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EFFECT OF THE ALCOHOLIC EXTRACT OF *TERFEZIA BOUDIERI* ON REPRODUCTIVE HORMONES IN MALE RATS

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ABSTRACT

Terfezia boudieri is a desert truffle belonging to the Pezizaceae family that enters into mycorrhizal associations with its hosts. The present study was undertaken to investigate the effect of Terfezia boudieri extract in male rat's fertility. Adult male rats weighing 180–230 g were divided into four groups of six rats each. The experimental groups (1 and 2) were administered (by intraperitoneal injection) the alcoholic extract of Terfezia boudieri in two doses, 200 and 300 mg/kg for 21 days. The placebo group (3) received distilled water for the same duration. The control group (4) did not receive anything. Animals were sacrificed and their blood was collected for biochemical analyses. The levels of LH and testosterone significantly increased in the experimental group (1 and 2), as compared with placebo group (p<0/05). The levels of FSH reduced significantly in the experimental group (p<0/05). The study indicates efficacy of Terfezia boudieri extract in doses of 200 and 300 mg/kg on pituitary-testis axis in rat.

KEY WORDS

Terfezia boudieri, FSH, LH, testosterone, rat.

INTRODUCTION

Infertility is one of the major health problems in life, and approximately 30% of this problem is due to male factors (Isidori *et al*., 2006). The percentage of infertility may be high among men in Arabic and Islamic countries due to the ancient habits and traditions that predominate among oriental men who always refuse going for medical consultation of their reproductive problems (Shalaby and Hamowieh, 2010).

In many developing countries, traditional medicines are widely utilized in the treatment of various ailments on an empirical basis (Vijay Kumar *et al*., 2012). A variety of plants including *Pongamia pinnata*, *Trachyspermum ammi, Semecarpus anacardium* (Vijay Kumar *et al*., 2012), *Hibiscus macranthus, Basella alba* (Moundipa *et al*., 1999), *Fumaria parviflora* (Heidari Nasrabadi *et al*., 2012), *Piper guineence* (Mbongue *et al*., 2005) and many others have had positive impacts on the male reproductive function.

Medicinal mushrooms have an established history of use in traditional oriental therapies. Contemporary research has validated and documented much of the ancient knowledge. Over the last three decades, the interdisciplinary fields of science that study medicinal mushrooms has sprung up and has increasingly demonstrated the potent and unique properties of compounds extracted from a range of species. Currently, the field is being developed into a very fruitful area. Modern clinical practice in Japan, China, Korea and other Asian countries rely on mushroom-derived preparations (Rahman and Choudhury, 2012).

Desert truffles are a type of an obligate hypogeous ascomycetes ectomycorrhizal fungi formed in association with host roots of Helianthemum spp and the soil inhabiting fungi Terfezia or Tirmania spp (Dundar *et al*., 2007). Desert truffles are seasonal and socioeconomically important fungi. The truffles usually appear in the deserts following the rainy season between February and April. Truffles are grown

naturally in large quantities in virgin lands in the Middle East and North Africa (Morocco, Tunisia, Algeria and Egypt), Southern Europe (Spain, France, Italy and Greece) and other Mediterranean bordering countries (Libya, Tunisia, Syria) as well as in Iran, Iraq, Kuwait and Turkey during the autumn rain and thunder storms (Al-Laith, 2010). Desert truffles are a rich source of protein, amino acids, fatty acids, minerals and carbohydrates (Bokhary and Parvez, 1993). Similar to other fungi, desert truffles comprise a vast and yet largely unexploited source of new pharmaceutical products. In search for new therapeutic alternatives, and most importantly for modern medicine, truffles represent an unlimited source of therapeutic compounds with anti-inflammatory, immunosuppressor, antimutagenic and anticarcinogenic (Hannan *et al*., 1989), antioxidant (Murcia *et al*., 2002), antimicrobial properties (Janakat *et al*., 2004) and steroidal glucoside with polyhydroxy ergosterol nucleus (tuberoside) (Dundar *et al*., 2011).

For centuries, truffles have been believed to possess mystical aphrodisiac powers. Food connoisseurs describe the scent of truffles as sensual and seductive. A survey has reported that, about 95% of the non-Bahraini respondents and 72% of the Bahraini respondents eat truffles for sexual reasons (Mandeel and Al-Laith, 2007).

The present study was undertaken to evaluate the effects of the *Terfezia boudieri* on reproductive hormone levels of male rats.

MATERIALS AND METHODS

Fungi and providing of their extract

Terfezia boudieri was collected from ranges of Ardabil province (Iran). The samples cleaned in the laboratory and the whole parts of the fungi were air-dried in shadow followed by grinding. Providing of Extract was performed by adding ethanol to 100 g of dried plant, heated in bain-marie (45ºC for 24 h), then leaved at room temperature (1h). The obtained extracts were concentrated by rotary evaporator apparatus *in vacuo* (60 rev/min, 64°C) and needed doses were prepared.

