International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

TOXICOLOGICAL STUDIES OF AN AYURVEDIC MEDICINE CHANDRANGSHU RAS

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Received on: 12-08-2017; Revised on: 25-09-2017; Accepted on: 28-09-2017

ABSTRACT

Chandrangshu Ras (CDR) is an ayurvedic preparation used as a traditional medicine in the treatment of vulvodynia. In this study, effect of CDR on organ toxicity profile was evaluated after chronic administration of this drug to male Sprague-Dawley rats. The acute pharmacological study of CDR recorded no death or any signs of toxicity even at the highest dose of 4000 mg/Kg body weight. For chronic pharmacological evaluation, the animals were divided into two groups. The first group was given CDR preparation at a dose of 100 mg/kg body weight for 32 days while the second group that served as the control received water for the same period. After 32 days of chronic administration of the CDR preparation, the following effects on the organ toxicity profile were noted. All throughout the experimental period the CDR treated animals were always maintaining negligible decrease in body weight, in the body weight study, but it was not significant. The drug (CDR) did not affect any absolute or relative percent weight of different organs of the body and also water content of these organs significantly. So, the results of the present prospective study showed that ayurvedic treatment with Chandrangshu Ras is safe for oral administration

Keywords: Chandrangshu Ras, organ toxicity, absolute weight, organ water content

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.

Chandrangshu Ras (CDR) is an Ayurvedic preparation used as a traditional medicine in the treatment of Vulvodynia in the rural female population [5-10]. Chandrangshu Ras is included (pages 275-276) 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, under the authority vested in the Board vide section 13(j) of the Bangladesh Unani

and Ayurvedic practitioners Ordinance, 1983. 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 CDR to Sprague-Dawley rats at a high dose (100 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, Chandrangshu Ras (CDR) 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 toxicological 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.

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, Chandrangshu Ras (CDR) 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 CDR administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with Chandrangshu Ras (CDR) 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 32 days. For all the pharmacological studies the drugs were administered per oral route at a dose of 100 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].

Growth Analysis: Careful monitoring of body weights of rats was performed throughout the 32 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 number 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 32 day treatment period, the animals were fasted for 18 hours. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia [14]. Rats of both CDR and Control groups were sacrificed after the completion of the 32-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. 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 weight of organ= ——×100

AOW= Absolute organ weight
BW= body weight

Water content in tissue= ————×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 of the Mean) and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significant.

RESULTS AND DISCUSSION

Acute toxicity study: The drug Chandrangshu Ras (CDR) administered up to a high dose of 4000 mg/kg produced no mortality. Thus the LD_{50} value was found to be greater than 4000 mg/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 (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 Chandrangshu Ras (CDR) when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic growth study

Effect of CDR on Overall Body Weight: The administration of ayurvedic preparations without any standard dosage along with insufficient scientific studies on their safety profile has raised concerns on their toxicity [15]. Alteration in weight is an indication of impairment in the normal functioning of the body. The total treatment period was of 32 days. All throughout the experimental period the CDR treated animals were always maintaining decrease in body weight, in the body weight study, the CDR administered animal were weighing 0.63 % (p=0.949) to 3.39 % (p=0.652) 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 [16]. All throughout the experimental period no statistically significant decrease was noted.

Effect of CDR 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 [16]. 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 [17].

In absolute weight determination results show, there is a (7.45 %) decrease in the absolute weight of the male rat lungs, the decrease though not significant yet it was *noticeable* (p=0.106). There is a statistically insignificant (p=0.657) (3.50 %) decrease in the absolute weight of the male rat liver. There is a negligible (1.50 %] increase in the absolute weight of the male rat kidney, which was statistically not at all significant (p=0.786). There is a statistically insignificant (p=0.544) (4.71 %) decrease in the absolute weight of the male rat spleen. There is a negligible (1.54 %) increase in the absolute weight of the rat testis, which was statistically not at all significant (p=0.822).

