Research Article

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A COMPARATIVE STUDY ON THE CARDIOPROTECTIVE EFFECT OF ARJUNA ARISHTA AND ARJUNA GHRITA: TWO CONVENTIONAL AYURVEDIC FORMULATIONS CONTAINING *TERMINALIA ARJUNA*

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ABSTRACT

In the present study, two Ayurvedic formulations, Arjuna arishta (Partha arishta), Arjuna ghrita were prepared as per traditional Ayurvedic methods and their cardioprotective effect was evaluated on healthy young adult male Wistar rats. Initially, the acute toxicity of prepared formulations was evaluated with three doses – 50 mg.kg^{-1}, 300 mg.kg^{-1} and 2000 mg.kg^{-1}. The cardioprotective effect was evaluated on animals with Isoproterenol induced Myocardial infarction and Digoxin induced arrhythmia. Quercetin treated animal group was used for comparative evaluation. At the end of 15 days long study, blood was collected, processed and utilized for the evaluation of biochemical parameters. Histopathological analysis of heart cells was done to observe the physiological alterations. The results of acute toxicity study of prepared formulations Arjuna arishta and ghrita showed zero percent mortality up to the final dose of 2000 mg.kg^{-1}. Analysis of biochemical parameters and histopathological evaluation strongly supports the cardioprotective effect of formulations, importantly, the Arjuna ghrita revealed more significant results comparing with Partha arishta. The outcome of this study is beneficial and further studies are needed to find out the effects of long-term use of these formulations.

Keywords: Arjuna arishta, Arjuna ghrita, Cardioprotective effect,

INTRODUCTION

Nowadays, globally, Ayurveda remedies gained great interest because of the growing awareness of modern medicines' adverse effects. Ayurveda system comprises different types of formulations including arishtas (a fermented form) Asavas and ghritas (medicated ghee)[1,2]. It is well known that Ayurvedic formulations have a rich history of effectiveness; moreover, modern researches also acknowledged the importance of this system[3].

Coronary heart disease (CHD) is one of the major causes of death in developed countries and disease burden in developing countries[4]. *Terminalia arjuna*, generally known in the name, Arjuna, belongs to the family of Combretaceae[5] is a well-known source plant of Ayurvedic formulations. According to Charaka Samhita, Arjuna is useful in the management of Udarda (urticaria). The Vrindamadhava (9th AD), a medieval compendium recommended the Arjuna for the treatment of Hridroga (heart disease)[6]. Recently, investigations about the mechanism of the cardioprotective effect of Arjuna have shown a dose-dependent regulation of blood pressure and heart rate. There was also a slight increase in the HDL to total cholesterol ratio and an overall improvement in the cardiovascular profile[7]. In the previous study in this laboratory, Partha arishta and Arjuna ghrita – the two conventional Ayurvedic formulations containing *Terminalia arjuna* were prepared as per the traditional Ayurvedic methods and their physicochemical properties and antioxidant activity were evaluated[8]. The present study was designed to evaluate the cardioprotective effect of these formulations.

MATERIALS AND METHODS

Preparation of Arjuna arishta and Arjuna ghrita

Arjuna arishta (Partha arishta) and Arjuna ghrita were prepared as per the standard procedure[8].

Animals

Healthy young adult male Wistar rats (150–200 g) were used for the experiments. They were obtained from the Central Animal House, Sreekrishna College of Pharmacy and Research Centre, Thiruvananthapuram, Kerala, India. All animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The institute animal ethical committee has approved the conduct for the animal experiments (SKCPRC/2014-15/IAEC/10).

Acute toxicity study

The study was designed in reference to the previous literature[9] and carried out as per the guidelines of Organization of Economic Co-Operation and Development (OECD 2001). Animals were randomly selected and divided into two groups of 3 each and kept for seven days prior to dosing to allow for acclimatization to the laboratory conditions. They were maintained in controlled laboratory conditions of 12 h dark/light cycle, 22 ± 3°C temperature and 45-60% humidity. Animals were allowed free access to standard pellet diet produced by Hindustan Animal Feeds and water *ad libitum*. The test substance was administered in a single dose by gavage using stomach tube. Initially, Partha arishta and Arjuna ghrita (50 mg.kg^{-1}) was administered to group-

1 and 2 animals respectively and the number of animals' death was noted after 24 h. The same procedure was repeated with next doses 300 mg.kg^{-1} and 2000 mg.kg^{-1}.

Cardioprotective activity

The cardioprotective effect of prepared Partha arishta and Arjuna ghrita was evaluated on animals with Isoproterenol-induced Myocardial infarction and Digoxin induced arrhythmia. The study was designed in reference to previous literature[10] and all animal experiments were carried out in accordance with the guidelines of the Organization of Economic Co-operation and Development (OECD 2001). Experimental animals were housed in standard environmental conditions like ambient temperature (25 ± 2°C), relative humidity (55 ± 5%) and 12 hours light / dark cycle. The animals were fed with standard pellet diet and water *ad libitum* and allowed to adapt to laboratory conditions for a week prior to the experiments.

