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The effects of celery leave extract on male hormones in rats

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ABSTRACT

Introduction: Celery (*Apium graveolens*) belongs to the Umbelliferae family, and has a plenty of nutritional and pharmaceutical applications. The presence of phytoestrogenic compounds has been reported in this plant. These compounds may affect the pituitary-gonad axis. The aim of the present study was to evaluate the efficacy of hydro-alcoholic extracts of celery leaves on serum levels of testosterone, LH and FSH in male rats.

Methods: In this experimental study, 32 male Wistar rats were divided into four groups, eight rats included in each. The control group did not receive any treatment. The placebo group received distilled water and the case groups received 200 and 300 mg/kg/B.W of hydro-alcoholic celery leaf extract for 20 consecutive days by oral administration. After completion of the treatment, the rats were anesthetized and blood sampling from their heart was carried out. Then, serum levels of testosterone, LH and FSH were measured using immunoassay methods. The obtained data were analyzed by the SPSS using the statistical ANOVA test.

Results: The level of LH in the case group receiving 200 mg/kg B.W of celery extract showed a significant decrease compared with the control and placebo groups ($P<0.05$). The level of FSH and testosterone in case groups did not show any significant difference in comparison with the control group ($P>0.05$).

Conclusion: The result of the present study shows that in the administered dose, celery extract does not have any considerable side effect on the secretion of hormones in male rats.

Implication for health policy/practice/research/medical education:
Celery (*Apium graveolens*) does not cause any hormonal impairment in male rats; so, as celery improves fertility, its consumption might be useful in the treatment of infertility.

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Introduction

The importance of fertility and procreation as a factor for survival of the human race is not hidden from anyone (1). According to documents the use of the herbs has a long history in order to fertility regulation (2). Nowadays the effect of plant-derived chemicals on the endocrine system and the activity of sexual organs have induced a great interest (3).

Among the medicinal herbs in indigenous systems of medicine it can be pointed to the numerous health benefits of celery. Celery (*Apium graveolens* L.) is a plant, belonging to the parsley descent (Umbelliferae), an herbaceous, biennial, and branched stem plant, with a height of 20 to 60 cm (4). This plant is native to the Mediterranean region that is also cultivated in other parts of the world (5). In laboratory studies celery has been referred for antifungal effects (6), anti-hyperlipidemic activities (7,8) and anti-inflammatory effects (9). This herb is rich in antioxidant compounds such as flavonoids (e.g. apiin and apigenin), as well as vitamins E and C (10,11). The sperm plasma membrane is susceptible to oxidative damage due to large amounts of polyunsaturated fatty acids leading to decreased sperm motility and viability (12). Antioxidant compounds may enhance the sperm function and also

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improve fertility. In this regard, celery extract, due to its antioxidant contents has been pointed for inhibiting the liposomal peroxidation (13) and protection of testes against sodium valproate (14). Also some reports suggest the efficacy of the extracts on the male reproductive system (15,16). In this study we aimed to evaluate hormonal impairment in male rats after oral administration of hydro-alcoholic extracts of celery leaves.

Materials and Methods

In this experimental study, 32 male Wistar rats, with a weight range of 170–200 g, purchased from the Animal Reproduction and Breeding Center of Ahvaz University of Medical Sciences were used. The animals were kept in plastic cages in a room with optimal temperature and appropriate environmental situations and with the environmental temperature of 22 to 24°C, and a period of 12 hours darkness and 12 hours light. The animals were provided food and water indefinitely. All other conditions of rat maintenance were remained identical during the investigation.

Preparation of hydro-alcoholic extraction of celery

Celery pant was purchased from a well-known store in Ahvaz, and then evaluated and confirmed as *A. graveolens*. The leaves were then dried in the shade and milled; soon after. Powders were stored in a refrigerator until extraction.

In order to prepare 50 g of hydro-alcoholic extract, the orally administrable celery were dissolved in 200 ml of 70% ethanol and the solution was kept at room temperature for three days. During these three days the solution was stirred several times to separate the extract, and after 72 hours, the mixture filtered with a filter paper. The extract solution was spread on a glass surface at room temperature to evaporate the solvent. The dried extract powder was obtained by scraping them from the glass surface, and then stored at 4°C until the use (17). The 200 and 300 mg/kg/B.W. concentrations were prepared from the powder of celery leaf extract, using distilled water as a solvent.

