Effect of Nettle Root Extract on Folliculogenesis and Estrogen and Progesterone Hormones in Rats

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ABSTRACT

Background and Objective: nettle is an herbaceous perennial plant, which has been used as a source of medicine since ancient times. It is reported that lignan, sterols, flavonoids, poly-saccharides, lectins, and fatty acids are responsible for pharmacological effects of nettle. The present study aimed to evaluate the effects of hydro alcoholic extract of nettle on folliculogenesis as well as estrogen and progesterone hormones in rats.

Materials and Methods: In this experimental study, 32 healthy and mature female rats were used. The rats were divided randomly into one control and three experimental groups. The first, the second and the third experimental groups orally received 75mg/kg, 150mg/kg and 300mg/kg extract of nettle root with respect to their body weights. Blood samples were taken from the animals to assess serum levels of estrogen and progesterone after three weeks. Their ovaries were isolated and weighed. Tissue sections were examined by an optical microscope. The collected data was analyzed using SPSS and one-way analysis of variance (ANOVA).

Findings: mean weight of ovaries and mean number of primary, secondary and tertiary follicles significantly increased in the second and the third experimental groups compared to the control group (P<0.05). Mean serum concentration of estrogen in the second and the third experimental groups significantly increased compared to the control group (P<0.05). Mean serum concentration of progesterone in the third experimental group significantly increased compared with the control group (P<0.05).

Conclusion: The extract of nettle root increases folliculogenesis due to presence of phytoestrols in rats.

Keywords: nettle, rat, ovary

INTRODUCTION

Nettle with scientific name of Urtica dioica belongs to the Urticaceae family, which is used to treat a variety of diseases (1 and 2). Nettle is a perennial herbaceous plant with of 8 to 10 inches in height. Nettle shoots are covered by hook-like or conical fluffs. Nettle contains 60 genera and more than 700 species. This plant is mostly distributed in northern, northwestern and central parts of Iran (3). Lignans, sterols, flavonoids, polysaccharides, lectins and fatty acids are effective compounds in nettle (4). Nettle is also rich in vitamins A, C and D and such minerals as magnesium and calcium (5).

Several effects of this plant have been reported in various global regions. This plant is widely used in traditional medicine and modern medicine for treatment of multiple diseases. Nettle is characterized by antioxidant (6), anti-inflammatory (4), anticancer (7), anti-endogenous properties (8) as well as blood glucose and lipid lowering effects (9 and 10). The results also suggested that nettle is used for local treatment of...
such dermal diseases as eczema as well as treatment of alopecia (3). The results also suggested anti-osteoporosis effects of nettle (5). In addition, nettle root extract products are offered as oral drops or coated tablets under the name of ortidin brand in pharmacies. These medication effectively treat benign prostatic hyperplasia (BPH). Scholars believe that nettle effectively treat alopecia and benign prostatic hyperplasia due to inhibition of 5-alpha reductase enzyme (3).

Numerous studies were conducted on the effects of nettle on reproductive function in males. Ghorbani Ranjbari et al. (11) found out that nettle leaf extract decreases testosterone levels and impairs spermatogenesis in rats. Scholars believe that testosterone levels are decreased due to presence of phytoestrogens in this plant. They believe that long-term administration of nettle extract increases plasma estrogen levels, which affects function of pituitary-gonadal axis and decreases secretion of estrogen from testes. Bagheri et al. (12) found out that nettle root extract has a dose-dependent effect on hypothalamic - pituitary-testes axis in rats. They showed that 300mg/kg dose of nettle root extract increased serum levels of LH and testosterone.

Thereby, the present study aimed to investigate the effects of nettle root extract on folliculogenesis and ovarian hormone levels given that no study was conducted on the effects of nettle on reproductive physiology of females and according to presence of phytoestrogens in this plant.

MATERIALS AND METHODS

In this study, 32 healthy and mature female rats were used who were between two and three months old and averagely weighted from 170g to 200g. The rats were kept at animal breeding room of Jahrom University of Medical Sciences for one week to adapt to the environment. The animals were kept under 12 hours of light and 12 hours of darkness. Ambient temperature was between 20°C and 25°C. The animals could freely access food and water. The animals were randomly divided into four groups. Each group consisted of eight animals (control and experimental groups receiving different doses of nettle root extract).

In order to prepare hydro alcoholic extract of nettle root, 100-gram dried powder of nettle root was dissolved in 80 ml of ethanol 95%. The solution was kept in a percolator at room temperature for 72 hours. Water was removed from the solution by a rotary device to increase concentration of the solution. The prepared material was placed in a diskator for 24 hours to dry out the extract. In addition, water, alcohol and other additional matters evaporated. To prepare desired doses of the extract, 75mg/kg, 150mg/kg and 300mg/kg of the concentrated extract was dissolved in one milliliter of distilled water (12). The control group was not given any medication. The first, the second and the third experimental groups were orally and respectively given 75mg/kg (minimum dosage), 150mg/kg (average dosage) and 300mg/kg (maximum dosage) nettle root extract with respect to their body weights in a daily manner for four weeks. At the end of the experiment, the animals were weighted and 5-cc blood samples were directly taken from the heart of the animals (anesthetized by ether). Their serums were collected by centrifugation (3000 rpm for 15 minutes). The collected serums were kept at (-20) °C in the freezer until the experiment. ELISA kits manufactured by Diametra in Italy were used to measure estrogen and progesterone hormones. After taking blood samples, an abdominal incision was made and the ovaries were removed from the surrounding fat tissues and fallopian tubes by scalpel and forceps. The ovaries were weighted using a digital scale and washed with saline. Then, each isolated ovary was kept in a tube containing 3% formalin for 14 days. At least, ten slides of the ovaries were prepared for evaluation. The number of primary, secondary and tertiary follicles were counted by an optical microscopy with 400X magnification. Mean number of follicles in each group was determined and compared with other groups.
One-way analysis of variance was used for data analysis. Statistical analysis was performed using SPSS version 21. Significance level was considered as $P<0.05$. The collected data was calculated and compared as Mean ± SEM.