In relative weight determination results show, there is an (3.23 %) increase in the relative percent weight of the male rat heart, the increase though not significant yet it was prominent (p=0.326).There is a statistically insignificant (p=0.551) (3.55 %] decrease in the relative percent weight of the male rat lungs. There is a negligible [0.27 %] increase in the relative percent weight of the male rat liver, which was statistically not at all significant (p=0.956).There is an [5.09 %] increase in the relative percent weight of the male rat kidney, the increase though not significant yet it was prominent (p=0.260). There is a negligible (1.77 %] decrease in the relative percent weight of the male rat spleen, which was statistically not at all significant (p=0.819). There is a negligible [3.58 %] decrease in the relative percent weight of the male rat thymus, which was statistically not at all significant (p=0.811). There is an [4.15 %] increase in the relative percent weight of the rat testis, the increase though not significant yet it was prominent (p=0.417).

Effect of CDR 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 (Popkin BM et al., 2010).

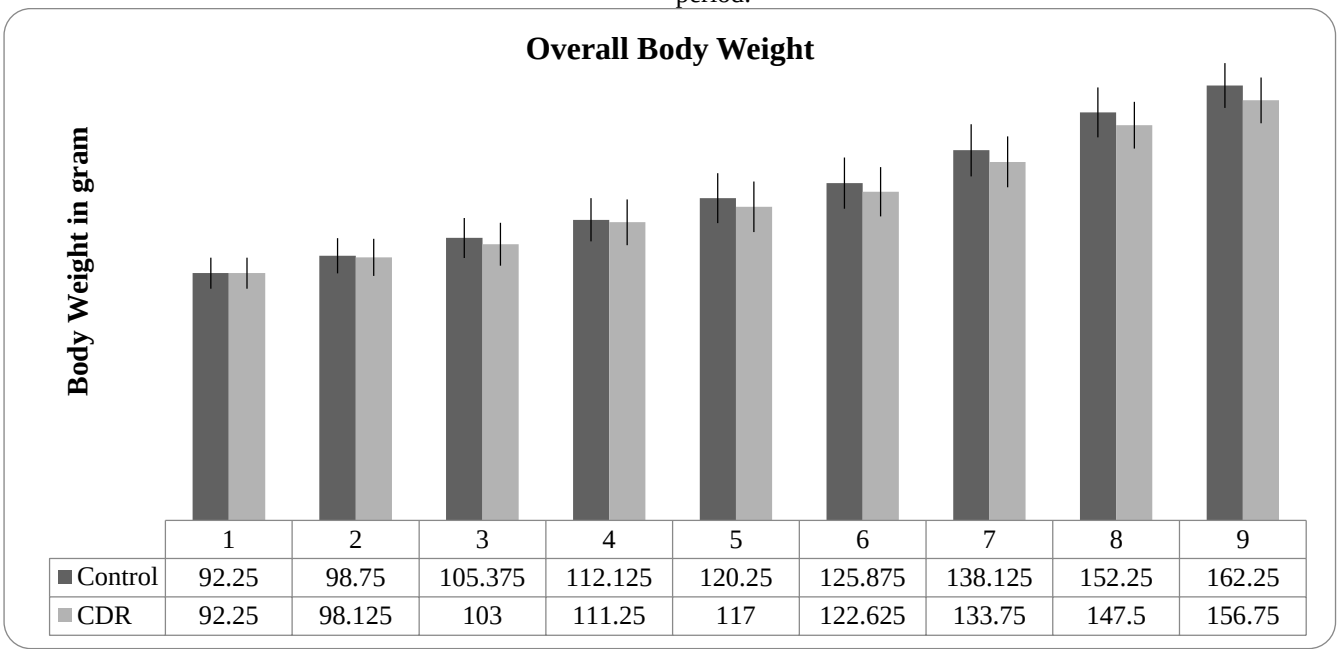
In the tissue hydration index determination, there is an [2.67 %] increase in the organ water content of the male rat heart, the increase though not significant yet it was prominent (p=0.168).There is a [0.60 %] decrease in the organ water content of the male rat lungs, the decrease though not significant yet it was prominent (p=0.496). There is an [0.84 %] increase in the organ water content of the male rat liver, the increase though not significant yet it was prominent (p=0.160). There is a statistically insignificant (p=0.676) [0.72 %] decrease in the organ water content of the male rat kidney. There is a [4.75 %] decrease in the organ water content of the male rat spleen, the decrease though not significant yet it was prominent (p=0.239). There is a statistically insignificant (p=0.634) [0.13 %] decrease in the organ water content of the rat testis.

