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ARTICLE

# Reducing Effect of a *Phaseolus vulgaris* Dry Extract on Food Intake, Body Weight, and Glycemia in Rats

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Extracts of kidney beans (*Phaseolus vulgaris*) are known to reduce food intake and glycemia in rodents and humans. This study evaluated the effect of a novel extract of *P. vulgaris* on food (regular food pellets, starch-enriched diet, and chocolate-flavored beverage) intake, body weight, and glycemia in rats. The effect of the combination of the colecistokinin (CCK) receptor antagonist, lorglumide, and *P. vulgaris* dry extract on food intake was also investigated. Administration of doses of *P. vulgaris* dry extract devoid of any behavioral toxicity dose-dependently decreased food intake (irrespective of the diet), body weight gain, and glycemia. Pretreatment with lorglumide blocked the reducing effect of *P. vulgaris* dry extract on food intake. The capacity of this *P. vulgaris* dry extract to reduce food intake, body weight, and glycemia in rats may be due to (a) inhibition of α-amylase, (b) stimulation of CCK release from the intestinal brush border cells, and/or (c) interference with the central mechanism(s) regulating appetite, food intake, and food palatability.

**KEYWORDS: Kidney beans; *Phaseolus vulgaris* dry extract (Beanblock); food intake; body weight; glycemia; chocolate; lorglumide; rat**

## INTRODUCTION

Extracts of kidney beans (*Phaseolus vulgaris*), as well as some of their isolated ingredients, have been reported to reduce food intake and body weight and to lower glycemia in lean and obese rats (*1*−*7*). For example, the repeated daily administration of an extract of *P. vulgaris* markedly reduced daily food intake in rats given access to a starch-enriched diet (*5*); this effect was associated with a reduction in body weight gain as well as a steady reduction in glycemia. Notably, these results were subsequently replicated in streptozotocin-treated diabetic rats (*6*). In another study (*2*), a *P. vulgaris* extract was mixed with rat chow; a restricted amount of food was subsequently made available to rats and was consumed entirely. Reduced body weight gain was reported for rat groups consuming chow mixed with the *P. vulgaris* extract.

Two mechanisms of action have been proposed for the reducing effect of *P. vulgaris* extracts on food intake, body weight, and glycemia. Both of these mechanisms are based on the presence of two lectins (a class of constituents present in different cereals and pulses, including *P. vulgaris*): phytohemoagglutinin and α-amylase inhibitors (four different isoforms have been isolated from common bean) (*8*). These lectins, together with a third type, named arcelins, possess high degrees (40−95%) of amino acid sequence similarity (*9*−*11*). Specifically, inhibition of the pancreatic enzyme α-amylase (a) suppresses starch metabolism, resulting in a decrease in glycemia (*5*, *12*), and (b) delays gastric emptying, producing satiety and in turn decreasing food intake (*13*, *14*). Additionally, lectins bind to the intestinal brush border, stimulating the release of colecystokinin (CCK) and glucagon-like peptides that modulate food intake (*7*, *15*−*18*).

The present study was designed to evaluate the effect of the acute and repeated administration of a new, standardized, and purified dry extract of *P. vulgaris* on food intake, body weight, and glycemia in rats. Starting from the literature results, where the *P. vulgaris* preparations were mainly enriched in either α-amylase inhibitors (*5*, *6*) or phytohemoagglutinin (*3*), the extract used in the present study was prepared in view of possible dual action: inhibition of α-amylase and a phytohemoagglutinin-induced anorectic effect. The present study also assessed the effect of this new *P. vulgaris* dry extract on the intake of a highly palatable food (a chocolate-flavored beverage), providing first data on the effect of a *P. vulgaris* dry extract on food palatability. An ancillary experiment also evaluated whether this *P. vulgaris* dry extract produced any sign of behavioral toxicity, resulting in a nonspecific inhibition of rat behavior capable of leading to false-positive conclusions on its anorectic activities. Finally, to assess the possible involvement of CCK, the effect of the CCK type A ($CCK_A$) receptor antagonist, lorglumide, on *P. vulgaris* dry extract induced reduction in food intake was evaluated.

## MATERIALS AND METHODS

The experimental procedures employed in the present study were in accordance with the European Communities Council Directive (86/609/EEC) and the subsequent Italian law on the

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protection of animals used for experimental and other scientific reasons.

**Animals.** Adult male Wistar rats (Charles River Laboratories, Calco, Italy), weighing approximately 375 g at the start of the study, were used. Rats were individually housed in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12:12 h light−dark cycle (lights on at 11:00 p.m.), at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Rats were extensively habituated to handling and intragastric infusion.

**Extract Preparation.** The *P. vulgaris* dry extract, named Beanblock, containing α-amylase inhibitor and phytohemeagglutinin, in established ratios (see below), was prepared by means of aqueous extraction and alcoholic precipitation from the common white bean (*P. vulgaris*) according to a subsequent manufacturing process of Indena (see also ref *19*).

In detail, the kidney bean extract was obtained by extraction with citrate buffer and precipitation with ethanol. A suspension of kidney bean flour in aqueous solution (1:10, w/w) of citric acid was stirred for 3 h at the temperature of 4 °C. The suspension was centrifuged, and the aqueous centrifugate was concentrated 7.5 times (dry residue, 15.0% w/w). The concentrate was diluted with 95% ethanol to a concentration of 65% ethanol to obtain a precipitate, which was recovered by centrifugation at a temperature of 20−22 °C. The obtained precipitate was centrifuged, filtered, redissolved in demineralized water, reprecipitated in 60% ethanol to reduce the saline part, and recentrifuged. The final collected solid was dried under vacuum at a temperature not exceeding 50 °C.

The obtained extract (yield = 2.5%, approximately) was characterized by a standardized composition in (a) 8.5% (w/w) α-amylase inhibitor, assessed by high-pressure liquid chromatography on a diethylaminoethyl cellulose (DEAE) column, with reference to a standard material purified to homogeneity from *P. vulgaris* dry extract by preparative scale chromatography on the same DEAE resin (*20*), with inhibiting activity of 1400 U/mg, by Marshall and Lauda (*21*) test, calculated as United States Pharmacopoeia (USP) 50% inhibited units per milligram of sample on pancreatin (Sigma, Milan, Italy) from porcine pancreas (not less than 25 USP units/mg) as source of α-amylase; (b) phytohemoagglutinin (hemoagglutinating activity = 16 HAU/mg) analyzed by hemoagglutinating activity on rabbit red blood cells, with hemoagglutinating unit defined as the reciprocal of the highest dilution, in a serial 2-fold dilution test, of a pH 7.4 phosphate-buffered saline buffer extract of the sample still showing, by eye evaluation, hemoagglutination of red cells, according to Trugo and von Baer (*22*).

