The Effect of the Hydro-alcoholic Extract of Ephedra Pachyclada on Serum Concentration of Hormones of Leptin, Cholecystokinin and Body Weight in Male Rats

Zahra Hasanpour Jahromi¹, Mahsa Sadat Sarvarzadeh¹, Hossein Kargar Jahromi²*, Zahra Kargar Jahromi³, Ava Hosseini⁴, Zeinab Esmaily⁴, Zahra Khabbaz Kherameh ⁵

¹Jahrom University of Medical Sciences, Jahrom, Iran.
²Research center for non-Communicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran.
³Zoonoses Research center, Jahrom University of Medical Sciences, Jahrom, Iran.
⁴Student Research Committee, Jahrom University of Medical Sciences, Jahrom, Iran.
⁵Young Researchers Club, Islamic Azad University, Jahrom Branch, Jahrom, Iran.
*Corresponding Author email: hossein.hargarjahromi@yahoo.com, Tel: (+98)9399711845

ABSTRACT
Background and objective: medicinal plants are considered as one of the main and popular options for the weight reduction and control among the people and researchers. The aim of the present research is to investigate the effect of hydro-alcoholic extract of Ephedra Pachyclada on serum level of hormones of leptin, cholecystokinin and body weight in male rats.

Materials and methods: In this Shamal research 40 adult male Wistar rats were selected and were divided randomly into 5 groups: control (without receiving any material), Sham (receiving 1 ml distilled water), the Shamal group 1 (receiving 250 mg.kg extract of hydro-alcoholic ephedra), the Shamal group 2 (receiving 500 mg.kg extract of ephedra hydro-alcoholic ephedra) and Shamal group 3 (receiving 1000 mg.kg extract of ephedra hydro-alcoholic ephedra). In the Shamal group the extract was prescribed in the form of gavage for 28 days. For investigating the serum level of hormones of leptin and cholecystokinin, on the twenty-ninth day bleeding from the mice was done. The weight of the mice was also measured daily during the course of Sham.

Findings: 500 and 1000 mg.kg doses of the hydro-alcoholic extract of Ephedra Pachyclada cause to increase significantly the serum levels of hormones of leptin and cholecystokinin as well as the reduction of body weight compared to the control group (p < 0.05).

Discussion and Conclusion: Through increasing the serum level of leptin and cholecystokinin hormones, the hydro-alcoholic extract of Ephedra Pachyclada causes to reduce the body weight.

Keywords: Orchis, leptin, cholecystokinin, body weight, rat

INTRODUCTION
Obesity is the most general metabolic disease as well as the factor causing and intensifying many diseases; it is associated with a reduction of the quality of life. Today, the question of adjusting the weight and energy is an important and interested topic for many researchers (1). In terms of availability, as well as the more effectiveness and safety in related to the produced drugs, themedicinal plants are regarded as one of the main and popular options for controlling and
reducing the weight in between people and researchers (2).

Ephedra Pachyclada is a plant from the family of Ephedraceae. This plant is a shrub with green, articulated, branchy, usually vertical stems. Ephedra Pachyclada grows in a dry and semi-arid climate, especially in the desert and rocky areas of South and South East of Iran and is known locally as Hoom and Armak. The reputation of the plants of this family is due to their abundant ephedrine alkaloids whose most important ones are ephedrine and pseudoephedrine and (3). This family of plants has many healing properties and for thousands of years in the traditional East Asian medicine is applied in the treatment of respiratory diseases such as asthma, bronchitis and allergies (3, 4, 5). Ephedra also has the anti-inflammatory, antioxidant, and hypoglycemic effects and is a sympathetic system emulator (6, 7). Although a study on the effects of Ephedra Pachyclada on weight control has not been carried out, but studies conducted on other species of ephedra indicate the effectiveness of this plant in weight control. In a study done by Hackman et al. in 2006, it was determined that taking supplements containing ephedra and caffeine in obese and overweight women causes to reduce body weight, fasting blood glucose and insulin levels (8). In 2011 Song et al. examined the effect of extract of Ephedra sinica (species of ephedra native to China and Korea) on glucose intolerance in rats having oily full nutritional regime. The results of this study showed that Ephedra causes to reduce the amount of triglyceride (TG) and increase the amount of HDL cholesterol. In addition, Ephedra decreases the increased levels of glucose, as well as the body weight in these animals (9).

