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ORGAN BODY WEIGHT RATIO TOXICITY STUDIES OF AN AYURVEDIC MEDICINE CHINTAMANICHATURMUKH RAS USED IN VERTIGO

Tasniya Nahiyan Zulfiquar, Md. Mamun Sikder, Tanmoy Sana, Imtiaj Hossain Chowdhury, Nilay Saha, Sagor Chandra Roy, Runa Masuma and M. S. K. Choudhuri*

Department of Pharmacy, Jahangirnagar University, Savar-1342, Dhaka, Bangladesh

***Corresponding author e-mail**: mskc1954@gmail.com

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ABSTRACT

Chintamanichaturmukh Ras (CMC) is an Ayurvedic preparation used as a traditional medicine in the treatment of vertigo in the rural population. To find out the toxicological characteristic of CMC, it was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg for 28 days. After 28 days chronic administration of the CMC preparation, the following toxicological changes were noted. All throughout the experimental period the CMC treated animals were always maintaining decrease in body weight but it was not significant. There was a statistically significant (p=0.039; 9.28 % decrease) decrease in the relative percent weight of the male rat heart. There was a statistically significant decrease in the absolute weight of the male rat liver (p=0.018; 19.04 % decrease) and a statistically highly significant decrease was noted in case of relative percent weight of the liver (p=0.01; 18.61 % decrease). There was also a statistically highly significant (p=0.002; 4.31 % decrease) decrease in the organ water content of the rat liver.

Key words: Chintamanichaturmukh Ras, vertigo, toxicology, absulate weight, organ ratio, organ water content

INTRODUCTION

Vertigo is a subtype of dizziness, defined as an illusion of movement caused by asymmetric involvement of the vestibular system [1-5]. The incidence of vertigo increases with age and is about two to three times higher in women than in men [2, 5]. In the developed world, Vertigo accounts for about 2-3% of emergency department visits [6]. The most common causes of vertigo are vestibular disorders including: benign paroxysmal positional vertigo (BBPV), Ménière's disease (MD), and vestibular neuritis [7, 8]. There is no single effective medication for vertigo and in clinical practice a combination of drugs are used, including antihistamines and anti-emetics [9, 10]. Acutely administered anti-vertiginous medications can be given to treat the attack; however, these have limited benefit and no effect in those patients where episodes only last a few seconds [11, 12].

Ayurvedic medicines have reputation as decent and effective remedies for a number of diseases [13]. 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 [14]. According to WHO, an estimated 1.5 billion people of the world are now getting treatment with these medicines [15, 16]. They have a good safety profile also [17]. Chintamanichaturmukh Ras is an Ayurvedic medicine in tablet form. It is used in the treatment of vertigo, giddiness, epilepsy, psychosis, etc [18-23]. Chintamanicaturmukha Ras is included (page 215) 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). 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 Chintamanichaturmukh Ras, used as a remedy of vertigo 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 toxicological study, Chintamanichaturmukh Ras (CMC) 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) [24]. 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 (CMC) 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 CMC administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with CMC 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 [25]. 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 [26].

***Overall Body Weight Analysis*:** Careful monitoring of body weights 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. 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.

Organ Toxicity Study: At the end of the 28 day 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 CMC 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. 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.

Relative weight of organ= ——×100

AOW= Absulate organ weight
BW= body weight

Water content in tissue= ————×100

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 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 significance.

RESULTS

Acute toxicity study: The drug (CMC) 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 CMC when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic growth study

***Effect of* CMC *on Overall Body Weight*:** The total treatment period was of 28 days. All throughout the experimental period the CMC treated animals were always maintaining decrease in body weight, in the body weight study, the CMC administered animal were weighing 1.43 % ($p=0.810$) less than their control counterpart. All throughout the experimental period no statistically significant decrease was noted.

Effect of* CMC *on Organ Toxicity Study: In absolute weight determination, results show that there is a statistically significant ($p=0.018$) decrease in the absolute weight of the male rat liver [19.04 % decrease]. In relative weight determination results show, there is a statistically significant ($p=0.039$) decrease in the relative percent weight of the male rat heart [9.28 % decrease]. There is a statistically highly significant ($p=0.01$) decrease in the relative percent weight of the male rat liver [18.61 % decrease].

Effect of* CMC *on Tissue Hydration Index: In the tissue hydration index determination, there is a statistically highly significant ($p=0.002$) decrease in the organ water content of the male rat liver [4.31 % decrease].

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 [27]. 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 1.43 % 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 [28].

Effect of* CMC *on Organ Toxicity Study: Relative organ weight may serve as indicator 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 [29]. 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 [30].

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 [31]. In this study we found, relative heart weight decrease significantly to the Chintamanichaturmukh 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 [32]. 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 dieresis 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 age-related change in both humans and laboratory rodents is a decline in liver volume [33]. Here we found significantly decrease of liver weight to the Chintamanichaturmukh Ras treated rats.

Effect of* CMC *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 [34]. In our study we found that CMC cause significant reduction in % water content of liver. It can be suggested that this drug has negative impact on maintaining cellular haemostasis.

CONCLUSION

The present study revealed that CMC should not be administered chronically at a higher dose. Further research will be needed to clarify the toxicological effect of CMC at higher dose as well as its effect at lower dose.

