Reducing effect of an extract of *Phaseolus vulgaris* on food intake in mice — Focus on highly palatable foods

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**Article info**

**Abstract**

Different lines of experimental evidence indicate that treatment with extracts from and derivatives of *Phaseolus vulgaris* reduces intake of food, including highly palatable foods and beverages, in rats. The present study was designed to extend to mice these lines of evidence. To this end, CD1 mice were treated acutely with a standardized extract of *P. vulgaris* and then exposed to unlimited access to regular food pellets (Experiment 1) or 1-hour limited access to three different palatable foods/beverages, such as butter cookies (Experiment 2), a condensed-milk beverage (Experiment 3), and a chocolate-flavored beverage (Experiment 4). Treatment with *P. vulgaris* extract resulted in a significant reduction in the intake of regular food pellets, that was still evident 24 h later, as well as of the three palatable nourishments. Together, these results (a) extend to mice several previous findings on the capacity of *P. vulgaris* extracts to suppress food intake in rats, (b) suggest that *P. vulgaris* extracts may interfere with the central mechanisms regulating appetite, food intake, palatability, and/or the rewarding and hedonic properties of food, and (c) *P. vulgaris* extracts may represent a potentially effective therapy for overeating, obesity, and food craving.

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1. Introduction

Previous investigations found that treatment with extracts from or derivatives of *Phaseolus vulgaris* (Fabaceae) beans resulted in substantial reductions in appetite, food intake, and body weight in rats exposed to multiple experimental procedures [1–5]. Interestingly, extracts of *Phaseolus vulgaris* were particularly effective in reducing intake and reinforcing properties of highly palatable foods and beverages. For instance, acute administration of a standardized extract of *P. vulgaris* virtually completely suppressed intake of (a) butter cookies in pre-satiated rats [3] and (b) a chocolate-flavored beverage in rats given unlimited, concurrent access to this beverage, water, and regular food pellets [5]. In a subsequent experiment, treatment with the same *P. vulgaris* extract markedly reduced responding for the chocolate-flavored beverage in rats trained to press a lever to access the beverage [6], providing evidence of the ability of the *P. vulgaris* extract to affect, besides mere consumption, also the reinforcing and motivational properties of the chocolate-flavored beverage.

The present study was aimed at extending the above results to another animal species, with the intent of providing a wider characterization of the anorectic properties of *P. vulgaris* extracts. To this end, CD1 mice were exposed to either regular food pellets (Experiment 1) or different, highly palatable foods and beverages, including butter cookies (Experiment 2), a condensed-milk beverage (Experiment 3), and a chocolate-flavored beverage (Experiment 4), and then treated acutely
with different doses of the same P. vulgaris extract tested in the previous rat studies [3–6].

2. Materials and methods

2.1. Animals

Adult male CD1 mice (Charles River Laboratories, Calco, Italy), weighing approximately 30 g at the start of each experiment, were used. Mice were individually housed in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12:12 hour light–dark cycle (lights on 11:00 a.m., at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%). Standard rodent chow (2.67 kcal/g; Mucedola, Settimo Milanese, Italy) and tap water were available ad libitum in the homecage, except as noted below (see Experiments 2–4). Independent sets of mice were used in each single experiment.

2.2. Extract preparation

The procedure applied in preparation of P. vulgaris dry extract (BeanBlock®) has been described in detail elsewhere [5]. Briefly, P. vulgaris dry extract was prepared by means of aqueous extraction and alcoholic precipitation from the common kidney bean (P. vulgaris). Bean extract was obtained by extraction with citrate buffer and precipitation with ethanol. The extract obtained was characterized by a standardized composition in: (a) 8.5% (w/w) α-amylase inhibitor, with inhibiting activity of 1400 U/mg; (b) phytohemagglutinin (hemagglutinating activity equal to 16 HAU/mg).

2.3. Experimental procedures

2.3.1. Experiment 1: effect on intake of regular food pellets

On the test day (occurring 30 days after the start of the single-housing period), mice were (a) divided into 3 groups of n = 9, matched for body weight and daily food intake over the 3 days preceding the test, and (b) deprived of food for the 120-min time period that preceded lights off (this fasting phase was included in the experimental design to ensure that mice had empty stomachs at the time of administration of the P. vulgaris extract). Mice were treated acutely with 0, 500, and 1000 mg/kg P. vulgaris extract; treatment was performed 60 min before lights off. P. vulgaris extract was suspended and administered as described above. Food and water intake (expressed in g/kg and ml/kg, respectively) was recorded 12 and 24 h after lights off, as well as the day after (first day of the post-treatment period), by weighing food pellets and bottles (0.1-g accuracy). Data on food and water intake at each recording time were statistically analyzed by separate 1-way ANOVAs, followed by the Newman–Keuls test for post hoc comparisons.

