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BIOCHEMICAL AND ELECTROPHYSIOLOGICAL MECHANISMS OF CARDIOVASCULAR ACTIVITY OF AYURVEDIC PREPARATION 'ARJUNARISTA' IN RAT MODEL

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ABSTRACT

Background: Ayurvedic drugs, among other methods of treatments, are getting importance and reputation day by day because of their required pharmacological actions with fewer side effects. But prolonged and excessive usage may lead to harmful impacts such as damage of the heart muscles leading to various types of arrhythmias and coronary artery diseases. We have chosen a cardioactive Ayurvedic drug "Arjunarista" which is mainly used for treatment of heart diseases. The active ingredient of this preparation is 'Arjuna' and it is mainly indicated for the treatment of coronary artery diseases, heart failure, edema, angina and hypercholesterolemia. **Methods:** In the present study, changes in electrocardiogram and serum lipid profile in Arjunarista pretreated rats at normal (0.28 mL/kg bw) and high dose (2.8 mL/kg bw) were studied before and after digoxin administration. **Results:** The study showed that pretreatment with Arjunarista had a definite, dose dependent modulatory effect on heart. Both normal (0.28 mL/kg bw) and high dose (2.8 mL/kg bw) of Arjuna pretreated groups offered better cardio-protection when subjected to digoxin induced arrhythmia. The results of lipid profile tests indicated that almost all the atherogenic indices were decreased along with total cholesterol level. **Conclusions:** It can be inferred that Arjuna pretreated rats demonstrated significant prevention of the risks of coronary heart diseases.

KEYWORDS: Arjunarista, Electrocardiography, Cardiovascular Disease, Rat Model, Traditional Medicines, Arrhythmia, Lipid Profile.

BACKGROUND

The traditional herbal medicine and their preparations have been widely used for thousands of years in many oriental countries, such as Bangladesh, India, China, Korea, Japan etc.[1] Day by day the importance and reputation of these traditional or alternative medicines are increasing in the treatment of various diseases such as diabetes, cardiovascular, cancer, gastrointestinal disorders etc. This is due to their fewer side effects, perceived effectiveness and relatively affordable cost. World Health Organization (WHO) and National Institute of Health (NIH, USA) also recommend the use of Ayurvedic drugs in the name of alternative or complementary medicine system, as these drugs have fewer side effects, better compatibility with the human body and give necessary pharmacological actions.[2,3,4] Bangladesh is considered as the home of medicinal/herbal plants where over 546 species of medicinal plants grow, out of which 206 plants are used in herbal medicines. Being a member of LDCs, most of the people of our country are unable to get the benefits of modern medicines for the treatment of various diseases. They mainly depend on these easily affordable, cost effective herbal drugs which have been traditionally used for thousands of years. Cardiovascular disorders are the most prevalent health problems in Bangladesh. Here a large number of plant-derived herbal drugs are known to be used in treating cardiovascular and related disorders. Few of these drugs also have lipid lowering and cardio protective activities. Among these, Arjunarista (*Terminalia arjuna*) has the distinct superiorities over other drugs.

In a review article it was observed that various *in vitro*, *in vivo* and clinical trials revealed the pleiotropic effects of *Terminalia arjuna* such as anti-atherogenic, hypotensive, inotropic, anti-inflammatory, anti-thrombotic and antioxidant actions for treatment of various cardiovascular disorders. It was documented that this plant has a good safety profile when used in conjunction with other conventional drugs. However, there is a paucity of data regarding the exact molecular mechanism of its action, appropriate form of drug administration, whether whole crude drug or aqueous or

alcoholic extract should be used, toxicological studies and its interaction with other drugs.[5]

The bark of *Terminalia arjuna* has been used for centuries in ayurvedic medicine as cardiotonics for treatment of cardiac disorders. The aqueous extracts of *Terminalia arjuna*-induced cardiotonic action via enhancing SR function, a unique action minimizing the occurrence of arrhythmias, makes arjuna a promising and relatively safe cardiotonic beneficial to the healthy heart and the treatment for chronic heart disease. However, the cellular mechanism of its cardiotonic effect remains undefined.[6]

To provide *in vitro* and *in vivo* evidence, Kapoor *et al.* evaluated the cardioprotective effects of *Terminalia arjuna* at a dose of 500 mg twice a day on classical and immuno-inflammatory markers in coronary artery disease (CAD) as an adjuvant therapy by microarray and in silico analysis in few representative samples. It was observed that *T. arjuna* significantly down-regulated TG, VLDL-C, and immuno-inflammatory markers in stable CAD versus placebo-treated subjects. Microarray and pathway analysis of a few samples from *T. arjuna*/placebo-treated groups and real-time PCR validation further confirmed these observations. It was also demonstrated that the anti-inflammatory and immunomodulatory effects of *T. arjuna* may attenuate ongoing inflammation and immune imbalance in medicated CAD subjects.[7]

There are a lot of studies on arjuna testing its cardioprotective effects. But digoxin induced arrhythmia model in rats has rarely been used. *Terminalia arjuna*, commonly known as *arjuna*, is used for anginal pain, hypertension, congestive heart failure, and dyslipidemia, based on the observations of ancient physicians for centuries. The utility of *arjuna* in various cardiovascular diseases needs to be studied further. Most of the studies, have suggested that the crude drug possessed anti-ischemic, antioxidant, hypolipidemic, and antiatherogenic activities. However, its long-term safety still remains to be elucidated. Though it has been found quite useful in angina pectoris, mild hypertension, and dyslipidemia, its exact role in coronary prevention is yet to be explored.[8,18]

