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Research Paper

Hypericum silenoides Juss. and *Hypericum philonotis* Cham. & Schlecht. extracts: in-vivo hypolipidaemic and weight-reducing effects in obese rats*

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Keywords
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Abstract

Objectives This study was carried out to assess the anti-obesity effect of *Hypericum silenoides* Juss. and *Hypericum philonotis* Cham. & Schlecht. in male Wistar rats fed with a cafeteria diet.

Methods Adult male Wistar rats with an initial body weight of 290–320 g were used in this trial. The rats were fed with a cafeteria diet for 77 days. *Hypericum* species were administered orally at a dose of 10, 30 or 100 mg/kg of body weight daily for 35 days. Body weight, food intake, anorexic effect and various biochemical parameters, such as serum glucose, lipid profile, alanine transaminase (ALT), aspartate transaminase (AST) and atherogenic index (AI), were assessed. Additionally, inhibitory lipase activity assay and forced swimming test were also carried out.

Key findings Oral administration of *H. silenoides* and *H. philonotis* extracts resulted in a significant decrease in body weight and serum glucose levels in obese male Wistar rats. Treatment with aqueous extract of *H. silenoides* showed anorexic and antidepressant effects and also significantly ($P < 0.05$) decreased total cholesterol, triglycerides and high-density lipoprotein-cholesterol, while low-density lipoprotein-cholesterol, AI, AST and ALT were not changed. The dichloromethane extract of *H. silenoides* (half maximal inhibitory concentration (IC_{50}) = 262.79 ± 0.09 μg/ml) and hexane extract of *H. philonotis* (IC_{50} = 162.60 ± 0.02 μg/ml) showed the most potent lipase inhibitory activity.

Conclusion Some *H. silenoides* and *H. philonotis* extracts showed a significant anti-obesity activity in cafeteria-diet-fed rats. This research provides the first scientific support for the use of the *Hypericum* genus for weight reduction in Mexican folk medicine.

Introduction

Recently, obesity has increased at an alarming rate and it is a worldwide health problem. It is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure. Additionally it is characterized by increased fat mass and elevated lipid concentration in blood.[1,2] Currently, more than 1.4 billion adults worldwide are overweight and at least 500 million of them are clinically obese.[3]

Obesity is considered to be a risk factor associated with the development of major human diseases, including cardiovascular diseases, hyperlipidaemia, cancer and diabetes.[4] Western diets are high in fat and tend to promote obesity. Increased intake of high-calorie (energy and fat) food promotes body-fat storage and greater body weight and adiposity in humans[5] and animals.[6] Cafeteria diet is a useful tool with which to study obesity in animals[7,8] and humans due to the similarity in development of obesity. In this model of obesity, the diet is self-selected, by animals, from a variety of supermarket foods that usually are high in fat and/or carbohydrates and low in proteins, vitamins and minerals.[9,10]

Despite important efforts made to develop anti-obesity drugs worldwide, only two agents–sibutramine (Reductil or Meridia, banned in some countries), an appetite

suppressant, and orlistat (Xenical), which reduces intestinal fat absorption through inhibition of lipases–have been successfully introduced into the market during recent years.[11,12] On the other hand, natural products for the treatment of obesity are actively under research, and could be an excellent alternative strategy for the development of effective and safe anti-obesity drugs.[13,14] Plant species of the genus *Hypericum* are well known for their therapeutic uses in the traditional medicine of several cultures. One of the most important and commercially recognized species of this genus is *Hypericum perforatum* L. (St John's wort) and this plant is mainly used as an antidepressant.[15,16] However, recent studies have demonstrated a significant antihyperglycaemic activity in diabetic rats treated with crude extracts of *H. perforatum*.[17,18] Another trial demonstrated that a hydroalcoholic extract of *H. perforatum* decreases body-weight gain, total cholesterol, low-density lipoprotein-cholesterol, triglycerides, glucose and insulin in rats fed with a high-fat diet.[19] It has been also been suggested that *Hypericum* spp. could provide an alternative therapy for obese patients.[19] The Mexican flora includes some *Hypericum* spp. that could be a natural treatment for obesity. Twelve species of the genus *Hypericum* are known to be present in Mexico (*H. denticulatum*, *H. fastigiatum*, *H. formosum*, *H. galinum*, *H. hypericoides*, *H. moranense*, *H. mutilum*, *H. paniculatum*, *H. pauciflorum*, *H. philonotis*, *H. silenoides* and *H. simulans*).[20] Of these 12 species of *Hypericum*, *H. silenoides* is the most abundant and frequently used species in Mexican folk medicine for the reduction of weight.[21,22] Its popular name is 'tlanchalagua'. The common name for *H. philonotis* is 'vinagrillo' and there are no reports describing its use in traditional medicine.[20] Additionally, in Mexico several commercial products exist for the reduction of weight, which include in their composition species of the *Hypericum* genus. However, there is no scientific evidence that accounts for the anti-obesity activity of these plants. In this study we show that some *H. silenoides* and *H. philonotis* extracts decreased body-weight gain, altered some serum variables and produced an anorexic effect when a novel experimental cafeteria diet was fed to rats. Additionally, both species showed an inhibitory effect on lipase activity *in vitro*.

Materials and Methods

Plant material

Hypericum silenoides Juss. was purchased at Sonora Market in Mexico City, during February 2010. A voucher specimen (No. 15542) was deposited in the Herbarium of the Instituto Mexicano del Seguro Social in Mexico City. Plant identification was confirmed by A. Aguilar.

Hypericum philonotis Cham. & Schlecht. was collected from Pátzcuaro, Michoacán de Ocampo, Mexico, during September 2010. Plant identification was carried out by a botanist at the Herbarium IEB of the Institute of Ecology, A. C., Mexico (IE-Bajío) where a voucher specimen was deposited (No. 14864).

Preparation of *Hypericum* extracts

Organic extracts

Five kilograms of the aerial parts of *H. silenoides* were dried at room temperature (~22°C) under shadow. After grinding, they were consecutively extracted by maceration at room temperature three times for three consecutive days, each time with 20 l of the following solvents: hexane, dichloromethane and methanol. After evaporation of the solvents under vacuum (Büchi B-480, Büchi Labortechnik AG, Flawil, Switzerland), 124.8 g of hexane extract, 74.8 g of dichloromethane extract and 678.1 g of methanol extract were obtained. The same procedure was applied to 3 kg of the aerial parts of *H. philonotis* and 70.7 g of hexane extract, 58.3 g of dichloromethane extract and 283.0 g of methanol extract were obtained. All extracts were kept in an airtight and waterproof containers and stored at 4°C until their use.

Aqueous extract

Five hundred g of dried *H. silenoides* was cut into small pieces and simmered for 30 min in a conical flask containing 2 l of distilled water. The decoction was allowed to cool at room temperature for approximately 1 h and then was filtered through a piece of clean white cotton pad. The filtrate was evaporated to complete dryness in a vacuum (Büchi V 850, Büchi Labortechnik AG) at 50 °C and 72 mbar. The semisolid residue (11.3 g) was kept in an airtight and waterproof container and stored at 4°C until its use. The same procedure was applied to 500 g of *H. philonotis* and 17.4 g of aqueous extract was obtained.