In case of evaluation on animals with Isoproterenol-induced Myocardial infarction, selected animals were randomly divided into five groups of six each. Group 1 served as the vehicle control received 1% sodium carboxymethyl cellulose (2 ml; p. o) for 21 days. Group 2 served as the MI control received the vehicle (2 ml; p. o) for 21 days and received Isoproterenol (85 mg.kg^{-1}; i. p.) on 20th and 21st days. Group 3 served as the positive control, treated with Quercetin triturated in 1% sodium carboxymethyl cellulose (50 mg.kg^{-1}; p. o) for the 21 days and received Isoproterenol mixed in normal saline (85 mg.kg^{-1}; i. p.) on 20th and 21st day. Group 4 served as treatment control received Partha arishta (4 ml.kg^{-1}; p. o) for the 21 days and received Isoproterenol (85 mg.kg^{-1}; i. p.) on 20th and 21st day. Group5 served as treatment control received Arjuna ghrita (0.9 ml.kg^{-1}; p. o) for the 21 days and received Isoproterenol (85 mg.kg^{-1}; i. p.) on 20th and 21st day.

In case of evaluation on animals with Digoxin induced arrhythmia, selected animals were randomly divided into four groups of six each and treated for 15 days. Group 1 served as the vehicle control received 1% sodium carboxymethyl cellulose (2 ml; p. o). Group 2 served as the arrhythmia control received the vehicle (2 ml; p. o) and received Digoxin (100 µg.kg^{-1}; p. o) for 15 days. Group 3 served as treatment control received Partha arishta (4 ml.kg^{-1}; p. o) and Digoxin (100 µg.kg^{-1}; p. o) for 15 days. Group 4 served as treatment control received Arjuna ghrita (0.9 ml.kg^{-1}; p. o) and Digoxin (100 µg.kg^{-1}; p. o) for 15 days.

At the end of both evaluations, all animals were anesthetized for blood collection by retro-orbital method. Blood was allowed to clot and centrifuged at 10,000 rpm for 10 minutes to separate the serum from the whole blood and used for the analysis of various biochemical parameters such as marker enzymes and antioxidant enzymes. Following the collection of blood, the animal was sacrificed by cervical decapitation; heart was removed and washed immediately with ice-cold saline. 100 mg of heart tissue was homogenized in 5 ml of 0.1 M Tris Hydrochloride Buffer (pH 7.4) in the ice-cold condition. The homogenate was centrifuged, and the supernatant obtained was used for the estimation of total protein, lactate dehydrogenase and lipid peroxidase. Histopathological analysis of heart cells of animals with Isoproterenol-induced Myocardial infarction was done to observe the physiological alterations.

RESULT

The results of acute toxicity study of prepared formulations Arjuna arishta and ghrita showed zero percent mortality up to the final dose of 2000 mg.kg^{-1}. Effect of Partha arishta and Arjuna ghrita on CPK, cytosolic calcium, and LDH of animals with Isoproterenol-induced MI is shown in Table 1. From the results, it was found that the CPK level was increased (164.00 ± 2.16) in group 2 animals which received Isoproterenol only which were significantly ($p < 0.001$) reduced to 121.00 ± 1.83 by Partha arishta in Group 4 animals and 107.75 ± 2.21 by Arjuna ghrita in Group 5 animals. Similarly, the cytosolic calcium level was increased to 0.39 ± 0.01 in Group 2 animals which were significantly ($P < 0.05$) restored to 0.24 ± 0.02 by Partha arishta in Group 4 animals and 0.22 ± 0.02 by Arjuna ghrita in Group 5 animals. LDH level was increased to 52.00 ± 2.16 in Group 2 animals, which were significantly ($p < 0.01$) reduced to 42.50 ± 1.29 by Partha arishta in Group 4 animals and 41.75 ± 1.50 by Arjuna ghrita in Group 5 animals.

Table 2 revealed the effect of Partha arishta and Arjuna ghrita on LPO, protein and SGOT levels on experimental animals with Isoproterenol-induced MI. The results indicated that the level of LPO was increased to 23.22 ± 6.279 in group 2 animals which received Isoproterenol only which were significantly ($p < 0.01$) reduced to 16.47 ± 1.752^{b} by Partha arishta in Group 4 animals and 17.76 ± 2.598^{b} by Arjuna ghrita in Group 5 animals. The protein level was increased to 0.56 ± 0.266 in Group 2 animals which were significantly ($P < 0.001$) brought back to 0.50 ± 0.36 by Partha arishta in Group 4 animals and 0.38 ± 0.251^{c} by Arjuna ghrita in Group 5 animals. SGOT level was increased to 176.50 ± 9.327 in Group 2 animals, which were significantly ($p < 0.05$) restored to 158.50 ± 7.416^{a} by Partha arishta in Group 4 animals and 141.50 ± 5.196^{a} by Arjuna ghrita in Group 5 animals.

