The Effect Of Hydro-alcoholic Extract of Urtica dioica Root on Testes In Adult Rats

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Abstract: Urtica dioica (UD) with a long history of usage in folkloric and science based herbal medicine is dioeciously and has therapeutic applications. This study was aimed to assess the effect of nettle root extract UD on the testes of adult rats. Eighty adult male rats were randomly divided into 8 groups. Three groups received 300, 600 and 1000 mg/kg/day of hydro-alcoholic extract of nettle root intraperitoneally for 3 days for determining lethal dose. One group, as control and another as sham and three others received 75, 150, 300 mg/kg/day for 20 days. The rats were then sacrificed and serum levels of FSH, LH and testosterone were determined by radioimmunoassay. The testes removed from the body and then stained tissue samples, changes of testicular tissue were studied and data collected were analyzed. There was a reduction in the mean body weight in the experiment group 3 (300 mg/kg/day) at the end of the treatment. The mean number of spermatogonia per tubular tubules, Leydig cells/mm2 in diameter tubules (µm) in the experiment group3 compared to experiment group1 (75 mg/kg/day) decreased significantly (P<0.05). However, the average diameter of the tubules of the experimental group 2 (150 mg/kg/day) and group 3 (300mg/kg/day) compared to control and sham groups decreased significantly (P<0.05). This study showed that administration of nettle root extract, at a dose of 75 mg/kg has had a positive effect on spermatogenesis and with 300 mg/kg dose has had a negative effect on spermatogenesis.

Keywords: Urtica dioica, Histometrical, Testes tissue, Rat

1. Introduction

Urtica dioica (UD) with a long history of usage in folkloric and science based herbal medicine is dioecious and has therapeutic applications (Morovvati et al., 2013). Nettle has antioxidant activity (Gulcin et al., 2004), anti-androgenic properties, anti-inflammatory, anti-diabetes and antimicrobial effects are indicated (Chrubasik et al., 2007). Nettle has antiproliferative effect on prostate epithelial cells, which may be due to its anti-androgen properties (Durak et al., 2004).

Nettle is used in treatment of prostate hyperplasia (Nahata et al., 2012), rheumatoid arthritis, hypertension and allergic rhinitis (Mittman et al., 2007).

It was shown that the UD phytochemical extracts exhibit strong antioxidant and anti-proliferative activities (Wagner., 1994). Also, it has been proposed that the additive and synergistic effects of phytochemicals in U. dioica are responsible for these potent anticancer and antioxidant activities (Thompson et al., 2006).

UD root contains tannins, coumarin (scopoletin), triterpens, lignans, lectins, sterols (sitosterol, campesterol, stigmasterol) and flavonoids (Hajhashemi et al., 2013).

As the use of herbal medicines has few side effects, they are highly regarded by researchers. According to the resources search, no study has been found to demonstrate the effect of UD extract on testes tissue. This study was aimed to evaluate the effect of UD extract on testes tissue in adult rat.

2. Material and Methods

UD was collected from Dehdasht, Iran, 2012 and confirmed by Herbarium department of Yasuj University of Medical Sciences, Yasuj, IRAN. After drying, nettle roots were milled. Two hundreds grams of dried nettle roots were dissolved in 1000 ml of 70% ethanol and then the resulting solution was kept at room temperature for 72 hours. After filtration, additional water of solution was collected by Rotary. Water and alcohol were separated from nettle roots extract by desiccators (Singh., 2008).

Eighty male Sprague-Dawley rats, weight 185-250 gram and 90-100 days old were housed in polypropylene cages at room temperature (22±2°C) and also were fed on standard pellet diet, water and libitum. After a week adaptation period to the new environment, the rats were weighed with a digital scale (a day before the starting of the treatment).

The rats were randomly divided into 8 groups of 10 members:
Three groups as lethal dose groups received intraperitoneal dose 300, 600 and 1000 mg/kg/day of hydro-alcoholic extract of nettle root to determine the concentration of drug for injection (LD50= lethal dose). Seventy two hours after injection, was observed in the group receiving dose of 600, fifty percent of the animals were dead, so this dose was selected as the lethal dose.

Three another groups as experimental groups received intraperitoneal injections doses of 75, 150 and 300 mg/kg/day hydro-alcoholic nettle root extract dissolved in distilled water for 20 days and injections were performed at 10 am.

One group as sham group received distilled water and the last group as control group received no drug or distilled water.

At 10:00 am, day 21, animals were weighed and anesthetized under light ether anesthesia and sacrificed. Blood sampling was done from the rats to determine the effect of nettle root extract on serum testosterone and gonadotropin hormones concentration. The rat’s testes were removed with a cut-off in the inguinal region. All blood sampling and other manipulation on the rats have been followed on the institutional or national guidelines for the care and use of laboratory animals. After removal of excess tissue, testes were dried with gas, weighed with digital scale (accuracy of 0.001 gram), then the rat’s left or right testes were removed randomly and fixed in 10% buffered formalin solution.

Serum testosterone, FSH and LH levels were measured by radioimmunoassay technique using commercial kits (Immunotech-Radiova, Prague, Czech Republic).