HPLC analysis

The composition of aqueous extract from *H. silenoides* was analysed by high-performance liquid chromatography (HPLC) according to Pharmacopeial Method (USP 30).[27] Briefly, the HPLC system consisted of a 600 Waters Delta pump (Waters, Milford, MA, USA) equipped with a Waters 600E automated gradient controller and a Waters 717 Plus automatic sample injection module. The detection was performed with a photodiode array detector (PDAD) Waters 2996 working in the range of 200–400 nm. The chromatograms were recorded and processed by Waters Empower 5.0 software. The concentration of the aqueous extract of *H. silenoides* extract was 1 mg/ml (methanol–water, 9 : 1, v/v).

Analyses were carried out at 30°C with a Symmetry C_{18} column (4.6 × 250 mm internal diameter, 5 μm; Waters,

Table 1 Linear gradient programme for HPLC analysis

Time (min)	Solvent A (%)[a]	Solvent B (%)[b]	Solvent C (%)[c]
Initial	100	0	0
10	85	15	0
30	70	20	10
40	10	75	15
55	5	80	15
56	100	0	0
65	100	0	0

[a]Solvent A, 0.3% phosphoric acid; [b]Solvent B, acetonitrile; [c]Solvent C, methanol.

Dublin, Ireland). Mobile phase (A = 0.3% phosphoric acid; B = acetonitrile; C = methanol) was used in a linear gradient programme shown in Table 1. The flow-rate was 1.0 ml/min and the injection volume was 10 µl. Peaks were detected at 270 nm.[27]

Identification of the components in *H. silenoides*

The identification of the compounds found by HPLC was based on the comparison of their retention time (t_R) with those obtained for the authentic reference samples.[27]

Drugs

Sibutramine hydrochloride (pharmaceutical grade) was from Helm de Mexico S.A. de C.V. (Naucalpan, Mexico). Type II porcine pancreatic lipase and *p*-nitrophenylpalmitate were from Sigma-Aldrich (St Louis MO, USA). Tris (hydroxymethyl)aminometane was from USBiological (Swampscott, MA, USA). Orlistat was isolated and purified from Xenical (Roche, Toluca, Mexico). Hyperforin, hypericin and pseudohypericin were purchased from CromaDex-kit-00019545-005 (CromaDex, Irvine, CA, USA). Quercetin was from Fluka Chemicals (Sigma-Aldrich Division, Milan, Italy). Rutin was from Sigma-Aldrich (St Louis, MO, USA). Chorogenic acid was from Extrasynthése (Genay, France). Acetonitrile and methanol (HPLC grade) were from J. T. Baker (Deventer, Netherlands). Phosphoric acid (85%, reagent grade) was from Ashland (Milan, Italy). Water was purified by a Milli-Q_{plus} system (Millipore, Milford, MA, USA). The other reagents used were of analytical grade. All drugs were prepared immediately before experimentation.

Experimental animals and diets

Ninety male adult Wistar rats with initial body weight between 290 and 320 g were obtained from Centro UNAM Harlan (Mexico City, Mexico). They were housed in groups of six in plastic cages in a temperature-controlled room at **22** ± 2°C under a 12-h light–dark cycle (lights on at 06:00 h). The rats were given free access to tap water and laboratory food (Teklad Global Rodent Diets, Harlan).

Two weeks later, the rats were randomly divided into two dietary groups, and allowed free access for 77 days to either a cafeteria diet (CF group; $n = 84$, six rats/cage) or a standard diet (S group; $n = 6$).[9,10] Food intake and body weight were recorded each week. All rats had access to drinking water or sucrose (25%).

Experiments were conducted according to the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999) and compliance with international rules on care and use of laboratory animals. Clearance for conducting the studies was obtained from the Ethics Committee for the Use of Animals in Pharmacological and Toxicological Testing, 'Facultad de Química, UNAM' (OFICIO/FQ/CICUAL/020/11) approval date 16 May 2011.

The control diet consisted of tap water and standard rat diet pellets (Teklad Global Rodent Diets, Harlan). The experimental cafeteria diet was a modification of a cafeteria diet described by Prats *et al.*:[10] standard rat diet pellet, bacon (Kir), salted peanuts (Mafer Premium), potato chips (Sabritas), cookies (Chokis, Gamesa), manchego-type cheese (Alpura), chocolate bars (Nestlé), sweet bread, Mexican bread roll (white wheat flour), tortillas (cooked maize dough), carrots and sucrose at 25%. All these foods were prepared in small pieces and presented inside the rat cages daily.

Measure of food consumption

The rats were periodically introduced into single metabolic cages for the measurement of their food intake (cafeteria or standard diet).[9] In this case 10 g of each components of the cafeteria diet (total weight 110 g) were mixed and offered in small pieces to allow the recovery the following day of at least part of all components provided. Water or sucrose (25%) consumption was measured by determining the weight of the remaining fluid contained in the drink bottles at the time of location change. The S group received 110 g of standard rat diet pellets.

The amount of each component consumed by each rat was calculated from the difference between the amount provided and the amount recovered the next day.[9,10] The energy content of each food was calculated by using the energy correlates of lipid (9 Kcal/g), protein (4 Kcal/g) and carbohydrate (4 Kcal/g).[23] The results from this study are shown in Table 2.

Administration of treatments

On day 77, body weights (476 ± 22 g) of the CF group were 24.6% higher than body weights (382 ± 19 g) of the S

Table 2 Energy content and composition of ingredients that constitute the cafeteria diet

Food	Energy (kcal/100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)	Lipid (g/100 g)
Bacon	655	4.1	0	71
Salted peanuts	572	24	20	44
Potato chips	528	4	56	32
Cookies	504	5.6	68	21.3
Manchego-type cheese	485	5.3	4.4	40.3
Chocolate	451	3.2	78	14
Sweet bread	445	7.9	48.3	24.5
Mexican bread roll (white wheat flour)	309	9.2	65.2	0.4
Tortillas (cooked maize dough)	224	5.8	49.5	1.1
Carrots	51	0.3	12.3	0.3
Standar chow pellets	320	23.9	50.8	4.4
Sugar	384	0	99.1	0
Total	4928	93.3	551.6	253.3

All data refer to the weight of the fresh materials. Nutrition values were taken from food label.

Table 3 Random assignment of rats to different groups

Group No.	Treatment	Dose (mg/kg/day)	*n* for each group
Group 1	Standard diet + Tween 80	–	6
Group 2	Cafeteria diet + Tween 80	–	6
Group 3	Cafeteria diet + sibutramine	5 and 10	3
Cafeteria diet + *Hypericum silenoides*			
Group 4–6	Hexane extract	10, 30 and 100	3
Group 7–9	Dichloromethane extract	10, 30 and 100	3
Group 10–12	Methanol extract	10, 30 and 100	3
Group 13–15	Aqueous extract	10, 30 and 100	3
Cafeteria diet + *Hypericum philonotis*			
Group 16–18	Hexane extract	10, 30 and 100	3
Group 19–21	Dichloromethane extract	10, 30 and 100	3
Group 22–24	Methanol extract	10, 30 and 100	3
Group 25–27	Aqueous extract	10, 30 and 100	3

group. On day 77, *Hypericum* crude extracts were suspended in 0.5% Tween 80 and administered orally through a gastric cannula at 10, 30 or 100 mg/kg per day for 35 days. The drugs/extracts were administered daily at 1700 h (1 h before the start of the dark period). The doses of extract were selected on the basis of pilot tests performed in our laboratory. Sibutramine was used as reference drug and dissolved in physiological saline (0.9%). The dose range (5 and 10 mg/kg) was determined from the existing literature.[24] A cafeteria diet and a standard diet control group were included.

