

Evaluation of pharmacological and toxicological studies of an ayurvedic medicine Rasaraj Ras on biological system of the male Sprague-Dawley rats

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ABSTRACT

Background: In this study, the pharmacological and toxicological effects along with possible side effects of the classical ayurvedic formulation Rasaraj Ras (RR) which is used as a traditional medicine in the treatment of hemiplegia in the rural population were evaluated.

Methods: During this study, various experiments on body growth rate, organ-body weight ratio and tissue hydration indices were performed to evaluate its efficacy and toxicity. To find out the toxicological characteristic of RR, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg. After 28 days chronic administration of the RR preparation the following toxicological changes were noted.

Results: All throughout the experimental period the RR treated animals were always maintaining negligible changes in body weight, but all throughout the experimental period no statistically significant increase or decrease was noted. There is a statistically significant decrease in the relative percent weight of the male rat heart. There is a statistically highly significant decrease in the absolute weight of the male rat liver. There is a statistically highly significant decrease in the relative percent weight of the male rat liver. There is a statistically significant decrease in the relative percent weight of the male rat kidney. In the tissue hydration index determination, no significant changes were noted in case of any organ.

Conclusions: As RR decreases abnormally weight of several organs in body of treated rats, so it should not be administered chronically at a higher dose.

Keywords: Absolute weight, Organ percent weight, Organ water content Pharmacological, Rasaraj Ras, Toxicological

INTRODUCTION

Ayurvedic medicines have reputation as decent and effective remedies for a number of diseases.¹ Currently, the World Health Organization (WHO) has officially recognized and recommended large-scale use of herbal (Unani and Ayurvedic) medicines, particularly in the developing countries, as an alternative system of medicine to deliver health care services at the primary health care level.² According to WHO, an estimated 1.5 billion people of the world are now getting treatment with these medicines.^{3,4} They have a good safety profile also.⁵

Rasaraj Ras (RR) an Ayurvedic preparation used as a traditional medicine in the treatment of hemiplegia in the rural populatio.^{6,7}

Rasaraj Rasa 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).

METHODS

Drugs, chemicals and reagents

For the toxicological study, Rasaraj Ras (RR) was collected from Sri Kundeswari Aushadhalaya 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 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 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.

Table 1: Name of the ingredients/herbs used in the preparation of Rasaraja Rasa.

Ingredients botanical/English name amounts
Parada Purified and processed Mercury 48 g
Abhrakastva Purified and processed Silica 12 g
Swarna Bhasma Gold Bhasma 12 g
Loha Bhasma Iron Bhasma 6 g
Rajata Bhasma Silver Bhasma 6 g
Vanga Bhasma Tin Bhasma 6 g
Vajigandha (Root) <i>Withania somnifera</i> 6 g
Lavanga (Flower) <i>Syzigium aromaticum</i> 6 g
Jatikosha (Ar.) <i>Myristica fragrans</i> 6 g
Ksheerakakoli (Root) <i>Fritillaria roylei</i> 6 g
Kakamachirasa (Juice) <i>Solanum nigrum</i> Quantity sufficient

Experimental design

Acute pharmacological 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).⁸ 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 Rasaraj Ras (RR) 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 RR administration.

Chronic pharmacological studies

Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with Rasaraj Ras (RR) 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.⁹ 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.¹⁰

Growth analysis

Careful monitoring of body weights of rats of both sexes 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. An equal numbers of animals of the same species were also maintained as the Control group and these were also kept under close observations. 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.

Body weight: Organ weight ratio analysis

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.¹¹ Rats of both Rasaraj Ras (RR) 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 histopathological 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.

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

AOW= Absolute organ weight; BW= body weight

$$\text{Water content in tissue} = \frac{\text{OW}_1 - \text{OD}}{\text{OW}_1 - \text{OF}} \times 100$$

OW₁ = organ wet weight; OD = organ dry weight; OF = organ foil weight

Statistical analysis

The data were analysed 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.

RESULTS

Acute pharmacological study

The Rasaraj Ras (RR) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD₅₀ 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 RR when administered at single dose is non-toxic and can be used safely in oral formulations.

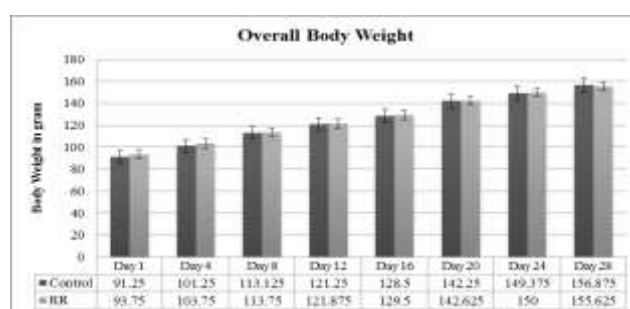
Effect of RR on overall body weight

The total treatment period was of 28 days. All throughout the experimental period the RR treated animals were always maintaining negligible (0.55% gain (p=0.929) – 0.80% loss (p=0.873)) changes in body weight, but all

throughout the experimental period no statistically significant increase or decrease was noted.

