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Feeding of Nigella sativa during lactation improved serum prolactin level of hypothyroid and euthyroid rats

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Abstract

This study was done to investigate the effects of feeding by the hydro-alcoholic extract of Nigella sativa (NS) during lactation on prolactin level of mothers and weight gain of offspring in propylthiouracil (PTU) induced- hypothyroid and euthyroid rats. Forty pregnant rats were randomly divided into 8 groups and treated by: (1) Normal drinking water as a control group; (2) 0.005% PTU in drinking water; (3-5) 100, 200, or 400 mg/kg hydro-alcoholic extract of NS plus PTU (6-8) 100, 200 or 400 mg/ kg of the plant extract in drinking water without PTU. Mothers received the experimental treatments from the first day after delivery through the lactation period. The offspring continued to receive the experimental treatments up to the first two months of their life. PTU decreased serum thyroxin concentration of mothers and their dams compared to the control ones ($P<0.001$) which improved by the plant extract ($P<0.001$). There was no significant difference between the groups in TSH concentration. There was no significant difference between PTU and control groups in the duration of the lactation period and also in serum prolactin level. All doses of the plant extract increased the duration of lactation period of both hypothyroid and euthyroid rats compared to both control and PTU groups which were accompanied by serum prolactin concentration ($P<0.001$). The extract also increased the body weight gain of offspring rats ($P < 0.01$- $P<0.001$). In the present study we showed hypothyroid status induced by PTU during the lactation period did not change serum prolactin level. Administration of NS extract during lactation period improved prolactin concentrations in both hypothyroid and euthyroid rats, prolonged lactation period, and improved weight gain of the offspring.

Key words: Nigella Sativa, Propylthiouracil, Hypothyroid, Lactation, Prolactin.

Introduction

Hyperthyroidism is a disease that needs a long duration of continuous therapy of thioamide anti-thyroid drugs such as propylthiouracil (PTU) [1]. PTU exerts its anti-thyroid effects through iodine oxidation inhibition and monoiodotyrosine ionization, preventing the coupling stage in the thyroxin production process, and peripheral inhibition of the conversion of T4 into T3 [2]. PTU is also frequently used to induce a hypothyroidism status in rodents [3]. On the other hand, thyroid hormones have critical roles in the regulation of energy metabolism, mitochondrial activity, oxygen consumption, and active oxygen metabolism [4,5]. Additionally, juvenile hypothyroidism has an insidious onset characterized by gradual slowing and eventual cessation of growth [6].

Hyperprolactinemia is suggested to develop in patients with primary hypothyroidism through a variety of mechanisms. In response to the hypothyroid state, a compensatory increase in the discharge of central hypothalamic thyrotropin releasing hormone (TRH) occurs, which results in stimulation of prolactin secretion. The role of TRH as a hypothalamic hypophysiotrophic

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hormone that stimulates thyroid-stimulating hormone (TSH) released from the anterior pituitary gland is well-known but its role in stimulating prolactin release from the anterior pituitary is still controversial [7]. Prolactin elimination from the systemic circulation is reduced in patients with primary hypothyroidism, which contributes to increased prolactin concentrations [8].

Many women with problems in milk secretion use traditional herbs to raise their milk production. Some studies have reported the beneficial effects of many plants on milk production. Several herbs have been shown to increase the production of milk because of the induction of the lactogenic hormones including prolactin, growth hormone, and casein accumulation in the mammary gland. Prolactin has vital functions and roles in lactogenesis. For sure, prolactin empowers the generation of milk proteins in the epithelial cells and induces the proliferation of secretory tissue [9]. Prolactin is a protein hormone of the anterior pituitary gland that was named for its ability to lactation promotion in response to the suckling stimuli of hungry young mammals. Its biological roles are not limited only to reproduction because it has been shown to control some behaviors and even have a role in homeostasis. The stimuli of prolactin-releasing not only include the nursing stimulus but audition, light, olfaction, and stress can serve as a stimulatory action. Although dopamine of hypothalamic origin provides inhibitory control over the prolactin secretion, other factors in the brain, pituitary gland, and peripheral organs have been shown to inhibit or stimulate secretion of prolactin as well [10].

