Evaluation of effect of tribulus terrestris extract on sex hormones in male rats after treatment with cyclophosphamide

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Abstract

Introduction:
Cyclophosphamide is an anti-cancer medication used in chemotherapy. This study aimed to evaluate the effect of Tribulus terrestris on sexual hormones in male rats after treatment with cyclophosphamide.

Material and Method:
In this experimental study, 56 male rats weighing 200-220 g and aged 10-12 weeks were randomly divided into control, sham control and experimental groups. Experimental group 1 received daily cyclophosphamide 5 mg/kg of body weight intraperitoneally and experimental groups 2 and 3 received daily 20 and 40 mg/kg of body weight Tribulus terrestris extract orally, respectively, and experimental groups 4 and 5 received cyclophosphamide and Tribulus terrestris extract. After eight weeks, sexual hormones were measured and the data were analyzed by one-way analysis of variance.

Results:
The results showed that serum levels of luteinizing hormone, follicle stimulating hormone and testosterone in a group that had received cyclophosphamide decreased compared to the control group, but they significantly increased in experimental groups 2 and 3 that had received Tribulus terrestris extract compared to the control group. The serum levels of these hormones in experimental groups 4 and 5 that had received cyclophosphamide and Tribulus terrestris extract increased compared to cyclophosphamide group.

Conclusion:
Tribulus terrestris extract lowered complications of cyclophosphamide on sex hormone producing cells. This effect can be attributed to the antioxidant property of Tribulus terrestris.

Keywords: cyclophosphamide, Tribulus terrestris, hormone, rat

Introduction
Cyclophosphamide is an anticancer medication used in chemotherapy. This medication is completely absorbed from the gastrointestinal tract and widely distributed in the tissues and the body fluids before passing the blood-brain barrier. The medication is converted to active metabolites in the liver and is eventually excreted through the kidney (1). Cyclophosphamide, which is commonly used as an anticancer medication and also as an immunosuppressant, has alkylating properties and is able to establish covalent bonds in the nucleophilic sites of DNA and protein strands and form cross-links that...
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lead to the breakage and activation of DNA strands, halting of their synthesis, inhibition of cell proliferation, formation of micronuclei and ultimately cell death (2). This medication was first synthesized in 1985 as a treatment for tumor, and is now widely being used in human medication therapy (3). Despite broad clinical applications, cyclophosphamide has many side effects, including reproductive toxicity (4).

Cyclophosphamide metabolism occurs in the liver, where it is decomposed by the liver microsomal enzymes into its active metabolites, namely, phosphoramide mustard and acrolein (5). Studies have shown that cyclophosphamide reduces luteinizing hormones, follicle-stimulating hormones, testosterone and spermatogenesis (6).

Tribulus terrestris is an herb native to warm temperate and tropical desert areas and grows wildly in Iran, particularly across its two deserts –Dasht-e Kavir and Dasht-e Lut. This plant has a four-nutlet soft fruit with sharp spines that comprise its most medically active ingredient (7, 8 & 9). Studies have shown tribulus terrestris to contain steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, potassium nitrate, aspartic acid and glutamic acid (10). As a plant containing testosterone-increasing and luteinizing-hormone-increasing protodioscins and saponins, tribulus terrestris has long been used in traditional Chinese and Indian medicine for treating sexual impotence and increasing sexual desire (11). Tribestan is a compound of tribulus terrestris having the effect of increasing sexual desire and fighting low sexual desire, infertility and menopausal disorders (12). The dioscin content of tribulus terrestris has also been found to increase sexual potency in men by increasing free testosterone and regulating estrogen and progrenolone levels(13). The few studies conducted on this topic have suggested that tribulus terrestris extract improves the folliculo genesis process in female rats after treatments with cyclophosphamid (14).

Since the treatment and prevention effects of tribulus terrestris have not yet been investigated on sexual hormones after treatment with cyclophosphamide medication, the present study aims to examine these effects.

