Investigation of Orchid root aqueous extract treatment on hormone cholecystokinin serum concentration and body weight in male rats

Kargar Jahromi Hossein¹, Hajiani Malihe*¹, Kargar Jahromi Zahra¹, Khabbaz Kherameh Zahra² Dowlatkhah HamidReza ³, Mahdiyar Maryam⁴

Abstract

Introduction:
The growing prevalence of obesity is one of the biggest health challenges across the world. Stimulating the body metabolism and increasing its energy use through herbal agents is one of the main methods used to treat obesity.

Materials and Methods:
This study was conducted on 50 adult male Wistar rats weighing between 180 and 200 g randomly divided into 5 groups of equal sizes, including a negative control group (receiving no drugs), a sham control group (receiving 1 ml of distilled water) and experimental groups 1, 2 and 3 (receiving 20, 40 and 80 mg/kg aqueous extract of Orchid roots, in respective order). The extract was injected intraperitoneally to the experimental groups for 28 days. On the 29th day, the rats' blood samples were taken to examine their serum concentration of cholecystokinin. The rats' body weight was measured on a daily basis throughout the experiment. Statistical analysis were performed in SPSS using the one-way ANOVA and Duncan's post-hoc test.

Results:
Orchid root aqueous extract injection in a dose-dependent led to significant increase in serum levels of cholecystokinin hormone and decrease in food intake and body weight than the control group (p<0.05).

Conclusion:
Through increased serum levels of cholecystokinin hormone, aqueous extract of orchid plant root reduces body weight.

Keywords: Orchid, Cholecystokinin, Body Weight, Rat

Introduction
In recent years, obesity has been raised as a global epidemic phenomenon. The main reasons for being epidemic are changes in human lifestyle based on which the lack of physical activity and the use of energy-dense diets have been increased. Obesity and increased adipose tissues in the body has been identified as a risk factor for several diseases, including diabetes mellitus, dyslipidemia, hypertension and
cardiovascular disorders (1). There are several strategies for controlling obesity, such as increasing the energy consumption or reducing the energy received. Recently, other methods have been taken into consideration, including stimulating metabolism and increasing the energy consumption through herbal ingredients (2).

Lancibracteata (C.Koch) Renz Dactylorhiza, previously called Orchis (maculate L.), belongs to the orchid family. It has several species and grows almost all over the world. The tuber of this herb can usually be exploited in early summer and retains its medicinal properties up to two years (3 and 4). This herb has the following constituents: glucomannan, nitrogen compounds, starch, protein, sugar, hydroxybenzaldehyde, ferulic acid, quercetin, daucosterol, cirsilineol, and steroids (5,6).

In traditional medicine, Orchis mascula is prescribed as a treatment for breast disorders, gastrointestinal disorders, tuberculosis, diarrhea, Parkinson’s disease, cancer, fever, and especially for enhancing sexual activities and treatment of erectile dysfunction, and increasing stamina and energy. This herb is also used in beverage, confectionary, and ice cream making industry (7, 8).

One of the main constituents of the orchid root extract is a water-soluble fiber called glucomannan, whose role has been identified in weight loss, blood sugar control, and reduced cholesterol (9,10). Studies have shown that fibers, especially water-soluble fibers are effective in controlling body weight by reducing the speed of evacuation and absorbing substances in the gastrointestinal tract, increasing the secretion of cholecystokinin (CCK), and regulating leptin secretion (11, 12).

The discovery of the peptides secreted by the gastrointestinal tract affecting the appetite highlighted the role of this tract in the energy balance and stomach is now considered an endocrine organ effective in energy balance, like adipose tissue, muscle, and liver. Cholecystokinin is among the hormones secreted by the gastrointestinal tract. Cholecystokinin is a 33 amino acid peptide produced by the endocrine cells of the small intestine and different gastrointestinal neurons and the central nervous system. This hormone can also act as a neuropeptide (13).

Cholecystokinin has various functions in humans and laboratory animals, including the ability to cause satiety and reduce food intake, prevention of gastric emptying, prevention of gastric acid secretion, and stimulating the peristalsis of the small intestine (14).

Since no direct scientific research has been conducted to examine the effect of the orchid extract on the body weight and hormones controlling appetite, this study aimed to examine the effects of this plant extract on the body weight and serum levels of cholecystokinin in male rats.

Materials and Methods

Sampling and extraction method:
Orchid samples were collected from around Yasouj city in early summer. After washing to remove soil, the tuberous roots of this herb were dried in shadow in the lab. Then, the fully dried samples were powdered using an electric grinder. The obtained powder was mixed with 96% ethanol at an extraction ratio of 5:1 and was fully stirred for 24 hours at room temperature until a uniform solution was achieved. Then, the solution was filtered and dried for 48 hours at ambient conditions to turn into an alcohol-free solid extract. Different amounts of the solid extract (20, 40, and 80 mg) were dissolved in 1 cc of double-distilled water and were refrigerated until used (15).

Animals and their Classification:
Throughout this study, ethical issues in relation to housing and use of laboratory animals were observed and the procedures were approved by the Ethics Committee of Jahrom University of Medical Sciences.
In this experimental study, 50 adult male Wistar rats with an average weight of 180-200 gr were used. The rats were housed for a week in the animal breeding room of Jahrom University of Medical Sciences to adapt to the environment. Throughout the study, the rats were housed in 12 hours of light/dark cycle and at ambient temperature of 20-25 °C and had free access to food and water. The rats were randomly divided into five groups of 10 rats. During the study, the control group received no substance. The sham group was intraperitoneally injected with 1 ml/bw of distilled water. The experimental groups 1, 2, and 3 were respectively injected intraperitoneally a minimal dose of 20 mg/kg, a medium dose of 40 mg/kg, and a maximum dose of 80 mg/kg aqueous extract of orchid root daily for 4 weeks based on the body weight. The injection volume was 0.2 cc in all groups.

