

Hepatotoxicological Studies of an Ayurvedic Medicine “Brihat Khadir Batika” on Biological System of Male Sprague–Dawley Rats

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ABSTRACT

“Brihat Khadir Batika” (BKD) is an ayurvedic preparation used as a traditional medicine in gingivitis in the rural population. To find out the toxicological characteristics of BKD on liver function, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg. After 28 days of chronic administration of the BKD preparation, the following effects on the hepatic function were noted. In this experiment, plasma protein, albumin, serum bilirubin, and various serum enzymatic parameters were evaluated. The results of the liver function tests are given below. There is a very highly statistically significant ($P=0.001$) decrease in the total protein in the serum of the male rat [12.67% decrease] and statistically insignificant ($P=0.558$) [2.24%] increase was observed in the albumin level in the serum of the male rat. There is a statistically highly significant ($P=0.002$) decrease in the globulin level in the serum of the male rat [28.44% decrease]. The albumin: globulin ratio is found to be statistically highly significant ($P=0.007$) increase in the serum of the male rat [39.04% increase]. The bilirubin level in the serum of the male rat is increased by [12.50%], the increase though not significant, yet it was prominent ($P=0.170$). The serum ALT level of the male rat is found to increase, which is statistically significant ($P=0.023$) [35.15% increase]. There is an [9.58%] increase in the serum AST level of the male rat, the increase though not significant, yet it was prominent ($P=0.231$). Moreover, there is a statistically significant ($P=0.027$) decrease in the serum LDH level of the male rat [35.76% decrease]. The serum alkaline phosphatase level of the male rat is found to show statistically insignificant ($P=0.514$) [9.32%] decrease level. There is a negligible [2.20%] increase in the serum GGT level of the male rat, which was statistically not at all significant ($P=0.892$). From the study on ratio of different enzyme activities it was noted that change in ALT was the most prominent, followed by AST and the GGT.

Keywords: Brihat Khadir Batika, Ayurvedic preparation, Gingivitis, Toxicological characteristic.

INTRODUCTION

Hepatotoxicity exposes chemical-driven liver damage and acute and chronic liver diseases are caused by drug-induced liver injury. The liver plays a significant role in transforming and removing chemicals and is efficient to the toxicity from these agents. Certain medicinal agents, if taken in large doses and sometimes even when established within therapeutic ranges, may damage

the organ. More than 900 drugs have been found in causing liver injury [1], so that it is commonly seen many drugs are being withdrawn from the market. Drug-induced liver injury is accountable for all hospital admissions and acute liver failures about 5% and 50%, respectively [2,3].

As herbal and homeopathic mediations, complementary and alternative medicines have been used dating as far back as 2100

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BC in ancient China and India. Their use has created a \$180 billion market in United States [4]. Ayurvedic medicines have great reputation for preventing number of diseases and currently, large-scale use of herbal (Unani and Ayurvedic) medicines has been officially recognized and recommended as an alternative system of medicine by the World Health Organization (WHO), particularly in the developing countries to deliver health care services at the primary health care level [5]. Day by day, ayurvedic medicines are getting more popular for its good therapeutic and safety profile [6]. According to WHO, approximately 1.5 billion people of the world are now getting treatment with these medicines [6,7]. Although drug-induced liver injury (DILI) are caused by some herbal medications, there are many others that may be implicated.

Brihat Khadir Batika (BKD) is an ayurvedic and herbal medication which is used for mouth ulcer, pharyngitis (sore throat) and other diseases of teeth, gums, tongue and throat. The formulation BKD contains Khadira Saar (Katha)—Catechu as main ingredients [8,9]. BKD 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).

MATERIALS AND METHODS

Drugs, chemicals, and reagents

For the toxicological study, BKD 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 were 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 are maintained at 12-hour day and 12-hour night cycle. All experiments on rats were carried out in absolute

Table 1: Name of the ingredients/herbs used in the preparation of Brihat Khadir Batika (MUKHAROGA)

Name of ingredients	Scientific/common names	Parts used	Amount used
Khadirasara	<i>Acacia catechu</i>	Heart wood	4.800 kg
Arimeda	<i>Acacia farnesiana</i>	Stem bark	9.600 kg
Water for decoction			49.152 L
Water for decoction		Reduced to	12.288 L
Candana (svetacandana)	<i>Santalum album</i>	Heart wood	12 g
Usira	<i>Vetiveria zizanoids</i>	Root	12 g
Manjistha	<i>Rubia cordifolia</i>	Stem	12 g
Dhataki	<i>Woodfordia fruticosa</i>	Flower	12 g
Ghana (musta)	<i>Cyperus rotundus</i>	Rhizome	12 g
Yastyahva (yastimadhu)	<i>Glycyrrhiza glabra</i>	Root	12 g
Tvak (St. Bk.)	<i>Cinnamomum zeylanicum</i>	Stem bark	12 g
Ela	<i>Elettaria cardamomum</i>	Seed	12 g
Padma (kamala)	<i>Nelumbo nucifera</i>	Flower	12 g
Kesara (nagakesara)	<i>Mesua ferrea</i>	Flower	12 g
Laksa	<i>Persicaria odorata</i>	Exd.	12 g
Rasanjana	<i>Berberis aristata</i>		12 g

