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Studying Effect of Extract of the Roots of Salep Plants on Serum Factors.

Maliheh Hajiani[1], Hossein Kargar Jahromi[1]*, Ali Ghorbani Ranjbary[2], Zahra Kargar Jahromi[1], Zahra Khabbaz Kherameh[2]

[1]Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran.
[2]Young Researchers and Elite Club, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
[3]Department of Biology, University of Payam-e-noor, Iran.

ABSTRACT

Obesity is a very common complication of human societies and the cause of many diseases. A wide variety of methods to treat obesity have been suggested, one of which is herbal therapy. The aim of the present study is to investigate the effect of aqueous extract of orchid plant roots on serum level of leptin and body weight in male rats. In this experimental study, 50 adult male rats from Wistar breed were selected and randomly divided into 5 groups: control (no drug) witness (which received 1 ml of distilled water), the experimental group 1 (received 20 mg/kg aqueous root extract of vanilla plant), experimental group 2 (received 40 mg/kg aqueous root extract of vanilla) and experimental group 3 (received 80 mg / kg aqueous root extract of vanilla plant). In the experimental group, the extract was injected intra peritoneal for 28 days. On the twenty-ninth, the mice were bled to measure serum concentrations of leptin and Cholecystokinin hormones. During the trial period the weight was also measured daily. Injection of orchid root extract in a dose-dependent manner caused significant increase in serum levels of leptin and Cholecystokinin and a significant decrease in food intake and body weight than the control group (p <0/05). The aqueous extract of the roots of orchid plants by increasing serum levels of leptin and Cholecystokinin decreased the body weight.

Keywords: orchid root extract, leptin, Cholecystokinin, body weight, male rats

Corresponding author

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INTRODUCTION

Obesity is a metabolic disorder characterized by excessive intake of energy and thereby increasing the weight of the body. Recent studies have shown that the prevalence of overweight and obesity is increasing worldwide at an alarming rate. Obesity is the root of many diseases such as diabetes, hypertension, cardiovascular disease and some cancers, including breast, colon and prostate cancer [1, 2]. Today, various treatments for obesity are used, such as drug therapy, diet, liposuction, exercise and physical activity. Stimulating metabolism and increasing energy using plant material are considered as other approaches to weight management and obesity treatment [3].

Orchid plants or woodland's tubers finger (Lancibracteata (C.koch) Renz Dactylorhiza) with the old name maculate L. (Orchis) belongs to the family of orchids and different species grow almost all over the world. Usually the tubers (glands) are harvested in early summer and maintain their medicinal properties for up to two years [4, 5]. The plant contains the chemicals including fiber glucomannan, Nitrogenous materials, starch, protein, sugar, benzaldehyde hydroxy , ferulic acid, Quercetin, Daucosterol, Cirsilineol and steroids [6, 7]. Orchid plant in traditional medicine is prescribed as a cure for breast disorders, intestinal disorders, tuberculosis, diarrhea, Parkinson's disease, cancer, fever, and in particular to strengthen the sexual activities, treatment of erectile dysfunction, increasing strength and energy and regeneration. Also, it is used in the ice cream manufacturing, soft drink and confectionery industry [8, 9].

One of the main compounds in root extract of vanilla is a water-soluble fiber called glucomannan that has a role in weight loss, controlling blood sugar and cholesterol [10, 11]. Studies have shown that fiber, especially water- soluble fiber slows the desorption and absorption of material through the gastrointestinal tract, increases the Cholecystokinin secretion, regulates leptin hormone secretion and controls body weight [12 and 13]. Cholecystokinin and leptin are two well-known hormones in appetite control and body weight [14]. The leptin hormone is16 kDa that is a product of ob gene, which is essential for regulating normal weight and weight loss. White adipose tissue is the major site of leptin synthesis and sparing can be built in the intestinal epithelium, placenta, skeletal muscle and brain. The main physiological role of leptin is to decrease body weight by reducing appetite and increasing the body's energy reserves [15].

Cholecystokinin: a peptide of 33 amino acids that is produced from endocrine cells of the small intestine, stomach- colon neurons in the central nervous system. These hormones can also act as a neuro peptide [16]. Cholecystokinin performs different functions in humans and laboratory animals, including the ability to create a feeling of satiety and reduce food intake, inhibition of gastric emptying, prevention of gastric acid secretion and stimulates peristalsis of the small intestine [17]. Since direct scientific research on impact of the vanilla extract on body weight and appetite control hormones has not been done until now, the present study aimed to investigate the effects of Cholecystokinin and leptin hormones in the rats.

