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The Effect of Aqueous Extract of *Dactylorhiza Maculate* Root on the Concentration of Hypothalamic-pituitary-thyroid Axis Hormones in Adult Female Rats

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ABSTRACT

Thyroid is a very important endocrine gland in living organisms which regulates almost all bodily functions. The aim of the presentstudy is to investigate the effect of aqueous extract of Dactylorhiza maculateroots on hypothalamic- pituitary-thyroidaxishormones in adult female rats. Fifty adult female rats were randomly divided into control group, sham group (receiving distilled water) and three experimental groups (receiving aqueous extract of Dactylorhiza maculaterootat a dose of 20, 40 and 80 mg kg^{-1}) each consisting of 10 rats. Intra-peritoneal injections were carried out for 28 days. The injection volume was 2.0 ml in all groups. On the twenty-ninth day, blood samples were taken from the rats to investigate the serum levels of the HPTaxis hormones. Data were analyzed bySPSS (Version 15). Comparisons were carried out employing one-way ANOVA and Duncan's test. The doses of 20 and 40 $mgkg^{-1}$ of aqueous extract of Dactylorhiza maculaterootshad no significant impact on the serum levels of TRH, TSH, T3 and T4 hormones. However, a dose of 80 mg kg^{-1} significantly reduced the serumlevels of TRH, TSH, T3 and T4 hormones as compared to the control group ($P < 0.05$). According to the results, reduction of hypothalamic-pituitary-thyroid axis hormones by aqueous extract of Dactylorhiza maculateis dose-dependent. Thus,Dactylorhiza maculatecan be usedas an alternative to chemical drugs in the treatment of hyperthyroidism.

Keywords: *Dactylorhiza maculate*, HPT axis, Female rat

INTRODUCTION

The ability to maintain body homeostasis is a key point in human health which is controlled by several factors, particularly pituitary-thyroid hormonal axis. Aside fromdisrupting the body's health,hypothyroidism and hyperthyroidismimpose large irrecoverable costs on patients in many cases [1]. Thyroid regulates the body's metabolism by secreting thyroxine (T3) and triiodothyronine (T4). Therefore, it is important to study the role of thyroid. One of the key factors to control the synthesis and secretion of thyroid hormones is regulatory effect of hypothalamic-pituitary hormonal axis[2, 3]. Thyrotropin-releasing hormone (TRH) is released from the paraventricular nucleus of hypothalamus and influences the anterior pituitary gland leading to secretion of thyroid-stimulating hormone (TSH) [4, 5]. Secretion of T3 and T4 hormones is influenced by TSH. Thyroid hormones stimulate the transcription of numerous genes and produce high levels of enzymes and structural and carrier proteins,and thereby increase basal metabolism[6, 7].

Herbal medicines are commonly prescribed by practitioners of traditional medicine in Iran and other countries for treatment of many patients. *Dactylorhiza maculate*is a widely used herbin India, Nepal, China, Europe and other regions of the world [8, 9]. *Dactylorhiza maculate*or *Lancibracteata (C.koch) Renz Dactylorhiza* with the old name of maculate L.Orchis belongs to the orchid family. It has different species and grows almost all over the world. *Dactylorhiza maculate*glands can be usually used early summers and maintains its optimum medicinal propertiesup to two years[9, 10]. The plant contains chemicals including nitrogenizedmaterials, starch, protein, sugar, hydroxy benzaldehyde, ferulic acid, quercetin, daucosterol, cirsilineol, steroids and glucomannan [9, 11, 12]. In traditional medicine, this plant is used as cough mixtureand is prescribed for the treatment of chestand bowel disorders,tuberculosis, diarrhea, Parkinson's disease, cancer, fever and,in particular, to strengthen sexual activities, treatment of erectile dysfunction and increased stamina and energy. The plant is also used in ice cream, beverages and confectionery industries[13, 14].

According to literature, fibers and flavonoids cause changes in the thyroid function. Flavonoids have an inhibitory effect on thyroid function and increase the risk of hypothyroidism [15]. Due to structural similarity with T3 and T4 hormones, flavonoids have a negative feedback on the hypothalamic-pituitary activity [16]. In addition,there are evidencesforthe therapeuticaleffect of water soluble glucomannanfiber on hyperthyroidism [17].

Due to the presence of these compounds in the root extract of *Dactylorhiza maculate*, it is likely to affect thyroid function. Accordingly, the effect of aqueous extract of *Dactylorhiza maculate*root on the hypothalamic-pituitary-thyroid (HPT) axis in female rats was examined.

