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Research Article

EVALUATION OF CARDIOTONIC ACTION OF ETHANOL EXTRACT OF *LAGENARIA SICERARIA* PULP ON FROG'S HEART

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ABSTRACT
The aim of this study is to the Herbal remedies are used worldwide for various diseases. They are safer and less expensive with lesser side effects than synthetic drugs. *Lagenaria siceraria*, the vegetable gourd, belonging to the family Cucurbitaceae is used as diuretic, cardio-tonic, cardio-protective and nutritive agent and traditionally in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure and skin diseases. The present study was carried out to evaluate the cardiotonic effect of ethanol extract of pulp of *Lagenaria siceraria* (EELSP) in Amphibians such as the grass frog *Rana pipiensin*. After toxicity evaluation in albino rats two doses of the ethanol extract of *Lagenaria siceraria* pulp extracted with 90 % methanol using a Soxhelts apparatus was selected for the experiment. One grass *Rana pipiensin* frogs were taken and double pitching was done. Frog heart in situ was setup following standard procedures. Frog's ringer, Acetyl choline, Adrenalin and ¼th Ca followed by two doses of EELSP was given one after the one and the graphing was recorded. The extract at 400 mg and 800 mg produced significant cardiotonic action. Thus the study elucidates that ethanol extract of *Lagenaria siceraria* pulp possess cardiotonic action at the doses of 400 and 800 mg.
Keywords: Amphibian, Acetyl choline, Adrenalin Frog's ringer, cardio-tonic, cardio-protective 1/4 Th Ca, *Lagenaria siceraria*.

INTRODUCTION

Cardiovascular disease is main cause of death due to rise in diabetes and obesity throughout the world. CVDs is the number one cause of death globally: more people die annually from CVDs than from any other cause[1]. An estimated 17.3 million people died from CVDs in 2008, representing 30 % of all global deaths[1]. Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke[2]. The number of people, who die from CVDs, mainly from heart disease and stroke, will increase to reach 23.3 million by 2030[1,3]. CVDs are projected to remain the single leading cause of death[3]. People in low- and middle-income countries who suffer from CVDs and other noncommunicable diseases have less access to effective and equitable health care services which respond to their needs (including early detection services). As a result, many people in low- and middle-income countries die younger from CVDs and other noncommunicable diseases, often in their most productive years. The poorest people in low- and middle-income countries are affected most. There is a large and increas ing global burden of cardiovascular disease. The continuous increase in incidences of cardiovascular disease is a manifestation of chronic poor diet and lifestyle choices, which lead to diabetes and obesity.[4] The plant, *Lagenaria siceraria* (Mol.) Standl. (Family: Cucurbitaceae), commonly known as bottle gourd, a common fruit vegetable used throughout the India.[5] The Bottle Gourd is a diploid, self-compatible monoecious annual (monoecious plants do not have separate male and female individuals). Two morphologically distinct subspecies of Bottle Gourd are recognized, *L. siceraria* (the African and American/New World gourds) and *L. siceraria asiatica* (the Asian gourds).[6] Traditionally, the fruit of *Lagenaria siceraria* is used for its cardio protective, cardiotonic, diuretic and aphrodisiac activities and as an antidote to certain poisons.[7,8] Ancient Ayurveda says that bottle gourd prevents premature graying hair. Drinking fresh bottle gourd juice in the morning on a regular basis can be very useful. Different parts of plant *Lagenaria siceraria* has been studied extensively for various disorders. *Lagenaria siceraria* juice prevents excessive loss of sodium, satiating thirst, and giving a cooling effect.[4] The fruit is also reported to have good source of vitamin B complex and choline along with fair source of vitamin C and β-carotene.[9] *Lagenaria siceraria* fruit has been reported to possess as immunosuppressant[10] and picarp exhibits maximum free radicals scavenging activity. The pulp of *Lagenaria siceraria* fruit is cool and has diuretic action.[11] cardio-tonic, cardio-protective,[12] and nutritive agent,[13] and hypolipidemic activity[14]. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness.[13,15]. *Lagenaria siceraria* juice is an excellent remedy for heart problems, digestive and urinary disorders, and in diabetes Cardio protective activity of *Lagenaria siceraria* fruit powder in rat against Doxorubicin induced cardiotoxicity at 200 mg/kg, p.o for 18 days was evaluated in one study,[12] The present investigation was undertaken to study the cardiotonic effect of ethanol extract of pulp of *Lagenaria siceraria* on intact frog heart in comparison with frog ringer, acetyl choline, adrenalin and 1/4th Ca frog ringer.

