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Original Research Article

Steroidal and gonadotropin hormone profile studies of a classical ayurvedic preparation of "Saraguna Balijarita Makardhwaja" after chronic administration to male Sprague-Dawley rats

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ABSTRACT

Background: Saraguna Balijarita Makardhwaja (SGM) is an Ayurvedic preparation used as a traditional antipyretic in the rural population. This research work was designed to get an overview of steroidal and gonadotropin hormone profiles after chronic administration of this drug.
Methods: The acute pharmacological test of SGM recorded no death or any signs of effectivity even at the highest dose of 4000 mg/kg body weight. For chronic pharmacological evaluation, sixteen healthy Sprague-Dawley male rats were randomly divided into two groups, one group was a control group and the other was an experimental group. The experimental group was assigned to receive the drug at a dose of 400 mg/kg of body weight orally. After 28 days of treatment, blood samples were collected for biochemical tests.
Results: The research showed the following effects on the steroidal and gonadotropin hormone profile. In this study, serum circulating level of dehydroepiandrosterone sulfate (DHEA-S), testosterone level, progesterone, 17-beta-estradiol (E2), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured to determine safety profile study of SGM after chronic administration. There were no significant differences in any parameters which is suggesting that SGM has no effect of the steroidal and hormone profile.
Conclusions: According to studies, SGM has no harmful effect on the steroid and hormone profiles after chronic treatment. Further studies are needed to establish the safety aspects of SGM.

Keywords: Antipyretic, Ayurvedic preparation, Gonadotropin, Hormone, Steroidal

INTRODUCTION

Ayurveda is a traditional medical system that originated in the Indian subcontinent. Ayurveda is acknowledged as a medical system in various countries like India, Nepal, Sri Lanka, Pakistan, the Bangladesh, Saudi Arabia, Serbia, Mauritius, Tanzania, Cuba, and Brazil, with only five European Union (EU) nations regulating its activities.[1] Ayurveda emphasizes disease prevention rather than disease treatment.[2] It originated on the Indian subcontinent and has been practiced for over 3,000 years. The term "Ayurveda" is derived from two Sanskrit words: "Ayur", which means "life", and "Veda", which means "knowledge or science". Ayurveda is known as the "science of life". Ayurveda is built on the idea of maintaining the interrelated interactions between the body and mind in check. It teaches patients the importance of knowing their bodies and minds, as well as spending time in nature.

Makardhwaja is a herbo-mineral formulation listed in India's Ayurvedic formulary. It has been used for centuries

and is said to be effective and safe in the treatment of chronic fever and as a rejuvenator. Heavy metal toxicity is now thought to be at an all-time high, yet sublimed mercury compounds commonly used in Ayurvedic therapies such as Makaradhwaja are safer than others. It has been confirmed by numerous researchers through toxicological studies.[3] Though the formulation is clinically effective, its preparation is difficult. Makaradhwaja may be divided into several subtypes according to the proportion of minerals and ingredients such as Dwiguna Balijarita Makaradhwaja, Triguna Balijarita Makaradhwaja (TBM) Shadguna Balijarita Makaradhwaja, Siddha Makardhwaja, Brihot Chandrodoy Makardhwaj and Swalpo Chandrodoy Makardhwaj.[3-6]

Saraguna Balijarita Makaradhvaja (SBM) is included (page 92) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 (approved by the Government of Bangladesh vide Ministry of Health and Family Welfare memo number Health-1/Unani-2/89/(Part-1) 116 dated 3-6-1991).

It has already been shown that chronic SBM treatment raises total cholesterol (TC), LDL, Non-HDL, and all atherogenic indices while decreasing HDL levels, implying that SBM has a negative effect on the lipid profile and could be responsible for the development of atherosclerosis or other cardiovascular diseases.[7] The current study was conducted to investigate the effect of Saraguna Balijarita Makaradhvaja on the steroidal and gonadotropin hormone profiles of rat serum following chronic administration of the medicine to male Sprague-Dawley rats.