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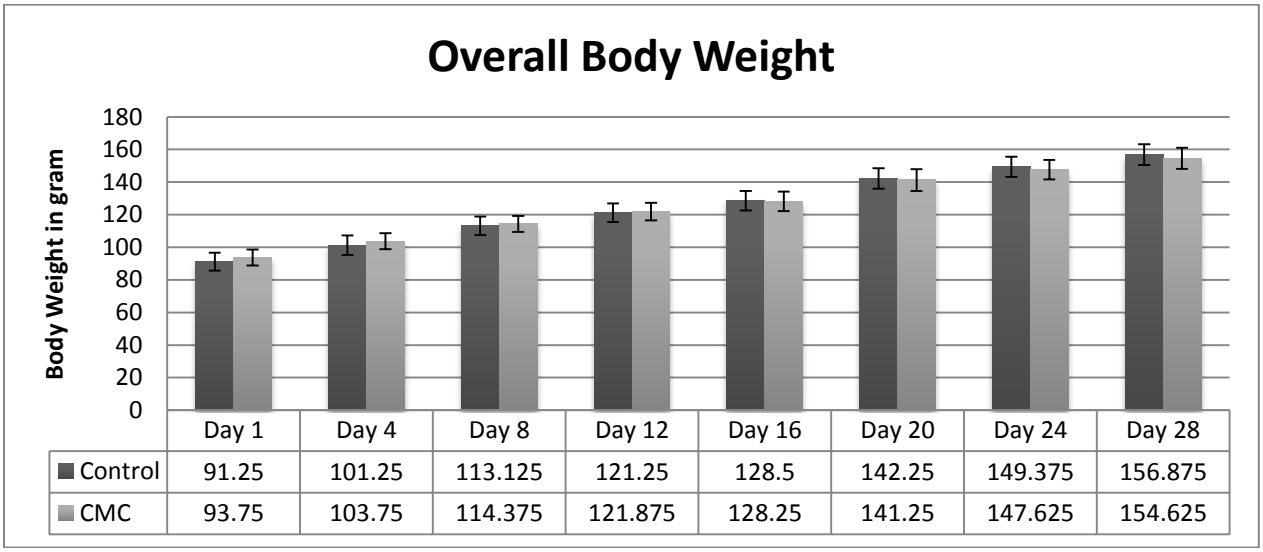

Figure 1: The effect CMC (40 mg/kg) on the body weights (g) of Spraque-Dawley rats with the time of treatment. Independent sample t-test was performed to analyze this weight variation in different days. All values are expressed as mean ± SEM and $p<0.05$ was taken as the level of significant.

Table 1: Name of the ingredients/herbs used in the preparation of Chintamanichaturmukh Ras

Name of ingredients	Plant part	Botanical name	Family	Amount
Rasa Sindura		Mercury sulphide (alpha-HgS, hexagonal)	Gandhaka yoga	20 g.
Lauha bhasma	Calyx	Purified Iron oxide Fe_3O_4	Mineral	10 g.
Abhraka bhasma	Calyx	Purified Mica oxide $K(Mg,Fe)_3AlSi_3O_{10}(Fe,OH)_2$	Mineral	10 g.
Svarna	Calyx	Gold (Au nanoparticles)	Mineral	5 g.
Kanya (ghrtakumari)	Exudate	Aloe barbadensis Mill.	Liliaceae	q.s. as paste for mardana
Eranda patra	Leaf	Ricinus communis L.	Euphorbiaceae	q.s. for avestana

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

Parameters	Control	CMC	p value	%increase/decrease
Heart	0.4646 ± 0.02222	0.4141 ± 0.01819	0.101	↓10.87
Lung	0.8841 ± 0.03987	0.9197 ± 0.04082	0.543	↑4.03
Liver	6.7825 ± 0.36095	5.4908 ± 0.31619	0.018	↓19.04
Kidney	0.5599 ± 0.02303	0.5162 ± 0.04036	0.363	↓7.80
Spleen	0.7074 ±0.04301	0.6838 ± 0.12021	0.864	↓3.34
Testis	1.0759 ± 0.03306	1.0261 ± 0.04141	0.363	↓4.63

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 Chintamanichaturmukh Ras (40 mg/kg) on the relative organ weights of male rats.

Parameters	Control	CMC	p value	%increase/decrease
Heart	0.2963 ± 0.00774	0.2688 ± 0.00927	0.039	↓ 9.28
Lung	0.564 ± 0.01312	0.5975 ± 0.02287	0.224	↑ 5.94
Liver	4.3542 ± 0.22992	3.5438 ± 0.10268	0.01	↓ 18.61
Kidney	0.3589 ± 0.01323	0.335 ± 0.02561	0.422	↓ 6.66
Spleen	0.4619 ± 0.02116	0.4423 ± 0.08055	0.829	↓ 4.24
Testis	0.6897 ± 0.01931	0.6666 ± 0.0238	0.464	↓ 3.35

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 Chintamanichaturmukh Ras (40 mg/kg) on various tissue hydration indices of male rats.

Parameters	Control	CMC	p value	%increase/decrease
Heart	76.7788 ± 1.77558	73.8714 ± 2.01312	0.305	↓ 5.73
Lung	73.9187 ± 6.85189	66.54 ± 10.90054	0.576	↑ 4.84
Liver	76.0269 ± 0.32347	72.7535 ± 0.79932	0.002**	↓ 4.78
Kidney	77.6486 ± 2.33869	76.8877 ± 0.34999	0.752	↓ 1.00
Spleen	76.8223 ± 1.26437	75.8441 ± 1.36876	0.612	↓ 2.72
Testis	86.5863 ± 0.56317	79.2571 ± 5.89848	0.236	↓ 5.83

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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