BODY ORGAN RATIO TOXICITY STUDIES OF AN AYURVEDIC MEDICINE ‘BASAKARISTA’ AFTER CHRONIC ADMINISTRATION TO FEMALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Basakarista (BSK), a well-known ayurvedic preparation, is widely used as a traditional medicine in the treatment of cough and respiratory trouble. In this study, effect of BSK on organ toxicity profile was evaluated after chronic administration of this drug to female Sprague-Dawley rats. The acute pharmacological study of BSK recorded no death or any signs of toxicity even at the highest dose of 80 ml/Kg body weight. For chronic pharmacological evaluation, the animals were divided into two groups. The first group was given BSK preparation at a dose of 40 ml/kg body weight for 35 days while the second group that served as the control received water for the same period. All throughout the experimental period, the BSK treated animals were always maintaining negligible decrease in body weight, in the body weight study, but it was not significant. There is a statistically significant ($p=0.05$) decrease in the absolute and relative weight of rat heart, kidney, spleen and thymus. The drug (BSK) also significantly decrease the tissue hydration index in heart and lung. These results demonstrate that BSK should not be administered chronically at a higher dose.

Keywords: Basakarista, toxicology profile, tissue hydration index, organ body weight ratio

INTRODUCTION

Cough is a common symptom of upper respiratory tract inflammation which has been found in most clinical investigations regularly. It is identified with three phases including- inspiratory phase, forced expiratory exertion against a closed glottis and glottis opening [1, 2]. It is considered as a defense reflex mechanism that protects from aspiration of foreign materials occurring as a consequence of aspiration or inhalation of pathogens, particulate matter, accumulated secretions, inflammation, postnasal drip and mediators associated with inflammation [3-5]. Although cough serves as an important protective role in the airways and lungs under normal conditions, it may become excessive and nonproductive, and could be troublesome and potentially harmful to the airway mucosa in chronic condition [6-8]. Nature and occurrences of coughs might be criteria for the diagnosis, pharmacological efficacy and a measure for the development of chronic disease [9-10].

Ayurvedic medicines can be a useful remedy for cough because plants are used as remedies for many diseases associated with cold from ancient times. Recently, the World Health Organization (WHO) has announced and suggested large-scale usage of alternative treatments, predominantly in the unindustrialized countries, as an alternate branch of medicine to provide services regarding primary health care [11-13].

Basakarista (BSK) is a well-known Ayurvedic liquid medicine. Its principle ingredient is Vasaka –Malabar nut. It is a potent mucolytic and anti-asthmatic [14-
15]. Hence, BSK is used in many respiratory conditions. It is used in the treatment of cold, cough, bronchitis, inflammation and bleeding diseases [16-19]. Though this preparation is conspicuously used in the rural areas, its toxicological profile is not established yet. Our aim was to find out the toxicological profile of Basakarista, used as a remedy of cough in ayurvedic medicine. Besides, growth study or growth pattern of experimental animals was also the matter of concern here in this research work.

MATERIALS AND METHODS

**Drugs, Chemicals and Reagents:** For the pharmacological study, Basakarista (BSK) 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 Abbott Laboratories, USA.

**Experimental Animals:** Six to eight-week old female Sprague-Dawley rats bred and maintained at the animal house of the Department of Pharmacy, Jahangirnagar University, were used in the pharmacological experiment. These animals were apparently healthy and weighed 60-70 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 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, Facultmacy, Jahangirnagar University.

**Experimental Design**

**Acute Toxicity Study:** The acute oral pharmacological test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425) [20]. Sixteen female mice (30-40 g body weight, non-pregnant) were divided into four groups of four animals each. Different doses (50 ml/kg, 60 ml/kg, 70 ml/kg and 80 ml/kg) of experimental drug (BSK) were administered by stomach tube. The dose was divided into two fractions and given within 12 hours. Then all the experimental animals were observed for mortality and clinical signs of toxicity (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 three days following BSK administration.

**Chronic Toxicity Studies:** Prior to the experiment, rats were randomly divided into 2 groups of 10 animals each. One group was treated with BSK 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 35 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 40 ml/kg body weight. After acclimatization, ayurvedic 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.

**Blood Samples Collection and Preparation of Serum:** At the end of the 35 days treatment period, after 18 hours fasting, rats from each group were anaesthetized by administration (i.p) of ketamine (500 mg/Kg body weight). 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 g 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.

