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# Evaluation of Pharmacological and Toxicological Studies of Ayurvedic Medicine Siddha Makardhwaja on Biological System of Male Sprague-Dawley Rats

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## Abstract

In this study, the pharmacological and toxicological effects along with possible side effects of the classical ayurvedic formulation Siddha Makardhwaja (SMD) which is used as a traditional medicine in the treatment in the rural population were evaluated. During this study, various experiments on organ body weight ratio and tissue hydration indices were performed to evaluate its efficacy and toxicity. SMD was administered chronically to the male Sprague-Dawley rats at a dose of 40 mg/kg to determine its toxicological characteristics. After 28 days of chronic administration of the prepared SMD, the following toxicological changes were noted: a statistically very highly significant ($p$=0.001) decrease in the absolute weight of the male rat liver [26.55% decrease]; a statistically highly significant ($p$=0. 002) decrease in the relative percent weight of the male rat liver [19.45% decrease]; a statistically highly significant ($p$=0.006) increase in the relative percent weight of the male rat kidney [17.73% increase]; and a statistically significant ($p$=0.041) decrease in the organ water content of the male rat kidney [4.50% decrease]. As SMD decreases and increases abnormally the weight of several organs in the body of treated rats, it should not be administered chronically at a higher dose.

**Keywords:** Siddha Makardhwaja; Pharmacological; Toxicological; Absolute weight; Relative percent 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, approximately 1.5 billion people of the world are now getting treatment with these medicines [3]. They have a good safety profile also [4].

Siddha Makardhwaj is an ancient Indian multipurpose ayurvedic medicine that acts as an alternative, stimulant, tonic, and rejuvenator. Its regular use prevents the wrinkles of skin and greying of hair due to old age. Siddha Makardhwaj is also an effective natural aphrodisiac; however, it should be taken only under strict medical supervision [5-9].

Being a natural aphrodisiac, this herbal product is known for calming cardiac muscles as well. It contains gold particles or Swarna Bhasma, which is known to have many good benefits for the human body. Ayurveda states that gold, in its element and medicinal formulation, can improve intelligence and sharpen memory [5-9] Table 1.

Siddha Makardhwaj 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) [5].

## Materials and Methods

### Drugs, chemicals, and reagents

For the toxicological study, Siddha Makardhwaja (SMD) 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

| Sl no | Ingredient | Plant part | Botanical/Zoological or Calyx name | Amount |
|---|---|---|---|---|
| 1 | Gandhaka | | Purified and processed sulfur | 160 g |
| 2 | Parada | | Purified and processed mercury | 80 g |
| 3 | Swarna Bhasma | | Gold Bhasma | 40 g |
| 4 | Rakta karpasa kusuma | Flower | Gossypium herbaceum | Q.S. (for mardana) |
| 5 | Kumari | Leaf | Aloe Vera Barbadensis | Q.S. (for mardana) |

**Table 1**: Name of the ingredients/herbs used in the preparation of Siddha Makardhwaja

Guideline 425) [10]. Sixteen male mice (30-40 g body weight) were divided into four groups of four animals each. Different doses (50, 60, 70, and 80 ml/kg) of experimental drug (SMD) were administered by stomach tube. The dose was divided into two fractions and given within 12 h. 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 h and thereafter once a day for the next three days following SMD administration.

*Chronic toxicity studies*

Prior to the experiment, rats were randomly divided into two groups of eight animals each. One group was treated with SMD, 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 [11]. After acclimatization, Ayurvedic medicinal preparation was administered to the rats by intra-gastric syringe between 10 am and 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 [12].

***Body weight:organ weight ratio analysis***

At the end of the 28-day treatment period, the animals were fasted for 18 h and also 24 h after the last administration. Ketamine (500 mg/kg i.p.) was administered for the purpose of anesthesia [13].

Rats of both SMD 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 as follows:

Heart, kidney, lungs, liver, spleen, thymus, stomach, cecum, 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 such as heart, lungs, liver, and spleen, and 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

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

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 Statistical Package for Social Science (SPSS) Statistics 11.5 package (SPSS Inc., Chicago IL). All values are expressed as mean ± standard error mean (SEM), and the level of statistical significance was indicated by $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

## Results

### Acute toxicity study

The drug (SMD) 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 SMD when administered at single dose is nontoxic and can be used safely in oral formulations.

### Chronic growth study

*Effect of SMD on organ toxicity study*

In absolute weight determination, Table 2 the results reveal that there is a [6.18%] decrease in the absolute weight of the male rat heart, the decrease though not significant yet it was prominent ($p = 0.313$). There is a statistically insignificant ($p = 0.966$) [0.24%] decrease in the absolute weight of the male rat lungs. There is a statistically very highly significant ($p = 0.001$) decrease in the absolute weight of the male rat liver [26.55% decrease]. There is a [7.54%] increase in the absolute weight of the male rat kidney, the increase though not significant yet it was prominent ($p = 0.330$). There is a statistically insignificant ($p = 0.670$) [3.50%] decrease in the absolute weight of the male rat spleen. There is a statistically insignificant ($p = 0.905$) [1.05%] decrease in the absolute weight of the male rat thymus. There is a [9.81%] decrease in the absolute weight of the rat testis, the decrease though not significant yet it was prominent ($p = 0.122$).