Effect of RR on organ pharmacological study

In absolute weight determination, results show that There is a statistically highly significant (p=0.007) decrease in the absolute weight of the male rat liver [19.94 % decrease]. In relative weight determination results show, There is a statistically significant (p=0.025) decrease in the relative percent weight of the male rat heart [8.07 % decrease]. There is a statistically highly significant (p=0.008) decrease in the relative percent weight of the male rat liver [19.62 % decrease]. There is a statistically significant (p=0.056) decrease in the relative percent weight of the male rat kidney [9.11 % decrease].



Independent sample t-test was performed to analyse 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.

Figure 1: The effect Rasaraj Ras (40 mg/kg) on the body weights (g) of Sprague-Dawley rats with the time of treatment.

Chronic growth study

Effect of RR on tissue hydration index

In the tissue hydration index determination, no significant changes were noted in case of any organ.

Table 2: The effect of Rasaraj Ras (RR) (40 mg/kg) on the absolute organ weights of male rats.

Parameters	Control	RR	p value	%increase/decrease
Heart	0.4646±0.02222	0.4244±0.01552	0.16	↓8.65
Lung	0.8841±0.03987	0.9104±0.03089	0.61	↑2.97
Liver	6.7825±0.36095	5.4299±0.22651	0.007**	↓19.94
Kidney	0.5599±0.02303	0.507±0.01553	0.078	↓9.45
Spleen	0.7074±0.04301	0.6337±0.09935	0.512	↓10.42
Testis	1.0759±0.03306	1.0458±0.01002	0.407	↓2.80

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

Table 3: The effect of Rasaraj Ras (RR) (40 mg/kg) on the relative organ weights of male rats.

Parameters	Control	RR	p value	%increase/decrease
Heart	0.2963±0.00774	0.2724±0.00555	0.025*	↓8.066
Lung	0.564±0.01312	0.5902±0.03325	0.476	↑4.65
Liver	4.3542±0.22992	3.4999±0.15534	0.008**	↓19.62
Kidney	0.3589±0.01323	0.3262±0.00834	0.05*	↓9.11
Spleen	0.4619±0.02116	0.4094±0.06939	0.489	↓11.37
Thymus	0.1466±0.01648	0.1265±0.0295	0.538	↓13.71
Testis	0.6897±0.01931	0.6749±0.01643	0.568	↓2.15

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

Table 4: The effect of Rasaraj Ras (RR) (40 mg/kg) on various tissue hydration indices of male rats.

Parameters	Control	RR	p value	%increase/decrease
Heart	76.7788±1.77558	74.5269±2.81526	0.525	↓2.93
Lung	73.9187±6.85189	73.9765±4.46345	0.994	↑0.078
Liver	76.0269±0.32347	75.0743±1.21306	0.461	↓1.25
Kidney	77.6486±2.33869	80.5775±1.84735	0.342	↓3.77
Spleen	76.8223±1.26437	70.1826±3.86954	0.148	↓8.64
Testis	86.5863±0.56317	119.74±33.96021	0.346	↓38.29

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

DISCUSSION

The administration of herbal preparations without any standard dosage along with insufficient scientific studies on their safety profile has raised concerns on their toxicity.¹² Change in body weight is a sign of impairment in the normal functioning of the body. All throughout the experimental period the RR treated animals were always maintaining negligible changes in body weight, but all throughout the experimental period no statistically significant increase or decrease was noted. Rapid body weight change may be due to feed and/or water consumption, disease, dental maladies, or specific toxic effects.¹³

Relative organ weight (ROW) may serve as a sign of pathological and physiological status in man and animals. Toxic substances induce abnormal metabolic reactions that affect primary organs (e.g. heart, liver, spleen, kidney and lung).¹⁴ Change in organ weight is a symbol of impairment in the normal body functioning. Organ-body weight ratio may indicate organ swelling, atrophy or hypertrophy.¹⁵

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.¹⁶ In this study we found, heart weight decrease significantly to the Rasaraj Ras 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.¹⁷ 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.

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.¹⁸ Here we found significantly decrease of liver weight to the Rasaraj Ras 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 Rasaraj Ras 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.¹⁹

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 RR 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 RR should not be administered chronically at a higher dose as it decrease weight of heart, liver, kidney. Further studies should be done by reducing the administered dose.

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Conflict of interest: None declared

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