Biology and Medicine
ISSN: 0974-8369

Masud *et al.*, Biol Med (Aligarh) 2017, 9:1
DOI: 10.4172/0974-8369.1000372

Research Article Open Access

# Steroidal and Gonadotropin Hormone Profile Studies of a Classical Ayurvedic Preparation of "Makardhvaja" after Chronic Administration to Male Sprague-Dawley Rats

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**Received:** Sep 30, 2016; **Accepted:** Nov 24, 2016; **Published:** Dec 16, 2016



## Abstract

In this study, the effect of the classical Ayurvedic formulation of Makardhvaja (MD) on steroidal and gonadotropin hormone was evaluated after chronic administration. MD is used as a traditional medicine in the treatment of Rasayan in the rural population. The acute pharmacological test of MD recorded no death or any signs of effectivity even at the highest dose of 80 ml/Kg body weight. For chronic pharmacological evaluation, animals were divided into two groups. The first group was given MD preparation at a dose of 40 mg/kg body weight for 28 days while the second group that served as the control received water for the same period. After 28 days of chronic administration of the MD preparation, the following effects on the steroidal hormone panel were noted: There is a statistically significant ($p = 0.040$) increase in the serum circulating progesterone level of the male rat. [20.38% increase]. The steroidal hormone indices such as serum circulating dehydroepiandrosterone sulfate (DHEA-S), serum circulating total testosterone, serum circulating 17-beta-Estradiol ($E_2$) does not change significantly. The significant effects on the gonadotropin hormone profile after chronic administration were thus: There is a statistically significant ($p = 0.047$) increase in the serum circulating luteinizing hormone (LH) level of the male rat [76.07% increase]. Serum circulating follicle stimulating hormone (FSH) level does not change significantly.

**Keywords:** Makardhvaja; Ayurvedic preparation; Rasayan; Steroidal hormone; Gonadotropin hormone; Progesterone hormone; Luteinizing hormone

## Introduction

Ayurvedic drugs remain as one of the most ancient and yet living traditions practiced widely in various parts of the world, including India and Sri Lanka, and has a sound philosophical and experiential basis [1,2]. Ayurvedic practice offers an integrated approach to the prevention and healing of disease through a system of lifestyle interventions and natural therapies.

Makaradhvaja (MD) is a well-known inorganic preparation of the Ayurvedic Pharmacopoeia used in Ayurvedic anti-aging and aphrodisiac treatment [3,4]. Chemically, it is red sulfide of mercury and gold in uncombined form. It is a sublimed product made from pure mercury, sulfur, and gold.

Eight parts of mercury and one part of gold leaf are mixed together to form an amalgam. To this mixture, 16 parts of sublimed sulfur are added, and the resulting mixture is ground very thoroughly in a stone mortar for 24 h or more until the mixture is converted into a lusterless, fine, impalpable powder of uniform consistence. This mixture is then placed in a narrow-mouthed bottle and is gradually heated on a sand bath. On heating, the bottle is filled with reddish fumes of various hues. On cooling, MD is found deposited in the inner surface of the neck of the bottle.

MD (447 p.) is included in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (approved by the Government of Bangladesh Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991).

## Materials and Methods

### Drugs, chemicals, and reagents

For the pharmacological study, MD was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Bangladesh. All the 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 were 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 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

| Name of ingredients | Used part | Botanical/ English name/ calyx name | Amount used |
|---|---|---|---|
| Shuddha Swarna | Calyx | Purified gold | 12 g |
| Shuddha Parada | Calyx | Herbal purified Mercury | 96 g |
| Shuddha Gandhaka | Calyx | Herbal purified sulfur | 288 g |
| Karpasa | Flower | *Gossypium herbaceum* | Quantity sufficient |
| Kumari | Leaf exudate | *Aloe barbadensis* | Quantity sufficient |

**Table 1:** Name of the ingredients used in the preparation of MD

(OECD Guideline 425) [5]. 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 (MD) 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 3 days following MD administration.

### *Chronic pharmacological studies*

Prior to the experiment, rats were randomly divided into two groups of eight animals each. One group was treated with MD 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 [6]. After acclimatization, the Ayurvedic medicinal preparation was administered to the rats by intragastric syringe between 10 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 experimental animals were marked carefully on the tail, which helped to identify a particular animal. By using the identification mark, responses were noted separately for a particular period prior to and after the administration [7].

### *Blood sample collection and preparation of serum*

At the end of the 28-day treatment period, after 18 h fasting, rats from each group were anesthetized by the administration (i.p) of ketamine (500 mg/Kg body weight) [8]. Blood samples were collected from post vena cava of rats into EDTA (Ethylene di-amine tetra acetic acid) sample tubes for hematological analysis and into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 min and centrifuged at 4,000 g for 10 min using benchtop centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were completed within 12 h of sample collection [9].

### *Determination of the steroidal and gonadotropin hormone profile*

Studies involved analysis of parameters such as DHEA-S, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, and 17-beta-Estradiol (E2) were assayed by the Architect i2000 analyzer (Abbott Laboratories, Chicago, IL), which employed chemiluminescent microparticle immunoassay (CMIA) method.

### 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 $p < 0.05$, $p < 0.01$, and $p < 0.001$ were taken as the level of significance.