**Experiment 1 (Effect of the Repeated Administration of *P. vulgaris* Dry Extract on Food Intake, Body Weight, and Glycemia).** Rats were maintained on a starch-enriched diet [63% (w/w) carbohydrate (100% starch), 4% fibers, 20% protein, and 5% fat (Rieper, Vandoies, Italy)] available 24 h/day. Water was always available. Rats were divided into four groups of $n = 9$, matched for body weight and food intake over the 3 days preceding the start of the experiment. Rats were treated with 0, 50, 200, and 500 mg/kg *P. vulgaris* dry extract once a day (immediately before lights off) for 10 consecutive days. *P. vulgaris* dry extract was suspended in distilled water plus 0.5% methylcellulose and administered by gavage at an infusion volume of 2 mL/kg. Daily food and water intake was recorded once a day (approximately 30 min before lights off) by weighing food pellets and water bottles within 0.1 g accuracy. Rat body weight was recorded once a day (approximately 30 min before lights off) within 0.5 g accuracy. Food and water intake as well as rat body weight were recorded for an additional 3 day period after the end of administration of the *P. vulgaris* dry extract (post-treatment period). On the first day of treatment, food and water intake was recorded 120, 240, and 360 min after lights off. The effect of the overall 10 day treatment with *P. vulgaris* dry extract on feed efficiency was also determined; feed efficiency was defined as the body weight gained per calorie provided by food: the lower the feed efficiency, the lower the quantity of energy converted from food into body weight.

Glycemia levels were measured once every other day. Specifically, 2 h after lights off (corresponding to 2 h after administration of *P. vulgaris* dry extract), a small (0.05 mL) blood sample was collected from the tip of the tail of each rat and analyzed enzymatically by means of GL5 Analox (Analox Ltd., London, U.K.). Glycemia was also recorded on days 1 and 3 of the post-treatment period.

Data on the effect of repeated administration with *P. vulgaris* dry extract on (a) daily food intake (expressed in g/kg), (b) daily water intake (mL/kg), (c) daily changes in body weight (percentage of change in comparison to baseline), and (d) glycemia (mg/dL) were analyzed by two-way (treatment; time) analyses of variance (ANOVAs) with repeated measures on the factor time. Two-way (treatment; time) ANOVAs with repeated measures on the factor time were also used to analyze data collected in the post-treatment period. Data on the effect of *P. vulgaris* dry extract administration on food and water intake during the first 360 min of the first day of treatment were analyzed by two-way (treatment; time) ANOVAs with repeated measures on the factor time. Feed efficiency was expressed as the number of milligrams of body weight gained throughout the 10 day treatment with *P. vulgaris* dry extract per kilocalorie deriving from ingested food; data were expressed in milligrams per kilocalorie and analyzed by one-way ANOVA.

**Experiment 2 (Effect of *P. vulgaris* Dry Extract on Locomotor Activity).** This experiment was conducted to assess the effect of *P. vulgaris* dry extract on spontaneous locomotor activity in rats; the experiment was designed to rule out the possibility that the suppressing effect of *P. vulgaris* dry extract on food intake, observed in experiment 1, may have been associated with or secondary to sedation induced by administration of *P. vulgaris* dry extract.

Horizontal locomotor activity was measured in each rat homecage. Homecages were located within the frames [480 × 480 × 400 (h) mm] of a computer-operated, photocell-equipped apparatus (Motil, TSE, Bad Homburg, Germany). Photocell counts were recorded in 60 min bins. Recording was performed for 24 consecutive hours, starting from the beginning of the dark phase of the light−dark cycle.

Rats were maintained on a starch-enriched diet (see above for details) available 24 h/day. Water was always available. Rats were divided into two groups of $n = 8$, matched for body weight and food intake over the 3 days preceding the start of the experiment. Rats were treated acutely with 0 and 500 mg/kg *P. vulgaris* dry extract immediately before lights off. The latter dose of *P. vulgaris* dry extract was selected in view of the greater efficacy demonstrated in reducing food intake in experiment 1. *P. vulgaris* dry extract was suspended and administered as described above. In addition to locomotor activity, food and water intake over the 24 h period was also recorded.

Data on the effect of *P. vulgaris* dry extract on locomotor activity were analyzed by two-way (treatment; time) ANOVA with repeated measures on the factor time. Data on the effect of *P. vulgaris* dry extract on food intake (g/kg) and water intake (mL/kg) were analyzed by unpaired, two-tailed Mann−Whitney tests.

**Experiment 3 (Effect of the Acute Administration of *P. vulgaris* Dry Extract on Postprandial Glycemia).** Rats were initially habituated to the restricted availability of food pellets for 1 h/day (the first hour of the dark phase of the light−dark cycle). A regular rodent diet [60% (w/w) carbohydrate (46% starch), 4% fibers, 16% protein, and 3% fat (Safe, Augy, France)] was used. Water was available 24 h/day. The experiment was performed when stable amounts of food were consumed in each daily session. On the test day, rats were divided into four groups of $n = 8-9$ and treated with 0, 50, 200, and 500 mg/kg *P. vulgaris* dry extract, suspended as described above. *P. vulgaris* dry extract was administered by gavage immediately before food presentation. At lights off, rats were given a restricted amount of food (15 g/kg); this amount was chosen on the basis of previous observations, corresponding to the quantity consumed fully when rats were treated with 500 mg/kg *P. vulgaris* dry extract under this specific experimental procedure. Glycemia was determined 0, 60, 120, 180, and 300 min after the start of the 60 min session. Blood samples were collected and analyzed as described above.

Data on the effect of *P. vulgaris* dry extract on glycemia over time were analyzed by a two-way (treatment; time) ANOVA with repeated measures on the factor time.

**Experiment 4 (Effect of the Acute Administration of *P. vulgaris* Dry Extract on Intake of a Highly Palatable Chocolate-Flavored Beverage).** Rats were exposed to a chocolate-flavored beverage (see below), regular rodent chow (see above for details), and water with unlimited access 24 h/day. Bottles were refilled every day with fresh solution and their left−right positions interchanged at random to avoid development of position preference. Bottles containing the chocolate-flavored beverage were shaken regularly to prevent development of any deposit. Intake of chocolate-flavored beverage, water,and food pellets was monitored by weighing the bottles and food pellets (0.1 g accuracy) once

daily immediately before the start of the dark phase. The chocolate-flavored beverage was prepared by diluting powdered Nesquik (Nestlè Italiana, Milan, Italy) in tap water (5%, w/v) (*23*). This beverage provided 0.19 kcal/g, approximately $^{1}/_{17}$ of the caloric supply provided by regular food pellets (3.3 kcal/g).

