Research Article

TOXICOLOGICAL STUDIES OF AN AYURVEDIC MEDICINE “KARPUR RAS” USED IN DIARRHOEA

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ABSTRACT

Karpur Ras (KRP) is an Ayurvedic preparation used as a traditional medicine in the treatment of diarrhea in the rural population. To find out the toxicological characteristic of KRP, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg for 28 days. All throughout the experimental period the KRP treated animals were always maintaining decrease in body weight in the overall body weight study compare to their control counterpart. There were a statistically significant (p=0.013) decrease in the absolute weight of rat heart and a statistically highly significant (p=0.009) decrease in the relative percent weight of the heart. There were a statistically highly significant decrease in the absolute weight of the liver (p=0.002) and the relative percent weight of the liver (p=0.005). Also there was a statistically very highly significant (p=0.001) decrease in the organ water content of the liver. There was a statistically highly significant (p=0.004) decrease in the absolute weight of the rat kidney but a statistically significant (p=0.015) decrease was noticed in relative percent weight of the rat kidney. There were a statistically very highly significant (p=0.001) decrease in the absolute weight of spleen relative percent weight of spleen.

Keywords: Karpur Ras, Absolute organ weight, Relative organ weight, Tissue hydration, Ayurvedic formulation.

INTRODUCTION

Diarrheal disease is a major problem in the developing countries. About 1.7 to 5 billion cases of diarrhea occur per year in the world [1, 2]. In developing countries, it is most common to the young children get diarrhea on average three times a year [3]. Approximately 1.5 million children die from diarrheal diseases each year. That’s why it the second most common cause of mortality in children under five [3]. Worldwide, as of 2012, it is the second most common cause of deaths in children less than five (0.76 million or 11%) [2]. Diarrheal diseases are one of the leading causes of death in children under 5, accounting for 20% of all infant deaths in Bangladesh [4]. It can be caused by contaminating drinking water, poor sanitation and hygiene, and more broadly to poverty [5, 6].

Karpur Ras (KRP) is an Ayurvedic medicine in tablet form. It is used in the treatment of gastro-enteritis, fever, diarrhoea, dysentery, bleeding per rectum, malabsorption syndrome and such other gastro intestinal conditions [7-12]. Karpur Ras is included 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). Bangladesh National Formulary of Ayurvedic Medicine is compiled by the National Unani and Ayurvedic Formulary Committee and published by the Bangladesh Board of Unani and Ayurvedic Systems of Medicine, 38, Bangabandhu
Avenue, Dhaka-1000 under the authority vested in the Board vide section 13(j) of the Bangladesh Unani and Ayurvedic practitioners Ordinance, 1983 in collaboration with the World Health Organization. Permission to manufacture at industrial scale is printed in page no. 535 (column 2: Product code 16.60). Directorate of Drug Administration has issued Notification DA/Admin/1-10/96/6212 dated 19th October 1996 has issued license under Drug Act, 1940 and Rules there under and Drug (Control) Ordinance 1982 for local manufacture and sale in Bangladesh. (Published Bangladesh Gazette #24 Part VI dated Thursday, June 11th 1998.) [7-12].

This research work on Ayurvedic formulation, Karpur Ras unfolds a field of its toxicological aspects by utilizing experimental animals. That is why; we designed our current experiment to observe the effect of chronic administration of KRP to Sprague-Dawley rats at a high dose. The objective is to have a better understanding of the potential toxicological profile of the drug. The study provides directions for further research as well. The research work has been carried out in order to characterize the organ toxicity study of the Ayurvedic medicinal preparation, KRP.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Karpur Ras (KRP) was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Bangladesh. All other reagents and chemicals used in this work were analytical grade.

Experimental Animals: Six to eight-week old male Sprague-Dawley rats bred and maintained at the animal house of the Pharmacology Lab, Department of Pharmacy, Jahangirnagar University, were used in the toxicological experiment. These animals were apparently healthy and weighed 80-100 g. The animals were housed in a well-ventilated clean experimental animal house under constant environment 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 h day and 12 h 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, 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 modifications (OECD Guideline 425) [13]. Sixteen male mice (30-40 g body weight) 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 (KRP) 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 toxicity signs (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 KRP administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with KRP 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 [14]. After acclimatization, Ayurvedic medicinal 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 [15].