**Findings**

Contents of Table 1 showed that 150mg/kg and 300mg/kg doses of nettle root extract significantly increased weight of ovaries, the number of primary, secondary and tertiary follicles compared to the control group ($P<0.05$). Mean weight of ovaries and mean number of ovarian follicles increased in the experimental group that received 75mg/kg dose of the extract but this increase was not statistically significant compared to control group ($P<0.05$).

Contents of Table 2 showed that 150mg/kg and 300mg/kg doses of nettle extract significantly increased serum concentrations of estrogen compared to the control group ($P<0.05$). Mean serum concentration of estrogen increased in the experimental group that received 75mg/kg dose of the extract but this increase was not statistically significant compared to the control group ($P<0.05$).

**DISCUSSION**

In the present study, nettle root extract increased ovarian weight and the number of ovarian follicles (primary, secondary and tertiary), which shows the positive effects of this herb in improving ovarian function and subsequently function of female genital system. Ovarian weight gain was associated with increased number of ovarian follicles in this study. Weight of ovaries increases as the number of small and medium follicles (secondary follicles) increases. These follicles contribute to a significant weight of ovaries (13 and 14). The results showed that nettle root extract
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causes dilation of blood vessels and increases blood supply to tissues by increasing nitric oxide (15 and 16). Therefore, nettle root extract reduces atresia in primary and secondary follicles by increasing blood supply to ovaries and consequently increasing diffusion of oxygen in granulosa cells. Folliculogenesis refers to developmental processes in the ovaries, which are regulated and maintained by endocrine, paracrine and autocrine factors as well as cell–cell and cell–matrix connections (17). Ovarian steroids are endocrine factors that affect the severity of folliculogenesis. It seems that positive effects of nettle root extract on folliculogenesis are associated with direct effects of this plant in increasing estrogen hormonal levels. Estrogen strongly stimulates growth of follicles in rodents and has a mitogenic effect on granulosa cells (18). Lignans, sterols and beta sitosterol are identified phytoestrogens in nettle root extract (4 and 5). Gupta et al. found out that nettle extract has anti-osteoporotic effects on ovariectomized rats in 2014. Osteoporosis is associated with estrogen deficiency in ovariectomized states. They suggested that nettle can be used in diet as an appropriate pharmaceutical alternative for synthetic prescription of estrogen hormone in patients with osteoporosis (5).

Gupta et al. also showed that nettle extract improves levels of such minerals as calcium and magnesium that were decreased in patients with osteoporosis (5). Calcium plays a major role in reproduction. Deficiency in this macro mineral can affect fertility (19). Calcium-dependent reproductive mechanisms include steroid biosynthesis in adrenal glands and ovaries. Calcium may also affect delivery of cholesterol or using cholesterol in mitochondria or stimulating conversion of pregnenolone to progesterone in steroidogenesis (20 and 21).

Fatty acids are the other constituents of nettle root extract. Unsaturated fatty acids with multiple double bonds and particularly omega-6 fatty acids can affect the expression of growth factors involved in folliculogenesis and increase the number of ovarian follicles and steroidogenesis in order to improve reproductive process (22).

Vitamins C and D are other constituents of nettle root extract that affect folliculogenesis. Scholars believe that vitamin C is a fertility regulator in females. They confirmed the importance of ascorbic acid in reproductive process (23). Ibrahimi Zarandi et al. identified significant effects of vitamin C on histopathologic changes in ovarian tissue in rats. They showed that vitamin C increases the number of primary follicles in growing and antral phases (24). The results also suggested that vitamin D affects estrogen biosynthesis in ovarian granulosa cells. Vitamin D receptors and active metabolites of vitamin D in ovaries also confirm the role of this vitamin in proper function of female reproductive system (25 and 26).

Conclusion
The results showed that nettle root extract has positive effects on fertility indices in females by increasing folliculogenesis and estrogen and progesterone hormones. However, it is essential to conduct studies in this area.

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References
1.Zare S, Nabiuni M, Tayunloo A, Hoseini S, Karimzadeh Bardei L. The effects of Urtica dioica extract on lipid profile, insulin
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11. ghorbani ranjbar A, ghorbani ranjbar N, ghorbani ranjbar Z, ghorbani ranjbar S. Study of nettle hydro alcoholic extraction (Urtica dioica) influence on spermatogenesis and Testosterone, spermatogenesis hormones, changes in rats. NCMBJ. 2014; 4 (14):31-39


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