In an *in vivo* model using rats Sing *et al.* studied the effects of butanolic fraction of *Terminalia arjuna* bark on Doxorubicin-induced cardiotoxicity. Extracts of arjuna was administered orally to Wistar rats at different doses (0.42 mg/kg, 0.85 mg/kg, 1.7 mg/kg, 3.4 mg/kg and 6.8 mg/kg) for 6 days/week for 4 weeks. Then all the animals except saline and arjuna-treated controls were administered 20mg/kg Doxorubicin intraperitonially. Co-treatment of arjuna and Doxorubicin resulted in an increase in the cardiac antioxidant enzymes, decrease in serum creatine kinase-MB levels and reduction in lipid peroxidation as compared to Dox-treated animals. It was suggested that butanolic fraction of *Terminalia arjuna* bark has protective effects against Dox-induced cardiotoxicity and may have potential as a cardioprotective agent.[19]

Maulik et al. in a double-blind, randomized controlled trial, studied the Clinical efficacy of water extract of stem bark of *Terminalia arjuna* in patients of chronic heart failure, at a dose of 750 mg or matching placebo twice daily and observed that Arjuna extract was well-tolerated, but did not change left ventricular ejection fraction or secondary outcome measures except preservation of RBC catalase activity compared to placebo. Significantly greater percentage increases occurred in distance covered in 6 min walk test, RBC-superoxide dismutase, RBC catalase, RBC glutathione and in symptom severity and stability domains of Kansas City Cardiomyopathy Questionnaire in patients on Arjuna extract versus those on placebo between subgroups of patients who improved in these outcomes.[20]

It is mainly manufactured as liquid by arista process.[21] Although these herbal drugs have been extensively used, their pharmacological efficacies, i.e. cellular and molecular mechanisms have not been scientifically explored. Moreover, to the best of our knowledge, no scientific evaluation of the safety level of these drugs has been done. The purpose of the present study was to investigate the pharmacological activities and safety profile of Arjunarista collected from the local market and to study the cardiovascular properties of this drug using electrocardiographic and biochemical tests on rat model. This work has a very strong correlation with the healthcare systems in Bangladesh as large number of our population are still dependent on traditional systems of medicines for their primary health care.

MATERIALS AND METHODS

Drug

Arjunarista was purchased from Shree Kundeshwari Oushadhalaya, Dhaka. It was presented as 500 mL in glass bottle.

Dose of the drug

Low dose pretreatment groups received recommended dose of drug (in bottle label), which was calculated for a 70 kg adult and re-estimated for rats to 0.28 mL/kg body weight. High dose refers to ten times of the recommended dose, 2.8 mL/kg body weight.

Instrument

ECG machine (Edan Vet ECG 300, China) with six channels, Humalyzer 3000 (Human, Germany).

Animals

Rats were purchased from animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. A total number of 16 Swiss Albino rats of either sex weighing about 80-120 gm, aged 2 month were used. All the rats were acclimatized to the new environment for a

period of one week. During the experimental period the rats were kept in a well ventilated animal house at room temperature of 25 ^{0}C and supplied with standard pellets purchased from local market and fresh drinking water *at libitum*. The rats were kept in separate cage and maintained with natural 12 h light and 12 h dark cycle in the animal house of the Institute of Nutrition and Food Science, University of Dhaka, Bangladesh.

Animals handling

Prior to the experiment, the rats were randomly divided into 4 groups of 4 rats each. These 4 groups were control group, digoxin control group, normal dose pretreated group and high dose pretreated group. The rats of control group were given normal food and water twice daily. In digoxin control rats digoxin (20 mg/kg, i.p.) was injected at the 36th day. Prescribed dose pretreated group were given normal food and water along with 0.28 mL/kg bw of Arjunarista twice daily for 35 days. At 36th day, digoxin (20 mg/kg bw, i.p.) was injected. Similarly high dose pretreated rats were given normal food and water along with 2.8 mL/kg bw of Arjunarista twice daily for 35 days. At 36th day, digoxin (20 mg/kg, i.p.) was injected. Arjunarista was administered to the rats by intra-gastric syringe between 10 am to 12 am daily throughout the study period. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. The experimental animals were marked carefully on the ear which helped to identify a particular animal. By using identification mark, responses were noted separately for a particular period prior to and after the administration of digoxin and Arjunarista, respectively.

Anesthetic agent

Ketamine HCl (50 mg/kg bw, intraperitoneal).

Recording of ECG tracings

To record ECG tracings, each rat was taken from the animal house to the laboratory. After an hour of acclimatization it was weighed by electronic balance for calculating the dose of ketamine and digoxin. The rat was then anesthetized with ketamine. After the rat was sufficiently anesthetized, it was placed on dissecting board filled with wax and pinned to it by small pins. Then electrodes, dipped in electrode gel, were connected to the designated positions, e.g. two forelimbs, two hind limbs and chest. When the rat was found stable ECG tracings were recorded. Auto readings were taken where the reading of seven leads were obtained together (I, II, III, avR, avL, avF, V). At the same time rhythm II tracings were taken. Auto and rhythm (lead ll) readings were taken every five minutes to detect the changes. For getting print, the machine took 60 sec for sampling. Readings were taken for 60 minutes. At first normal ECG tracings were taken for 30 minutes in the same procedure mentioned above. Then digoxin, at a dose of 20 mg/kg bw, was injected which had been found as arrhythmogenic dose by a trial and error based approach. Intraperitoneal administration of digoxin induced arrhythmia and different changes were recorded for 60 minutes. The above procedure was repeated for every rat.[22,23]