MATERIALS AND METHODS

L. siceraria fruits were collected from the local vegetable market of Khammam District, Andhra Pradesh, India in the month of October-November, 2013 and the botanical authentication was done by the Head of the Department of Botany, Govt degree college, Khammam. Andhra Pradesh, India.

Preparation of extract

The fruits were cut in to small pieces and were dried under shade. The dried pieces were crushed in to fine powder. The extraction of powdered plant material was done with 90 % methanol using a Soxhelts apparatus. The extract obtained was evaporated at 45°C to get a semisolid mass. The extractive value of aqueous extract of *L. siceraria* was 23.07g w/w. To detect chemical constituents of LSME phytochemical screening was carried out for the presence of steroids, saponins and polyphenols, carbohydrates, proteins.

Drugs used

Adrenalin (Mercury Medicare, Chennai, India), Acetyl choline (Nilkanth Organics, Mumbai, India), ¼ Ca frog ringer and normal frog ringer

Instruments

Sherrington Rotating Drum, Sterling's heart lever

Animals

Frogs of *Rana hexadactyla* species maintained in the animal house were used for the studies. The animals were maintained as per the norms of CPCSEA and the experiments were cleared by CPCSEA and the local ethics committee, the clearance number being (5/IAEC/MMC/2013-14)

Toxicity Evaluation in Albino Rats

The ethanol extract was tested for its acute toxicity in albino rats. Acute oral toxicity was performed as per OECD-423 guide lines.[16] It was observed that the test extract was not lethal to rats up to 2000 mg/kg. So the doses of 400 and 800 mg/kg were taken arbitrarily for the study.

Evaluation of Cardiotonic Activity

Preparation of the Frog

The intact heart of *Rana hexadactyla* was used in the experiment. Double-pithed frogs were placed on their dorsal surface. Using the sharp end of a pair of blunt/sharp scissors made a small penetration into the abdominal cavity of the frog. Carefully cut abdominal wall with a pair of scissors and cut towards the sternum. Precaution was taken to prevent cutting of the heart bloods vessels or other internal organs. The pectoral girdle was cut through to expose the heart in the pericardial sac. The pericardial sac was carefully removed. Connective tissue attachments (not the vessels around the atria) were cut so that the heart beats freely. Using forceps grasped the apex of the ventricle and pushed the point of the 'J'-hook into the apex of the ventricle wall until the bend of the hook is inside the heart. The thread from the frog heart was connected to the starling's heart lever and tied it twice to make sure it won't come loosen when the heart contracts, which was fixed to a stand. A proper tension was adjusted by altering the height of the lever. The inferior venacava was traced, put a thread around it and given a small cut in order to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure the cannula in place which is in turn connected to a saline bottle containing frog ringer solution. A small cut in one of the aorta was given for the ringer to come out. The frog was positioned so the thread from the heart is vertical. If it is pulling at an angle much of the contraction of the frog heart will not be observed. The normal heart rate was noted. All drugs namely acetyl choline, adrenalin 10 ug/ml were given through the catheter inserted in to inferior vena cava and tracings were noted for 60 seconds. The frog heart was washed with the Ringer solution after every administration of extracts and drugs till it was brought back to the normal state. That is followed by 1/4th Ca frog ringer. When hypo dynamic state is obtained, 400 mg/ml of EELSP was administered and the rate and force of heart contractions were noted on kymograph. The experiment was repeated with 800 mg/ml of EELSP and the recordings were noted. The frog's heart was bathed in Ringer periodically. The results were expressed as % increase in force of contraction over control.

