Toxicological studies of an ayurvedic medicine Yogendra Ras used in epilepsy

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ABSTRACT

Yogendra Ras (YR) is an ayurvedic preparation used as a traditional medicine in the treatment of epilepsy. To find out the toxicological characteristic of YR, it was administered chronically to the Sprague-Dawley rats at a dose of 40 mg/kg for 28 days. After 28 days chronic administration of the YR preparation, the following toxicological changes were noted. All throughout the experimental period, the YR treated animals were always maintaining negligible changes in overall body weight. There is a statistically very highly significant (p=0.001; 25.50% decrease) decrease in the absolute weight of the male rat liver and a highly significant (p=0.006; 23.53% decrease) decrease in the relative percent weight of the male rat liver. There is also a statistically highly significant (p=0.002; 18.90% decrease) decrease in the absolute weight of the male rat kidney and a statistically significant (p=0.015; 16.69% decrease) decrease in the relative percent weight of the kidney. There is a statistically significant decrease in the absolute weight of the male rat spleen (p=0.017; 31.10% decrease) and the relative percent weight of the male rat spleen (p=0.021; 30.48% decrease).

Keywords: Yogendra Ras, Ayurvedic preparation, Toxicology, Relative Organ Weight

INTRODUCTION

Yogendra Ras is an Ayurvedic medicine, with herbal and mineral ingredients, in tablet form. It is used in treating neuro-muscular conditions (Epilepsy) and diabetes [1-4]. It is also called as Yogendra Rasa. This medicine is used more in North Indian Ayurvedic treatment method and should only be taken strictly under medical supervision. Epilepsy is a serious neurological disorder affecting about 65 million people of the world [5, 6]. It is more common in males than females with the overall difference being small [7, 8]. Most of the people with this disease (80%) are in the developing countries [9]. In the developed world epilepsy most commonly starts either in the young or in the old. In the developing world its onset is more common in older children and young adults due to the higher rates of trauma and infectious diseases [10].

Yogendra Ras is included (pages 422-423) 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). The use of herbal preparations with inadequate scientific studies on their safety profile has raised concerns on their toxicity. That is why; we designed our current experiment to observe the effect of chronic administration of YR to Sprague-Dawley rats at a high dose (40 mg/kg). 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.
MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the toxicological study, Yogendra Ras (YR) was collected from Sri Kundeswari Aushadhlaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animals: Six to eight-week old male Sprague-Dawley rats 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 80-120 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 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) [11]. Sixteen male mice (30-40 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 (YR) 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 YR administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with YR 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 mg/Kg body weight [12]. 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 [13].

Overall Body Weight Analysis: Careful monitoring of body weight of rats was performed throughout the 28 days drug administration period. Body weights were recorded at regular intervals (2-3 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, expressed as percent increment in the body weight. 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 [14]. Rats of both YR 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 thus subjected to histo-pathological evaluation are: Heart, kidney, lungs, liver, spleen, thymus, stomach, caecum, pancreas, adrenal glands, urinary bladder, reproductive organs, which include testis, seminal vesicles, prostate gland and epididymis in case of males and ovaries, fallopian tube and uterus in case of females.

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) = \[
\frac{BW}{BW} \times 100
\]

AOW = Absolute organ weight

BW = body weight
RESULTS

Acute toxicity study: The drug (YR) administered up to a high dose of 4000 mg/kg produced no mortality. Thus the LD₅₀ value was found to be greater than 4000 mg/kg body weight. The animals did not manifest any sign of clinical toxicity. Since YR is in the clinical use for treating neuro-muscular conditions and diabetes 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 (4000 mg/kg body weight) was conducted. There were no mortality and toxicity signs observed at 4000 mg/kg body weight. Therefore, it can be concluded that YR when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic growth study

Effect of YR on Overall Body Weight: The total treatment period was of 28 days. All throughout the experimental period the YR treated animals were always maintaining negligible changes in body weight, but all throughout the experimental period no statistically significant increase or decrease was noted.

