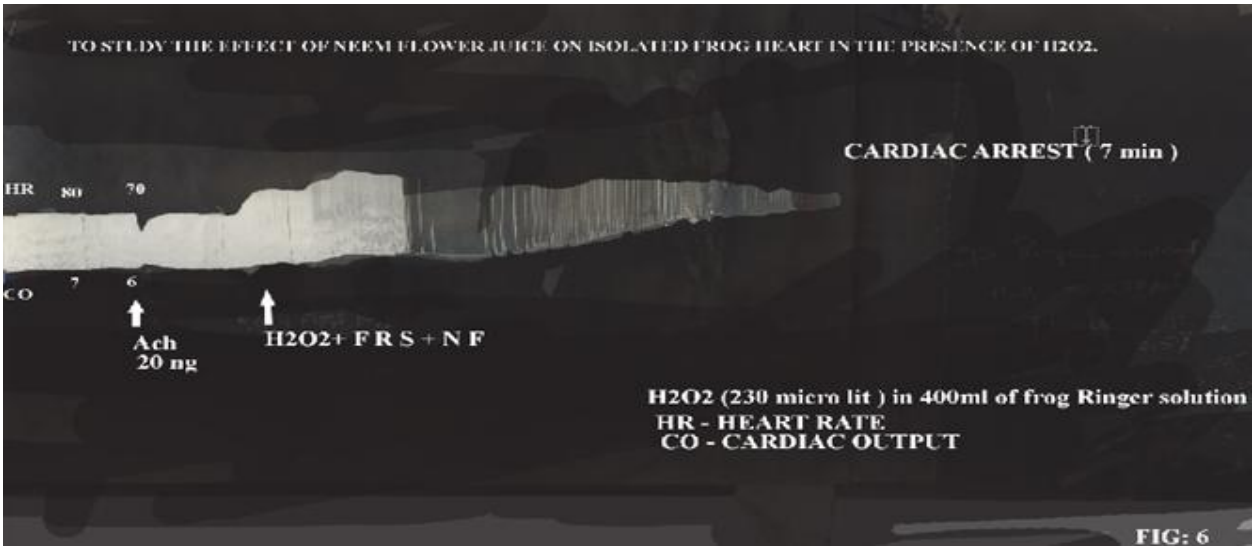
Study of the effect juice of *Neem flower* juice on isolated frog heart in the presence of H_2O_2

FIG: 6

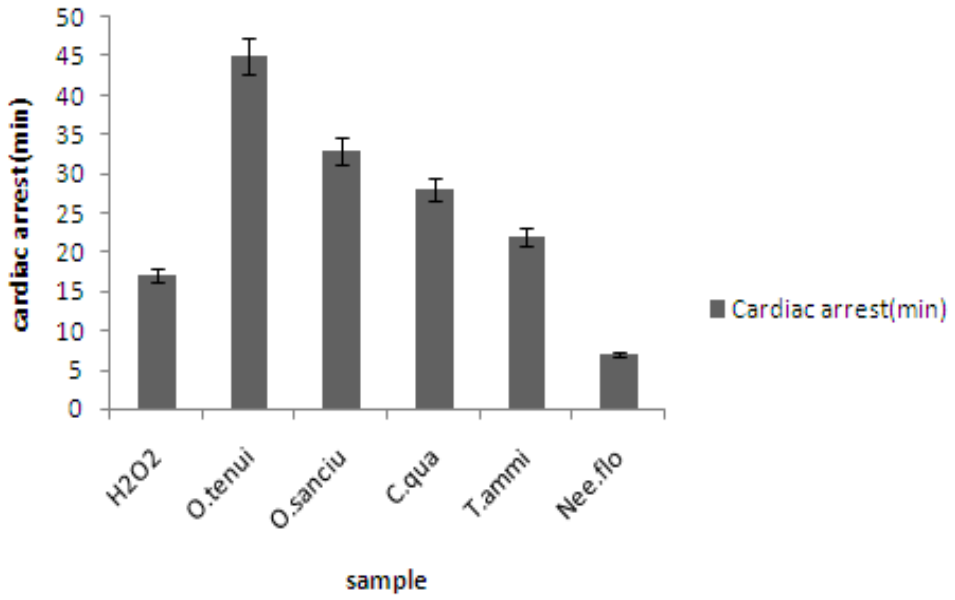


ANTI-OXIDANT ACTIVITY

Table: 1 Protective effect of sample juice on cardiac arrest

Sample	Cardiac arrest(min)
H_2O_2	17
O.tenui(1%)	Above 45
O.sanciu(1%)	33
C.qua (1%)	28
T.ammi (1%)	22
Nee.flo (1%)	7