MATERIALS AND METHODS

Sample Collection and Extraction

Dactylorhiza maculate samples were collected from areas aroundYasuj, Iran early in the summer. The gland roots of *Dactylorhiza maculate* were washed to remove the soil and then were dried in a laboratory environment. The completely dried samples were powdered by an electric mill. The resulting powder was mixed with ethyl alcohol 96% in a volume ratio of 1/5. The mixture was thoroughly stirred for 24 hrs. using a stirrer at ambient temperature to obtain a homogeneous solution. The solution was then filtered out and dried for 48h at ambient temperature to obtain an alcohol-free solid extract. Finally, 20, 40 and 80 mg of the solid extract were dissolved in 1 ml of double-distilled water and were kept in the refrigerator [18].

Animal groups

All ethical issues regardingthe maintenance and the use of laboratory animals wereobserved. The project was registered by the Ethics Committee of JahromUniversity of Medical Sciences on 2014.03.04 (Registration Number: 2991/C/D).In this experimental study, 50 adult male Wistar rats with the average weight of 180-200 g were used. Rats were kept at animal room of Jahrom University of Medical Sciences for a week to adapt conditions. Throughout the investigation,animals were kept at 12 hours of light and 12 hours of darknessat room temperature of 20-25°C.The rats had free access to food and water. The animals were randomly divided into 5 groups of 10 rats. The control group did not receive any substance.The sham group received 0.2ml $g^{-1}$of distilled water through intraperitoneal injection. Experimental groups 1, 2 and 3respectively received the minimum (20 mg kg^{-1}), medium (40 mg kg^{-1}) and maximum dose (80 mg kg^{-1}) of aqueous extract of*Dactylorhiza maculate*root through daily intraperitoneal injection for 4 weeks.

Blood samples and hormonal tests

At the end of study (day 29), the animals were weighed and blood samples were directly taken from the heart of animals using 5 ml syringes (under anesthesia with diethyl ether). The blood serums were separated by centrifugation (3000 rpm for 15 min) and stored in the freezer at -20˚C. ELISA kits for rats (Biovendor, Czechoslovakia) were used to measure TRH, TSH, T3 and T4 hormones.

Statistical analysis

One-way analysis of variance (ANOVA) was employedto analyze the collected data. In cases, where there was significant statistical difference between different groups, Duncan's test was used to understand the difference between the means. Statistical analysis was performed using SPSS21 considering a significance level of $P < 0.05$. The results were given as Mean ± SEM.

RESULTS

The effect of*Dactylorhiza maculate* root extract on the serum level of TRH

There was no significant difference between the control and sham groups. Doses of 20 and 40 mg $kg^{-1}$of aqueous extract of *Dactylorhiza maculate*roothad no significant effect on the serum level of TRH. A dose of 80 mg kg^{-1} of aqueous extract of *Dactylorhiza maculate*rootsignificantly reduced TRH serum level as compared to the control and sham groups (P<0.05)(Table 1).

The effect of *Dactylorhiza maculate* root extract on the serum level of TRH

There was no significant difference between the control and sham groups. Doses of 20 and 40 mg $kg^{-1}$of aqueous extract of *Dactylorhiza maculate*roothad no significant effect on the serum level of TSH. A dose of 80 mg kg^{-1} of aqueous extract of *Dactylorhiza maculate*rootsignificantly reduced TSHserum level as compared to the control and sham groups (P<0.05)(Table 1).

The effect of *Dactylorhiza maculate* root extract on the serum level of T3

There was no significant difference between the control and sham groups. Doses of 20 and 40 mg $kg^{-1}$of aqueous extract of *Dactylorhiza maculate*roothad no significant effect on the serum level of T3. A dose of 80 mg kg^{-1} of aqueous extract of *Dactylorhiza maculate*rootsignificantly reduced T3serum level as compared to the control and sham groups (P<0.05)(Table 1).

The effect of *Dactylorhiza maculate* root extract on the serum level of T4

There was no significant difference between the control and sham groups. Doses of 20 and 40 mg $kg^{-1}$of aqueous extract of *Dactylorhiza maculate*roothad no significant effect on the serum level of T4. A dose of 80 mg kg^{-1} of aqueous extract of *Dactylorhiza maculate*rootsignificantly reduced T4serum level as compared to the control and sham groups (P<0.05)(Table 1).