Effect of YR on Organ Toxicity Study: In absolute weight determination, there was a statistically very highly significant (p=0.001; 25.50% decrease) decrease in the absolute weight of the male rat liver. There was a statistically highly significant (p=0.002; 18.90% decrease) decrease in the absolute weight of the male rat kidney. There was also a statistically significant (p=0.017; 31.10% decrease) decrease in the absolute weight of the male rat spleen. In the relative weight determination, there is a statistically highly significant (p=0.006; 23.53% decrease) decrease in the relative percent weight of the male rat liver. There is a statistically significant (p=0.015; 16.69% decrease) decrease in the relative percent weight of the male rat kidney. There is also a statistically significant (p=0.021; 30.48% decrease) decrease in the relative percent weight of the male rat spleen.

Effect of YR on Tissue Hydration Index: In the tissue hydration index determination, no significant changes were noted in case of any organ.

DISCUSSION

Effect of YR on Overall Body Weight: All throughout the experimental period, the YR treated animals were always maintaining negligible changes in body weight, and no statistically significant increase or decrease was noted, Body weight (BW) or body weight gain (BWG) can influence the choice of the doses.

Effect of YR on Organ Toxicity Study: Drug-induced alterations in blood pressure, heart rate or cardiac conduction in animal studies may have implications for safety of a novel drug, even if they are devoid of any morphological correlate [15]. In this study we found that, heart weight decrease significantly to the YR 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 [16]. It was postulated that the reductions in heart weight were the result of the effect of atriopeptin III on fluid volume by an enhanced passage of fluid from the intramuscular to extra muscular compartment, or diuresis with subsequent alterations to cardiac workload.

Fresh lung weight is also a helpful measure in lung assessment, although passive vascular engorgement can significantly affect this value. There is no significant result of lung weight found in this study. Nevertheless, studies in the normal Fischer 344 rat have shown that after exsanguinations, wet lung weights show a close relationship to body weight and that dry weight of lungs consistently represents about 20% of the wet weights regardless of age or body weight [17]. An increase in wet weight over dry weight appears to be a good index of pulmonary edema [18]. 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 [19]. Here we found significantly decrease of liver weight to the YR treated rats.

Renal weight in laboratory animals appears not to show a close relationship with body weight. Here we found significantly decrease of kidney weight to the YR 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 pharma-cokinetics and pharmacodynamics of administered drugs [20].

In this study we found, spleen weight decrease very highly significantly to the YR treated rats. Although weighing of the spleen is easy, 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. This occurs with increasing age in rodents. 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 xenobiotics, notably corticosteroids, immunosuppressive and anticancer drugs.

It can be difficult to make a clear distinction in routine toxicity studies from the thymus weight loss and here we found that thymus weight decrease to the YR treated rats but the result is not significant. Atrophy produced by agents with immune modulating properties from those compounds that produce similar morphological changes as a result of a generalized, high dose stress response. Indeed thymic involution decreased responsiveness to Concanavalin A and lowered NK cell activity can be produced by a generalized stress response in a manner similar to that produced by the administration of glucocorticosteroids [21, 22]. The dose-response relationship however is of some help in deciding whether thymic atrophy is a direct result of immunosuppression or a non-specific result of stress. Powerful immunosuppressive drugs such as cyclophosphamide produce thymic effects in a dose-related manner, with thymic weight loss and atrophy occurring at essentially non-toxic doses. By contrast, atrophy resulting from stress is usually limited to high doses where there is other clear evidence of stress-related phenomena such as general clinical depression, weight loss or other overt evidence of intoxication [22].

Testicular weight remains fairly constant in sexually mature laboratory animals and in human adults. Its weight is relatively unaffected compared with organs such as liver and kidney or even accessory sex organs by under-feeding and low protein diets [18-20]. In this study, we found that testis weight decrease to the YR treated rats but the result is not significant. Dietary restriction of rats, however, reduces testicular weight only minimally compared with organs such as the heart, kidney and liver [20-22]. Drug induced effects on the seminiferous epithelium or hormonal control mechanisms may also reduce testicular weight. This is usually in association with histological evidence of cell loss.

**Effect of YR on Tissue Hydration Index:** Several physiological disorders can be caused by dehydration. It comprises from 75% body weight in infants to 55% in elder people and it is essential for maintaining cellular homeostasis. In our study, we found that YR did not cause any significant change in water content of any organ of our body. It can be suggested that this drug has no impact on maintaining cellular hemostasis.