Animals

Healthy, adult male Wistar albino rats weighing 180-230g, aged 3-4 months were used in this study. Male Wistar rats were reared on a standard laboratory diet and given tap water. They were kept in a room where humidity (65 to 70%), temperature (22±2°C) and day/night cycle (12:12 light/dark) were controlled. Rats were divided into 4 groups: control, placebo, experimental 1, and experimental 2 (n=6). The control group received nothing but placebo group received distilled water; the experimental 1 and 2 groups received 200 and 300 mg/kg *Terfezia boudrieri* alcoholic extract for 21 days through intraperitoneal injection.

Sample preparation

Rats were anaesthetized with ether and then decapitated for blood collection. Serum was separated by centrifugation at 3000 rpm for10 min. The serum levels of FSH, LH and testosterone were determined by radioimmuno-method according to the procedures provided in the kits (North Biochemistry Kit Center, Beijing).

Statistical analysis

The data were expressed as Mean±SEM. Statistical analysis was performed with ANOVA followed by Post-Hoc Tukey multiple range tests using the SPSS 16. The values were considered statistically significant if $p < 0.05$.

RESULTS

A comparison of sexual hormone levels between control and Terfezia-treated groups indicated significant differences in the testosterone and LH levels ($p < 0.05$). In addition, in a similar comparison, in the experimental group 1 (200mg/kg Terfezia treatment), serum levels of LH (**Fig. 1**) and testosterone (**Fig. 2**) showed significant increase compared to the group 2 (300mg/kg Terfezia treatment). On the other hand, as it is shown in **Fig. 3**, in Terfezia-treated groups compared to non-treated group, serum levels of FSH has been significantly decreased ($p<0.05$). In a similar comparison, in the group 2 (300 mg/kg Terfezia treatment), serum levels of FSH have significantly decreased compared to the group 1 (200 mg/kg) ($p<0.05$). Data also showed that the serum levels of testosterone hormone in control and placebo groups were totally different.

Figure 1. Effect of Terfezia on LH levels in control and treatment groups

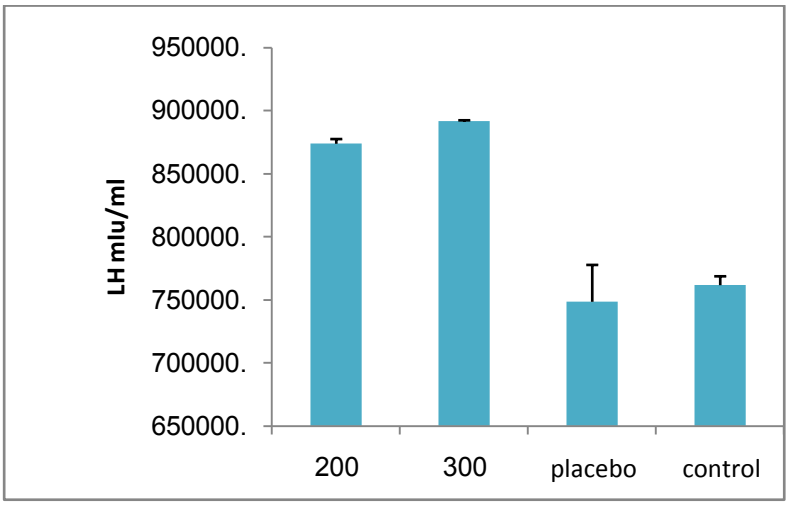


Figure 2. Effect of Terfezia on testosterone levels in control and treatment groups.

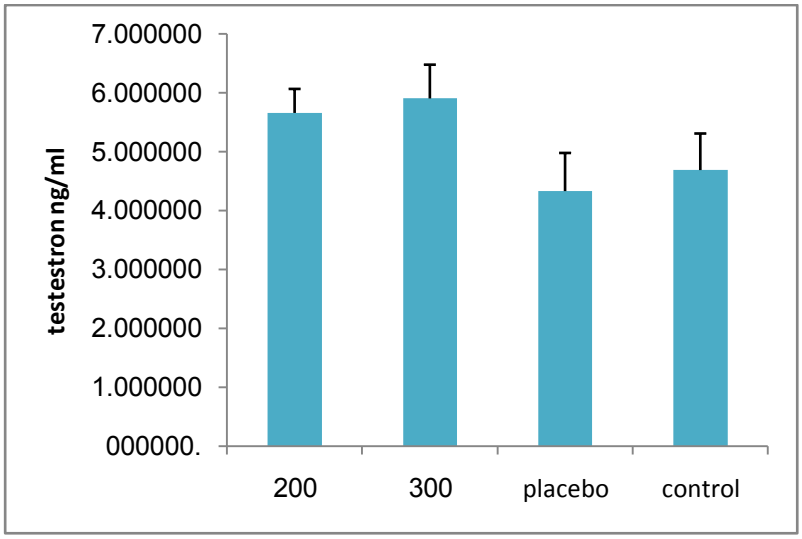
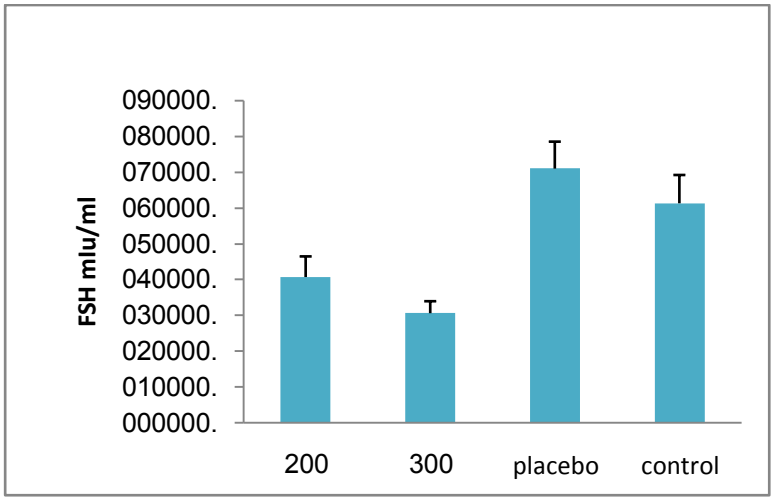