Lipid Profile Test

The rats were sacrificed and blood samples were collected by aorta puncture using 5 mL hypodermic syringe and dispensed into 1.5 mL microcentrifuge Eppendorf tubes. The samples were allowed to stand for 30 minutes at room temperature to clot. Serum for the assays was thereafter separated from the clot by centrifugation at 4000 rpm for 5 minutes. The supernatant, i.e. serum was collected by simple aspiration with Pasteur pipette and transferred into another microcentrifuge tube. All the biochemical determinations were carried out immediately after separation of the serum from the clot. The above procedure was repeated for every rat. Diagnostic kits for the lipid profile (with the exception of low density lipoprotein-cholesterol, LDL-C) were purchased from Exim GmbH (Germany). The assays were performed according to the manufacturer's instruction. LDL-C concentrations were estimated using the methods of Friedewald (1972).[24]:

$$LDL\ \text{cholesterol (mg/dl)} = \frac{\text{Total cholesterol - (HDL cholesterol - Triglyceride)}}{5}$$

Serum total cholesterol, triglyceride, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol were analyzed by using commercial kits that were purchased from Exim GmbH, using Blood analyzer. The atherogenic indices were calculated as follows.[24]:

$$Cardiac Risk Ratio (CRR) = \frac{TC}{HDL - C}$$

$$\text{Castelli's Risk Index (CRI - II)} = \frac{LDL - C}{HDL - C}$$

$$\text{Atherogenic Coefficient (AC)} = \frac{(TC - HDL)}{HDL - C}$$

$$\text{Atherogenic Index of Plasma (AIP)} = \log \frac{TG}{HDL - C}$$

Data Analysis

Data were expressed as mean±S.E.M. Differences in mean values between experimental groups were analyzed by two tailed student's t-test. A probability value less than 0.05 ($p<0.05$) was defined to be significant and probability value less than 0.001 ($p<0.001$) was defined to be highly significant.

RESULTS AND DISCUSSION

Electrocardiographic study

ECG tracings of digoxin control rats

Digoxin induced various types of arrhythmias in the digoxin-treated group of rats such as ventricular flutter, P wave inversion and ventricular fibrillation (**Figure 1**).

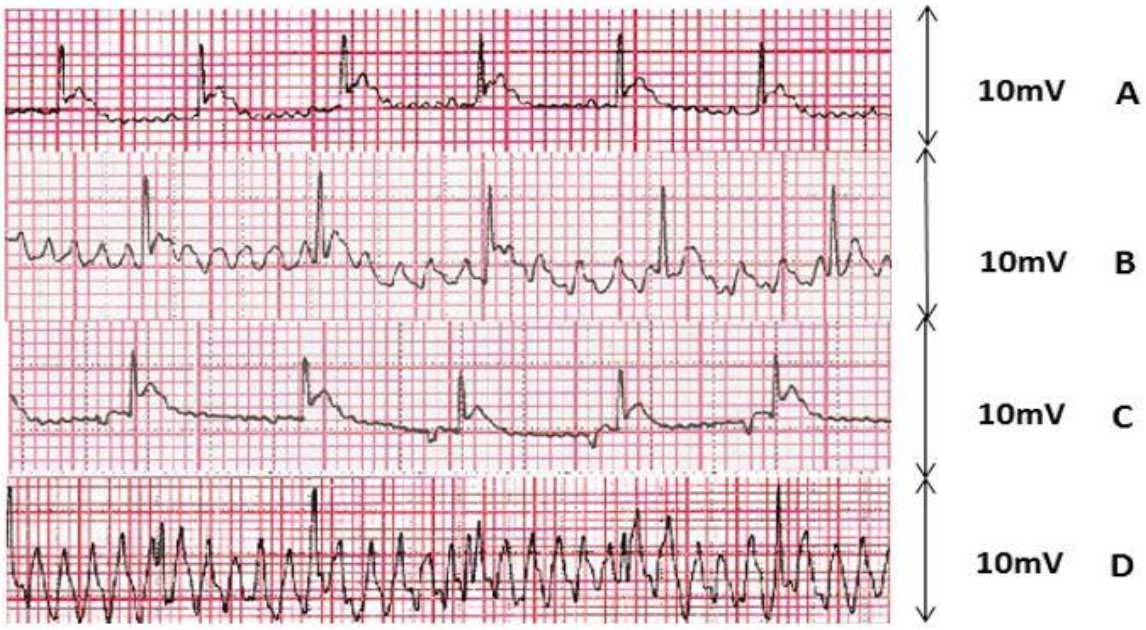

Figure 1: Typical ECG tracings showing various types of changes after digoxin (20 mg/kg). Panel (A) shows normal sinus rhythm (NSR), (B) ventricular flutter, (C) P wave inversion, (D) Ventricular fibrillation. The recording speed from panel A-D is 50 mm/sec. ECG tracings are chosen from one of the four (n = 4) similar and representative experiments.

ECG tracings of Arjunarista normal dose pretreated rats after digoxin injection

In the normal dose (0.28 mL/Kg) pretreated animals, digoxin was administered and the changes in ECG tracings were noted. The result is shown in **Figure 2**.