Nigella sativa (NS) has been used in folk medicine because of its many useful effects, especially during lactation time. NS is an annual plant of the Ranunculaceae family and is found in various countries bordering the Mediterranean Sea, Pakistan, India, and Iran [11]. Also, its seeds, which are commonly known as black seeds, are eaten with honey, sweet foods, bread, and cheese. In Arabic countries, the black seeds are known as black caraway seeds, Habbatu Sawda, and Habatul Baraka [12].

NS is protective in some situations associated with neuronal damage [13]. NS is used as healing medicine for treatment and amelioration of various diseases such as asthma [14], infections [15], diabetes [16], renal damages [17,18], hypertension [19], memory impairment [20] and gastrointestinal problems [21]. The major bio-actives of NS seeds are thymoquinone (TQ), alkaloids (nigellone, nigellimine, and nigericin), riboflavin, vitamin-like thiamine, pyridoxine, folic acid, niacin, minerals, and proteins [22]. Also, NS is used as a galactagogic in traditional medicine. Studies showed that the extract of NS can stimulate milk production in rats [23].

Due to the use of this plant as a galactagogue in traditional medicine and the presence of a few reports on its effect on milk production, the purpose of this study was to explain the effects of feeding by Nigella sativa during lactation on serum prolactin level of hypothyroid and euthyroid rats. The effects of the plant on the duration of the lactation period and the body weight gain of newborns were also evaluated.

Materials and Methods

Animals and treatments

Forty pregnant female Wistar rats (12 week-old and weighing 220-250 g) were kept in separate cages at 22 ± 2 °C in a room with a 12 h light/dark cycle (light on at 7:00 am). Animals were randomly divided into 8 groups and treated by: (1) Normal drinking water as a control group, (2) 0.005% propylthiouracil (PTU) in drinking water to induce hypothyroidism, (3-5) 100, 200, or 400 mg/ kg hydro-alcoholic extract of NS plus PTU, (6-8) 100, 200 or 400 mg/ kg of the plant extract in drinking water without PTU [24-26]. After delivery through the lactation period, mothers received the experimental treatments from the first day. To examine the effects of the plant extract on weight gain, the offspring continued to receive the experimental treatments in their drinking water for the first two months of their life. Blood samples were collected from mothers after the lactation period to determine serum TSH, thyroxin, and prolactin levels. After the lactation period, ten male offspring of each group were randomly selected. They were

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weighed at days 10, 30, and 60 after delivery. On day 60, post-delivery, the blood samples were collected from offspring to measure serum thyroxin level. The serum thyroxin and prolactin levels were assessed using the radioimmunoassay method. Animal handling and all related procedures were carried out in accord with the procedures approved by the Mashhad University of Medical Sciences ethical committee.

Statistical analysis

All data were expressed as means ± SEM. The data of TSH, thyroxin, and prolactin levels were compared by one-way ANOVA followed by Tukey's post hoc comparisons test. The data of body weight of offspring were compared using repeated-measures ANOVA followed by Tukey's post hoc comparisons test. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Administration of PTU in drinking water during the lactation period decreased serum thyroxine concentration of dams compared to the control ones (Figure 1; P<0.001). Co-administration of all three doses of the plant extract with PTU improved the serum thyroxine level of lactating rats (Figure 1; P<0.001). Additionally, continuing PTU administration to the offspring rats decreased serum thyroxine levels compared to the control ones (Figure 2; P<0.001). Treatment by all three doses of the plant extract significantly attenuated the PTU-induced reduction in serum thyroxine (Figure 2; P<0.001).

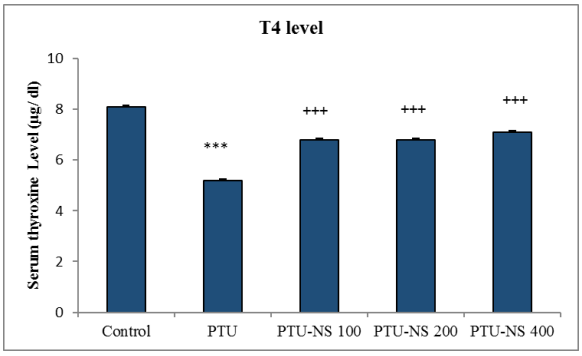

Figure 1: The mother serum thyroxin concentrations. Data are presented as mean±SEM. (n = 10). ***P<0.001 vs. control group, +++P<0.001 vs. PTU group. Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively. The thyroxin level in PTU group was lower while in all PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups was higher than PTU group.

