Original Article

Exploring Toxicological and Safety Status Evaluation of an Ayurveda Medicine used for Indigestion in South Asian Region

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Abstract

Aim: Brhad Agnikumara Rasa (BAR), an Ayurvedic preparation used as a traditional medicine in the treatment of indigestion in the Asian population. **Sample:** For this present study, Brhad Agnikumara Rasa (BAR) had been collected from Sri Kundeswari Aushadhalaya Ltd., Chittagong. **Study design:** Healthy albino rats were used for the toxicological evaluation of BAR. During this experiment, rats were randomly divided into two groups of five rats/group and a dose equlibrium to 400 mg/kg body weight was selected for this toxicological evaluation. **Place and duration of Study:** This study was carried out between June 2015 and July 2016 at Pharmacy department of Atish Dipankar University of Science and Technology. **Methodology:** To established safety status of BAR, healthy albino rats (Sprague-Dawley strain) (50-70 g) were used. Rats were randomly divided into two groups of 5 rats/group. BAR was administered chronically to the experimental rats at a dose equlibrium to 400 mg/kg body weight for 51 days. To assess the function of the liver and kidney various biochemical analysis were carried out by using rats serum. **Results:** Our findings showed that total protein content was increased (7.493%) in the BAR treated rats. The increase in total protein though not found statitically significant (p=0.088). Interestingly, the serum albumin content was statistically insignificantly (p=0.141) increased (14.657%) and the globulin content was insignificantly (p=0.281) decreased (11.373%) in BAR treated rats. There had been found a prominent increase in the total cholesterol (247.5%), and a decrease in HDL (35.835%) level within the rat serum. A very highly significant (p=0.001) increase (247.5%) of serum total cholesterol level had been observed, while a statistically highly significant decrease was observed in a case of HDL (35.835%) (p=0.008). After chronic administration of BAR to the rats, an increase of bilirubin level (14.015%) was noted in comparison to their control group (p=0.793). Finally, There was found an insignificant decrease in the plasma urea (10.922%) in the BAR treated rats (p=0.436), while BAR caused 9.862% increase in plasma uric acid. **Conclusion:** Therefore, Brhad Agnikumar Rasa will have been thought of safe for human therapeutic use at the counseled therapeutic doses.

Keywords: Brhad Agnikumara Rasa; Albumin; Lipid profiles; Liver function; Kidney function test; Uric acid

Introduction

The phrase Ayurveda has been derived from Sanskrit phrases: Ayur means existence, and Veda means science. Thus the meaning of Ayurveda is stand "science of life". This is an ancient system for healing the numerous diseases. It is a mixture of frame, soul, and mind. As Ayurveda tells that no two individuals aren't same in their physical status, so according to the body type Ayurveda recommends the special treatment to them. This clinical system tells us about the absolute source that is 'Paramatma' that's the main foundation for all the awareness and intelligence. [1] The fundamental precept of Ayurveda is the sum of three vital factors inside the human body. These elements are Vata (wind element), Kapha (water element), and Pitta (hearth element). The Ayurvedic clinical system believes that each and every illnesses of the human body arise from an imbalance in these three critical factors. [2,3] Ayurveda is very plenty beneficial for bringing about nicely-being and exact fitness. It additionally performs an essential role in treating disorder such as coronary heart disorder, diabetes, and cancer in many nations like Japan, Singapore and Finland. It is far tested and may be frequently generate many ways for treating

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critical disorders. [4] Besides, there's an incredible development of contemporary medicine; there are some areas within which synthetic medicine have didn't prove its effectiveness like, side effects, the price of medicine, relapse, and lack of curative treatment. So, it attracts our attention towards a various system of drugs. [5] In India, 80% population depends on ancient drugs, out of which, nearly 70-75% depends directly on the ayurvedic medicine. [6] Our present ayurvedic preparation falls on the group rasashastra, which deals with differing kinds of metals like Copper (Cu), Gold (Au), Mercury (Hg), Arsenic (As), and Iron (Fe). Hence, demonstrating the protection against metal based ayurvedic preparations is of preponderant importance. [7,8] However, there's a scarcity of information on the protection and effective profiling of Ayurvedic preparations. [1] In this condition, there's a requirement of carried out tactical toxicity study over metals containing ayurvadic preparations. [9] Ayurvedic preparations are largely poly or mono flavored herbal-mineral formulations. In recent years, there's exaggerated utilization of flavored preparations have been found which underneath dietary supplements. [5,10] Keeping the above-mentioned facts and factors in mind, this research work has been carried out to characterize the chronic toxicological profile of the marketed Ayurvedic medicinal preparation, Brhad Agnikumara Rasa (BAR). Bhaisajyaratnavali. Prasarani Sandhan is included (page 359) 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). Its preferred medically in heaviness in the abdominal region, gas, accumulation of stool within viscous, loose motions, etc. At present, a good number of Ayurvedic manufacturers are manufacturing and marketing this classical Ayurvedic medicinal preparation. Brhad Agnikumara Rasa (BAR) unfolds its toxicological aspects as this preparation contains some undesireable metals in toxic concentration. Thus aim of our study was to had a better understanding of the authentic safety and toxicological profile of this Ayurvedic drug and also to decide how the use of this drug is behave within the body under the stated conditions.