Formaldehyde-fixed samples were embedded in paraffin and sliced (slice thickness: 5 micrometer). After deparaffining with xylol, histologic observations were performed by the hematoxylin–eosin method staining. For histometric study, the sections were observed under a light microscope, and the following parameters were evaluated in testes of all groups:

The mean of diameter seminiferous tubules/micrometer and the number of leydig cells/mm², the mean number of spermatogonia, spermatocytes, spermatid and sertoli cells of seminiferous tubules. Diameter of round or nearly round seminiferous tubuli in micrometers was obtained with software Dino Lite placed in the ocular of the light microscope (Olympus BX51). Ten sections were randomly chosen for each testis, the total number of Leydig cell nuclei was counted, and the mean of Leydig cells/mm² was scored. All cells were counted at ×1000 magnification, and at least ten round or nearly round seminiferous tubule cross-sections were chosen at random for each testes. The total number of spermatogonia, spermatocytes, spermatid and sertoli cells per seminiferous tubule were determined from the corrected counts of cells nuclei and sertoli cells nucleiol per seminiferous tubule cross section (Franca et al., 2005).

All results are expressed as the mean±SD, Comparisons between groups were performed using the Student’s t test, ANOVA and Duncan post hoc test. P value less than 0.05 was considered to be statistically significant.

3. Results

In experiment group 2 and 3, mean body weights (BW) (226.6±7.09 gr and 218.1±11.68 gr respectively) decreased considerably after 20 days of treatment with the nettle root extract in comparison to a day before the starting of the treatment (234.2±9.02 gr and 249.1±14.6 gr respectively), while in experiment group 1, the control and the sham groups BW were significantly increased after 20 days (p≤0.05).

In experiment group 3, the control and the sham groups, serum luteinizing Hormone (LH) level was increased significantly, 0.105±0.006 IU/ml, 0.074±0.0102 IU/ml and 0.076±0.007 IU/ml respectively, (p≤0.05).

The serum level of follicular stimulating hormone (FSH) decreased but testosterone serum level was increased, however, difference between groups was not significant.

In experimental group 3, mean testicular weight/BW ratio was 0.0044 (0.969±0.081 gr/ 218.8±11.68 gr) and in the control group this ratio was 0.0056 (1.26±0.055 gr/ 207.6±8.11 gr).

These results showed that in experimental group 3 (high dose), in addition to BW, testicular weight reduced. In experimental group 3 in comparison to group 1, mean number of spermatogonia per tubular of seminiferous tubules (ST), Leydig cells/mm2 and diameter of seminiferous tubules (µm) decreased significantly (p≤0.05). However, mean diameter of the seminiferous tubules of the experimental groups 2 and 3 in comparison to the control and the sham groups decreased and the difference between groups were significant (p≤0.05) (Table-1).
Table - Comparison of testicular parameters (mean ± SD) between groups (n = 10)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Sham</th>
<th>EX1</th>
<th>EX2</th>
<th>EX3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatogonia cells/ST</td>
<td>66.76 ± 1.66</td>
<td>65.85 ± 2.50</td>
<td>69.73 ± 1.40*</td>
<td>67.03 ± 1.65</td>
<td>64.68 ± 1.24**</td>
</tr>
<tr>
<td>Spermatocyte cells /ST</td>
<td>92.24 ± 5.11</td>
<td>91.78 ± 4.64</td>
<td>94.02 ± 2.68</td>
<td>92.29 ± 4.01</td>
<td>89.82 ± 3.64</td>
</tr>
<tr>
<td>Spermatids /ST</td>
<td>85.29 ± 5.8</td>
<td>76.7 ± 6.81</td>
<td>86.10 ± 2.92</td>
<td>81.68 ± 4.62</td>
<td>72.92 ± 10.19</td>
</tr>
<tr>
<td>Sertoli cells/ST</td>
<td>17.89 ± 0.62</td>
<td>17.07 ± 1.11</td>
<td>18.24 ± 0.49</td>
<td>17.61 ± 0.67</td>
<td>17.56 ± 0.68</td>
</tr>
<tr>
<td>Leydig cells/mm2</td>
<td>17.96 ± 1.20</td>
<td>17.92 ± 1.21</td>
<td>21.12 ± 2.46*</td>
<td>19.03 ± 2.94</td>
<td>15.02 ± 1.73**</td>
</tr>
<tr>
<td>ST diameter (lm)</td>
<td>0.38 ± 0.01*</td>
<td>0.374 ± 0.01*</td>
<td>0.377 ± 0.01*</td>
<td>0.34 ± 0.01**</td>
<td>0.33 ± 0.05**</td>
</tr>
</tbody>
</table>

EX1: Experimental 1 (rNRE, 75 mg/kg/day)
EX2: Experimental 2 (rNRE, 150 mg/kg/day)
EX3: Experimental 3 (rNRE, 300 mg/kg/day)
** Parameters showed a significant difference with
* Parameters (P<0.05)
rNRE: receive Nettle root extract, ST: Seminiferous tubule

4. Discussion

In groups received extract 150, 300 mg/kg/day respectively, mean BW showed a considerable decrease after 20 days of treatment with the nettle root extract in comparison to a day before the starting of the treatment. This decrease of body weight in these groups may be due to nettle antilipemic activity that decreased HMG-COA reductase activity. Decrease of this enzyme is caused reduction in total cholesterol, low density/high density cholesterol (LDL/HDL) ratios via lower concentrations of LDL (Malini et al., 2004).