The rats were randomly allocated to groups (Table 3). The number of rats in each group was determined based on a 3-factor design (treatments, doses and plants), which allowed for statistically valid comparison. Additionally, the number of rats in each group was approved by the Ethics Committee for the Use of Animals in Pharmacological and Toxicological Testing, 'Facultad de Química, UNAM' (OFICIO/FQ/CICUAL/020/11).

Effect of *Hypericum* extracts on body weight and food intake

Treatments (drugs/extracts) were begun on day 77 and administered daily for 35 days. The CF control group and the S control group received the same volume of vehicle (0.5% Tween 80). Body weight was recorded each week. All groups continued on the same dietary regimen (cafeteria or standard diet). At the end of the treatments, the rats were introduced into single metabolic cages for the measurement of their food intake. The procedure was described in the measure of food consumption section.

Blood glucose and lipid profile determination

After 35 days on treatments and 12 h of fasting, rats were anaesthetized by ether inhalation and 5 ml of blood was collected from the femoral vein. Blood samples were centrifuged at 2400 rpm for 15 min and serum was separated.

Glucose, total serum cholesterol (total-C), triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), alanine transaminase (ALT), aspartate transaminase (AST) and atherogenic index (AI) were analysed by the University Clinical Laboratory of School of Pharmacy (FES Zaragoza UNAM), using a Reflotron Plus apparatus (Roche, LABSSA, Mexico City, Mexico).

Forced swimming test

This test was performed according to the method described by Sánchez-Mateo *et al*.[25] with slight modifications. After 35 days on treatments (drugs, extracts or vehicles) and 1 h after oral administration, rats were individually forced to swim in a plastic cylinder (42 cm high, 33 cm in diameter) filled with 35 cm of water at 25 ± 1°C. The total duration of immobility (s) was measured during the last 4 min of a single 6-min test session. Rats were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.[25] The number of rats in each group was determined based on a 3-factor design (treatments, doses and plants), which allowed for statistically valid comparison.

Pancreatic lipase activity assay

The procedure described by Lee *et al*.[26] to test lipase inhibition activity was used. Briefly, the following solutions were mixed in a microplate (added in order): 10 µl of *p*-nitrophenylpalmitate (5.6 mg dissolved in 1.6 ml acetonitrile and adjusted to 5 ml with ethanol to reach a final concentration of 3 mM), 162 µl of 75 mM Tris-HCl buffer (pH 8.5), 16 µl of test solution and 12 µl of type II porcine pancreatic lipase (5 mg/ml in 75 mM Tris-HCl buffer). This mixture was incubated at 37°C for 25 min and then the absorbance was determined at 405 nm (BIO-RAD microplate reader model 680, Philadelphia, PA, USA). In the positive control, the extracts were replaced with the vehicle (dimethyl sulfoxide or water). A blank used the same mixture but without the substrate. Orlistat was used as a positive lipase inhibitor and was dissolved in ethanol, which alone served as vehicle control. The dose range 0.024, 0.08, 0.24, 0.8 and 2.4 µg/ml was determined from the literature.[26] The final concentrations of each extract evaluated were 3, 30, 100, 300 and 562 µg/ml.

Statistical analyses

All values are reported as mean ±standard error of mean (SEM). Two-way analysis of variance followed by the Bonferroni test was used to determine the significance ($P < 0.05$). GraphPad Prism (version 5.0 for Mac OS X, GraphPAd Software Inc., La Jolla, CA, USA) software was used for statistical analysis.

Results

Effect on body weight and food intake

On day 77, the body weights (476 ± 22 g) of the rats in the CF group were 24.6% higher than the body weights (382 ± 19 g) of the S group (Figure 1). The cafeteria diet consumed by the rats was hypercaloric, hyperlipidic, hyperglycaemic and lower in proteins. Figure 2 shows the total energy intake (kcal per day) and the energy intake from lipid, carbohydrate and protein consumed by feeding on a cafeteria diet or standard diet.

The crude extracts of *H. silenoides* significantly reduced the body-weight gain induced by cafeteria diet (Figure 3). On day 35 of treatment, hexane (10 mg/kg), dichloromethane (10 mg/kg) and aqueous (10, 30 and 100 mg/kg) extracts of *H. silenoides* reduced body-weight gain with

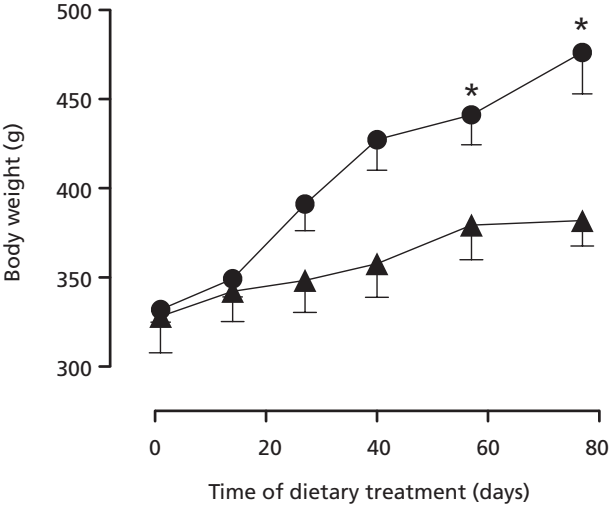

Figure 1 Body weight increase during cafeteria diet feeding in rats. Standard diet group (▲) and cafeteria diet group (●). Values are mean ± SEM for six rats. *$P < 0.05$ compared with standard diet group.

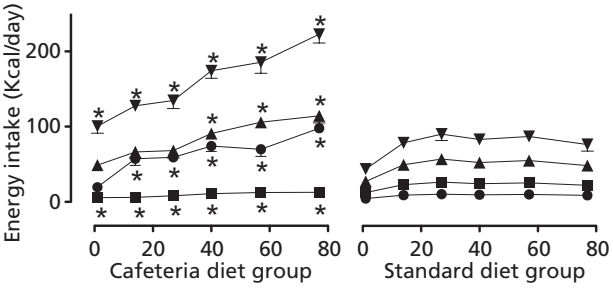

Figure 2 Total energy intake (▼), carbohydrate (▲), lipid (●) and protein (■) contribution throughout the feeding on a cafeteria diet. Values are mean ± SEM for six rats. *$P < 0.05$ compared with standard diet group.

