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PRECLINICAL BLOOD CHEMISTRY SAFETY PROFILE STUDIES OF "BASANTA KUSUMAKAR RAS" AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Basanta Kusumakar Ras (BKR), an Ayurvedic preparation is used as a traditional medicine in the treatment of Diabetes mellitus. We were eager to know the effect of chronic administration of BKR on the lipid profile, liver function test and kidney function test. To find out the toxicological characteristic of BKR, it was administered chronically to the male Sprague-Dawley rats at a dose of 400 mg/kg. After 28 days chronic administration of the BKR preparation the following toxicological changes were noted. There was a statistically significant increase in serum total cholesterol (TC) and LDL-C level besides a statistically significant decrease in serum HDL-C level; thus leading to a statistically significant increase of both Cardiac Risk Ratio (TC/HDL-C) and Castelli's Risk Index (LDL-C/HDL-C). There was a statistically significant increase in both bilirubin and creatinine level. Chronic BKR administration revealed dyslipidemia, possible loss of liver function and kidney function.

Keywords: Basanta Kusumakar Ras, Cardiac Risk Ratio, Atherogenic Index of Plasma, Atherogenic Coefficient, Ayurvedic formulation.

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 signifies good health. Any imbalance due to internal or external factors may cause disease [2]. 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.

Diabetes mellitus (Madhumeha) was known to ancient Indian physicians and an elaborate description of its clinical features and management appears in Ayurvedic texts [3]. Ayurvedic practitioners treat diabetes with a multi-pronged approach, using diet modification, Panchkarma to cleanse the system, herbal preparations, breathing exercises and yoga. The herbs which are used to treat diabetes include shilajit, turmeric, neem, *coccinea indica*, amalaki, triphala, bitter gourd, aloe vera, leaves of bilva, cinnamon, gymnema, fenugreek, bay

leaf and rose apple. Decoctions of triphala, fenugreek and Shilajit are commonly used. Powders (Churana) used include Amalaki Churna, Haldi powder (Turmeric powder) and Naag Bhasma [4]. The Ayurvedic preparations 'Basanta Kusumakar Ras'and 'Chandra prabhavati' are believed to lower sugar levels [5]. Proprietary Ayurvedic medications are also used to treat diabetes.

It is postulated that Ayurvedic medications may act through potential pancreatic as well as extra pancreatic effects. The probable mechanisms of action include: delaying gastric emptying, slowing carbohydrate absorption, inhibition of glucose transport, increasing the erythrocyte insulin receptors and peripheral glucose utilization, increasing glycogen synthesis, modulating insulin secretion, decreasing blood glucose synthesis through depression of the enzymes glucose-6-phosphatase, fructose-1, and 6-bisphosphatase and enhanced glucose oxidation by the enzyme glucose-6-phosphatase-dehydrogenase pathway [6].

Basanta Kusumakar Ras (BKR) is an ayurvedic formulation which is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (enlarged 2nd edition 2011) (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). It is indicated in the treatment of Diabetes mellitus (Madhumeha in Ayurvedic terminology) [7]. There are reports of heavy metal contamination (such as lead) in herbal preparations resulting in toxicity [8]. There are also reports of spurious herbal products contaminated with oral hypoglycemic agents which could lead to adverse effects, such as hypoglycemic episodes [9]. The safety profile of these drugs has not been fully investigated. It is also not clear, whether these preparations might interact with other drugs.

That is why we designed our present study to explore the following effect of the drug: protein parameters, lipid profile, liver function test, kidney function test and uric acid level of rat plasma after the chronic administration of BKR to the male Sprague-Dawley rats.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Basanta Kusumakar Ras (BKR) was collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Experimental Animals: Six to eight-week old male Albino rats (*Rattusnorvegicus*; Sprague-Dawley strain), 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 hygienic 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 hour day and 12 hour 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, 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, TG 425 with minor modifications [10]. Sixteen female mice (non-pregnant, 25-30 g body weight) were divided into four groups of four animals each. Different doses (1000 mg/kg, 2000 mg/kg, 3000 mg/kg and 4000 mg/kg) of experimental drug (BKR) were administered by stomach tube. Then all the experimental animals were observed for mortality and clinical signs of toxicity (general behaviour, 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 BKR administration. Body weight was recorded daily. LD_{50} cut-off value of BKR was determined in accordance with Globally Harmonised System of Classification and Labelling of chemicals [11].

