Anti-obesity and Anorectic Effects of Saffron and its Constituent Crocin in Obese Wistar Rat

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Abstract

Background: Obesity is pandemic worldwide and a strong risk factor for cardio-metabolic disorders. The few approved anti-obesity drugs have poor efficacy and safety profile. Thus, there is an urgent need for new anti-obesity agents. According to the traditional medicine and a clinical trial, saffron may have anorexigenic and anti-obesity effects which need further investigation.

Objectives: Evaluation of the effects of saffron and crocin on body weight, food intake and blood leptin levels in obese Wistar rat.

Methods: In the present study, saffron methanolic extract (25, 50, 100, 200 mg/kg) and its active constituent crocin (5, 15, 30, 50 mg/kg), sibutramine (5 mg/kg) and saline were gavaged daily to obese Wistar rats for 2 months and their effects on the body weight, food intake and blood leptin levels were evaluated.

Results: The saffron extract and crocin at all doses as well as sibutramine reduced body weight, food intake and leptin levels significantly compared to saline and baseline ($p < o.o5$). The extract and crocin effects were comparable to sibutramine.

Conclusion: Saffron has anti-obesity and anorectic effects in the obese Wistar rat. The lowered leptin levels indicate that saffron reduces fat mass and increases insulin sensitivity. Crocin may be one of the active constituents involved in the effects of saffron. The effects of saffron and crocin may have important clinical implications in terms of treatment and prevention of obesity in humans.

Keywords: ***Crocus sativus*, Crocin, Obesity, Rat, Saffron**

Introduction

Obesity and overweight are rapidly growing health hazards around the world. Obesity/overweight is associated with many related co-morbid conditions such as cardiovascular disease [1, 2]. Orlistat, lorcaserin and phentermine-topiramate combination are currently the anti-obesity drugs approved for long-term use. Gatsrointestinal side effects of orlistat (abdominal pain/discomfort and oily spotting) and abuse potential of lorcaserine and phentermine-topiramate combination are their main limitations [3, 4]. Historically, anti-obesity drugs have been unsafe and minimally efficacious. Hence, there is increasing need for new drugs that feature higher efficacies and improved safety profiles for the prevention and therapy of obesity [5, 6].

A variety of natural products, including crude extracts and isolated compounds from plants, can reduce body weight and prevent diet-induced obesity. Moreover, natural products may be used as a source for developing future effective, safe anti-obesity drugs [7]. Spices are functional foods and have been used medicinally for millennia [8, 9]. Spices may reduce body weight through modification of energy balance in the body [9, 10]. Moreover, chronic inflammation and oxidative stress caused by obesity are responsible for its co-morbidities like cardiovascular disease [8, 11]. Anti-inflammatory and anti-oxidative effects of spices can slow progression of the obesity co-morbidities [8, 12, 13]. Saffron is obtained from the flowers (dried, dark red stigmata) of *Crocus sativus* L. (Iridaceae) and is cultivated principally in Iran and on a small scale in Morocco, India, Greece, Italy, Spain and France. Saffron is the most expensive spice known and is employed mainly to give color and flavor to foods. It also has many traditional uses such as appetite suppression and treatment of cancer, asthma, menstruation disorders, liver disease, pain and mental depression [14]. Mohammad Ibn Zakariya Razi (Rhazes) and Hossein Ibn Ali Ibn Sina (Avicenna) have pointed out the anorexigenic effect of saffron [14, 15]. The chief constituents of saffron are the carotenoids including crocetin and crocin (crocetin glycoside) and the monoterpene aldehydes picrocrocin and safranal. Saffron and its active constituents have had anti-inflammatory, anti-oxidative [16, 17], anti-hyperglycemic, insulin sensitizing, blood insulin elevating and anti-hyperlipidemic effects [18-22] in previous studies. Further, saffron is cardio-protective [17]. There has been only one study into the effect of saffron on body weight. In that study, saffron extract administered at only one dose had body weight reducing and satiating effects in mildly overweight healthy women. However, the active constituents responsible for the effects of saffron were not determined in the study [23] and the results of the study have not been replicated so far. Besides, the blood leptin level is a good index of body fat mass and insulin sensitivity. The effects of saffron and its constituents on the leptin level have not been examined. Given the data about saffron, further preclinical and clinical research on its possible anti-obesity effect is warranted [24]. Notably, animal models have remarkably good predictive validity for the clinical efficacy of anti-obesity drugs. Anti-obesity agents produce similar effects on food intake and body weight in laboratory rodents and humans [6, 25]. Thus, in the present study, the effects of gavage of various doses of saffron and its active constituent crocin and sibutramine on the body weight, food intake

and serum leptin level were evaluated and compared with saline in obese Wistar rats.