Mamsi (jatamamsi)	<i>Nardostachys jatamansi</i>	Rhizome	12 g
Haritaki	<i>Terminalia chebula</i>	Fr Patel	12 g
Bibhitaka	<i>Terminalia bellirica</i>	Fr Patel	12 g
Amalaki	<i>Phyllanthus emblica</i>	Fr Patel	12 g
Lodhra	<i>Symplocos racemosa</i>	Stem bark	12 g
Balaka (hrivera)	<i>Pavonia odorata</i>	Root	12 g
Haridra	<i>Curcuma longa</i>	Rhizome	12 g
Daruharidra	<i>Berberis aristata</i>	Stem	12 g
Phalini (priyangu)	<i>Callicarpa macrophylla</i>	Flower	12 g
Samanga (lajjalu)	<i>Mimosa pudica</i>	Plant	12 g
Katphala	<i>Terminalia bellirica</i>	Flower	12 g
Vaca	<i>Acorus calamus</i>	Rhizome	12 g
Yavasa	<i>Alhagi maurorum</i>	Root	12 g
Agaru	<i>Aquilaria malaccensis</i>	Heart wood	12 g
Pattanga	<i>Caesalpinia sappan</i>	Root	12 g
Gairika	Oxide of iron (Fe ₂ O ₃)		12 g
Anjana (srotanjana)			12 g
Lavanga (Fl.)	<i>Syzygium aromaticum</i>	Flower	48 g
Nakha	<i>Martynia annua</i>		48 g
Kakkola (kankola)	<i>Piper cubeba</i>	Rhizome	48 g
Jatikosa (jatipatri)	<i>Myristica fragrans</i>	Ar.	48 g
Karpura	<i>Cinnamomum camphora</i>	Exd.	192 g

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) [10]. Sixteen male mice (30-35 g body weight) were divided into 4 groups of 4 animals each. Different doses (1000, 2000, 3000, and 4000 mg/kg) of the experimental drug (BKD) were administered by a stomach tube. The dose was divided into 2 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 3 days following BKD administration.

Chronic toxicity studies

Before the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with BKD 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 [11]. After acclimatization, ayurvedic medicinal preparation was administered to the rats by intragastric syringe

between 10 am and 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 before and after the administration [12].

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) [13]. 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 minutes 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 [14].

Determination of biochemical parameters

Biochemical analysis was carried out on serum to assess the state of the liver [15]. Biochemical studies involved analysis of parameters such as total protein, albumin, globulin, bilirubin, ALT, AST, ALP, LDH, GGT were done with available kits (Dhaka City Hospital, Dhaka, Bangladesh). 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 IL, USA). 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

Acute toxicity study

The drug (BKD) 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 LD50 value was found to be greater than 4000 mg/kg body weight. 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, BKD can be classified as Category 5 and this provides direct relevance for protecting human and animal health. Since BKD 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) were conducted. There were no mortality and toxicity signs observed at 4000 mg/kg. BKD can be classified under category-5 and LD50 value was greater than 4000 mg/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 BKD when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic toxicity studies

BKD is an ayurvedic preparation used as a traditional medicine in gingivitis in the rural population. To find out the effect of BKD on liver function, it was administered chronically

Table 2: Effect of Brihat Khadir Batika on different liver parameters

Parameters	Control	BKD	P	Overall output (%)
Total protein	54.25 \pm 1.01	47.38 \pm 0.92	0.001	decr 12.67
Albumin	27.88 \pm 0.78916	28.50 \pm 0.68	0.558	incr 2.24
Globulin	26.38 \pm 1.76	18.88 \pm 0.88	0.002	decr 28.44
A/G	1.11 \pm 0.11	1.54 \pm 0.09	0.007	incr 39.04
Bilirubin	0.20 \pm 0.00	0.23 \pm 0.02	0.17	incr 12.5
ALT	41.25 \pm 4.11	55.75 \pm 3.89	0.023	incr 35.15
AST	107.12 \pm 4.51	117.38 \pm 6.84	0.231	incr 9.58
ALP	140.88 \pm 14.22	127.75 \pm 13.50	0.514	decr 9.32
LDH	89.13 \pm 0.12	57.25 \pm 4.61	0.027	decr 35.76
GGT	11.38 \pm 1.03	11.63 \pm 1.49	0.892	incr 2.20