MATERIALS AND METHODS

Sample collection and extraction methods

Orchid plant or tuber fingered woodlands samples were collected around Yasooj city in early summer. Tuber roots of orchid plants after rinsing, soil removal were dried in a shadow laboratory environment. The dried samples were powdered by an electric mill. The resultant powder were mixed with 96% ethyl alcohol at a ratio of 5 times the size of the plant and mixed thoroughly in a Rutuduksy device for 24 hours at room temperature until a uniform solution was obtained. Then the solution was filtered and dried for 48 h at ambient temperature to become solid non-alcohol extract. Solid extracts with the value of 20, 40 and 80 mg were dissolved in 1 ml of distilled water twice and refrigerated until use [18].

Animals and their category

In this study, all ethical issues related to the care and use of laboratory animals during the work were followed and recorded by the Ethical Committee of Jahrom Medical University with 2991/ p/d number and dated 13/12 /1392. In this experimental study, 50 adult Wistar male rats with an average weight of 200-180 g were used. To ensure the mice adapted with the environment they were maintained for a week in the animal rooms at Jahrom Medical University. Throughout the study, animals were kept under conditions of 12 hours

light/12 hours darkness, at room temperature 25-20 ° C and free access to water and food.. Animals were randomly divided into 5 groups of 10 rats: control group which received no material, the witness group in terms of body weight were intraperitoneally injected 1 ml of distilled water, experimental groups 1, 2 and 3, respectively, were injected minimal dose (20 mg kg), medium dose (40 mg kg) and maximum dose (80 mg kg) of aqueous extracts of orchid roots intraperitoneally daily for 4 weeks, depending on body weight.

Blood sampling and hormone assays

At the end of the study (day 29) after weighing the animals, blood was collected directly from the heart of the animals using 5 cc syringe (under anesthesia with diethyl ether) and the blood serum by means of centrifugation (3000 rpm for 15 min and min) was collected and kept in the freezer at $-20°c$ until used. Rat specific Elisa kits that were manufactured in Biospes Company,China were used to measure the leptin and Cholecystokinin hormones.

Statistical Analysis

For analysis of information, Analysis variance ANOVA was used. In the cases where there was significant difference between different groups, Duncan test was used to obtain the difference between the means. Statistical analysis was performed using SPSS version 21 and the significance level (P <0.05) was considered. The results shown in Mean ± SEM were calculated and compared.

Findings

Based on these results, the injection of moderate doses (40 mg / kg / day) and maximum (80 mg/kg/day) aqueous extract of the roots of orchid plants over a period of 28 days causes a significant increase in serum leptin levels than the control group (p <0.05). But in least dose (20mg / kg) a significant difference is not observed in serum leptin levels compared with the control group (Fig. 1).

The injection of maximum dose (80mg/kg/day) from aqueous extract of the roots of orchid plants for 28 days resulted in a significant increase in Cholecystokinin serum levels compared with the control group (p <0.05). But in the least dose (20mg / kg) and medium (40mg/kg/day) extract no significant differences were observed in Cholecystokinin hormone serum level (Fig. 2). In this study the results of the measurement of body weight in male rats showed that injection of moderate doses (40mg/kg/day) and maximum (80mg /kg/day) aqueous extract of the roots of orchid plants for 28 days resulted in a significant reduction body weight compared with the control group (p <0.05). But in the least dose (20mg/kg) extracts a significant reduction in body weight compared to controls was not observed (Fig. 3).

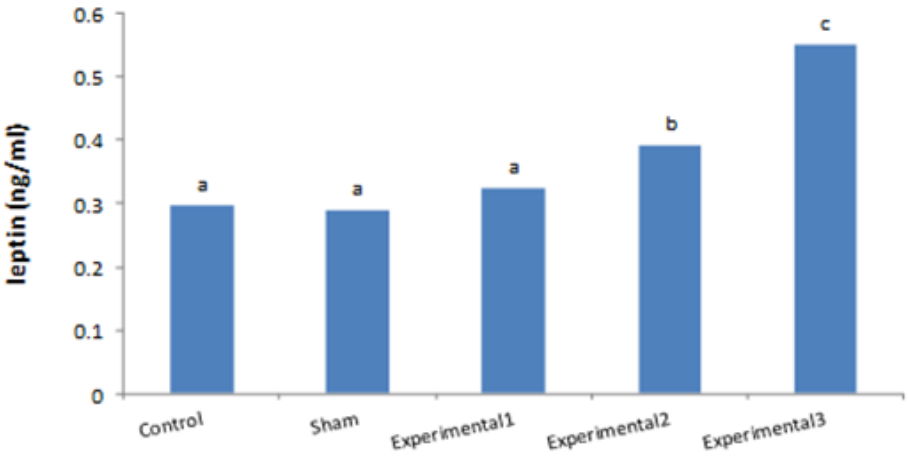

Figure 1: Comparison of serum levels of leptin hormone in the control groups that received different doses of vanilla extract for 28 days

- According to Duncan test, the sum of groups which have dissimilar characters are significant.
- Means are presented as Mean ± SEM. - P <0.05 was considered statistically significant.