METHODS

Drugs, chemicals and reagents

For this study, SBM was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong, Bangladesh (formulation in Table 1). All other reagents, assay kits, and chemicals used in this work were purchased from Human GmbH, Wiesbaden, Germany.

Table 1: Name of the ingredients used in the preparation of SGM.

Name of ingredients	Botanical/calyx name	Used part	Amount used
Shuddha Swarna	Purified gold	Calyx	20 gm
Shuddha Parada	Herbal purified mercury	Calyx	160 gm
Shuddha Gandhaka	Herbal purified sulphur	Calyx	960 gm
Karpasa	*Gossypium herbaceum*	Juice	Quantity sufficient
Kumari	*Aloe barbadensis*	Juice	Quantity sufficient

Experimental animals

This toxicological experiment used eight-week-old male Sprague-Dawley rats raised and kept at department of pharmacy, Jahangirnagar University, Bangladesh. The animals appeared healthy, weighing between 50-70 grams. Throughout the experiment, the animals were housed in a well-ventilated, clean experimental animal home, with consistent environmental and nutritional circumstances. They were fed rat chow produced using a formula devised by the Bangladesh Council of Scientific and Industrial Research (BCSIR). Water was provided ad libitum, and the animals were kept on a 12-hour day/12-hour night cycle. The animals were handled in accordance with the ethical guidelines and the study protocol was approved by the Biosafety, Biosecurity and Ethics Committee of Jahangirnagar University [Ethics approval no: BBEC, JU/M 2018 (5)2], Savar, Dhaka, Bangladesh.

Experimental design

Acute toxicity study

The acute oral pharmacological test was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for evaluating chemicals with minor changes (OECD guideline 425).[8] Sixteen male rats (50-70 gm body weight) were placed into four drug-specific groups of four rats each. Different doses of experimental medications (1000 mg/kg, 2000 mg/kg, 3000 mg/kg, and 4000 mg/kg) were delivered by stomach tube. The dose was d administered within 12 hours. The experimental animals were then evaluated for mortality and clinical symptoms of toxicity (general behavior, breathing pattern, cardiovascular signs, motor activities, reflexes, and changes in skin and fur texture) at 1, 2, 3, and 4 hours, and then once a day for the next three days following administration.

Chronic toxicity studies

Prior to the investigation, rats were randomly assigned into two groups of eight, one for each medication. One group was given medications, while another served as a control. For 28 days, the control animals received the same volume of distilled water as the drug-treated group. In all pharmacological investigations, the medicines were supplied orally at a dose of 400 mg/kg in the case of SBM. Following acclimation, an ayurvedic medicinal concoction was delivered to the rats by intra-gastric syringe between 10 am and 12 am daily during the study period. All rat tests were conducted in strict accordance with the ethical

guidelines for the care and use of laboratory animals. The experiment rats were marked carefully on the tail which helped to identify a particular animal. Responses were recorded individually for a specific period of time before and after administration using an identification mark.

Blood samples collection and preparation of serum

At the end of the 28-day treatment period, following 18 hours of fasting, rats from each group were anaesthetized with ketamine (500 mg/kg body weight) administered intravenously. Blood samples were obtained from rats' post vena cava and placed in simple sample tubes for serum production and biochemical examination. Serum was collected by allowing blood to clot for 30 minutes and centrifuged at 4000 g for 10 minutes using a bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected with a dry Pasteur pipette and stored in the refrigerator for further analysis. All assays were done within 12 hours of the sample collection.[9]

Determination of the steroidal and gonadotropin hormone profile

DHEA-S, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, and 17-beta-estradiol (E2) were measured using the Architect i2000 analyzer (Abbott Laboratories, Chicago, IL), which used the chemiluminescent microparticle immunoassay (CMIA) method.

