

Short Communication

Reducing effect of a *Phaseolus vulgaris* dry extract on operant self-administration of a chocolate-flavoured beverage in rats

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Extracts from or derivatives of *Phaseolus vulgaris* beans reduce body weight and food intake, including highly palatable foods and fluids, in multiple rodent models of overeating and obesity. The present study was designed to assess whether a standardised *P. vulgaris* dry extract was effective in reducing also the operant self-administration of a chocolate-flavoured beverage. To this end, rats were initially trained to lever-press for a chocolate-flavoured beverage under a fixed ratio 10 schedule of reinforcement in daily 60 min sessions. Once lever-responding reached stable levels, the effect of a *P. vulgaris* dry extract on the number of lever-responses for the chocolate-flavoured beverage was determined. Pretreatment with 50, 200 and 500 mg (intra-gastric) *P. vulgaris* dry extract per kg produced an approximate 15, 35 and 40% reduction, respectively, in lever-responding for the chocolate-flavoured beverage. These results indicate the capacity of a *P. vulgaris* preparation to reduce the reinforcing properties of a highly palatable fluid in rats.

***Phaseolus vulgaris* dry extract (Beanblock[®]): Food intake: Chocolate-flavoured beverages: Operant self-administration: Rats**

A growing body of experimental evidence suggests that extracts from or derivatives of *Phaseolus vulgaris* (*Fabaceae*) beans may have the capacity of reducing food intake, body weight, lipid deposit and glycaemia in multiple animal models of overeating, obesity, diabetes and the metabolic syndrome^(1–3). The results of preliminary clinical surveys apparently extend these results to humans, as *P. vulgaris* preparations have been reported to reduce appetite, food consumption and glycaemia in healthy, overweight, or obese individuals^(4–10).

Recently, this laboratory has conducted a series of experiments aimed at characterising the anorectic effects of a newly produced, standardised *P. vulgaris* dry extract, named Beanblock^{®(3,11)}. Its acute and repeated administration dose-dependently reduced intake of food (both regular and starch-enriched diets) in rats and mice. Notably, this extract resulted to be more effective in reducing the intake of highly palatable foods and fluids, including butter cookies and a chocolate-flavoured beverage, than regular or starch-enriched food pellets, suggesting that *P. vulgaris* derivatives may have the capacity of selectively reducing appetite for and intake of palatable (solid and liquid) nourishments.

The present study was designed to further investigate the effect of this *P. vulgaris* dry extract on the intake of palatable foods and fluids. To this end, the present study employed an operant procedure of self-administration of a chocolate-flavoured beverage. At variance with the previous study⁽¹¹⁾, where rats were exposed – within their own homecage – to the choice between two bottles containing the chocolate-flavoured beverage and water, respectively, in the present study rats were required to perform a specific effort or ‘task’ (i.e. pressing a lever) to access the chocolate-flavoured beverage. Specifically, rats had to press the lever ten times to gain a brief (5 s) access to the chocolate-flavoured beverage; after consumption of the chocolate-flavoured beverage, rats could repeat several times, over the daily 60 min session, this sequence of actions. In comparison with the previous, non-operant study⁽¹¹⁾, this procedure provided a more detailed analysis of the rats’ behaviour – and, of course, of the effect of the *P. vulgaris* extract – as it was not merely limited to its consumption aspects (i.e. the amount of chocolate-flavoured beverage ingested) but it examined also the reinforcing aspects (i.e. the capacity of the chocolate-flavoured beverage to direct and maintain this specific behaviour).

Abbreviation: FR, fixed ratio.

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Materials and methods

The experimental procedures employed in the present study were in accordance with the Italian Law on the 'Protection of animals used for experimental and other scientific reasons'.

Animals

Ten adult male Wistar rats (Charles River Laboratories, Calco, Italy), weighing approximately 400 g at the start of the study, were used. Rats were housed four per cage in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12 h light–12 h dark cycle (lights on at 19.00 hours), constant temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of approximately 60%. Standard rat chow (Mucedola, Settimo Milanese, Italy) and tap water were always available in the homecage, except as noted below. Rats were extensively habituated to handling and intragastric infusion.

Extract preparation

The procedure for preparing the *P. vulgaris* dry extract has been described in detail elsewhere⁽¹¹⁾. Briefly, the *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 obtained extract was characterised by a standardised composition in: (a) 8.5% (w/w) α -amylase inhibitor, with inhibiting activity of 1400 U/mg; (b) phytohaemagglutinin (haemagglutinating activity equal to 16 haemagglutinating units/mg).