Materials and Methods

Collection of Brhad Agnikumara Rasa (BAR)

For this toxicological study, Brhad Agnikumara Rasa (BAR), tablet, had been collected from local supplier named Sri Kundeswari Aushadhalaya Ltd., Chittagong, Bangladesh.

Dose of administration

For this toxicological experiment, the tablet had been administered at a dose such it would allow best dose accuracy while not manifested to the entire increase within the bodily fluid. For these current studies, the drug was administered orally at a dose of 400 mg/kg body weight. [1]

Route of administration

During this toxicological study, the solid dosage form of BAR had been administered orally. [1]

Experimental animals

For this toxicological evaluation of BAR, eight-week-old (50-70 g) healthy albino rats (Sprague-Dawley strain) were used. The rats had been nourished at the well-ventilated experimental animal house of Department of Pharmacy, Atish Dipankar Science and Technology University. Then, rats were randomly divided into two groups of five rats/group. Four plastic cage having dimensions of 30 x 20 x 13 cm used for nursing all of the rats and soft wadding was given as bedding within the cage. Feeding of animals had been maintained at the natural day-night cycle. The rat was fed with "mouse chow" (prepared in step with the formula developed at BCSIR, Dhaka). All experiments on rats had been applied in absolute compliance with the moral guide for the care and use of laboratory animals. The experimental animals were marked rigorously on the tail that helped in identification severally for a selected rate before the administration of drugs.

Control groups

This group administered with distilled water as a placebo as par the same volume as the drug treated group for the same number of days.

Toxicological experiment

The intragastric syringe has been used for the administration of the Ayurveda medicinal preparation. Administration of drug has been carried out between the hours of 10 AM and 12 PM.

Animal treatment

At the end of the 51-day treatment period, the animals were fasted for 18 hours for the initial dose and also 24 hours for every other dose. Ketamine (500 mg/kg i.p.) was administered for the purpose of anaesthesia.

Blood sample collection

Blood samples were withdrawn from post vena cava and transferred into heparinized tubes in order avoid early coagulation.

Preparation of serum

Blood samples were then centrifuged at 4,000 g for 10 min by the using bench pinnacle centrifuge (MSE Minor, England) machine, which facilitate get rid from red blood cells and to recover serum. Serum samples were separated by using dry Pasteur pipette and stored within the refrigerator for further analyses. All analyses have been finished within 24 h of serum collection.

Determination of biochemical parameters

Various biochemical analyses were carried out on serum, to assess the function of the liver and kidney. Biochemical studies involved analysis of parameters such as total protein content, serum albumin, blood urea nitrogen (BUN), and bilirubin (total and direct). Biuret method was used for the determination of total protein content of the samples, [11] while serum albumin concentration was determined by using the method of Doumas et al. [12] Total cholesterol concentration were evaluated by

using assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). Serum level of high-density lipoprotein (HDL) was determined by utilizing Randox Laboratory kit reagents. The method of Evelyn and Malloy was employed to determine the serum bilirubin concentration. [13] Finally, serum urea concentration was determined by following the method of Kaplan. [14] The absorbance's of all the tested samples were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PCP).

Statistical analysis

The experimental data are expressed as Mean ± SEM (Standard Error of the Mean). Paired "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 when value of $p < 0.05$, 0.01 and 0.005.

Results

Acute toxicity study

When drug (BAR) administered at a dose of 4000 mg/kg and produced no mortality of the experimental animals. Thus the LD50 value was found to be greater than 4000 mg/kg body weight. The animals did not manifest any sign of fever, diarrhea, dysentery, bleeding on rectum, mal-absorption syndrome. Since BAR is in the clinical use for fever, diarrhea, dysentery, bleeding on rectum, malabsorption syndrome treatment for many years, a limit test was performed in acute oral toxicity study.

Effect on total serum protein and albumin content

After 51 day's chronic administration of the BAR preparation the total protein and albumin content in serum were determined in the experimental rats group. In the study, the total protein content in the serum was increased (7.493%) in the BAR treated rats. The increase in total protein though not significant yet it was noticeable (p=0.088). Interestingly, the albumin content was statistically insignificantly (p=0.141) increased (14.657%) and the globulin content was insignificantly (p=0.281) decreased (11.373%) in BAR treated rats. The increase in the Albumin / Globulin ratio (21.239%) though were not significantly different from their corresponding control value (p=0.235) [Table 1].

