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The effects of *Asian Red Ginseng* ethanolic extract on serum concentration of leptin hormone in male wistar rat

Amirhossein Mansourabadi[1], Nastaran Razavi[2*], Mohammad Hassan Zadeh[3], Maryam Moogooei[1]

[1]*Immunology Dept., Shahid Sadoughi University of Medical Sciences, Yazd, I.R. Iran;* [2]*English Foreign Language Dept., Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran;* [3]*Immunology Dept., Shiraz University of Medical Sciences, Shiraz, I.R. Iran.*

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ABSTRACT

Background and aims: Leptin is a hormone known to control appetite and body weight. The leptin Levels in obese animals are always more than animals with normal size. A wide variety of methods have been offered to treat obesity of which herbal therapy is considered as one of these methods. The aim of this study was to investigate the effect of ethanolic extract of *Asian Red Ginseng* on leptin serum level in male wistar rats.

Methods: In this "in vitro" experimental study, 30 adult male wistar rats were randomly divided into 5 groups equally: control (no drug), SHAM (received 1ml of physiology serrum), Interventional group 1 (received 20mg/kg ethanolic extract of Asian red ginseng), Interventional group 2 (received 40mg/kg) and Interventional group 3 (receiver 80mg/kg). The extract was injected intraperitoneally for 20 days. On the twentieth day, blood samples were collected. Dataset of experiments were collected and analyzed with Graphpad Prism 5 software. Data were analyzed using one-way ANOVA and $P<0.05$ was considered significant.

Results: The dose-dependent injection of the ethanolic extract of *Asain Red Ginseng* root did not show a significant effect on leptin hormone serum level, but it showed a significant decrease in food intake compared to the control group.

Conclusion: The ethanolic extract of Asian red ginseng root does not reduce the serum level of leptin hormone.

Keywords: Ginseng, Male Rat, Leptin.

Original article

INTRODUCTION

Leptin is a hormone known to control appetite and body weight which came from the Greek word (Leptos) means lean. Leptin is a small protein with 167 amino acids which is produced in fat cells and by entering through the bloodstream to the brain and connecting with its receptors on the hypothalamic can affect the appetite. Leptin is identified as the product of a gene in mice and it is shown by OB (it means obese). Mice with two copies of the defective gene genotype (ob/ob), physiology have stable starvation behavior: with increasing the amounts of corticosterone hormone, they are not able to tolerate the heat, but they maintained the normal growth and appetite. Because of the later reason, some of the mice are obese and weighed 3

[*]**Corresponding author:** Shahrekord Univerasity of Medical Sciences, Shahrekord, I.R. Iran, Tel: 00989131807055, E-mail: nastaran.razavi@ymail.com

times more than a normal mice.[1] These mice also have some metabolic disorders such as diabetes and are not able to use insulin. The level of leptin in obese animals is always more than that in animals with normal size (with the exception of animals ob/ob which have mutation in leptin gene and therefore they do not produce leptin). In very rare cases, excessive obesity in the individuals who have leptin gene deficiency reduce the body weight when they have leptin injection, although leptin levels increase by increasing the fat level in the animals with normal OB gene. Obesity leads to many diseases such as diabetes, cardiovascular diseases, hypertension, etc. Stimulating the metabolism and increasing the energy using plant extraction is a routine method to reduce the weight and obesity.[2]