Overall Body Weight Analysis: Careful monitoring of body weights of rats of both groups was performed throughout the 28 days drug administration period. Body weights were recorded at regular intervals (3-4 days) until the treatment period was completed. All rats were kept under close observation throughout the experimental period. Statistical analysis of the initial and final growth rates was performed. The growth rate of the treatment group was compared with that of the Control group.

Organ Toxicity Study: At the end of the 28 day treatment period, the animals were fasted for 18 hours and also twenty-four hours after the last administration. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia [16]. Rats
of both KRP and Control groups were sacrificed after the completion of the 28-day 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 and sent for the evaluation of histological anomalies, if any. The tissues (Heart, kidney, lungs, liver, spleen, thymus, stomach, cecum, pancreas, adrenal glands, reproductive organs which include testis, seminal vesicles, epididymis etc.) thus subjected to histo-pathological evaluation. 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.

Relative organ weight (ROW) and water content in tissue were calculated using the following formula:

Relative organ weight = \( \frac{\text{AOW}}{\text{BW}} \times 100 \)

Here,

AOW=Absolute organ weight

BW=body weight

Water content in tissue = \( \frac{\text{OW1-OF}}{\text{OW1-OF}} \times 100 \)

Here,

OW1=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 Il). 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.

RESULTS

Acute Toxicity Study: The drug (KRP) administered up to a high dose of 80 ml/kg produced no mortality of the experimental animals. Thus the LD_{50} value was found to be greater than 80 ml/kg body weight. The animals did not manifest any sign of fever, diarrhoea, dysentery, bleeding per rectum, mal-absorption syndrome. Since KRP is in the clinical use for fever, diarrhoea, dysentery, bleeding per rectum, mal-absorption syndrome treatment for many years, a limit test was performed in acute oral toxicity study. 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 KRP when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Toxicity Study

Effect of KRP on Overall Body Weight: The total treatment period was of 28 days. All throughout the experimental period the KRP treated animals were always maintaining decrease in body weight, in the body weight study, the KRP administered animal were weighing 2.76 % to 4.60 % less than their control counterpart. All throughout the experimental period no statistically significant decrease was noted.

Effect of KRP on Organ Toxicity Study: In case of absolute organ weight, there was a statistically significant decrease in the absolute weight of heart (16.44 % decrease; p=0.013) but there were a statistically highly significant decrease in the absolute weight of liver (24.45 % decrease; p=0.002) and absolute weight of the kidney (18.22 % decrease; p=0.004). There was also a statistically very highly significant (p=0.001) decrease in the absolute weight of the male rat spleen (42.25 % decrease; p=0.001).

In case of relative organ weight of male rat, there were a statistically highly significant decrease in the heart (13.26 % decrease; p=0.009) and liver (22.06 % decrease; p=0.005). There was a statistically significant decrease in the relative percent weight of rat kidney (15.30 % decrease; p=0.015) and a statistically very highly significant decrease in the rat spleen (42.84 % decrease, p=0.001).

Effect of KRP on Tissue Hydration Index: In case of tissue hydration index, There was a statistically very highly significant (p=0.001) decrease in the organ water content of the male rat liver (4.78 % decrease; p=0.001). No significant increase or decrease was noticed in case of water content of other organs of KPR treated male rats.

DISCUSSION

Overall Body Weight: The administration of herbal preparations without any standard dosage along with insufficient scientific studies on their safety profile has raised concerns on their toxicity [17]. Alteration in weight is an indication of impairment in the normal functioning of the body. In this study we found decrease in body weight about 2.76 % to 4.60 % less than their control counterpart. Rapid body weight loss may be due to decreased feed and/or water consumption, disease, dental maladies, or specific toxic effects [18].
Effect of KRP on Organ Toxicity Study: Relative organ weight may serve as indication of pathological and physiological status in man and animals. Toxic substances induce abnormal metabolic reactions that affect primary organs such as heart, liver, spleen, kidney and lung [19]. Alteration in organ weight is a sign of impairment in the normal functioning of the body organs. Organ-body weight ratio may indicate organ swelling, atrophy or hypertrophy [20].