**Materials and methods**

The present research is an experimental study that abides by all the ethical codes of conduct in working with laboratory animals. Animals tested in this study were 56 adult male Wistar rats weighing approximately 200-220 g and aged 10-12 weeks. During the 56 days of the experiment, the animals were housed at a constant temperature of 22±4 °C in a 12-hour light/dark cycle. The animals were given ad libitum food and tap water during the entire experiment. The rats were randomly divided into eight groups of seven, consisting of, the negative control group, the sham control groups 1 and 2 and the experimental groups 1, 2, 3, 4 and 5, according to the following conditions.

The negative control group was given their standard water and food. The sham control groups 1 and 2 received, in respective order, an intraperitoneal dose of 1 ml of distilled water and 1 ml of distilled water + alcohol; the experimental group 1 received a daily intraperitoneal dose of 5 mg/kg of body weight cyclophosphamide; the experimental groups 2 and 3 received, in respective order, a daily oral dose of 20 and 40 mg/kg of body weight tribulus terrestris extract; and the experimental groups 4 and 5 first received a daily intraperitoneal dose of 5 mg/kg of body weight cyclophosphamide, and then after an hour, received an oral dose of 20 and 40 mg/kg of body weight tribulus terrestris extract, in respective order.

After 56 days, the rats were anesthetized using ether and a sample of their blood was taken directly from the heart. Their blood serum was then separated and their luteinizing hormone, follicle-stimulating
hormone and testosterone concentrations were measured using the ELISA method. Data were analyzed in SPSS software using the one-way ANOVA and Duncan’s follow-up tests. The level of significance was set at $P<0.05$.

**Results**

Findings of the study are presented in Table 1 and Figures 1, 2 and 3. According to Table 1, the serum concentration of the luteinizing hormone, the follicle-stimulating hormone and testosterone significantly decreased in experimental group 1 (the cyclophosphamide group) compared to the negative control group ($P<0.05$), while the serum concentration of the luteinizing hormone significantly increased in the experimental groups 2 and 3 (the tribulus terrestris 20 and 40 groups) compared to the negative control group ($P<0.05$). The serum concentration of the luteinizing hormone decreased insignificantly in the cyclophosphamide + tribulus terrestris 20 group and the cyclophosphamide + tribulus terrestris 40 group compared to the negative control group.

The serum concentration of testosterone significantly decreased in the cyclophosphamide + tribulus terrestris 20 group compared to the negative control group ($P<0.05$), while it increased insignificantly in the cyclophosphamide + tribulus terrestris 40 group compared to the negative control group.

The serum concentration of the follicle-stimulating hormone and testosterone increased in the experimental groups 2 and 3 compared to the negative control group, though an increase only significant in the experimental group 3 ($P<0.05$). The serum concentration of the follicle-stimulating hormone decreased insignificantly in the cyclophosphamide + tribulus terrestris 20 group and increased insignificantly in the cyclophosphamide + tribulus terrestris 40 group compared to the negative control group.

The serum concentration of testosterone significantly decreased in the cyclophosphamide + tribulus terrestris 20 group compared to the negative control group ($P<0.05$), while it increased insignificantly in the cyclophosphamide + tribulus terrestris 40 group compared to the negative control group.