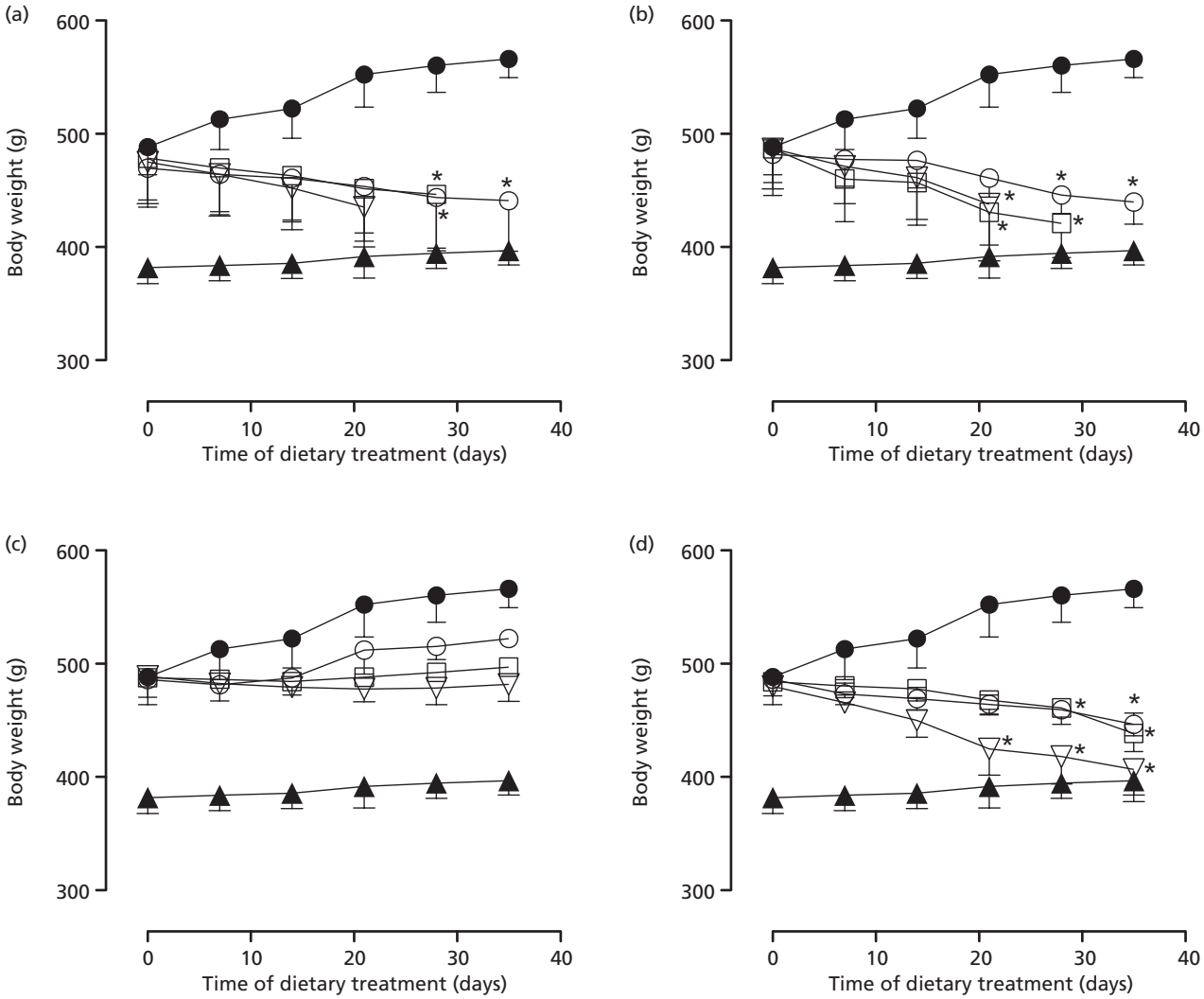

Figure 3 Effect of (a) hexane, (b) dichloromethane, (c) methanol and (d) aqueous extracts of *Hypercum silenoides* on body-weight change in cafeteria diet overweight rats. Cafeteria diet control group (●), 10 mg/kg extract (○), 30 mg/kg extract (□), 100 mg/kg extract (▽) and standard diet control group (▲). Values are mean ± SEM for three rats in each group. *$P < 0.05$ compared with cafeteria-diet-fed rats.

respect to the CF group ($P < 0.05$). In contrast, the methanol extract had no effect (Figure 3c). The order of the extracts' efficacy on maximum percentage of reduction of body-weight gain was: aqueous (15.4 ± 5.2%) > dichloromethane (8.7 ± 1.5%) > hexane (6.4 ± 3.0%) > methanol (no effect). Additionally, hexane (30 and 100 mg/kg) and dichloromethane (30 and 100 mg/kg) extracts of *H. silenoides* induced diarrhoea and premature death of the rats. In this matter, we detected some symptoms such as steatorrhoea, abdominal bloating and swelling. In both cases, we observed petechiae in abdominal muscle and an increase in heart size.

The oral administration of dichloromethane (10 and 30 mg/kg) and aqueous (30 and 100 mg/kg) extracts of *H. philonotis* (Figure 4) resulted in statically significant reduction in body weight ($P < 0.05$), but the methanol extract did not show significant effects. The hexane extract of *H. philonotis* also induced toxic effects and the death of the rats on day 15 of its oral administration (symptoms included steatorrhoea, abdominal bloating, swelling and petechiaes in abdominal muscle).

The order of the extracts' efficacy on maximum percentage of reduction of body-weight gain produced by *H. philonotis* was: dichloromethane (7.3 ± 2.5%) > aqueous (5.6 ± 1.0%) > methanol (no effect). Sibutramine at 5 and 10 mg/kg reduced body-weight gain by 17.0 ± 12.3% and 18.0 ± 9.9%, respectively (Figure 5).

The aqueous extract of *H. silenoides* showed the greatest effect in reducing food intake (by 51.1%) followed by dichloromethane (36.0%) and hexane (34.8%)