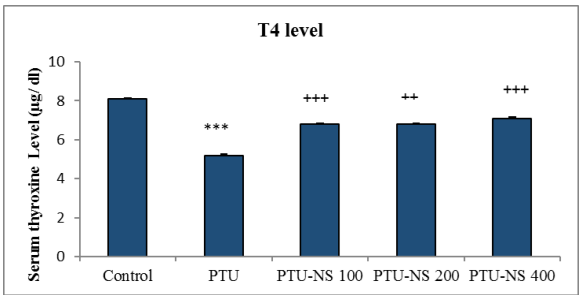

Figure 2: Offspring serum thyroxin concentrations. Data are presented as mean±SEM. (n = 10). ***P<0.001 vs. control group, +++P<0.001 vs. PTU group. Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively. The thyroxin level in PTU group was lower while in all PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups was higher than PTU group.

There was no significant difference between groups in serum TSH concentration (Figure 3). There was no significant difference between PTU and control groups in the duration of the lactation period. Interestingly, all doses of the plant extract increased the duration of the lactation period compared to both the control and PTU group (Figure 4, p<0.001). Additionally, compared to the control group all doses of the plant extract increased the duration of the lactation period when administered without PTU (Figure 5, p<0.001). Also, when the mother prolactin level in the PTU group compared to the control group, no significant difference was found but in three treatment groups with NS the prolactin level significantly increased compared to both PTU and control groups (Figure 6; P<0.001). Also, the prolactin level significantly increased in all three NS treated groups compared to the control group (Figure 7; P<0.001).

The bodyweight of the rats in the PTU group was significantly lower than that of the control group (p<0.001). The body weight in treated groups with PTU plus NS 100, 200, and 400 was higher than that of the PTU group in all three times of

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weighting (Figure 8; P<0.05 - P<0.001). The body weight in the treated groups by 200 and 400 mg /kg NS significantly increased compared to the control group (Figure 9; P<0.01 - P<0.001)

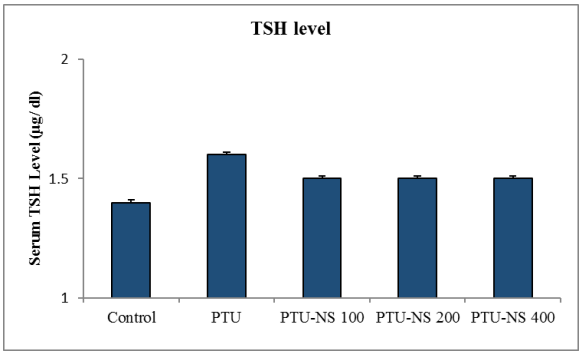

Figure 3: There was no significant difference between groups. Data are presented as mean±SEM. (n = 10). Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively.

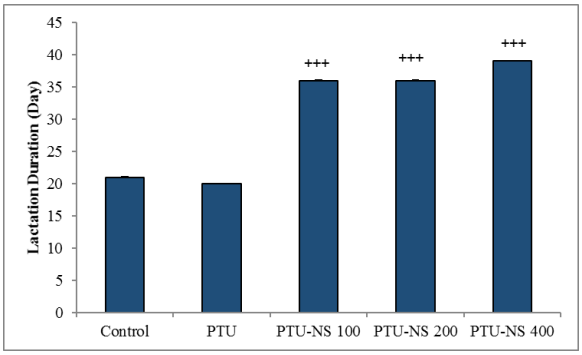

Figure 4: lactation duration**.** Data are presented as mean±SEM. (n = 10). +++P<0.001 vs. PTU group. Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively. Lactation duration was higher in NS treated groups compared PTU group.

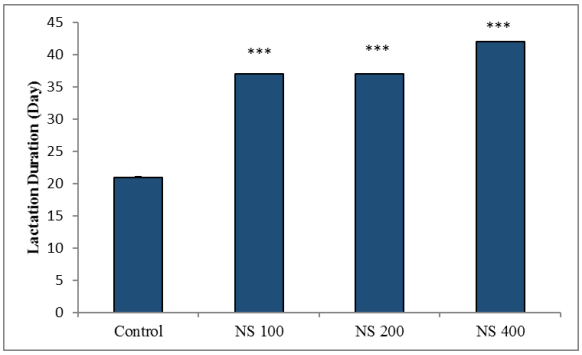

Figure 5: lactation duration. Data are presented as mean±SEM. (n = 10). ***P<0.001 vs. control group. Rats in control group received tap drinking water, NS 100, NS 200 and NS 400 groups received normal drinking water and 100, 200 and 400 mg/kg of NS extract respectively. Lactation duration was higher in NS treated groups compared PTU group (p<0.001).