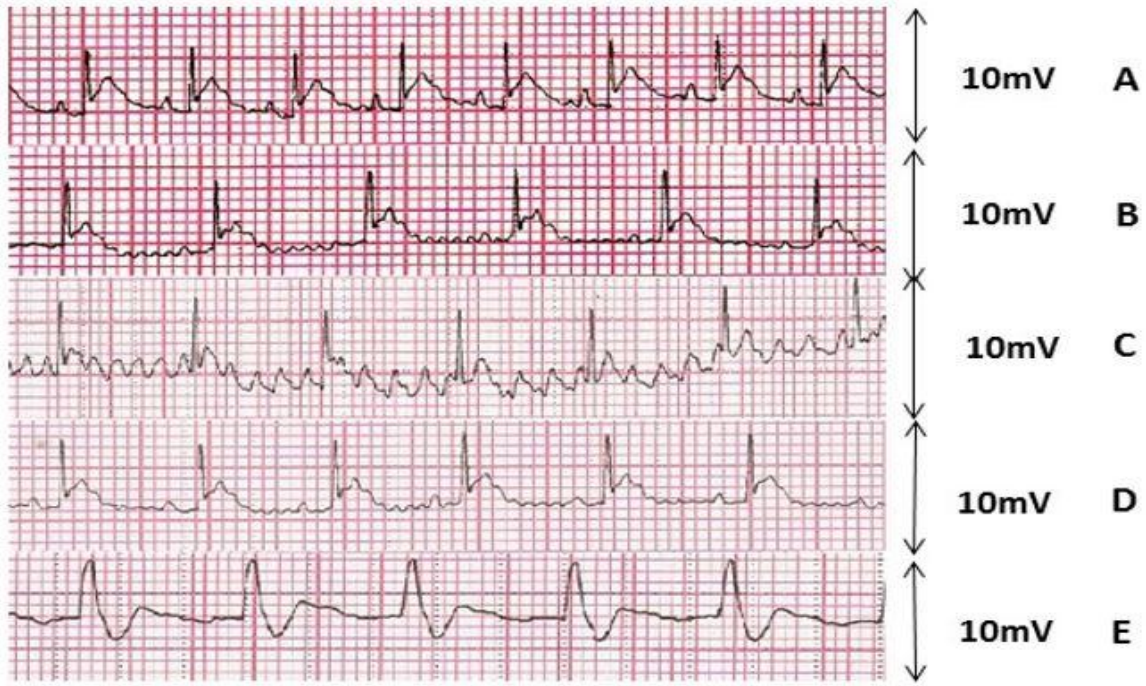

Figure 2: Typical ECG tracings showing various arrhythmic effect of digoxin (20 mg/kg) on Arjuna pretreated rats (normal dose, 0.28 mL/kg). Panel (A) shows normal sinus rhythm (NSR), (B) negative chronotropic effect of Arjunarista before digoxin injection, (C) ventricular flutter, (D) bradycardia, (E) wide QRS syndrome (indication of ventricular abnormality). The recording speed was 50 mm/sec. ECG tracings are chosen from one of the four (n=4) similar and representative experiments.

ECG tracings of Arjunarista high dose pretreated rats after digoxin injection

In the high dose (2.8 mL/Kg) treated animals, digoxin was injected and changes in ECG tracings were noted and presented in **Figure 3.**