Chronic toxicity studies: Prior to the experiment, Rats were randomly divided into two groups of ten animals. One group was treated with BKR 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 45 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 400 mg/kg body weight [12]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between the 10 am and 12 pm 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 response were noted separately for a particular rat prior to and after the administration [13].

Blood Samples Collection and Preparation of Plasma: At the end of 45 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 heparinized tubes immediately [14]. Blood was then centrifuged at 4,000 *g* for 10 min 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 [15].

Determination of Biochemical Parameters: Biochemical studies involved analysis of parameters such as total protein (TP) concentration was determined by Biuret method [16]; albumin was determined based on its reaction with bromocresol green (Binding method) [17]; triglyceride (TG) level was determined by GPO-PAP method [18]; total cholesterol (TC) level was determined by CHOD-PAP method [19]; LDL-cholesterol level was determined by CHOD-PAP method [20]; HDL-cholesterol level was determined by CHOD-PAP method [21]; total bilirubin was determined by Jendrassik and Grof method [22]; creatinine was determined by the Jaffe's reaction method [23] and Uric acid level measured by Uricase/PAP method [24]. The absorbances of all the tests were determined using spectrophotometer (UV-visible Spectrophotometer Model No. UV-1601 PC).

Plasma VLDL and LDL cholesterol concentrations were calculated using the Friedewald equation [25] as follows:

i. LDL cholesterol (mg/dL) = Total cholesterol – HDL cholesterol – (Triglyceride / 5)
ii. VLDL cholesterol (mg/dL) = Triglyceride / 5.

While the plasma Non-HDL cholesterol concentration was determined as reported by Brunzell [26]: Non-HDL cholesterol = Total cholesterol − HDL cholesterol.

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) =TC/HDL-C [27].
Atherogenic Coefficient (AC) = (TC -HDL-C)/HDL-C [28].
Atherogenic Index of Plasma (AIP) = log (TG/HDL-C) [29].
Castelli's Risk Index (CRI-II) = LDL-C/HDL-C [30].
(Note: for calculation of atherogenic indices we have converted mg/dl values of TC, HDL-C, LDL-C and TG into mmol/L)

Statistical Analysis: The data were analyzed using unpaired t-test as described by Glasnapp and Poggio with the help of SPSS (Statistical Package for Social Science) Statistics 17.0 package (SPSS Inc., Chicago Ill) [31]. 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.

RESULT AND DISCUSSION

Ayurvedic medicines have achieved greater importance as an alternative to conventional therapy. To enhance the safe use of a plant-based medicine, one should take into account their historical applications on humans and animals as well as toxicity evaluation of the medicinal herbs and their active components [32]. Many screening methods are employed to determine the safety and efficacy of these ayurvedic medicines and also to establish the active component of the herbal products [33].

Acute toxicity study: The drug (BKR) administered up to high dose (4000 mg/kg) produced no mortality of experimental animals. No significant difference in body weight gain was also observed. Thus the LD_{50} value was found to be greater than 4000 mg/Kg body wt. The animals did not manifest any sign of restlessness, respiratory distress, general irritation, coma or convulsion. With reference to the Globally Harmonised System of Classification and Labelling of chemicals, BKR can be classified as Category 5 and this provides direct relevance for protecting human and animal health. Since BKR is in clinical use for diabetes mellitus treatment for many years, a limit test was performed in acute oral toxicity study. According to the OECD test guideline 423 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 (4000 mg/Kg body weight) was conducted. There were no mortality and toxicity signs observed at 4000mg/Kg. BKR can be classified under category-5 and LD_{50} value was greater than 4000mg/Kg in accordance with Globally Harmonized System of Classification and Labelling of chemicals and this provides us a direct relevance for protecting human and animal health. Therefore, it can be concluded that BKR when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic toxicity studies

Effect of BKR on plasma protein of male rate: In the study, the total protein content in the plasma was decreased (4.197 %), p=0.361 in the BKR treated male rats. The result showed no significant difference between the control and the BKR treated groups. Interestingly, the albumin content was increased (3.154 %), p=0.565 and the globulin content was decreased (13.986 %), p=0.276 in BKR treated male rats. The Albumin / Globulin ratio was increased (4.096%), p=0.800. None of the increase or decrease noted in the parameters was statistically significantly different from their corresponding control value (Table-2). These proteins are important liver function markers. Plasma albumin is well known to decrease in response to inflammation [34].