Statistical analysis

The group data are expressed as Mean±SEM (Standard Error of the Mean). Independent sample 't' tests were done for statistical significance analysis. SPSS (Statistical Package for Social Science) for WINDOWS (version 16.0) was applied for the analysis of data. Differences between groups were considered significant at $p<0.05$*, 0.01** and 0.001***.

RESULTS

Acute pharmacological study

The drug, when supplied individually at a high dose of 4000 mg/kg, produced no mortality (Table 2). Thus, the LD50 (median lethal dose) value was discovered to be larger than 4000 mg/kg body weight. The rats showed no signs of restlessness, respiratory distress, general discomfort, or convulsions. Because medications have been used clinically for many years to treat disorders, a limit test was performed in the acute oral toxicity research. According to OECD test guideline 425, when there is evidence of low or non-toxicity and immortality of the test material, the limit test at the highest beginning dose level (4000 mg/kg body weight) is performed. There were no mortality and toxicity signs observed at 4000 mg/kg body weight. Therefore, it can be concluded that SBM administered at a single dose is non-toxic and can be used safely in oral formulations.

Table 2: Acute oral toxicity study of SGM in rats.

	1000 mg/kg		2000 mg/kg		3000 mg/kg		4000 mg/kg	
	A	D	A	D	A	D	A	D
SGM % mortality	4	0	4	0	4	0	4	0
	0		0		0		0	

Here, n = 4; A = Alive; D = Death

Chronic pharmacological study

Table 3 shows the effects on the steroid hormone profile following chronic treatment. The serum circulating dehydroepiandrosterone sulfate (DHEA-S) level in the male rat increased by 4.52%, which is statistically insignificant. The male rat's serum circulating total testosterone level has decreased by 11.72%; while not statistically significant, the decline is noticeable. The male rat's serum circulating progesterone level decreased by 2.86%, which was statistically insignificant. The male rat's serum circulating 17-beta-estradiol (E2) level was increased by 4.11%; while not statistically significant, the increase was noticeable.

The effects on the gonadotropin hormone profile following chronic dosing were as follows: The male rat's serum circulating LH level increased by 21.58%, which was statistically significant. The male rat's serum circulating follicle stimulating hormone (FSH) level increased by just 3.62%, which was statistically insignificant.

Table 2: Effect of SGM (400 mg/kg) on steroidal and gonadotropin hormone in male rats.

Parameters	Control mean±SEM	SGM mean±SEM	P value	Overall output
Serum DHEA-S	2.89±0.136	3.02±0.21	0.58	↑4.52%
Serum total testosterone	30.98±1.91	27.32±2.25	0.24	↓11.72%
Serum progesterone	68.91±3.66	66.94±2.58	0.75	↓2.86%
Serum E2	58.41±3.03	60.51±5.10	0.70	↑4.11%
Serum LH	0.03±0.01	0.04±0.01	0.73	↑21.58%
Serum FSH	0.08±0.01	0.08±0.01	0.60	↑3.62%

Independent sample 't' tests were done for statistical significance analysis. Values are expressed as Mean±SEM, n=8. Increase ↑; Decrease↓

DISCUSSION

Ayurvedic remedies are becoming increasingly popular as an alternative to mainstream therapy. Their historical usage on humans and animals, as well as the toxicity assessment of medicinal herbs and active components are considered to improve the safety profile of plant-based medications.[10] From several chronic investigations in rats it has been reported that only plant-based ayurvedic remedies have long-term deleterious effects on organs such as the liver, kidney, and spleen, as well as the glycemic index.[11-14] More importantly, there are extremely limited toxicity investigations on Ayurvedic drugs. Thus, it is necessary to determine a safety profile assessment of Ayurvedic drugs. To determine the safety and efficacy of Ayurvedic medications and to identify the active component of herbal products, many screening methods are used.[15]

Steroid hormones are lipophilic molecules that organisms utilize as chemical messengers. They act on a wide range of tissues and biological functions and play an important role in a variety of physiological functions such as growth, development, energy metabolism, homeostasis, and reproduction.[16] A variety of pathological disorders such as cancer, steroids insensitivity, poor infertility result from issues with steroid hormone function. Progesterone acts as a physiological regulator of the menstrual cycle, reproduction and steroid hormone biosynthesis.[17] Other physiological functions of progesterone in the central neurological and immunological systems support the idea that progesterone is essential for life, and a greater understanding of this crucial hormone aids in its widespread therapeutic implications for human health.