On reaching a stable daily intake of the chocolate-flavored beverage, rats were divided into four groups ($n$ = 8), matched for body weight as well as daily intake of chocolate-flavored beverage and regular food pellets over the 3 days immediately preceding the experiment. Rats were treated acutely with 0, 50, 200, and 500 mg/kg *P. vulgaris* dry extract, suspended as described above. *P. vulgaris* dry extract was administered by gavage 60 min before lights off. Recording of the intake of chocolate-flavored beverage, food pellets, and water was performed 24 h after lights off.

Data on the effect of *P. vulgaris* dry extract on intake of chocolate-flavored beverage (expressed in mL/kg), food pellets (g/kg), total calories (kcal/kg), and water (mL/kg) were evaluated by one-way ANOVAs with repeated measures on the factor time, followed by the Newman−Keuls test for post hoc comparisons.

**Experiment 5 (Effect of the $CCK_A$ Receptor Antagonist, Lorglumide, on *P. vulgaris* Dry Extract Induced Reduction of Food Intake).** As described for experiment 1, rats were maintained on a starch-enriched diet (see above) that was made available for 24 h/day. Water was also always available. Rats were divided into four groups of $n$ = 7−8, matched for body weight and food intake over the 3 days preceding the experiment, and treated with 0 and 10 mg/kg lorglumide (Sigma-Aldrich, Milan, Italy); this dose of lorglumide was chosen on the basis of preliminary experiments (this laboratory, unpublished results) indicating that it did not alter food intake per se. Lorglumide was suspended in saline with 0.1% Tween 80 (injection volume = 2 mL/kg) and administered intraperitoneally 15 min before the ig administration of *P. vulgaris* dry extract (0 and 50 mg/kg), suspended as described above; *P. vulgaris* dry extract was administered 15 min before food presentation. Food intake was recorded 120 min after food presentation.

Data on the effect of the combination of lorglumide and *P. vulgaris* dry extract on food intake were statistically analyzed by a one-way ANOVA, followed by the Newman−Keuls test for post hoc comparisons.

## RESULTS

**Experiment 1 (Effect of the Repeated Administration of *P. vulgaris* Dry Extract on Food Intake, Body Weight, and Glycemia).** ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 33.54$, $P < 0.0001$] and days of treatment [$F_{time}(9;288) = 2.48$, $P < 0.01$] and a significant interaction between the two factors [$F_{interaction}(27;288) = 4.88$, $P < 0.0001$] on daily food intake. Specifically, the repeated administration of *P. vulgaris* dry extract produced a dose-dependent reduction in daily food intake (**Figure 1**, top panel). Over the first 4 days of treatment, the magnitude of the reducing effect produced by 50, 200, and 500 mg/kg *P. vulgaris* dry extract averaged approximately 10, 20, and 30%, respectively, with respect to vehicle-treated rats. On continuing treatment, a progressive reduction of the inhibitory effect of *P. vulgaris* dry extract on daily food intake was observed, suggesting development of some degree of tolerance; as a result, from day 8 onward daily food intake did not differ among rat groups. ANOVA revealed the presence of significant differences in daily food intake also in the post-treatment phase [$F_{treatment}(3;32) = 9.47$, $P < 0.0005$; $F_{time}(2;64) = 12.38$, $P < 0.0001$; $F_{interaction}(6;64) = 1.28$, $P > 0.05$]. Specifically, after treatment completion, daily food intake tended to be higher in the rat group treated with 500 mg/kg *P. vulgaris* dry extract than in the vehicle-treated rat group (**Figure 1**, top panel).

ANOVA revealed a significant effect of the first treatment (corresponding to an acute treatment) with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 8.56$, $P < 0.0005$] and time [$F_{time}(2;64) = 60.12$, $P < 0.0001$] and a significant interaction between the two factors [$F_{interaction}(6;64) = 2.40$, $P < 0.05$] on food intake over the first 6 h.

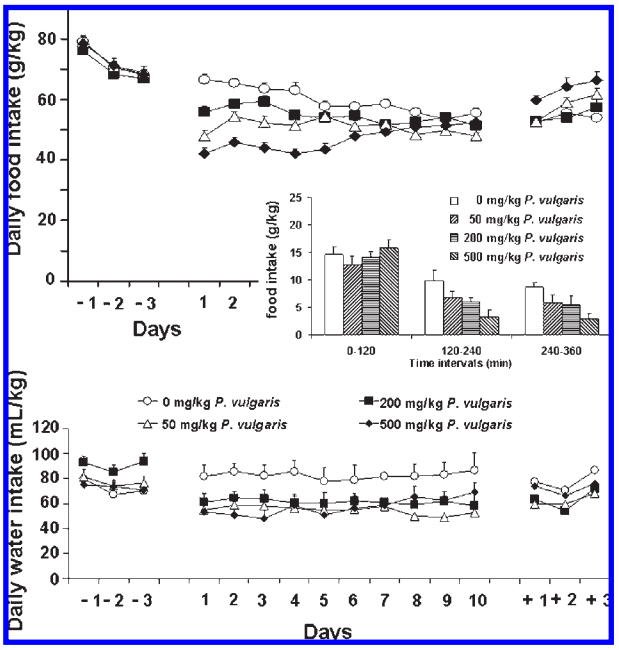


**Figure 1.** Effect of the repeated, daily, and intragastric administration of the *P. vulgaris* dry extract on daily food (top panel) and water (bottom panel) intake in Wistar rats given unlimited access to a starch-enriched diet and water. The dashed line indicates the end of the 10 day treatment period and the beginning of the 3 day post-treatment period. Each point is the mean ± SEM of $n$ = 9 rats. (Inset) Effect of the first administration of *P. vulgaris* dry extract on food intake over the first 6 h. Each bar is the mean ± SEM of $n$ = 9 rats.

Specifically, *P. vulgaris* dry extract exerted its reducing, dose-dependent effect on food intake starting from the second time interval (120−240 min); this effect was also maintained in the third time interval (240−360 min) (**Figure 1**, inset). These data suggest that the reducing effect of *P. vulgaris* dry extract on food intake had a relatively long onset.

ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 4.12$, $P < 0.05$], but not of days of treatment [$F_{time}(9;288) = 1.41$, $P > 0.05$], on daily water intake; the interaction between the two factors was, however, significant [$F_{interaction}(27;288) = 1.96$, $P < 0.01$]. Specifically, daily water intake was steadily reduced in all *P. vulgaris* dry extract treated rat groups with respect to the vehicle-treated rat group (**Figure 1**, bottom panel). *P. vulgaris* dry extract treated rats likely had a lower need of fluids than vehicle-treated rats, as they consumed lower amounts of food. No difference in daily water intake was observed among the four rat groups during the 3- day post-treatment period [$F_{treatment}(3;32) = 1.21$, $P > 0.05$; $F_{time}(2;64) = 7.40$, $P < 0.01$; $F_{interaction}(6;64) = 0.37$, $P > 0.05$] (**Figure 1**, bottom panel).

ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 9.98$, $P < 0.0001$] and days of treatment [$F_{time}(9;288) = 318.16$, $P < 0.0001$] and a significant interaction between the two factors [$F_{interaction}(27;288) = 5.36$, $P < 0.0001$] on rat body weight. Specifically, during the 10 day treatment period, vehicle-treated rats increased their body weight by approximately 9%; in contrast, body weight gain in rats treated with 50, 200, and 500 mg/kg *P. vulgaris* dry extract averaged 7.3, 6.5, and 4.8%, respectively (**Figure 2**). ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 6.16$, $P < 0.005$] and time of treatment [$F_{time}(2;64) = 94.68$, $P < 0.0001$] on body weight also in the 3 day post-treatment period; the interaction between the two factors

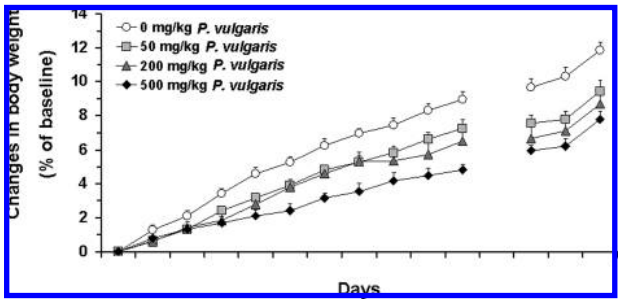


**Figure 2.** Effect of the repeated, daily, and intragastric administration of the *P. vulgaris* dry extract on changes in body weight (expressed as percent of baseline values, recorded immediately before the start of the treatment with *P. vulgaris* dry extract) in Wistar rats given unlimited access to a starch-enriched diet and water. The dashed lines indicate the end of the 3 day pretreatment period and the beginning of the 10 day treatment period, and the end of the treatment period and the beginning of the 3 day post-treatment period. Each point is the mean ± SEM of $n = 9$ rats.

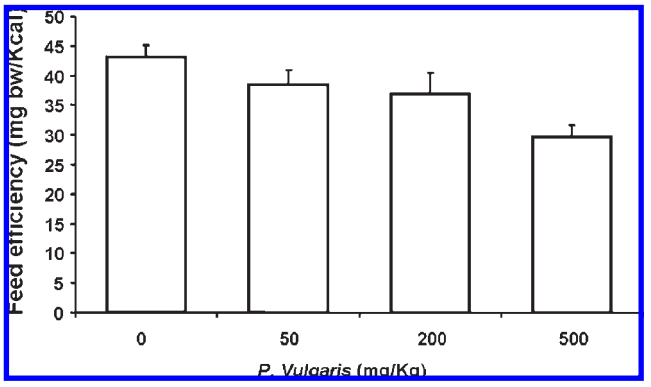


**Figure 3.** Effect of the repeated, daily, and intragastric administration of the *P. vulgaris* dry extract on feed efficiency (defined as mg of body weight gained over the 10 day treatment/kcal from ingested food) in Wistar rats given unlimited access to a starch-enriched diet and water. Each bar is the mean ± SEM of $n = 9$ rats.

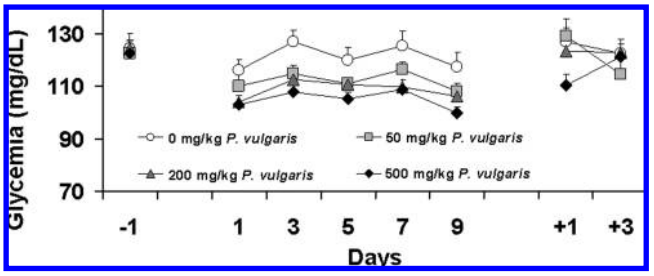


**Figure 4.** Effect of the repeated, daily, and intragastric administration of the *P. vulgaris* dry extract on glycemia in Wistar rats given unlimited access to a starch-enriched diet and water. Glycemia was assessed every other day (specifically on days 1, 3, 5, 7, and 9 of the 10 day treatment period as well as on days 1 and 3 of the post-treatment period) 2 h after administration of the *P. vulgaris* dry extract. The dashed lines indicate the beginning of the 10 day treatment period and the end of the treatment period and the beginning of the 3 day post-treatment period. Each point is the mean ± SEM of $n = 9$ rats.

was not significant [$F_{interaction}(6;64) = 1.25$, $P > 0.05$]. Specifically, differences in rat body weight in the post-treatment period were consonant with those recorded during the 10 day treatment period, and the overeating observed in *P. vulgaris* dry extract treated rats during the post-treatment period resulted only in a tendency toward a more rapid increase in body weight (**Figure 2**).

ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract on feed efficiency [$F(3;32) = 4.50$, $P < 0.01$]. In the vehicle-treated rat group, feed efficiency averaged approximately 43 mg/kcal, whereas in the rat groups treated with 50, 200, and 500 mg/kg *P. vulgaris* dry extract feed efficiency was approximately 10, 15, and 30% lower, respectively, than that calculated in vehicle-treated rats (**Figure 3**).

ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract [$F_{treatment}(3;32) = 8.58$, $P < 0.0005$] and days of treatment [$F_{time}(4;128) = 4.86$, $P < 0.005$] on glycemia, whereas the interaction between the two factors was not significant [$F_{interaction}(12;128) = 0.25$, $P > 0.05$]. Specifically, repeated treatment with *P. vulgaris* dry extract produced a marked decrease in glycemia, apparently not subject to development of tolerance (**Figure 4**). Glycemia in *P. vulgaris* dry extract treated rats returned to control values immediately following completion of treatment [$F_{treatment}(3;32) = 1.89$, $P > 0.05$; $F_{time}(1.32) = 0.36$, $P > 0.05$; $F_{interaction}(3;32) = 0.93$, $P > 0.05$] (**Figure 4**).

**Experiment 2 (Effect of *P. vulgaris* Dry Extract on Locomotor Activity).** ANOVA failed to reveal any effect of acute treatment with 500 mg/kg *P. vulgaris* dry extract on cumulative recording of spontaneous locomotor activity [$F_{treatment}(1;14) = 0.01$, $P > 0.05$; $F_{time}(23;322) = 60.39$, $P < 0.0001$; $F_{interaction}(23;322) = 0.12$, $P > 0.05$]. Specifically, *P. vulgaris* dry extract did not produce any change in the rat's spontaneous locomotor activity at any time interval (**Figure 5**). In close agreement with the results of experiment 1, the acute administration of *P. vulgaris* dry extract was associated with an approximately 35% reduction, with respect to vehicle-treated rats, in daily food intake ($P < 0.0005$, Mann−Whitney test).

