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EFFECT OF SWARNA SINDURA ON THYROID HORMONES IN SPRAGUE-DAWLEY RATS

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ABSTRACT

Swarna Sindura (SS) is an ayurvedic preparation used as a traditional medicine in the treatment of neurasthenia. In this study, effect of SS on thyroid hormone profile was evaluated after chronic administration of this drug to male Sprague-Dawley rats. The acute pharmacological test of SS recorded no death or any signs of toxicity even at the highest dose of 4000 mg/Kg body weight. For chronic pharmacological evaluation, the animals were divided into two groups. The first group was given SS 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 SS preparation the following effects on the thyroid hormone profile were noted. There was a statistically highly significant (p=0.008, 15.62 % increase) increase in the serum circulating free Triiodothyronine (fT3) level. The drug (SS) did not affect serum circulating total Thyroxine (tT4) level, total Triiodothyronine (tT3) level, free Thyroxine (fT4) level and Thyroid stimulating hormone (TSH) significantly.

Keywords: Swarna Sindura, Ayurvedic preparation, Acute toxicity, Thyroxine, Triiodothyronine

INTRODUCTON

Thyroid hormones are essential for normal mammalian development and are well known to play fundamental roles in the cardiovascular, nervous, immune and reproductive systems [1-4]. It is produced by the thyroid gland, which consists of follicles in which thyroid hormone is synthesized through iodination of tyrosine residues in the glycoprotein thyroglobulin [5, 6]. Thyroid hormone regulates metabolic processes essential for normal growth and development as well as regulating metabolism in the adult [7-9]. It is well established that thyroid hormone status correlates with body weight and energy expenditure [10-12]. Hyperthyroidism, excess thyroid hormone, promotes a hypermetabolic state characterized by increased resting energy expenditure, weight loss, reduced cholesterol levels, increased lipolysis, and gluconeogenesis [13, 14]. Conversely, hypothyroidism, reduced thyroid hormone levels, is associated with hypometabolism characterized by reduced resting energy expenditure, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis [15]. Thyroid hormone stimulates both lipogenesis and lipolysis, although when hormone levels are elevated, the net effect is fat loss [16]. It influences key metabolic pathways that control energy balance by regulating energy storage and expenditure [8, 11, 17].

Swarna Sindhura is an Ayurvedic medicine in powder or tablet form. It is a herbo-mineral formulation mentioned in Ayurvedic formulary of India [18]. It consists of *Ficus benghalensis, Aloe vera,* and purified and processed Mercury, Sulphur and Gold. Swarna Sindhura is used in Ayurvedic treatment of neurasthenia, oligospermia, emaciation,

lack of concentration and intelligence [19-24]. Many drugs and medications can affect thyroid function. Thyroid hormone levels can be altered by drugs at many levels including the hypothalamus, thyrotropes in the anterior pituitary gland, synthesis and secretion from the thyroid gland and metabolism of thyroid hormones through deiodination, sulfation and glucuronidation [25]. Drugs may also affect thyroid hormone levels by altering affinity for or levels of thyroxine binding globulin. Finally, drugs may affect absorption of thyroid hormone in patients who are dependent on exogenous levothyroxine [26]. That is why; we designed our current experiment to observe the effect of SS on thyroid hormone profile in Sprague-Dawley rats. This research work on ayurvedic formulation, Svarna Sindura (SS) unfolds a field of its toxicological aspects. The study provides directions for further research as well.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents: For the pharmacological study, Swarna Sindura (SS) 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 Abbott Laboratories, USA.

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 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 pharmacological 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) [27]. 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 (SS) 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 SS administration.

Chronic Toxicity Studies: Prior to the experiment, rats were randomly divided into 2 groups of 8 animals each. One group was treated with SS 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. 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.

Blood Samples Collection and Preparation of Serum: At the end of the 28 days treatment period, after 18 hours fasting, rats from each group were anaesthetized by administration (i.p) of ketamine (500 mg/Kg body weight). Blood samples were collected from post vena cava of rats into plain sample tubes for serum generation for biochemical analysis. Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuged at 4000 g for 10 minutes using bench top 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 hours of sample collection [28].

Determination of the Thyroid Hormone Profile: We measured serum circulating thyroid stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), total Triiodothyronine (tT3) and total Thyroxine level. Thyroid function tests were analyzed in the Department of Endocrinology, Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine & Metabolic Disorders

(BIRDEM), Dhaka, Bangladesh. Serum fT3, fT4, tT3, tT4 and TSH were determined by Chemiluminescent Microparticle Immunoassay (Architect system; Abbott Laboratories, USA).

Statistical Analysis: The data were analyzed using independent sample t-test with the help of SPSS (Statistical Package for Social Sciences) 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 significant.