CONCLUSION

From the above experiment it can be concluded that CDR is devoid of any intensive adverse effect as revealed from the body organ ratio studies. Further studies should be done on heart, lung, liver, kidney, spleen, testis to have a clear picture about organ toxicities after chronic administration of CDR.

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.



	1	2	3	4	5	6	7	8	9
Control	92.25	98.75	105.375	112.125	120.25	125.875	138.125	152.25	162.25
CDR	92.25	98.125	103	111.25	117	122.625	133.75	147.5	156.75

Figure 1: The effect Chandrangshu Ras (CDR) (100 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 used in the preparation of Chandrangshu Ras (CDR)

Name of ingredients	Used part	Botanical name	Amount Used
Rasa (parada)	Kajjali	Hydragentum	10 g
Abhraka Bhasma	Calcined	Purified Mica oxide $K(Mg,Fe)_3AlSi_3O_{10}(Fe,OH)_2$	10 g
Lauha bhasma	Calcined	Purified Iron oxide Fe_3O_4	10 g
Vanga Bhasma	Calx	Tin	10 g
Shuddha Gandhaka	Kajjali	Herbal purified Sulphur	10 g
Kanya(ghrtakumari)	Exudate	Aloe barbadensis Mill.	Q.S.

Table 2: The effect of Chandrangshu Ras (CDR) (100 mg/kg) on the absolute organ weights of male rats

Parameters	Control	CDR	p value	%increase/decrease
Heart	0.4255±0.0184	0.4226±0.01464	0.904	↓0.68
Lung	0.8844±0.03365	0.8185±0.01803	0.106	↓7.45
Liver	5.2442±0.32213	5.0609±0.24501	0.657	↓3.50
Kidney	0.4931±0.01812	0.5005±0.01959	0.786	↑1.50
Spleen	0.4611±0.02856	0.4394±0.02023	0.544	↓4.71
Testis	1.0184±0.03086	1.0341±0.06123	0.822	↑1.54

Values are presented as mean ± SEM (n=8). 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 Chandrangshu Ras (CDR) (100 mg/kg) on the relative organ weights of male rats.

Parameters	Control	CDR	p value	%increase/decrease
Heart	0.2632±0.00521	0.2717±0.00649	0.326	↑3.23
Lung	0.5498±0.02052	0.5303±0.02445	0.551	↓3.545
Liver	3.2368±0.12244	3.2457±0.10447	0.956	↑0.27
Kidney	0.3063±0.00928	0.3219±0.00951	0.26	↑5.09
Spleen	0.2876±0.01912	0.2825±0.01101	0.819	↓1.77
Thymus	0.1451±0.01363	0.1399±0.01679	0.811	↓3.58
Testis	0.6341±0.02297	0.6604±0.02159	0.417	↑4.14

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. $p<0.05$ was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

Table 4: The effect of Chandrangshu Ras (CDR) (100 mg/kg) on various tissue hydration indices of male rats.

Parameters	Control	CDR	p value	%increase/decrease
Heart	75.9169±1.35881	77.942±0.30702	0.168	↑2.67
Lung	79.9438±0.53312	79.4666±0.42563	0.496	↓0.60
Liver	72.6499±0.3112	73.263±0.27097	0.16	↑0.84
Kidney	77.8321±1.28889	77.2718±0.25348	0.676	↓0.71
Spleen	76.7662±0.47337	73.1224±2.92552	0.239	↓4.74
Testis	86.8774±0.18917	86.7666±0.12645	0.634	↓0.13

Values are presented as mean ± SEM (n=8). Independent sample t-test was performed to analyze this dataset. $p<0.05$ was considered statistically significant when compared against control. ↑: increase, ↓: decrease.

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