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Research Article

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The effect of hydro-alcoholic extract of ephedra pachyclada on serum concentrations of neuropeptide Y and ghrelin hormones and body weight in male rats

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ABSTRACT

Background and purpose: *Ephedra pachyclada has always been considered as a natural weight loss option. The aim of this study is to investigate the effect of hydro-alcoholic extract of Ephedra pachyclada on serum concentrations of neuropeptide Y and ghrelin hormones and body weight in male rats.*

Materials and Methods: *In this experimental study, 40 adult male Wistar rats were selected. These were randomly divided into 5 groups: control (without receiving any substance), testator (receiving 1 ml of distilled water), the experimental group 1 (recipient of 250 mg/kg hydro-alcoholic extract of Ephedra pachyclada), experimental group 2 (recipient of 500 mg/kg hydro-alcoholic extract of Ephedra pachyclada), and the experimental group 3 (recipient of 1000 mg/kg hydro-alcoholic extract of Ephedra pachyclada). In the experimental groups, the extract was administered by gavage method for 28 days. On the day twenty-ninth, blood samples were taken from the rats to study serum levels of neuropeptide Y and ghrelin hormones. The rats weight was also measured daily during the experiment.*

Findings: *500 and 1000 mg/kg concentrations of hydro-alcoholic extract of Ephedra pachyclada (HAEEP) significantly reduce serum levels of neuropeptide Y, ghrelin, and body weight compared to the control group ($p < 0.05$).*

Conclusion: *HAEEP reduces body weight through reducing serum levels of neuropeptide Y and ghrelin.*

Keywords: *Ephedra pachyclada, neuropeptide Y, ghrelin, body weight, rat*

INTRODUCTION

Neuropeptide Y (NPY) is considered as the most abundant peptide hypothalamus and its most important effect is stimulating food behavior. In other words, NPY gene is the candidate gene for obesity; it is the appetizer peptide, and strong stimulator of appetite that plays a significant role in food intake, food choices, adjusting weight, and energy homeostasis (1). NPY also reduces the consumed energy (2). Many factors, including leptin, insulin, and ghrelin affect the amounts of NPY blood circulation (1).

Ghrelin is a 28-peptide amino acid that is mainly separated from the stomach of humans and animals. Although ligand ghrelin is known as the receptor of growth hormone secretion (3), this hormone is involved in the regulation of food intake behavior (4), energy homeostasis, and weight adjustment through mechanisms independent of growth

hormone (5). Long-term administration of ghrelin in rodents increases food intake and reduces energy consumption and ultimately leads to weight gain (4). On the other hand, temporary inhibition of ghrelin signaling by different methods reduces food intake and body weight (6).

Ephedra Pachyclada (EP) is a plant of Ephedraceae genus. This plant is as shrubs with green stems, with knots, hyperbranched and usually vertical. Ephedra Pachyclada grows in arid and semiarid climates, especially in desert and rocky areas of South and South East Iran and is known with local names of "Houm" and "Ermek." The fame of the plants of this family is due to having abundant ephedrine alkaloids, the most important of which are ephedrine and pseudoephedrine (7). This genus has many health benefits and has been used for thousands of years in traditional East Asian medicine to treat respiratory diseases such as asthma, bronchitis, and allergies (7, 8, and 9). EP also has anti-inflammatory, weight reducing, antioxidant, and hypoglycemic effects, and is a follower of the sympathetic nervous system (10 and 11).

Although the effectiveness of different species of EP in weight loss has been confirmed in several studies (12, 13, 14), there are few reporting the effect of this plant on hormones involved in appetite control and body weight.

Thus, given the existence of evidence of the relationship between NPY and ghrelin, in the present study, the effect of Pachyclada extract on serum levels of these two hormones and body weight are studied.

Materials and Methods

In this experimental study, 40 adult male Wistar rats with an average weight of 180-200 grams were used. The rats were kept in animal breeding room of Jahrom University of Medical Sciences for a week to adapt to the environment. Throughout the study, the animals were kept under 12 hours of light, 12 hours of darkness, ambient temperature of 20-25°C, and had free access to food and water. According to previous articles, prescribed concentrations of HAEEP in quantities of 250, 500, and 1000 mg per kg body weight were determined (15), and the rats were randomly divided into 5 groups of 8 animals as follows:

Control group: this group received no treatment during the experiment (28 days).

Testator group: this group received one ml of distilled water by gavage according to body weight during the experimental period (28 days).

Experimental groups 1, 2, and 3 received 250, 500, and 1000 mg/kg doses of HAEEP by gavage for 28 days according to body weight.

