The effect of hydroalkoholic extract urtica dioica on concentrations of sex hormones in adult male rats

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Abstract

Introduction:
Nowadays using herbal products as an alternative or supplement to contraceptive synthetic drugs is common. There are reports of anti-estrogenic effects of nettle resulting in sterility in mammals. We asked if hydro-alcoholic extract of the nettle root (Urtica dioica) could alter serum concentrations of hormones; gondotropins and testosterone, in adult male rats.

Materials and Methods:
80 adult male rats were randomly divided into 8 groups of 10. Three groups received 300, 600 and 1000 mg/kg body weight of hydro-alcoholic extract of nettle root intraperitoneally for 3 days to lethal dose. In remaining groups, one as control, another as sham, and three others received 75, 150, 300 mg/kg body weight for 20 days. The rats were then sacrificed and FSH and LH serum concentrations were determined by ELISA.

Results:
The nettle extract increased LH significantly (P<0.05), whereas the effect on FSH was variable and nonsignificant. The extract tended to increase testosterone serum concentration but did not reach significant level.

Conclusion:
Hydro-alcoholic extract of nettle root has a positive effect on LH hormone at the dose of 300 mg/kg body weight.

Keywords: Urtica dioica, Sex Hormones, Rat

Introduction
Normal endocrine condition is required for successful reproduction. None of the endocrine systems in vertebrates is as complex as the reproductive system. Although genetic sex of an individual is determined by the chromosomal composition the fertilized ovum, the path that gonads differentiate to create ovaries or testes depends on the hormones secreted by them. Therefore, gonadal sex controls and regulates gender phenotypes. Even brain development toward masculine or feminine type depends on the gonadal steroid hormones (1). Given the World Health Organization support in upkeep of community health, population control, and healthy reproduction, today herbal

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products are being considered as complementary or alternative to synthetic contraceptive medications. Nettle is a valuable plant species that has been considered as a medicinal plant for a long time, and its flowers, leaves, stem, and roots have been used for medicinal purposes (2). There are many compounds in the nettle plant including phenolic compounds such as caffeic acid, ferulic acid, fistin, cynapic acid, scholtin, locoanthocyanidin, flavones, flavonol. Isoflavonoids have been reported to exist in root of this plant (3). Flavonoids are the largest group of plant phenols that include; anthocyanins, flavones, flavonols, and isoflavonoids. Anthocyanins help attract animals for pollination and scattering of seeds. As well as attraction, flavones and flavonols have a duty to defend and protect against ultraviolet rays. Isoflavonoids have multi-task functions; some have strong insecticidal activities and some have anti-estrogenic effects, and thus, cause sterility in mammals (4). In traditional medicine, nettle was used as an anti-inflammatory and aphrodisiac. Traditionally, nettle has been considered as a blood cleanser, diuretic, hemorrhage stopper, and sexual stimulant (5). Nettle has been reported to cause increased lipid peroxidation and liver enzymes, and decreased enzymatic antioxidant levels (6). In 1996, Scout Tanner et al. investigated the possibility of binding a variety of nettle root extract ligands to prostate and reported that there was a high affinity of tetra-hydro-furan dianilil 3 and 4 for sex hormone binding globulin, which prevents binding to the receptor on the prostate gland membrane (7). The effect of nettle’s polysaccharide on castrated rats with prostatic hyperplasia, induced by testosterone propionate was studied, and the results showed a reduction in weight and volume indices of prostate. Histopathologic examination revealed a significant reduction in number of prostate cells (7).

According to Guyton, unlike the adrenal gland, hydroxlase α-17 enzyme through conversion of pregnenolone to hydroxyl-pregnenolone-17 and hydroxyl-progesterone α-17 provides the precursor of estrogen for testosterone biosynthesis. In some organs such as the external genitalia and the prostate, testosterone is converted to di-hydro-testosterone by the reductase α-5 enzyme, which is responsible for the development of these organs (1). About 97% of the testosterone secretion from testes, either loosely binds to plasma albumin, or tightly binds to a beta globulin called the sex hormone receptor globulin. After about an hour’s circulation in blood, it is either transferred to the tissues or degraded into inactive products, which are later excreted (8). Given the lack of a comprehensive and detailed study of the effect of nettle extract on male sex hormones, this study was designed to assess the effect of nettle root extract on serum testosterone concentration and gonadotropin in rats.