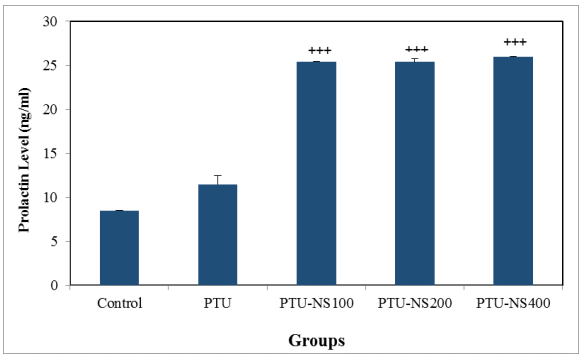

Figure 6: Serum prolactin concentrations. Data are presented as mean±SEM. (n = 10). ***P<0.001 vs. PTU group. Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively. The prolactin level in PTU group was lower while in all PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups was higher than PTU group.

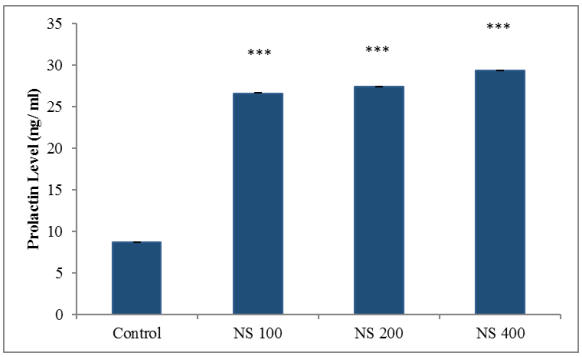

Figure 7: Serum prolactin concentrations. Data are presented as mean±SEM. (n = 10). ***P<0.001 vs. Control group. Rats in control group received tap drinking water, NS 100, NS 200 and NS 400 groups received normal drinking water plus 100, 200 and 400 mg/kg of NS extract respectively. The prolactin level in in all NS 100, NS 200 and NS 400 groups was higher than control group.

The results of the present study showed that exposure to the anti-thyroid agent PTU, during the lactation period resulted in a typical hypothyroid status associated with weight changes in the present study. In confirm with our results, another study showed that PTU treatment during pregnancy and lactation was associated

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with low birth weight [27,28]. Also, in another study of the rats that received PTU during pregnancy, the bodyweight of their dams was significantly lower than the control group [29].

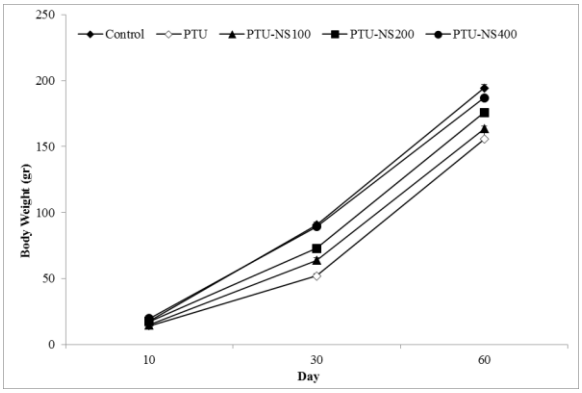

Figure 8: **Body weight rate.** Data are presented as mean±SEM. (n = 10). Rats in control group received tap drinking water, PTU group 0.005% PTU, PTU-NS 100, PTU-NS 200 and PTU-NS 400 groups 0.005% PTU plus 100 , 200 and 400 mg/kg of NS extract respectively. The body weight in PTU group was lower ($p<0.001$) while in all PTU-NS 100, PTU-NS 200 ($p<0.01$) and PTU-NS 400 ($p<0.001$) groups was higher than PTU group.