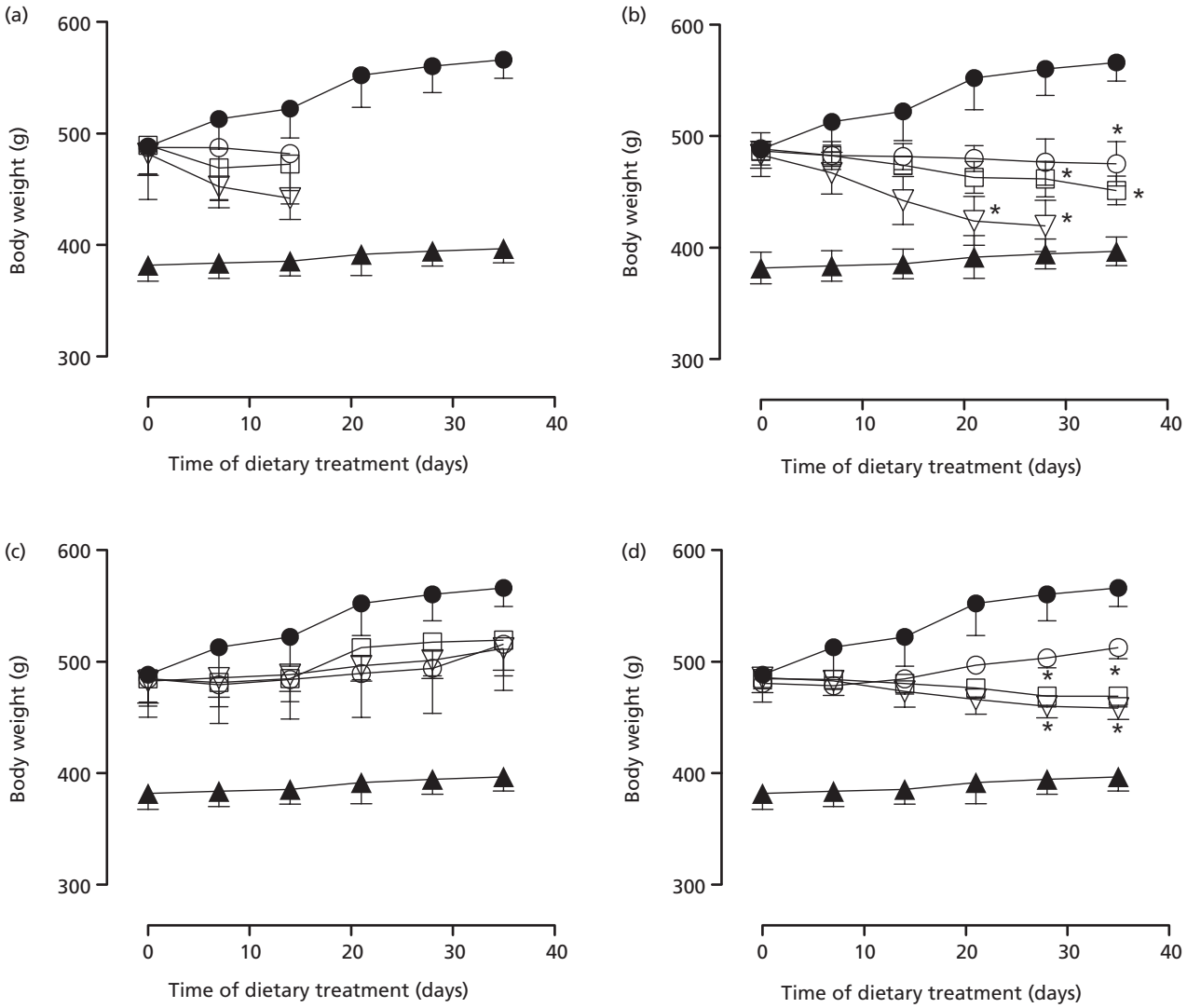

Figure 4 Effect of (a) hexane, (b) dichloromethane, (c) methanol and (d) aqueous extracts of *Hypericum philonotis* on body weight-change in cafeteria diet overweight rats. Cafeteria diet control group (●), 10 mg/ kg extract (○), 30 mg/kg extract (□), 100 mg/kg extract (▽) and standard diet control group (▲). Values are mean ± SEM for three rats in each group. *$P < 0.05$ compared with cafeteria-diet-fed rats.

extracts (Table 4). With respect to *H. philonotis* only the dichloromethane extract reduced the food intake (by 36.4%) (Table 4).

Blood glucose and lipid profile

Cafeteria diet induced significant increase of plasma total cholesterol, triglycerides and HDL-C compared with standard diet control group of rats (Tables 5, 6). AI, AST and ALT were decreased by cafeteria diet treatment, while LDL-C and glucose did not change (Tables 5, 6). Rats treated with *H. silenoides* (Table 5) crude extracts showed an important decrease in glucose levels (52.0–75.5%) and this decrease was dose dependent. The best hypoglycaemic activity was presented by the aqueous extract at 100 mg/ kg. Additionally, this extract decreased ($P < 0.05$) total cholesterol, triglycerides and HDL-C while LDL-C, AI, AST and ALT were not significantly altered ($P > 0.05$) (Table 5).

Crude extracts of *H. philonotis* (Table 6) significantly reduced blood glucose levels (between 48% and 58%). Methanol and dichloromethane extracts of this plant caused a significant decrease in AST levels and increased total cholesterol, HDL-C and LDL-C levels (Table 6). The aqueous extract decreased triglyceride levels ($P < 0.05$). Rats treated with sibutramine showed a significant decrease in plasma glucose, but increased total cholesterol, HDL-C and LDL-C levels (Tables 5, 6).

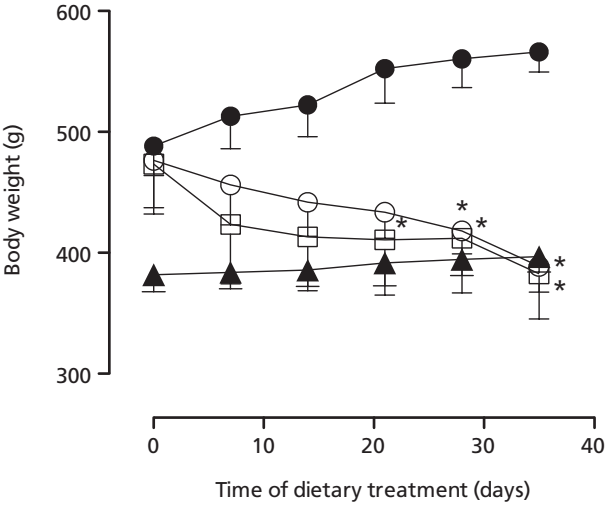

Figure 5 Effect of 5 mg/kg (○) or 10 mg/kg (□) of sibutramine on body-weight change in cafeteria diet overweight rats. Cafeteria diet control group (●) and standard diet control group (▲). Values are mean ± SEM for three rats in each group. *$P < 0.05$ compared with cafeteria-diet-fed rats.

Forced swimming test

In the forced swimming test, the cafeteria diet induced significant increase in the duration of immobility time compared with the standard diet control group of rats (Table 7). Only hexane (10 mg/kg) and aqueous (30 and 100 mg/kg) extracts of *H. silenoides* decreased the duration of immobility time in comparison with the cafeteria diet control ($P < 0.05$). In contrast *H. philonotis* extracts did not show an effect (Table 7). Sibutramine at 5 and 10 mg/kg reduced the duration of immobility time (63.8 ± 32.3 s and 2.0 ± 2.0 s, respectively) (Table 7).

Effect on pancreatic lipase activity

Hypericum silenoides and *H. philonotis* showed an inhibitory effect on lipase activity *in vitro* (Figure 6). The hexane extract of *H. philonotis* was the most active extract in inhibiting pancreatic porcine lipase *in vitro* (half maximal inhibitory concentration (IC_{50}) 162.60 ± 0.02 μg/ml), followed by dichloromethane (IC_{50} 197.24 ± 0.11 μg/ml) and aqueous (IC_{50} 341.45 ± 0.07 μg/ml) extracts of this plant (Table 8). Only the dichloromethane (IC_{50} 262.79 ± 0.09 μg/ml) and aqueous (IC_{50} 403.70 ± 0.08 μg/ml) extracts of *H. silenoides* showed inhibitory lipase activity (Table 8). Orlistat, used as reference inhibitor lipase drug, presented an IC_{50} value of 0.041 ± 0.0028 μg/ml (Table 8).

Constituents of *H. silenoides*

HPLC analyses were performed to identify the constituents of aqueous extract of *H. silenoides*; a typical chromatogram is reported in Figure 7. The t_R values for chlorogenic acid (peak 1), rutin (peak 2), quercetin (peak 3) and hyperforin (peak 4) were 19.42, 26.31, 40.71 and 49.71 min, respectively. *H. silenoides* did not contain hypericin and pseudohypericin.