It was reported that stinging nettle aqueous extract (150 mg/kg/day) given for 30 days to rats, improved the blood lipid profile. Significant lowering in total cholesterol, low density/high density cholesterol (LDL/HDL) ratios via lowering concentration of LDL, was reported (Daher et al., 2006). It was showed that the ethanolic extract of stinging nettle leaf increased serum HDL cholesterol and lowered serum LDL cholesterol, (Avci et al., 2006). Nassiri-Asl showed that Urtica dioica extract at 100 and 300 mg/kg significantly reduced the levels of total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C). It was suggested that the extract may have a direct role in lipoprotein synthesis and metabolism (Nassiri-Asl et al., 2009). Meguro et al. reported that plant sterols which are structurally similar to cholesterol could displace cholesterol from mixed micelles, since they are more hydrophobic than cholesterol. This replacement causes a reduction of micellar cholesterol level and consequently lowers cholesterol absorption (Meguro et al., 2001). Thus, it is possible that UD extract could decrease the storage of cholesterol through this mechanism (Nassiri-Asl et al., 2009).

In the present study, decrease the testicular weight in group received extracts 300 mg/kg/day, is probably due to decrease in number of testicular cells. Another study showed that weight loss in testes of rats is due to reduction in spermatogenic cells (Nuiser et al., 2007). Decrease in number of cells in this group (300 mg/kg/day) is probably due to increase in level of serum testosterone and increase of this hormone is probably due to high concentration of nettle root extract flavonoid in this group (300 mg/kg/day).

Nettle contains compounds such as sterols, flavonoids and polysaccharides that these compounds have antiandrogen (Ganzera et al., 2005). Flavonoid with secretion of the enzyme 5-alpha reductase inhibitors, prevents from the conversion of testosterone to dihydrotestosterone, so level of serum testosterone was increased. Nahata et al reported that nettle prevents from formation of the active form of testosterone, dihydrotestosterone by inhibition the enzyme 5-alpha reductase (Nahata et al., 2012). Chrubasik et al reported nettle root extract with inhabitation of aromatase, prevents the conversion of testosterone to estrogen, and also prevents androgen binding to androgen receptors (Chrubasik et al., 2007). Probably because of nettle root extract flavonoid secretion of the enzyme 5-alpha reductase inhibitors with inhibitory properties, prevents the conversion of testosterone to dihydrotestosterone (kavalali et al., 2003).

In present study, in group received extract 300 mg/kg/day, the levels of serum LH and testosterone (T) were increased and level of serum FSH was decreased. These changes probably are due to prevention from the conversion of testosterone to dihydrotestosterone by nettle root extract 300 mg/kg/day. In study of Middleton et al were reported isoflavonoid phytoestrogens and mammalian lignans, are diphenolic compounds with molecular weights similar to those of steroid estrogens (Middleton et al., 2000). In another study was reported that the mechanism of the phytooestrogen bioactivity might be the possible binding to estrogen receptors (ERs) because of its structural similarity with estradiol (E2,
17 β-estradiol) (Turner et al., 2007). E2 not only modulates the function of reproductive organs, but also has positive and negative feedback roles on gonadotropin (Turner et al., 2007). Therefore, similar to the structure of E2, phytoestrogen plays a role in mimicking or antagonizing the E2 function (Middleton et al., 2000). So in present study probably because of positive feedback roles of phytoestrogen in low dose of extract (75 mg/kg/day) testicular parameters were increased. While in high dose of extract (300 mg/kg/day) because of negative feedback roles of phytoestrogen testicular parameters were decreased. But to comparison to control, no significant difference between groups was observed.

Previous study showed phytoestrogen had various effects on steroidogenesis (Zhao et al., 2011). In rat testicular Leydig cells, isoflavone decreased the cellular production of steroid hormone but increased serum levels of testosterone under a high-dose and long-time treatment (Akingbemi et al., 2007). In comparison with the phytoestrogen effect on oocyte generation, the administration of dietary phytoestrogen at a high level in rats blocked spermatogenesis and induced germ cell apoptosis, possibly due to disruption of estrogenic regulation in testes (Assinder et al., 2007). Morovvati reported that the prescription of nettle extract in the group of hamsters receiving nettle-testosterone has enhancing effect on some parameters of testicular structure, so has significant protective effect on the epithelial thickness of the seminiferous tubules, spermatogenic cells and sertoli cells (Morovvati et al., 2013).

This study showed that administration of nettle root extract, at a dose of 75 mg/kg has had a positive effect on spermatogenesis and with 300 mg/kg dose has had a negative effect on spermatogenesis.

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