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PRECLINICAL LIPID PROFILE STUDIES OF A CLASSICAL AYURVEDIC PREPARATION "ARJUNARISTA" AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

Marjana Khalil, Swagata Sarker Lopa, Md. Rakib Hasan*, Md. Mahmudur Rahman Mamun, Paritosh Chakma, Arjyabrata Sarkar, Mohammed Motaher Hossain Chowdhury and M. S. K. Choudhuri

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka - 1342, Bangladesh

Corresponding author e-mail: rakibju38@gmail.com

ABSTRACT

Arjunarista (RJN) is an Ayurvedic preparation used as a traditional medicine in the south Asian countries for the treatment of cardiac diseases. We were eager to know the effect of chronic administration of RJN on the lipid profile test. To find out the effect of chronic administration of RJN on the lipid profile it was administered chronically to the male Sprague-Dawley rats at a dose of 40 ml/Kg. After 28 days of chronic administration of the RJN preparation, the following effects on the lipid profile were noted. There was a very highly significant increase in LDL Cholesterol (p=0.001). A very highly significant (0.001) decrease was noticed in triglyceride (TG), VLDL-C & HDL-C level; thus leading to a statistically very highly significant (p=0.001) increase in Cardiac Risk Ratio (CRR) (TC/HDL-C), Castelli's Risk Index-II (CRI-II) (HDL-C/LDL-C) and Atherogenic Coefficient (AC) [(TC/HDL-C)/HDL-C]], but a statistically significant (p=0.033) decrease in case of Atherogenic Index of Plasma (AIP) (log (TG/HDL-C)).

Keywords: Arjunarista, Lipid profile, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient.

INTRODUCTION

Ayurveda which means 'Science of life' is derived from the Sanskrit words 'Ayur' meaning life and 'Veda' meaning knowledge. It takes an integrated view of the interactions of the physical, mental, spiritual and social aspects of the life of human beings. Ayurveda was first referred to in the Vedas (Rigveda and Atharva Veda 1500 BC). It was originally composed by Agnivesa around 1000 BC and subsequently comprehensively documented in the Charaka Samhita around 300 BC [1]. Ayurveda aims to keep the structural and physiological entities in a state of equilibrium which signify good health. Any imbalance due to internal or external factors may cause disease [2]. Ayurvedic treatment aims to re-establish the equilibrium through various techniques, procedures, regimens, diet and medicines. Ayurvedic treatment consists of drugs, diet, exercise and general mode of life. Ayurveda largely uses plants as raw material for the manufacture of drugs though materials of animal and marine origin, metals and minerals are also used. The safety profile of this drug has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests.

Arjunarishta is one of the ancient liquid oral formulations prescribed for cardiovascular disorders. It nourishes and strengthens heart muscle and promotes cardiac functioning by regulating blood pressure and cholesterol.

The formulation is manufactured by producing a decoction of three plants in specified amounts as listed in AFI (Ayurvedic Formulary of India). Crushed jaggery and the flowers of *Woodfordia fruticosa* are then added and preparation is kept for a specified period of time during which it undergoes fermentation process generating alcohol that helps

extraction of active principles and also serves as preservative for these formulations [3]. Arjunarista is included (page 92) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991). The present study was undertaken to explore the effect of Arjunarista in the lipid profile of rat serum after chronic administration of the drug to male Sprague-Dawley rats.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Arjunarista was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Pharmaceuticals Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 50-70 g. The animals were housed in a well-ventilated clean experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. They were fed with rat chow prepared according to the formula developed at Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided *ad libitum* and the animals maintained at 12 hours day and 12 hours night cycle. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals approved by Ethical Review Committee, Faculty of Life Sciences, Department of Pharmacy, Jahangirnagar University.