Discussion

The experimental cafeteria diet used in this study was an adequate diet to make the rats overweight (Figure 1). The body-weight gain reached with this diet was in agreement with the weight gain reported for similar cafeteria diets in rats in other studies.[9,10,28,29] It has been reported that palatable human foods included in cafeteria diets stimulate energy intake[10,28] and induce hyperphagia, which results in an increase of heat production,[29] as well as increase fat stores in rats.[30] After standardization of the overweight rat model, we started the evaluation of plant extracts on overweight rats. Daily oral administration of *H. silenoides* and *H. philonotis* extracts for 35 consecutive days clearly reduced body-weight gain and induced hypoglycaemic and hypolipidaemic effects in rats (Figures 3, 4). Methanol extracts of both plants were the exception. Recent studies have demonstrated that hydroalcoholic extract of *H. perforatum* decreases body-weight gain, total cholesterol, LDL-cholesterol, triglycerides, glucose and insulin in rats fed a high-fat diet.[19] These results were attributed to increased serotonin levels due to *H. perforatum* inhibiting synaptosomal uptake of this neurotransmitter.[19] It is a well-known fact that, there is an inverse relationship between the level of brain serotonin signalling and food intake–when brain serotonin signalling is augmented, food intake is reduced, and vice versa.[31] Therefore, manipulation of endogenous serotonin synthesis, bioavailability and metabolism provides important evidence for the role of endogenous serotonin in the regulation of food intake and body weight.[31] It is possible that active extracts of *H. silenoides* and *H. philonotis*, like *H. perforatum* extract, can increase brain serotonin, resulting in reduced food intake and finally promoting weight loss. Additionally, the swimming test in the current study demonstrated that oral administration of hexane and aqueous extracts from *H. silenoides* produced a reduction of immobility time when compared with the CF group. This suggested that *H. silenoides* produced a possible antidepressant effect in obese rats. The forced swimming test is a widely accepted pharmacological tool for the evaluation of antidepressant activity.[25] In this assay, rats are forced to swim in a restricted space from which there is no escape, and will, after periods of agitation, cease attempts to escape and become immobile. The characteristic behaviour scored in this test is termed as immobility, reflecting behaviour despair as seen in human depression and it is well known that antidepressant drugs are able to reduce the

Table 4 Anorexic effect of *Hypericum silenoides* and *Hypericum philonotis*

Treatment	Dose (mg/kg/day)	Average food intake (g)	Food intake (%)
Standard diet + Tween 80	–	21.6 ± 1.0	
Cafeteria diet + Tween 80	–	45.7 ± 4.1*	111.8 ± 19.1*
Cafeteria diet + sibutramine	5	36.2 ± 0.7	–21.0 ± 1.4
	10	27.3 ± 3.5†	–40.3 ± 7.7†
Cafeteria diet + *Hypericum silenoides*			
Hexane extract	10	29.8 ± 6.1†	–34.8 ± 13.4†
	30	+	+
	100	+	+
Dichloromethane extract	10	29.3 ± 1.2†	–36.0 ± 2.7†
	30	+	+
	100	+	+
Methanol extract	10	40.5 ± 5.3	–11.5 ± 11.5
	30	39.8 ± 3.6	–12.9 ± 7.8
	100	39.1 ± 5.3	–14.5 ± 11.5
Aqueous extract	10	32.0 ± 3.8	–29.9 ± 8.4
	30	30.1 ± 4.3†	–34.1 ± 9.5†
	100	22.3 ± 1.2†	–51.1 ± 2.6†
Cafeteria diet + *Hypericum philonotis*			
Hexane extract	10	+	+
	30	+	+
	100	+	+
Dichloromethane extract	10	42.2 ± 2.5	–7.6 ± 5.4
	30	29.1 ± 5.7†	–36.4 ± 12.5†
	100	+	+
Methanol extract	10	41.1 ± 4.0	–10.0 ± 8.7
	30	41.4 ± 1.9	–9.4 ± 4.1
	100	44.9 ± 1.1	–1.9 ± 2.3
Aqueous extract	10	44.9 ± 4.5	–1.8 ± 9.9
	30	41.2 ± 3.8	–9.9 ± 8.2
	100	41.7 ± 9.4	–8.8 ± 16.7

Data are means ± SEM, $n = 3$. *$P < 0.05$ vs standard diet group; †$P < 0.05$ vs cafeteria diet group; + = death before end the experiments.

immobility time in rats.[25] Hyperforin has been identified as one of the main components of *H. perforatum* responsible for its antidepressant effects.[32–35] This compound inhibits the reuptake of serotonin, dopamine and noradrenaline.[32–35] Hyperforin, rutin, chlorogenic acid and quercetin were identified in the aqueous extract from *H. silenoides*. It is possible that hyperforin present in this extract is able to increase serotonin levels and as a consequences this may explain the reduce food intake in our rat model and the final promotion of weight loss.

On the other hand antidiabetic[17] and antihyperglycaemic activity[18] have been observed in a standardized *H. perforatum* extract. Hyperforin has been considered to be one of the antihyperglycaemic bioactive components of *H. perforatum*. This compound is a potent releaser of diverse types of neurotransmitters.[32–35] Hyperforin has also been reported to activate cation channels of the transient receptor potential currents (TRPC6) changing intracellular sodium and calcium concentration.[36] Acetylcholine is released from the intrapancreatic parasympathetic nerve endings during food intake. It potentiates insulin secretion through stimulation of muscarinic G protein-coupled receptors (M3) on the pancreatic beta cell. M3 stimulation by acetylcholine can activate TRP-like channels in neurons and beta cells.[37,38] Activation of TRP-like channels by muscarinic stimulation may contribute to beta cell depolarization and play a role in the insulin-potentiating effects of acetylcholine during feeding.[39] Hence it is possible that hyperforin present in the aqueous extract from *H. silenoides* can activate TRPC6 channels and as a result increase insulin release, resulting in an antihyperglycaemic activity.[38,39]

The hypolipidaemic mechanism(s) for these two *Hypericum* species is not known, but could involve blockade of biosynthesis of cholesterol or decrease intestinal absorption.[40] However, it is necessary to carry out additional experiments to clarify this effect.

