Preclinical study of an antiobesity phytotherapic compound obtained from Cactus Cereus plantas in male and female rats fed with high-fat diet: comparison with sibutramine

Estudo pré-clínico de um composto fitoterápico antiobesidade, obtido de plantas Cactus Cereus em ratos machos e fêmeas alimentados com dieta hipercalórica: comparação com a sibutramina

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Resumo

Abstract

Objective – To assess the effectiveness of a phytotherapic compound developed from a Cereus peruvianus, plants as an adjuvant treatment of obesity in rats fed with a high-fat diet. Phytotherapy is becoming increasingly popular both for the results it yields in several pathologies and because of a growing sense of mistrust toward conventional medical treatments. **Methods** – Male and female rats were fed with a high-fat diet for one month. The diet was then replaced by a chow diet and a phytotherapic compound (8 mg/kg; KOUBO™) was orally administered twice a day over 30 d. Body weight gain was assessed weekly and, at the end of treatment, total body weight gain was calculated. A positive control with sibutramine (7.5 mg/kg, twice a day, orally, over 30 d) was also included. **Results** – A significant reduction in weekly body weight gain, as well as in total weight gain, in both male and female rats after phytotherapic compound administration. The index of body weight loss showed that the phytotherapic compound was more effective in reducing body weight in female than in male rats. The sibutramine treatment showed the same profile as the phytotherapic compound treatment. **Conclusion** – The present data indicate that KOUBO™ phytotherapic compound was effective in decreasing body weight in both, male and female rats, submitted to a high-fat diet, and showed a similar profile to that of sibutramine.

Descriptors: Phytotherapy; Obesity; Medicinal plant; Body weight loss

Resumo

Objetivo – Avaliar a eficácia de um medicamento desenvolvido a partir de um grupo de plantas do gênero *Catus Cereus* no tratamento adjuvante da obesidade em ratos alimentados com uma dieta hipercalórica. A fitoterapia está se tornando cada vez mais popular, tanto pelos resultados positivos em diversas doenças e porque eles estão crescendo estudos de medicina de ervas que mostram a sua eficácia. **Métodos** – Ratos machos e fêmeas foram alimentados com uma dieta rica em gordura durante um mês. A dieta foi então substituída por uma ração normal do biotério. Todos os ratos e ratas foram com 8mg/kg de KOUBO™ ou água. O tratamento foi administrado por via oral, duas vezes por dia durante 30 dias. O ganho de peso corporal foi avaliado semanalmente e, no final do tratamento, o ganho de peso total foi calculado. Como controle positivo empregou-se a sibutramina (7,5mg/kg, duas vezes por dia, por via oral durante 30 dias. **Resultados** – Observou-se redução significativa no ganho de peso corporal semanal, bem como do ganho de peso total, tanto nos ratos macho e fêmeas, após administração do medicamento à base da planta. O índice de perda de peso corporal mostrou que o fitoterápico KOUBO™ foi mais eficaz na redução do peso corporal nas fêmeas do que em ratos machos. O tratamento com sibutramina mostrou o mesmo perfil do fitoterápico. **Conclusão** – Os presentes dados indicam que o fitoterápico KOUBO™ foi eficaz em diminuir o peso corporal em ratos machos e fêmeas submetidos a uma dieta rica em gordura, e mostrou um perfil semelhante ao da sibutramina.

Descritores: Fitoterapia; Obesidade; Medicina natural; Perda de peso

Introdução

Obesity is a major health problem facing the developed and developing world. Efforts by individuals, health professionals, educators, and policy makers to combat the escalating trend of growing obesity prevalence have been multifaceted and mixed in outcome. Various dietary supplements have been marketed to reduce obesity. These products have been suggested to accomplish this by decreasing energy intake and energy absorption, and/or increasing metabolic rate. Obesity and overweight are associated with several disorders, including cancer, diabetes, and heart disease, and have become two of the most important risk factors for morbidity and mortality in both men and women[1]. The evidence that obesity is a health problem that is difficult to control is apparent not only in statistical data but also in observations of the general public. The development of new medicines to control weight is the object of much recent attention by the food and drug industries.

