Original Article

J Babol Univ Med Sci
Vol 19, Issu 4; Apr 2017. P:54-59

The Effects of a Hydroalcoholic Extract of Terfezia boudieri on serum level of Estrogene and Progesterone in female Rats

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J Babol Univ Med Sci; 19(4); Apr 2017; PP: 54-9
Received: Dec 15th 2016, Revised: Jan 25th 2017, Accepted: Feb 22th 2017

ABSTRACT

BACKGROUND AND OBJECTIVE: Terfezia Boudieri (TB), has flavonoids and antioxidant compounds. The aim of this study was to investigate the possible effects of ethanol extracts of TB on serum levels of estrogen and progesterone in adult female rat.

METHODS: 35 female Wistar rats were used in this experimental study. Animals weighing 180 ± 20 g were divided into 5 groups (each group consisted of7 rats. normal control group with no treatment, sham group received normal saline and experimental groups (1,2,3groups) were injected i.p. with 110, 220 and 330 mg/kg of TB for 14 days respectively. All groups at the end of the 14th day were bled and the sex hormones estrogen and progesterone hormone levels were measured.

FINDINGS: According to the results of serum levels of progesterone and estrogen, 220 mg/kg extract of TB (16±0.75), estrogen concentrations increased compared with the control group (12.2±0.98) and sham (12±1.14) ($p<0.01$). Also 220 mg/kg of TB (39.1±1.55) compared to controls (15±0.83) and sham (15.1±1.2) increased significantly ($p<0.01$).

CONCLUSION: The results of this study showed that TB can increase significantly the serum levels of estrogen and progesterone.

KEY WORDS: *Mushroom, Infertility, Estrogen, Progesterone, Rat.*

Please cite this article as follows:

Zabihi E, Sheikhkanloui Milan H, Motavallibashi SE, Pilevaribadi F, Mahmoudzade Y, Pourmohammad P. The Effects of a Hydroalcoholic Extract of Terfezia boudieri on serum level of Estrogene and Progesterone in female Rats. J Babol Univ Med Sci. 2017;19(4):54-9.

[DOR: 20.1001.1.15614107.1396.19.4.7.4]

[DOI: 10.22088/jbums.19.4.54]

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Introduction

Today, population growth and infertility treatment have great importance. There are many factors involved in infertility, of which women account for 45% of infertility. One of the causes of infertility is a disorder in the functioning of the female reproductive system that occurs as ovarian disorders, uterine factors, and ... (1). In most cases, infertile women, for the sake of excessive expectations, consider unproductive life as life-threatening (2). In this regard, the treatment of this problem seems very important. Today, finding new drugs with the least side effects in the treatment of diseases is very important (3). Because of the recommendation of the World Health Organization (WHO) and having numerous effective compounds, the use of plants and traditional medicine has become widespread [4]. Various materials are used in traditional medicine for fertility (5). One of them is the Terfezia boudieri used in traditional medicine for enhancing sexual desire and treating impotency in men and women. Terfezia boudieri increases the levels of testosterone and LH (6). Previous studies have shown that Terfezia boudieri is rich in essential amino acids, fatty acids and vitamins (8, 7). Terfezia boudieri reduces blood sugar in diabetes mellitus patients (9). The antioxidant and antibiotic effects of the Terfezia boudieri against germs and various types of cancer have been proven (11, 10). Previous research suggests that Terfezia boudieri have an important sexual force that is used for sexual stimulation and sexual enhancement (12). Recent studies have shown that flavonoids have estrogenic properties (13). Due to phenolic and flavonoid compounds in Terfezia boudieri (10), its effect on ovarian hormones can be important. Therefore, this study was conducted to investigate the possible effects of hydro alcoholic extract of Terfezia boudieri on serum estrogen and progesterone concentrations.

Methods

Animals: This study was approved by the Ethics Committee of Ardabil University of Medical Sciences with 85.1395 IR.ARUMS.REC on 35 male Wistar rats weighing 180±20 g. The animals were kept at 22±2°C, 12 hours of light and 12 hours of darkness, and in a bedding room in separate cages with free access to urban water and animal feed (platform). Animals were randomly divided into 5 groups (7) and stored. The first group (normal control) did not receive anything; the second group (sham group) received only normal saline for 14 days; the third group received 14 mg / kg of extract for 14 days; the fourth group received extract 20 mg / kg , and the fifth group received extract of 330 mg / kg for 14 days. Extract was administered daily in the groups and intraperitoneally with volume of 0.25 ml for 14 days.

Cycle Unification: One of the most important steps in this experiment was the cycling of female rats. Given that the rats have 4 different phases (including estrous, diestrous, proestrous and metastroses phases) and their serum levels of sexual hormones vary throughout each cycle, all rats should be monitored in one specified cycle (estrus). To simulate the cycle of the mice, 100 µg of estradiol Valerate was dissolved in 0.2 ml of olive oil and injected intramuscularly. After 42 hours, 50 µg of progesterone was injected intramuscularly. Six hours later, vaginal smear was prepared from mice. The diagnosis of the stages of the estrus cycle was determined based on the ratio of the three types of cellular populations (epithelial cells, horn cells and leukocytes) observed in vaginal smear. Microscopic observations showed that all the mice were cycled at the Estros stage.