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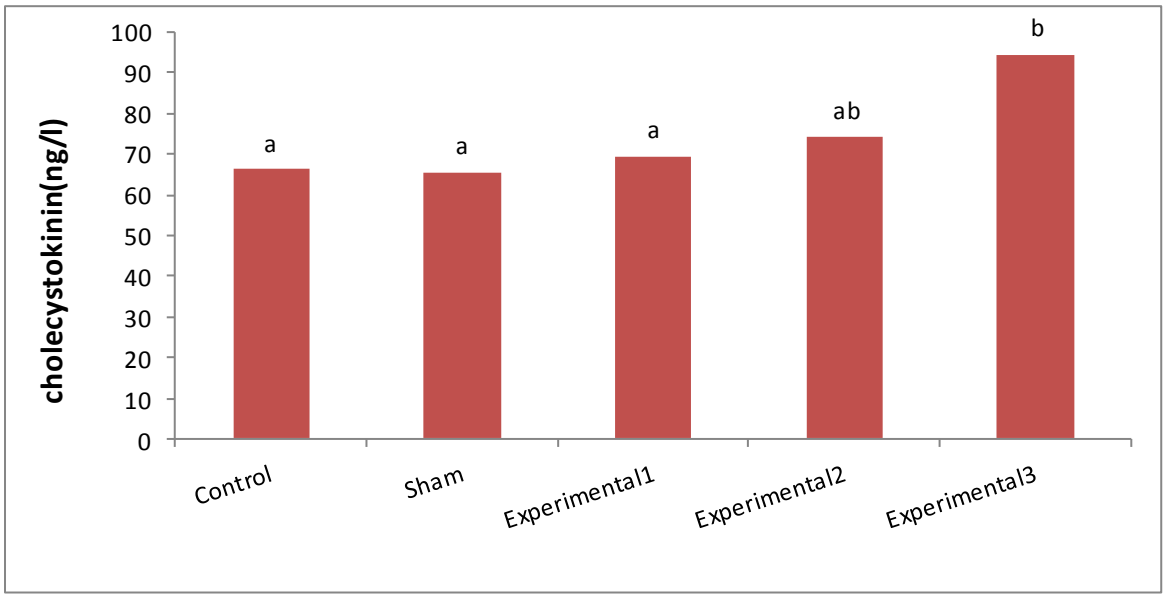

Figure 2: Comparison of serum levels of Cholecystokinin hormone in the experimental group and control group that received the vanilla extract for 28days.

- According to Duncan test, the sum of groups which have dissimilar characters are significant.
- Means are presented as Mean ± SEM.- P <0.05 was considered statistically significant.

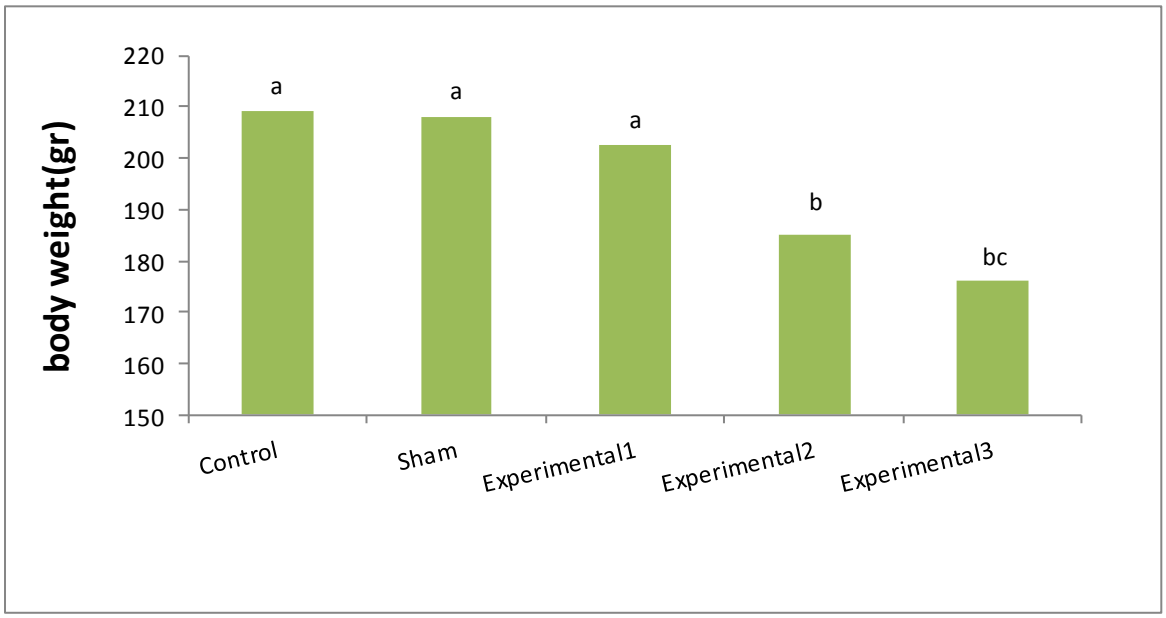

Figure 3: Comparison of changes in body weight in the experimental group and the control group that received the vanilla extract for 28 days.