Materials and Methods

Saffron

The stigmas of *Crocus sativus* were collected from the lands of Ghaen in the Iranian province of southern Khorasan in December and dried in shade followed by grinding. The identity of *Crocus sativus* was authenticated by a botanist (Yousef Ajanii) and a voucher specimen of the plant (number 15064) was deposited in the Tehran University Central Herbarium.

Preparation of the saffron extract:

The dried stigmas powder (260 g) was extracted with methanol/water (80/20) as the solvent in a percolator three times, the solvent was completely removed from the methanolic extract at 42 ºC by Rotavapor and 30 g dried extract was produced [26].

Drugs

Crocin and sibutramine hydrochloride (purity above 99%) were purchased from Sigma. For dilution, crocin, sibutramine and the extract were dissolved in physiological saline. All drugs were prepared immediately before use.

Animals

Adult male Wistar rats weighing 400-500 g from our own breeding colony were used. The rats had readily become obese on a standard diet. The rats were individually housed in metabolic cages. The animal facility had a 12:12 h light-dark cycle (lights on at 6:00 a.m.), a constant temperature of 23-25 ˚C and relative humidity of 40-45%. The rats had free access to standard rodent feed and water.

Experimental protocol:

The animals were randomly divided into 10 groups (N=10 in each group). The groups were matched with regard to body weight:

Group I: Rats received physiological saline.
Group II: Rats received the extract (25 mg/kg).
Group III: Rats received the extract (50 mg/kg).
Group IV: Rats received the extract (100 mg/kg).
Group V: Rats received the extract (200 mg/kg).
Group VI: Rats received crocin (5 mg/kg).
Group VII: Rats received crocin (15 mg/kg).
Group VIII: Rats received crocin (30 mg/kg).
Group IX: Rats received crocin (50 mg/kg).
Group X: Rats received sibutramine (5 mg/kg)

The data given here relate to the doses that not only did not cause any mortality in the rats after 2 months daily oral administration but also the effects of each dose on the body weight and food intake at the endpoint were significantly different from the control group. Each animal was used only once in all experiments. Animals were treated by oral gavage once a day for 2 months. To avoid irritation of the animal throat, the gavage needle was very thin with 1.25 mm diameter round tip and gavage was performed carefully. The extract and crocin were dissolved and administered in physiological saline in a volume of 5 ml/kg. At the beginning and the end of the study, body weight (g), daily food intake (g) and blood leptin level (pg/mL) of each animal were determined [27]. Blood was drawn from rat tail vein after overnight fasting and serum leptin level was measured using rat leptin ELISA kit (BioVendor, Brno, Czech Republic).

Statistical analyses

The data were presented as mean ± S.D. (standard deviation) and analyzed with the paired samples t test and One-Way ANOVA

followed by the tukey post hoc test. $p < 0.05$ was taken as significant.

Results

The rat groups receiving saffron, crocin or sibutramine were not significantly different from the control group with regard to body weight, food intake and leptin level at the baseline ($p > 0.05$). The saffron extract and crocin at all doses as well as sibutramine reduced the body weight, food intake and leptin levels significantly compared with the control group and baseline after 2 months of administration ($p < 0.05$). The percent body weight, food intake and leptin reduction from baseline by saffron and crocin is comparable to sibutramine (Figures 1 - 6).

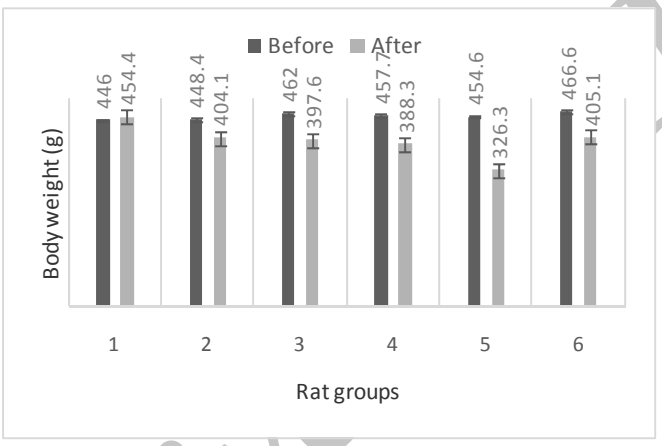

Figure 1- The body weight (g) before and after receiving oral saline (group 1), saffron extract (25, 50, 100, 200 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