Cereus peruvianus (L.) Miller (apple cactus, known also as koubo) is a large thorny columnar cactus, native to the subtropical southeastern coast of South America[2]. *C. peruvianus* is also common as an ornamental plant[2] and a commercially grown columnar cactus that produces an apple sized, berry like, edible fruit. An important attribute to fruit quality and consumer acceptability is its overall flavor[3-4]. An increase in sugar content, a decrease in organic acids, enhanced accumulation of aroma vo-

latiles, and changes in fruit color characterize the ripening of fruits. The unique aroma of this fruit is largely due to S-linalool and linalool derivatives[4]. Enzyme activity levels were negligible in green immature fruits and increased with the fruit development and during storage, concomitant with the timing of linalool accumulation in fruits[5]. This cactus is consumed in many countries it is rich source of protein, fiber, vitamin C, in its fresh form, fatty acids, amino acids, sugars and other substances.

Phytotherapy is becoming increasingly popular both for the results it yields in several pathologies and because of a growing sense of mistrust toward conventional medical treatments. Nowadays, it is possible to find herbal formulations that maintain the plant-specific characteristics and have undergone microbiological and analytical tests. In this preclinical study, we assessed the effectiveness of a phytotherapic compound developed from *Cactus Cereus* as adjuvant treatment of obesity in rats fed with a high-fat diet.

Methods

Animals

Adult male (200-250 g and 60 d old) and female (150-200 g and 60 d old) Wistar rats (Department of Pathology, School of Veterinary Medicine, University of São Paulo, Brazil) were used. The animals were housed in polypropylene cages (40 × 50 × 20 cm) at a regulated temperature (20 ± 2°C) and humidity (70 ± 5%) on a controlled light schedule (12 h light: 12 h dark), with lights on at 6:00 AM. The animals used in this study were kept in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo (protocol N.º 2041/2010 in 27/10/2010, FMVZ-USP). These guidelines are based on those of the U.S. National Institutes of Health. The experiments were performed in accordance with good laboratory practice protocols and with quality-assurance methods.

Phytotherapic Compound

The following manipulated product was employed: capsules with 200 mg of KOUBO™ (X'tract Vetorized) + excipients: 30 mg mannitol, 0.75 mg aerosil, 1.5 mg magnesium estearate, cellulose/talc (1:1) qs 100%. The capsules were manipulated in the Pharmacopeia® CIL laboratories (Brazil), specially developed for this assay. The gelatin capsules were made with *pullulan* to protect the extract and increase its shelf-life. This herbal preparation was registered in ANVISA KOUBO n.º 25352. 403822/2010-60.

Drug

Sibutramine was presented as 15 mg capsules plus excipients, qs 100%. The capsules were manipulated in an existing pharmacy (São Paulo, SP, Brazil – CNPJ 61.744.595/0001-84).

Treatments and Experiment Design

Thirty male and 30 female rats were fed with a high-fat diet (60% kcal, Rhoster Industria e Comércio Ltda, Rua José Egon Knittel, 120 – Jardim Tonelli, Araçoiaba da Serra/SP – CEP 18190-000, Brazil) for 30 d. Then, the rats were divided into 6 groups (3 male and 3 female groups) with 10 rats in each group. The high-fat diet was replaced with a chow diet (3.3 kcal) and the 3 groups of male rats received twice-a-day oral administrations of water (control group), 8 mg/kg of KOUBO™, or 7.5 mg/kg of sibutramine. The same procedure was performed with the remaining 3 groups of female rats. These treatments were administered for 30 d. The rats in all groups were weighed daily during treatments and observed for gross signs of toxicity. Weekly weight gain was calculated and at the end of the experiment and the delta of weight loss (DWL) in vivo was computed as

$$DWL = \frac{(WIT - WFT) \text{ X } 100 - (WIP - WFP) \text{ X } 100}{WIT \text{ X } WIP},$$

where

WIT = Initial body mass on the first day of the phytotherapic treatment,
WFT = Final body mass on the last day of the phytotherapic treatment.
WIP = Initial body mass on the first day of the placebo treatment, and
WFP = Final body mass on the last day of the placebo treatment.

At the end of the treatments, the rats were euthanized in $C0_2$ and examined for lesions or other alterations.

Statistical Analysis

Repeated measures two-way ANOVA was used to compare data of weight gain. One-way ANOVA was employed to compare the total weight gain between the control and experimental groups of male or female rats. In all cases, values of $P < 0.05$ were considered statistically significant. The statistical analyses were performed using GraphPad Prism software, version 5 (GraphPad, San Diego, CA, USA).