In 2012 Correa et al. also showed that the extract of Ephedra sinica dose-dependently causes to decrease the food intake and the body weight in rats (10). In the cited studies, the effects of ephedra on weight control in diabetic patients or in other words, obese and patient individuals have been investigated. In the study of Correa et al. the hormonal factors involved in weight control, such as leptin and cholecystokinin have not been measured and the probable effect of ephedra on the hormone of leptin has been only mentioned (10).

Since a direct scientific research has not been carried out so far about the impact of extracts of ephedra on the body weight and the appetite controlling hormones, the present study was done in order to evaluate the effects of the extract of this plant on body weight, the leptin hormone and cholecystokinin in male rats.

MATERIALS AND METHODS

In this Shamal research 40 adult male Wistar rats were used with an average weight of 200-180 g. For the sake of their compatibility with the environment, the mice were kept for a week in a breeding room of animals in Jahrom University of medical sciences. During the research, the animals were kept in conditions of 12 hours light and 12 hours darkness and the ambient temperature 20-25 ° C; they accessed freely to food and water.

According to the previous articles, the prescribed concentration of the hydro-alcoholic extract of Ephedra Pachyclada was determined in the values of 250 and 500 and 1000 mg per each kg of body weight (11); the mice were divided randomly into 5 groups of eight:

Control group: this group did not receive any caring during the Sham (28 days).

Sham group: this group received one ml of distilled water during the Sham (28 days), in the form of Gavage and according to body weight.

Experimental groups 1, 2, 3: they received respectively 250, 500 and 1000 mg.kg doses of hydro-alcoholic extract of Ephedra Pachyclada, in the form of Gavage and according to body weight for 28 days.

At the end of the research (twenty ninth day), after the weighing of animals the bleeding is done directly from the heart of the animals by the help of the 5 CC syringe (under anesthesia by diethyl ether); their serum is collected by centrifuge.
apparatus (for 15 minutes and 3000 rounds per minute) and is kept in the freezer-20 °. For measuring the hormones of leptin and cholecystokinin theElisa Kits specific to the rat is used. For analyzing data the variance one-way analysis ANOVA was used. In cases where the statistical difference of different groups was significant, Duncan test was used for understanding the difference between the averages. Statistical computation was carried out by SPSS software version 21 and (P < 0.05) was considered as the significant level. Data were calculated and compared as Mean ± SEM on results section.

Findings
The results of current study showed that the doses of 500 and 1000 mg.kg of hydro-alcoholic extract of ephedra cause to increase significantly the serum level of the hormones of leptin and cholecystokinin in comparison with the control study.

Table 1: comparison of the serum level changes of hormones of leptin, cholecystokinin and body weight in the Shamal groups receiving the different doses of ephedra hydro-alcoholic extract with the control group

<table>
<thead>
<tr>
<th>Group Variable</th>
<th>Hydro-alcoholic Extract of Ephedra(1000 mg.kg)</th>
<th>Hydro-alcoholic Extract of Ephedra(500 mg.kg)</th>
<th>Hydro-alcoholic Extract of Ephedra(250 mg.kg)</th>
<th>Sham</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>leptin (ng.ml)</td>
<td>C 4.50±0.27</td>
<td>BC 3.87±0.41</td>
<td>AB 3.58±0.12</td>
<td>A 3.02±0.09</td>
<td>A 3±0.15</td>
</tr>
<tr>
<td>Cholecystokinin (mg.L)</td>
<td>B 1149.523.11</td>
<td>B 1092.23±0.94</td>
<td>AB 1073.5±19.48</td>
<td>A 992.37±21.15</td>
<td>A 1001±31.89</td>
</tr>
<tr>
<td>Average of body weight changes (g)</td>
<td>C 19.87±0.81</td>
<td>C 22.37±0.59</td>
<td>BC 24.62±0.77</td>
<td>AB 25.87±1.14</td>
<td>A 25.75±1.29</td>
</tr>
</tbody>
</table>

- Based on Duncan test, in each row that contains an at least common letter, the available averages at the level of 5% of the Duncan test do not have a significant difference with each other.
- The averages have been offered as Mean±SEM.
- Statistically speaking, P < 0.05 has been regarded meaningful.