**Experiment 3 (Effect of the Acute Administration of *P. vulgaris* Dry Extract on Postprandial Glycemia).** All rats from each group consumed the 15 g of food provided at the beginning of the test session. ANOVA failed to reveal an effect of treatment with *P. vulgaris* dry extract [$F(3;29) = 2.28$; $P > 0.05$] on the time course of glycemia; however, ANOVA displayed a significant effect of time [$F(3;87) = 15.79$; $P < 0.0001$] and a significant interaction between treatment and time [$F(9;87) = 1.99$; $P < 0.05$] on the time course of glycemia (**Figure 6**). Specifically, mean glycemia in vehicle-treated rats rose from the initial, fasting value of approximately 75 mg/dL to approximately 95 mg/dL at the end of the meal (corresponding to the 60 min time interval) and up to approximately 110 mg/dL at the 120 min time interval. Administration of *P. vulgaris* dry extract resulted in a dose-dependent reduction in glycemia, which was maximally evident at the 120 min time interval, when glycemia in 50, 200, and 500 mg/kg *P. vulgaris* dry extract treated rats was approximately 10, 15, and 20% lower than that recorded in vehicle-treated rats (**Figure 6**).

**Experiment 4 (Effect of the Acute Administration of *P. vulgaris* Dry Extract on Intake of a Highly Palatable Chocolate-Flavored Beverage).** ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract on the 24 h intake of the chocolate-flavored beverage [$F(3;28) = 15.62$, $P < 0.0001$]. Vehicle-treated rats displayed an extremely high intake of the chocolate-flavored beverage, averaging approximately 240 mL/kg over 24 h (**Figure 7A**). Treatment with *P. vulgaris* dry extract resulted in a dose-dependent reduction in intake of the chocolate-flavored beverage. Specifically, the magnitude of this reduction when values were compared to those of vehicle-treated rats averaged approximately 20, 55, and 60% in the rat groups treated with 50, 200, and 500 mg/kg *P. vulgaris* dry extract, respectively (**Figure 7A**).

As expected, on the basis of the results of experiment 1, ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract on the 24 h intake of regular food pellets [$F(3;28) = 8.15$,

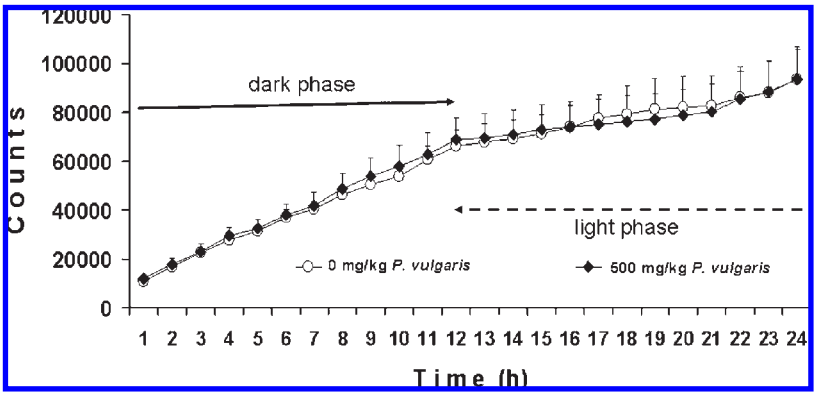


**Figure 5.** Effect of the acute, intragastric administration of the *P. vulgaris* dry extract on spontaneous locomotor activity (expressed as cumulative number of photocell counts) over a 24 h period in Wistar rats living in their homecage with unlimited access to a starch-enriched diet and water. Each point is the mean ± SEM of $n = 8$ rats.

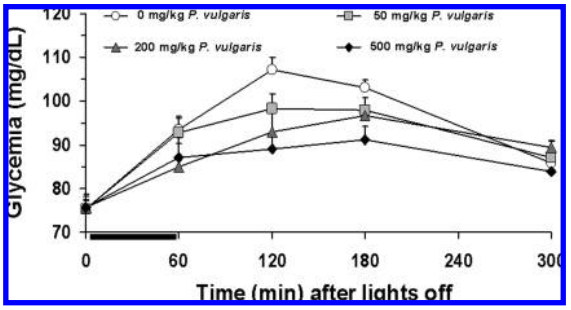


**Figure 6.** Effect of the acute, intragastric administration of the *P. vulgaris* dry extract on the time course of postprandial glycemia in Wistar rats having a restricted amount of regular food (15 g/kg) with a 1 h/day limited access (coinciding with the 0−60 min time interval and depicted by the horizontal black line). Each point is the mean ± SEM of $n = 8-9$ rats.

$P < 0.0005$]. Specifically, food intake over 24 h in 50, 200, and 500 mg/kg *P. vulgaris* dry extract treated rats was approximately 5, 15, and 25% lower, respectively, than that recorded in vehicle-treated rats (**Figure 7B**).

ANOVA failed to reveal any effect of treatment with *P. vulgaris* dry extract on the 24 h water intake [$F(3;28) = 1.54$, $P > 0.05$]. Water intake tended to be higher in the rat groups treated with the two highest doses of *P. vulgaris* dry extract, probably because these rat groups consumed a lower amount of the chocolate-flavored beverage (**Figure 7C**).

Finally, ANOVA revealed a significant effect of treatment with *P. vulgaris* dry extract on the 24 h total caloric intake (i.e., the sum of calories provided by the chocolate-flavored beverage and the regular food pellets) [$F(3;28) = 26.13$, $P < 0.0001$]. Specifically, total caloric intake over 24 h in 50, 200, and 500 mg/kg *P. vulgaris* dry extract treated rats was approximately 5, 25, and 35% lower than that calculated in vehicle-treated rats (**Figure 7D**).

**Experiment 5 (Effect of the $CCK_A$ Receptor Antagonist, Lorglumide, on *P. vulgaris* Dry Extract Induced Reduction of Food Intake).** ANOVA revealed the presence of significant differences among the rat groups [$F(3;27) = 4.76$, $P < 0.01$]. Administration of 10 mg/kg lorglumide, which did not alter food intake per se, resulted in the complete prevention of *P. vulgaris* dry extract-induced reduction in food intake. In the 120 min session, the reduction of food intake induced by 50 mg/kg *P. vulgaris* dry extract averaged approximately 40% with respect to the vehicle-treated rats ($P < 0.01$, Newmann−Keuls test); conversely, no differences in food intake were observed among the rat groups treated with 10 mg/kg lorglumide alone, 10 mg/kg lorglumide plus 50 mg/kg *P. vulgaris* dry extract, and vehicle (**Figure 8**).

