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Toxicological Studies of an Ayurvedic Medicine "Swalpo Chandrodoy Makardhwaj" Used as Geriatric Rejuvenator

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ABSTRACT

Swalpo Chandrodoy Makardhwaj (SCM) is an Ayurvedic geriatric rejuvenator used as a traditional medicine in the rural area of Bangladesh. To find out the toxicological characteristic of SCM, it was administered chronically to the male Sprague-Dawley rats at a dose of 100 mg/kg for 32 days and the following toxicological changes were noted. All throughout the experimental period the SCM treated animals were always maintaining negligible changes in body weight. There is a statistically significant (p=0.012) decrease in the organ water content of the male rat lungs whereas a statistically significant (p=0.044) increase was noted in case of organ water content of liver. There is a statistically significant increase in relative percent weight of rat kidney (p=0.028) and absolute weight of rat spleen (p=0.024). There is also a statistically significant (p=0.034) increase in the relative percent weight of the male rat spleen.

Keywords: Swalpo Chandrodoy Makardhwaj Toxicology, Rejuvenator, 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 [5]. Swalpo Chandrodoy Makardhwaj (SCM) is an Ayurvedic geriatric rejuvenator used in the old age in the rural population [6-11]. Swalpa Chandrodaya Makardhwaja 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 SCM 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, Swalpo Chandrodoy Makardhwaj (SCM) was collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh. Ketamine injection was purchased from ACI Limited, Dhaka, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Experimental Animal: 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) [12]. 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 (SCM) 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 SCM administration.

Chronic toxicity studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with SCM 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 ml/Kg body weight [13]. 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 [14].

Overall Body Weight Analysis: Careful monitoring of body weights of rats of both sexes 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 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 32-days 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 [15]. Rats of both SCM and Control groups were sacrificed after the completion of the 32-days 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.

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

AOW= Absolute organ weight

BW= body weight

$$\text{Water content in tissue} = \frac{OW1 - OD}{OW1 - OF} \times 100$$

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 Mean) and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significant.

RESULTS

***Acute toxicity study*:** The drug (SCM) administered up to a high dose of 80 ml/kg produced no mortality. Thus the LD_{50} 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 SCM when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic growth study

***Effect of* SCM *on Overall Body Weight*:** The total treatment period was of 32 days. All throughout the experimental period the SCM 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* SCM *on Organ Toxicity Study*:** Absolute weight determination study show that, there is an [10.04 %] increase in the absolute weight of the male rat kidney, the increase though not significant yet it was noticeable ($p=0.067$). But there is a statistically significant ($p=0.024$) increase in the absolute weight of the male rat spleen [42.07 % increase]. Relative weight determination results show that there is a negligible [0.90 %] decrease in the relative percent weight of the male rat liver, which was statistically not at all significant ($p=0.915$). But there is a statistically significant ($p=0.028$) increase in the relative percent weight of the male rat kidney [12.90 % increase]. There is also a statistically significant ($p=0.034$) increase in the relative percent weight of the male rat spleen [47.84 % increase].

Effect of* SCM *on Tissue Hydration Index: In the tissue hydration index determination study, there is a statistically significant ($p=0.012$) decrease in the organ water content of the male rat lungs [5.78 % decrease]. There is also a statistically significant ($p=0.044$) increase in the organ water content of the male rat liver [2.35 % increase].

DISCUSSION

Effect of SCM on over all body growth: The administration of Ayurvedic preparations without any standard dosage accompanied by inadequate scientific studies on their toxicological safety profile has raised concerns on their toxicity [16]. Change in body weight is a sign of impairment in the normal functioning of the body. All throughout the experiment, SCM administered animal showed no statistically significant increase in body weight.

Effect of* SCM *on Organ Toxicity Study: Change in organ weight is a symbol of impairment in the normal body functioning. 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) [17]. Organ-body weight ratio may indicate organ swelling, atrophy or hypertrophy [18].

Xenobiotics may alter renal weight and as a consequence any renal weight changes in toxicity studies should be assessed with care. In this study we found that relative weight of kidney significantly increases to the SCM treated rats. When increases in renal weight are manifestations of toxicity, they are frequently associated with macroscopic appearances of swelling and pallor of the kidney and evidence of significant damage on histological examination. When increases in renal weight occur in the absence of histopathological alterations, it is reasonable to assume that the changes are a manifestation of adaptive responses to increased physiological demands placed on the renal tissue in the elimination of the xenobiotic. Some xenobiotics, notably angiotensin-converting enzyme (ACE) inhibitors, have been associated with a reduction in renal weight without evidence of renal cellular damage, presumably as a result of reduced renal demand.

In this study we found, spleen weight significantly increases to the SCM treated rats. In rodents, evidence of increased red cell turnover in the spleen is provided by increased splenic weight, changes in splenic pigmentation, presence of foam cells and intense erythropoiesis. The red pulp may expand and develop marked haematopoiesis under a variety of circumstances. In rodents many of these stimuli are non-specific and occur sporadically in long term studies. Drugs and chemicals that affect blood cells may activate intense haematopoiesis in the spleen, the cytological nature of which varies with the type of cell affected. Thus, increased haematopoiesis as a result of increased red cell demand shows

predominantly cells of the red cell series in the spleen whereas less specific processes such as infections tend to be associated with a more diverse cell population, including megakaryocytes. However, increased erythropoiesis may be difficult to distinguish from plasma cell hyperplasia that also occurs in the red pulp. The presence of pigment-laden macrophages as well as numerous erythropoietic cells in the red pulp implies that there is increased red cell turnover in the spleen.

***Effect of** SCM **on Tissue Hydration Index*:** A lot of physiological disorders can be caused by dehydration [19]. Water 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 SCM causes significant decrease in % water content of lung and increase in % water content of liver. It can be suggested that this drug has impact on maintaining cellular haemostasis.