Materials and Methods
A total of 80 male Wistar rats weighing approximately 185 to 250 grams, and aged 90 to 100 days were studied. Rats were given a week to adapt to their new environment. They were then weighed using a digital scale with accuracy of 0.001 gram, and randomly divided into 8 groups of 10 each. Three groups were selected for determination of the lethal dose, one as the control, one as case, and also three others as experimental groups. To determine the required drug concentration (Lethal Dose=LD50), each members of each of the specially selected three groups were injected with 1000, 600, and 300 mg per kg body weight. 72 hours after injections, 50% of the rats from the 600 mg dose had died. Therefore, this dosage was selected as the lethal dose. Then, 3 doses of; 75, 150, and 300 mg per kg body weight were prepared for injecting experimental groups 1 to 3. The control group was fed and watered according to the standard during the experiment period and received no shots of the extract. In addition to ample
food and water, the control group members had peritoneal injections of distilled water every day for 20 days. The experimental groups 1, 2 and 3 were intraperitoneally injected with 75, 150, and 300 mg per kg body weight, respectively, of nettle root extract dissolved in distilled water every day at 10 AM for 20 days. After the treatment period, on the 21st day between 9 and 10 AM, rats were first weighed and then, to assess the effect of nettle root extract on concentration of serum testosterone hormones and gonadotropins, blood samples were taken from the rats’ hearts. Then the blood samples were refrigerated in test tubes without anticoagulant for one hour. After coagulation and serum separation by centrifuge, blood serum was carefully transferred to microcentrifuge tubes with a sampler. After labeling containing information about each group, before measuring Luteinizing hormone, the follicle-stimulating hormone and testosterone, the sera were kept frozen at -20 °C. The serum samples were sent to a specialized laboratory to determine concentration of serum testosterone hormones and gonadotropins.

For preparation of nettle root extract, one kilogram of fresh nettle roots were collected from Dehdasht region in Kohgiluyeh and Boyer Ahmad Province, approved by the herbal medicine specialists at Yasuj University of Medical Sciences. The roots were dried and milled to a powder. 100 grams of this powder was dissolved in 800 ml of ethanol 95%, and the solution was percolated for 72 hours at room temperature. To thicken the solution, excess water was extracted by a rotary machine. To dry the extract, alcohol and water were evaporated in a desiccator vacuum pump for 24 hours. To prepare doses of 75, 150, and 300 mg per kg body weight, these amounts were dissolved separately in 1 cc of distilled water.

Data analysis: The SPSS software was used for data analysis and the means were assessed with variance analysis (ANOVA) and Duncan post hoc tests.

**Results**

The mean weight of rats in nettle extract receiving groups (experiment group 2, 226.6±7.09 grams, and group 3, 218.1±11.68 grams) compared to their body weight before treatment (experiment group 2, 234.2±9.02 grams, and group 3, 249.1±14.6 grams) had significantly decreased, in spite of their older age. While in experiment group 1, the mean weight had slightly increased, and in control and case groups, body weight had significantly increased with age by the end of treatment period (P≤0.05).

Serum luteinizing hormone concentration had increased in experimental groups compared to the case and control groups, but the difference was only significant between experimental group 3 and case and control groups (P≤0.05). Also, serum testosterone hormone concentration had increased in experimental groups compared to the case and control groups and, follicular stimulating hormone levels had reduced in experimental groups 2 and 3 compared to the case and control groups. But, the difference was insignificant (P≤0.05) (table 1).

### Table 1 - Mean and standard deviation of stimulating follicles, luteinizing, and testosterone hormones in male rats in study groups

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Control</th>
<th>Sham (control)</th>
<th>Experimental 1</th>
<th>Experimental 2</th>
<th>Experimental 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/L)</td>
<td>0.074±0.0102*</td>
<td>0.76±0.007*</td>
<td>0.084±0.005</td>
<td>0.082±0.007</td>
<td>0.105±0.006*</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>0.067±0.0066</td>
<td>0.061±0.0069</td>
<td>0.080±0.022</td>
<td>0.047±0.0052</td>
<td>0.055±0.005</td>
</tr>
<tr>
<td>Testesteron (ng/ml)</td>
<td>1.52±0.177</td>
<td>1.58±0.213</td>
<td>1.67±0.196</td>
<td>2.08±0.50</td>
<td>3.90±1.33</td>
</tr>
</tbody>
</table>