2.3.2. Experiment 2: Effect on intake of butter cookies

After a 15-day period of habituation to the single-housing condition, mice were exposed to daily sessions of 1 h (the first hour of the dark phase), during which butter cookies (Ripensa, Ribe, Denmark) and water were available. Regular food pellets were available, together with water, during the remaining 23 h. Intake of butter cookies and water was monitored by weighing cookies and bottles (0.1-g accuracy) immediately after termination of each session. Butter cookies provided 4.84 kcal/g (approximately 80% more than the caloric supply provided by regular food pellets).

On the test day (occurring after 15 daily sessions, during which intake of butter cookies reached stable levels), mice were (a) divided into 3 groups of n = 9, matched for body weight and intake of butter cookies over the 3 sessions preceding the test, and (b) deprived of food for the 120-min time period that preceded lights off. Mice were treated acutely with 0, 500, and 1000 mg/kg P. vulgaris extract; treatment occurred 60 min before lights off. P. vulgaris extract was suspended and administered as described above. Intake of butter cookies and water (expressed in g/kg and ml/kg, respectively) was recorded at the end of the 60-min session. Data on the intake of butter cookies and water were statistically analyzed by separate 1-way ANOVAs, followed by the Newman–Keuls test for post hoc comparisons.

2.3.3. Experiment 3: Effect on intake of a condensed-milk beverage

After a 15-day period of habituation to the single-housing condition, mice were exposed to daily sessions of 1 h (the first hour of the dark phase), during which the condensed-milk beverage (see below) and water were available. Regular food pellets were available, together with water, over the remaining 23 h. Bottles containing the condensed-milk beverage and water were refilled every day with fresh solutions and their left-right position interchanged at random to avoid development of position preference. Intake of condensed-milk beverage and water was monitored by weighing bottles (0.1-g accuracy) immediately after termination of each session.

The condensed-milk beverage was prepared by diluting condensed milk (Nestlé Italiana, Milan, Italy) in tap water (10%, w/v). This beverage provided 0.32 kcal/g (approximately 1/8 of the caloric supply provided by regular food pellets).

On the test day (occurring after 15 daily sessions, during which intake of the condensed-milk beverage reached stable levels), mice were (a) divided into 3 groups of n = 9, matched for body weight and intake of condensed-milk beverage over the 3 sessions preceding the test, and (b) deprived of food for the 120-min time period that preceded lights off. Mice were treated acutely with 0, 500, and 1000 mg/kg P. vulgaris extract; treatment occurred 60 min before lights off. P. vulgaris extract was suspended and administered as described above. Intake of the condensed-milk beverage and water (both expressed in ml/kg) was recorded at the end of the 60-min session. Data on the intake of the condensed-milk beverage and water were statistically analyzed by separate 1-way ANOVAs, followed by the Newman–Keuls test for post hoc comparisons.

2.3.4. Experiment 4: Effect on intake of a chocolate-flavored beverage

After a 15-day period of habituation to the single-housing condition, mice were exposed to daily sessions of 1 h (the first hour of the dark phase), during which the chocolate-flavored beverage (see below) and water were available.
Regular food pellets were available, together with water, over the remaining 23 h. Bottles containing the chocolate-flavored beverage and water were refilled every day with fresh solutions and their left-right position interchanged at random to avoid development of position preference. Intake of chocolate-flavored beverage and water was monitored by weighing bottles (0.1-g accuracy) immediately after termination of each session.

The chocolate-flavored beverage was prepared by diluting powdered Nesquik® (Nestlé Italiana, Milan, Italy) in tap water (5%, w/v). This beverage provided 0.19 kcal/g (approximately 1/14 of the caloric supply provided by regular food pellets).

On the test day (occurring after 15 daily sessions, during which intake of the chocolate-flavored beverage reached stable levels), mice were (a) divided into 3 groups of n = 9, matched for body weight and intake of chocolate-flavored beverage over the 3 sessions preceding the test, and (b) deprived of food for the 120-min time period that preceded lights off. Mice were treated acutely with 0, 500, and 1000 mg/kg P. vulgaris extract; treatment occurred 60 min before lights off. P. vulgaris extract was suspended and administered as described above.