Results

Fig. 1 (A and B) shows the weekly weight gain of male and female rats fed with hypercaloric or normal diets. In male rats (Fig. 1A), the two-way ANOVA showed that treatments [$F_{2/108} = 2{,}214.43$, $P < 0.0001$] and number of weeks [$F_{3/108} = 137.72$, $P < 0.0001$] influenced the results, with significant interactions between the factors [$F_{6/108} = 301.87$, $P < 0.0001$]. In relation to the control group, the Bonferroni post hoc test revealed that weight gain of male rats treated with KOUBO™ and sibutramine was significantly reduced ($P < 0.0001$); KOUBO™ and sibutramine data did not differ ($P > 0.05$). Also, the

weight gain in both experimental groups was lower than that in the control group ($P < 0.0001$) throughout all four weeks of the treatments.

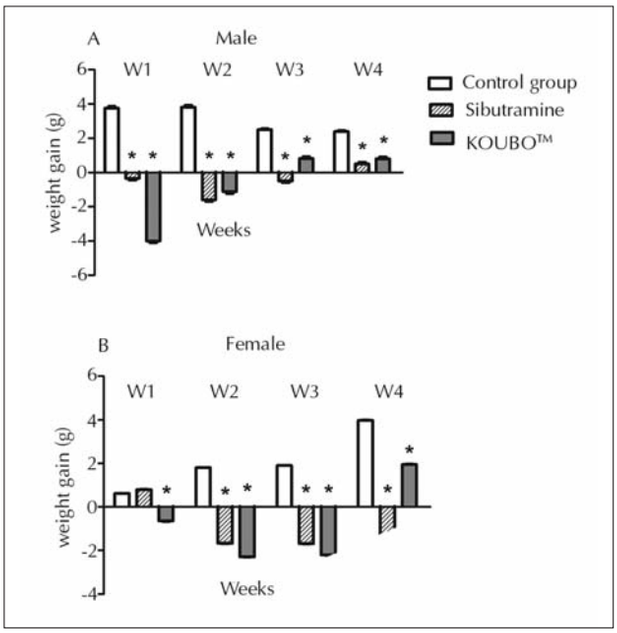

Figure 1. Weekly body weight gain (g) in male (A) and female (B) rats fed previously with a high-fat diet (1 month) and treated with KOUBO™ (8 mg/kg) or sibutramine (positive control group – 7.5 mg/Kg) or water (control group) twice a day. During treatments, rats received the normal chow. Data are presented as means ± SEM. * p< 0.0001, two way ANOVA followed by the Bonferroni test, in relation to control group.

In female rats (Fig. 1B), the two-way ANOVA showed that treatments [$F_{2/108}$ = 117,037.76, $P < 0.0001$] and number of weeks [$F_{3/108}$ = 33,204.75, $P < 0.0001$] influenced the results, with significant interactions between the factors [$F_{6/108}$ = 19,871.42, $P < 0.0001$]. In relation to the control group, the Bonferroni post hoc test revealed that weight gain of female rats treated with KOUBO™ and sibutramine was significant reduced ($P < 0.0001$); also weight gain with KOUBO™ was decreased in relation to that with sibutramine in all weeks of treatments ($P < 0.0001$).

Fig. 2 shows the data for total weight gain (A) and the DWL index (B). In relation to the control group, male rats treated with KOUBO™ and sibutramine had significantly reduced total weight gain [$F_{2/29}$ = 38.77, $P < 0.0001$]; no differences were detected between treatments ($P < 0.05$).

Treatments significantly reduced the total weight gain in female rats in relation to the control group [$F_{2/29}$ = 53.27, $P < 0.0001$]. Data from the treated female rats differed, with total weight gain with KOUBO™ being lower than that with sibutramine ($P < 0.05$).

The DWL index obtained was 6.5% in male rats and 7.37% in female rats. Finally, no gross signs of toxicity were observed during the experiment and no lesions were detected at the end of the treatments.