- According to Duncan test, the sum of groups which have dissimilar characters are significant.
- Means are presented as Mean ± SEM.- P <0.05 was considered statistically significant

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Table 1: Comparison of serum levels of leptin, Cholecystokinin and body weight in rats receiving vanilla extract for 28 days

Group/variable	Control	Sham	Experimental1	Experimental2	Experimental3
Leptin(ng/ml)	0.29±0.008a	0.28±0.007a	0.32±0.013a	0.39±0.013b	0.55±0.022c
Cholecystokinin(ng/l)	66.30 ± 1.285 a	65.66 ± 0.792 a	69.52±2.677 a	74.38 ± 2.091 ab	94.50 ± 1.558 b
Body weight (gr)	209.11±5.313a	208.09±3.189 a	202.50±1.995a	185.20±9.324b	176±3.003bc

- According to Duncan test, averages in each row having at least one common letter are not significantly different at 5% level of Duncan test. - Means are presented as Mean ± SEM. - P <0.05 was considered statistically significant.

DISCUSSION AND CONCLUSIONS

This study showed that aqueous extracts of orchid plant roots in a dose-dependent manner increase serum levels of leptin, Cholecystokinin and body weight loss can be explained. In this study body weight loss was linked to increased serum levels of the leptin and Cholecystokinin hormones by aqueous extract of vanilla plant root. It is known that leptin controls food intake by a mechanism involved in the negative roll [19].

Leptin, by binding to specific receptors in the hypothalamus causes changes in expression of regulating neuropeptides that receive and consume energy such as neuropeptide Y (NPY). Leptin is able to direct the expression of neuropeptide Y (NPY), which increases food intake and reduces energy consumption [20].

Basically the function of leptin is as a signal that prevents obesity because in ob/ob and db/db mice which had leptin deficiency and resistance to leptin, obesity was observed (21). With injection of recombinant leptin to these mice, a state of satiety, increased energy consumption and decreased body weight was observed [22]. On the other hand, the amount of energy stored in adipose tissue and serum leptin levels and leptin act in a paracrine/autocrine, stimulation of lipolysis by inhibiting lipogene directly involved in setting the metabolism of adipose tissue [23].

On the other hand, prescription of CCK to human and animals inhibits food intake by reducing the amount of food and the time. Cholecystokinin effect on appetite control via CCK-A receptors and via the vagus nerve, and injecting antagonists before meals increases meal size in humans and other species [24].

There is evidence that in some circumstances, a partnership between Systvkynyn pack and leptin to reduce body weight is associated with reduced calorie intake [25]. The long term effects of CCK on body weight is a result of its association with Adiposity signals like leptin which increases satiety [26]. Messages generated by peripheral CCK via vagy afferents are transmitted to the CNS and interact with leptin afferent to regulate body weight, and thus interfering signals from the top center of the efferent pathways to control nutrition, thermogenic, metabolic rate and consumption energy [27]. Inhibitory effects of leptin on food intake by CCK-A receptor antagonists stop [28]. Parabranshyal nuclei (BPN) is one of the places in pons that synergistic effects of leptin and Cholecystokinin are applied on body weight loss [29]. The core receives entries from hypothalamic arcuate nucleus and nuclear of isolating stem cells (NTS). Arcuate nucleus contains neuropeptide Y secreting neurons and isolated stem cells contain the vagus nerve (stimulating the secretion of Cholecystokinin) [30].

Presence of Glucomannan in the aqueous root of extract vanilla can be a possible cause of increased serum levels of leptin, Cholecystokinin hormones and then a decrease in body weight. Glucomannan is a water soluble fiber and its content in different species of orchid has been observed between 7 to 61 percent. The role of glucomannan on weight loss, blood sugar control and cholesterol reduction has been verified in experimental studies. [31, 32]. One of the mechanisms of glucomannan for weight loss is stimulating Cholecystokinin hormone from intrinsic. Slowing down the exhaust and intake on gastrointestinal, increases the Cholecystokinin hormone secretion in this area. Also, Cholecystokinin hormone with cooperation of leptin causes weight loss [33].

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CONCLUSION

It seems that the fiber composition of the aqueous extract of roots of the orchid plants by stimulating the secretion of Cholecystokinin from the digestive system and regulating the leptin hormones secretion results in effective reduction of food intake and body weight.

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