



Research Article

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TOXICOLOGICAL SCREENING OF BALAJIRAKADI KVATHA CURNA (BLJ) ON THE BASIS OF LIVER AND KIDNEY FUNCTION IN ALBINO RAT PLASMA

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ABSTRACT

The study was devised to evaluate the effect on liver and kidney in rats plasma after chronic administration of Balajirakadi Kvatha Curna (BLJ), a classical Ayurvedic preparation that was included in the Bangladesh National Formulary of Ayurvedic Medicine 1992. BLJ is widely used in cough. For the toxicological studies the drug was administered per oral route at a dose of 40 ml/kg of the body weight for 45 consecutive days. Eight-week old albino rats (*Rattus norvegicus*) of both sexes, bred and maintained at the animal house in the Department of Pharmacy, Jahangirnagar University, were used for the experiment. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals. Forty rats were randomly grouped into four where one male and one female were treated as control group and others were used as test group. After chronic administration of BLJ to the both male and female rats a statistically very highly significant ($p = 0.001$) decrease of Bilirubin level in the plasma was noted in comparison to their control group. In the male rats there was a noticeable increase ($p = 0.087$) in the Creatinine and a very highly significant ($p = 0.001$) decrease in the Urea content in plasma. In the female rats there was a statistically a very highly significant decrease ($p = 0.001$) in creatinine and urea content in plasma. In addition, there was a statistically a very highly significant increase in the Uric acid content in the plasma of the male rats while a statistically significant decrease in the Uric acid content found in the plasma of female rats.

Keywords: Ayurvedic, albino, Creatinine, chronic, plasma

INTRODUCTION

Ayurvedic medicine aims to integrate and balance the body, mind and spirit. This balance is believed to lead to happiness and health, and to help prevent illness. A chief aim of Ayurvedic practices is to cleanse the body from substances that can cause disease, thus helping to reestablish harmony and balance. Believing this, People avoid expensive and extensive procedures of clinical investigations in many cases and in many areas they have the choice to get treatment at a cheaper price; considering the widespread use of Ayurveda as the popular form of treatment in Bangladesh. One cannot emphasize enough the need for establishing the safety profiles of Ayurvedic drugs¹⁻³. Ayurvedic medicine (also called Ayurveda) is one of the world's oldest medical systems. It originated in India and has evolved there over thousands of years. Ayurvedic medicine is considered complementary and alternative medicine (CAM) and is based on experience since ancient time. These medicines are widely used but some of them are experimentally proved. Ayurvedic medicines are multi-components mixture containing plants, animal derived products, minerals and metals⁴⁻⁷. Keeping this in mind, we are interested to present scientific base of this Ayurvedic formulation, Balajirakadi Kvatha Curna (BLJ) which is widely used in cough⁸⁻⁹. Balajirakadi Kvatha Curna was included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 which was approved by the Government of Bangladesh via Ministry of Health and Family Welfare¹⁰. Balajirakadi Kvatha Curna (BLJ) is combination of ten medicinal plants with a equal ratio (Table 1). We tried to explore a spectrum of its toxicological aspects utilizing experimental animals. The present study is the combined effort where the toxicological effect of BLJ on liver and

kidney function of rats plasma after chronic administration is scrutinized.

MATERIALS AND METHODS

Chemicals and Reagents

Various chemicals and reagents were used. All chemicals and reagents were of analytical grade and these were collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. These chemicals and reagents were prepared with glass-distilled water. The extract (known as kwath) was prepared from dried powder according to the procedure mentioned in Bangladesh National Ayurvedic Formulary (BNAF), 1992. The kwath was prepared by adding 160 ml of distilled water with 5 g of the powder and it was thoroughly mixed to make a uniform suspension, it was then boiled till the volume was reduced to 40 ml and was finally filtered. This filtrate was collected (collection I). Then residue was again boiled with 160 ml of water till the volume was reduced to 40 ml and was then filtered. This filtrate was collection II. The two filtrates (collection I and II) were mixed and reduced to 20 ml and this mixture was known as kwath and was used for the toxicological study. For the toxicological experiment, the Kwath was administered at a volume such that it would permit optimal dosage accuracy.

Formulary of Balajirakadi Kvatha Curna (BLJ)

For the preparation of Balajirakadi Kvatha Curna (BLJ), ingredients were taken as per Table 1 with their classified family and botanical name. Which parts and what amount were used are listed in the Table 1.

Route of Administration

For the toxicological studies, the drug was administered per oral route (p. o.) at a dose of 40 ml/kg of the body weight. 500 mg/kg of Ketamine were administered intraperitoneal (i. p).

Management of Experimental Animal

Eight-week old albino rats (*Rattus norvegicus*: Sprague - Dawley strain) of both sexes were bred and maintained at the Animal House of the Department of Pharmacy, Jahangirnagar University. These animals were used in the toxicological experiment. These were apparently healthy and weighed 50 - 70 g.

