Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Effect of Urtica Dioica Extract on Histological and Histometrical Changes of Testis of Hamster after Testosteron Administration

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Article information

Article history:
Received: 21 Oct 2012
Accepted: 16 Feb 2013
Available online: 20 May 2013
ZJRMS 2013; 15(11): 4-8

Keywords:
Testes tissue
Testosterone
Urtica dioica extract
Hamster

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Abstract

***Background*:** Hyperactivity of testosterone is one cause of infertility and its incorrect use can produces reproductive disorders. Nettle (*Urtica dioica*) has antiandrogenic effect and may antagonized effect of testosterone. In present study structure of testes of golden hamster was evaluated after testosterone and extract.

***Materials and Methods*:** In this experimental and animal modeling study, twenty male mature hamsters were divided to 4 groups, group 1 was control, group 2 received testosterone at dose 3 mg/kg subcutaneously, group 3 received nettle extract dose 30 mg/kg orally and group 4 received testosterone and nettle for 30 days daily. The hamsters were euthanized and testes were removed and detected macroscopic parameters (weight, height, wide and volume) and fixed with formalin. The samples were sectioned and colored with H & E.

***Results*:** The volume, weight, length and wide of testes was at least in testosterone group and statistically was lesser than control and testosterone -nettle group ($p<0.05$), but did not the height epithelium of seminifer tubules, compact of spermatogenic cells and number of serotolli cells in testosterone group was lesser than control group significantly ($p<0.05$).

***Conclusion*:** The nettle extract decreased histological changes of testes by testosterone and improved its structure.

Introduction

Effects of drug and hormonal factors on reproduction and infertilities process have always been the case in order to find suitable drugs in the treatment of hormonal disorders. Heretofore, research on the production of anti-androgen drugs appropriate for diseases caused by increase in testosterone activity have been somewhat successful. These studies have often led to the production of anti-androgens that have insufficient effect and anti-androgenic adverse side effects on fertile males and females. Testosterone is the main hormone in the testicles that is made in leydig cells of the testis from cholesterol and also is produced from androstenedione secreted by the adrenal glands. In addition to their role during embryo development, testosterone and other androgens apply a feedback inhibition on LH secretion from pituitary that causes development and maintenance of male secondary sex characteristics and have important anabolic effect [1]. In many countries, testosterone is used as a hormonal drug of infertility that satisfies many of men and women. Zhang et al. have studied infertility caused by testosterone and concluded that testosterone level increase can lead to infertility [2].

Hamsters are more sensitive to steroids than other animals. Hamster has also appropriate androgenic properties that can be used as a model for evaluation of antiandrogenic drugs. The structure of the hamster testis from morphological point of view was an appropriate model and has been used in different studies [3].

Nettle is a member of the Urticaceae nettle family and is an important genus of the family. Urtica has 50 species. Urtica dioica nettle species is dioecious and has therapeutic applications [4]. Nettle has antioxidant activity [5] and antiandrogenic properties and also its analgesic, anti-inflammatory, anti-diabetes and antimicrobial effects are indicated [6]. It is believed that the boiled plant and its tincture roots in the form of lotions are effective in hair growth. Also nettle root extract improves some cases of benign prostatic hypertrophy [7]. Nettle has antiproliferative effect on prostate epithelial cells, which may be due to its anti-androgen properties [8]. The use of herbal medicines due to their few side effects, are highly regarded by researchers. According to the resources search, no study has been performed to demonstrate protective effects of nettle in testis tissue structure following prescription of testosterone on hamster and no improper influence or effect on the testis tissue have been reported; therefore this study was carried out to check it.

Materials and Methods

In this fundamental empirical investigation, 20 adult male golden hamsters weighing 70 to 120 g were used.

One week prior to the experiment, all animals were kept in the right conditions of temperature and light.

During the experiment, animals were fed with fresh vegetables and pelleted commercial diet and had free access to water. Animals were divided into four groups of 5 members each as follows: Group 1 as control group received no drugs. Group 2 received a daily dose of testosterone (produced by Aburayhan Company, Iran) of 3 mg/kg subcutaneously for 30 days. Group 3 received a daily dose of nettle ethanol extract (produced by Poursina Company, Iran) of 30 mg/kg in edible form for 30 days. Group 4 received a daily dose of testosterone of 3 mg/kg with a dose of nettle of 30 mg/kg for 30 days.

