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Preclinical Lipid profile studies of "Balarista" after chronic administration to male Sprague-Dawley rats

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Abstract: *Balarista (BLR) is an Ayurvedic preparation used as a traditional medicine in the South Asian countries for the treatment of dyspepsia, debility and arthritis. The effect of chronic administration of Balarista (BLR) on the serum Lipid profile was studied in this experiment. After 28 days of chronic administration of the BLR preparation to the male Sprague-Dawley rats at a dose of 40 ml/kg the following biochemical changes were noted. There was a statistically very highly significant (p=0.001) increase in serum Non-HDL-C and LDL-C level whereas a significant increase (p=0.005) was noted in case of total cholesterol (TC) level. Besides, a statistically very highly significant decrease was noted in case of serum triglyceride (TG), VLDL-C and HDL-C level; thus leading to a statistically very highly significant increase (p=0.001) in Cardiac Risk Ratio (CRR) (TC/HDL-C), Castelli's Risk Index-II (CRI- II) (LDL-C/HDL-C) and Atherogenic Coefficient (AC) ((TC - HDL-C)/HDL-C) but a non-significant decrease (p=0.091) in case of Atherogenic Index of Plasma (AIP) ((log (TG/HDL-C)).*

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

I. Introduction

Ayurvedic medicine also recognized as Ayurveda is one of the world's oldest holistic (whole-body) healing systems. It developed thousands of years ago in India. It is based on the belief that health and wellness depend on a delicate balance between the mind, body and spirit. The primary focus of Ayurvedic medicine is to promote good health, rather than fight disease. Ayurvedic medicines are regarded as a part of complementary and alternative medicine recognized by World Health Organization (WHO), National Institutes of Health (NIH) and others [1]. Ayurvedic treatment aims to restore 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.

Balarishta (BLR) is a liquid Ayurvedic medicine used mainly in the rural area of Bangladesh. It contains 5–10% of self-generated alcohol in it. This self-generated alcohol and the water present in the drug acts as a media to deliver water and alcohol soluble active herbal components to the body. It is used in the treatment of Vata imbalance diseases such as neuralgia, hemiplegia, paraplegia, arthritis etc. It is also a very good tonic. It improves strength of nerves, muscles and bones. Balarista (BLR) is included (page 106) 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). Traditionally this medicine is used to treat dyspepsia, debility and arthritis.

There are reports of heavy metal contamination (such as lead) in herbal preparations resulting in intoxication [2]. The safety profile of these drugs has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs or diagnostic tests. The present study was undertaken to explore the effect of the drug in the lipid profile of rat serum after chronic administration of the drug.

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

Name of ingredients or plants	Botanical or scientific names	Parts used	Amount used
Bala	*Sida cordifolia* Linn.	Root	4800 g
Asvagandha	*Withania somnifera*	Root	4800 g
Water for decoction Reduced to	-	-	49.152 L 12.288 L
Guda	*Saccharum officinarum* Linn.	-	14400 g
Dhataki	*Woodfordia fruticosa* Kurz	Flower	768 g
Payasa	*Fritillaria voylei* H.	Root	96 g
Pancangula	*Ricinus communis* Linn.	Root	96 g
Rasna	*Pluchea lanceolata*	Leaf or Root	48 g
Ela	*Elettaria cardamom*	Seed	48 g

Prasarani	*Paederia foetida Linn*	Leaf	48 g
Devapuspa	*Syzgium aromatic*	Flower bud	48 g
Usira	*Vetiveria zizaniodes Linn.*	Root	48 g
Svadamstra	*Tribulus terrestris Linn*	Fruit	48 g

II. Materials And Methods

Drugs, Chemicals and Reagents

For the toxicological study, Balarista (BLR) 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

Six to 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 60-70 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 modifications (OECD Guideline 425) [3]. Sixteen female mice (non-pregnant, 30-40 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 (BLR) 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 signs of toxicity (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 BLR administration.

Chronic toxicity studies

Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with BLR 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 drugs were administered per oral route at a dose of 40 ml/Kg body weight [4]. 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 tail 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 [5].

Blood Samples Collection and Preparation of Plasma

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 tubes immediately [6]. Blood was let to clot and was then centrifuged at 4,000 *g* for 10 min using bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 12 hour of sample collection [7].

Determination of Lipid Profile Parameters

Lipid profile studies involved analysis of parameters such as triglyceride (TG) level determined by GPO-PAP method [8]; total cholesterol (TC) level determined by CHOD-PAP method [9]; LDL-cholesterol level determined by CHOD-PAP method [10]; HDL-cholesterol level determined by CHOD-PAP method [11]. 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 [12] 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 [13]:
Non-HDL cholesterol = Total cholesterol − HDL cholesterol.