to the male rats at a dose of 40 mg/kg. After 28 days of chronic administration of the BKD preparation the following toxicological changes were noted. In this experiment the liver function profile was studied. The results of the Liver function studies was given below There is a statistically very highly significant ($P=0.001$) decrease in the total protein in the serum of the male rat [12.67% decrease] and statistically insignificant ($P=0.558$) [2.24%] increase was observed in the albumin level in the serum of the male rat. There is a statistically highly significant ($P=0.002$) decrease in the globulin level in the serum of the male rat [28.44% decrease]. The albumin:globulin ratio is found to be statistically highly significant ($P=0.007$) increase in the serum of the male rat [39.04% increase]. The bilirubin level in the serum of the male rat is increased by [12.50%], the increase though not significant yet it was prominent ($P=0.170$). The serum ALT level of the male rat is found to increase which is statistically significant ($P=0.023$) [35.15% increase]. There is an [9.58%] increase in the serum AST level of the male rat, the increase though not significant yet it was prominent ($P=0.231$). Moreover, there is a statistically significant ($P=0.027$) decrease in the serum LDH level of the male rat [35.76% decrease]. The serum alkaline phosphatase (ALP) level of the male rat is found to show statistically insignificant ($P=0.514$) [9.32%] decrease level. There is a negligible [2.20%] increase in the serum GGT level of the male rat, which was statistically not at all significant ($P=0.892$). From the study on ratio of different enzyme activities it was noted that change in ALT was the most prominent, followed by AST and the GGT.

DISCUSSION

The liver which is a significant organ of human body, plays a central role in transforming and cleansing chemicals and is also susceptible to the toxicity from foreign agents. Certain medicinal agents, when taken in large doses or sometimes even when their therapeutic range is neglected, may injure the organ. Also, chemical agents used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal medicines can also introduce hepatotoxicity which may cause liver failure. The liver may be considered as the most important organ in drug toxicity for 2 contentions: (1) it is functionally mediated between the site of absorption and the systemic circulation and is a fundamental site of metabolism and elimination of foreign substances; (2) these features also render it a preferred target for drug toxicity. Drug-induced liver injury therefore poses a major clinical problem [16]. In our study, an ayurvedic medicine, BKD is a traditional medicine used in gingivitis changes different parameters after administration which is related hepatotoxicity and BKD increased the HbA1c level which is indicator of diabetes mellitus [17].

Serum total protein is made up of albumin and globulin. Biochemical test for measuring the total amount of protein in serum is an important liver function marker and may indicate hepatotoxicity. Albumin and globulin which is the fundamental stuffs of serum proteins, have an emergent effect on the systemic inflammatory response. Previous examinations had demonstrated that low serum albumin, a marker of nutrition

state and chronic inflammation, is an independent predictor of mortality and recurrence rate in several types of malignancies [18]. Furthermore, globulin is shown significant importance not only for host immunity and inflammation but also as a reaction for cumulative exposure of different cytokines [19]. Studies even deemed that globulin might be an independent risk factor for predicting long-term mortality in colorectal or gastric cancer [20]. Alpha and beta globulins contain lipoproteins which may be markedly increased in cholestatic lesions of the liver. In cholestasis, serum lipid values are correlated with the increase in alpha and beta globulin components and may be a useful point in identification between biliary obstructive lesions and other non-obstructive types of jaundice and gamma globulins are commonly markedly increased in chronic hepatic disease [21]. Low or high albumin and globulin ratios can also help healthcare providers to identify health problems including certain cancers, autoimmune diseases, or some genetic disorders. In our study, the total protein content in the plasma was significantly decreased in the BKD-treated male rats. Interestingly, the albumin content was increased though not significant and the globulin content was significantly decreased as well as albumin and globulin ratios were significantly increased in the BKD-treated male rats.

Bilirubin is a yellowish compound which is appeared in the normal catabolic pathway that breaks down heme in vertebrates. This catabolism is an essential process in the body's clearance of waste products that springs from the destruction of aged or abnormal red blood cells [22]. Bilirubin is evacuated in bile and urine, and elevated levels may reveal certain diseases [23]. In our study bilirubin level increased in treated rats though result was not significant. According to Naganna [24] increase in bilirubin indicates the abnormal liver function which may be the result of higher synthetic function of the liver. ALT is found in plasma and in various body tissues but is most common in the liver which catalyzes the 2 parts of the alanine cycle. Serum ALT level, serum AST (aspartate transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health which are elevated due to liver injury [25, 26]. In our study, ALT significantly increased to the BKD-treated male rats.

Lactate dehydrogenase (LDH or LD) is an enzyme which is found in nearly all living cells (animals, plants, and prokaryotes). LDH catalyzes the conversion of lactate to pyruvate and back and because it is released during tissue damage, it is a marker of common injuries and disease such as liver disease [25]. Moreover, LDH is an essential enzyme for anaerobic respiration, and its production has been shown to be increased under hypoxic conditions in various cell lines [27,28]. Interestingly, in our study, LDH significantly decreased to the BKD-treated male rats.

CONCLUSION

From the above data it can be concluded that BKD should not be administered chronically as it fluctuates different parameters which are related hepatotoxicity. So, an integrated study required to focus on the fluctuation of those parameters after this ayurvedic dug (BKD) administration.

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