Ginseng is a native plant of eastern Asia and North America. Over thousands of years, the people of China, Korea, Thailand, Vietnam, Manchuria and American Indians have consumed its roots as the medication. This herb is used for centuries to improve memory, reduce stress, relieve impotence, decrease lethargy and other symptoms like fatigue and aging to increase life expectancy.[3,4] The plants has long been used in traditional medicine to deal with body weakness and fatigue. It increases energy and physical strength in addition to improving the immune system, calming the nervous system and supercharging the sex role.[5,6] Ginseng is prescribed as a cure for breast disorders and a treatment for intestinal disorders, cancer, treatment of erectile dysfunction, it increase stamina and energy.[7,8] Ginseng root with high level of antioxidant has a powerful role in improving brain function and helps to regulate blood sugar and general health.[9,10] Studies have shown that some traditional herbs like *asafoetida* can also regulate the secretion of the leptin hormone, which is effective in controlling body weight[11] and in balancing the intake and energy consumption applying by multiple and complex hormonal and neural mechanisms. These mechanisms are based on the amount of food molecules in the blood and body fat, energy metabolism and appetite.[12] White adipose tissue and sparing the intestinal epithelium, placenta, skeletal muscle and brain are major sites of leptin synthesis.[13] Since it is believed that ginseng is effective as a dietary supplement for slimming and fitness and no scientific research studies have directly worked on the effect of ginseng on leptin hormone secretion, the present study was designed to investigate the effect of ethanolic extract of Asian red ginseng on leptin serum level in wistar male rats.

METHOD

This study was an experimental "in vitro" study. Soxhlet method was used. *Asian Red Gginseng* roots were purchased from a reputable grocery. Then, their quality and originality were approved by the experts of agricultural department. After washing, roots were dried and, the vacuum dried roots were powdered (60 g). They were soaked in 300 ml of 80 % ethanol for 72 hours. The extract was filtered and dried by using a rotary. Dried ethanolic extract with doses of 20, 30, 40 and 80 mg were dissolved in 1 ml of distilled water and were refrigerated until use.[14]

Thirteen male wistar rats, with weight of 250-300 gr were bought from Pasteur Institute, Karaj, Iran. The mice were put in clean cages for 12 hours light and 12 hours darkness, the temperature was 22-24°C degree and relative humidity was 60-40% in the research Laboratory of Ghazvin University of Medical Sciences. Animals had access to sufficient food and water.

The animals were randomly divided into 5 groups, 6 in each group: Control group did not receive any substance, but 1

mL of distilled water per kg were injected intraperitoneally to the sham group, ethanolic ginseng root extract were injected intraperitoneally to the interventional groups 1, 2 and 3, daily for 4 weeks, depending on body weight; minimal dose (20 mg/kg), medium dose (40 mg/kg), and maximum dose (80 mg/kg).

On the day twentieth, 5cc of blood from the animals heart (anesthesia with chloroform), was taken with syringe and serum was separated by centrifuging at 2500 rpm for 15 min and samples were kept in -20°C until the test day. For measuring the leptin hormone level in blood samples, crystal chem ELISA kits (purchased from crystal chem. co, USA) have been used.

The dataset of experiments were collected and analyzed with Graphpad Prism 5 software. The results were obtained from the samples and they were compared with each other and with the control and sham groups. Significant level of data was examined with one-way ANOVA method and $P<0.05$ was considered significant.

RESULT

According to the results, injection of average dose (40 mg/kg/day) and maximum dose (80 mg/kg/day) of Asian red ginseng root ethanolic extract and also the minimum dose (20 mg/kg) over a period of 20 days, didn't show a significant increase in serum leptin levels compared to the control ($P<0.05$) and sham group (Figure 1). No significant differences in absorption at different doses were observed compared to the controls. $P<0.05$ was considered statistically significant.

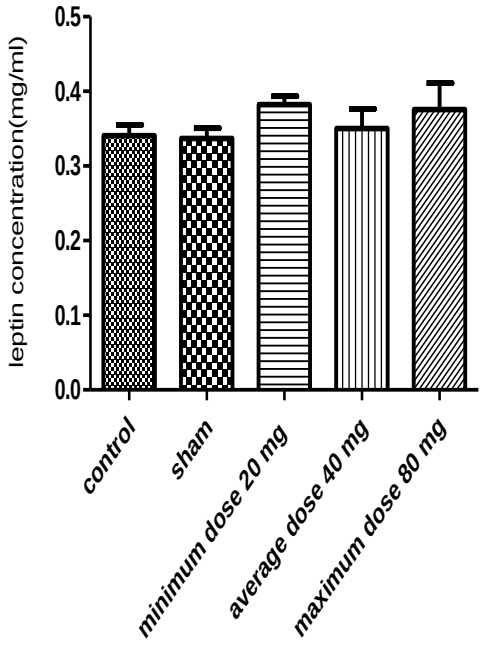

Fig 1: Comparison of changes in serum levels of leptin in the control group and experimental groups receiving different doses of ginseng extract for 20 days.