Intake of the chocolate-flavored beverage and water (both expressed in ml/kg) was recorded at the end of the 60-min session. Data on the intake of the chocolate-flavored beverage and water were statistically analyzed by separate 1-way ANOVAs, followed by the Newman–Keuls test for post hoc comparisons.

3. Results

3.1. Experiment 1: Effect on intake of regular food pellets

Treatment with the P. vulgaris extract significantly reduced intake of regular food pellets at both 12-hour [F(2,24) = 22.05, P < 0.0001] and 24-hour [F(2,24) = 15.13, P < 0.0001] recording times. At the 12-hour recording time, food intake in 500 and 1000 mg/kg P. vulgaris extract-treated rats was approximately 30% and 40% lower, respectively, than that recorded in vehicle-treated rats (Fig. 1, panel A). At the 24-hour recording time, food intake in 500 and 1000 mg/kg P. vulgaris extract-treated rats was approximately 15% and 30% lower, respectively, than that recorded in vehicle-treated rats (Fig. 1, panel B). Conversely, food intake was not altered on the first day of the post-treatment period [F(2,24) = 2.27, P > 0.05] (Fig. 1, panel C).

Water intake paralleled food intake, with significant reductions in P. vulgaris extract-treated rat groups at 12-hour [F(2,24) = 15.33, P < 0.0001] (Fig. 1, panel D) and 24-hour [F(2,24) = 11.60, P < 0.0001] (Fig. 1, panel E) recording times, but not on the first day of the post-treatment period [F(2,24) = 331, P > 0.05] (Fig. 1, panel F).

3.2. Experiment 2: Effect on intake of butter cookies

Treatment with the P. vulgaris extract significantly reduced intake of butter cookies [F(2,24) = 4.91, P < 0.05]. Treatment with 1000 mg/kg P. vulgaris extract produced an approximately 40% reduction in intake of butter cookies in comparison to treatment with vehicle (Fig. 2, left panel).

Water intake was not affected by treatment with the P. vulgaris extract [F(2,24) = 0.57, P > 0.05] (Fig. 2, right panel).

3.3. Experiment 3: Effect on intake of a condensed-milk beverage

Treatment with the P. vulgaris extract produced a highly significant reduction in intake of the condensed-milk beverage [F(2,24) = 17.14, P < 0.0001]. In comparison to treatment with vehicle, treatment with 500 and 1000 mg/kg P. vulgaris extract resulted in a 35–40% reduction in intake of the condensed-milk beverage (Fig. 3, left panel).

Water intake was not affected by treatment with P. vulgaris extract [F(2,24) = 0.72, P > 0.05] (Fig. 3, right panel).

3.4. Experiment 4: Effect on intake of a chocolate-flavored beverage

Acute treatment with the P. vulgaris extract significantly reduced intake of the chocolate-flavored beverage [F(2,24) = 3.52, P < 0.05]. When compared to treatment with vehicle, treatment with 1000 mg/kg P. vulgaris extract produced an approximately 25% reduction in intake of the chocolate-flavored beverage (Fig. 4, left panel).

Treatment with the P. vulgaris extract significantly altered also water intake [F(2,24) = 4.18, P < 0.05]. A tendency toward an increase in water intake was observed in the rat group treated with 1000 mg/kg P. vulgaris extract (Fig. 4, right panel).

4. Discussion

The results of Experiment 1 indicate that acute treatment with a standardized extract of P. vulgaris reduced intake of regular food pellets in mice given unlimited access to food. The reducing effect on food intake exerted by the two tested doses was still evident at the 24-hour time recording time (i.e., 25 h after extract administration), suggesting that the anorectic effect of the P. vulgaris extract is relatively long-lasting. The reducing effect of the P. vulgaris extract on food intake was associated with a proportional decrement in water intake (available ad libitum as well as food pellets); it is highly likely that this decrement in water intake was secondary to a reduced fluid need, because of the intake of a lower amount of the dry pellets, rather than to any malaise or sedative effect produced by the P. vulgaris extract. Indeed, previous experiments had not revealed any sign of behavioral toxicity in mice treated with even higher doses of this P. vulgaris extract (this laboratory, unpublished results).