Table 1: The changes in hypothalamic-pituitary-thyroid axis hormones in the control group and the experimental groups received different doses of *Dactylorhiza maculate*root extract

Parameter	TRH Pg/ml	TSH Iu/ml	T4 Ng/ml	T3 Pg/ml
Control	723.25±39.35 [b]	0.98±0.04 [b]	3.53±0.13 [b]	362.72±17.32 [b]
Sham	704.92±33.64 [b]	0.95±0.02 [b]	3.45±0.13 [b]	356.05±17.72 [b]
20 mg kg^{-1}	709.73±16.45 [b]	0.96±0.05 [b]	3.58±0.27 [b]	362.48±12.14 [b]
40 mg kg^{-1}	678.17±13.60 [b]	0.85±0.03 [ab]	3.34±0.23 [ab]	342.45±11.61 [b]
80 mg kg^{-1}	600.71±7.91 [a]	0.76±0.05 [a]	2.77±0.07 [a]	294.33±7.24 [a]

-According to Duncan's test,the means in each column with at least one common letter have no significant difference at significance level of 5%.

-Means are given as Mean ±SEM.

P<0.05 was considered statistically significant.

DISCUSSION

According to the results, the maximum dose of aqueous extract of *Dactylorhiza maculate* root(80 mg kg^{-1}) reduced the serum levels of TRH, TSH, T3 and T4hormones. However, the minimum and mediumdoses had no effect on the serum levels of these hormones. Therefore, the depressing effect of *Dactylorhiza maculate* root extract on HPT axis is dose-dependent.

Dactylorhiza maculate contains several compounds including glucomannan, flavonoids and steroids. These constituents can affect the serum levels of thyroid hormones.

Glucomannan content varies in different species of *Dactylorhiza maculate*from 7 to 61%. Glucomannan is a water soluble fiber which plays an important role in weight loss, blood sugar control and reducingcholesterol [18, 19]. The results also indicated the effectiveness of glucomannan in the treatment of hyperthyroidism. According to Azezli*et al.*, glucomannan reduces the serum levels of thyroid hormones (thyroxine and triiodothyronine) in patients with hyperthyroidism[17].

As previously mentioned,secretion of thyroid-stimulating hormone (TSH) is stimulated by thyrotropin-releasing hormone (TRH) in the paraventricularnucleus of hypothalamus. Several neurotransmitter structures including Y-neuropeptide control TSH-secreting neurons in the hypothalamus [20]. Glucomannan fiber in *Dactylorhiza maculate*extract has an inhibitory effect on the activity of Y neuropeptide-secreting neurons through increased secretion of leptin. Accordingly, when the activity of Y neuropeptide-secreting neurons (which have stimulating effect on the secretion of thyrotropin-releasing hormone (TRH)) decreases, the serum level of TRH and thereby

secretion of TSH reduces[21, 22]. Given the stimulating role of thyroid hormones on the secretion of thyroxine and triiodothyronine hormones, the plasma levels of thyroid hormones are also reduced.

Literature suggests antithyroid properties of flavonoids [15, 23]. Flavonoids inhibit TPO enzyme as a key for biosynthesis of thyroid hormones and decrease the serum levels of thyroid hormones [24]. Flavonoids also inhibit activation of type I deiodinase enzymewhich is specifically activated by TSH. They also prevent mineralization of iodine in thyroid cells and cause changes in thyroid hormone levels [25]. Quercetin is a flavonoid found in *Dactylorhiza maculate*. Giuliani *et al.*investigated the effects of quercetin on thyroid functionand found that this flavonoid impairs thyroid function through reduced expression of thyrotropin receptor, thyroid peroxidase and thyroglobulin genes [26].

Flavonoids inhibit monoamine oxidase enzyme[27, 28] leading to increased levels of catecholamine neurotransmitters (dopamine, epinephrine and norepinephrine) and indole-amine (serotonin) [29]. Serotonin is a neurotransmitter inhibiting secretion of thyrotropin-releasing hormone [30, 31]. Accordingly, it seems that*Dactylorhiza maculate*is able to reduce the levels of thyroid hormones by increasing serotonin level.

Dopamine level increases by inhibiting monoamine oxidase enzyme[29]. Dopamine reduces secretion of thyrotropin-releasing hormonein hypothalamus and pituitary and directly prevents secretion of thyroid-stimulating hormone and decreases serum levels of thyroid stimulating hormone either free or bound to plasma proteins. Dopamine prevents secretion of TSH in the anterior pituitary through D2 receptors. Dopamine also decreases secretion of TSH by stimulating secretion of somatostatin [32].

Steroids found in *Dactylorhiza maculate* may also affect the serum levels of thyroid hormones. According to literature, steroidal compounds decrease serum levels of thyroid hormone transport proteins and thus reduce thyroxine and triiodothyroninehormones [33].

CONCLUSION

Thereduction ofserum levels of hypothalamic-pituitary-thyroid axishormonesby*Dactylorhiza maculate*root extract is dose-dependent. However, further studies on the effect of *Dactylorhiza maculate*root extract on HPT axis are required to use this plant as a suitable alternative to chemical drugsin the treatment of hyperthyroidism.

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