ANTIOXIDANT ACTIVITY OF ANIMAL BILE USING FROG HEART AS A MODEL

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Summary

The present study was aimed at evaluation of isolated frog heart as a model for induction of oxidative stress and antioxidant activity of different animal bile secretions, which were collected from slaughter house. Perfusion of hydrogen peroxide containing frog Ringer solution to isolated frog heart results cardiac arrest at 15th minute indicated the induction of oxidative stress. In the presence of animal bile, cardiac arrest was prolonged upto 38 minutes indicated their antioxidant activity which was comparable with standard ascorbic acid.

Key Words: Frog heart, bile secretions, antoxident activity.

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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: 1) an increase in oxidant generation, 2) a decrease in antioxidant protection, or 3) 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], was chosen for induction of oxidative stress on isolated frog heart. Bile is, essential for the digestion of fat, secreted by the hepatic cells into the bile capillaries. It is a clear, viscous fluid and is stored in the gall bladder between the periods of digestion; bile is diverted via the cystic duct into the gall bladder where it is concentrated and stored. Bile consists of inorganic and organic compounds. In inorganic compounds the main constituents are bile acids, bile pigments, lipids, fatty acids, cholesterol and mucin [5]. The present study was aimed to develop a model of isolated frog heart for the induction of oxidative stress using H_2O_2 and evaluate the antioxidant activity of different animal bile secretions.

Methods

Materials: Acetyl choline bromide, $CaCl_2$ 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.

Animals used: Herbivorous animals like ox, cow, goat and sheep were used to collect the bile samples from the slaughter houses in Warangal, India. In this study all the animal bile samples were diluted to 100 times with frog's Ringer solution.

Collection of bile: Bile was collected by carefully isolating gall bladder and pressing out into small flask and stored in ice jacketed flask and avoided the samples contamination with blood. All the bile samples were stored at -80^{o} C until analysis.

Isolated frog heart preparation using symes technique:
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 to prevent drying. 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 [6]. The study protocol was approved by Institutional Animal Ethical Committee, UCPSc, Kakatiya University, Warangal, India.

H_2O_2 induced oxidative stress on isolated frog heart:
1mM of H_2O_2 in 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 10 ng, 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 Ringer solution containing H_2O_2 to the heart preparation and observed the parameters studied. The time taken to induce cardiac arrest was taken as the control.

Effect of animal bile on oxidative stress: Influence of diluted animal bile (ox, cow, sheep and goat) on oxidative stress was studied by perfusing frog Ringer solution containing animal bile 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). The time taken for cardiac arrest was noted by continuously perfusing frog Ringer solution containing animal bile and H_2O_2 solution.

Results

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

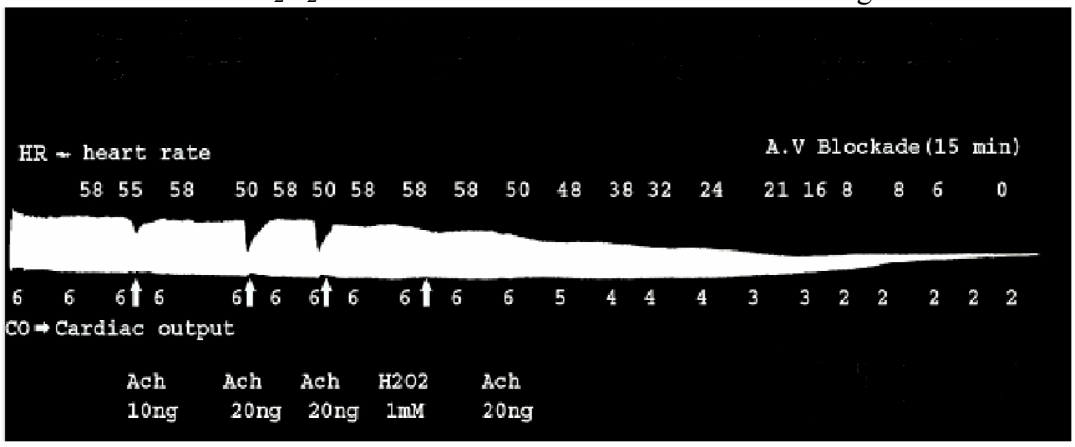

Figure 1. Influence of ascorbic acid on H_2O_2 induced oxidative stress.