The serum concentration of the follicle-stimulating hormone and testosterone increased in the cyclophosphamide + tribulus terrestris 20 and the cyclophosphamide + tribulus terrestris 40 groups compared to the cyclophosphamide group, though an increase only significant in the cyclophosphamide + tribulus terrestris 40 group ($P<0.05$).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental 1</th>
<th>Experimental 2</th>
<th>Experimental 3</th>
<th>Sham Control 1</th>
<th>Sham Control 2</th>
<th>Negative Control</th>
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<tbody>
<tr>
<td>LH</td>
<td>0.05±0.006 a</td>
<td>0.05±0.009 ab</td>
<td>0.25±0.13 c</td>
<td>0.23±0.12 c</td>
<td>0.03±0.005 a</td>
<td>0.06±0.004 b</td>
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<td>0.07±0.007 b</td>
<td>0.07±0.007 b</td>
<td>0.25±0.09 b</td>
<td>0.10±0.006 b</td>
<td>0.18±0.071 b</td>
<td>0.19±0.072 b</td>
</tr>
<tr>
<td>FSH</td>
<td>0.22±0.08 b</td>
<td>0.13±0.009 ab</td>
<td>0.48±0.05 d</td>
<td>0.25±0.09 b</td>
<td>0.18±0.071 b</td>
<td>0.20±0.07 b</td>
</tr>
<tr>
<td></td>
<td>1.82±0.63 b</td>
<td>1.90±0.64 b</td>
<td>2.48±0.56 bc</td>
<td>0.22±0.08 a</td>
<td>1.82±0.63 b</td>
<td>2.00±0.64 b</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.12±0.31 b</td>
<td>0.54±0.11 a</td>
<td>3.54±1.41 d</td>
<td>2.48±0.56 bc</td>
<td>0.22±0.08 a</td>
<td></td>
</tr>
</tbody>
</table>

* The means with at least one letter in common are not significantly different at the $P<0.05$ level.

**Discussion and Conclusion**

The present study examined the effects of tribulus terrestris extract on sexual hormones in male rats after treatment with cyclophosphamide. Based on measurements, testosterone, luteinizing hormone and follicle-stimulating hormone concentrations have decreased in the group receiving cyclophosphamide compared to the negative control group, which is also consistent with results of other studies [6, 16 & 16].
Figure 1: The mean plasma LH concentrations in the different experimental groups compared to the negative control group by IU/L.

Figure 2: The mean plasma testosterone concentrations in the different experimental groups compared to the negative control group by IU/L.

Figure 3: The mean plasma testosterone concentrations in the different experimental groups compared to the negative control group by IU/L.
Studies conducted by Cao et al showed that, by increasing oxidative stress, major enzymatic and non-enzymatic antioxidants have decreased in concentration in the interstitial cells, reducing synthesis and leading to the secretion of testosterone and effectively contributing to the impairment of spermiogenesis and thus significantly reducing the epididymal sperm count (17). The toxic effects of chemotherapy medications such as cyclophosphamide can be directly or indirectly imposed on the interstitial cells through seminiferous epithelium cell damage (18). Given that the steroid hormone testosterone, which plays an important role in the evolution and differentiation of sperm cells, is secreted by interstitial cells, sexual cell loss and Sertoli cell destruction cause the interstitial cells to undergo atrophy and thus lead to reduced synthesis and testosterone secretion (19). On the other hand, oxidative damages following treatment with cyclophosphamide decrease cellular processes and steroidogenesis of the interstitial cells (20). Studies show that, in addition to disrupting spermatogenesis, low testosterone levels also have negative effects on the epididymal tissue function and lead to impaired sperm maturation and quality (21).

Decreased serum concentrations of the luteinizing and the follicle-stimulating hormones after treatment with cyclophosphamide can be caused by active metabolites such as acrolein, which are themselves produced through cyclophosphamide metabolism in the body. This medication can develop many complications by impacting the DNA molecule and breaking it and also by impacting the RNA molecule and protein synthesis (22, 23 & 24).

Results of the present study show that, during the experiment, the concentration of testosterone increased in the animals that received 40 mg/kg of body weight tribulus terrestris extract, which is consistent with results of several other studies (25 & 26). Kalamegam et al showed that tribulus terrestris can also increase blood testosterone concentrations in castrated rats, which is consistent with results of the present study (27). Tribulus terrestris increases testosterone due to its estradiol glycoside content –mainly protodioscin. Natural estradiols in this compound may act as a mediator, facilitating the androgen production pathway through estradiol and thus increase testosterone, which then results in increased spermatogenesis (28).