**Blood Sample Collection and Hormone Tests:**
At the end of the study (the 29th day), after weighing the rats, 5 ml of blood was directly taken from their hearts (under anesthesia with diethyl ether). Blood sera were separated by centrifugation (for 15 minutes at 3000 rpm) and were kept at -20 °C in the freezer until tested. To measure cholecystokinin, ELISA kits (Biospes, China) made specifically for rats were used.

**Statistical Analysis:**
One-way ANOVA was used to analyze the data. In cases that different groups had statistically significant differences, the Duncan’s test was used to find out the points of differences between the means. Statistical analysis was performed using SPSS-15 and P<0.05 was considered as the significance level. The data are presented in the results section as Mean±SEM.

**Results**
Based on the results of this study, injection of the maximum dose of the aqueous extract of orchid root caused a significant increase in the serum levels of cholecystokinin compared with the control group (P<0.05), while no significant difference was observed in the serum levels of cholecystokinin in the group receiving the minimum and medium doses of the extract (Figure 1).

In addition, the results of the measurement of the female rats’ body weight indicated that the injection of the medium and maximum doses of the aqueous extract of orchid root leads to significant reduction in the body weight compared with the control group (P<0.05), while no significant reduction in the body weight was observed compared with the control group in the group receiving the minimum dose of the extract (Figure 2).

![Graph showing the level of cholecystokinin](image-url)
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Figure 1: Comparison of changes in serum levels of cholecystokinin in the experimental groups receiving different doses of the orchid extract with the control group
According to the Duncan’s test, the difference between the means of groups with dissimilar letters is significant at P<0.05.

![Graph showing changes in serum levels of cholecystokinin](image)

Figure 2: Comparison of changes in body weight of the experimental groups receiving different doses of orchid extract with the control group

Table 1. Comparison of changes in serum levels of cholecystokinin and the body weight in the experimental groups receiving different doses of orchid extract with the control group

<table>
<thead>
<tr>
<th>Groups variables</th>
<th>Control group</th>
<th>Sham group</th>
<th>Experimental group 1</th>
<th>Experimental group 2</th>
<th>Experimental group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystokinin (ng/L)</td>
<td>66.30 ± 1.28 a</td>
<td>65.66 ± 0.79 a</td>
<td>69.52 ± 2.67 a</td>
<td>74.38 ± 2.09 ab</td>
<td>94.50 ± 1.56 b</td>
</tr>
<tr>
<td>Body weight (gr)</td>
<td>209.11 ± 5.31 a</td>
<td>208.09 ± 3.19 a</td>
<td>202.50 1.99 a</td>
<td>185.20 9.32 b</td>
<td>176.00 ± 3.00 bc</td>
</tr>
</tbody>
</table>

- According to the Duncan’s test, the means in each row with at least one similar letter are not significantly different at the level of 5%.

Discussion
According to the results of this study, the aqueous extract of orchid root depending on the dose increased the serum levels of cholecystokinin and decreased the body weight. The results of this study are consistent with other studies conducted on the relationship between the effects of cholecystokinin (CCK) on body weight. It has been known that administration of cholecystokinin to animals and humans inhibits food intake by reducing the amount of food and its duration. Cholecystokinin controls appetite through CCK-A receptors and the vagus nerve, and injection of antagonists of this receptor before eating food increases the meal size in humans and other species (16). There is evidence that in some circumstances, there is a cooperation between cholecystokinin and leptin to reduce body weight through reduced calorie intake (17). The long-term effects of CCK on body weight are the result of its relationship with adiposity signals, such as leptin which increase the CCK-induced satiety (18). The messages produced by the peripheral CCK are transmitted to the CNS through vagal afferents and interferes with the afferent information related to leptin to regulate body weight and as a result, the interfered signals in top regions of efferent pathways...
control nutrition, thermogenesis, metabolic rate, and energy consumption (19). Inhibitory effects of leptin on food intake are stopped by CCKA- receptor antagonists (20). Parabrachial nucleus (BPN) in the pons is one of the places where the synergistic effects of leptin and cholecystokinin on the body weight loss are applied (21). This nucleus receives inputs from the arcuate nucleus of the hypothalamus and the nuclei of the solitary tract (NST). The arcuate nucleus contains neurons secreting neuropeptide Y and the nuclei of the solitary tract contain vagus nerves (stimulating the secretion of cholecystokinin) (22). The presence of glucomannan in the aqueous extract of the orchid root can be considered a possible reason for an increase in serum levels of cholecystokinin and as a result, the weight loss. Glucomannan is a water-soluble fiber, the amount of which has been reported as 7% to 61% in different types of orchids. The role of glucomannan in weight loss, blood sugar control, and lowering cholesterol has been confirmed in different experimental studies (23, 24).

Stimulation of cholecystokinin secretion by the enteric nervous system is one of the mechanisms of glucomannan for weight loss. Reduced speed of evacuation and absorption of substances in the gastrointestinal tract increases the secretion of cholecystokinin and ultimately leads to weight loss (25).

**Conclusion**

The aqueous extract of the orchid root reduces weight probably by stimulating the secretion of cholecystokinin.

**Conflict of Interest**

The authors declare no conflicts of interest regarding the compilation/publishing of this article.

**Acknowledgements**

This article is the result of a research project approved by the Jahrom University of Medical Sciences and Health Services. We would like to sincerely thank the vice chancellor for Research and Technology at Jahrom University of Medical Sciences for his cooperation and helping researchers conduct this study.

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