* Only the difference between the experimental groups 3 mean serum Luteinizing hormone and the case and control groups was significant (P≤0.05).
Discussion
Based on the results obtained, it can be concluded that mean body weight in groups receiving nettle root extract (experimental groups 2 and 3) compared to body weight before treatment reduced, despite increasing age. Body weight increased slightly in experimental group 1, but this increase was significant in the case and control groups after the end of treatment period. These findings reveal the effect of moderate and maximum doses of nettle root extract on weight reduction. However, considering the young age of the rats, body weight should have normally increased with increasing age. According to a report by Candasumi, the extract effect on the body weight is due to the presence of compounds like flavonoids, which cause lipid hydrolysis by competitive inhibition of phosphodiesterase, and also by inhibition of 3-hydroxy-glutaril CoA, which is the key cholesterol biosynthesis in the liver that can reduce body weight, or that flavonoids adjust the energy metabolism and body weight by binding to the site where adenosine tri-phosphate binds to enzymes and receptors (9).

Results of the present study showed that serum Luteinizing hormone concentration level increased in experimental group 3. This increase may have been due to the phyto-estrogen properties of flavonoids. Zaho reports that phyto-estrogens are plant-derived natural compounds that are similar to estrogens in terms of structural performance.

With both estrogenic and anti-estrogenic effects, phyto-estrogens influence the brain-pituitary-gonad axis as well as the external genitals (10). Gamma-amino-butyrlic acid and gonadotropin-releasing hormone affect secretion of gonadotropins of follicle-stimulating and luteinizing hormones, and they in turn affect the reproductive system. Follicle-stimulating and luteinizing hormones produce E2 (E2, 17 β-estroidal) and testosterone. E2 has a positive or negative feedback on gonadotropins (11). Thus, phyto-estrogens with similar structure to E2, function the same as E2 (12). Hence, the reason for increase in luteinizing hormone in the high dose nettle extract receiving group (300 mg/kg weight) could be the high concentration of phyto-estrogen in plasma with a negative feedback, causing inhibition of gamma-amino-butyrlic acid secretion. According to a study by Mytsashama et al., gamma-amino-butyrlic acid release inhibits secretion of luteinizing hormone (13). Based on Rachun report, high dose of dietary equol that is a metabolite of isoflavone increases luteinizing hormone level (14), which is in agreement with results of this study.

According to the hormone assessment results, mean concentration of serum follicle-stimulating hormone in high dose nettle extract receiving groups has reduced compared to the control group, but this difference was not significant. Tresomebone reports that a high dose of a plant extract containing phyto-estrogen caused a clear decrease in follicle-stimulating hormone, due to the ability of phyto-estrogen to regulate secretion of this hormone and by affecting pituitary gland (15), which is in agreement with the results of this study, though the difference was not significant between the two groups. With regards to the follicle-stimulating hormone, a feedback mechanism like luteinizing hormone, does not just apply to the testicular steroids.

The results of the present study indicate that serum testosterone hormone concentration has increased in the experimental group 3, but the difference is insignificant. This may have been due to the increase in luteinizing hormone and the presence of flavonoid compounds that, by inhibition of enzymes involved in testosterone metabolism such as aromatase and reductase α-5 cause an increase in testosterone. Since phyto-estrogens inhibit aromatase and reduce conversion of testosterone to estrogen, as estrogen production inhibitors, they function competitively and bind to estrogen...
receptors, and elevate testosterone levels (16). It is most likely that, since nettle root extract contains flavonoid compounds with reductase $\alpha$-5 inhibition properties, it prevents conversion of testosterone to dihydro-testosterone. Also, phyto-estrogens present in nettle extract stimulate production of binding globulin to liver steroidal hormone, and with increasing productive levels of binding globulin to steroidal hormone; reduction in di-hydro-testosterone can be expected (16, 17).

**Conclusion**

Given that low intake of hydro-alcoholic nettle root extract could have a positive effect and its high consumption a negative one, and since hormones play a significant role in a person’s fertility, after further studies, probably some doses can be used to improve fertility and some to inhibit it.

**References:**