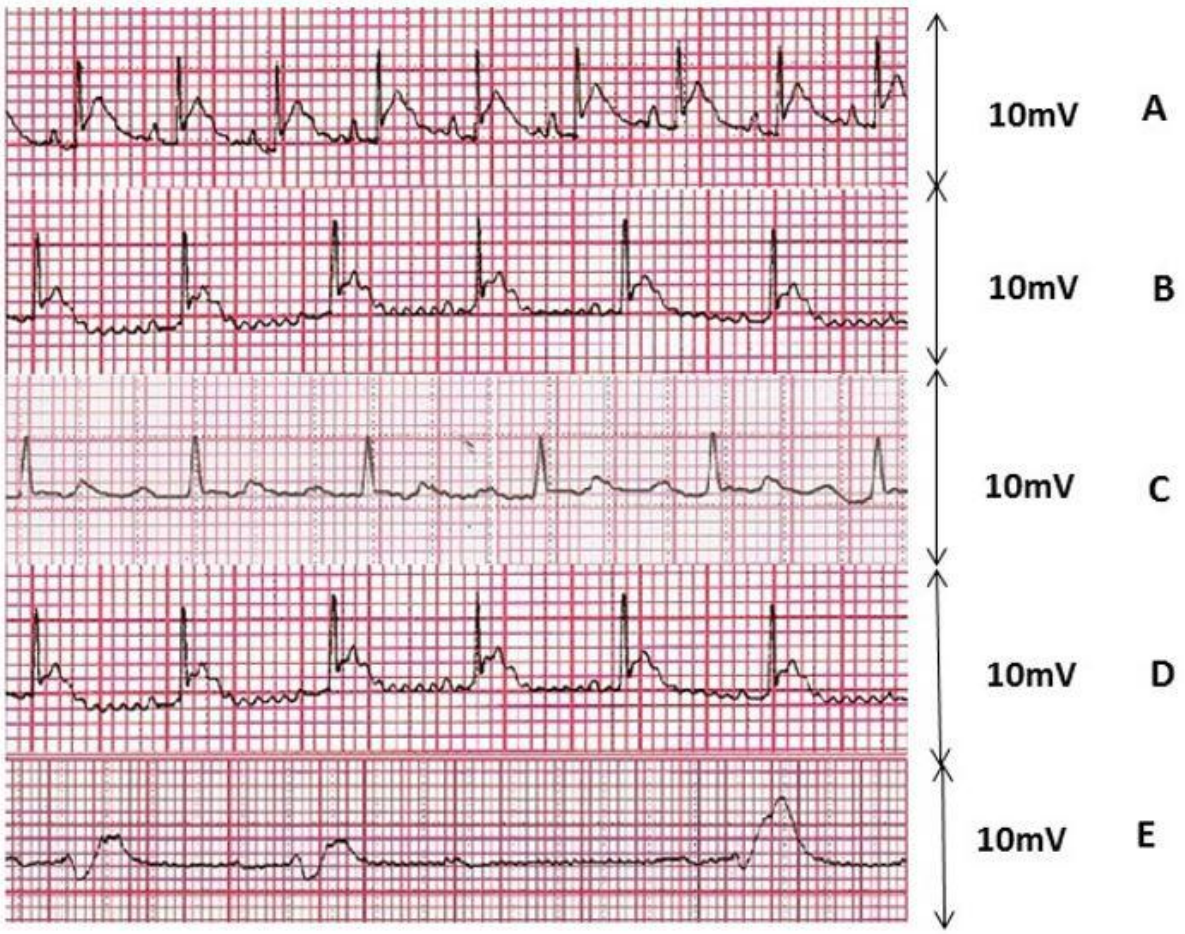

Figure 3: Typical ECG tracings showing various arrhymic effect of digoxin (20 mg/kg) on Arjuna pretreated rats (high dose 2.8 mL/kg). Panel (A) shows normal sinus rhythm (NSR), (B) negative chronotropic effect of Arjunarista before digoxin injection, (C) atrial flutter, (D) bradycardia, (E) ventricular fibrillation. The recording speed was 50 mm/sec. ECG tracings were chosen from one of the four (n=4) similar and representative experiments.

Observed arrhythmias with their duration in Arjunarista pretreated rats after digoxin injection

The duration of various types of cardiac disorders found in ECG tracings after administration of digoxin were calculated and presented in **Figure 4**.

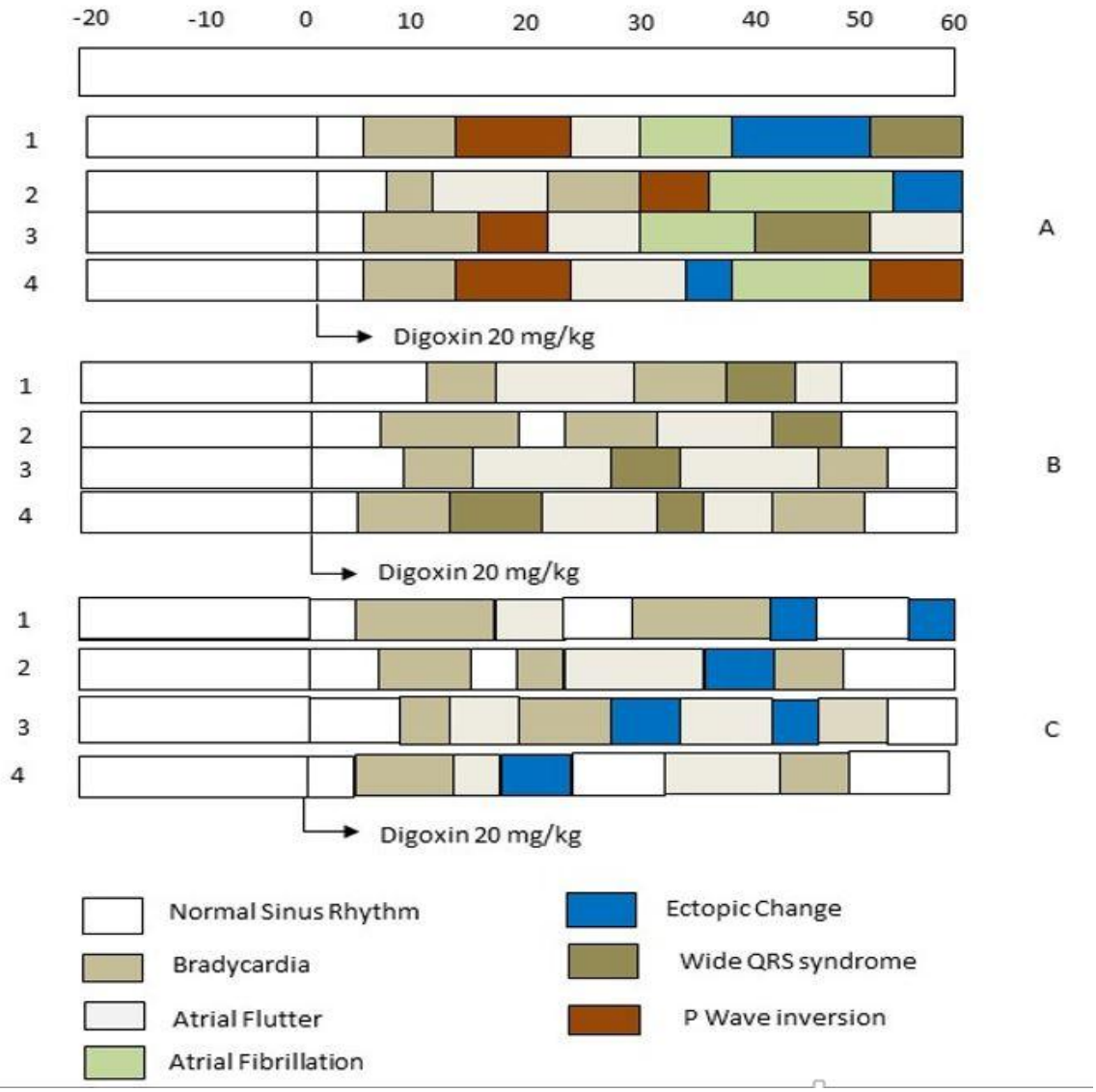

Figure 4: Effect of Arjunarista on digoxin-induced arrhythmia. Panel A-C show each type of arrhythmia with their duration in individual experiment induced by digoxin (20 mg/kg) and effects of normal and high dose of Arjunarista. Panel (A) shows duration of time (min) for the various types of arrhythmias induced by digoxin and their frequent changes from one state to another, panel (B) and (C) show the effects of normal and high doses of Arjunarista on those arrhythmias in each experiment (n=4 for all groups).