Grouping of animal and extract administration

Rats were divided into four groups including eight rats in each: control, placebo, case 1 and case 2 groups. The control group did not receive any treatment. The placebo group received 1 ml distilled water (as the solvent of the extract); and the case groups 1 and 2 received 200 and 300 mg/kg/B.W of hydro-alcoholic extract of celery, for 20 consecutive days, by gavage, respectively (18). One day after the last administration, rats were anesthetized with xylazine and ketamine, thereafter, blood sampling from the heart was done. Then each sample centrifuged at 3000 RPM for 15 minutes to separate the serum from the clot. After separation the serum from the clot, using a sampler, the samples were frozen and maintained in -20°C. Serum levels of LH, FSH and testosterone were measured by immunohistochemistry methods.

Statistical analysis

The data obtained from the hormone measurement were analyzed by variance one-way ANOVA using SPSS 15 software and the comparative LSD test, considering the significance level of $P \leq 0.05$.

Results

The effect of hydro-alcoholic extract of celery leaf on serum level of FSH

The comparison of the mean FSH serum level (mIU/ml) in case groups receiving different doses of hydro-alcoholic extract of celery leaf with the placebo and control groups did not show any significant difference ($P>0.05$; Figure 1).

The Effect of hydro-alcoholic extract of celery leaf on serum level of LH

The investigation of mean serum level of LH (mIU/ml) in different groups involved in this research demonstrated that the administration of the celery leaf extract at the dose of 200 mg/kg causes a significant reduction of LH in comparison with the placebo and control groups ($P<0.05$). However, any significant difference was not seen between the 300 mg/kg extract receiving group with the control and placebo groups ($P>0.05$; Figure 2).

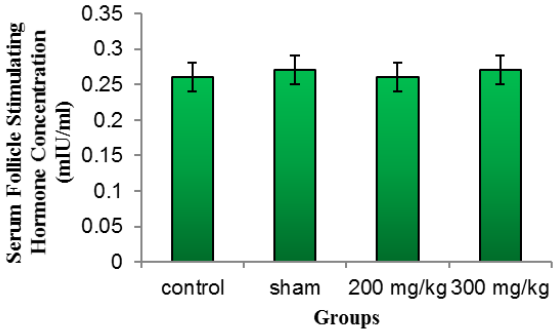

Figure 1. The comparison of the effect of celery leaf hydro-alcoholic extract on the average serum level of FSH in case and control groups

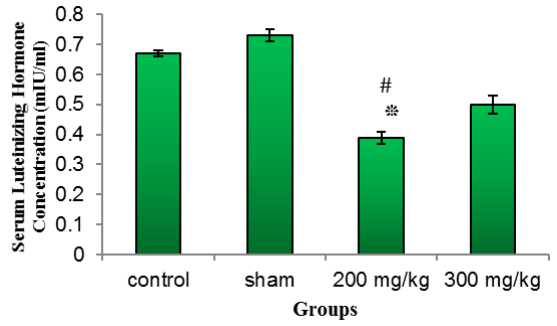

Figure 2. The comparison of celery hydro-alcoholic extract effect on serum level of LH in case and control groups; *Significant difference with respect to the control group ($P<0.05$); #Significant difference with respect to the sham group ($P<0.05$)

The Effect of hydro-alcoholic extract of celery leaf on serum level of testosterone

Based on the presented results in Figure 3, the investigation of the effect of different doses of celery leaf hydro-alcoholic extract on serum level of testosterone (ng/ml) in the 200 and 300 mg/kg extract receiving groups did not show any significant difference with the control and placebo groups (P>0.05).