In relative weight determination, Table 3 the results reveal that there is a statistically insignificant ($p = 0.621$) [1.70%] increase in the relative

| Parameters | Control | SMD | *p* value | % increase/decrease |
|---|---|---|---|---|
| Heart | 0.3913 ± 0.01427 | 0.3671 ± 0.01819 | 0.313 | ↓ 6.18 |
| Lung | 0.7623 ± 0.02870 | 0.7605 ± 0.03128 | 0.966 | ↓ 0.24 |
| Liver | 6.5072 ± 0.28197 | 4.7797 ± 0.16359 | 0.001*** | ↓ 26.55 |
| Kidney | 0.4166 ± 0.02671 | 0.4480 ± 0.01609 | 0.33 | ↑ 7.54 |
| Spleen | 0.5549 ± 0.02400 | 0.5355 ± 0.03736 | 0.67 | ↓ 3.50 |
| Thymus | 0.1814 ± 0.01217 | 0.1795 ± 0.00966 | 0.905 | ↓ 1.05 |
| Testis | 1.0308 ± 0.03346 | 0.9297 ± 0.05146 | 0.122 | ↓ 9.81 |

↑: increase, ↓: decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

**Table 2:** Effect of Siddha Makardhwaja (SMD) (40 mg/kg) on absolute organ weights of male rats

| Parameters | Control | SMD | *p* value | % increase/decrease |
|---|---|---|---|---|
| Heart | 0.2817 ± 0.00585 | 0.2865 ± 0.00761 | 0.621 | ↑ 1.70 |
| Lung | 0.5502 ± 0.01997 | 0.5976 ± 0.02647 | 0.175 | ↑ 8.62 |
| Liver | 4.6830 ± 0.14344 | 3.7723 ± 0.19568 | 0.002** | ↓ 19.45 |
| Kidney | 0.2983 ± 0.01281 | 0.3512 ± 0.00998 | 0.006** | ↑ 17.73 |
| Spleen | 0.3988 ± 0.01014 | 0.4150 ± 0.01007 | 0.276 | ↑ 4.06 |
| Thymus | 0.1284 ± 0.00785 | 0.1477 ± 0.00992 | 0.152 | ↑ 15.03 |
| Testis | 0.7452 ± 0.02660 | 0.7253 ± 0.02399 | 0.589 | ↓ 2.67 |

↑: increase, ↓: decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

**Table 3:** Effect of Siddha Makardhwaja (SMD) (40 mg/kg) on relative organ weights of male rats

| Parameters | Control | SMD | *p* value | % increase/decrease |
|---|---|---|---|---|
| Heart | 78.080 ± 0.2740 | 79.399 ± 1.3931 | 0.337 | ↑ 1.69 |
| Lung | 79.151 ± 0.1988 | 78.295 ± 2.5358 | 0.723 | ↓ 1.08 |
| Liver | 73.917 ± 0.4434 | 73.384 ± 1.3032 | 0.689 | ↓ 0.72 |
| Kidney | 77.038 ± 0.2507 | 80.508 ± 1.5170 | *0.041 | ↑ 4.50 |
| Spleen | 75.589 ± 1.6915 | 76.185 ± 0.9423 | 0.775 | ↑ 0.79 |

↑: increase, ↓: decrease; $p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

**Table 4:** Effect of Siddha Makardhwaja (SMD) (40 mg/kg) on various tissue hydration indices of male rats

percent weight of the male rat heart. There is a [8.62%] increase in the relative percent weight of the male rat lungs, the increase though not significant yet it was prominent ($p = 0.175$). There is a statistically highly significant ($p = 0.002$) decrease in the relative percent weight of the male rat liver [19.45% decrease]. There is a statistically highly significant ($p = 0.006$) increase in the relative percent weight of the male rat kidney [17.73% increase]. There is a [4.06%] decrease in the relative percent weight of the male rat spleen, the decrease though not significant yet it was prominent ($p = 0.276$). There is a [15.03%] decrease in the relative percent weight of the male rat thymus, the decrease though not significant yet it was prominent ($p = 0.152$). There is a statistically insignificant ($p = 0.589$) [2.67%] decrease in the relative percent weight of the rat testis.

### *Effect of SMD on tissue hydration index*

In the tissue hydration index determination, Table 4 there is a [1.69%] increase in the organ water content of the male rat heart, the increase though not significant yet it was prominent ($p = 0.337$). There is a statistically insignificant ($p = 0.723$) [1.08%] decrease in the organ water content of the male rat lungs. There is a statistically insignificant ($p = 0.689$) [0.72%] decrease in the organ water content of the male rat liver. There is a statistically significant ($p = 0.041$) decrease in the organ water content of the male rat kidney [4.50% decrease]. There is a statistically insignificant ($p = 0.775$) [0.79%] decrease in the organ water content of the male rat spleen.

## Discussion

### Effect of SMD on various organ:body weight ratios

The evaluation of organ weights is fundamental to many biological studies. This is particularly true in the field of toxicological drug testing. To eliminate the well-known deviations found in absolute organ weights, the ratio of organ-to-body weight (in percent) is often used; whereas, other reference parameters are sometimes preferred, brain or heart weight, for example [14-16]. However, a survey of the relevant literature reveals equally wide deviations in studies of relative organ weights.

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 make 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 [17]. Here, we found significant decrease of liver weight to the SMD-treated rats.

Administration of 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 kidney weight increases to the SMD-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.

### Effect of SMD on tissue hydration index

The water content of tissues is essential to know the development of physiologically based pharmacokinetic modeling [18] and the interpretation of drug tissue distribution data [19,20]. Changes in tissue water content can also be used to evaluate alterations in tissue physiology that are associated with an increase in tissue weight, such as the development of tissue edema [21,22]. In our study, we found that SMD causes significant increase in % water content of kidney.

## Conclusion

From the above experiment, it can be concluded that SMD should not be administered chronically at a higher dose as it decreases and increases the weight of several organs. Further studies should be done by reducing the administered dose.

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