Effect on lipid profiles

In the rats, there was a signficant increase within the total cholesterol (247.5%) and a decrease in HDL (35.835%) content within the serum. When the chronic administration of BAR during this investigation statistically very highly significant (p=0.001) increase (247.5%) of plasma total cholesterol level was discovered as compared to control. Statistically highly significant decrease was discovered in the case of HDL level (35.835%) (p=0.008). The increase in total cholesterol /HDL ratio (356.171%) was statistically significant (p=0.034) [Table 2].

Table 1: Effect of Brhad Agnikumar Rasa on total serum protein, albumin, globulin content and A/G ratio in rats.

Data	Total Protein (TP)		Albumin		Globulin		A/G	
	Control	BAR	Control	BAR	Control	BAR	Control	BAR
Mean ± SEM	5.79 ± 0.15	6.22 ± 0.17	2.37 ± 0.18	2.72 ± 0.13	3.87 ± 0.34	3.43 ± 0.15	0.68 ± 0.09	0.82 ± 0.06
t/p	-1.836/0.088		-1.543/0.141		1.118 /0.281		1.240/0.235	
95% CI of difference	-0.941 to 0.073		-0.825 to 0.128		-0.399 to1.280		-0.396 to0.105	
Percentage of increment or reduction	↑7.493%		↑14.6572%		↓11.37325%		↑21.2389%	

Here, BAR stands for Brhad Agnikumar Rasa, N-con and N-drug stands tor number of rates used for control and BAR treated group. All the values are expressed in Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of Brhad Agnikumar Rasa on total cholesterol, HDL level and total cholesterol/HDL ratio in rats.

Parameters		Control vs. BAR
Statistical findings	N-Con N-Drug	Control ± SEM Drug mean ± SEM t/p 95% CI of difference(Lower-Upper) Percentage of increment or reduction
Total Cholesterol (TCHO)	5 5	12.2951 ± 3.51190 42.7254 ± 5.25175 -4.817 /0.001 -43.98062to-16.88004 ↑247.5%
HDL	5 5	12.2168 ± 0.81250 7.8389 ± 0.45056 4.240/0.008 1.72347to7.03238 ↓35.83517%
Total Cholesterol/HDL	5 5	0.5275 ± 0.14130 1.8788 ± 0.32971 -2.559/.034 -2.56910to-0.13346 ↑356.171%

Here, BAR stands for Brhad Agnikumar Rasa, N-con and N-drug stands for number of rates used for control and BAR treated group. All the values are expressed in Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Table 3: Effect of Brhad Agnikumar Rasa on serum level of bilirubin in rats.

Parameters		Control vs. BAR
Statistical findings	N-con N-drug	Control ± SEM Drug mean ± SEM t/p 95% CI of difference(Lower-Upper) Percentage of increment or reduction
Bilirubin	5 5	0.2357 ± 0.08462 0.2688 ± 0.08890 -0.269/0.793 -0.30381to0.23774 ↑14.0152%

Here, BAR stands for Brhad Agnikumar Rasa, N-con and N-drug stands for number of rates used for control and BAR treated group. All the values are expressed in Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Table 4: Effect of Brhad Agnikumar Rasa on serum urea level in rats.

Parameters		Control vs. BAR
Statistical findings	N-con N-drug	Control mean ± SEM Drug mean ± SEM t/p 95% CI of difference(Lower-Upper) Percentage of increment or reduction
Urea	5 5	51.4888 ± 6.06257 45.8652 ± 4.03125 0.799/0.436 -9.29308to20.54027 ↓10.922%

Here, BAR stands for Brhad Agnikumar Rasa, N-con and N-drug stands for number of rates used for control and BAR treated group. All the values are expressed in Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Table 5: Effect of Brhad Agnikumar Rasa on serum Uric acid level of rats.

Parameters		Control vs. BAR
Statistical findings	N-con N-drug	Control mean ± SEM Drug mean ± SEM t/p 95% CI of difference(Lower-Upper) Percentage of increment or reduction
Uric acid	5 5	2.0898 ± 0.60176 2.2959 ± 0.66059 -0.227/0.825 -2.22457 to 1.81242 ↑9.86218%

Here, BAR stands for Brhad Agnikumar Rasa, N-con and N-drug stands for number of rates used for control and BAR treated group. All the values are expressed in Mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

Effect on liver and kidney function

After chronic administration of Brhad Agnikumar Rasa (BAR) to the rats an increase of bilirubin level (14.015%) in the serum was noted in comparison to their control group which was not statistically significant (p=0.793) [Table 3]. Kidney function test performed to measure the urea content in the serum. The urea content provides information about the effectiveness of the kidney function. There was a decrease in the serum urea (10.922%) in the BAR treated rats, and this decrease was not significant (p=0.436) [Table 4].