Results of the analysis of the effect of Partha arishta and Arjuna ghrita on CPK, cytosolic calcium and LDH of animals with Digoxin induced arrhythmias showed that the CPK level was increased in Group 2 animals (treated with Digoxin) to 186.31 ± 2.35, which were significantly ($p < 0.001$) reduced to 109.75 ± 5.19 by Partha arishta in Group 3 animals and 103.00 ± 6.33 by Arjuna ghrita in group 4 animals. The cytosolic calcium level was increased in Group 2 animals to 0.42 ± 0.02, which were significantly ($P < 0.05$), restored to 0.13 ± 0.02 by Partha arishta in Group 3 animals and 0.16 ± 0.02 by Arjuna ghrita in group 4 animals. In case of LDH, its level was increased in Group 2 animals to 75.01 ± 1.02, which were significantly ($p < 0.05$), reduced to 45.75 ± 3.59 and 50.00 ± 4.83 in Group 3 and group 4 animals by Partha arishta and Arjuna ghrita respectively.

Evaluation of histopathology of heart cells in normal control group animals (Group 1) showed the presence of normal myocardial cells. The MI control group animals (Group 2) showed scattered myocytic degeneration with some showing cytoplasmic vacuolation. Stroma showed lymphocytic and histiocytic infiltration. Stroma also showed edema and congested vessels. Group 3 animals (positive control treated with quercetin) showed that normal myocytes with no significant pathology. Stroma showed congested vessels. No lymphocytic infiltration/myocytic degeneration. In case of group 4 animals (treated with Partha arishta) showed mild focal myocytic degeneration with infiltration of lymphocytes in the stroma. The focal area showed extravasated RBCs. Group 5 animals (treated with Arjuna ghrita) showed normal myocytes with no significant pathology. Stroma showed mild focal lymphocytic infiltration and congested vessels. No evidence of myocytic degeneration (Figure 1).

Table 1: Effect of Partha arishta and Arjuna ghrita on CPK, cytosolic calcium and LDH of experimental animals with Isoproterenol-induced MI

Analyzed parameters	Group 1	Group 2	Group 3	Group 4	Group 5
CPK (µmol/l)	71.00 ± 1.826^{c}	164.00 ± 2.160	91.75 ± 2.754^{c}	121.00 ± 1.826^{c}	107.75 ± 2.217^{c}
Cytosolic calcium	0.20 ± 0.012	0.39 ± 0.010	0.23 ± 0.029^{a}	0.24 ± 0.016^{a}	0.22 ± 0.018^{a}
LDH	34.00 ± 3.162^{c}	52.00 ± 2.160	43.25 ± 2.754	42.50 ± 1.291^{c}	41.75 ± 1.500^{c}

CPK – Creatine phosphokinase; LDH – Lactate dehydrogenase; Group 1 – Normal control; Group 2 – MI control; Group 3 – Positive control; Group 4 – Treatment control (Partha arishta); Group 5 – Treatment control (Arjuna ghrita); Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by Dunnett's $^{c}P < 0.001$, $^{b}P < 0.01$,$^{a}P < 0.05$

Table 2: Effect of Partha arishta and Arjuna ghrita on LPO, protein and SGOT levels of experimental animals with Isoproterenol-induced MI

Analyzed parameters	Group 1	Group 2	Group 3	Group 4	Group 5
LPO	15.82 ± 5.024	23.22 ± 6.279	20.33 ± 7.545	16.47 ± 1.752^{b}	17.76 ± 2.598^{b}
Protein	0.38 ± 0.281	0.56 ± 0.266	0.39 ± 0.266	0.50 ± 0.359^{c}	0.38 ± 0.251^{c}
SGOT	161.25 ± 6.652^{a}	176.50 ± 9.327	132.50 ± 7.047^{a}	158.50 ± 7.416^{a}	141.50 ± 5.196^{a}

LPO – Lipid peroxidase; SGOT – Serum glutamic oxaloacetic transaminase Group 1 – Normal control; Group 2 – MI control; Group 3 – Positive control; Group 4 – Treatment control (Partha arishta); Group 5 – Treatment control (Arjuna ghrita); Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's $^{c}P < 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$

Table 3: Effect of Partha arishta and Arjuna ghrita on CPK, cytosolic calcium and LDH of experimental animals with Digoxin induced arrhythmia