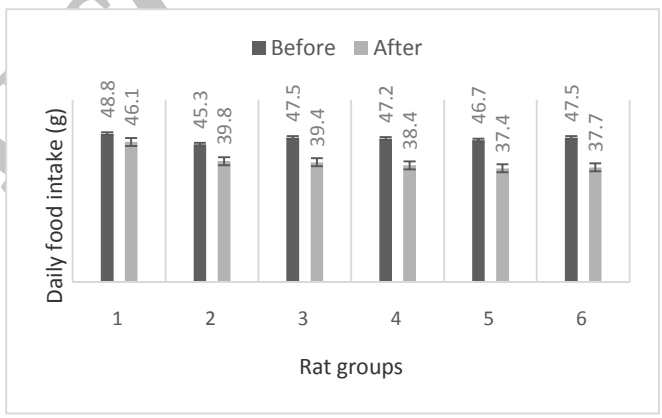

Figure 2- The daily food intake (g) before and after receiving oral saline (group 1), saffron extract (25, 50, 100, 200 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

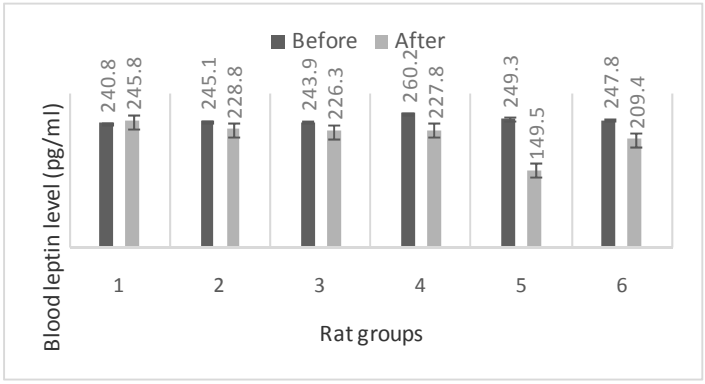

Figure 3 - The blood leptin level (pg/mL) before and after receiving oral saline (group 1), saffron extract (25, 50, 100, 200 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

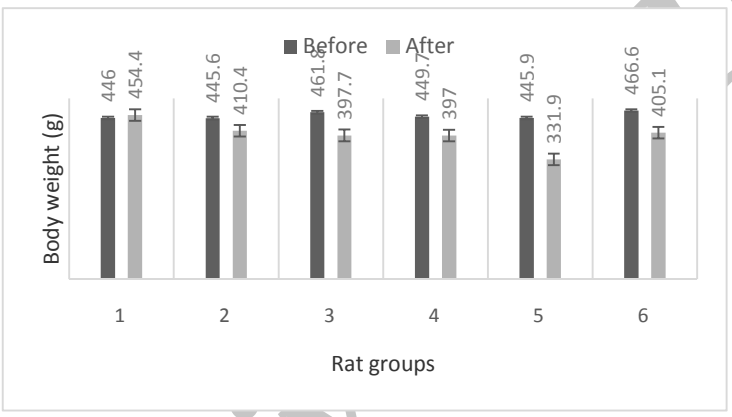

Figure 4- The body weight (g) before and after receiving oral saline (group 1), crocin (5, 15, 30, 50 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

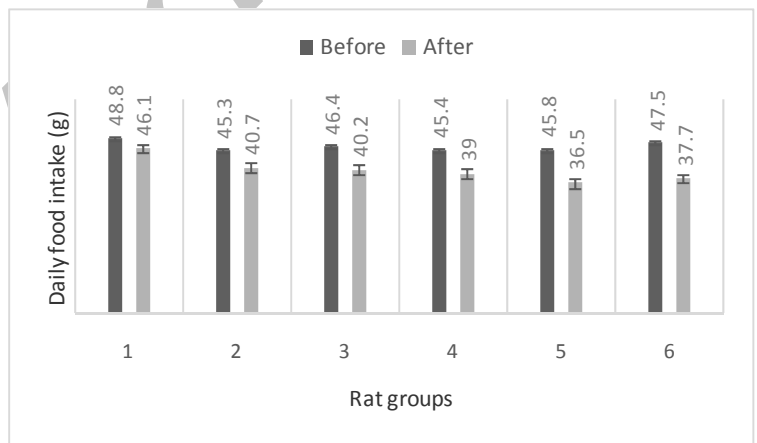

Figure 5- The daily food intake (g) before and after receiving oral saline (group 1), crocin (5, 15, 30, 50 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