Effect of BKR on lipid profile of male rate: In the male rats there was all throughout increase in the triglyceride level, total cholesterol, VLDL-C, LDL-C, Non HDL-C and all the atherogenic indices. The only exception was a decrease in HDL-C content in the plasma.

After chronic administration of BKR the triglyceride level was (3.569 %, p=0.727) increased in male rats group which was not statistically significant. In this investigation a statistically significant increase (23.219%, p=0.008) of plasma total cholesterol level in the BKR treated male rats was observed in comparison to control (Table-3). Also an increase in the VLDL-C and LDL-C and Non HDL-C content in the plasma was noted. Statistically insignificant increase was observed in case of VLDL-C (3.569%, p=0.727) but a significant increase was observed in case of Non HDL-C (32.41%, p=0.003) and a very highly significant increase was observed in case of LDL-C (42.528%, p=0.001) from their corresponding control. Elevated plasma total cholesterol level is a familiar and well-known risk factor for developing atherosclerosis and other cardiovascular diseases [35]. Therefore BKR may have been responsible for the hyper-cholesterolemic effect, observed in this study. High level of plasma LDL and VLDL cholesterol are risk factors for cardiovascular disease and often accompany hypertension and obesity [36-39]. In this study, significantly higher plasma LDL cholesterol levels were observed in the animals treated with BKR, representing the cardio-toxic effect of BKR. Numerous studies have presented that non-HDL cholesterol is a better predictor of cardiovascular disease risk than is LDL cholesterol [40, 41]. 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.

HDL level was decreased 46.54% in this study which was statistically significant (p=0.012). Reduced plasma HDL cholesterol is a risk factor for cardio-vascular diseases [42] and is often found in hypertension [38, 43].

In this study, BKR augmented almost all the atherogenic indices. The increase in Cardiac Risk Ratio (CRR) (123.45 %) was statistically very highly significant (p=0.001) and the increase in Castelli's Risk Index- II (CRI-II) (139.19 % incr.) was statistically very highly significant (p=0.001). The increase in Atherogenic Index of Plasma (AIP) (60.98%) was statistically significant (p=0.034) and statistically very high significant increase (140.53%, p=0.001) of Atherogenic Coefficient (AC) was also observed. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular complications and vice versa [28, 29].

Effect of BKR on liver function tes:The liver function test was performed to assess the state of liver by determining the plasma bilirubin level. After chronic administration of BKR to the male rats a statistically highly significant (p=0.002) increase of bilirubin level (165.237% incr.) was noted in comparison to their control group. According to Naganna [44] increase in bilirubin indicates the abnormal liver function which may be the result of higher synthetic function of the liver. So, increase level of bilirubin in BKR treated male rats indicates that it has toxic effect on liver. As reported by [45] Basanta Kusumakar Ras was found to have adverse effects on organs like kidney, liver and heart of the mice.

Effect of BKR on kidney function test: Creatinine is a breakdown product of creatine, which is an important part of muscle. The laboratory test is performed to measure the amount of creatinine in the blood. The test is done to evaluate kidney function. If kidney function is abnormal, creatinine levels will increase in the blood [46-52]. There was a statistically significant increase in the creatinine (37.154% incr.) (p=0.011) content in plasma in the BKR treated male rats. So, after chronic administration of BKR, it may cause kidney problem.

