Original Article

The effect of 4-week treatment with the aqueous extract of Dactylorhiza maculate roots on serum leptin levels and body weight in male rats

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Abstract

Introduction:
Obesity is a chronic disease with a globally increasing incidence that can contribute to the development of several diseases, such as diabetes, cardiovascular diseases, hypertension and some types of cancer. A wide range of methods have been proposed and adopted for treating obesity, including herbal therapy. The present study was conducted to investigate the effect of the aqueous extract of Dactylorhiza maculate roots on serum leptin levels and body weight in male rats.

Materials and Methods:
In the present experimental study, 50 adult male Wistar rats were selected and randomly divided into 5 groups of equal size, including a negative control group (without receiving any substances), the sham control group (receiving 1 ml of distilled water), experimental group 1 (receiving 20 mg/kg aqueous extract of Dactylorhiza maculate roots), experimental group 2 (receiving 40 mg/kg aqueous extract of Dactylorhiza maculate roots) and experimental group 3 (receiving 80 mg/kg aqueous extract of Dactylorhiza maculate roots). The extract was intraperitoneally injected to the experimental groups for 28 days. On the 29th day, blood samples were taken from the rats for assessing serum leptin levels. The rats' weight was also measured daily throughout the experiment. Data were analyzed using the ANOVA and Duncan's range test.

Results:
The dose-dependent injection of the aqueous extract of Dactylorhiza maculate roots led to a significant increase in serum leptin levels and a significant decrease in food intake and body weight compared to the control group ($P<0.05$).

Conclusion:
The aqueous extract of Dactylorhiza maculate roots reduces body weight by increasing serum leptin levels.

Keywords: Dactylorhiza maculate, Leptin, Body Weight, Male Rat

Introduction

Obesity is a chronic disease with a globally increasing incidence that can lead to many diseases including diabetes, cardiovascular diseases, hypertension, and some cancers (1). Today, many different methods are used worldwide to treat obesity such as medication, diet, liposuction, exercise, and physical activities. Other weight control and obesity treatment methods include metabolic

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stimulation and increased energy consumption using herbal substances (2).
Dactylorhiza maculate, Lancibracteata (C.koch) Renz Dactylorhiza, previously known as Maculate L. (Orchis), belongs to the orchid family, and with many different species, and grows throughout the world. Normally, the roots of this plant can be harvested in early summer, and maintains its medicinal property for two years (3, 4). This plant contains compounds such as glucomannan, nitrogen, starch, protein, sugar, hydroxybenzaldehyde, ferulic acid, quercetin, daucosterol, cirsilineol and steroids (5, 6). In traditional medicine, Dactylorhiza maculate is administered for lung diseases, intestinal disorders, tuberculosis, diarrhea, Parkinson's disease, cancer, and fever, and especially to enhance libido, treat erectile dysfunction, increase stamina and energy. Dactylorhiza maculate is also used in ice-cream, drinks, and confectionary industries (7, 8).
One of the main components in aqueous root extract of Dactylorhiza maculate is an aqueous fiber called glucomanan, with long known effects on weight loss, blood sugar control and cholesterol reduction (9, 10). Studies show that fibers, particularly aqueous ones, are effective in weight control through reducing bowel movement and absorption rates, increasing secretion of cholecystokinin and regulation of leptin secretion (11, 12).
Receiving and consuming energy is balanced by several complex hormonal and neuronal mechanisms that affect metabolism and appetite according to nutrient molecules in blood and body fat. Leptin is a known as a weight and appetite controlling hormone (13). Leptin is a 16kDa hormone produced by ob gene that is essential in regulating and reducing normal weight. Leptin is mainly synthesized in white adipose tissue, and to a lesser extent, in intestinal epithelium, placenta, muscles and the brain. The main physiological role of leptin is to reduce body weight and increase energy production from body reserves (14).
Since the effect of Dactylorhiza maculate on body weight and appetite-controlling hormones has not been directly studied so far, the present study aimed to assess the effects of this plant extract on body weight and leptin concentration in male rats.

Materials and methods

Sample collection and extraction method:
Dactylorhiza maculate was collected from around Yasuj town in early summer, and its glandular roots were cleaned, washed, and dried in shade in vitro. Using electric mill, dried samples were powdered, and the resulting powder was mixed with ethanol 96% at 5:1 ratio, and thoroughly stirred in a rotary at room temperature for 24 hours to obtain a homogeneous solution. Next, the solution was filtered, and left to dry at room temperature to turn into an alcohol-free solid extract. The solid extract was dissolved at dosages of 20, 40, and 80 mg in 1 cc of double-distilled water, and kept refrigerated until use (15).