**Overall body weight Analysis**

Careful monitoring of body weights of rats were performed throughout the 35 days of drug administration period at regular intervals (2-3 days) until the treatment period was completed and under close observation throughout the experimental period. An equal number of animals of the same species were also maintained as the control group and under close observations. Statistical analysis of the initial and final growth rates were performed which expressed as percent increment in the body weight. The growth rate of the treatment group was compared with the control group.
Organ toxicity study
At the end of the 35 days treatment period, the animals were fasted for 18 hours. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia. Rats of both BSK and control groups were sacrificed after the completion of the 35 days period and examined macroscopically for external lesions. Necropsy was performed to examine gross pathological lesions of various internal organs. Specific organs of interest were then detached and preserved in 13% formalin (HCHO) and sent for the evaluation of histological anomalies. Organs like heart, lungs, liver and spleen, portions of these tissues were excised and preserved for histological examination. The remaining portions were dried for determination of water content (tissue hydration index).

Relative weight of organ=$\frac{AOW}{BW} \times 100$
AOW= Absolute organ weight
BW= body weight

Water content in tissue=$\frac{OW_1-OD}{OW_1-OF} \times 100$
OW$_1$ = organ wet weight
OD = organ dry weight
OF = organ foil weight

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 ± SEM (Standard Error Mean) and p<0.05, p<0.01, p<0.001 was taken as the level of significant.

RESULT

Acute toxicity study
The drug Basakarista (BSK) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD$_{50}$ value was found to be greater than 80 ml/kg body weight. According to the OECD test guideline 425 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 (80 ml/kg body weight) was conducted. There were no mortality and toxicity signs observed at 80 ml/kg body weight. Therefore, it can be concluded that when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic toxicity study
Effect of BSK on Overall Body Weight
The total treatment period was of 35 days. All throughout the experimental period, the BSK treated animals were always maintaining decrease in body weight. In the body weight study, the BSK administered animal were weighing 1.78 % (p=0.670) to 4.78 % (p=0.480) less than their control counterpart. All throughout the experimental period no statistically significant decrease was noted.

Figure 1: The effect Basakarishta (BSK) (40 ml/kg) on the body weights (g) of Spraque-Dawley rats with the time of treatment. Independent sample t-test was performed to analyze this weight variation in different days. All values are expressed as mean ± SEM and p<0.05, were taken as the level of significant.
**Effect of BSK on Organ Toxicity Study:**
Average absolute weight of the heart of control animal was 0.4059 gm, whereas after treatment with BSK, a statistically very highly significant (p=0.001) decrease in the absolute weight of the female rat heart was found [26.93% decrease]. Absolute weight of the lung was also decreased after using BSK at a constant rate to the experimental rats. There was a statistically significant (p=0.028) decrease in the absolute weight of the female rat lungs [16.19% decrease]. Similar to other inner organs, the absolute weight of the kidney also decreased in the study group rats compared to control group. There was a statistically highly significant (p=0.002) decrease in the absolute weight of the kidney [20.03% decrease]. Absolute weight of the spleen in BSK group was found in a reduced manner comparing with the control group. Statistically very highly significant (p=0.001) decrease in the absolute weight of the spleen was found in the BSK group [35.41% decrease]. Significant (p=0.025) decrease in the absolute weight of the thymus was also seen in the BSK group. A very highly significant (p=0.001) decrease in the relative percent weight of the heart [23.41% decrease] was found in the BSK treated female rats. There was a highly significant (p=0.004) decrease found in the relative percent weight of the female rat kidney, nearly 16.04% decrease was found in the BSK treated group. There was a statistically very highly significant (p=0.001) decrease in the relative percent weight of the female rat spleen. A total 31.70% decrease was found when value was compared with control group rats. A significant (p=0.024) decrease in the relative percent weight of the female rat thymus was seen in the thymus-somatic index.

**Effect of BSK on Tissue Hydration Index:**
In this study, we found statistically significant (p=0.027) decrease in the organ water content of the female rat heart in cardio-hydration index of the BSK treated group. Similar to this in pulmuno-hydration index, there was a statistically significant (p=0.034) decrease in the organ water content of the female rat lungs. A different scenario came forward in renato-hydration index. Unlike other organs, a negligible increase was found in renato-hydration index [1.49%], which was statistically not at all significant (p=0.887).