In this study we found, heart weight decrease significantly to the KRP treated rats. Reduced heart weight has been reported in toxicity studies in which dogs and rats were treated with high doses of angiotensin-converting enzyme (ACE) inhibitors. Reductions in total ventricular weight, left ventricular weight and right ventricular weight normalized for body weight and reductions in mean arterial blood pressure were also reported in Sprague-Dawley rats receiving continuous infusions of the synthetic atriopeptin-III [21]. Dose-related increases in liver weight are commonly observed in repeat-dose toxicity studies performed in rodents, although in dog or other large animal studies, the individual variations and the small numbers of animals used makes assessment of liver weight changes less certain. The causes of liver weight changes are diverse. One documented aged-related change in both humans and laboratory rodents is a decline in liver volume [22].

Here we found significantly decrease of liver weight to the KRP treated rats. Kidney weight of laboratory animals appears not to show a close relationship with body weight. Here we found significant decrease of kidney weight to the KRP treated rats. However in humans, renal weight appears to decrease with advancing age. This has been linked to thickening of the intra-renal vascular intima, sclerosis of the glomeruli, infiltration by chronic inflammatory cells and stromal fibrosis associated with altered renal tubular function. These changes may modify the pharmacokinetics and pharmacodynamics of administered drugs [23]. In this study we found, spleen weight decrease very highly significantly to the KRP treated rats. The interpretation of treatment-related splenic weight changes is more difficult in view of the complexity of the vascular, tissue and cellular responses that can occur. The lymphocyte population of the splenic parenchyma becomes depleted under a number of circumstances in rodents and dogs. In mice and hamsters, lymphoid cells may be displaced by the accumulation of amyloid. Atrophy or loss of lymphocytes also occurs in all species as a non-specific reaction to stress, severe weight loss or as an agonal change. Lymphocytes are also depleted as a result of treatment with xenobiotic, notably corticosteroids, immunosuppressive and anticancer drugs.

Effect of KRP on Tissue Hydration Index: Water comprises from 75% body weight in infants to 55% in elder people and it is essential for maintaining cellular homeostasis. Dehydration can cause several physiological disorders [24]. In our study we found that KRP cause significant reduction in % water content of liver. It can be suggested that this drug has negative impact on maintaining cellular haemostasis.

CONCLUSION

From the above experiment it can be concluded that KRP should not be administered chronically at a higher dose as it decrease weight of heart, lung, liver, kidney, spleen. Further studies should be done by reducing the administered dose. Thus KarpurRas is to be taken only at a dosage of 125 mg once or twice a day usually advised after food. If needed, it can be mixed with equal quantity of water.

ACKNOWLEDGMENT

The authors are thankful to Focused Research on Ayurvedic Medicine and Education (F.R.A.M.E) Laboratory, Department of Pharmacy and all faculty members and the technical staffs of the Department of Pharmacy, Jahangirnagar University for their kind co-operation. We would express our special thanks to Mr. Shafiqul Islam for ensuring a constant supply of animals followed by proper maintenance and care of these animals during all throughout the experimental period.
Figure 1: The effect KRP (40 mg/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, p<0.01, p<0.001 were taken as the level of significant.

Table 1: Name of the ingredients/herbs used in the preparation of Karpur Ras (KRP)