Experimental Design
Acute toxicity study: The acute oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modification (OECD Guideline 425) [4]. Sixteen female mice (non-pregnant, 35-40 g body weight) were divided into four groups of four animals each. Different doses (50 ml/Kg, 60 ml/Kg, 70 ml/Kg and 80 ml/Kg) of experimental drug (RJN) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all

the experimental animals were observed for mortality and clinical toxicity signs (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 1, 2, 3 and 4 hours and thereafter once a day for the next three days following RJN administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with RJN and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 28 days. For all the pharmacological studies the drug was administered per oral route at a dose of 40 ml/Kg body weight [5]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 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 experiment 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 [6].

Blood Samples Collection and Preparation of Serum: At the end of 28 days treatment, after 18 hours fasting, blood samples were collected from post vena cava of the rats anaesthetizing with Ketamine (500 mg/Kg body, intra peritoneal) and transferred into plain sample tubes immediately for serum generation [7]. Blood was then centrifuged at 4,000 g for 10 minutes using bench top centrifuge (MSE Minor, England). The supernatant plasma samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hours of sample collection [8].

Determination of Lipid Profile Parameters: Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [9]; total cholesterol (TC) level determined by CHOD-PAP method [10]; LDL-cholesterol level determined by CHOD-PAP method [11]; HDL-cholesterol level determined by CHOD-PAP method [12]. The absorbance of all the tests was determined using Humalyzer, Model No-3500 (Human GmbH, Wiesbaden, Germany).
Serum VLDL and LDL cholesterol concentrations were calculated using the Friedewald equation [13] as follows:

i. **LDL cholesterol** (mg/dl) = Total cholesterol − (HDL cholesterol −Triglyceride / 5)
ii. **VLDL cholesterol** (mg/dl) = Triglyceride / 5.

While the serum non-HDL cholesterol concentration was determined as reported by Brunzell [14]:
Non-HDL cholesterol = Total cholesterol − HDL cholesterol.
The atherogenic indices were calculated as follows:
Cardiac Risk Ratio (CRR) =TC/HDL-C [15].
Castelli's Risk Index (CRI-II) = LDL-C/HDL-C [16].
Atherogenic Coefficient (AC) = (TC -HDL-C)/HDL-C [17].
Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [18].
(Note: for calculation of atherogenic indices mg/dl values of TC, HDL-C, LDL-C and TG were converted into mmol/l).

Statistical Analysis: The data were analyzed using independent sample *t*-test with the help of SPSS (Statistical Package for Social Science) Statistics 11.5 package (SPSS Inc., Chicago Ill). All values are expressed as mean ± SEM (Standard error of the mean) and $p < 0.05$, $p < 0.01$, $p < 0.001$ was taken as the level of significance.

RESULTS
Acute toxicity study: The drug (RJN) administered up to a high dose of 80 ml/Kg produced no mortality of the experimental animals. Thus the LD$_{50}$ (Median Lethal Dose) value was found to be greater than 80 ml/Kg body weight. The animals did not manifest any sign of restlessness, respiratory distress, general irritation or convulsion. Since RJN is in the clinical use for treatment of cardiovascular diseases for many years, a limit test was performed in acute oral toxicity study. According to the OECD test guideline 425 when there is information in support of low or non-toxicity and immortality nature of the test material, then the limit test at the highest starting dose level (80 ml/Kg body weight) was conducted. There were no mortality and toxicity signs observed at 80 ml/Kg body weight. Therefore, it can be concluded that RJN when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Lipid Profile Studies

Effect of RJN on lipid profile of male rats: In the male rats there was a very highly significant increase in the LDL-C level and a very highly significant decrease in the triglyceride (TG) and VLDL-C & HDL-C level in the serum. Statistically not significant increase was noticed in case of total cholesterol (TC) level.
After chronic administration of RJN the total cholesterol level was (9.54%, p=0.128) increased in male rats group which was not statistically significant. In this investigation, a statistically very highly significant (p=0.001) decrease was observed in case of HDL-C (32.53%), VLDL-C (52.61%) and triglyceride (52.61%) level in the RJN treated male rats in comparison to control. A statistically very highly significant (p=0.001) increase was observed in case of LDL-C (175.25%) level in the RJN treated male rats. All the results are presented in Table 2.