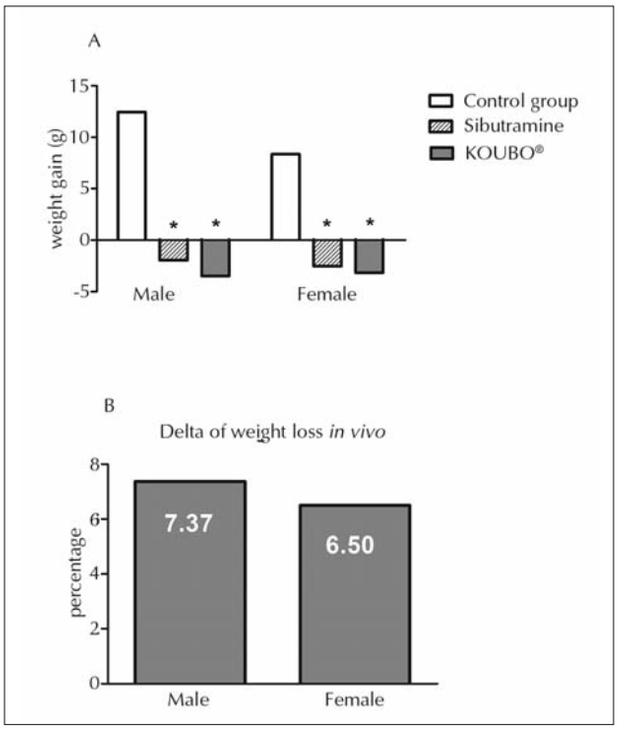

Figure 2. Total weight gain (A) and the delta of weight loss in vivo (B) of male and female rats fed previously with a high-fat diet (1 month) and treated with KOUBO™ (8 mg/kg) or sibutramine (positive control – 7.5 mg/Kg) or water (control group) twice a day. During treatments, rats received the normal chow. Data of total weight gain are presented as means ± SEM. * p< 0.0001, one way ANOVA followed by the Bonferroni test, in relation to control group. Data of delta of weight loss in vivo are presented in percentage.

Discussion

The phytotherapic compound evaluated in the present study significantly reduced the weight gain of male and female rats. The loss in weight gain of the phytotherapic group was examined weekly and at the end of treatment, and showed a high level of efficacy. These data, when compared with that of the anti-obesity drug sibutramine, were very similar in both, male and female rats. No gross signs of toxicity, organs lesions, or hemorrhage were detectable by visual examination at the end of the experiment.

Male and female rats received a hypercaloric diet for one month to induce weight gain and then it was tested whether KOUBO™ had an anti-obesity effect. The weekly weight gain data revealed that in male rats there was an abrupt decrease in body weight in the experimental group relative to the control group, in the first week of treatment; this decrease in body weight gain was attenuates in the remained weeks. Female rats treated with KOUBO™ presented significant reductions in body weight gain in the second and third weeks of treatment. Despite in the last week of treatment these female rats did not showed a reduced weight gain this weight gain was lower than those of the control rats.

These different profiles of the anti-obesity properties of KOUBO™ in male and female rats could be attributed to sexual dimorphism on pharmacokinetics. In fact,

sex-based differences in pharmacokinetics and pharmacodynamics are widely recognized[6-8] and can be important sources of individual differences in drug responses. Sex-based differences in pharmacokinetics reflect differences in bioavailability, distribution, metabolism, and/or excretion. Sex hormones influence bioavailability through effects on gastrointestinal motility; for example, estrogen inhibits gastric emptying. Sex differences in pharmacokinetics can result from sex differences in distribution, which can be caused by differences in body weight (lower in women), body fat (higher in women), plasma volume (lower in women, but varies throughout the menstrual cycle and during pregnancy), and organ blood flow (higher in women)[6].

The total weight gain data showed that, in relation to the respective control group, male and female experimental rats treated with the phytotherapic compound had a similar decrease in total weight gain. However, this apparent similarity may not reflect the actual weight loss because the sexual dimorphism interferes on body weight. Thus, we employed the DWL index, which allowed the minimization of these interferences. Thus, when we accounted for the initial body weight of the control and experimental groups, the DWL showed that the phytotherapic compound was more effective in male than in female rats in inducing body weight loss. Interestingly, sibutramine, a drug used to control overweight and obesity and employed here as a positive control, showed the same profile of reduction in body weight as the phytotherapic compound.

Although the present results do not provide data on the mechanism by which this phytotherapic compound reduced the weight of the animals, some indications attributed the antiobesity properties of *Cactus Cereus* to the presence tyramine e N-methyltryamine increasing the satiety[9].

Conclusions

The present data indicate that KOUBO™ phytotherapic compound was effective in decreasing body weight in male and female rats submitted to a high-fat diet and showed a similar profile to that of sibutramine. Although this compound has been commercially available in Brazil, its efficacy has never been assessed in a preclinical trial until now.

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