Data from Experiment 1 extend to mice several previous observations on the capacity of extracts/derivatives of P. vulgaris (including the same P. vulgaris extract tested in the present study) to reduce the intake of regular food pellets in lean [1–3,5] and obese [4] rats. Together, these data strengthen the hypothesis that P. vulgaris extracts/derivatives may represent potentially effective therapies for use in the treatment of overeating and obesity. Notably, the results of two recent, double-blind, placebo-controlled, clinical studies provide at least partial support to this hypothesis: (a) single administration of the same P. vulgaris extract tested in the present study decreased postprandial glycemia, insulinemia, and ghrelin secretion, increased the feeling of satiation, and decreased the desire to eat (both measured by visual analogue...
scales) in healthy human subjects [7]; (b) a 2-month treatment with a dietary supplement made up of the same \textit{P. vulgaris} extract tested in the present study, together with an extract of \textit{Cynara scolymus}, increased the feeling of satiation (measured by the Haber’s scale for hunger/satiety scoring) in healthy overweight and obese subjects [8].

Experiments 2–4 were designed to investigate the effect of the \textit{P. vulgaris} extract on the intake of three different highly palatable foods and beverages in mice. Palatability of the three selected nourishments is indicated by the extremely high intakes that mice made in the brief (1 h), daily sessions during which each specific food was available; data collected in control mice provide clear evidence of their palatability: indeed, vehicle-treated mice consumed an average of approximately 50 g/kg butter cookies (Experiment 2), 170 ml/kg condensed-milk beverage (Experiment 3), and 130 ml/kg chocolate-flavored beverage (Experiment 4). Notably, mice were not starving when the palatable food was offered, as regular food pellets were always available in the mouse homecage, with the sole exception of a brief time period (2 h) preceding the start of the test session.

In all three experiments, acute treatment with the \textit{P. vulgaris} extract invariably resulted in a significant reduction in intake of the palatable food. Treatment with 1000 mg/kg \textit{P. vulgaris} extract reduced intake of (a) butter cookies by approximately 40% (Experiment 2), (b) condensed-milk beverage by approximately 40% (Experiment 3), and (c) chocolate-flavored beverage by approximately 25% (Experiment 4).
Conversely, intake of water – concurrently available together with the palatable food – was totally unaltered (Experiments 2 and 3) or even tended to be increased (Experiment 4) by treatment with the *Phaseolus vulgaris* extract; the lack of any decreasing effect on water intake is consistent with the notion that doses of this *P. vulgaris* extract up to 1000 mg/kg do not produce any sedation or sign of malaise in mice.

The reducing effect of the *P. vulgaris* extract on palatable foods acquires additional relevance taking into account that three different tastants were used. Further, these foods widely varied in terms of caloric content, ranging from a beverage with a low nutritive value (the chocolate-flavored beverage, the caloric content of which was approximately 14 times lower than that provided by regular food pellets) to a food rich in fat and sugar (butter cookies, with a caloric content approximately 80% higher than that provided by regular food pellets). Together, these data suggest that – besides the likely effects on the mechanisms regulating appetite and satiation (*e.g.* glycemia, insulinemia, ghrelin secretion, release of colecystokinin and glucagon-like peptides [3,7]) – the *P. vulgaris* extract interfered also with the central mechanisms regulating the rewarding and hedonic properties of food.

Data from Experiments 2 to 4, as well as those from Experiment 1, represent strong confirmation and generalization to mice of experimental data collected in rats. Previous lines of experimental evidence have indeed demonstrated that the same *P. vulgaris* extract tested in the present study reduced (a) intake of butter cookies in pre-satiated rats (specifically, after a period of food-deprivation, rats were given unlimited access to regular food pellets until satiation; immediately after, they were treated with the *P. vulgaris* extract and exposed to the butter cookies) [3], (b) intake of the chocolate-flavored beverage in rats given the choice between regular food pellets, water, and the chocolate-flavored beverage with unlimited access for 24 h/day [5], and (c) operant, self-administration of the chocolate-flavored beverage in rats trained to lever-press, under a Fixed Ratio 10 schedule of reinforcement, to access the chocolate-flavored beverage [6].

Together, these data are suggestive of the possible use of *P. vulgaris* extracts or derivatives for the treatment of overeating.
including longing and craving for highly palatable foods (e.g., “chocoholism”), and obesity in humans.

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