Because of the unsaturated fatty acid content of tribulus terrestris, 17-beta-hydroxysteroid dehydrogenase enzyme activity increases, which in turn increases testosterone production (29 & 30). These acidic compounds are found to inhibit aromatase enzyme activity. Given that this enzyme converts androgens to estrogens, inhibiting its activity increases androgens (testosterone) levels in the blood (31). Studies show that taking the alcoholic extract of a plant of the same family as tribulus terrestris at a dose of 50 mg/kg significantly increases free testosterone serum in the body. This extract is also an aphrodisiac that likely increases androgens (32).

Results of the present study showed that 20 and 40 mg/kg doses of tribulus terrestris increase the serum concentrations of the luteinizing hormone, while at a 40 mg/kg dose, it increases the serum concentrations of the follicle-stimulating hormone in rats receiving the hydroalcoholic extract of this plant. In a study conducted on primates, rabbits and rats, Gauthaman et al showed that, due to its protodioscin content, this extract can increase certain sexual hormones (33). Due to its saponin content, tribulus terrestris increases luteinizing hormone through the pituitary gland. The luteinizing hormone is a particular stimulus for testosterone production, and can thus improve sexual function, including increased sperm production, improved erectile function and enhanced sexual function.
desire (34). Furostanol is a saponin of tribulus terrestris with stimulating effects on spermatogenesis through increasing gonadotropins production by the pituitary gland, which itself triggers the testosterone hormones. Saponin significantly improves the quality and quantity of sperm (35). Increased gonadotropin concentration might also be due to the active ingredients of the extract.

Researchers found that initiating and maintaining spermatogenesis requires normal levels of the luteinizing and the follicle-stimulating hormones before and after sexual maturation (36), in such a way that the differentiation between the intermediate pachytene spermatocytes and the seventh stage spermatids are directly affected by testosterone (37). In other words, the absence of the luteinizing and the follicle-stimulating hormones might indirectly affect them (36). Thus, as already mentioned, tribulus terrestris extract contains spermatogenesis-stimulating furostanol, which functions through increasing gonadotropin production by the pituitary gland (35). Therefore, increased concentrations of the follicle-stimulating hormone are most likely due to the effects of the extract on the pituitary-gonadal axis in the form of increased gonadotropin secretion.

Findings of the study show that testosterone levels have decreased in the cyclophosphamide + tribulus terrestris 20 group compared to the negative control group. Based on findings of previous studies, cyclophosphamide causes biochemical changes and tissue damage in the testicles (38), which perhaps also contribute to testosterone reduction. On the other hand, because testosterone is secreted from testicular interstitial cells, its concentration significantly increased in the cyclophosphamide + tribulus terrestris 40 group compared to the cyclophosphamide group, which is admittedly due to improved testicular tissue(39). In the present study, the follicle-stimulating hormone concentration has significantly increased in the cyclophosphamide + tribulus terrestris 40 group compared to the cyclophosphamide group, indicating the antioxidant and positive effects of tribulus terrestris extract. Reviews show that cyclophosphamide also reduces gonadal function and sexual desire (21). In studies conducted on the effects of cyclophosphamide on rat ovarian follicles, this medication was found to increase apoptotic cells in the ovary on the one hand, and cause dysfunctions in the oxidative system of the ovaries on the other. The major oxidative system of sex organs is glutathione peroxidase, the activity of which is reduced by taking cyclophosphamide (40 & 41). Based on results of a study conducted by Sabik et al, catalase and superoxide dismutase activities decrease by taking cyclophosphamide while malondialdehyde levels increase, indicating free radical production, oxidative stress and lipid peroxidation. These researchers evaluated the protective effects of vitamin E and ginger as antioxidants and introduced ginger as a more effective herbal medicine than vitamin E in reducing cyclophosphamide-induced oxidative stress (42). The administration of antioxidants thus seems a crucial component of chemotherapy for reducing cyclophosphamide-induced oxidative stress and for tissue detoxification (43).

It can be concluded that the use of tribulus terrestris can reduce the toxic effects of cyclophosphamide on the gonads and the sexual hormones.

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