Means of absorption in the control group and the groups of 20 mg/ml, 40 mg/ml and 80 mg/ml of the extract, had not a significant difference with each other. Average is presented as Mean ± SD. $P<0.05$ was considered statistically significant. Comparison of changes in serum leptin levels in the experimental group and control group is shown in Table 1.

Table 1: Averages of absorption presented as Mean ± SD

Variable / Group	Control	SHAM	Group 1	Group 2	Group 3
Leptin (mg/ml)	0.00734±0.3217	0.00638±0.3998	0.0129±0.3221	0.013±0.3219	0.02114±0.3232

DISCUSSION

Today, medicinal plants have an important role in diet of people.[11] The results showed that the ginseng ethanolic extract in dose dependent manner, despite the reduction in food intake in animals and reduction of appetite, did not increase the serum leptin level. Stabilizing the body weight in this study may be related to stabilizing the leptin serum level according to using of ginseng ethanolic extract with different doses. It has been found that the leptin hormone has a role in controlling the food intake by negative feedback mechanism.[15] Leptin can change gene expression of neuropeptide like fuel intake neuropeptide Y (NPY) by binding to specific receptors in the hypothalamus. Leptin is able to prevent the expression of neuropeptide Y (NPY) which increases food intake and reduces fuel and energy directly.[16] Leptin can also increase corticotropic releasing hormone (CRH) gene expression in the paraventricular hypothalamic nuclei where CRH reduces appetite in this region.[17] Basically, the function of leptin is as a message to prevent obesity because the ob/ob and db/db mice with the lack of leptin resistance, obesity were observed.[18] Prescription of recombinant leptin to these mice increases energy consumption, satiety and lost weight.[19] On the other hand, serum leptin levels reflect the amount of energy stored in fat tissue and by lipogenesis prevention and lipolysis stimulation with paracrine/autocrine action it has a role in adipose tissue metabolism directly.[20] Physiological factors and hormones such as cortisol, insulin, estrogen, glucocorticoids are involved in the regulation of leptin. Many documents from "in vitro" studies on human and murine adipose tissue indicate that insulin stimulates the expression of mRNA and secretion of leptin in adipose tissue.[21] Studies in mice have shown that insulin increases the ob gene and increased plasma leptin levels in normal and diabetic rats.[22] Possible mechanism to explain how leptin stimulates insulin secretion can be expressed in this way that insulin causes glucose transport in adipocytes via GLUT4 (glucose transporter proteins), then glucose acts as an intracellular signal and stimulates the secretion of leptin from fat cells.[23] The results of researches show that prescription of Ferulic acid increases insulin secretion in diabetic rats and decreases the blood sugar.[24] Quercetin also prevents from glycosylation of insulin, which keeps them active.[25] The ingredients of ginseng root extract has a little effect on the inhibition of obesity, although very effective antioxidants are at the root of this plant.[26] On the other hand, the stimulatory sexual power except increasing in the testicular weight and body weight has not been reported.[15] Research studies also suggest that the ginseng reduces serum lipids,[9,10] although studies on the effects of ginseng on the weight loss in mice have been reported.[23] In the present study, there was no significant change in the weight of the mice and it may be because of consumption of dietary supplements and food which were used in the described studies. There are some evidences in some circumstances that show the association between cholecystokinin and leptin, caused losing the weight by reducing calorie intake.[27,28] This trend did not occur in the study of ginseng extract.

CONCLUSION

The results didn't show a significant increase in serum leptin levels compared to the control and sham group after the treatment. It looks like that ingradients of Asian red ginseng root ethanolic extract has no effect on regulating of the secretion of the leptin hormone, and in contrary to

popular belief, they are not effective in reducing body weight.

CONFLICT OF INTREST

The authors declare that they have no conflict of interests.

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