Estrogens are another type of steroid hormone that control the growth, development, and physiology of the human reproductive system. Estrogens also play roles in the neuroendocrine, skeletal, adipogenic, and cardiovascular systems.[18] The greatest favorable effect is found from the combination of estrogen and progesterone.[19] Testosterone is responsible for basic sexual development, which includes testicular descent, spermatogenesis, penile and testicular hypertrophy, and increased libido. Testosterone regulates secondary male features, which are associated with masculinity.[20] In this investigation, we discovered that the blood progesterone, estrogen, and testosterone levels did not alter significantly in SGM-treated rats compared to the control group.

In this work, we discovered that the serum circulating LH and follicle stimulating hormone (FSH) levels did not alter significantly in SGM-treated rats. For both males and females, LH is necessary for reproduction. This “LH spike” causes ovulation, releasing the egg while simultaneously commencing the conversion of the remnant follicle into a corpus luteum, which releases progesterone to prepare the endometrium for possible implantation.[21] To maintain luteal function for the first two weeks, LH is required. In the event of pregnancy, luteal function will be enhanced by the action of hCG (a hormone very similar to LH) from the newly formed pregnancy. LH helps thecal cells in the ovary to produce androgens and acts as hormonal precursor for estradiol synthesis. LH is also known as interstitial cell-stimulating hormone (ICSH) in males that promotes testosterone production from Leydig cells.[21,22] In human males, LH and testosterone blood levels as well as pulse secretions changes are results from changes in sexual excitement.[23]

Ayurvedic medicine, like any other system of medicine, has its own ideas and practices regarding toxicity. Ayurveda stresses the use of natural ingredients such as herbs, minerals, and animal products for healing and wellness. Some substances like alkaloids and heavy metals like mercury, lead, and arsenic may be found in ayurvedic medicines that can be hazardous to the liver and kidney if not properly manufactured, prescribed, or used.[24-29] Heavy metals such as mercury in different concentrations is found in all kind of Makaradhwaja formulations and so prolonged use of these medications has a deleterious impact on the liver, kidney, and lipid profile.[30-33] Previously reported Makardhwaja, which contains sulfur and mercury (24:1), induces considerable elevations in progesterone and luteinizing hormone levels following chronic dosing.[30] In this investigation, rats were administered with SGM to assess their steroidal and gonadotropin hormone profiles, and no significant differences were identified between the treated and control groups, implying that this treatment had no effect on serum circulating steroidal and gonadotropin hormone levels.

However, drug safety profile studies are critical for assessing potential toxicity as well as identifying the optimal dose. The possible toxicity of certain chemicals is identified and also proper guidelines are provided for their safe usage from drug safety profile.[34-37] Like any other type of treatment, ayurvedic medicine has the potential for harm if not used properly. It is reported from a study that heavy metals such as mercury, lead, arsenic, and chromium were present in the majority of Ayurvedic formulations.[38] Heavy metals commonly found in Ayurvedic medicines have a deleterious impact on the body. Due to insufficient research, it is unclear whether heavy metals are the primary cause of toxicity of Ayurvedic medicines.[38-41]

There are few research protocols and toxicity studies in ayurvedic medicine research field which are limitations behind this investigation.

CONCLUSION

This investigation demonstrates that SBM had no influence on the levels of steroidal hormone and gonadotropin hormone during chronic pharmacological study. This study enriches our knowledge about toxicological profile of ayurvedic medicine. Furthermore, the results must be confirmed with other biochemical markers in order to evaluate the drug’s safety profile.

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