RESULT

Acute Pharmacological Study: The drug (SS) administered up to a high dose of 4000 mg/kg produced no mortality. Thus the LD_{50} (Median Lethal Dose) value was found to be greater than 4000 mg/Kg body weight. The animals did not manifest any sign of restlessness, respiratory distress, general irritation or convulsion. Since SS is in the clinical use for treatment of diseases for many years, a limit test was performed in acute oral toxicity study. 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 SS when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic Pharmacological Study: There is an increase in the serum circulating total Thyroxine (tT4) (0.29% increase, p=0.971) level, total Triiodothyronine (tT3) (9.36% increase, p=0.335) level and free Thyroxine (fT4) (13.55% increase, p=0.195) level of the male rat, which was not statistically significant. There is a statistically highly significant (15.62% increase, p=0.008) increase in the serum circulating free Triiodothyronine (fT3) level. There is also a negligible increase in the serum circulating Thyroid stimulating hormone (TSH) level, which was statistically not at all significant (3.37% increase, p=0.904).

DISCUSSION

Thyroid hormones (THs), particularly triiodothyronine (T_3), are potent regulators of multiple physiological activities, including cellular metabolic rate, heart and digestive functions, muscle function, brain development, and bone maintenance [29, 30]. In addition to their crucial roles in maintaining cellular homeostasis, THs can cause multiple disorders, including cardiovascular disease [31, 32], diabetes mellitus [33, 34], and chronic liver disease [35-37], when their levels in the body are out of balance. In our study we focused on thyroid hormonal effect after chronic administration of Swarna Sindura. We found free Triiodothyronine (fT3) level significantly increased in the SS-treated rats. Increase in the serum circulating free Triiodothyronine (fT3) level reveals hyperthyroidism, T3 toxicosis or peripheral resistance syndrome. T3 circulates in the blood as an equilibrium mixture of free and protein bound hormone [38]. T3 is bound to thyroxine binding globulin (TBG), prealbumin, and albumin. The binding of these proteins is such that only 0.2-0.4% of the total T3 is present in solution as unbound or free T3. This free fraction represents the physiologically active thyroid hormone [38]. Free T3 is typically elevated to a greater degree than free thyroxine (T4) in Graves' disease and in toxic adenomas. Occasionally, free T3 alone is elevated (T3 thyrotoxicosis) in about 5% of the hyperthyroid population [39].

T4 and its associate thyroid hormone T3 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal metabolic and neural activity. Clinically, T4 measurements have long been recognized as an aid in the assessment and diagnosis of thyroid status. Thyroid-stimulating hormone (also known as TSH or thyrotropin) is a glycoprotein synthesized and secreted by thyrotrope cells in the anterior pituitary gland which regulates the endocrine function of the thyroid gland. TSH stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3). Elevated levels of T3 and T4 suppress the production of TSH via a classic negative feedback mechanism. The mechanism controlling thyroid function in rats is exactly analogous to the mechanism operating in humans. This means that thyrotropin-releasing hormone stimulates the release of TSH from the pituitary gland as well as the serum concentrations of T4 and T3 influence the action of the pituitary gland. Usually an increase in the serum circulating Thyroid stimulating hormone (TSH) level is noted in case of Hypothyroidism. The negligible increase in Thyroid stimulating hormone (TSH) level was not that prominent to have any importance to notice.

CONCLUSION

From the above experiment it can be concluded that Swarna Sindura should not be administered chronically at a higher dose as it significantly increase serum circulating free Triiodothyronine

(fT3) level. Further studies should be done by reducing the administered dose.

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Table1: Name of the ingredients/herbs used in the preparation of Swarnasindura (SS)

Name of ingredients	Scientific name	Amount
Rasendra (parada)	Purified and processed Mercury	48 g.
Gandhaka	Purified and processed Sulphur	48 g.
Hema (svarna)	Gold Bhasma	12 g.
Vatapraroha rasa	juice extract of Ficus benghalensis	Q. S. (for mardana)
Kumarika rasa	Juice extract of Aloe vera	Q. S. (for mardana)

Table 2: Effect of Swarna Sindura (SS) on Serum circulating thyroid hormone level in Male Rats

Parameters	**Control**	**SS**	**p value**	**%increase/decrease**
Serum total T4	6.381±0.345	6.400±0.369	0.971	0.29% Increase
Serum total T3	0.668±0.023	0.730±0.057	0.335	9.36% Increase
Serum free T4	0.876±0.056	0.995±0.067	0.195	13.55% Increase
Serum free T3	1.640±0.028	1.896±0.077	0.008	15.62% Increase
TSH	0.018±0.004	0.018±0.004	0.904	3.37% Increase

Independent sample t-test was performed to analyze this data set. All values are expressed as mean ± SEM and $p<0.05$, $p<0.01$, $p<0.001$ were taken as the level of significant

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