**Table 1: The effect of BSK (40 ml/kg) on the absolute organ weights of female rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (gm)</th>
<th>BSK (gm)</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.301± 0.011</td>
<td>0.231± 0.008</td>
<td>0.001</td>
<td>↓23.41</td>
</tr>
<tr>
<td>Lung</td>
<td>0.618± 0.045</td>
<td>0.540± 0.025</td>
<td>0.164</td>
<td>↓12.53</td>
</tr>
<tr>
<td>Liver</td>
<td>2.464± 0.469</td>
<td>2.381± 0.072</td>
<td>0.865</td>
<td>↓3.36</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.294± 0.012</td>
<td>0.247± 0.008</td>
<td>0.004</td>
<td>↓16.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.296± 0.020</td>
<td>0.202± 0.011</td>
<td>0.001</td>
<td>↓31.70</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.111± 0.044</td>
<td>0.016± 0.002</td>
<td>0.061</td>
<td>↓85.91</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.172± 0.017</td>
<td>0.120± 0.012</td>
<td>0.024</td>
<td>↓29.96</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=10). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

**Table 2: The effect of BSK (40 ml/kg) on the relative organ weights of female rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (gm)</th>
<th>BSK (gm)</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.406±0.012</td>
<td>0.297± 0.011</td>
<td>0.001</td>
<td>↓26.93</td>
</tr>
<tr>
<td>Lung</td>
<td>0.826± 0.047</td>
<td>0.692± 0.026</td>
<td>0.028</td>
<td>↓16.19</td>
</tr>
<tr>
<td>Liver</td>
<td>3.275± 0.635</td>
<td>3.080± 0.151</td>
<td>0.771</td>
<td>↓5.97</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.398± 0.017</td>
<td>0.318± 0.012</td>
<td>0.002</td>
<td>↓20.03</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.401± 0.031</td>
<td>0.259± 0.013</td>
<td>0.001</td>
<td>↓35.41</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.157± 0.064</td>
<td>0.020± 0.002</td>
<td>0.060</td>
<td>↓87.29</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.233± 0.025</td>
<td>0.157± 0.017</td>
<td>0.025</td>
<td>↓32.73</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM (n=10). Independent sample t-test was performed to analyze this dataset. p<0.05 was considered statistically significant when compared against control. ↑: increase, ↓: decrease.
Table 3: The effect of BSK (40 ml/kg) on various Tissue Hydration Indices of female rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>BSK</th>
<th>p value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>79.355± 0.410</td>
<td>69.809± 4.148</td>
<td>0.027</td>
<td>↓12.03</td>
</tr>
<tr>
<td>Lung</td>
<td>85.709± 4.234</td>
<td>75.854±0.496</td>
<td>0.034</td>
<td>↓11.50</td>
</tr>
<tr>
<td>Liver</td>
<td>72.257± 2.713</td>
<td>63.369±6.736</td>
<td>0.220</td>
<td>↓12.30</td>
</tr>
<tr>
<td>Kidney</td>
<td>72.480± 6.969</td>
<td>73.557±1.312</td>
<td>0.887</td>
<td>↑1.49</td>
</tr>
<tr>
<td>Spleen</td>
<td>67.331± 5.015</td>
<td>79.109±8.762</td>
<td>0.248</td>
<td>↑17.49</td>
</tr>
</tbody>
</table>

DISCUSSION

Absolute organ weight is one of the most sensitive drug toxicity indicators to the animal model and its changes often precede morphological changes of that animal. A number of factors involved in the influence of animal organ weights including strain of animal, age of the animal, sex, environmental and experimental conditions [21, 22]. The establishment of organ weight reference values at each testing facility for laboratory animals used in toxicological studies has become a standard practice. In this study we found that all internal organs in the BSK treated rats possessed less absolute weight compared to the control group. Which indicates that, this preparation has direct physiological alteration capability in animal. A number of reasons may involve in the reduction of the weight of the organ, such as stagnant of the growth of the organ tissue, tissue dehydration, tissue collapse and lack of proper nutrition [23, 24]. Organ body ratio is another vital tool to measure the relative toxicity of the preparation [25, 26]. As the absolute organ weight decreased in the BSK treated rats, the relative body weight ratio of the ratio also found in descending orders in every steps. The most decrease found in ovary of the female rats compared to other organ which indicates long term taking this preparation may involve in the fetus development anomalies. Decreased ratio of heart, lung and kidney was also noticeable, which may cause cardiac, pulmonary or renal abnormalities in the future of the test animals.

Dehydration may lead to several physiological disorders as water is one of the main element for cell functioning [27]. Water comprises from nearly three-fourth of total body weight of any human and it is essential for maintaining cellular homeostasis [28]. In our study, we found that BSK caused some significant change in the water content of some organs like heart, lung and liver. But a different outcome occurred in case of kidney and spleen. In both cases it increased and for spleen the hydration index was quiet high.

CONCLUSION

From this experimental research work, it could be concluded that BSK should not be administered chronically at a higher dose as it significantly reduces the weight of heart, kidney, spleen and ovary as well as has influence in the hydration index of the body organ of female rats, though it is one of the good medicine for Cough treatment. Further studies should be done to find the molecular mechanism of the drugs.

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CONFLICTS OF INTERESTS

The authors have no conflicts of interests.

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