RESULT

The ethanol extract of *Lagenaria siceraria* pulp showed dose dependent cardiotonic activity on frog's heart as shown in Table 1. The ethanol extract of *Lagenaria siceraria* mg/ml shows moderate increase in the force of contraction and significant change in the heart rate at 400 mg/ ml dose. At 800 mg/ml concentration test drug shows significant increase in the force of contraction with decrease in heart rate after producing hypo dynamic state with 1/4th Ca frog ringer; whereas acetyl choline 10 ug/ml produced significant decrease in force of contraction and heart rate. There was significant increase in the height of force of contraction, and heart rate with adrenalin 10 ug/ml. There was minimal force of contraction and highly significant fall in heart rate with ¼th Ca frog ringer which produces hypo dynamic state of the heart. The plant extract exhibits wide therapeutic index and does not show any kind of cardiac toxicity at higher doses tested up to 500 g/ml.

DISCUSSION

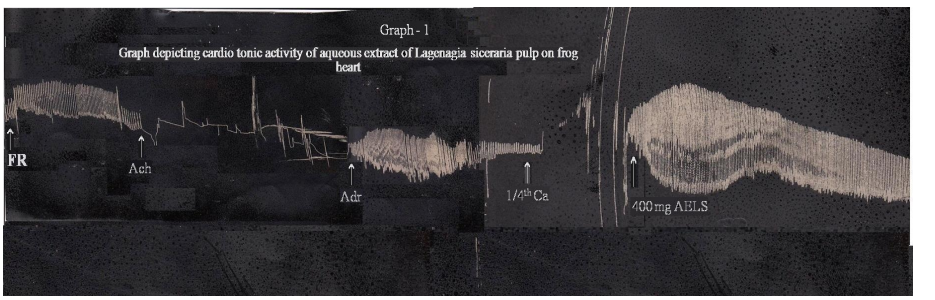
Most of the plants have cardio protective properties. Different phytoconstituents such as carotenoids, triterpenes, flavonoids, cardiac glycosides, alkaloids saponins, polyphenols, terpenoids, fatty acids etc are present in different plants. The phytoconstituents from plants were responsible for cardio protective activity including carotenoids (*Eugenia uniflora*), triterpenes (*Ganoderma lucidum*), flavonoids (*Anacardium occidentale*, *Nelumbo nucifera*) cardiac glycosides (*Digitalis purpurea*, *Antiaris toxicaria*) alkaloids (*Desmodium gangeticum*, Erythroxylon coca *Tinospora cordifolia*), saponins (*Asparagus racemosus*, *Vaccaria pyramidata*); terpenoids (*Ginkgo biloba*), fatty acids (*Elaeis guineensis*) etc[17-19]. Various studies proved that cardio protective plants significantly prevented the altered biochemical variation such as marker enzymes serum glutamate pyruvate transaminase (SGPT) or alanine transaminase (ALT), serum glutamate oxaloacetate transaminase (SGOT) or aspartate transaminase (AST), creatine phosphokinase (CPK), alkaline phosphatise (ALP), lactate dehydrogenase (LDH), lipid profile including low density lipoprotein (LDL), VLDL (very low density lipoprotein), triglycerides (TGs), high density lipoprotein (HDL), total cholesterol and antioxidant parameters including Superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), Glutathione peroxidase (GPx), MDA (malonaldialdehyde) and glutathione reductase (GR) come to near normal status. Cardio protective activity was evaluated using various pharmacological screening models like isoprenaline induced myocardial necrosis in rats, doxorubicin (DOX) induced cardiotoxicity in albino rats, Cyclophasphamide induced oxidative myocardial injury in a rat model, ischemia -reperfusion induced myocardial

infarction in albino rats, cigarette smoke exposed Rats, adriamycin induced cardiomyopathy in rats etc[20-27]. In one study ethanolic extract of *Lagenaria siceraria* fruits also showed increased in force of contraction and decrease in rate of contraction from 66 to 44 in isolated frog heart when perused with normal ringer solution.[12] The fruit traditionally used as a cardiotonic was confirmed in our study also.