Chocolate-flavoured beverage

The chocolate-flavoured beverage was prepared by diluting powdered Nesquik[®] (Nestlé Italiana, Milan, Italy) in tap water. The concentration of Nesquik[®] chocolate powder was 5% (w/v) throughout the study. This concentration was selected on the basis of the results of previous experiments in which it had been largely preferred over a wide range of concentrations⁽¹²⁾. The chocolate-flavoured beverage was prepared daily and sipper bottles (see below) were shaken immediately before the start of each session to prevent development of any deposit. The chocolate-flavoured beverage provided 0.8 kJ/g.

Apparatus

Operant sessions were conducted in modular chambers (Med Associates, St Albans, VT, USA), located in sound-attenuated cubicles, with fans for ventilation and background white noise. The front panel of each chamber was equipped with: (a) one retractable response lever; (b) one green stimulus light mounted above the lever; (c) the retractable spout of a liquid sipper bottle (250 ml capacity) located outside the chamber. A white house light was centred at the top of the back wall of each chamber. Achievement of the response requirement (see below) resulted in exposure of the sipper bottle spout (lasting for 5 s in each phase of the experiment) and illumination of the green light for the period of exposure of the sipper bottle spout.

Experimental procedure

In all the three experimental phases (see below), self-administration sessions were conducted on 5 d per week (Monday to Friday) during the first 4 h of the dark phase of the light–dark cycle. All self-administration sessions lasted 60 min.

Training phase

To facilitate the acquisition of lever-pressing behaviour, rats were water-deprived in their homecage in the 18 h preceding the first two operant sessions. During the first five daily sessions, rats were trained to lever-press on a fixed ratio (FR) 1 (FR1) schedule of reinforcement for the chocolate-flavoured beverage. This means that each time the FR requirement was met, the drinking spout of the sipper bottle was introduced into the chamber and the chocolate-flavoured beverage was available. Over the nine subsequent daily sessions, the FR schedule of requirement was progressively increased from FR1 to FR10; specifically, FR2 for two daily sessions, FR5 for two daily sessions, FR8 for two daily sessions, and FR10 for the final three daily sessions.

Maintenance phase

The maintenance phase started immediately after the training phase. The maintenance phase was made up of fifteen daily sessions; these sessions were identical to the final three sessions of the training phase. During this phase, the behaviour (both in terms of number of lever-responses and intake of the chocolate-flavoured beverage) stabilised in all rats. This was considered a necessary condition before the start of the test sessions with the *P. vulgaris* dry extract.

Testing phase

Test sessions were conducted on Fridays; four consecutive (Monday–Thursday) daily baseline sessions elapsed between test sessions; these baseline sessions were (a) identical to those of the maintenance phase, as no treatment with the *P. vulgaris* dry extract was given, and (b) included in the experimental design to maintain stable levels of self-administration between test sessions. All doses of the *P. vulgaris* dry extract were tested in each rat under a Latin-square design; specifically, each rat received one of the four tested doses (see below) in each of the four different test sessions in order to complete, over 4 weeks, the entire dose–response curve. The *P. vulgaris* dry extract was suspended in distilled water plus 0.5% methylcellulose and administered by intragastric administration (infusion volume: 2 ml/kg) at the doses of 0, 50, 200 and 500 mg/kg, 120 min before the start of the test sessions. The dose range of the *P. vulgaris* dry extract and pretreatment time were chosen on the basis of previous results⁽¹¹⁾.

Measured variables and data analysis

Measured variables were (a) number of lever-responses and (b) amount of self-administered chocolate-flavoured beverage (expressed in ml/kg and determined by weighing the sipper

bottle (0.1 g accuracy) before and after the session). Data on the effect of the *P. vulgaris* dry extract on number of lever-responses and amount of self-administered chocolate-flavoured beverage were analysed by separate one-way ANOVA with repeated measures, followed by the Newman–Keuls test for *post hoc* comparisons.

Results

All rats easily acquired and steadily maintained self-administration of the chocolate-flavoured beverage. During the 15 d maintenance phase, the number of lever-responses and amount of self-administered chocolate-flavoured beverage over each daily session averaged approximately 2000 and 60 ml/kg, respectively.