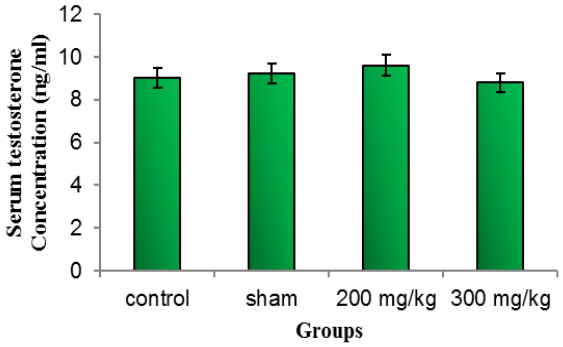

Figure 3. The comparison of celery leaf hydro-alcoholic extract effect on the average serum level of testosterone in the case and control groups

Discussion

The present study was carried out with the purpose of investigation of celery hydro-alcoholic extract effect on pituitary-gonad hormonal axis. The results of this study demonstrated a significant reduction in serum level of LH in case group compared with the control group. These findings were in accordance with the results of the study performed by Modaresi *et al.* (19). In a research carried out by Mokhtari *et al.*, on *Coriandrum sativum* L plant belonging to Apiaceae family, it was shown that the hydro-alcoholic extract of *Coriandrum sativum* L seed has led to a reduction in serum gonadotropins in the treatment group compared with the control group (20). Moreover, in the research done by Hemayatkhah Jahromi *et al.* the reduction of serum LH was observed after treatment with the hydro-alcoholic extract of *Citrus aurantifolia* peel. Among the important compounds of these two plants are flavonoids and coumarins (21).

The existence of phytoestrogenic compounds such as flavonoids and coumarins was demonstrated in celery extract by phytochemical studies (22). Phytoestrogens are natural plant-derived compounds with the structure and function similar to estrogen (23). These compounds result in inhibition of the human and animal gonadotropin secretion and the hypothalamus-pituitary-gonad axis arrest, by affecting the hypothalamic gonadotropin producing cells (24,25). Therefore, the decreased LH secretion can be attributed to extract of the phytoestrogenic compounds (11).

According to the findings of the present study, FSH serum level in the case groups did not show any significant difference in comparison with the control and placebo groups that is in agreement with the findings of Kerishchi *et al.* (26). It could be due to the modulatory effects of some factors such as inhibin, activin and follistatin, which play a role in regulation and modulation of FSH via a central effect on gonadotropin releasing hormone (27). On the other hand, the results of the researches carried out by the other scientists showed that the probability of occurrence of a mutation in LH receptors in Leydig cells is high, however, these mutations occur merely in Sertoli cells (28,29). Therefore, another reason for the lack of the changes in FSH, may be probably due to the changes in receptor activity after the administration of the extract.

In this study, serum level of testosterone was measured, as well. The findings demonstrated that serum level of testosterone did not have any significant difference in case group compared with the placebo and control groups. Kerishchi *et al.* showed that the administration of 100 and 300 mg/kg of the extract does not have any effect on serum levels of testosterone and gonadotropins through the investigation of the effect of celery seed extract on the pituitary-gonad axis in Syrian mouse (26). This result is in agreement with the results of the present study. However, the increase in serum level of testosterone in the group receiving 200 mg/kg celery seed extract has been also another result of this research.

Moreover, the results of the various studies might be due to the differences in the methods of experiments, such as the used extract, the routes of administration the extract, the extraction techniques, the duration of treatment and the animal species of the experiment.

The secondary phenol metabolites of plants affect the hormonal networks such as pituitary-gonad axis. Lack of the changes in higher doses may be the result of the feedback regulation of this network on LH and FSH hormones, followed by the modulation of testosterone, as well (30). Furthermore, the antioxidants lead to the modulation of the hormone concentrations via changing the levels of O2 in the body and ATP metabolism (31). FSH and LH hormones act synergistically. These two hormones affect Sertoli cells, and lack of each hormone leads to impaired spermatogenesis (32). Considering the role of testosterone in spermatogenesis and the important secondary effects on sexual organs, it may be concluded that it is the most important reproduction factor in testis (20).

Conclusion

Since in the present study serum levels of FSH and testosterone did not show any considerable changes, it seems that consumption of this plant does not cause any hormonal impairment in males; and probably does not have any side effect on the fertility. However, more investigations are required in this field. In order to improve the validity of the obtained results, it is recommended that the effects of other celery extracts on sexual hormone levels be evaluated using other solvents. On the other hand, the clinical effects could be investigated through preparation of the appropriate formulation of the effective extracts.

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Authors' contributions
All the authors wrote the manuscript equally.

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