Analyzed parameters	Group 1	Group 2	Group 3	Group 4
CPK (µmol/l)	71.00 ± 1.826^{c}	186.31 ± 2.35^{c}	109.75 ± 5.188^{c}	103.00 ± 6.325^{c}
Cytosolic calcium	0.20 ± 0.012	0.42 ± 0.012^{a}	0.13 ± 0.022^{a}	0.16 ± 0.017
LDH	34.00 ± 3.162^{c}	75.01 ± 1.02^{a}	45.75 ± 3.594^{a}	50.00 ± 4.830

CPK – Creatine phosphokinase; LDH – Lactate dehydrogenase; Group 1 – Normal control; Group 2 – Arrhythmia control; Group 3 – Treatment control (Partha arishta); Group 4 – Treatment control (Arjuna ghrita); Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA followed by Dunnett's $^{c}P < 0.001$, $^{b}P < 0.01$, $^{a}P < 0.05$

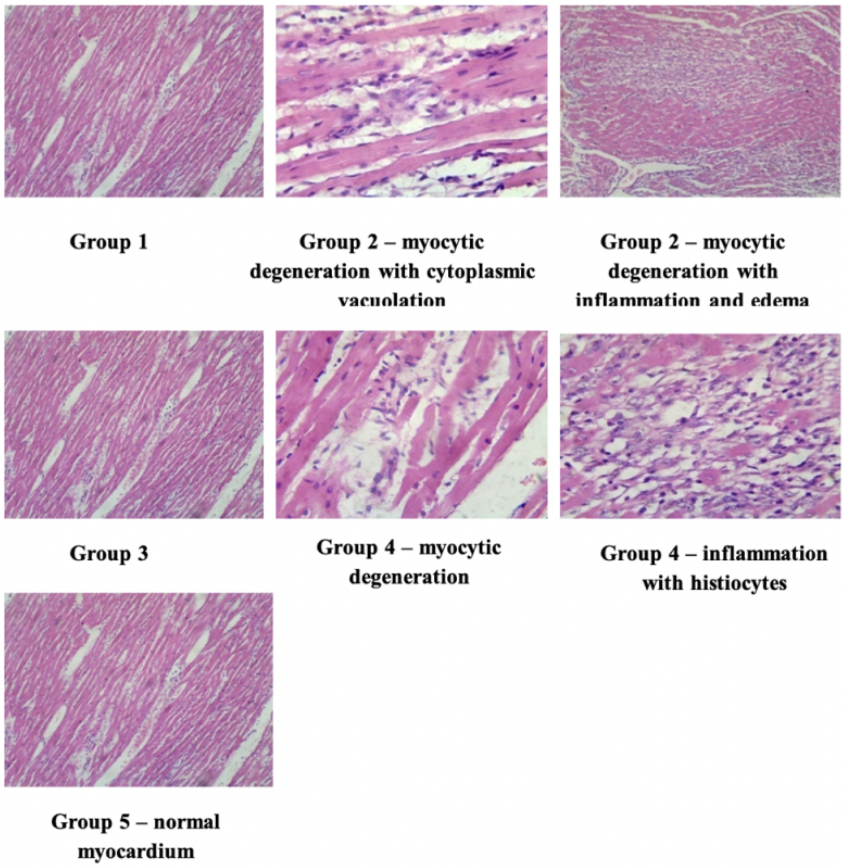

Figure 1: Histopathology of heart cells of experimental animals

DISCUSSION

Nowadays, cardiovascular diseases (CVDs) have become one of the leading causes of mortality in India. Ischemic heart disease and stroke are important causes and responsible for more than 80% of cardiovascular disease deaths[11]. In the present study, the Ayurvedic formulations Arjuna arishta (Partha arishta) and Arjuna ghrita were prepared by traditional Ayurvedic methods and initially, the acute toxicity of these formulations was evaluated which revealed that LD_{50} values of Partha arishta and Arjuna ghrita were high and apparently showed the safety. Evaluation of the effect of Partha arishta in the dose of 4 $ml.kg^{-1}$and Arjuna ghrita in the dose of 0.9 $ml.kg^{-1}$on animals with Isoproterenol-induced MI and Digoxin induced arrhythmia gave significant results compared with control group animals. Analysis of biochemical parameters and histopathological evaluation strongly supports the cardioprotective effect of these formulations, importantly, the Arjuna ghrita revealed more significant results comparing with Partha arishta.

CONCLUSION

In the present study, two Ayurvedic formulations, Arjuna arishta and Arjuna ghrita were prepared as per traditional methods and the cardioprotective effect of these formulations was evaluated on experimental animals with Isoproterenol-induced Myocardial infarction and Digoxin induced arrhythmia which revealed that both the formulations have the cardioprotective effect but the Arjuna ghrita showed significant effect comparing with Partha arishta against myocardial infarction and arrhythmia. However further studies are needed to find out the effects of long term use of these formulations.

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