Additionally, one of the most promising strategies in the effort to reduce energy intake, without altering the central mechanisms, is the development of inhibitors of intestinal absorption of nutrients.[41] Pancreatic lipase inhibition is one of the studied mechanisms for natural products as

Table 5 Plasma composition of rats treated with *Hypericum silenoides*

Treatment	Dose (mg/kg/day)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	AI	AST (IU/l)	ALT (IU/l)
Standard diet + Tween 80	–	109.0 ± 10.0	42.3 ± 4.4	77.2 ± 7.6	28.7 ± 2.8	4.3 ± 1.0	1.48 ± 0.03	305.0 ± 64.0	133.0 ± 22.0
Cafeteria diet + Tween 80	–	133.3 ± 11.3	76.8 ± 6.3*	99.4 ± 6.6*	56.5 ± 5.0*	5.8 ± 0.7	1.35 ± 0.02*	179.4 ± 9.5*	69.1 ± 9.7*
Cafeteria diet + sibutramine	5	139.0 ± 15.5	110.0 ± 9.1†	87.3 ± 4.8	76.0 ± 6.1	12.7 ± 2.3†	1.43 ± 0.03	311.3 ± 22.7	132.3 ± 35.3
	10	26.0 ± 8.4†	88.7 ± 16.3	84.7 ± 21.2	67.7 ± 12.5	12.3 ± 1.3†	1.30 ± 0.06	76.7 ± 36.3	78.3 ± 3.8
Hexane extract	10	52.0 ± 2.9†	55.3 ± 5.8	80.0 ± 7.2	51.0 ± 7.8	6.0 ± 1.2	1.13 ± 0.18†	240.7 ± 21.9	74.0 ± 11.6†
	30	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Dichloromethane extract	10	62.3 ± 4.5†	77.0 ± 5.3	110.7 ± 15.1	58.7 ± 3.8	10.3 ± 1.2†	1.33 ± 0.03	111.3 ± 21.0	52.3 ± 1.3
	30	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Methanol extract	10	37.0 ± 25.2†	118.0 ± 13.4[b]	100.7 ± 13.4	84.0 ± 13.4	20.0 ± 3.0†	1.43 ± 0.09	14.0 ± 6.0[b]	100.3 ± 9.0
	30	42.0 ± 14.3†	102.0 ± 21.3	73.3 ± 8.0	79.3 ± 15.8	15.0 ± 2.4[b]	1.30 ± 0.00	52.3 ± 39.3[b]	70.0 ± 16.0
	100	96.3 ± 10.7	94.3 ± 4.7	102.3 ± 17.0	71.3 ± 2.7	11.0 ± 3.0	1.30 ± 0.00	172.0 ± 78.3	67.7 ± 15.0
Aqueous extract	10	64.0 ± 9.3†	47.0 ± 1.1†	61.3 ± 8.7†	37.3 ± 0.7†	4.3 ± 0.3	1.27 ± 0.03	197.0 ± 26.0	49.0 ± 5.5
	30	55.3 ± 15.3†	86.7 ± 3.3	89.3 ± 21.3	64.0 ± 7.0	9.3 ± 0.7	1.40 ± 0.10	109.3 ± 6.0	160.0 ± 81.0
	100	32.7 ± 19.7†	57.3 ± 2.2	70.0 ± 4.7	43.0 ± 3.5	8.3 ± 1.5	1.37 ± 0.07	57.3 ± 45.0	69.0 ± 8.6

AI, atherogenic index; ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are means ± SEM, $n = 3$. *$P < 0.05$ vs standard diet group; †$P < 0.05$ vs cafeteria diet group; + = death.

Table 6 Plasma composition of rats treated with *Hypericum philonotis*

Treatment	Dose (mg/kg/day)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Tryglicerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	AI	AST (IU/l)	ALT (IU/l)
Standard diet + Tween 80	–	109.0 ± 10.0	42.3 ± 4.4	77.2 ± 7.6	28.7 ± 2.8	4.3 ± 1.0	1.48 ± 0.03	305.0 ± 64.0	133.0 ± 22.0
Cafeteria diet + Tween 80	–	133.3 ± 11.3	76.8 ± 6.3*	99.4 ± 6.6*	56.5 ± 5.0*	5.8 ± 0.7	1.35 ± 0.02[a]	179.4 ± 9.5[a]	69.1 ± 9.7[a]
Cafeteria diet + sibutramine	5	139.0 ± 15.5	110.0 ± 9.1†	87.3 ± 4.8	76.0 ± 6.1	12.7 ± 2.3†	1.43 ± 0.03	311.3 ± 22.7	132.3 ± 35.3
	10	26.0 ± 8.4†	88.7 ± 16.3	84.7 ± 21.2	67.7 ± 12.5	12.3 ± 1.3†	1.30 ± 0.06	76.7 ± 36.3	78.3 ± 3.8
Hexane extract	10	+	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+	+
Dichloromethane extract	10	96.3 ± 24.4	84.0 ± 10.4	104.0 ± 23.6	59.3 ± 6.6	11.3 ± 4.4	1.45 ± 0.04	49.7 ± 16.4	173.0 ± 79.0
	30	58.3 ± 3.2†	96.0 ± 14.5	89.0 ± 2.7	72.3 ± 11.2	9.8 ± 2.3	1.27 ± 0.03	34.6 ± 20.3[b]	110.3 ± 7.0
	100	+	+	+	+	+	+	+	+
Methanol extract	10	64.7 ± 3.2†	105.0 ± 13.0	88.7 ± 14.7	78.3 ± 5.0†	13.0 ± 2.7†	1.43 ± 0.07	30.7 ± 0.7[b]	103.3 ± 13.4
	30	69.0 ± 16.0†	78.3 ± 0.9	82.0 ± 12.1	58.0 ± 1.0	9.7 ± 1.8	1.47 ± 0.12	136.0 ± 59.2	112.3 ± 10.5
	100	89.3 ± 23.7	89.0 ± 10.5	86.3 ± 14.2	65.7 ± 7.6	10.3 ± 2.2	1.37 ± 0.03	57.7 ± 23.8	120.0 ± 15.9
Aqueous extract	10	48.3 ± 17.6†	58.7 ± 4.4	68.7 ± 3.0[b]	46.7 ± 4.0	5.3 ± 0.3	1.27 ± 0.03	129.0 ± 56.0	54.3 ± 4.7
	30	61.3 ± 10.0†	57.3 ± 1.5	76.0 ± 8.0	43.3 ± 1.2	5.7 ± 0.3	1.27 ± 0.03	197.0 ± 14.4	52.7 ± 4.0
	100	56.0 ± 15.0†	89.5 ± 11.5	70.5 ± 3.5	66.5 ± 4.5	10.5 ± 4.5	1.35 ± 0.05	243.5 ± 47.5	51.5 ± 10.5

AI, atherogenic index; ALT, alanine transaminase; AST, aspartate transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data are means ± SEM, $n = 3$. *$P < 0.05$ vs standard diet group; †$P < 0.05$ vs cafeteria diet group; + = death.

Table 7 Effect of *Hypericum silenoides* and *Hypericum philonotis* on the forced swimming test in rats

Treatment	Dose (mg/kg/day)	Duration of immobility (s)
Standard diet + Tween 80	–	94.5 ± 14.9
Cafeteria diet + Tween 80	–	174.3 ± 9.0*
Cafeteria diet + sibutramine	5	63.8 ± 32.3†
	10	2.0 ± 2.0†
Cafeteria diet + *Hypericum silenoides*		
Hexane extract	10	89.8 ± 6.1†
	30	+
	100	+
Dichloromethane extract	10	174.6 ± 2.4
	30	+
	100	+
Methanol extract	10	208.6 ± 21.6
	30	184.8 ± 15.8
	100	195.8 ± 13.4
Aqueous extract	10	146.2 ± 17.1
	30	106.4 ± 4.1†
	100	103.6 ± 9.0†
Cafeteria diet + *Hypericum philonotis*		
Hexane extract	10	+
	30	+
	100	+
Dichloromethane extract	10	178.8 ± 19.5
	30	187.6 ± 24.2
	100	+
Methanol extract	10	131.6 ± 23.8
	30	133.0 ± 22.4
	100	184.8 ± 17.2
Aqueous extract	10	199.2 ± 18.3
	30	188.6 ± 9.8
	100	164.4 ± 12.2

Data are means ± SEM, $n = 3$. *$P < 0.05$ vs standard diet group; †$P < 0.05$ vs cafeteria diet group; + = death.