After the 30-day period, hamsters were transferred to the laboratory and then killed painlessly by the chloroform with regarding research ethics and right and left testicle were removed with a cut-off in the inguinal region. After removal of excess fat and tissue, physical characteristics such as weight (with digital scale), volume (with changes in volume and water displacement), the length and width of the testes (by caliper) were studied in each group. To investigate with light microscopy, tissue sections with a thickness of 5 micrometers were prepared using microtome and sections were stained with hematoxylin and eosin. Counting spermatogonia, spermatocytes, sertoli, round spermatid cells (using a calibrated slide in 5 microscopic fields per slide, 5 microscopic slides were prepared from each hamster), measurement of seminifrous tubule diameter and epithelium height of seminifrous cells (by using a calibrated slide) were performed.

Data were analyzed by SPSS-16 software. To compare the groups under study, one-way ANOVA and LSD Supplementary test were used and average differences with $p \leq 0.05$ was considered significant.

Results

There were no significant difference in the testicular weight between control, nettle extract and testosterone-nettle group. The control and nettle group compared to the testosterone group showed significant superiority for testicular weight (p=0.04). Maximum and minimum testicular weights were measured in the control and testosterone group respectively. Testicular volume was highest in the control group that compared to the nettle and nettles-testosterone group had no significant difference, however, it was significantly higher than the testosterone group (p=0.012). The testosterone group had lowest testicular volume. Testicular length in the control group compared to the nettle extract group had no significant difference but compared to the two groups of testosterone and testosterone-nettle was significantly higher (p=0.038). There was no significant difference between the lowest in testosterone groups and lowest in the nettle extract, nettle-testosterone groups. Testis width was highest in the control group that compared to the nettle extract had no significant difference, but compared to the other two groups had significant difference (p=0.001). This parameter in the testosterone group had the lowest value and compared to the testosterone-nettles group was not significant (Table 1).

In the microscopic examination, the highest number of sertoli was for the control group that showed a significant difference compared to the testosterone group (p=0.035). Effect of nettle extract and nettle-testosterone blend did not significantly differ in the number of sertoli but compared to the testosterone group had significant superiority. The minimum number of sertoli was counted in the testosterone group. Number of spermatogonia in the control and nettle groups were not significantly different but have significant difference with nettle-testosterone and testosterone group (p=0.001). Nettle and testosterone groups had also no significant differences. The maximum and minimum numbers of spermatogonia were in the control and testosterone group respectively. The control and nettle groups had no significant difference in the number of spermatocytes. The nettle and nettle-testosterone group did not differ significantly, but all three groups compared to the group receiving testosterone showed a significant difference (p=0.041). So that the highest and lowest numbers were counted in the control and testosterone groups, respectively. There were no significant differences between control, nettle, nettle-testosterone groups in the number of spermatid, and a mixture of nettle nettle-not testosterone but all three groups showed significant superiority compared to testosterone group (p=0.022). The maximum and minimum numbers of spermatid were counted in the nettle and testosterone group, respectively. There was no significant difference in epithelial thickness between control and nettle groups. The nettle and nettle-testosterone groups did not differ significantly, but all three groups compared to the group receiving testosterone showed a significant difference (p=0.014). So that the maximum and minimum heights of the epithelium were measured in the control and testosterone groups, respectively. The control group had significant superiority in tubules diameter in terms of micrometer compared to other groups (p=0.05). There was significant priority in the nettle group compared to testosterone and testosterone-nettle groups (p=0.05). The minimum tubules diameter is related to the testosterone group (Table 2). Seminifrous tubules are seen arranged and dense, as can be seen in figure 1. Histological structure of seminifrous tubules had high diameter and epithelial height and spermatogenic cells had ordered arrangement. In the group receiving testosterone, tiny seminifrous tubules were taken apart irregularly and can be seen low-dense and in the histological structure of some seminifrous tubules, diameter and epithelium height decreased and spermatogenic cells appeared disordered (Fig. 2). Simultaneous use of nettle with testosterone prevented changes in testis tissue caused by testosterone meant that tubules lysis was minimal. Seminifrous tubules are more active than groups receiving testosterone in terms of diameter, epithelium height, density and spermatogenic cells arrangement (Fig. 3). In the group receiving nettle alone, arrangement and density of seminifrous tubules and histological structure of tubules in terms of diameter,

epithelial height and spermatogenic cells density are similar to the control group (Fig. 4).