It is also evident from the **Figure 4** that the digoxin-induced arrhythmias such as ventricular fibrillation, ventricular flutter, P wave inversion, ectopic change which are frequently transformed to one another, Arjunarista prevented those arrhythmias and the most fatal form of arrhythmias, the VF (ventricular fibrillation) did not appear after treatment with both normal and high doses of Arjunarista.

Changes in heart rate before and after digoxin injection in Arjunarista pretreated rats

In normal dose pretreated rats, the ECG tracings before digoxin injection showed sinus bradycardia (~265 bpm) and the heart rate decreased even further after digoxin administration (~236 bpm). It showed delayed appearance of arrhythmia at 18 minutes (HR varies from 228 to 204) compared to control group (13 minutes). Heart went through different arrhythmic changes and ultimately recovered from arrhythmia by 48 minutes. The heart rate gradually increased and stabilized to 257 bpm by 60 minutes. No P-wave inversion was observed (**Figure 5** and **Figure 6**).

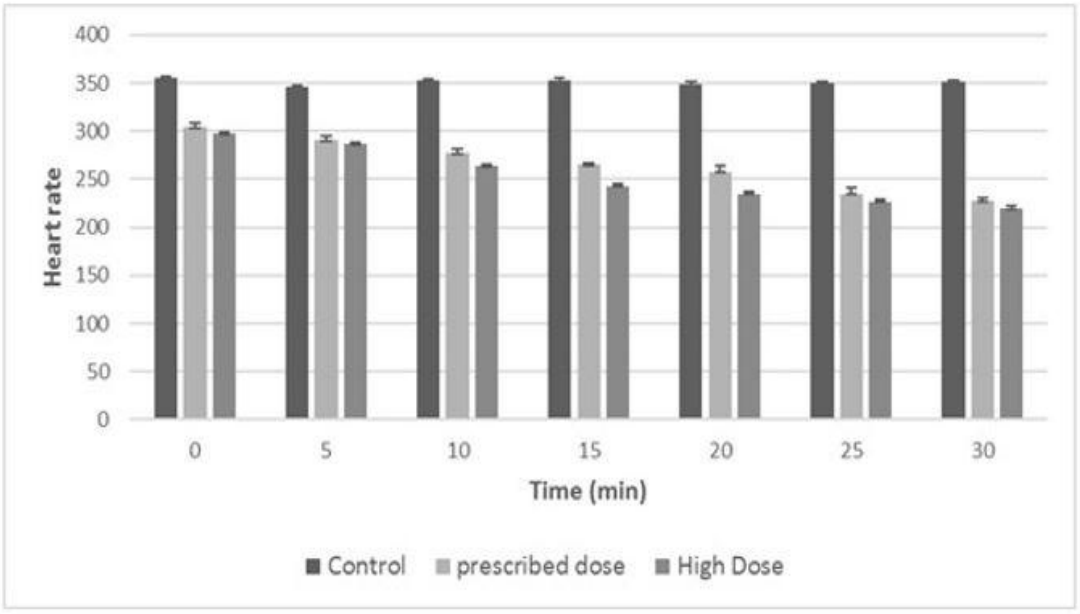

Figure 5: Effect of Arjunarista (normal dose=0.28 mL/kg, High dose=2.8 mL/kg) on heart rate before digoxin injection. ECG tracings before digoxin injection showed sinus bradycardia.

In high dose pretreated rats, the ECG tracings before digoxin injection revealed sinus bradycardia (~254 bpm) and decrease in heart rate after digoxin administration (~244 bpm). It showed delayed appearance of arrhythmia at 25 minutes (HR varies from 212 to 207 bpm), compared to control group (13 minutes) and low dose pretreated group (18 minutes). The duration of normal sinus rhythm was increased. The heart was seen to go through different arrhythmic changes and ultimately recovered from arrhythmia by 45 minutes. Heart rate gradually increased and stabilized to 262 bpm by 60 minutes. No P-wave inversion was observed (**Figure 5** and **Figure 6**).