Animals and their grouping:
All ethical issues concerning working with laboratory animals were observed and registered at the ethics committee of Jahrom University of Medical Sciences, No D/P/2991, dated 4.3. 2014. In this experimental study, 50 adult male Wistar rats of mean weight 180-200 grams were used, and initially housed in the animal house of Jahrom University of Medical Sciences for one week to adapt to the new environment. In the course of study, animals were housed in 12 hourly alternate light/dark cycles at 20-25 °C, with free access to food and water. Animals were randomly divided into 5 groups (10 each): negative control group that received no substances, sham group that received intraperitoneal injection of 1ml of distilled water according to body weight, and trial groups 1, 2, and 3 that received intraperitoneal injection of 20, 40, and 80 mg/kg body weight daily doses of aqueous Dactylorhiza maculate extract respectively for 4 weeks.

Blood sampling and hormone tests:
By the end of the study (on the 29th day), after weighing, blood samples were taken from the heart using 5 cc syringes (under anesthesia using diethyl-ether). Then, blood serum was separated by centrifuging at 3000 rpm for 15 minutes, and kept frozen at -20 °C until testing. Leptin level was measured using ELISA kit (Biospes Company, China) for rats.

Statistical analysis:
Data were analyzed in SPSS-21 software using one-way variance analysis, and Duncan test (when difference between groups was significant) at significance level $P<0.05$. Results obtained are presented as mean ± SEM.

Results

According to results, injection of moderate (40mg/kg/day) and maximum (80mg/kg/day) doses of Dactylorhiza maculate extract over a course of 28 days caused a significant increase in serum leptin concentration compared to the control group ($P<0.05$). However, no significant difference in serum leptin concentration was observed at minimum dose (20mg/kg/day) compared to the control group (figure 1).

Weighing male rats showed that moderate and maximum doses of Dactylorhiza maculate extract over a course of 28 days caused a significant weight loss compared to the control group ($P<0.05$), but no significant weight loss was observed at minimum dose compared to the control group (figure 2).

Therefore, with increased concentration of leptin after 28 days of injecting moderate and maximum doses of Dactylorhiza maculate extract, a significant weight loss was achieved (figures 1 and 2) (table 1).

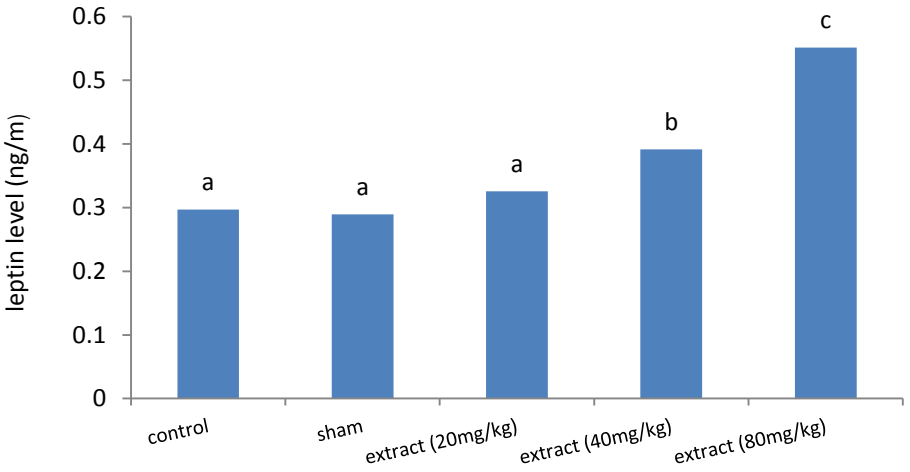

Figure 1: Changes in serum leptin concentration in experimental groups receiving different doses of Dactylorhiza maculate extract over 28 days compared to control group

Table 1: Changes in serum leptin concentration and body weight in experimental groups receiving different doses of Dactylorhiza maculate extract over 28 days compared to control group

Group/variable	Negative control	Sham	Trial 1	Trial 2	Trial 3
Leptin (ng/ml)	0.2971±0.0084a	0.2896±0.00748a	0.3254±0.01329a	0.3915±0.01361b	0.5513±0.02271c
Body weight (gr)	209.1111±5.31362a	208.0909±3.18934a	202.5000±1.99583a	185.2000±9.32476b	176.0000±3.00370bc

-According to Duncan test, mean values in each row with at least one common letter have no significant difference at 5% level