## Results

### Acute pharmacological study

The drug (MD) 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. The animals did not manifest any sign of fever, chronic skin diseases, diabetes, urinary tract disorders, sinuses, nonhealing wounds, fistula, obesity, rheumatoid arthritis, ascites, headache, gynecological disorders, and diseases of ear, nose, throat, and eyes. According to the OECD test guideline 425, when there was information in support of low or nontoxicity and immortality nature of the test material, the limit test at the highest starting dose level (80 ml/kg body weight) was conducted. There were no mortality and effective or toxic signs observed at 80 ml/kg body weight. Therefore, it can be concluded that MD when administered at a single dose is nontoxic and can be used safely in oral formulations.

### Chronic pharmacological study

#### *Effect of MD on steroidal hormone*

The effects on steroidal hormone profile after chronic administration were thus: There is a statistically insignificant decrease ($p = 0.562$, 7.26%) in the serum circulating dehydroepiandrosterone sulfate (DHEA-S) level of the male rat. There is a decrease ($p = 0.367$, 8.15%) in the serum circulating total testosterone level of the male rat; though the decrease is not significant, it is prominent. There is a statistically significant increase ($p = 0.040$, 20.38%) in the serum circulating progesterone level of the male rat. There is an increase ($p = 0.422$, 8.09%) in the serum circulating 17-beta-Estradiol ($E_2$) level of the male rat; though the increase is not significant, it is prominent.

#### *Effect of MD on gonadotropin hormone*

The effects on the gonadotropin hormone profile after chronic administration were thus: There is a statistically significant increase ($p = 0.047$, 76.07%) in the serum circulating LH level of the male rat. There is a negligible increase ($p = 0.789$, 2.28%) in the serum circulating follicle stimulating hormone (FSH) level of the male rat, which was statistically not at all significant.

| Parameters | Control | MD | *p* value | % increase/ decrease |
|---|---|---|---|---|
| Serum DHEA-S | 2.7538 ± 0.12963 | 2.5538 ± 0.30610 | 0.562 | Decr 7.262692 |
| Serum total testosterone | 29.5000 ± 1.82248 | 27.0950 ± 1.82195 | 0.367 | Decr 8.152542 |
| Serum progesterone | 65.6250 ± 3.48434 | 79.0000 ± 4.78091 | *0.04 | Incr 20.381 |
| Serum E2 | 55.6250 ± 2.88430 | 60.1250 ± 4.61920 | 0.422 | Incr 8.08989 |

$p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

**Table 2:** Effect of MD (40 mg/kg) on steroidal hormone in male rats

| Parameters | Control | MD | *p* value | % increase/ decrease |
|---|---|---|---|---|
| Serum LH | 0.0305 ± 0.00911 | 0.0537 ± 0.00557 | *0.047 | Incr 76.0656 |
| Serum FSH | 0.0745 ± 0.00383 | 0.0762 ± 0.00516 | 0.789 | Incr 2.28188 |

$p^* \leq 0.05$, $p^{**} \leq 0.01$, $p^{***} \leq 0.001$.

**Table 3:** Effect of MD (40 mg/kg) on gonadotropin hormone in male rats

## Discussion

Ayurvedic medicines have achieved greater importance as an alternative to conventional therapy. To enhance the safe use of plant-based medicines, one should take into account their historical applications on humans and animals as well as the toxicity evaluation of the medicinal herbs and their active components [10]. Many screening methods are employed to determine the safety and efficacy of these Ayurvedic medicines and also to establish the active component of herbal products [11].

In this study, we found serum circulating progesterone level significantly increased in the MD-treated rats. Progesterone offers neuroprotection [12], contributes to cardiovascular health, assists normal brain development [13], and provides protection from some types of cancer. Progesterone at a concentration similar to that seen during the third trimester of pregnancy exhibited a strong antiproliferative effect on at least two breast cancer cell lines [14,15]. Progesterone has a stimulating effect on bone building osteoblasts, resulting in increased bone building activity [16-21]. This is due to a direct stimulation of progesterone receptors in osteoblast bone cells [22,23] as well as an increased secretion of IGF-1 and other growth factors by the bone cells exposed to progesterone [24-26]. The most positive effect is seen when estrogen and progesterone are used in combination [27].

In this study, we found that the serum circulating LH level significantly increased in the MD-treated rats. In both males and females, LH is essential for reproduction. In females, menstrual cycle is divided by a midcycle surge of both LH and FSH into a follicular phase and a luteal phase. This "LH surge" triggers ovulation, thereby not only releasing the egg [28] but also initiating the conversion of the residual follicle into a corpus luteum, which, in turn, produces progesterone to prepare the endometrium for a possible implantation. LH is necessary to maintain luteal function for the first 2 weeks. In case of pregnancy, luteal function will be further maintained by the action of hCG (a hormone very similar to LH) from the newly established pregnancy. LH supports thecal cells in the ovary, which provide androgens and hormonal precursors for estradiol production. In males, where LH also called interstitial cell–stimulating hormone (ICSH) [29], stimulates Leydig cell production of testosterone [28]. Changes in LH and testosterone (T) blood levels and pulse secretions are induced by changes in sexual arousal in human males [30]. As MD significantly increases the level of progesterone hormone and luteinizing hormone to the treated animals, it may be concluded that MD have a positive effect on both progesterone hormone and luteinizing hormone.

## Conclusion

This experiment shows that MD significantly increases progesterone and luteinizing hormone levels; thus, it necessitates further close investigation to figure out the reason of this discrepancy in the case of different parameters which represent the proper functioning of steroidal hormone and gonadotropin hormone.

## 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. They express their special thanks to Mr. Shafiqul Islam for ensuring a constant supply of animals followed by proper maintenance and care of these animals throughout the experimental period.

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