Pretreatment with the *P. vulgaris* dry extract resulted in a reduction in the number of lever-responses for the chocolate-flavoured beverage ($F(3,39) = 6.50$; $P < 0.005$) (Fig. 1(a)). Specifically, the number of lever-responses in the rats treated with 50, 200 and 500 mg *P. vulgaris* dry extract per kg was approximately 15, 35 and 40% lower, respectively, than that recorded in vehicle-treated rats. *Post hoc* tests revealed that the number of lever-responses in the rats treated with 200 and 500 mg *P. vulgaris* dry extract per kg was significantly lower than that recorded in vehicle-treated rats. The *P. vulgaris* dry extract-induced reduction in the number of lever-responses for the chocolate-flavoured beverage was associated with a proportional decrease in the amount of self-administered chocolate-flavoured beverage ($F(3,39) = 3.51$; $P < 0.05$) (Fig. 1(b)).

Fig. 2 depicts the cumulative lever-response patterns after pretreatment with the *P. vulgaris* dry extract. It is noted that: (a) latency to the first lever-response was extremely short and virtually identical for each dose; (b) over the first 5–6 min of the session, lever-responding was intense and differed minimally among the four doses; (c) pretreatment with 200 and 500 mg *P. vulgaris* dry extract per kg resulted, in comparison with pretreatment with vehicle, in lower values at which responding for the chocolate-flavoured beverage reached its plateau.

Discussion

The chocolate-flavoured beverage employed in the present study proved to possess strong motivational and reinforcing properties, as indicated by the intense operant behaviour (approximately 2000 responses on the lever) that rats performed to access the beverage during each daily 60 min session of the maintenance phase. Consumption of the chocolate-flavoured beverage averaged approximately 60 ml/kg per session, which – to some extent – appears to be the maximal volume of a fluid that rats can ingest over such a short period of time⁽¹²⁾. These large amounts of self-administered chocolate-flavoured beverage resemble the polydipsic-like consumption of the same beverage (approximately 250 ml/kg over 24 h) displayed by Wistar rats exposed to the non-operant, homecage two-bottle ‘chocolate-flavoured beverage v. water’ choice regimen⁽¹¹⁾.

The results of the present study also indicated that administration of a standardised dry extract of *P. vulgaris* beans reduced the self-administration, and thus the reinforcing

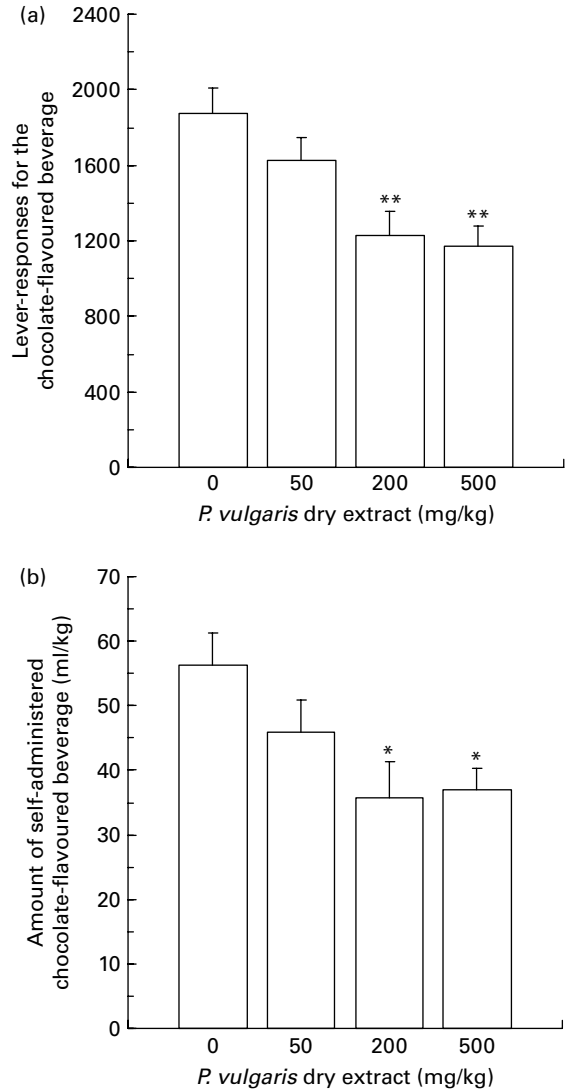


Fig. 1. Effect of the administration of a *Phaseolus vulgaris* dry extract on number of lever-responses (a) and amount of self-administered chocolate-flavoured beverage (Nesquik® chocolate powder diluted 5% (w/v) in tap water; expressed in ml/kg) (b) in Wistar rats exposed to daily 60 min self-administration sessions with a fixed ratio 10 schedule of reinforcement for 5 s accesses to the chocolate-flavoured beverage. All doses of the *P. vulgaris* extract were tested in each rat under a Latin-square design; four non-drug sessions elapsed between test sessions. Values are means for ten rats, with standard errors represented by vertical bars. Mean value was significantly different from that of the vehicle-treated rats: * $P < 0.05$, ** $P < 0.01$ (Newmann–Keuls test).