Effect on serum uric acid

From Table 5, it was ascertained that about 9.862%, increase in serum uric acid content of BAR treated rats in comparison to their control rats which was not statistically significant (p=0.825).

Discussion

In the Asian region, Ayurveda is one among the oldest systems of drugs practicing over 5000 years. However, the most important downside of this technique of drugs may be a lack of standardization, safety, and effectuality and there is an absence of supporting knowledge concerning its safety and effectuality in clinical trials [5]. Serious metals toxicity may be a major concern over the Ayurveda formulations because of the presence of Lead, Arsenic, and Mercury, etc. [15] Nowadays, as per World Health Organization list of ancient medicines, in Asia there are inflated a variety of herbs-mineral preparations to treat numerous ailments like anemia, cancer, diabetes, and skin diseases, etc. [15] There are variety of herbo-mineral preparations are available, which are utilized since many years based on their therapeutic value. [5,16] The biochemical indices monitored within the liver and kidney are useful 'markers' for evaluation of tissue damage. The examination of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis, [17] disturbance on the various enzymes in the tissues and body fluids to a reasonable extent reflect the toxicity of the drug. [18] Tissue enzymes can also indicate cellular tissue damage caused by chemical compounds before structural damage that

can be picked by conventional histological techniques. [19] Brhad Agnikumara Rasa (BAR), an Ayurvedic preparation used as conventional drugs in the treatment of stomach upset in the rural population. To find out the toxicological characteristic of BAR, it was administered chronically for 51 consecutive days to the male rats group. Present study concerned determination of toxicological aspects, such as- total protein content, serum albumin content, lipid profiles, hepatic function test, urinary function test, and serum uric acid level. The total protein content was increased in the BAR treated rats. The increase in total protein though is not significant. Interestingly, the albumin content was statistically insignificantly increased and the globulin content was insignificantly decreased in BAR treated rats. In the rats there was a prominent increase in the total cholesterol and a decrease in HDL content in the serum. Statistically very highly significant increase of serum total cholesterol level was observed, while statistically highly significant decrease was observed in the case of HDL. As, our examined Ayurvadic increase serum cholesterol and decrease beneficial HDL, so we can say that this preparation should not be given to patients with cardiac abnormalities or can be given in after dose adjustment. In a prospective study based on western population moderate and highly significant association was observed between triglyceride or cholesterol values and coronary heart disease risk factor. [20] In another examine, it become determined that incidences of coronary heart disease (CHD) and stroke per 1000 character/years have been 9.59 and 7.45 respectively and myocardial and brain infections had been 3.84 and 6.29 respectively. As CHD risk became linearly and continuously improved through triglycerides and low density lipoprotein (LDL) cholesterol, they were reported as the 0.33 robust hazard aspect for CHD and their blended outcomes might be additive. [21] From the present study, it is observe that BAR increase statistically very significantly cholesterol level on rat serum. Apart from this, the present drug may create problem in patients with coronary heart disease (CHD). As our present study was undertaken to discover safety status of BAR. So, we can say that this Ayurvadic preparation may be a bad choice for patients with CHD. After chronic administration of BAR to rats, an increase of bilirubin level in the serum was noted in comparison to their control group which was not statistically significant. There was found an insignificant decrease in the plasma urea in the BAR treated rats. If the body produces too much uric acid or doesn't remove enough of it, a person becomes sick. So the test was performed to measure the effect of BAR on serum uric acid level. BAR caused an increase in plasma uric acid, which was not statistically significant. Thus, oral administration of BAR at 400 mg/kg body wt., that is equivalent to 5 times the therapeutic dose, didn't show any toxicological signs. During this study, weight and calculable feed consumption weren't found affected, when no hematological abnormalities were noted. No mortality was determined and didn't observe. Therefore, Brhad Agnikumar Rasa will be thought of safe for human therapeutic use at the counseled therapeutic doses, except CHD.

Conclusion

Analysis of these data of toxicity study reveals these BAR preparations have no serious deleterious effect on body function as a whole, but only exert patient unfriendly impact on the serum lipid profile. That is why, caution should be taken while calculating the dose and duration for patients with CHD, and otherwise it is safe even at high dose. The study may also create scopes and directions for further research as well.

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Conflict of Interest

All authors disclose that there was no conflict of interest.

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We do not have any private or public organizational founding. All members of research group having personal contributions for found.

Availability of data and materials

Data and materials are available in the Department of Pharmacy, Atish Dipankar University of Science and Technology.

Contribution statement

AK, MI and MSH were directly related to conducting this research work. MSH, DD, MNA, FH and MLN were also contributed during data generation, manuscript preparation and collection of the plant part. All of these authors are acknowledged about the publication of this research work.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethical committee of Atish Dipankar University of Science and Technology.

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