DISCUSSION AND CONCLUSION
The effectiveness of the different species of ephedra in reducing the weight has been confirmed in several studies (8, 9, 10), but none of these studies have examined the effect of this plant on the serum levels of hormones controlling the body weight.

The results of the current research showed that the comestible prescription of 500 and 1000 mg.kg doses of hydro-alcoholic extract of Ephedra Pachyclada causes to increase the serum level of the hormones of leptin, cholecystokinin and also the reduction of body weight in healthy rats. The main compounds of the plant ephedra are the alkaloids such as ephedrine and pseudoephedrine (3). The results of the studies show that the alkaloid compounds present in plants cause to reduce the body weight (12, 13). The mechanisms through which the alkaloids cause to reduce the body weight are the adjustment of fatty acid metabolism, reduction of appetite and increase in energy expenditure. The reduction of body weight is due to the reduction of food intake and increase in energy expenditure.
metabolism, control of pancreatic lipase and activation of the enzyme AMPK\(^1\) (14, 15). Leptin is considered also as one of the main hormones controlling the fatty metabolism; it is mainly secreted from the adipocyte tissue. This hormone is mentioned as the factor adjusting the amount of energy consumption and reception and controlling the weight; its operation depends on its receptor in the center of nutrition in hypothalamus. In rodents, the leptin is essential for the feedback loop related to satiety in hypothalamus centers (16). Through inhibition of the synthesis of noropeptide Y (NPY), Leptin causes to suppress the appetite. It stimulates also the sympathetic processin CNS. Through neurotransmitters of apinlarin and norapinlarin and by receptors of beta-adrenergic and cyclase adenylyl system, the sympathetic terminals that enter into the peripheral tissues such as adipose tissue, cause to produce the Cytosol cAMP in the adipocyte, and suppress the production of leptin, and by hormone-sensitive lipase it increases the lipolysis to the amount of serum leptin. Thus, the response of CNS byafeedback arc leads to a reduction of the adipose tissue size and a control of the production of leptin (17). So it seems that one of the possible mechanisms of ephedrine alkaloids found in the plant ephedra is the reduction of weight, increase in leptin hormone secretion and consequently an increase in Lipolysis. The results of the present study also show an increase in serum level of hormone of cholecystokinin by ephedra. Polypeptide hormone of cholecystokinin (CCK) is the first intestinal hormone whose role in the control of appetite is known (18). Cholecystokinin has several performances in human and laboratory animals, including the ability to create a feeling of satiety and decrease of food consumption, prevention of gastric emptying, prevention of acid secretion and stimulating the small intestine andvisible peristaltic waves (19).

It has been known that the synergetic effects of two hormones of leptin and cholecystokinin have a key role in reducing the weight (20). The results of researches show that the controlling effects of leptin hormone on the amount of receiving food are stopped by the receptor antagonists CCK-A (21). Synergetic effects of leptin and cholecystokinin on the reduction of body weight are applied through the central nervous system. Parabrachial nucleus (BPN) in the brain area is one of the places wherein the synergistic effects of these two hormones are applied (22). This nucleus receive someinputs from the arcnucleus of hypothalamus as well as the nuclei of the isolated brainstem (NTS). The arcnucleus containsthe neurons secreting Neuropeptide Y and the nuclei of the isolated brainstem contain the vagus nerves that (secretion stimulant of cholecystokinin) (23). On the other hand, the existence of leptin hormone receptors in the arcnucleus and the nuclei of isolated brainstem has been proven (24).

**General conclusion**

Through increasing the secretion of leptin and cholecystokinin hormones, the hydro-alcoholic extract of Ephedra Pachyclada causes to control the appetite and consequently reduce the body weight.

**ACKNOWLEDGEMENTS**

This study performed with IR.JUMS.REC.1394.217 code number under investigation of the committee of ethics in research in Jahrom University of Medical Sciences. The authors would like to thank all the staff and officials of research deputy and technology deputy of this university who sincerely helped us in the course of this research.

**CONFLICTS OF INTEREST**

The authors declare no conflicts of interest to them.

---

\(^1\)Adenosine Monophosphate-Activated Protein Kinase
The Effect of the Hydro-alcoholic Extract of Ephedra Pachyclada on Serum Concentration

REFERENCE