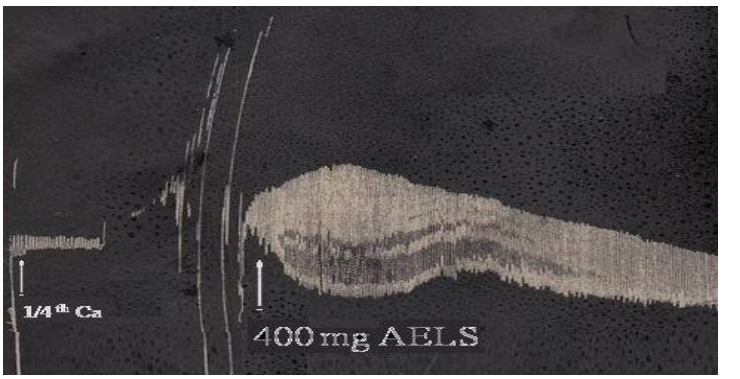
Table 1: Cardiotonic activity of ethanolic extract of *Lagenaria siceraria* pulp on Frog's heart

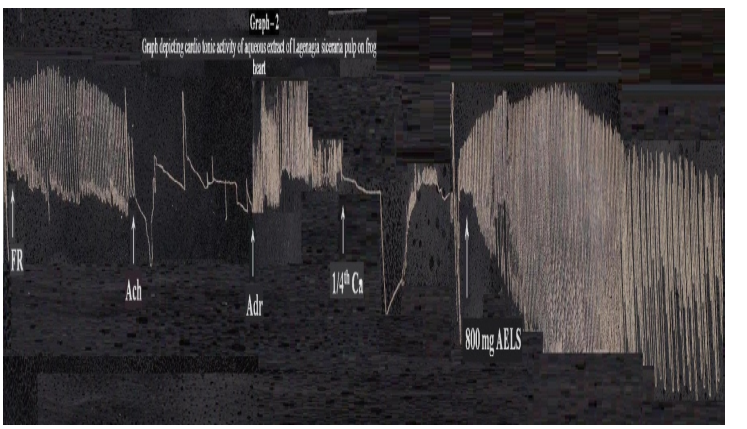
Drug concentration	HFC (cm)	HR/mt
Control (Frog Ringer)	1.5	40
Acetyl choline 10 ug/ml	0.8	6
Adrenalin 10 ug/ml	1.6	42
1/4 th Ca Frig ringer	0.4	21
EELSP-400 mg/ml	3.5	45
EELSP-800 mg/ml	4.7	36

HFC: Height of force of contraction, HR: Heart rate. EELSP- ethanolic extract of *Lagenaria siceraria*

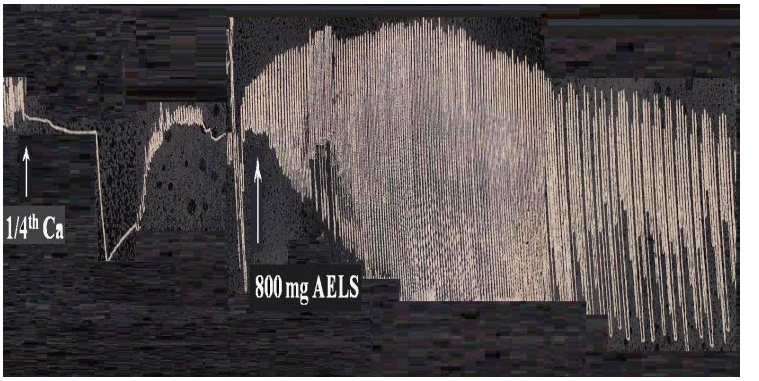


Graph 1





Graph 2



CONCLUSION

This study has confirmed that, *Lagenaria siceraria* pulp having cardiotonic activity which confirms the traditional use of bottle gourd in cardiac ailments as cardio protective. Secondary metabolites like carotenoids, triterpenes, flavonoids, cardiac glycosides, alkaloids saponins, polyphenols, terpenoids, fatty acids etc were responsible for cardio protective activity at a particular dose which was evaluated using appropriate pharmacological screening approach. Further research is needed to elucidate the bioactive ingredients that are responsible for the observed activity in the *Lagenaria siceraria* pulp.

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