Effect of RJN on atherogenic indices of male rats: In this study, RJN augmented almost all the atherogenic indices except Atherogenic Index of Plasma (AIP). The increase in Cardiac Risk Ratio (CRR) (63.55 % increase), Castelli's Risk Index-II (CRI-II) (310.18 % increase) and Atherogenic Coefficient (AC) (135.92 % increase) was statistically very highly significant (p=0.001). A statistically significant (p=0.033) decrease in case of Atherogenic Index of Plasma (AIP) (1611.11 %) was noticed. All the results are presented in Table 3.

DISCUSSION

Effect of RJN on lipid profile of male rats: A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases and is often related with hypertension, obesity and diabetes mellitus [19-21]. In this study, a significantly lower serum triglyceride level was observed in the animals treated with RJN. Low triglyceride levels may be due to hyperthyroidism and mal-absorption syndrome [22].
High level of plasma LDL and VLDL cholesterol are risk factors for cardiovascular disease and often accompany hypertension and obesity [23-26]. In this study, significantly higher plasma LDL and significantly lower VLDL cholesterol levels were observed in the animals treated with RJN.
Reduced serum HDL cholesterol is a risk factor for cardio-vascular disease [27] and is often found in hypertension [20, 25]. So, in the present study, the low serum HDL cholesterol level, recorded for the treated groups is suggestive of the cardio-toxic effect of the drug.

Effect of RJN on atherogenic indices of male rats: In this study, RJN augmented almost all the atherogenic indices except AIP. The increase in Cardiac Risk Ratio (CRR), Castelli's Risk Index-II (CRI-II) and Atherogenic Coefficient (AC) was statistically highly significant. The decrease in

Atherogenic Index of Plasma (AIP) was statistically significant. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular problems and vice versa [16-17].

CONCLUSION

From the above experiment it can be concluded that RJN should not be administered chronically at a higher dose as it increase total cholesterol (TC), LDL-C, almost all atherogenic indices except AIP and decrease HDL-C level. Further studies should be done by reducing the administered dose. Thus Arjunarista is to be taken only at a dosage of six teaspoonfuls mixed with equal quantity of water twice a day usually advised after food.

Table-1: Name of the ingredients/herbs used in the preparation of "Arjunarista" (RJN)

Snaskrit Name	Botanical or Scientific name of Plant	Parts Used	Quantity Used
Partha (arjun)	*Terminalia arjuna*	Stem bark	4.800 kg
Mrdvika (draksa)	*Vitis vinifera*	Dry fruit	2.400 kg.
Madhuka puspa	*Madhuca longifolia*	Flower	960 g
Water for decoction			49.152 L.
Reduced to	-	-	12.288 L
Dhataki	*Woodfordia fruticosa*	Flower	960 g
Guda	-	-	4.800 kg

Table 2: Effect of RJN on lipid profile of rat serum.

Parameters	Control	RJN	p values	% Change
Triglycerides (TG)	74.125±6.06935	35.125±1.74681	0.001	↓52.61%
Total Cholesterol (TC)	57.625±2.91509	63.125±1.6414	0.128	↑9.54%
VLDL-C	14.825±1.21387	7.025±0.34936	0.001	↓52.61%
LDL-C	12.625±2.85318	34.75±2.0244	0.001	↑175.25%
HDL-C	31.125±1.56339	21±1.10195	0.001	↓32.53%

Table 3: Effect of RJN on atherogenic indices of rat serum.

Parameters	Control	RJN	p values	% Change
CRR	1.8780±0.11796	3.0714±0.19706	0.001	↑63.55 %
CRI-II	0.4166±0.09886	1.7088±0.17195	0.001	↑310.18 %
AC	0.8780±0.11796	2.0714±0.19706	0.001	↑135.92 %
AIP	0.0090±0.04540	-0.136±0.04116	0.033	↓1611.11 %

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