**CONCLUSION**

From the above experiment it can be concluded that YR 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.
Figure 1: The effect YR (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 Yogendra Ras (YR)

<table>
<thead>
<tr>
<th>Name of ingredients</th>
<th>Amount Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parada</td>
<td>10 grams</td>
</tr>
<tr>
<td>Gandhaka</td>
<td>10 grams</td>
</tr>
<tr>
<td>Loha Bhasma</td>
<td>5 grams</td>
</tr>
<tr>
<td>Swarna Bhasma</td>
<td>5 grams</td>
</tr>
<tr>
<td>Abhraka Bhasma</td>
<td>5 grams</td>
</tr>
<tr>
<td>Shuddha Mukta</td>
<td>5 grams</td>
</tr>
<tr>
<td>Vanga bhasma</td>
<td>5 grams</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>YR</th>
<th>P value</th>
<th>% increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.465±0.022</td>
<td>0.422±0.011</td>
<td>0.107</td>
<td>↓ 9.19</td>
</tr>
<tr>
<td>Lung</td>
<td>0.884±0.039</td>
<td>0.798±0.020</td>
<td>0.073</td>
<td>↓ 9.78</td>
</tr>
<tr>
<td>Liver</td>
<td>6.783±0.361</td>
<td>5.053±0.215</td>
<td>0.001***</td>
<td>↓ 25.50</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.559±0.023</td>
<td>0.454±0.015</td>
<td>0.002**</td>
<td>↓ 18.90</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.707±0.043</td>
<td>0.487±0.066</td>
<td>0.017*</td>
<td>↓ 31.10</td>
</tr>
<tr>
<td>Testis</td>
<td>1.076±0.033</td>
<td>0.936±0.087</td>
<td>0.155</td>
<td>↓ 12.98</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease; \( p* \leq 0.05, p** \leq 0.01, p*** \leq 0.001 \)
Table 3: The effect of Yogendra Ras on the relative organ weights of male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>YR</th>
<th>P value</th>
<th>%increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.296±0.008</td>
<td>0.278±0.014</td>
<td>0.256</td>
<td>↓ 6.34</td>
</tr>
<tr>
<td>Lung</td>
<td>0.564±0.013</td>
<td>0.524±0.023</td>
<td>0.147</td>
<td>↓ 7.15</td>
</tr>
<tr>
<td>Liver</td>
<td>4.354±0.229</td>
<td>3.329±0.220</td>
<td>0.006**</td>
<td>↓ 23.53</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.359±0.013</td>
<td>0.299±0.017</td>
<td>0.015*</td>
<td>↓ 16.69</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.462±0.021</td>
<td>0.321±0.046</td>
<td>0.021*</td>
<td>↓ 30.48</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.147±0.0165</td>
<td>0.124±0.011</td>
<td>0.333</td>
<td>↓ 15.14</td>
</tr>
<tr>
<td>Testis</td>
<td>0.689±0.019</td>
<td>0.607±0.055</td>
<td>0.176</td>
<td>↓ 11.96</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease; *p*≤0.05, **p**≤0.01, ***p***≤0.001

Table 4: The effect of Yogendra Ras on various tissue hydration indices of male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>YR</th>
<th>P value</th>
<th>%increase/decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>76.779±1.776</td>
<td>62.358±14.426</td>
<td>0.372</td>
<td>↓ 18.78</td>
</tr>
<tr>
<td>Lung</td>
<td>73.919±6.852</td>
<td>78.227±0.201</td>
<td>0.55</td>
<td>↑ 5.83</td>
</tr>
<tr>
<td>Liver</td>
<td>76.027±0.323</td>
<td>70.951±3.351</td>
<td>0.154</td>
<td>↓ 6.68</td>
</tr>
<tr>
<td>Kidney</td>
<td>77.649±2.339</td>
<td>76.457±0.810</td>
<td>0.638</td>
<td>↓ 1.53</td>
</tr>
<tr>
<td>Spleen</td>
<td>76.822±1.264</td>
<td>80.224±3.107</td>
<td>0.340</td>
<td>↑ 4.43</td>
</tr>
<tr>
<td>Testis</td>
<td>86.586±0.563</td>
<td>85.637±0.718</td>
<td>0.311</td>
<td>↓ 1.10</td>
</tr>
</tbody>
</table>

↑: increase, ↓: decrease; *p*≤0.05, **p**≤0.01, ***p***≤0.001

REFERENCES:


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