Animal Care

The animals were housed in a well ventilated hygienic experimental animal house under constant environmental and adequate nutritional conditions throughout the period of the experiment. All of the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done by *ad libitum*, along with drinking water and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka, Bangladesh). Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular rat prior to and after the administration could be noted separately.

Controls

A group of equal number of rat as the drug treated group was simultaneously employed in the experiment. They were administered with distilled water as placebo as per the same volume as the drug treated group for the same number of days and this group served as the control. Prior to the experiment, they were randomly divided into 4 groups of 10 animals according to sex. Thus ten rats were taken for each group for both control and the experimental group.

Toxicological experiment

After acclimatization, administration of the Ayurvedic medicinal preparation was done by intra-gastric syringe. Administration of the extract was between the hours of 10 am and noon. At the due of the 45-days treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration, the animals were anaesthetized using intra peritoneal Ketamine (500 mg/kg). Blood samples were collected from post vena cava and transferred into heparinized tubes immediately. Blood was then centrifuged at 4,000 g for 10 minutes using bench top centrifuge (MSE Minor, England) to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteurized pipette and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection. All other reagents and chemicals that were used in this work were of analytical grade and were prepared in all glass-distilled water. All experiments on

rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals.

Determination of Total Bilirubin, Creatinine, Urea and Uric acid

Biochemical analysis was carried out on serum, to assess the state of the liver and kidney. Biochemical studies involved analysis of parameters such as bilirubin, creatinine, blood urea nitrogen (BUN) and Uric acid. The method of Evelyn and Malloy (1938) was employed to determine the serum bilirubin concentration of the samples. The procedure of Tietz *et al* (1994) was used to determine serum creatinine concentration while the serum urea concentration was determined by the method of Kaplan (1965). For working procedure only one blank is required for a series of tests¹¹⁻¹³.

Statistical Analysis

The group data were expressed as Mean \pm SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 11) was applied for the analysis of data. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001 . These are denoted as accordingly * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ¹⁴ (noticeable, high and very high significant respectively).

Test procedure for Total Bilirubin

For sample blank 1000 μ l blank reagents, for tests 500 μ l DCA (Dichloroaniline) and 500 μ l NIT are mixed and allowed to wait at least 15 minutes at room temperature protected from light. Then 100 μ l of Serum/Plasma for sample Blank and 100 μ l of Serum/Plasma for test sample were mixed well and then incubated at 20°C to 25°C for 10 minutes. Finally, read the result with Analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

Test Procedure for Creatinine

For standard 500 μ l of Picric acid and diluted NaOH 500 μ l, for test sample 500 μ l of Picric acid and diluted NaOH 500 μ l were mixed well and allowed to wait for 5 minutes at 37°C. 100 μ l of standard and 100 μ l of Serum/Plasma were mixed well and read immediately after 30 sec, recorded the absorbance, A_1 . Then read and recorded the absorbance A_2 exactly after 2 minutes. Finally, read the result with Analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

Test Procedure for Urea

Only one blank is required for a series of tests. enzyme reagent 1a is the enzyme concentrating 1 ml + 100 ml Reagent-1 But, For one test enzyme concentrating 10 μ l + 1 ml reagent -1(1 ml = 1000 μ l). For blank 1000 μ l enzyme reagent - 1a (Above proportionate to enzyme + reagent - 1) and for standard 1000 μ l of enzyme reagent - 1a with 10 μ l of standard reagent were mixed and incubated for 5 minutes at room temperature or 3 minutes at 37°C. For sample 1000 μ l enzyme reagent - 1a and 10 μ l of Sample (Serum) were mixed and incubated for 5

minutes at room temperature or 3 minutes at 37° C. Then 1000 µl (1 ml) of reagent -2 was mixed and incubated for 10 minutes at room temperature and 5 minutes at 37°C for each blank, standard and sample. Finally, read the result with analyzer/Colorimeter/Spectrophotometer against Reagent Blank (Normal Range: 10 - 50 mg/dl).

Test Procedure for Uric Acid

Only one blank is required for a series of tests. For blank 1000 µl total protein reagents, for test sample 1000 µl of total protein reagents and 20 µl of Serum/Plasma and for standard 1000 µl total protein reagents with 20 µl of protein standard are mixed and incubated at 20°C to 25°C for 10 minutes. Finally, read the result with analyzer/Colorimeter/Spectrophotometer against Reagent Blank.