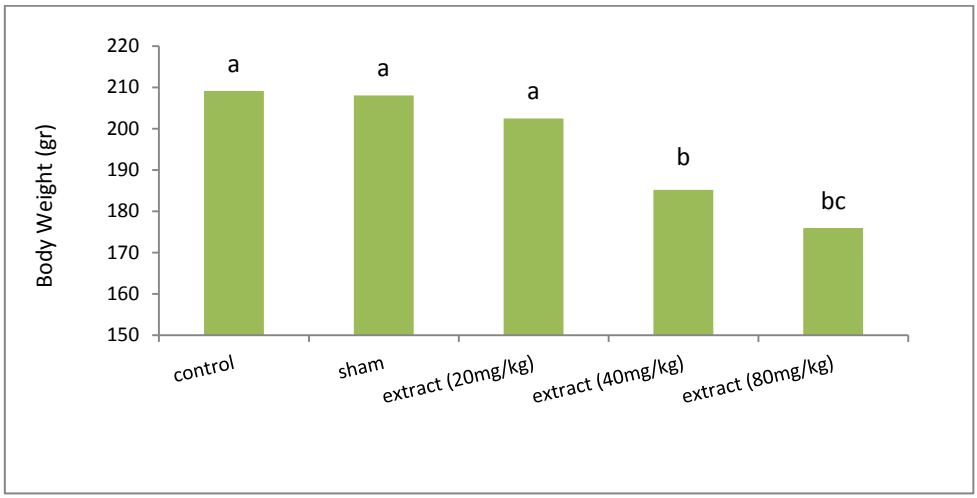

Figure 2: Changes in body weight in experimental groups receiving different doses of Dactylorhiza maculate extract over 28 days compared to control group

Discussion

The present study results showed that dose-dependent aqueous Dactylorhiza maculate extract caused increased serum leptin concentration, reduced food intake, and weight loss.

In this study, weight loss may be attributed to the increase in serum leptin concentration due to Dactylorhiza maculate extract. Leptin has been shown to be involved in control of food intake through a negative after-effect mechanism (16), by connecting to its specific receptors in hypothalamus, and changing expression of neuropeptides that regulate receiving and consuming energy, such as neuropeptide Y (NPY). Leptin is able to directly inhibit expression of NPY that increases food intake and reduces energy consumption (17). It also increases expression of CRH gene in paraventricular nucleus of hypothalamus that is responsible for reducing appetite (18). Leptin function is considered as obesity prevention message, so that obesity is observed in ob/ob and db/db rats with leptin deficiency or resistance (19). Administration of recombinant leptin in rats caused satiety, increased energy consumption and weight loss (20). On the other hand, concentration of leptin is indicative of stored energy in adipose tissue, and leptin is directly involved in regulating adipose tissue metabolism through a paracrine/autocrine function with inhibition of lipogenesis and and stimulation of lipolysis (21).

Various physiological factors and hormones such as cortisol, insulin, estrogen, and glucocorticoids are involved in regulating leptin level. Evidence from in vitro studies on human and rat adipose tissue suggest that insulin stimulates leptin mRNA expression and its secretion from adipose tissue (22). Studies conducted on rats indicate that insulin increases both ob gene and plasma leptin in healthy and diabetic rats (23). Mechanism of insulin-induced leptin secretion is as follows: insulin causes transfer of glucose into adipose cells through glucose-transferring protein (GLUT4), and glucose acts as an intracellular signal, causing stimulation of leptin secretion from adipose cells (24).

Many compounds in Dactylorhiza maculate root affect increased secretion of insulin. Studies show that administration of ferulic acid in diabetic rats reduces blood sugar by increasing insulin secretion (25, 16).

Quercetin maintains insulin active by preventing its glucosylation (27). Thus, in this study, increased insulin level due to Dactylorhiza maculate extract is a possible leptin-increasing mechanism.

Furthermore, stimulating effects of Dactylorhiza maculate have been

demonstrated on pituitary-testes axis, increased testosterone, spermatogenesis and libido, but increased testicular and body weights have not been reported. Researchers have attributed this to the presence of glucomannan in Dactylorhiza maculate (15). Glucomannan is an aqueous fiber that constitutes 7% to 61% of various Dactylorhiza maculate species, and has a major role in weight loss, blood sugar control and cholesterol reduction (9, 10). Studies on the effect of glucomannan on weight loss have reported that it increases viscosity of gastric contents, reduces emptying rate and absorption of contents, increases cholecystokinin secretion, and thus controls body weight (24). It seems glucomannan indirectly affects leptin level by increasing secretion of cholecystokinin. There is evidence that in certain circumstance, cooperation between cholecystokinin and leptin leads to weight loss and reduced calorie intake (28).

Conclusion

It appears that components in aqueous root extract of Dactylorhiza maculate reduce food intake and thus body weight by regulating secretion of leptin.

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Conflict Of Interest

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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