properties, of the chocolate-flavoured beverage. Pretreatment with 50, 200 and 500 mg *P. vulgaris* dry extract per kg resulted indeed in (a) an approximately 15, 35 and 40% reduction, respectively, in the number of responses on the lever that supplied the chocolate-flavoured beverage and (b) a proportional decrement in the amount of self-administered chocolate-flavoured beverage. These results extend to an operant procedure, the results of a previous study demonstrating that the same *P. vulgaris* dry extract employed in the present study reduced the intake of an identical chocolate-flavoured beverage in rats exposed to the two-bottle ‘chocolate-flavoured beverage v. water’ choice regimen⁽¹¹⁾. In both

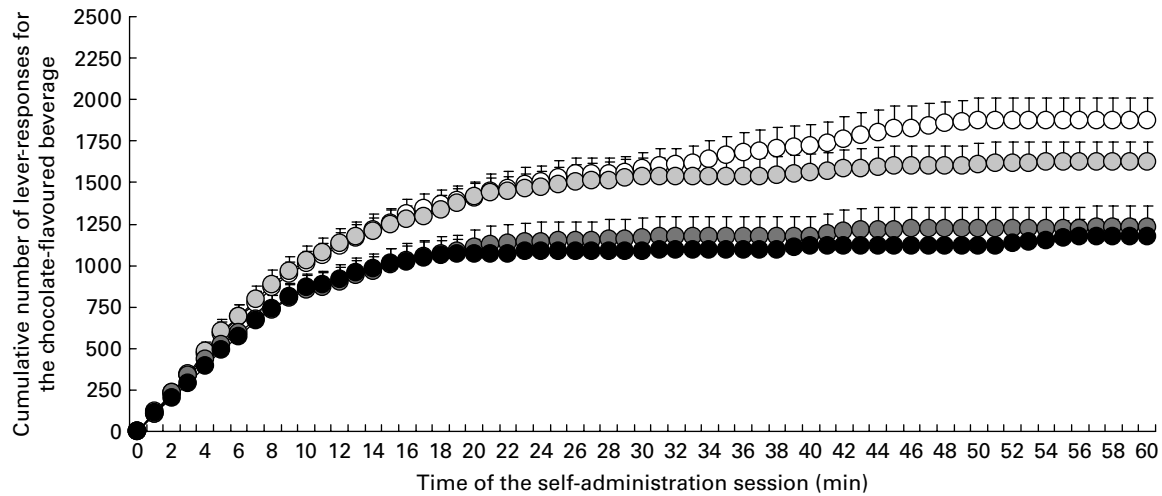


Fig. 2. Effect of the administration of a *Phaseolus vulgaris* dry extract on cumulative lever-response patterns of self-administration for a chocolate-flavoured beverage (Nesquik® chocolate powder diluted 5% (w/v) in tap water) in Wistar rats exposed to daily 60 min self-administration sessions with a fixed ratio 10 schedule of reinforcement for 5 s accesses to the chocolate-flavoured beverage. All doses of the *P. vulgaris* extract were tested in each rat under a Latin-square design; four non-drug sessions elapsed between test sessions. Values are means for ten rats, with standard errors represented by vertical bars. In the graph, session length was divided into sixty intervals of 1 min each. (—○—), *P. vulgaris* extract at 0 mg/kg; (—□—), *P. vulgaris* extract at 50 mg/kg; (—●—), *P. vulgaris* extract at 200 mg/kg; (—■—), *P. vulgaris* extract at 500 mg/kg.

non-operant⁽¹¹⁾ and operant (present study) investigations, the reducing effect of the *P. vulgaris* dry extract reached its plateau at the dose of 200 mg/kg (55–60 and 35–40% in the non-operant and operant studies, respectively), as administration of higher doses (for example, 500 mg/kg) did not result in any additional, appreciable increase in magnitude of the effect of the *P. vulgaris* dry extract on intake and self-administration of the chocolate-flavoured beverage.