CONCLUSION

From the above data it can be concluded that BKR should not be administered chronically as it increase Triglyceride, VLDL-C, LDL-C, Non-HDL-C and decreases HDL-C and it also increases Bilirubin, creatinine and Uric acid. Further studies should be done by reducing the administered dose. Thus

Basanta Kusumakar Ras is to be taken under Medical Supervision only at a dosage of 125–250 mg once in the morning, before or after food which is traditionally administered along with honey, sugar or ghee. It should not be used more than 1–2 months based on doctor's prescription. Self medication with this medicine may prove to be dangerous, since it contains heavy metal ingredient.

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Table 1: Name of the ingredients/herbs used in the preparation of Basanta Kusumakar Ras (BKR).

Name of ingredients	Parts used	Botanical or scientific names	Amount used
Hataka (svarna) bhasma	Calx.	Gold	9.62 mg
Candra (rajata) bhasma	Calx.	Silver	9.62 mg
Vanga (bhasma)	Calx.	Tin	14.42 mg
Ahi (naga) bhasma	Calx.	Lead	14.42 mg
Kantaka (kantaloha) bhasma	Calx.	Iron	14.42 mg
Abhra (bhasma)	Calx.	Mica	19.23 mg
Pravala (bhasma)	Calx.	Coral	19.23 mg
Mauktika (bhasma)	Calx.	Pearl	19.23 mg
Gavyadugdha	Milk	Cow milk	Q.S. (for bhavana)
Iksu rasa (St.)	Stem	*Saccharum officinarum*	Q.S. (for bhavana)
Vasa rasa (Lf)	Leaf	*Adhatoda vasica*	Q.S. (for bhavana)
Laksa rasa (Exd.)	Exudate	Lacciferlacca	Q.S. (for bhavana)
Udicya (sugandha balaka) rasa (Rt.)	Root	*Andropogan vetiveria*	Q.S. (for bhavana)
Rambha kanda (kadali kanda) rasa (St. Tr.)	Stem	*Musa paradisiacal*	Q.S. (for bhavana)
Sata patra prasunaka rasa (gulaba puspa svarasa)	Flower ext	*Nelumbium speciosum*	Q.S. (for bhavana)
Malatikusumodaka (Fl.)	Flower	*Jasminum grandiflorum*	Q.S. (for bhavana)
Mrgamada (kasturi)	Exudate	Musk	4.81 mg (for bhavana)

Table 2: Effect of BKR on protein parameter of rat.

Parameters	Control	BKR	p values	% Change
Total Protein (TP)	5.45 ± 0.14	5.22 ± 0.20	0.361	↓4.197268%
Albumin	2.93 ± 0.12	3.03 ± 0.10	0.565	↑3.15431%
Globulin	2.51 ± 0.20	2.16 ± 0.24	0.276	↓13.98624%
A/G	1.27 ± 0.16	1.32± 0.11	0.800	↑4.09597%

Table 3: Effect of BKR on lipid profile of rat.

Parameters	Control	BKR	p values	% Change
Triglycerides (TG)	35.33 ± 2.40	36.59 ± 2.61	0.727	↑3.57%
Total Cholesterol (TC)	47.18 ± 3.15	58.14 ± 1.74	0.008	↑23.22%
HDL-C	6.29 ± 0.87	3.36 ± 0.35	0.012	↓46.54%
Non HDL-C	1.08±.085	1.43±.053	0.003	↑32.41%
LDL-C	34.13 ± 1.20	48.64 ± 1.76	0.001	↑42.53%
VLDL-C	7.07 ± 0.48	7.32 ± 0.52	0.727	↑3.57%
CRR	8.23±1.16	18.39±1.87	0.001	↑123.45%
CRI-II	6.43±1.08	15.38±1.73	0.001	↑139.19%
AIP	0.41±0.08	0.66±0.08	0.034	↑60.98%
AC	7.23±1.16	17.39±1.87	0.001	↑140.53%

Table 4: Effect of BKR on liver function of rat.

Parameters	Control	BKR	p values	% Change
Bilirubin	0.0653 ± 0.01347	0.1732± 0.02706	0.002	↑165.237%

Table 5: Effect of BKR on kidney function of rat.

Parameters	Control	BKR	p values	% Change
Creatinine	0.4737 ± 0.05201	0.6497 ± 0.01720	0.011	↑37.1543%
Uric acid	1.2018±0.18996	1.6933±0.66096	0.463	0.463

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