## DISCUSSION

Repeated daily administration of *P. vulgaris* dry extract produced a dose-dependent decrease in daily food intake in rats exposed to a starch-enriched diet (experiment 1). Some degree of tolerance to this effect tended to develop after the first 4−5 days of treatment. The effect of *P. vulgaris* dry extract on food intake was associated with a concurrent reduction in rat body weight. These data are in close agreement with several literature reports describing the reducing effect of different *P. vulgaris* extracts, or their isolated components, on food intake and body weight in rats (*1*−*7*, *18*).

Reduction in food intake was not secondary to any sedative effect or possible malaise induced by *P. vulgaris* dry extract. Indeed, the highest dose of *P. vulgaris* dry extract tested in experiment 1 failed to affect, even minimally, spontaneous locomotor activity (a parameter highly sensitive to alterations in the state of well-being of rodents) in rats (experiment 2). Furthermore, no evident sign of malabsorption, such as diarrhea and altered number of fecal boluses, was observed in any rat used in experiments 1 and 2. This tends to rule out the arrival in the ileum of large amounts of undigested carbohydrates, as hypothesized following the administration of an α-amylase inhibitor (*2*).

Treatment with *P. vulgaris* dry extract significantly and dose-dependently reduced feed efficiency, defined as the body weight gained with each calorie provided by food. A reduction in feed efficiency suggests that food was less efficaciously converted into energy (and, in turn, into body mass). Thus, rats treated with *P. vulgaris* dry extract, compared to vehicle-treated rats, consumed less food and the latter produced fewer anabolic effects. The reduced absorption of complex carbohydrates, along with the capability of lectins to interfere with body metabolism and nutrient availability (*1*,*2*), may explain the effect of *P. vulgaris* dry extract on feed efficiency.

Repeated treatment with *P. vulgaris* dry extract resulted also in a marked and dose-dependent decrease in glycemia. At variance with the pattern of the effect of repeatedly administered *P. vulgaris* dry extract on daily food intake, the reducing effect of *P. vulgaris* dry extract on glycemia did not apparently undergo development of tolerance. These results are consistent with those of a recent study by Tormo et al. (*5*), who demonstrated that the chronic (21 consecutive days) daily administration of a *P. vulgaris* extract resulted in a steady reduction in glycemia. The results of the present study, indicating the development of tolerance to the anorectic, but not hypoglycemic, effect of *P. vulgaris* dry extract may suggest the existence of independent mechanisms of action underlying these two effects.

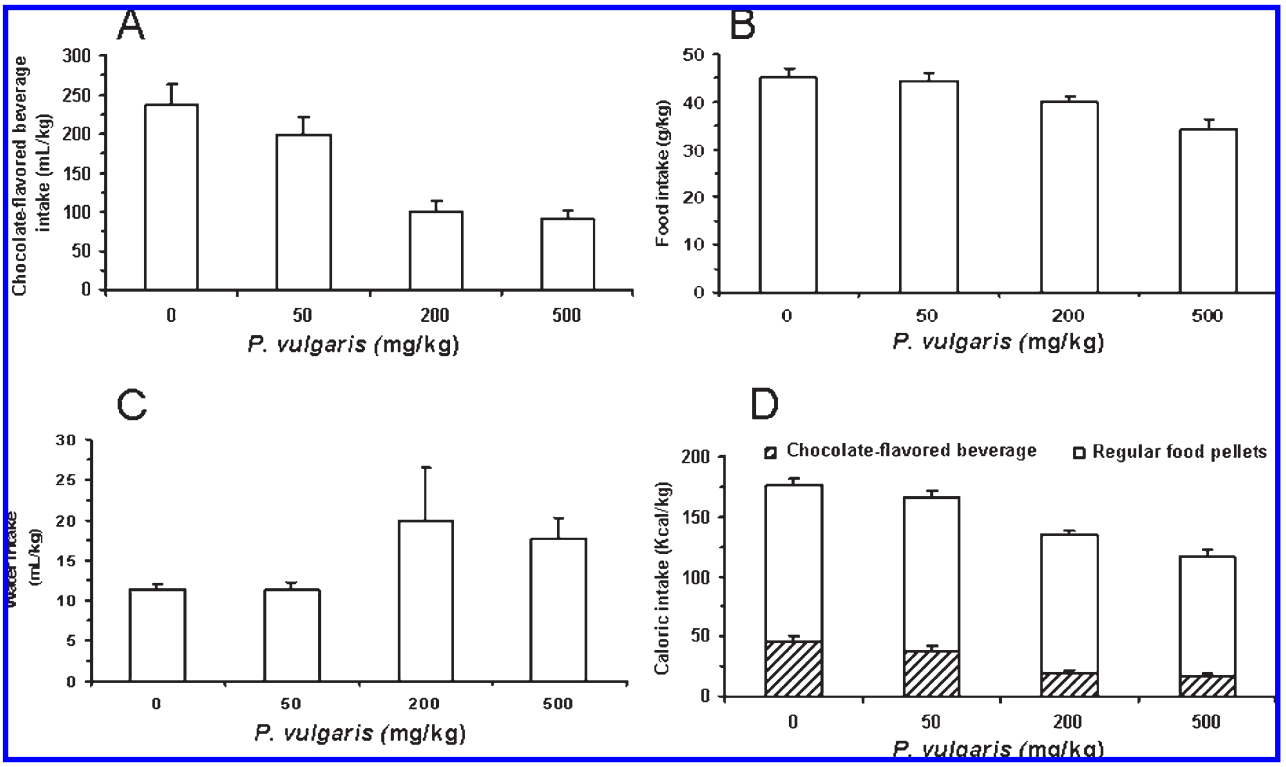


**Figure 7.** Effect of the acute, intragastric administration of the *P. vulgaris* dry extract on intake of a chocolate-flavored beverage (**A**), food (**B**), water (**C**), and calories (**D**) in Wistar rats given the choice between the chocolate-flavored beverage, regular food pellets, and water for 24 h/day. In panel **D**, hatched and plain portions represent calories from the chocolate-flavored beverage and calories from regular food pellets, respectively. Each bar in the mean ± SEM of $n = 8$ rats.