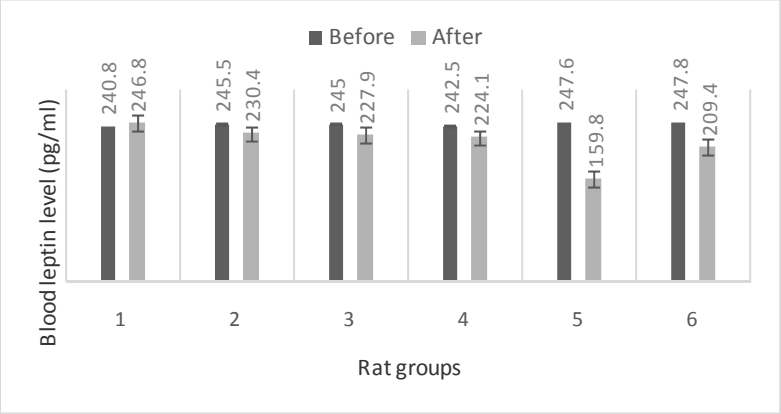

Figure 6- The blood leptin level (pg/mL) before and after receiving oral saline (group 1), crocin (5, 15, 30, 50 mg/kg; groups 2-5) and sibutramine (5 mg/kg; group 6) for 2 months. N = 10 rats in each group. The data are given as mean ± S.E. The number above each column represents mean.

Discussion

Prevalence of obesity and its associated co-morbidities and poor efficacy and safety profile of the few approved anti-obesity drugs necessitate finding new anti-obesity agents. Saffron and crocin have wide therapeutic indexes. The oral administration of hydro-alcoholic (ethanol 80%) extract of saffron was non-toxic at the doses up to 5 g/kg with LD_{50} above 5 g/kg in mice [28]. Acute (up to 3 g, p.o. and i.p.) and chronic (15-80 mg/kg, i.p.) administration of crocin did not cause any biochemical, hematological and histopathogic toxicity in mice and rats [29]. Saffron and crocin have high safety [28, 29]. Thus, the doses of saffron and crocin in the current study were non-toxic. Considering the information on the properties of saffron, this study was conducted. The obese Wistar rat used in this study can be regarded as a rodent model of polygenetic obesity. The results suggest that saffron and crocin reduce the body weight, food intake and blood leptin levels significantly compared to the control group and baseline. The effects of saffron and crocin are comparable to sibutramine. Thus, the results are in keeping with the anorexigenic effect of saffron in the traditional medicine and the earlier findings [23]. Moreover, it shows that crocin is at least one of the ingredients involved in the effects of saffron. Leptin is secreted mainly by white adipose tissue, and levels are positively related to the amount of body fat and adiposity [30]. Hyperleptinemia is a result of obesity [31]. Hence, the lowered leptin levels in this study also indicate that saffron and crocin reduce body fat. Leptin secretion improves insulin sensitivity and glycemic control in rodents and humans [32]. Hyperleptinemia is indicative of insulin resistance. The fasting plasma leptin level may be a more robust surrogate measure of insulin sensitivity than insulin [33]. Accordingly, the present study conforms to the reports of insulin sensitizing effects of saffron and crocin [19-21]. The results also show that saffron and crocin cause body weight loss due to their anorectic effect. Parameters affecting body weight include food intake, energy expenditure (thermogenesis), nutrient absorption and fat modulation [1]. Therefore, besides reduced food intake, other mechanisms such as anti-oxidative action and increased lipid and glucose metabolism may have a role

in the anti-obesity effect of saffron and crocin [24]. Crocetin existing in the saffron or produced by crocin disintegration in the gut and crocin may reduce fat absorption and as a result energy intake and body weight through pancreatic lipase inhibition [34, 35]. The mechanisms controlling food intake are numerous and complex. Here, the mechanisms of the anorectic effects of saffron and crocin and also the saffron and crocin effects on the energy expenditure (thermogenesis), nutrient absorption and fat modulation were not evaluated, which can be regarded as the shortcomings of the present study. Crocin is hydrolyzed to crocetin before or after absorption in the rat intestine and crocetin but not crocin is found in the bloodstream [36]. Low bioavailability of plant bioactives does not preclude their anti-obesity effect. Plant bioactives may suppress appetite by action in the gut or brain [13]. Thus, crocin may act in the gut or through the blood crocetin in the brain to reduce food intake. However, active ingredients of saffron in addition to crocin may also be involved in the effects of saffron on the food intake and the body weight in the rat. Finally, considering the results of the present study, further research on the mechanisms and constituents involved in the anti-obesity effect of saffron seem necessary. Moreover, conduction of clinical trials regarding anti-obesity efficacy, pharmacokinetics, optimal dosage, long term safety and potential side effects of crocin use in the treatment of obese individuals is warranted.

Conclusion

Saffron has anti-obesity and anorectic effects in the obese Wistar rat. The lowered leptin levels indicate that saffron reduces fat mass and increases insulin sensitivity. Crocin may be one of the active constituents responsible for the effects of saffron. The effects of saffron and crocin may have important clinical implications in terms of treatment and prevention of obesity in humans.

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