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Research Article

Effect of Pijusaballi Rasa on Thyroid Hormones in Sprague-Dawley Rats

Mahbub MR[1], Sikder MM[2]*, Sony TA[1], Jahan NN[1], Faruque MO[1], Zaman T[1], Nawar A[1] and Choudhuri MSK[1]

[1]Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh; [2]Department of Biochemistry, Kagawa University School of Medicine, Miki, Kagawa, Japan

ABSTRACT

Pijusaballi Rasa (PJB) is an Ayurvedic preparation used as a traditional medicine in the treatment of constipation in the rural population in Bangladesh. In this study, the effect of PJB on thyroid hormone profile was evaluated after chronic administration of this drug to male Sprague-Dawley rats. The acute pharmacological test of PJB 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 PJB preparation at a dose of 400 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 PJB preparation, the following effects on the thyroid hormone panel were noted. There was a statistically insignificant ($p = 0.523$; 5.20%) decrease in the serum-circulating total thyroxine (tT4) level of the male rats. There was a statistically insignificant ($p = 0.667$; 3.35%) decrease in the serum-circulating total triiodothyronine (tT3) level of the male rats. There was a statistically insignificant ($p = 0.551$; 4.40%) decrease in the serum-circulating free thyroxine (fT4) level of the male rats. There was a statistically highly significant ($p = 0.003$; 16.36%) increase in the serum-circulating free triiodothyronine (fT3) level of the male rats. There was a statistically insignificant ($p = 0.691$; 51.03%) increase in the serum-circulating thyroid-stimulating hormone (TSH) level of the male rats. Ratios of other hormone levels with respect to fT3 level showed a more deviating trend.

Keywords: Pijusaballi Rasa; Ayurvedic Preparation; Acute Toxicity; Thyroxine; Triiodothyronine

INTRODUCTION

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 adults [7-9]. It is well established that thyroid hormone status correlates with body weight and energy expenditure [10-12]. Hyperthyroidism, that is, overproduction of 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, that is, reduced production of thyroid hormone, 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].

Pijusaballi Rasa (PJB) is an Ayurvedic preparation used as a traditional medicine in the treatment of constipation in the rural population in Bangladesh [18,19]. PJB has been included (pages 246-247) 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).

Correspondence to: Md Mamun Sikder, Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa, 761-0793 Japan, Tel: +8801671826017; E-mail: mamunshikder1678@gmail.com

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Table 1: Name of the ingredients/herbs used in the preparation of Pijusaballi Rasa.

Name of ingredient	Scientific/common name	Parts used	Amount used
Sutaka (suddha parada)	Mercury (purified)		6 g
Gandhaka (suddha)	Sulfur (purified)		6 g
Abhra (abhraka bhasma)	Calcined purified mica ash		6 g
Tara (rajatabhasma)	Silver ash (calcined silver)		6 g
Lauha (bhasma)	Iron, cinnabar		6 g
Tangana (suddha tankana)	Borax		6 g
Rasanjana (daruharidra) (Ext.)	*Berberis aristata*	Extract	6 g
Maksika (bhasma)	Chalcopyrite		6 g
Lavanga (Fl.)	*Syzygium aromaticum*	Flower	6 g
Candana (svetacandana) (Ht. Wd.)	*Santalum album*	Wood	6 g
Musta (musta) (Rz.)	*Cyperus rotundus*	Rhizomes	6 g
Patha (Rt.)	*Cissampelos pareira*	Root	6 g
Jiraka (Sveta jiraka) (Fr.)	*Cuminum cyminum*	Flower	6 g
Dhanyaka (Fr.)	*Coriandrum sativum*	Flower	6 g
Samanga (Lajjalu) (Pl.)	*Mimosa pudica*	Plant	6 g
Ativisa (Rt.)	*Aconitum heterophyllum*	Root	6 g
Lodhra (St. Bk.)	*Symplocos racemosa*	Stem bark	6 g
Kutaja (St. Bk.)	*Holarrhena antidysenterica*	Stem bark	6 g
Indrayava (Kutaja) (Sd.)	*Holorrhena antidysenterica*	Seed	6 g
Tvaca (Tvak) (St. Bk.)	*Cinnamimum zeylanicum*	Stem bark	6 g
Jatiphala (Sd.)	*Myristica fragrans*	Seed	6 g
Cirabilva (Fr. P.)	*Holoptelea integrifolia*	Fr Patel	6 g
Kanaka bija (Suddha dhattura) (Sd.)	*Datura stramonium*	Seed	6 g
Dadimachada (dadima) (Fr. P.)	*Punica granatum*	Fr Patel	6 g
Samanga (Lajjalu) (Pl.)	*Mimosa pudica*	Plant	6 g
Dhataki (Fl.)	*Woodfordia fruticosa*	Flower	6 g
Kustha (Rt.)	*Saussurea lappa*	Root	6 g
Kesaraja Rasa (bhrngaraja) (Pl.)	*Eclipta alba*	Plant	Q.S. for bhavana
Chagi dugdha (ajaksira)	Goat's milk		Q.S. for bhavana

MATERIALS AND METHODS

Drugs, chemicals, and reagents

For the pharmacological study, PJB was collected from Sri Kundeswari Aushadhalaya Limited, Chittagong. Ketamine injection was purchased from ACI Limited, Dhaka, 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 each 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. Water was provided ad libitum and the animals were maintained at 12 hours day and 12 hours night cycle. All experiments on the 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 as per the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals with minor modifications (OECD Guideline 425) [20]. Sixteen male Sprague-Dawley rats (30-40 g body weight) were divided into four groups, each group comprising four animals. Different doses (1000, 2000, 3000, and 4000 mg/kg) of the experimental drug (PJB) were administered to the rats 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 3 days following PJB administration.