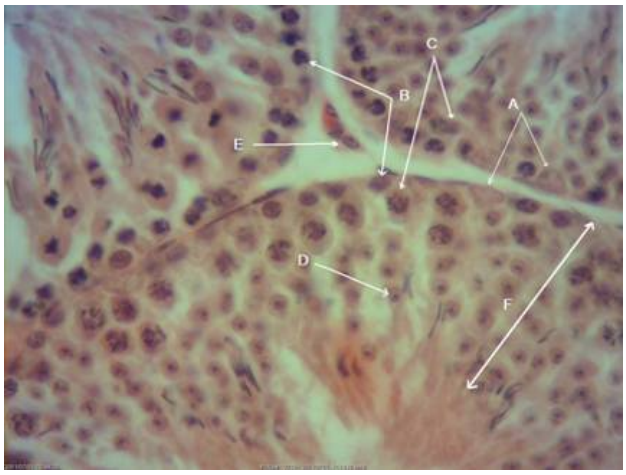

Figure 1. Testicular tissue in the control group: thick epithelium and arranged cells. Sertoli (A), spermatogonia (B), spermatocytes (C), round spermatid (D), Leydig (E), epithelial height (H & E ×40) (F)

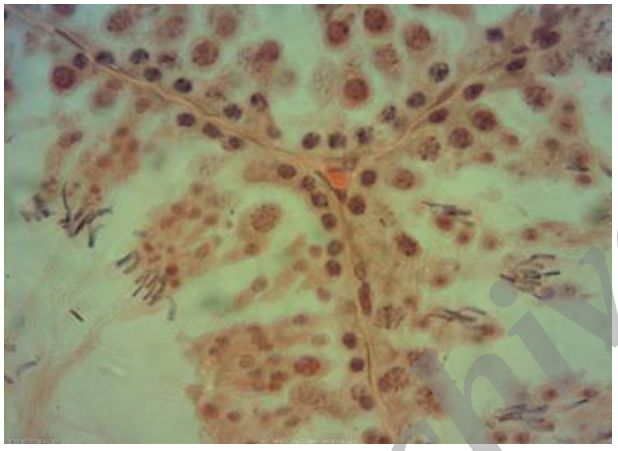

Figure 2. Seminiferous tubules lysis and spermatogenic cells taking away from each other and cells being non-arranged (H & E ×40)

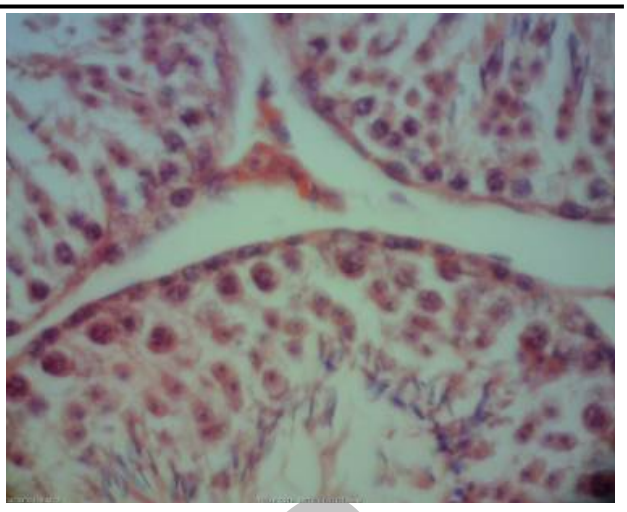

Figure 3. The testes tissue in the nettle-testosterone. Epithelium thickness compared to the group receiving testosterone increases and spermatogenic cells can be seen more arranged and dense (H & E ×40)