When isolated frog heart was perfused with normal 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 Ringer solution containing H_2O_2 to the heart preparation, the muscarinic actions were not observed, indicated the damage of muscarinic receptors by H_2O_2 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 15th minute and was taken as a control (n=6). This investigation supported the model of induction of oxidative stress on isolated frog heart.The influence of animal bile (ox, cow, sheep and goat) on H_2O_2 induced oxidative stress was showed in figures 2, 3, 4 and 5 respectively.

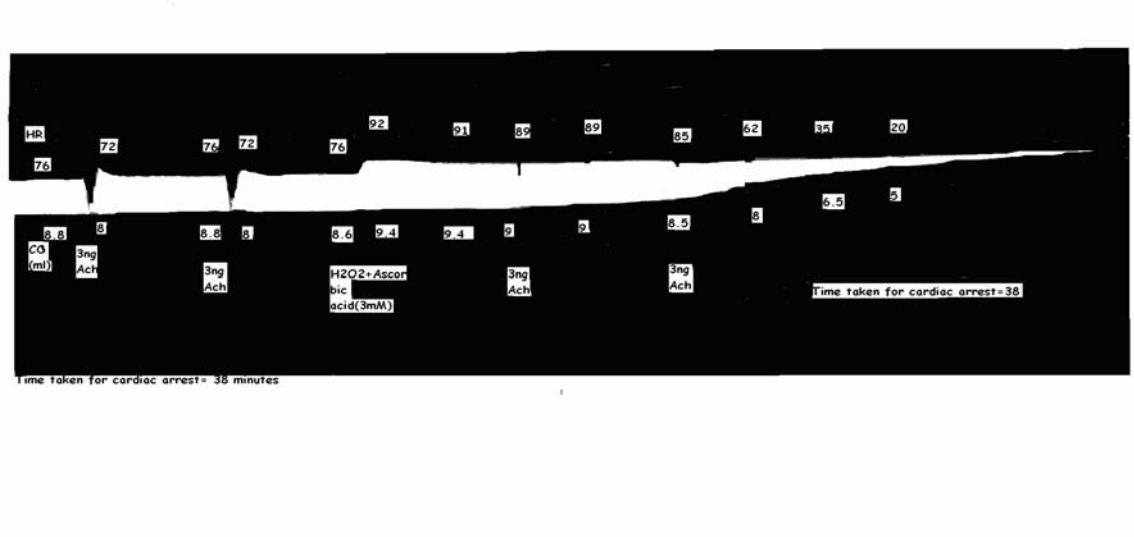

Figure 2. Influence of ox bile on H_2O_2 induced oxidative stress

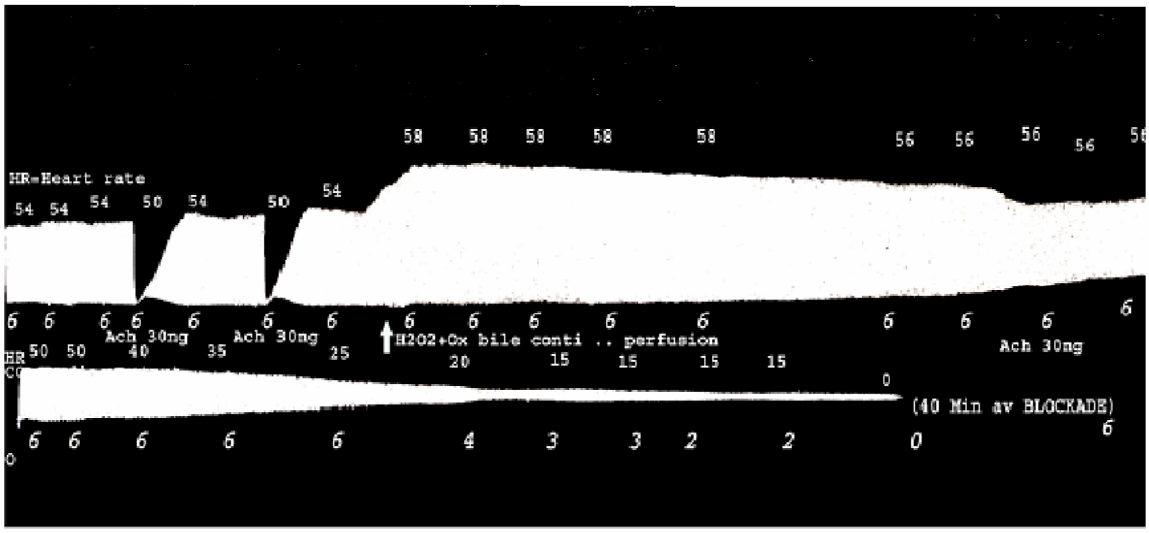

Figure 3. Influence of cow bile on H_2O_2 induced oxidative stress

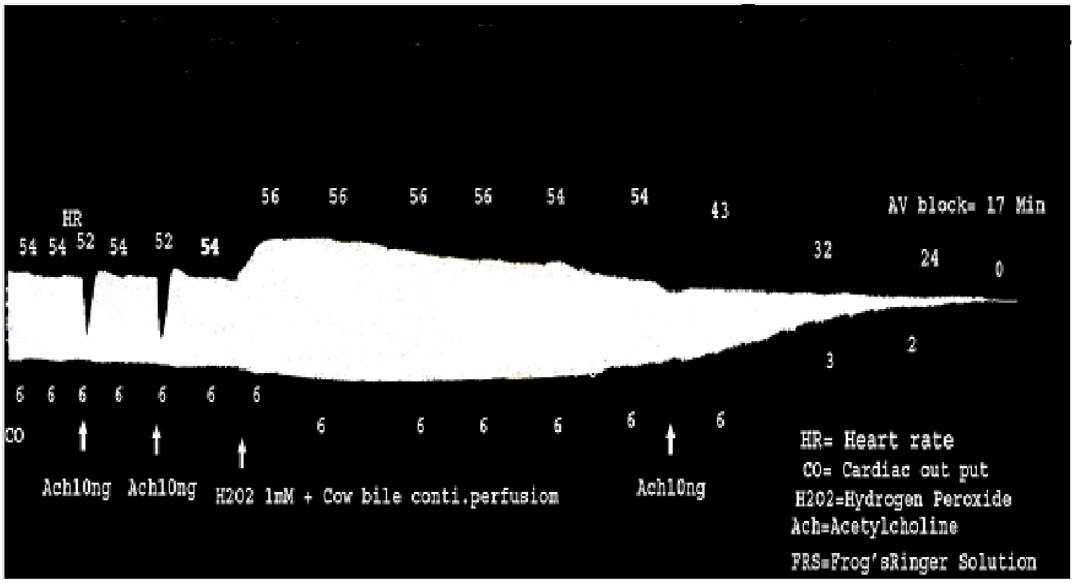

Figure 4. Influence of sheep bile on H_2O_2 induced oxidative stress

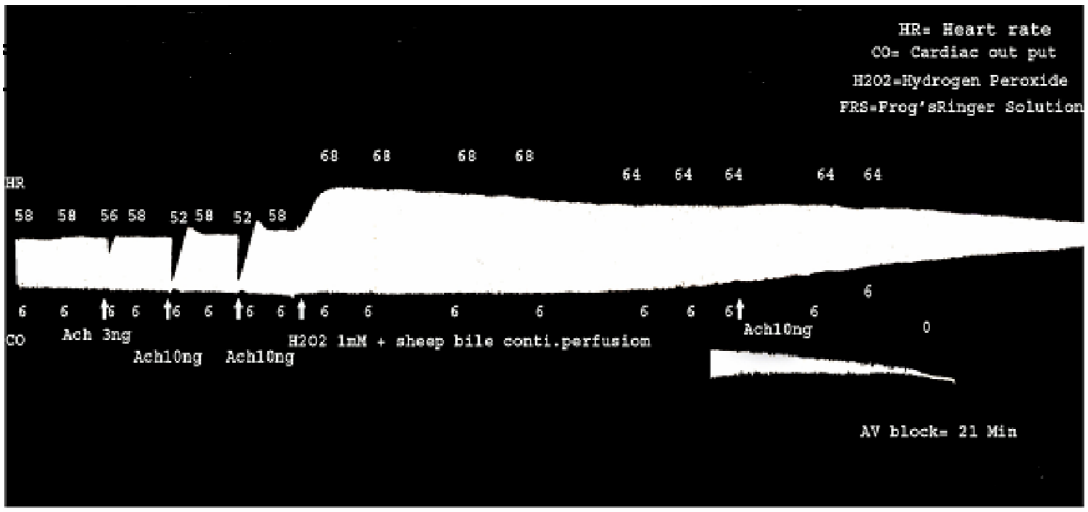

Figure 5. Influence of goat bile on H_2O_2 induced oxidative stress