Table 1: Formulary of Balajirakadi Kvatha Curna (BLJ)

Ayurvedic/ Traditional Name	Parts Used	Botanical Name	Family	Amount used
Bala	Root	<i>Sida cordifolia</i>	Malvaceae	1 Part
Jiraka	Fruit	<i>Cuminum cyminum</i>	Apiaceae	1 Part
Bilva	Root	<i>Aegle mermelos</i>	Rutaceae	1 Part
Abda	Rhizome	<i>Cyperus rotundus</i>	Cyperaceae	1 Part
Vrsa	Root	<i>Adhatoda vasaka</i>	Acanthaceae	1 Part
Visva (sunthi)	Rhizome	<i>Zingiber officinalis</i>	Zingiberaceae	1 Part
Suradruma (devadaru)	Heart Wood	<i>Cedrus deodara</i>	Pinaceae	1 Part
Guha (salaparni)	Pulp	<i>Pseudarthria viscida</i>	Fabaceae	1 Part
Iksu	Root	<i>Saccharum officianarum</i>	poaceae	1 Part
Laja	Stem	<i>Lathyrus japonicas</i>	Fabaceae	1 Part

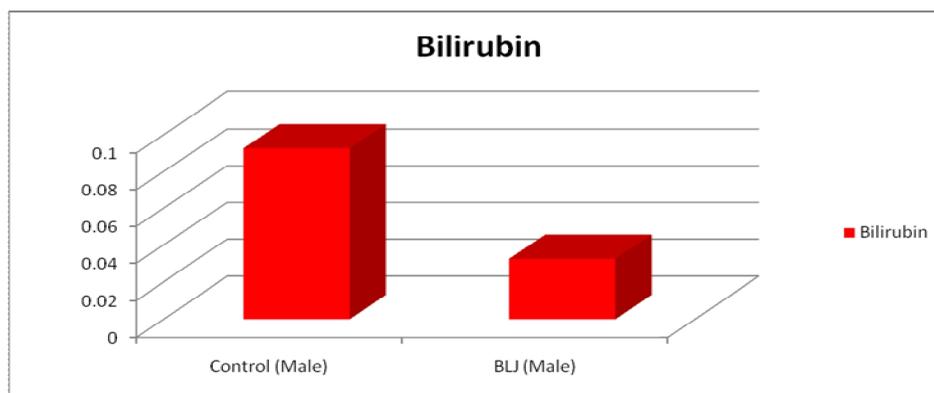


Figure 1: Comparative presentation of liver function test (Bilirubin) of Balajirakadi Kvatha Curna (BLJ) between control group male rats and drug treated male rats

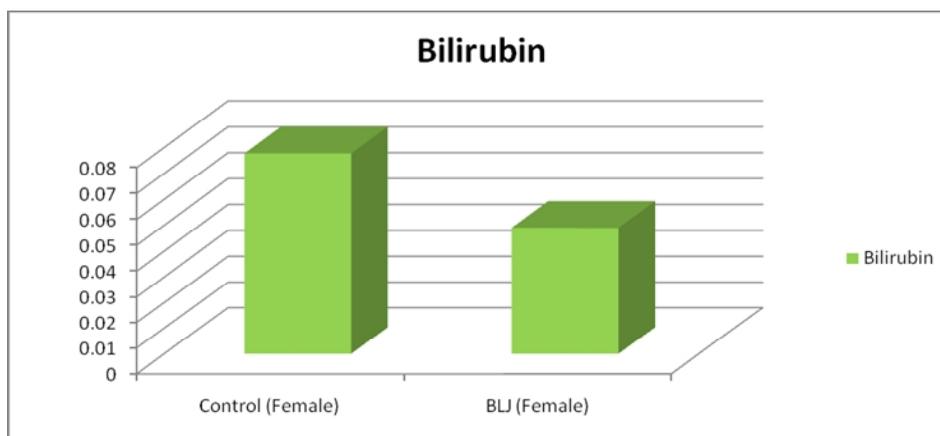


Figure 2: Comparative presentation of liver function test (Bilirubin) of Balajirakadi Kvatha Curna (BLJ) between control group female rats and drug treated female rats

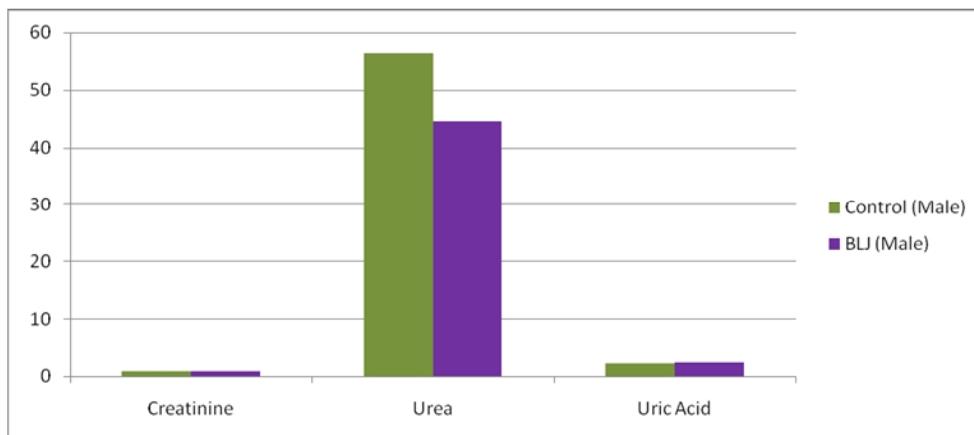


Figure 3: Comparative presentation of kidney function test (Creatinine, Urea and Uric acid) of Balajirakadi Kvatha Curna (BLJ) between control group male rats and drug treated male rats