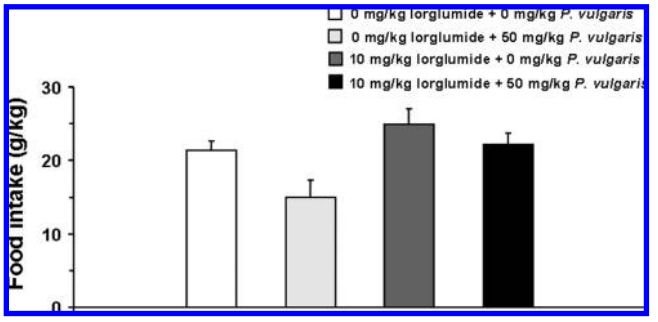


**Figure 8.** Effect of the acute, intraperitoneal administration of lorglumide on the decrease, induced by acute intragastric administration of the *P. vulgaris* dry extract, of food intake (over the first 120 min of the dark phase of the light−dark cycle) in Wistar rats given unlimited access to a starch-enriched diet and water. Each bar is the mean ± SEM of $n = 7-8$ rats.

Consistent results were collected in the experiment testing the effect of acutely administered *P. vulgaris* dry extract on glycemia (experiment 3). Acute administration of *P. vulgaris* dry extract resulted indeed in a dose-dependent decrease in the time course of postprandial glycemia in rats given a restricted amount of food over a limited time interval. All rats consumed the same amount of food, ruling out the possibility that the reducing effect of *P. vulgaris* dry extract on glycemia observed during the first phase of experiment 1, that is, before development of tolerance, was merely due to a reduced intake of food. It should be emphasized that experiment 3 was conducted using regular rodent chow and not a starch-enriched diet, as was the case in experiment 1, suggesting that the reducing effect of *P. vulgaris* dry extract on glycemia may be unravelled with different diets.

The mechanism of action of *P. vulgaris* dry extract on food intake and glycemia is currently not completely understood. It may be hypothesized that the reducing effect of *P. vulgaris* dry extract on glycemia is due to an inhibitory effect on pancreatic α-amylase, capable of decelerating starch metabolism and absorption (*5*, *12*). The active constituents of the *P. vulgaris* dry extract responsible for the lowering effect on glycemia might not include phytohemeagglutinin, as a study by Bardocz et al. (*3*) reported that rats' plasma glucose levels were unaffected by treatment with a purified *P. vulgaris* phytohemeagglutinin.

Conversely, the reducing effect of *P. vulgaris* dry extract on food intake might be secondary to the action of lectins on the intestinal brush border; it is known that lectins may alter the release of CCK and glucagon-like peptides at the level of the intestinal wall in the small intestine (*15*−*18*). Accordingly, data from this study indicated that the CCK receptor type$_A$ antagonist, lorglumide, blocked the reducing effect of *P. vulgaris* dry extract on food intake in rats (experiment 5), suggesting that the appetite-reducing effect of *P. vulgaris* dry extract may indeed be mediated, at least in part, by CCK$_A$ receptors. However, it cannot be excluded that inhibition of α-amylase may also contribute to the reducing effect of *P. vulgaris* dry extract on food intake, as Tormo et al. (*5*) recently reported that a *P. vulgaris* extract devoid of any lectin content was still effective in reducing food intake in rats.

As predicted (*24*), data from an additional experiment (experiment 4) indicate that undrugged rats displayed a daily, polydipsic-like (approximately equal to one-fourth their body weight) consumption of a highly palatable, chocolate-flavored beverage. Acute treatment with *P. vulgaris* dry extract produced a marked and dose-dependent suppression of intake of this chocolate-flavored beverage. *P. vulgaris* dry extract was more potent and effective in reducing intake of the chocolate-flavored beverage than of regular food pellets. The chocolate-flavored beverage was prepared so as to provide a relatively modest caloric supply, approximately $^1/_{17}$ that provided by regular food pellets. This

should have resulted in a consumption of the chocolate-flavored beverage mainly for its palatability (or hedonic value) rather than its caloric properties (or nutritive value). Accordingly, and taking into account the observed greater potency and efficacy of *P. vulgaris* dry extract on intake of the chocolate-flavored beverage than of regular food pellets, *P. vulgaris* dry extract appears to exert a stronger reducing effect on the hedonic properties of foods rather than on their caloric properties. These data further support the hypothesis that mechanisms other than the inhibition of α-amylase are responsible for the reducing action of *P. vulgaris* dry extract on food intake. Indeed, it is likely that the suppressing effect of *P. vulgaris* dry extract on intake of the chocolate-flavored beverage is due to an interference of some of its ingredient(s) with the central mechanisms regulating appetite, palatability, and/or the rewarding properties of foods.

Lectins have been proposed to exert some degree of toxicity (*16*, *25*, *26*), which might limit the potential utility of those products with some lectin content. However, additional lines of evidence suggest that lectins may exert beneficial and protective effects on intestinal functions (*7*). Notably, the *P. vulgaris* dry extract used in the present study was devoid of any apparent harmful consequence. A recent toxicological study demonstrated indeed that its chronic administration, once a day and for 4 consecutive weeks, at doses as high as 2 g/kg, did not result in any toxic effect, even in target organs such as the pancreas, in rats (Daniela Gallo, Catholic University, School of Medicine, Rome, Italy, personal communication).

The results of the present study, indicating the capability of *P. vulgaris* dry extract to reduce food intake (including a highly palatable chocolate-flavored beverage), body weight, and glycemia in rats, confirm previous preclinical data (*1*−*7*, *18*), as well as some clinical observations (*27*−*30*), collected with different extracts of *P. vulgaris*. Preliminary clinical studies, currently ongoing at different sites, will assess whether the observed effects of the *P. vulgaris* dry extract tested in the present study may be extended to human patients with overweight and metabolic syndrome.

## ABBREVIATIONS USED

CCK, colecystokinin; DEAE, diethylaminoethyl cellulose, USP, United States Pharmacopoeia; HAU, hemoagglutinating unit.

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## LITERATURE CITED

(1) Grant, G.; Dorward, P. M.; Buchan, W. C.; Armour, J. C.; Pusztai, A. Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: effects on body composition and organ weights. *Br. J. Nutr.* **1995**, *73*, 17–9.

(2) Pusztai, A.; Grant, G.; Duguid, T.; Brown, D. S.; Peumans, W. J.; Van Damme, E. J.; Bardocz, S. Inhibition of starch digestion by α-amylase inhibitor reduces the efficiency of utilization of dietary proteins and lipids and retards the growth of rats. *J. Nutr.* **1995**, *125*, 1554–1562.

(3) Bardocz, S.; Grant, G.; Pusztai, A.; Franklin, M. F.; De, F. F. U.; Carvalho, A. The effect of phytohaemagglutinin at different dietary concentrations on the growth, body composition and plasma insulin of the rat. *Br. J. Nutr.* **1996**, *76*, 613–626.