<table>
<thead>
<tr>
<th>Name of ingredients</th>
<th>Botanical names</th>
<th>Parts used</th>
<th>Amounts Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shuddha Hingula</td>
<td>Purified and processed Cinnabar (Mercury)</td>
<td>10g</td>
<td></td>
</tr>
<tr>
<td>Ahiphena</td>
<td><em>Papaversomniferum</em></td>
<td>seeds, fruits</td>
<td>10g</td>
</tr>
<tr>
<td>Musta</td>
<td><em>Cyperus rotundus</em></td>
<td>root</td>
<td>10g</td>
</tr>
<tr>
<td>Indrayava</td>
<td><em>Holarrhena antidysenterica</em></td>
<td>Connessi seed</td>
<td>10g</td>
</tr>
<tr>
<td>Jatiphala</td>
<td><em>Myristica fragrans</em></td>
<td>fruit</td>
<td>10g</td>
</tr>
<tr>
<td>Karpoora</td>
<td><em>Cinnamomum camphora</em></td>
<td>Whole plant</td>
<td>10g</td>
</tr>
</tbody>
</table>

Table 2: The effect of KRP on the absolute organ weights of male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>KRP</th>
<th>P value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.4646 ± 0.022</td>
<td>0.3882 ± 0.015</td>
<td>0.013</td>
<td>↓16.44</td>
</tr>
<tr>
<td>Lung</td>
<td>0.8841 ± 0.039</td>
<td>0.8499 ± 0.020</td>
<td>0.461</td>
<td>↓3.87</td>
</tr>
<tr>
<td>Liver</td>
<td>6.7825 ± 0.361</td>
<td>5.1239 ± 0.244</td>
<td>0.002</td>
<td>↓24.45</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.5599 ± 0.023</td>
<td>0.4579 ± 0.019</td>
<td>0.004</td>
<td>↓18.22</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.7074 ± 0.043</td>
<td>0.4085 ± 0.035</td>
<td>0.001</td>
<td>↓42.25</td>
</tr>
<tr>
<td>Testis</td>
<td>1.0759 ± 0.033</td>
<td>0.9876 ± 0.037</td>
<td>0.098</td>
<td>↓8.21</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease

Table 3: The effect of Karpur Ras on the relative organ weights of male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>KRP</th>
<th>P value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.2963 ± 0.0077</td>
<td>0.2570 ± 0.0105</td>
<td>0.009</td>
<td>↓13.26</td>
</tr>
<tr>
<td>Lung</td>
<td>0.5640 ± 0.0131</td>
<td>0.5644 ± 0.0219</td>
<td>0.988</td>
<td>↑0.07</td>
</tr>
<tr>
<td>Liver</td>
<td>4.3542 ± 0.2299</td>
<td>3.3936 ± 0.1689</td>
<td>0.005</td>
<td>↑22.06</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.3589 ± 0.0132</td>
<td>0.3040 ± 0.0148</td>
<td>0.015</td>
<td>↓15.30</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.4619 ± 0.0212</td>
<td>0.2640 ± 0.0134</td>
<td>0.001</td>
<td>↓42.84</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.1466 ± 0.0165</td>
<td>0.1142 ± 0.0072</td>
<td>0.097</td>
<td>↑22.10</td>
</tr>
<tr>
<td>Testis</td>
<td>0.6897 ± 0.0193</td>
<td>0.6540 ± 0.0264</td>
<td>0.293</td>
<td>↓5.18</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>KRP</th>
<th>P value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>76.7788 ± 1.7756</td>
<td>72.3821 ± 4.4834</td>
<td>0.403</td>
<td>↓ 5.73</td>
</tr>
<tr>
<td>Lung</td>
<td>73.9187 ± 6.8519</td>
<td>77.4971 ± 0.1108</td>
<td>0.618</td>
<td>↑ 4.84</td>
</tr>
<tr>
<td>Liver</td>
<td>76.0269 ± 0.3235</td>
<td>72.3924 ± 0.3966</td>
<td>0.001</td>
<td>↓ 4.78</td>
</tr>
<tr>
<td>Kidney</td>
<td>77.6486 ± 2.3387</td>
<td>76.8712 ± 0.1940</td>
<td>0.745</td>
<td>↓ 1.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>76.8223 ± 1.2644</td>
<td>74.7353 ± 0.9236</td>
<td>0.207</td>
<td>↓ 2.72</td>
</tr>
<tr>
<td>Testis</td>
<td>86.5863 ± 0.5632</td>
<td>81.5403 ± 4.0622</td>
<td>0.263</td>
<td>↓ 5.83</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease

REFERENCES