At the end of the study (day 29), after weighing the animals, taking blood samples directly from the heart of the animals with the help of 5 cc syringe (anesthetized by ether) is performed, and their serum is collected by centrifugation (15 minutes, 3000 rpm) and kept in freezer of -20° until examination. To measure NPY and ghrelin hormones, ELISA kits for rats are used.

ANOVA analysis of variance was used to analyze the data. In cases where the statistical difference between groups was significant, Duncan's test was used to find the differences between means. Statistical analysis was performed using SPSS version 21, and significance level was considered as ($P<0.05$). In the results, the data were calculated and compared as Mean ± SEM.

Findings

Based on the results listed in Table 1, mean serum concentrations of NPY and ghrelin in the groups receiving doses of 500 and 1000 mg/kg of HAEEP showed significant decrease compared to control and testator groups at the level of $P\leq0.05$. In comparison of groups receiving different doses of EP, it was determined 1000 mg/kg compared to other doses has greater effect in reducing NPY and ghrelin hormones. The results of measuring the mean change in body weight also showed that doses of 500 and 1000 mg/kg of HAEEP significantly reduce body weight compared with the control group ($P\geq0.05$). In comparison of groups receiving different doses of EP, it was determined 1000 mg/kg compared to other doses has greater effect in reducing body weight (Table 1).

Table 1: Comparison of changes in serum levels of neuropeptide Y, ghrelin, and body weight in experimental groups receiving different doses of HAEEP with the control group

Group / Variable	Control	Testator	HAEEP 250 mg/kg	HAEEP 500 mg/kg	HAEEP 1000 mg/kg
Neuropeptide Y (ng/ml)	**b8.64 ±204.20**	**202.12±2.56b**	**19.87±4.74ab**	**184.55±2.88a**	**177.32±2.46a**
Ghrelin	**1775.25±46.26b**	**1837±41.88b**	**1725.37±60.81ab**	**1688.87±85.10a**	**1599.87±27.80a**
Average changes of body weight	**25.75±1.292c**	**25.87±1.140c**	**24.62±0.777bc**	**22.37±0.595ab**	**19.87±0.811a**

- According to Duncan's test, the means in each row that have at least one letter in common are not significantly different in Duncan's test at 5% level.

- Means have been presented as Mean ± SEM.

- $P<0.05$ is considered statistically significant.

Discussion and conclusion:

EP has always been considered as a natural weight loss option. About 80% of natural supplements used for weight loss contain ephedrine (main alkaloid extracted from EP) (16). By increasing the noradrenaline release and inhibiting its absorption, ephedrine decreases food intake and induces satiety. In addition, through thermogenesis, ephedrine increases the consumed energy and helps to reduce body weight. Thermogenesis effects of ephedrine occur through effects on beta-adrenergic receptors (17).

Kim et al. (2004) showed that the aqueous extract of Ephedra Intermedia is able to stop the increase of induced expression of neuropeptide Y by food deprivation in the hypothalamus of rats (17). Therefore, due to the effect of EP on neuropeptide Y hormone and the existence of evidence of a link between NPY and ghrelin, in the present study, the effect of Ephedra Pachyclada extract on all serum levels of these two hormones and its association with body weight were examined. The results showed that HAEEP, in dose-dependent form, reduces serum levels of neuropeptide Y, ghrelin, and decreases body weight in rats under normal diet (free access to food and water).

Most central and peripheral peptides involved in the regulation of energy balance, such as ghrelin, leptin, and insulin exercise their stimulating or inhibitory effects on food intake through increase or decrease of the expression of NPY respectively (18 and 19). The results of our previous study showed that HAEEP increases serum levels of leptin in male rats receiving normal diet (20). Leptin suppresses appetite through inhibition of synthesizing NPY (21). Therefore, it seems that, in this study, the reduction in the serum levels of NPY is connected to increase in serum levels of leptin by the extract. Using electron microscope and immunohistochemistry methods, recent studies have proven the interaction between neurons that express ghrelin and neuropeptides that express NPY in the hypothalamic arcuate nucleus (ArcN) (22). Central or peripheral administration of ghrelin in addition to increasing secretion of growth hormone increases food intake and body weight in humans and rodents through the increase in expression of NPY (23). Moreover, it has become clear that peripheral administration of NPY antagonist Y stops appetite-forming effects of ghrelin (24). These findings suggest that NPY neurons in ArcN of hypothalamus can be an important effector for oxygenic function of ghrelin.

Therefore, due to the interaction of NPY and ghrelin, it seems that EP extract has also affected the serum levels of ghrelin hormone by inhibiting NPY secretion.

Conclusion

HAEEP suppresses appetite and reduces body weight through reducing serum levels of NYP and ghrelin.

Acknowledgment

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Disclosure of interest

None of the authors have any conflict of interest to declare.

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