Extraction method: In this study, Terfezia boudieri was collected by a researcher from Yamchi district of Ardebil province. To obtain a watery and alcoholic extract, Terfezia boudieri was dried at 37 ° C and in a shade, and then powdered with an electric mill. 1 liter of distilled water and 96% ethanol with a 3 to 7 ratio were added to100 grams of Terfezia boudieri powder to be soaked for 72 hours. The extract was passed through an extraction to obtain a pure extract. To evaporate the alcohol and obtain dry extract, it was placed at 37 ° C for 15 hours. Then, the extract was mixed with physiologic serum (extract solvent) to prepare different concentrations.

Sampling and measuring hormones: At the end of the 14th day, all animals were weighed by digital scales and then anesthetized with ketamine (100mg / kg) and xylacin (10mg / kg) and blood samples were collected from their hearts. Blood samples were stored at -20 ° C after 3500 centrifuges for 5 minutes and until hormones were monitored. The Biovendor kit was used to measure estrogen and progesterone hormones. According to the kit's manufacturer's instructions, after adding samples, the added buffer was incubated for 120 minutes at room temperature. By adding the conjugate enzyme diluted to the wells, it was again incubated for 60 minutes. After washing 4

[DOR: 20.1001.1.15614107.1396.19.4.7.4]
[DOI: 10.22088/jbums.19.4.54]

times, at this stage the substrate solution was added and incubated for 30 minutes in a dark environment without shaking. Finally, with the addition of the stopper, the results were read at 150 nm in 15 minutes.

Statistical analysis: After collecting data, data was analyzed using SPSS software using one way ANOVA and Tukey's post hoc test. $p<0.05$ was considered significant

Results

The results of this study showed that the mean concentration of estrogen hormone in the 14-day treatment of animals with 202 mg/kg of hydroalcoholic extract of TB (16±0.75 pg/ml) than control group (12.2±0.98 pg/ml) and sham (12±1.14 pg/ml) had a significant difference ($p<0.01$). There was no significant difference in estrogen hormone levels in other experimental groups (Fig 1). The results of this study showed that the group receiving 20 mg/kg hydroalcoholic extract of truffle fungus (mean 39.1±1.55 µg/ml) than the control group (mean±15.8±15 ng/ml) Liter) and sham (mean 15.1±1.2 ng/ml) had a significant difference ($p<0.01$) (Fig 2).

In this study, the results of the analysis of the data of this study, indicated no changes in the body weight of the animals tested in any of the experimental groups receiving the extract in comparison with the sham group and the control, including reduction or increase in weight, was not significantly (Fig. 3). During the 14-day study with 330 mg/kg and 220, 110 doses of Terfezia boudieri, head loss, hair loss, hair loss, tremor, and sweating, diarrhea and weight loss were not observed in rat.

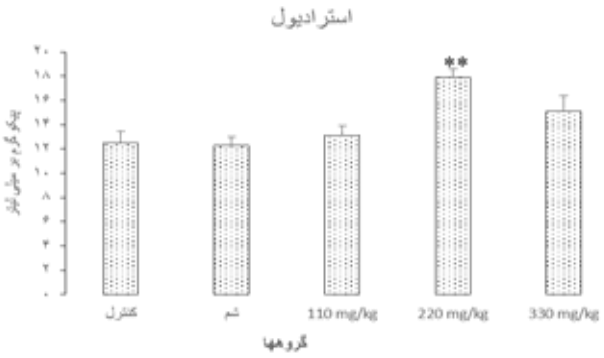


Figure 1. Effect of different concentrations of hydroalcoholic extract of Terfezia boudieri on serum estrogen levels (picograms per ml) in female rats

** showed a significant difference ($p<0.01$) in comparison with sham and control group

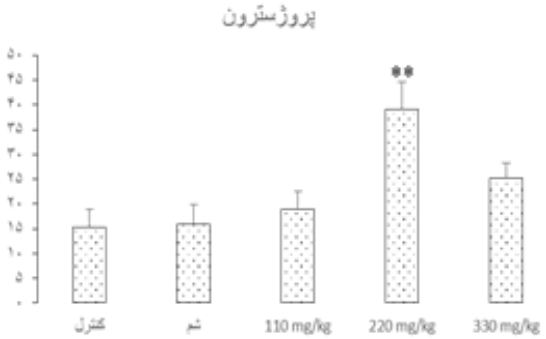


Figure 2. Effect of Different Concentrations of hydroalcoholic Extract of Terfezia boudieri on Serum Progesterone Hormone Level (ng / ml) in female Rat

** showed a significant difference ($P<0.01$) compared to the control group and sham

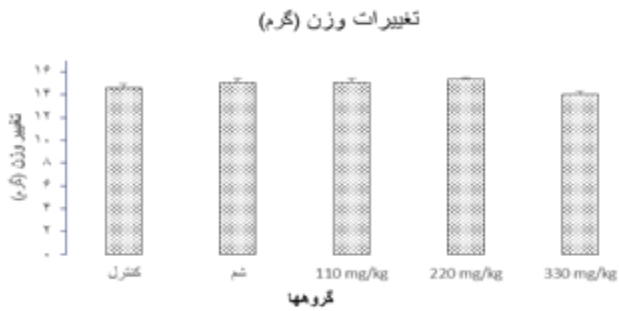


Figure 3. Effect of Different Concentrations of Hydroalcoholic Extract of Terfezia boudieri on Average Weight of Rats