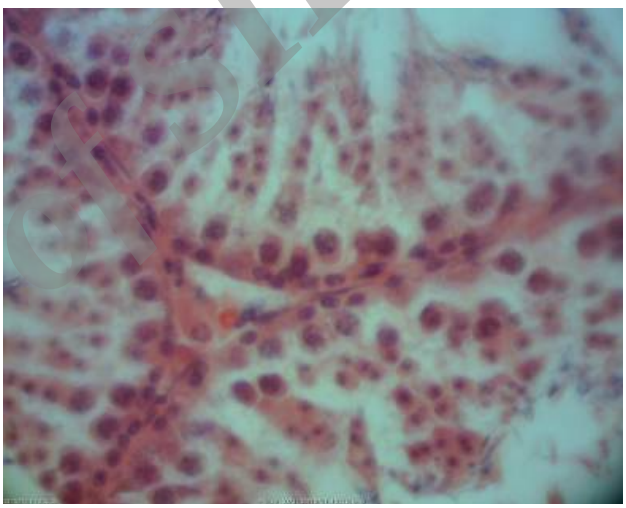

Figure 4. Testicular tissue in the nettle group, tubules epithelial thickness spermatogenic cells activity and cells arrangement is similar to the control group (H & E ×40)

Table1. Average weight and testicular size numbers with a same letter within each column have no significant statistical difference

Characteristics / Treatments	Testicular weight(g)	Testicular volume(mL)	Testicular length(mm)	Testicular width (mm)
Control	1.36±0.24[a]	1.47±0.07[a]	15.35±0.12[a]	10.05±0.13[a]
Testosterone	0.87±0.32[b]	1.1±0.35[b]	12.38±0.75[b]	8±1.55[b]
Nettle extract	1.34±0.11[a]	1.4±0.25[ab]	14.25±0.65[ab]	9.73±0.36[a]
Nettle+testosterone	1.06±0.22[ab]	1.18±0.19[ab]	12.38±2.75[b]	8.18±1.26[b]

Numbers with the same words within each column, dose ot significant statistical difference

Table 2. Cellular changes in the testes structure in different groups (1 to 4). Numbers with a same letter within each column have no significant statistical difference

	Charactristics					
	The number of Sertoli	The number of spermatogonia	The number of Spermatid	The number of Spermatocytes	Epithelium (µm)	Tubule diameter (µm)
1	10.73±2.1[a]	55.35±3.2[a]	201.19±7.8[a]	60.15±6.9[a]	84.9±11.1[a]	304±10.5[a]
2	7.38±1.6[b]	44.65±0.8[b]	155.3±12.5[b]	42.13±6.7[c]	66.86±4.9[c]	242±5.88[c]
3	9.98±1.34[ab]	52.78±4.6[a]	202.1±15.38[a]	55.63±2[ab]	79.3±4.1[ab]	281.6±15.4[b]
4	10.05±2.4[ab]	48.78±4.7[b]	192.26±29.9[a]	51.83±2.8[b]	76.19±4.1[b]	262±19.9[c]

Numbers with the same words within each column, dose ot significant statistical difference

Discussion

In our study testosterone caused testicular tissue atrophy, this effect could be negative feedback due to a variety of reasons. Testosterone hormone impresses hypothalamic-pituitary-gonadal axis by different ways. FSH and LH secretion is under the control of gonadotropin-releasing hormone from the hypothalamus. Gonadotropin-releasing hormone binding to pituitary gonadotropin receptors caused release of LH and FSH to LH. These hormones are secreted in pulses.

Increase in testosterone levels can lead to infertility [2]. Sheckter et al. showed that prescription of testosterone with direct effects on pituitary inhibits FSH and LH secretion, so that high doses of testosterone leads to aligospermia or azoospermia [9]. Matsumoto et al. stated that LH has a known direct effect on seminifrous tubules epithelium, but FSH is a major factor stimulating the growth of seminiferous tubules during evolution. So when counting tubules, which have occupied 80% of testicular volume, FSH levels can be measured as a determinant factor in the initiation of spermatogenesis and testicular volume. In adult men with decrease in FSH serum level, sperm production rates decreased largely, but never reaches zero, however LH serum level is normal. They also showed that increasing the amount of testosterone in the testicles has a significant impact on sertoli cells and spermatogonia cells [10].