Analysis of cumulative lever-response patterns (Fig. 2) suggests that the *P. vulgaris* dry extract was minimally effective on the rats' motivation to start lever-pressing for the chocolate-flavoured beverage, as (a) latency to the first lever-response was extremely short and (b) the rate of lever-responding over the first 5–6 min was virtually identical in all rat groups. The rat behaviour tended to differentiate, among the four rat groups, after the first 8–10 min, when rats treated with the two highest doses of the *P. vulgaris* dry extract (200 and 500 mg/kg) rapidly slowed down and then ceased their lever-pressing behaviour, while it was maintained, at relatively high rates and for an additional 10 min, in the rat groups treated with 0 and 50 mg *P. vulgaris* dry extract per kg. These data may be interpreted as suggesting that 200 and 500 mg *P. vulgaris* dry extract per kg facilitated the achievement of a satiety state for the chocolate-flavoured beverage after an initial period of sustained intake.

Reduction in lever-responding for and self-administration of the beverage were probably not due to any unspecific, sedative effect or possible malaise induced by the *P. vulgaris* dry extract. A recent study⁽¹¹⁾ demonstrated indeed that doses of this *P. vulgaris* dry extract up to 500 mg/kg failed to affect, even minimally, spontaneous locomotor activity in Wistar rats exposed to an open-field arena; notably, spontaneous locomotor activity of rats in an unfamiliar (never explored) environment constitutes a parameter highly sensitive to alterations in the state of wellbeing of rats.

As mentioned above, the chocolate-flavoured beverage employed in the present study was prepared as it (a) was

highly palatable (being the most widely preferred over a large range of concentrations⁽¹²⁾) and (b) provided a relatively modest energy supply (0.8 kJ/g, approximately 17 times lower than that provided by regular food pellets (13.8 kJ/g)). These two features should have resulted in a beverage with a low energy, or nutritive, value and high hedonic value; it is therefore conceivable that the chocolate-flavoured beverage was probably consumed mostly for its palatability rather than energy supply.

The exact mechanism of the reducing effect of the *P. vulgaris* dry extract on self-administration of the chocolate-flavoured beverage is presently unknown. Lectins are the probable active ingredients of *P. vulgaris* beans responsible for the reducing effect on appetite, food intake and body weight^(2,3). Specifically, lectins inhibit pancreatic α -amylase, resulting in (a) reduced carbohydrate absorption and metabolism and (b) delay of gastric emptying, which, in turn, promotes feelings of satiety^(6,13–15). However, pertaining to the effect on carbohydrate absorption and metabolism, the negligible content of starch and complex sugars in the chocolate-flavoured beverage employed in the present investigation makes it unlikely that intraluminal inhibition of α -amylase played a relevant role in *P. vulgaris* dry extract-induced reduction in self-administration of the chocolate-flavoured beverage. Additional mechanisms may involve the lectin, phytohaemagglutinin, known to modulate the activity of cholecystokinin and glucagon-like peptides and, therefore, interfering with the central regulation of appetite, satiety and food consumption^(1–3).

To summarise, the results of the present study demonstrated the capacity of a *P. vulgaris* dry extract to reduce the robust reinforcing properties of a chocolate-flavoured beverage in rats. These results are in close agreement with previous data indicating that the same *P. vulgaris* dry extract was effective in (a) reducing the daily consumption of an identical chocolate-flavoured beverage offered to rats in free choice with water under the two-bottle regimen⁽¹¹⁾, and (b) suppressing the

overeating of highly palatable butter cookies in presatiated rats⁽³⁾. A recently completed, double-blind placebo-controlled trial found that a 2-month treatment with a dietary supplement made up of the *P. vulgaris* dry extract employed in the present study together with an extract of *Cynara scolymus* increased the feeling of satiation (measured by the Haber's scale for hunger/satiety scoring) in healthy overweight and obese subjects⁽¹⁰⁾. Should the capacity of the *P. vulgaris* dry extract to reduce the consumption and reinforcing properties of highly palatable foods and beverages extend to humans, *P. vulgaris* dry extract would possess an interesting and promising therapeutic potential for treating overeating and its consequences.

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P. Maccioni, G. C. and M. A. M. C. conceived of the study, designed the experiment, and managed the literature searches and summaries of previous related work. A. R., P. Morazzoni and E. B. prepared and analysed the plant extract. P. Maccioni and M. A. M. C. conducted the experiments and analysed the data. G. C. drafted the manuscript. G. L. G. supervised the study. All authors contributed to and approved the final draft of the manuscript. G. C. takes responsibility for the paper as a whole.

A. R. and P. Morazzoni are employees at Indena S.p.A., Milan, Italy. E. B. is a consultant for Indena S.p.A., Milan, Italy.

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