Discussion

The results of this study showed that Terfezia boudieri with a dose of 202 mg/kg could affect the level of estrogen and progesterone. Given that there is little research about Terfezia boudieri extract, it will be very difficult to discuss how it works. Terfezia boudieri is effective due to phytoestrogenic compounds on the ovary and the pituitary-gonad axis, and it is possible that its phytoestrogenic compounds are beneficial for people with low estrogen and progesterone levels.

Terfezia boudieri (TB) can have many effects due to the presence of several compounds such as minerals such as zinc and calcium, and many other active substances such as antioxidants (10,11). It seems that this fungus, while having many biological and pharmacological properties, such as anti-diabetic activity (9) and antioxidant activity (7), has been able to increase levels of estrogen and progesterone hormones in the blood. The results of this study showed that hydro-alcoholic extract of Terfezia boudieri can increase serum progesterone levels. Studies by Shakshak and colleagues suggest that the

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[DOI: 10.22088/jbums.19.4.54]

administration of the Terfezia boudieri extract reduces blood sugar. The blood glucose may have been lowered by the insulin hormone, which acts as an antidiabetic agent on beta cells in the pancreas. Also, studies on mice recieving ferulic acidhave have shown that blood sugar decreases with increasing insulin levels. Ferulic acid, by increasing the level of insulin as well as IGF-1 (14,15), have an effect on the response of the ovary to the follicular stimulatory hormone followed by increased progesterone levels. Due to the fact that the insulin-like growth factor IGF-1, after production in the liver, goes to the follicular fluid and affects the follicular maturation (16,17), and ferulic acid is one of the active compounds in the tidal age, it is likely that increased progesterone levels due to the presence of Ferulic acid in the ternate and with the mechanism of IGF-1 effect. Another result of this study was an increase in serum estrogen concentrations. Flavonoids in plants called phytoestrogens have estrogenic powers. Phytosterols in the Terfezia boudieri have estrogenic powers. It seems that these compounds play an important role in estrogen uptake by their biological activity (18,19). Studies have shown that aloe vera increases the level of serum estrogen by increasing the production of steroid from the ovary (20). Therefore, it's possible that Terfezia boudieri with the same active ingredients, and with increased growth and further development of follicles, can increase estrogen secretion. Another finding of this study was a decrease in the level of sex hormones at a higher dose. The activity of phytoestrogens depends on factors such as the saturation status of sex hormones receptors, the concentration of estrogen present in the body and the time it takes to attach phytoestrogens to the receptors. Given that phytoestrogens have a structure similar to estradiol, it can act as estrogen and antiestrogen, which can be attributed to the antiestrogenic effect (21). Flavonoids, such as camproforol, in both the alpha and beta receptor at high doses have anti-estrogenic effects and exhibit weak estrogenic effects at a minimal dose (22) and, on the other hand, coumarins can show anti-aromatase activity and in higher doses reduce estrogen levels (23). Also, various studies have shown that the extract at low concentrations is mediated by the estrogen receptor and acts at high concentrations independently of the estrogen receptor (24). Therefore, it is possible that the Terfezia boudieri extract with these mechanisms reduces the level of sex hormones at higher doses. Due to the minerals found in the Terfezia boudieri including Zinc, Calcium, Iron, Manganese, it is likely that these substances have also been effective in increasing the level of sex hormones in this study. Minerals act not only as a common factor in the activation of various enzyme systems for oocyte growth and maturation, but also affect ovarian function and hence fertility ability (25, 26). Calcium increases the production and metabolism of steroids by using cholesterol in mitochondria or by stimulating the conversion of perigonolone to progesterone (25, 27) and zinc element increases production and metabolism of steroids by increasing biosynthesis of 17 beta-hydroxy steroid dehydrogenase (28).

It seems that due to the increased levels of serum estrogen and progesterone levels by Terfezia boudieri due to phytoestrogenic compounds, to treat the consequences of reducing progesterone and even estrogen during menopause, and to compensate for this deficiency in the luteal phase, this fungus can be considered as one of the treatments for traditional medicine. Terfezia boudieri could increase the level of estrogen and progesterone hormones at a dose of 220 mg/kg, which is probably due to the presence of flavonoids and the mechanism of increasing IGF-1 and its effect on the ovary, which has been shown to increase hormones. It is suggested that in the future studies, the effect of Terfezia boudieri on ovarian tissue over a longer period (more than 14 days) sould be investigated.

Acknowledgments

We would like to thank the Student Research Committee of Ardabil University of Medical Sciences for the financial support of this research and Dr. Saadati for cooperation.

[DOR: 20.1001.1.15614107.1396.19.4.7.4]
[DOI: 10.22088/jbums.19.4.54]

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