

**PRECLINICAL BLOOD CHEMISTRY SAFETY PROFILE STUDIES OF “BALARISTA” ON THE KIDNEY FUNCTION AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS**

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ABSTRACT

Balarista (BLR) is an Ayurvedic preparation used as a traditional medicine to treat debility in the rural population of Bangladesh. To find out the effect of chronic administration of BLR on serum blood chemistry profile, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 ml/kg for 28 days. In this study, the total protein (TP) content was increased (16.15 %) in BLR treated male rats and it was statistically significant ($p=0.022$). The globulin content was highly significantly ($p=0.003$) increased (62.83 %) and as a result the decrease (45.16%) in the Albumin / Globulin (A/G) ratio was noticed and it was also statistically highly significantly ($p=0.004$) different from their corresponding control. There were also a statistically significant decrease of blood urea nitrogen (BUN) level (22.69% decrease; $p=0.039$) and BUN/Creatinine ratio (26.96% decrease; $p=0.021$).

Keywords: Balarista, Total protein, Blood urea nitrogen, Uric acid, Creatinine

INTRODUCTION

Balarishta (BLR) is a liquid Ayurvedic medicine used mainly in the rural area of Bangladesh to treat debility. 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 debility, dyspepsia, neuralgia, hemiplegia, paraplegia, arthritis etc. It is also a very good tonic. It improves strength of nerves, muscles and bones^[1-5]. 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 and debility^[6-12].

The use of herbal preparations without any standard dosage along with inadequate scientific studies on their safety profile has raised concerns on their toxicity^[13]. That is why; we designed our current experiment to observe the effect of chronic administration of BLR to Sprague-Dawley rats at a high dose. The objective is to have a better understanding of the potential toxicological profile of the drug and to decide how justifiable the use of this drug is under the stated conditions. The study provides directions for further research as well. The research work has been carried out in order to characterize the kidney function profile of the Ayurvedic medicinal preparation, BLR.

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: Eight to ten 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 100-120 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 h day and 12 h 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

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^[14]. 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^[15].

Blood Samples Collection and Preparation of Serum: At the end of the 28 days treatment period, after 18 hour fasting, rats from each group were

anaesthetized by administration (i.p) of ketamine (500 mg/kg body weight)^[16]. Blood samples were collected from post vena cava of rats into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuged at 4000 rpm 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 analysis. All analyses were completed within 12 hours of sample collection^[17].

Determination of Biochemical Parameters: Biochemical analysis was carried out on serum to assess the state of the liver^[18] and kidney^[19]. Biochemical studies involved analysis of parameters such as Total Protein^[20], Albumin by Bromocresol green method^[21], Creatinine^[22], Blood Urea Nitrogen (BUN)^[23] and Uric Acid^[24]. The absorbances of all the tests were determined using Humalyzer Model No-3500.

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 \pm SEM (Standard error of the mean) and $p < 0.05$, $p < 0.01$, $p < 0.001$ was taken as the level of significance.

RESULTS

Daily oral dose of BLR (40 ml/Kg) did not cause any physical abnormalities or death after four week treatment period. In a previous study, Hasan et al (2014) performed the acute toxicity of this drug. Administration of doses up to 80 ml/Kg (the highest dose) produced no mortality of animals which was accompanied by normal physical activity of the tested animals^[25]. This study strengthens the safety of the drug.

Effect of BLR on Total Serum Protein, Albumin, Globulin content and A/G ratio in male rats: After 28 days of chronic administration of the BLR preparation the total protein, albumin content and the calculated ratio of albumin to globulins, termed the A/G ratio in serum were determined in the male rats. In the study, the total protein content in the serum was increased (16.15 %) in the BLR treated male rats. The increase in total protein was statistically significant ($p = 0.022$). On the contrary, the albumin content was decreased (5.83 %) in BLR treated male rats though the decrease was not statistically significant ($p = 0.193$), the globulin content was highly significantly ($p = 0.003$) increased (62.83 % incr.) as a

result the decrease (45.16%) in the Albumin / Globulin ratio was statistically highly significantly different from their corresponding control values ($p=0.004$) (Table-2).

Effect of BLR on Creatinine, BUN, Urea, Uric Acid level in male rats: Kidney function test was performed to measure the creatinine and blood urea nitrogen content in the serum. These two contents can provide information about how effective the kidney function is. There was a statistically insignificant increase in the creatinine (5.80% incr; $p=0.312$) content in serum in the BLR treated male rats. On the contrary, a statistically significant ($p=0.039$) decrease of blood urea nitrogen (BUN) level (22.69% decr.) in the serum was noted in comparison to their control group. The decrease in BUN / Creatinine ratio (26.96 % decr) was also statistically significant ($p=0.021$). It was observed that a negligible 0.71% decrease in serum uric acid content of BLR treated male rats in comparison to their control male rats which was not statistically significant ($p=0.915$).

DISCUSSION

Proteins are important parts of all cells and tissues. The total protein test measures the total amount of two classes of proteins found in the fluid portion of blood: albumin and globulin. Albumin helps prevent fluid from leaking out of blood vessels and globulins are an important part of immune system^[26, 27]. Drugs that can increase total protein measurements include anabolic steroids, androgens, corticosteroids, dextran, growth hormone, insulin, phenazopyridine, and progesterone^[28]. The significant increase of total protein in the BLR treated experimental population can be due to chronic inflammation, adrenal cortical hypofunction, liver dysfunction, hypersensitivity states.