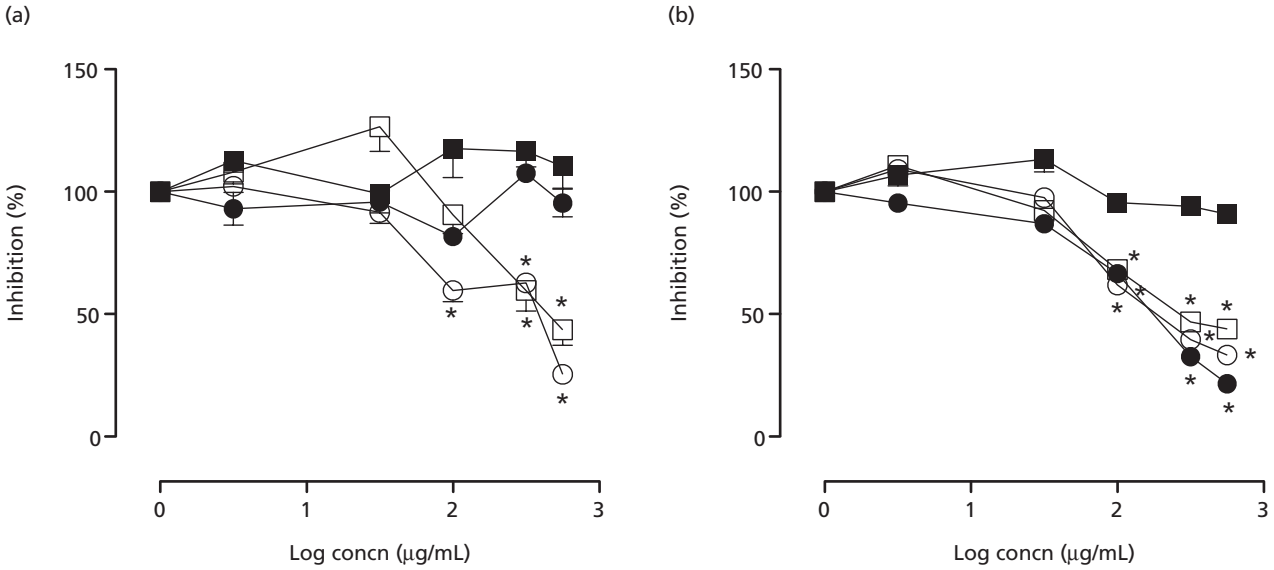

Figure 6 Pancreatic lipase inhibitory effect of *Hypericum silenoides* (a) and *Hypericum philonotis* (b). Hexane extract (●), dichloromethane extract (○), methanol extract (■) and aqueous extract (□). Values are mean ± SEM for nine determinations. *$P < 0.05$ compared with control.

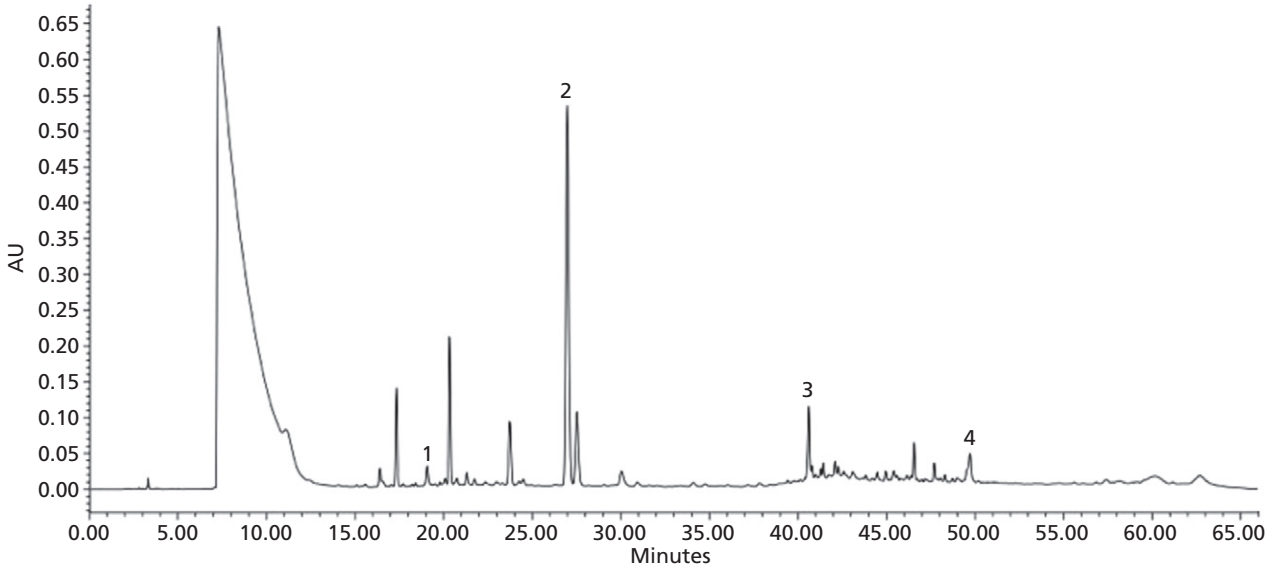

Figure 7 Profile of *Hypericum silenoides* aqueous extract with high-performance liquid chromatography attributions of the components detected. Peak1, chlorogenic acid; peak 2, rutin; peak 3, quercetin; peak 4, hyperforin.

Table 8 Inhibitory effect of *Hypericum silenoides* and *Hypericum philonotis* on pancreatic lipase

Treatment	IC_{50} (μg/ml)
Hypericum silenoides	
Hexane extract	NA
Dichloromethane extract	262.79 ± 0.09*
Methanol extract	NA
Aqueous extract	403.70 ± 0.08*
Hypericum philonotis	
Hexane extract	162.60 ± 0.02*
Dichloromethane extract	197.24 ± 0.11*
Methanol extract	NA
Aqueous extract	341.45 ± 0.07*
Orlistat	0.04 ± 0.03*

IC_{50}, half maximal inhibitory concentration; NA, not active. Data are means ± SEM, $n = 9$. *$P < 0.05$ vs control.

potential anti-obesity agents.[42] Some of the *H. silenoides* and *H. philonotis* extracts showed modest lipase inhibitory activity (Figure 6). This activity could be an additional explanation for the effect of *H. silenoides* and *H. philonotis* in reducing the body-weight gain induced in cafeteria-diet-fed rats.

Conclusions

This study demonstrated that *H. silenoides* and *H. philonotis* extracts show a significant anti-obesity activity, promoting antihyperglycaemic and hypolipidaemic activity in rats fed an experimental cafeteria diet. Moreover, both species showed an inhibitory effect on lipase activity *in vitro*. This trial provides the first scientific basis to support the popular use in Mexico of the *Hypericum* genus as an anti-obesity treatment.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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