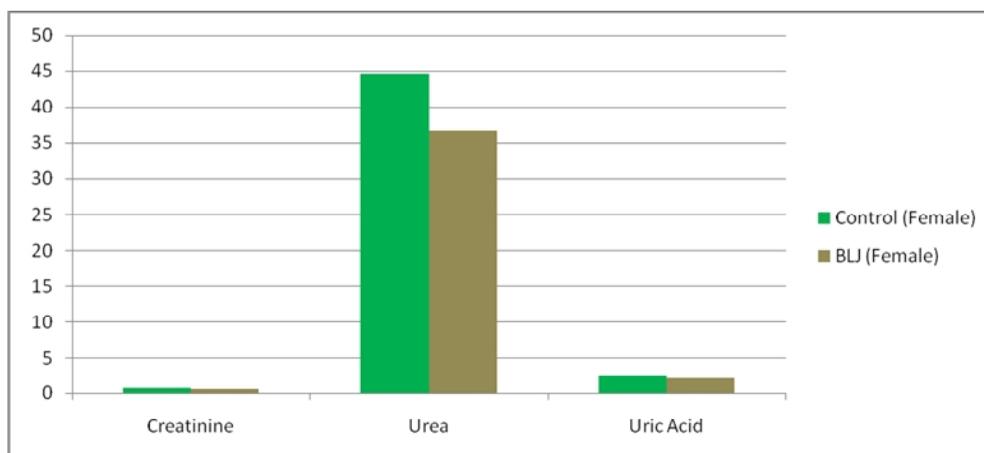


Figure 4: Comparative presentation of kidney function test (Creatinine, Urea and Uric acid) of Balajirakadi Kvatha Curna (BLJ) between control group female rats and drug treated female rats

RESULT AND DISCUSSION

Balajirakadi Kvatha Curna (BLJ) was studied for its toxicological aspects after chronic administrations for 45 consecutive days.

Liver function test (Male and Female)

After chronic administration of BLJ to the male rats a statistically very highly significant ($p = 0.001$) % decrease of Bilirubin level in the plasma was noted in comparison to their control group. There was a statistically a highly significant decrease in bilirubin content in plasma in the female rats. There was a statistically a highly significant decrease in bilirubin content in plasma in the female rats. Result is showed in Figure 1 and 2.

Kidney function test

Serum Creatinine and Urea

In the male rats there was an increase in the creatinine and a decrease in the Urea content in the plasma. Though the increase in creatinine was not statistically significant yet it was noticeable ($p = 0.087$). In the male rats there was a statistically very highly significant ($p = 0.001$) decrease in the Urea content in the plasma. In the female rats there was a statistically a very highly significant decrease in the creatinine and urea content in the plasma. There is a

statistically very highly significant ($p = 0.001$) decrease in the Urea content in the plasma. Results are shown in Figure 3 and 4.

Serum Uric acid

Most uric acid dissolves in blood and travels to the kidneys, where it passes out in urine. If the body produces too much uric acid or doesn't remove enough if it, one can get sick. High level of uric acid in the body is called hyperuricemia. High levels of uric acid can also cause gout. In the male rats there was a statistically very highly significant increase in the Uric acid content in the plasma. In the female rats there was a statistically significant decrease in the Uric acid content in the plasma. Results are shown in Figure 3 and 4.

CONCLUSION

By the evaluation of toxicological study of Balajirakadi Kvatha Curna we get some significant result in both male and female rats in case of liver function and kidney function. After chronic administration of BLJ to the both male and female rats a statistically very highly significant decrease of bilirubin and urea level in the plasma was noted in comparison to their control group. Though the increase in creatinine level change was not statistically

significant yet it was noticeable. Uric acid content behaved very strangely, highly significant rise in male while significant fall in female. However a few observed results are not congruent in case of both male and female rats which may trigger out extensive further investigation to decipher more valid result to claim exemption from discrepancy. Since many years in the Asian subcontinent Ayurvedic medicine has been used for treatment of many diseases but till now the scientific authenticity of this type of traditional medicine is not completely identified and investigated. Our study may attribute to this exploration and realization of scientific basis of such type commonly used Ayurvedic medicine.

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