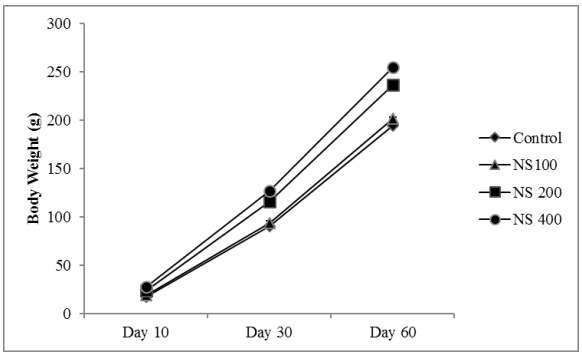

Figure 9: **Body weight rate.** Data are presented as mean±SEM. (n = 10). Rats in control group received tap drinking water, NS 100, NS 200 and NS 400 groups received normal drinking water plus 100, 200 and 400 mg/kg of NS extract respectively. The body weight in NS 200 ($p<0.01$) and NS 400 ($p<0.001$) groups was higher than PTU group.

Hyperprolactinemia is suggested to develop in patients with primary hypothyroidism [30]. In the current study PTU- induced hypothyroidism didn't change the serum prolactin level. The responsible mechanism(s) to elucidate this discrepancy was not evaluated in the present study. The prevalence of hyperprolactinemia in overt hypothyroidism has been reported to be as high as 40% however, its prevalence and clinical significance in subclinical hypothyroidism have only been reported in case reports, and few studies [31]. Additionally, hyperprolactinemia in hypothyroidism status has been suggested to be due to an increased level in serum TSH concentration [7]. In the present study, the TSH level of mothers was not significantly increased during the lactation period to induce hyperprolactinemia which seems probably to be due to a short period of treatment of the mothers (about 20 days). In confirm with this result, it was previously shown that induction of hypothyroidism by PTU in rats did not affect prolactin production [32].

NS is widely grown as an annual herb in different parts of the world [33]. The seeds of the plant have been used as a food additive and medicinal herb for many years [11]. In traditional medicine, NS has been shown to have a galactagogue effect [12]. In the current study, NS extract increased serum prolactin level and duration of lactation period in both hypothyroid and euthyroid conditions. To the best of our knowledge, there was no report on this issue to compare with the results of the present study. The responsible mechanism (s) for the effects of the plant extract was not evaluated in the present study. In some studies, it was meant that several plants influenced milk production in animals through the stimulation of lactogenic hormone (prolactin) [34]. Fennel (Foeniculum vulgare) and anise (Pimpinella anisum), which are containing estrogenic constituents, for example, anethole, increase milk production, promote the feminine cycle and make easier birth. Anethole is like dopamine and exerts a competitive antagonism at the dopamine receptor site. So it may stimulate prolactin release and increase milk generation [35]. Additionally, the results showed that the plant extract improved the weight gain of the pups in hypothyroid and euthyroid groups. In another study of chicks feeding by 40 g/kg NS increased weight gain, feed intake, and weight of different body organs [36].

The results of the present study also showed that administration of NS increased the serum thyroxin level in mothers and their offspring. It has also been previously reported that NS oil

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(NSO) given orally significantly increases the concentration of T4 and T3 and decreases the TSH in hypothyroid rats as compared to untreated rats. Recovery of thyroid parenchyma due to the protective effect of NSO against hyperplasic changes was suggested to be responsible for the beneficial effects of NS. The therapeutic effect of NSO against PTU-induced hypothyroidism was most probably related to its antioxidant effect [37, 38]. So it could be suggested that the mechanism of action could be in part because of an antioxidant defense system that may protect the gland against PTU toxicity.

In the present study, we showed hypothyroid status induced by PTU during the lactation period did not change serum prolactin level. The results also showed that administration of NS extract during the lactation period improved prolactin concentrations in both hypothyroid and euthyroid rats which was accompanied by prolonging of the lactation period. The hydro-alcoholic extract of NS also showed an improving effect on the weight gain of the offspring of both euthyroid and hypothyroid rats.

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Footnotes

Authors' Contribution: F. Beheshti and A. Basiri collected and analyzed the data and contributed to the interpretation of the results. S. Saadat wrote the manuscript with input from all authors.

Conflict of Interests: The authors declared no conflict of interest.

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Informed Consent: No informed concent.

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