Chronic toxicity studies: Prior to the experiment, the rats were randomly divided into two groups of eight animals each. One group was treated with PJB and another was used as a control. For 28 days, the control animals were administered with distilled water only, the volume of water being the same as the volume of drug administered to the study group. For all the pharmacological studies, the drugs were administered per oral route at a dose of 400 mg/kg body weight. After acclimatization, the 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 the 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 uniquely identify each of them. Using identification mark, responses were noted separately for a particular period prior to and after the administration.

Blood sample collection and preparation of serum: At the end of the 28 days treatment period, after 18 hours fasting, rats from each group were anesthetized by administration (i.p.) of ketamine (500 mg/kg body weight). For biochemical analysis, blood samples were collected from posterior vena cava of the rats into plain sample tubes for serum generation. Serum was obtained after allowing blood to coagulate for 30 minutes and centrifuging it at 4000 rpm for 10 minutes using a benchtop centrifuge (MSE Minor, UK). The supernatant serum samples were collected using a dry Pasteur pipette and stored in the refrigerator for further analysis. All analyses were completed within 12 hours of sample collection [21].

Determination of the thyroid hormone profile: We measured the serum-circulating thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), total triiodothyronine (tT3), and total thyroxine (tT4) levels. 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, IL). All values are expressed as mean ± SEM and $p < 0.05$, $p < 0.01$, and $p < 0.001$ were taken as the levels of significance.

RESULT

Acute pharmacological study

The drug (PJB) administered up to a high dose of 4000 mg/kg caused no mortality. Thus the LD50 (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 PJB has been 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 toxicity or non-toxicity and NIL mortality 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 PJB when administered at single dose is non-toxic and can be used safely in oral formulations.

Chronic pharmacological study

From Table 2, there is a statistically insignificant ($p = 0.523$; 5.20%) decrease in the serum-circulating tT4 level of the male rats. There is a statistically insignificant ($p = 0.667$; 3.35%) decrease in the serum-circulating tT3 level of the male rats. There is a statistically insignificant ($p = 0.551$; 4.40%) decrease in the serum-circulating fT4 level of the male rats. There is a statistically highly significant ($p = 0.003$; 16.36%) increase in the serum-circulating

Table 2: Effect of Pijusaballi Rasa (PJB) on serum-circulating thyroid hormone level in male rats.

Parameters	Control	PJB	p value	% Increase/decrease
Serum total T4	6.180 ± 0.3798	5.859 ± 0.316	0.523	Decr 5.197411
Serum total T3	0.707 ± 0.0343	0.684 ± 0.0418	0.667	Decr 3.349823
Serum free T4	0.906 ± 0.0454	0.866 ± 0.0472	0.551	Decr 4.403002
Serum free T3	1.597 ± 0.0492	1.859 ± 0.0519	0.003	Incr 16.3568
TSH	0.0243 ± 0.00612	0.0367 ± 0.02667	0.691	Incr 51.0288

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 levels of significance.

fT3 level of the male rats. There is a statistically insignificant ($p = 0.691$; 51.03%) increase in the serum-circulating TSH level of the male rats.

DISCUSSION

Thyroid hormones, particularly triiodothyronine (T3), are potent regulators of multiple physiological activities, including cellular metabolic rate, heart and digestive functions, muscle function, brain development, and bone maintenance [22,23]. In addition to their crucial roles in maintaining cellular homeostasis, when their levels in the body are out of balance, thyroid hormones can cause multiple disorders, including cardiovascular disease [24,25], diabetes mellitus [26,27], and chronic liver disease [28-30]. In our study we focused on thyroid hormonal effect after chronic administration of PJB.

We found fT3 level to be significantly increased ($p < 0.01$) in the PJB-treated rats. Increase in the serum-circulating 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 [31]. T3 is bound to thyroxine-binding globulin, 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 fT3. This free fraction represents the physiologically active thyroid hormone. fT3 is typically elevated to a greater degree than fT4 in Graves' disease and in toxic adenomas. Occasionally, fT3 alone is elevated (T3 thyrotoxicosis) in about 5% of the hyperthyroid population [32].

TSH (or thyrotropin) is a glycoprotein synthesized and secreted by thyrotropic cells in the anterior pituitary gland that 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 and the serum concentrations of T4 and T3 influence the action of the pituitary gland. Usually an increase in the serum-circulating TSH level is noted in case of hypothyroidism. The negligible increase in TSH level was not that prominent to have any importance to be noted.

CONCLUSION

From the above experiment it can be concluded that PJB should not be administered chronically at a higher dose as it significantly increase serum-circulating fT3 level. Further studies should be done by reducing the administered dose.

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