Figure 3. Effect of Terfezia on FSH levels in control and treatment groups.



DISCUSSION

Truffle extract is used as a nourishing and invigorating preparation for convalescents in Mediterranean countries (Singer, 1961). Furthermore, truffle aqueous extract is used as a folk medicine in Iraq, Saudi Arabia and eastern Jordan to ameliorate eye ailments (Abu-Rabia, 1983). Some enzymes involved in the quality of desert truffles are known (Janakat, 2004).

In the present study the effects of Terfezia extracts on levels of follicle-stimulating hormones (FSH), luteinizing hormone (LH) and testosterone in adult male rats was studied. The results showed significant increase in hormone levels in the experimental group receiving 300 and 200 mg/kg Terfezia extract compared to the control and placebo groups. According to the results of this research simultaneity raise in LH and testosterone levels showing a positive effect of Terfezia extract on axis of pituitary-testicles. Because so far there is no research on the evaluation of the effects of Terfezia extract on hormone levels that affect the reproductive system such as the LH, FSH and testosterone hormone, increased levels of these hormones can be due of compounds exist in the Terfezia extract that affected the hypothalamus-pituitary-testicles (Raton and James, 2000).

Hypothalamic-pituitary- testicles axis could be influenced by a variety of useful positive and negative control's factor. One of factors that affecting this axis is nitric acid. High levels of existing arginine in Terfezia could become to nitric acid. Nitric acid can increase the release of gonadotropin-releasing hormone (Gonzalez *et al*., 2001; Davies *et al*., 1995). Cyclic guanosine monophosphate that cause to the release of nitric oxide cycle enzyme Guanylate be active and increase secretion of gonadotropins and LH and FSH, quit raising sperm and induce erection in men (Yeoman *et al*., 1998).

In female, Nitric oxide plays an important role in stimulus of ovaries and ovulation (Bonavera *et al*., 1993).

Aspartic acid as the amino acids existing in Terfezia has stimulatory effect on gonadotropin-releasing hormone secretion and LH. Experiments showed that this amino acid, increase LH and testosterone synthesis through cyclic guanosine monophosphate-mediated and adenosine monophosphate cyclic as second messenger adjustment at the pituitary and hypothalamus (Pampillo *et al*., 2002). Iincrease of testosterone in present study could be secondary to increase of gonad-stimulating hormone levels, especially LH.

Terfezia contains large amounts of polyunsaturated fatty acids such as linolenic acid, linoleic acid and oleic acid (Dundar *et al*., 2011). Some studies have shown that in many responder organs to androgen like prostate, testosterone converts to 5-alpha-Di-Hydro testosterone by 5-alpha-reductase. Some of natural unsaturated fatty acids are capable to inhibit 5-alpha-reductase in cell culture and in cell-free systems resulting to increase of testosterone levels in serum (Lang and Liao, 1992).

In other hand, it seems that another probable reason of increase in serum testosterone levels at higher doses of Terfezia administration is direct effect on Leydig cells and biosynthesis of testosterone in these cells, because existing arachidonic acid in Terfezia can play an important role in testicular steroidogenesis. It has cleared that prostaglandins found in mushrooms stimulates testosterone production in testicles (Gobbetti and Zerani, 1995; Wade and Van der Kraak, 1993).

Other existing steroidal compounds in mushrooms like androstenol recognized as a pheromone that could increase the sexual arousal of human female, adjust moods as submissive rather than aggressive in female menstrual cycle and antagonize anxiety and convulsion by positively modulating the GABA receptors. Due to its pleasant odour and pharmaceutical function, androstenol has been developed to perfume with high value in market. Also, it has potential in drug development for anti-depression (Patel, 2012).

CONCLUSION

The alcoholic extracts of *Terfezia boudieri* produced a significant increase in the levels of serum testosterone and LH levels at dose 200 and 300 mg/kg. It is concluded that *Terfezia boudieri* extract appears to possess aphrodisiac activity due to its androgen increasing properties.

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