CONCLUSION

From the above experiment to determine toxicity, it can be concluded that Swalpo Ch**a**ndrod**o**y Makardhwaz should not be administered chronically at a higher dose. Further studies should be done by reducing the administered dose.

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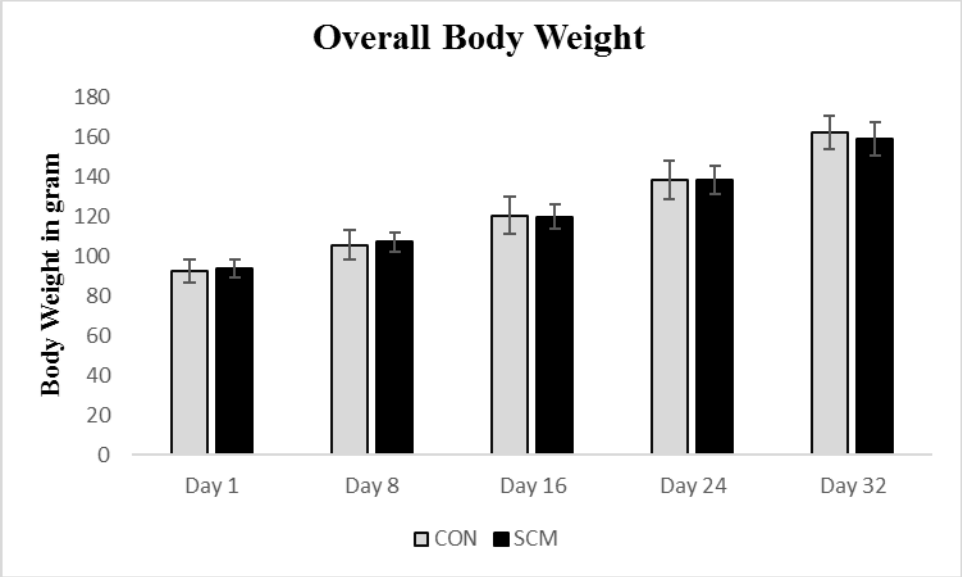

Figure 1: The effect Swalpo Chandrodoy Makardhwaj (SCM) (100 mg/kg) on the body weights (g) of Sprague-Dawley rats with the time of treatment. Independent sample t-test was performed to analyze this weight variation in different days. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant.

Table 1: Name of the ingredients/herbs used in the preparation of Swalpo Chandrodoy Makardhwaj (SCM)

Name of ingredients	Amounts
1. Svarnadala (suksma svarna patra)	48 g.
2. Rasendra (suddha parada)	348 g.
3.Gandhaka (suddha)	768 g.
4. Sona Sukarpasaprasuna (Fl.)	Q.S. for mardana
5. Kumarika (kumari) (Lf.)	Q.S. to mardana
6. Karpura raja	48 g.

7. Jatiphala (Sd.)	4 g.
8. Usna (marica) (Fr)	4 g.
9. Indra-puspa (lavanga) (Fl.)	4 g.
10. Mrgandaja (mrgamada)	4 g.

Table 2: The effect of Swalpo Chandrodoy Makardhwaj (SCM) (100 mg/kg) on the absolute organ weights of male rats:

Parameters	Control	SCM	p value	%increase/decrease
Heart	0.4255 ± 0.0184	0.459 ± 0.01234	0.153	↑7.87
Lung	0.8844 ± 0.03365	0.8891 ± 0.0336	0.922	↑0.53
Liver	5.2442 ± 0.32213	5.0841 ± 0.42138	0.767	↓3.05
Kidney	0.4931 ± 0.01812	0.5426 ± 0.01714	0.067	↑10.04
Spleen	0.4611 ± 0.02856	0.6551 ± 0.0664	0.024*	↑42.07
Testis	1.0184 ± 0.03086	1.0585 ± 0.10343	0.716	↑3.94

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

Table 3: The effect of Swalpo Chandrodoy Makardhwaj (SCM) (100 mg/kg) on the relative organ weights of male rats.

Parameters	Control	SCM	p value	%increase/decrease
Heart	0.2632 ± 0.00521	0.294 ± 0.01508	0.087	↑11.70
Lung	0.5498 ± 0.02052	0.5658 ± 0.02157	0.601	↑2.91
Liver	3.2368 ± 0.12244	3.2076 ± 0.23864	0.915	↓0.90
Kidney	0.3063 ± 0.00928	0.3458 ± 0.01321	0.028*	↑12.90
Spleen	0.2876 ± 0.01912	0.4252 ± 0.05541	0.034*	↑47.84
Thymus	0.1451 ± 0.01363	0.1694 ± 0.01458	0.244	↑16.75
Testis	0.6341 ± 0.02297	0.6662 ± 0.06035	0.626	↑5.06

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

Table 4: The effect of Swalpo Chandrodoy Makardhwaj (SCM) (100 mg/kg) on various tissue hydration indices of male rats.

Parameters	Control	SCM	p value	%increase/decrease
Heart	75.9169 ± 1.35881	77.5931 ± 0.31893	0.25	↑2.21
Lung	79.9438 ± 0.53312	75.3199 ± 1.51394	0.012*	↓5.78
Liver	72.6499 ± 0.3112	74.3606 ± 0.70957	0.044*	↑2.35
Kidney	77.8321 ± 1.28889	77.2543 ± 0.40686	0.676	↓0.74
Spleen	76.7662 ± 0.47337	76.134 ± 0.22632	0.248	↓0.82
Testis	86.8774 ± 0.18917	86.6979 ± 0.13069	0.448	↓0.20

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

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