Globulins are the key building block of antibodies. Globulins include gamma globulins (antibodies), beta globulins, alpha-2 globulins, and alpha-1 globulins and a variety of enzymes and carrier or transport proteins. Since the gamma fraction usually makes up the largest portion of the globulins, antibody deficiency should always come to mind when the globulin level is low^[28]. Chronic infections, liver disease (biliary cirrhosis), fatty necrotic liver, kidney dysfunction (Nephrosis), ulcerative colitis, rheumatoid arthritis, leukemia, multiple myelomas, increased amount of nonspecific protein, and autoimmune disorders such as collagen diseases can affect globulin level. The increase of globulin in the

BLR treated experimental population can be due to any of the factors mentioned above.

The liver can function adequately on 20% of liver tissue, thus early diagnosis by lab methods is difficult. A reversed A/G ratio may be a helpful indicator. Normally this ratio exceeds 1.0 but in disease conditions which selectively affect albumin levels, are associated with lesser ratios^[28]. A low A/G ratio may reflect overproduction of globulins such as seen in multiple myeloma or autoimmune diseases or underproduction of albumin such as may occur with cirrhosis or selective loss of albumin from the circulation as may occur with kidney disease (nephrotic syndrome), liver dysfunction. The decrease of Albumin/Globulin ratio (A/G ratio) in the BLR treated experimental population can be due to any of the factors mentioned above.

BUN stands for blood urea nitrogen. The BUN test is often done to check kidney function. BUN increases by 10-20 mg/dl/day if renal function absent. Serum creatinine is a better measure of renal function and BUN is reabsorbed at renal tubules^[29-31]. Decrease of BUN level may be seen in severe liver disease, malnutrition, and sometimes when a person is over-hydrated. A decrease of BUN level may indicate lower risk of kidney disease. BUN-to-creatinine ratio is considered a reliable test that helps in detecting kidney problems. BUN and creatinine are two compounds found in the blood and the amount of these substances is directly governed by the functioning of the kidneys. The principle behind this ratio is the fact that both urea (BUN) and creatinine are freely filtered by the glomerulus, however urea reabsorbed by the tubules can be regulated (increased or decreased) whereas creatinine reabsorption remains the same (minimal reabsorption)^[29-31]. Any dysfunction of the kidneys can increase or decrease the quantity of these compounds in the blood. In this study, Balarista noticeably decrease the BUN level and BUN/Creatinine ratio. So the drug may have nephroprotective effect.

CONCLUSION

From the above data it can be concluded that BLR should not be administered chronically at a higher dose as it may cause liver disease. Further studies should be done by reducing the administered dose. Thus BLR is to be taken under medical supervision 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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Table 1: Name of the ingredients/herbs used in the preparation of Balarista

Name of ingredients	Scientific names	Parts used	Amount used
Bala	<i>Sida cordifolia</i> Linn.	Root	4800 g
Asvagandha	<i>Withania somnifera</i>	Root	4800 g
Water for decoction	-	-	49.152 L
Reduced to			12.288 L
Guda	<i>Saccharum officinarum</i> Linn.		14400 g
Dhataki	<i>Woodfordia fruticosa</i> Kurz	Flower	768 g
Payasa	<i>Fritillaria voylei</i> H.	Root	96 g
Pancangula	<i>Ricinus communis</i> Linn.	Root	96 g
Rasna	<i>Pluchea lanceolata</i>	Leaf or Root	48 g
Ela	<i>Elettaria cardamom</i>	Seed	48 g
Prasarani	<i>Paederia foetida</i> Linn	Leaf	48 g
Devapuspa	<i>Syzygium aromatic</i>	Flower bud	48 g
Usira	<i>Vetiveria zizanioides</i> Linn.	Root	48 g
Svadamstra	<i>Tribulus terrestris</i> Linn	Fruiti	48 g

Table 2: Effect of Balarista (BLR) on Total Serum Protein, Albumin, Globulin content and A/G ratio in male rats

Parameters	Control	BLR	p values	% Change
Total Protein (TP)	44.125±2.1584	44.125±2.1584	0.022	↑16.15 %
Albumin	30.0±1.06904	28.25±0.7008	0.193	↓5.83 %
Globulin	14.125±1.7262	23±1.7928	0.003	↑62.83 %
A/G ratio	2.3447±0.2819	1.2859±0.1135	0.004	↓45.16 %

Table 3: Effect of Balarista (BLR) on Creatinine, BUN, BUN/Creatinine ratio, Uric Acid level in male rats

Parameters	Control	BLR	p values	% Change
Creatinine	0.8625±0.0375	0.9125±0.0295	0.312	↑5.80 %
Blood Urea Nitrogen (BUN)	28.033±1.2914	21.667±1.66	0.039	↓22.69%
BUN/Creatinine	32.5159±1.5063	23.7497±1.8163	0.021	↓26.96%
Uric Acid	1.75±0.0866	1.7375±0.07545	0.915	↓0.71 %

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