By exposing frog Ringer solution containing animal bile and H_2O_2 solution to heart preparation, the parameters were studied. Initially there was a slight increase in force of contraction and heart rate, but no significant change in cardiac output was observed with 10 ng, 20ng dose levels indicating the cardiac stimulant activity of animal bile. Upon continuous perfusion, the cardiac arrest was observed at 40, 17, 21 and 25 minutes respectively for ox, cow, sheep and goat bile, on average of 6 experiments. Figure 6 represents the comparison of antioxidant activity of animal bile with ascorbic acid.

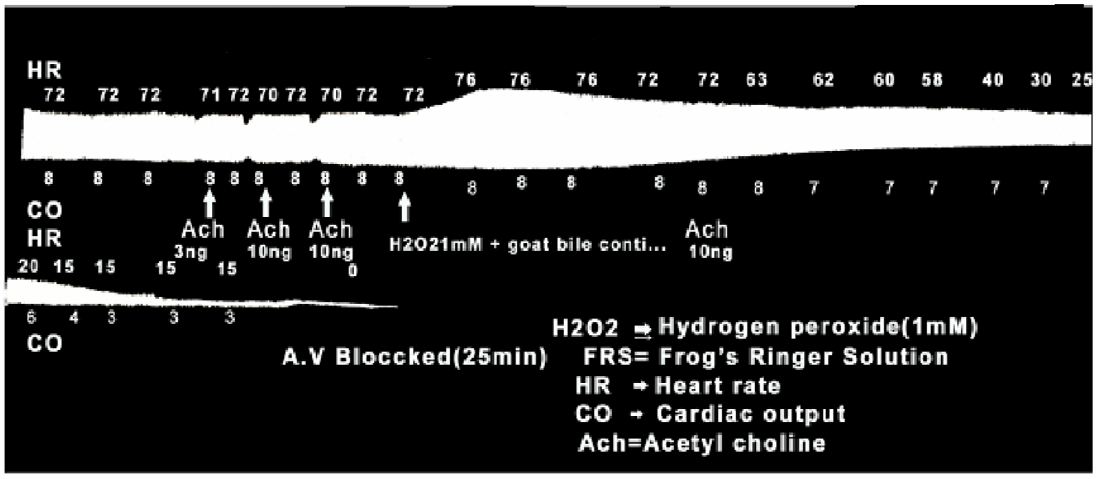

Figure 6. Comparison of antioxidant activity of animal bile with ascorbic acid.

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 potential 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 [7]. It was reported that an oxidant burden established by hydrogen peroxide overload may elicit post-ischemic myocardial damage [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]. 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 [10]. So far, there was no report on isolated frog heart as a model for induction of oxidative stress. 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 15^{th} minute. This result supports the frog heart model for induction of oxidative stress by H_2O_2. In the presence of animal bile the cardiac arrest was observed at 40, 17, 21, 25 and 38 minutes respectively for ox, cow, sheep, goat bile and standard ascorbic acid (3 mM), were showed in figures 1- 6. Therefore the cardiac arrest time

was prolonged by 25, 2, 6, 10 and 23 minutes respectively, i.e. heart was protected longer period with ox bile, against H_2O_2 induced oxidative stress when compared with the control. This indicates the antioxidant activity of animal bile which was comparable with ascorbic acid. Ox bile had more activity than ascorbic acid on H_2O_2 induced oxidative stress.

In conclusion, the present investigation supported the isolated frog heart model for the induction of oxidative stress and animal bile secretion might have antioxidant activity which was comparable with ascorbic acid.

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