The atherogenic indices were calculated as follows:
Cardiac Risk Ratio (CRR) =TC/HDL-C [14].
Atherogenic Coefficient (AC) = (TC -HDL-C)/HDL-C [15].
Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [16].
Castelli's Risk Index (CRI-II) = LDL-C/HDL-C [17].
(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 Mean) and p<0.05, p<0.01, p<0.001 was taken as the level of significance.

III. Results

Acute toxicity study

The drug (BLR) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD_{50} 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 BLR is in the clinical use for dyspepsia, debility and arthritis treatment 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 BLR when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Lipid Profile Studies

Effect of BLR on Lipid Profile of male rats

In the male rats there was noticeable increase in the total cholesterol (TC), LDL-C, Non-HDL-C level and a very highly significant decrease in the triglyceride (TG), HDL-C and VLDL-C level in the serum.

After chronic administration of BLR the total cholesterol level was (22.78%, p=0.005) increased in male rats group which was statistically significant. In this investigation, statistically very highly significant (p=0.001) increase was observed in case of Non-HDL-C (80.66%) and LDL-C (191.94%) level in the BLR treated male rats in comparison to control. Statistically very highly significant (p=0.001) decrease was observed in case of triglyceride (43.17%), HDL-C (26.51%) and VLDL-C (43.17%) level in the BLR treated male rats. All the results are presented in Table 2.

Table 2: Effect of BLR on Lipid Profile of rat serum.

Parameters	**Control**	**BLR**	**p values**	**% Change**
Triglycerides (TG)	74.125±6.069	42.125±2.022	0.001	↓43.17 %
Total Cholesterol (TC)	57.625±2.915	70.750±2.691	0.005	↑22.78 %
VLDL-C	14.825±1.214	8.425±0.404	0.001	↓43.17 %
LDL-C	12.625±2.853	36.857±1.857	0.001	↑191.94 %
HDL-C	31.125±1.563	22.875±1.260	0.001	↓26.51 %
Non HDL-C	26.5±3.094	47.875±2.894	0.001	↑80.66%

Effect of BLR on Atherogenic Indices of male rats

In this study, BLR augmented almost all the atherogenic indices except Atherogenic Index of Plasma (AIP). The increase in Cardiac Risk Ratio (CRR) (67.92% increase), Castelli's Risk Index (CRI-II) (322.59% increase) and Atherogenic Coefficient (AC) (145.27% increase) was statistically very highly significant (p=0.001). The decrease in Atherogenic Index of Plasma (AIP) (1140%) was not statistically significant (p=0.091). All the results are presented in Table 3.

Table 3: Effect of BLR on Atherogenic Indices of rat serum.

Parameters	Control	BLR	p values	% Change
CRR	1.878±0.118	3.1535±0.20191	0.001	↑67.92 %
CRI-II	0.4166±0.099	1.7605 ±0.17873	0.001	↑322.59 %
AC	0.8780±0.1180	2.1535 ±0.20191	0.001	↑145.27 %
AIP	0.0090±0.045	-0.0936±.03347	0.091	↓1140%

IV. Discussion

Effect of BLR on lipid profile of male rats

Elevated serum total cholesterol level is a familiar and well-known risk factor for developing atherosclerosis and other cardiovascular diseases [18]. Therefore BLR may have been responsible for the hyper-cholesterolemic effect, observed in this study. Numerous studies have presented that non-HDL cholesterol is a better predictor of cardiovascular disease risk than is LDL cholesterol [19-20]. Therefore, the significantly higher plasma non-HDL cholesterol levels observed in the treated groups is indicative of the ability of the drug to increase cardiovascular risk. High level of plasma LDL and VLDL cholesterol are risk factors for cardiovascular disease [21-22] and often accompany hypertension [23] and obesity [24]. In this study, significantly higher plasma LDL and significantly lower VLDL cholesterol levels were observed in the animals treated with BLR.

A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases [25] and is often related with hypertension [26], obesity and diabetes mellitus [27]. In this study, significantly lower serum triglyceride level was observed in the animals treated with BLR. Therefore BLR may have been responsible for the hypo-triglyceridemic effect.

HDL level was decreased in this study which was statistically very highly significant. Reduced serum HDL cholesterol is a risk factor for cardio-vascular diseases [28] and is often found in hypertension [23, 26]. 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 BLR on atherogenic indices of male rats

In this study, BLR augmented almost all the atherogenic indices except AIP. The increase in Cardiac Risk Ratio (CRR), Castelli's Risk Index (CRI-II) and Atherogenic Coefficient (AC) was statistically very highly significant. The decrease in Atherogenic Index of Plasma (AIP) was not 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 [15-16].

V. Conclusion

From the above experiment it can be concluded that BLR should not be administered chronically at a higher dose as it increase Total Cholesterol (TC), LDL-C, Non-HDL-C, almost all atherogenic indices except AIP and decrease HDL-C level. Further studies should be done by reducing the administered dose. Thus Balarista is to be taken only at a dosage of 12–24 mL once or twice a day usually advised after food. If needed, it can be mixed with equal quantity of water.

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