Walczak-Jedrzejowska et al. investigate effect of testosterone on the growth of seminiferous tubules and sertoli cells in neonatal rats. They showed that testosterone inhibits testicle growth and reduces the number of sertoli cells. This reduction is significant in the first 5 days after birth [11]. In this study, a significant reduction in volume, testis weight, epithelium height as well as spermatogenic cell density was seen in the group receiving testosterone. Pac et al. showed that in swissmice and hamsters compared to other rodents, the hypothalamus-pituitary-gonadal axis is specifically affected by testosterone and estradiol. These hormones delay puberty in both rodents. They also reported that testosterone caused suppression of testis tissue structure in these rodents that all of these results are similar to the present study [12].

Mitchem et al. investigates effects of testosterone, dihydrotestosterone and estradiol on hamster's spermatogenesis. They showed that these hormones cause reduction in the number of spermatids and increase in spermatogonias [13]. It's necessary to note that these reports are opposed with the present study results that the conflicts are perhaps due to differences in species, duration and method of prescription of testosterone. In the survey was conducted by Hardy et al., it was found that increase in Leydig cells while reduction in testosterone hormone is due to inappropriate function of Leydig cells. Furthermore, FSH increase causes increase in the diameter of seminifrous tubules and spermatogenesis activity [14]. By subcutaneous injection of testosterone in rats for 3 weeks, Udagawa et al. observed that exogenous testosterone caused restoration of spermatogenesis in the testes of rats [15], another study showed that weight loss in testis of rats is due to reduction in spermatogenic cells [16]. Changes in testicular weight in the present study groups are probably due to changes in number of cells. In the study of Mitchem et al., the prescription of exogenous testosterone has no significant effect on serum testosterone level in hamsters [13].

Since the nettle extract are currently used as drugs for the disease prostate hyperplasia, therefore more understanding of the effect of this herbal product on other tissues can be useful. By review of the available literature in Iran no histological studies has been done on hamster testes. Also no study has been observed on nettle effect on testicular tissue structure in the world. Nettle extract contains linoleic acid, which can reduce cholesterol. Nettle extract may lower testosterone by cholesterol reduction. Decrease in testosterone increases LH and FSH. LH affects on the leydig cells and cause increase in their number and activity. Nettle has many effects including the case which canter et al. demonstrated that the nettle with antioxidant properties can help prevent lipid oxidation and liver cell damage by carbon tetrachlorid [17]. Since the antioxidants increase sperm count and fertility by affecting directly or indirectly on the hypothalamus-pituitary-testicular axis [18], maybe antioxidants found in nettle extract in the group receiving nettle cause such an effect [5]. Safarinejad reported positive effect of nettle extract in reducing symptoms of patients with benign prostatic hyperplasia [19]. Nettle contains compounds such as sterols, flavonoids and polysaccharides that these compounds have antiandrogen and deal with the testosterone function [20, 21].

Nettle root extract has at least 18 types of sterols and 8 types of lignan [22]. Nettle prevents formation of the active form of testosterone, dihydrotestosterone by inhibition the enzyme 5-alpha reductase [23, 24]. In the study of Nahata and Dixit, effect of nettle extract was investigated followed by prescription of testosterone for 28 days in a subcutaneous form and with dose of 3 mg/Kg of rat's body weight that was similar to the present study [24]. Nettle root extract inhibits aromatase and thus prevents the conversion of testosterone to estrogen, and also prevents androgen binding to androgen receptors [6].

Results showed that in the group of hamsters received 30 days of testosterone, testicular tissue has changes such as atrophy, reduction of the epithelium height and thus reduction in the testicular spermatogenic cells. While the prescription of nettle extract in the group receiving nettle-testosterone has enhancing effect on some parameters of testicular structure, so that has significant protective effect on the epithelial thickness of the seminifrous tubules, spermatogenic cells and sertoli cells.

Acknowledgements

The authors hereby express their gratitude and thank to the Shahid Chamran University Research Council for providing grant for this study. This paper was prepared

from Master Thesis No. 9014616 in Shahid Chamran University by Mr. Khodabakhsh Rashidi.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Chamran University-Ahvaz.

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Please cite this article as: Morovvati H, Najafzadehvarzi H, Rashidi K. Effect of Urtica dioica extract on histological and histometrical changes of testis of hamster after testosteron administration. Zahedan J Res Med Sci (ZJRMS) 2013; 15(11): 4-8.