(4) Pusztai, A.; Grant, G.; Buchan, W. C.; Bardocz, S.; de Carvalho, A. F.; Ewen, S. W. Lipid accumulation in obese Zucker rats is reduced by inclusion of raw kidney bean (*Phaseolus vulgaris*) in the diet. *Br. J. Nutr.* **1998**, *79*, 213–221.

(5) Tormo, M. A.; Gil-Exojo, I.; Romero de Tejada, A.; Campillo, J. E. Hypoglycaemic and anorexigenic activities of an α-amylase inhibitor from white kidney beans (*Phaseolus vulgaris*) in Wistar rats. *Br. J. Nutr.* **2004**, *92*, 785–790.

(6) Tormo, M. A.; Gil-Exojo, I.; Romero de Tejada, A.; Campillo, J. E. White bean amylase inhibitor administered orally reduces glycaemia in type 2 diabetic rats. *Br. J. Nutr.* **2006**, *96*, 539–544.

(7) Pusztai, A.; Bardocz, S.; Ewen, S. W. B. Uses of plant lectins in bioscience and biomedicine. *Front. Biosci.* **2008**, *13*, 1130–1140.

(8) Ishimoto, M.; Suzuki, K.; Iwanaga, M.; Kikuchi, F.; Kitamura, K. Variation of seed - amylase inhibitors in the common bean. *Theor. Appl. Genet.* **1995**, *90*, 425–429.

(9) Moreno, J.; Chrispeels, M. J. A lectin gene encodes the α-amylase inhibitor of the common bean. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7885–7889.

(10) Lee, S. C.; Gepts, P. L.; Whitaker, J. R. Protein structures of common bean (*Phaseolus vulgaris*) α-amylase inhibitors. *J. Agric. Food Chem.* **2002**, *50*, 6618–6627.

(11) Sharma, V.; Surolia, A. Analyses of carbohydrate recognition by legume lectins: size of the combining site loops and their primary specificity. *J. Mol. Biol.* **1997**, *267*, 433–445.

(12) Santimone, M.; Koukiekolo, R.; Moreau, Y.; Le Berre, V.; Rouge, P.; Marchis-Mouren, G.; Desseaux, V. Porcine pancreatic alpha-amylase inhibition by the kidney bean (*Phaseolus vulgaris*) inhibitor (α-AI1) and structural changes in the α-amylase inhibitor complex. *Biochim. Biophys. Acta* **2004**, *1696*, 181–190.

(13) Jain, N. K.; Boivin, M.; Zinsmeister, A. R.; Brown, M. L.; Malagelada, J. R.; Di Magno, E. P. Effect of ileal perfusion of carbohydrates and amylase inhibitor on gastrointestinal hormones and emptying. *Gastroenterology* **1989**, *96*, 377–387.

(14) Jain, N. K.; Boivin, M.; Zinsmeister, A. R.; Di Magno, E. P. The ileum and carbohydrate-mediated feedback regulation of postprandial pancreatico-biliary secretion in normal humans. *Pancreas* **1991**, *6*, 495–505.

(15) King, T. P.; Pusztai, A.; Grant, G.; Slater, D. Immunogold localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in epithelial cells of the rat small intestine. *Histochem. J.* **1986**, *18*, 413–420.

(16) Bardocz, S.; Grant, G.; Ewen, S. W.; Duguid, T. J.; Brown, D. S.; Englyst, K.; Pusztai, A. Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. *Gut* **1995**, *37*, 353–360.

(17) Herzig, K. H.; Bardocz, S.; Grant, G.; Nustede, R.; Fölsch, U. R.; Pusztai, A. Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. *Gut* **1997**, *41*, 333–338.

(18) Baintner, K.; Kiss, P.; Pfuller, U.; Bardocz, S.; Pusztai, A. Effect of orally and intraperitoneally administered plant lectins on food consumption of rats. *Acta Physiol. Hung.* **2003**, *90*, 97–107.

(19) World-wide Patent Appl. WO2007071334, **2007**.

(20) Powers, J. R.; Whitaker, J. R. Purification and some physical and chemical properties of red kidney bean (*Phaseolus vulgaris*) α-amylase inhibitor. *J. Food Biochem.* **1977**, *1*, 217–238.

(21) Marshall, J. J.; Lauda, C. M. Purification and properties of Phaseolamin, an inhibitor of α-amylase from the kidney bean (*Phaseolus vulgaris*). *J. Biol. Chem.* **1975**, *250*, 8030–8037.

(22) Trugo, L. C.; von Baer, D. Analytical methods for the analysis of antinutritional factors in legume seeds. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed*; Jansman, A. J. M., Hill, G. D., Huisman, J., van der Poel, A. F. B., Eds.; Wageningen Pers: Wageningen, The Netherlands, 1998; pp 11−23.

(23) Maccioni, P.; Pes, D.; Carai, M. A. M.; Gessa, G. L.; Colombo, G. Suppression by the cannabinoid $CB_1$ receptor antagonist, rimonabant, of the reinforcing and motivational properties of a chocolate-flavoured beverage in rats. *Behav. Pharmacol.* **2008**, *19*, 197–209.

(24) Gessa, G. L.; Orrù, A.; Lai, P.; Maccioni, P.; Lecca, R.; Lobina, C.; Carai, M. A. M.; Colombo, G. Lack of tolerance to the suppressing effect of rimonabant on chocolate intake in rats. *Psychopharmacology* **2006**, *185*, 248–254.

(25) Gabriel, C. Erkrankungen durch Bohenflocken (*Phaseolus vulgaris*) und Platterbsen (*Lathyrus tingitanus* L.). *Z. Lebensm. Unters. Forsch.* **1950**, *90*, 191–197.

(26) Noah, H. D.; Bender, A. E.; Reaidi, G. B.; Gilbert, R. J. Food poisoning from raw red kidney beans. *Br. Med. J.* **1980**, *281*, 236–237.
(27) Layer, P.; Go, V. L.; Di Magno, E. P. Carbohydrate digestion and release of pancreatic polypeptide in health and diabetes mellitus. *Gut* **1989**, *30*, 1279–1284.
(28) Layer, P.; Zinsmeister, A. R.; Di Magno, E. P. Effects of decreasing intraluminal amylase activity on starch digestion and postprandial gastrointestinal function in humans. *Gastroenterology* **1986**, *91*, 41–48.
(29) Celleno, L.; Tolaini, M. V.; D'Amore, A.; Perricone, N. V.; Preuss, H. G. A dietary supplement containing standardized *Phaseolus vulgaris* extract influences body composition of overweight men and women. *Int. J. Med. Sci.* **2007**, *4*, 45–52.
(30) Udani, J.; Singh, B. B. Blocking carbohydrate absorption and weight loss: a clinical trial using a proprietary fractionated white bean extract. *Altern. Ther. Health Med.* **2007**, *13*, 32–37.

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