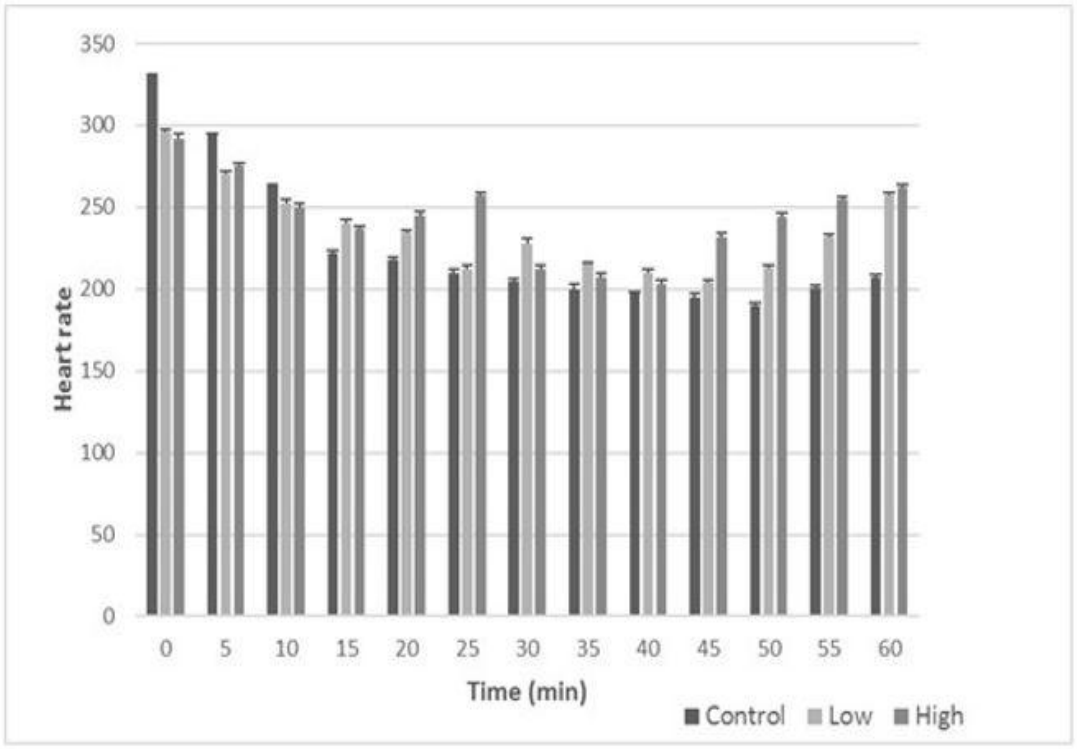

Figure 6: Effect of Arjunarista (normal dose=0.28 mL/kg, High dose=2.8 mL/kg) on heart rate after digoxin injection.

It has been suggested that Arjuna induces myocardial heat shock protein 72 and augments myocardial endogenous antioxidants which offer cardio protection.

Hematological Test for Serum Lipid Profile

Serum total cholesterol, triglyceride, high density lipoprotein-cholesterol and low density lipoprotein-cholesterol were analyzed by using spectrometric assay. **Figure 7** showed the changes in serum lipid profile on rats pretreated with Arjunarista. Control values were 75.5±1.2, 66.9±2.4, 27.7±1.7 and 34.2±2.8 mg/dL for total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein, respectively. After pretreatment with normal dose (0.28mL/kg) of Arjunarista for 35 days these values became 44.7±3.1, 55.03±2.5, 42.55±1.7 and 25.19±2.0 mg/dl and after treatment with high dose (2.8 mL/Kg body) these values changed to 39.98±2.6, 52.13±1.8, 39.4±1.5 and 21.67±3.5 mg/dL for total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein, respectively.

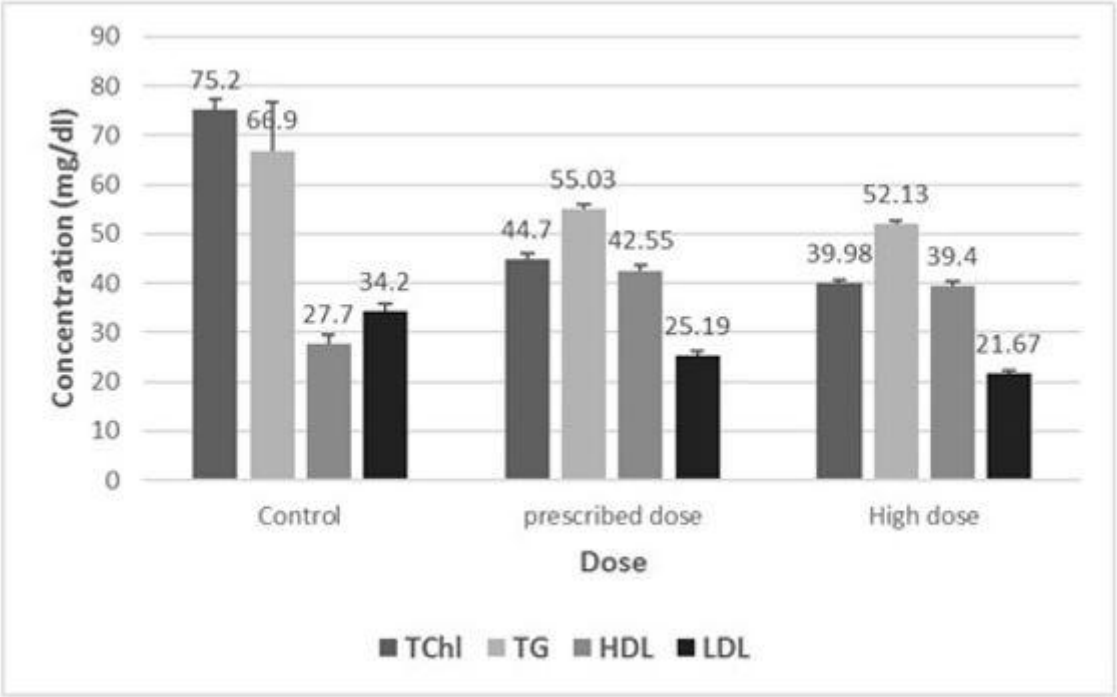

Figure 7: Changes in serum lipid profile of rats pretreated with Arjunarista.