ISSN: 0975-8585



EVALUATION OF ANTIOXIDANT CAPACITY (By using analytical reagents) [7]

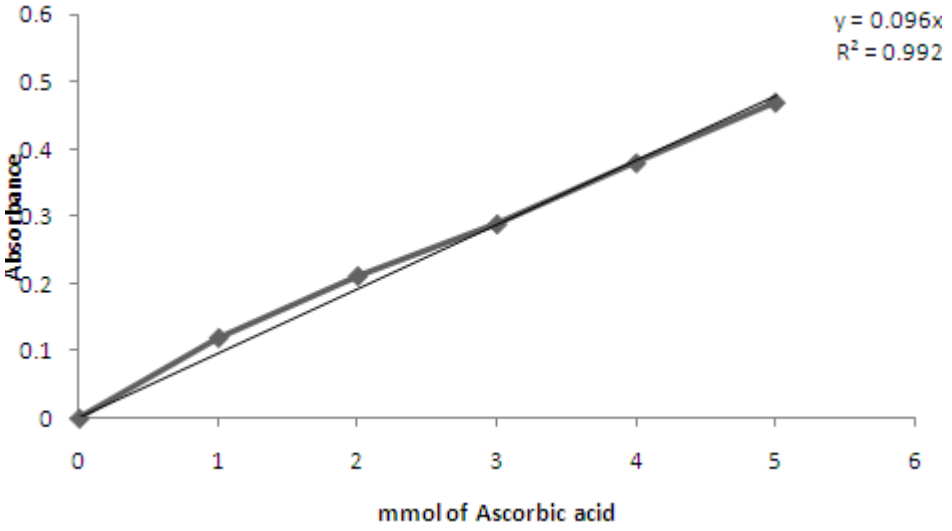
RESULTS

The reaction between phosphor molybdenum and test drug (Ascorbic acid /plant extract) gave blue colour after incubation for 90 min in water bath at 95ºc. Ascorbic acid at concentration ranging from 0.5 mmol was selected for standard curve preparation.

Standard curve of ascorbic acid at 695 nm shows linearity with y = 0.091x + 0.016 and R^2 = 0.995

Concentration (%)	Absorbance
0	0
1	0.12
2	0.212
3	0.289
4	0.38
5	0.47

ISSN: 0975-8585



Plant extract	Equivalent to mmol of ascorbic acid
Aqueous *O.tenuiflorum (3%)*	0.61
Methanolic *O.tenuiflorum (3%)*	0.986
Ethanolic *C.quadrangularis (2.5%)*	0.931
Ethanolic *T.ammi (3%)*	0.8

DISCUSSION

Oxidative stress induced by hydrogen peroxide (H_2O_2) may contribute to the pathogenesis of ischemic-reperfusion injury in the heart. For the purpose of investigating directly the injury spotential of H_2O_2 on heart muscle, a cellular model of H_2O_2 induced myocardial oxidative stress was developed using monolayer rat cardiomyocyte cultures [8]. It was reported that an oxidant burden established by hydrogen peroxide overload may elicit post-ischemic myocardial damage [9]. 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 [10]. It was reported that exposure of high concentration of H_2O_2 increases apoptotic signals, eventually inducing apoptosis, which resulted in mitochondrial membrane potential disruption [11]. In this study we induced the oxidative stress on isolated frog heart by perfusing frog Ringer solution containing H_2O_2. When Ringer solution containing H_2O_2 perfused to heart preparation, the muscarinic actions of acetylcholine were not observed indicating the oxidative stress on frog heart induced by H_2O_2,

this might be due to the desensitization of receptors. The cardiac arrest was produced at 17th minute. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of juice of plants the cardiac arrest was observed at above 40, 33, 28, 22 and 7 minutes respectively for *O.tenuiflorum, O.sancium, C.quadrangularis, T.ammi* and *Neem flowers* were showed in figures 1- 6. Therefore the cardiac arrest time was prolonged by 25, 17,11,and 5 minutes respectively, i.e. heart was protected longer period with plant juice, against H_2O_2 induced oxidative stress when compared with the control.

CONCLUSION

The order of protection of the heart with juice of plants is as follows:

Ocimum tenuiflorum > Ocimum sancium > Cissus quadrangularis (stem) > Trachyspermum ammi.

The order of antioxidant property of plant extract by using phosphomolybdenum reagent is *Ocimum tenuiflorum > Cissus quadrangularis (stem) > Trachyspermum ammi.*

The order of antioxidant property of plant extract by using phosphomolybdenum reagent is *Ocimum tenuiflorum > Cissus quadrangularis (stem) > Trachyspermum ammi.*

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