In Arjunarista pretreated rats, there was a significant decrease in the total cholesterol (TC) level at both normal (p=0.0001) and high dose (p=0.0002). The triglyceride (TG) level was increased but statistically not significant (p=0.31 in normal dose and 0.23 in high dose). A statistically significant increase was also observed in HDL-C level in the serum at both doses (p=0.001 in normal dose and 0.005 in high dose). LDL-C level was decreased significantly (p=0.003 in low dose and 0.002 in high dose).

The decrease in total cholesterol may be attributed to possible inhibition of hepatic cholesterol biosynthesis by down regulation of HMG-CoA reductase and inhibiting mevalonic acid pathway.

Atherogenic index

It was observed that Arjuna decreased almost all the atherogenic indices (**Figure 8)** in rats. The decrease in Castelli's Risk Index-II (CRI-II) (p=0.01 in normal dose and 0.009 in high dose), atherogenic coefficient (AC) (p=0.001 in low dose and 0.001 in high dose) and cardiac risk ratio (CRR) (p=0.001 in low dose and 0.0009 in high dose) was statistically significant at both low and high doses. A statistically insignificant decrease in case of Atherogenic Index of Plasma (AIP) was also noticed (p=0.18 in low dose and 0.19 in high dose). The hypolipidemic action of Arjunarista is thought to be mediated through (i) inhibition of hepatic cholesterol biosynthesis, (ii) increased hepatic clearance of cholesterol, (iii) increased fecal bile excretion, (iv) down-regulation of lipogenic enzymes, (v) stimulation of receptor-mediated catabolism of LDL cholesterol and (vi) inhibition of HMG-CoA reductase.

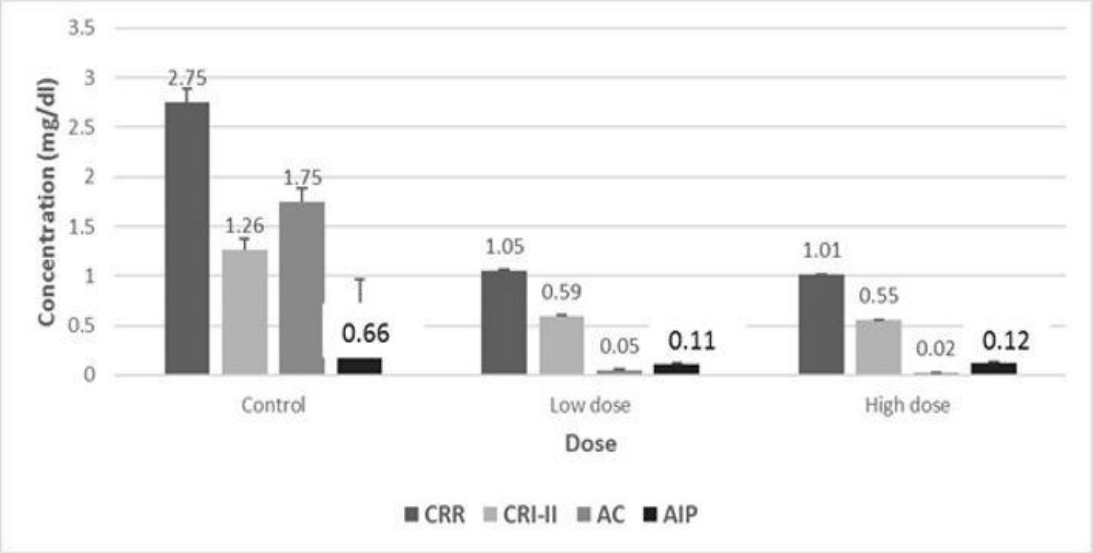

Figure 8: Changes in atherogenic indices of rats pretreated with Arjunarista.

CONCLUSION

The cardio protective effect of the active phyto constituents of *Arjuna*, is probably due to its induction of myocardial heat shock protein 72 which augments myocardial endogenous antioxidants that offer cardio protection. Arjunarista's lipid lowering activity may be attributed to possible inhibition of hepatic cholesterol biosynthesis by down regulation of HMG-CoA reductase.

Ethical Approval

We hereby declare that all experiments were examined and approved by the Ethical Review Committee, Faculty of Pharmacy, University Dhaka, Dhaka-1000, Bangladesh and has, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Authors' Contributions and Consent for Publication

This work was carried out in collaboration among all authors. Authors MSA and AAC designed and wrote the research protocol. Authors SZ, RR equally performed the experiments, managed the literature searches. Authors JAC, SK and AR helped to the experimentations and performed the statistical analysis. Author MSA has taken care of the whole project during the research period. All authors read and approved the final manuscript and give their consent for publication of manuscript in the esteemed journal BMC Complementary and Alternative Medicine.

Availability of Data and Materials

Rats were purchased from animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka.

Arjunarista was purchased from Shree Kundeshwari Oushadhalaya (Shree Kundeshwari Pharmaceutical Company), Dhaka, Diagnostic kits for the lipid profile (with the exception of low density lipoprotein-cholesterol, LDL-C) were purchased from Exim GmbH (Germany), Ketamine HCl was purchased from local market.

ECG machine (Edan Vet ECG 300, China) and Humalyzer 3000 (Human, Germany).

Consent

All authors agreed to submit this article.

ACKNOWLEDGEMENT

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2. The experiments were carried out in the Molecular Pharmacology and Herbal Drug Research Laboratory established through the HEQEP grant (CP no.:3258).
3. Authors are thankful to Professor Dr. Sheikh Nazrul Islam, Institute of Food and Nutrition Science, University of Dhaka, Bangladesh for his kind permission to use their animal house.

Competing Interests

Authors have declared that no competing interests exist.

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