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Effect of orally and intraperitoneally administered plant lectins on food consumption of rats

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A panel of orally administered lectins (100 mg/kg b.w.) of different binding specificities was tested for suppression of voluntary food consumption in prefasted rats. PHA isolectins (*Phaseolus vulgaris*) and RPA-I (*Robinia pseudoacacia*) were found to exert a marked and significant effect, but two other gut-binding lectins, i.e. SBA (*Glycine max*) and WGA (*Triticum vulgare*) and several non-binding lectins were ineffective. In cannulated rats PHA infused into the duodenum induced food suppression, i.e. binding of the lectin to the mouth or stomach was unnecessary. Suppression of food consumption lasted through the whole nocturnal feeding period, control (BSA) and experimental (PHA) curves of cumulative food consumption showed a V-like divergence. Suppression by PHA or RPA-I showed very similar time courses, but a long-lasting inhibition of gastric emptying was only observed in the RPA-treated animals. Intraperitoneally administered lectins suppressed food consumption much more effectively than the oral ones, whereas *Galanthus nivalis* agglutinin (GNA) had little or no effect. It is concluded that lectins can be used as effective tools for the modulation of food consumption and gastric emptying in experimental animals.

Keywords: lectin, food consumption, gastric emptying, GNA, PHA, WGA

Plant lectins are carbohydrate-binding proteins that do not make covalent modifications in the carbohydrate receptor. If fed to rats, several lectins are able to resist digestion and to bind to the surface of gastrointestinal epithelial and endocrine cells and less than 5 to 10% of the oral dose is transmitted into the circulation and to the tissues. These oral

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lectins damage the intestinal brush border locally, induce the growth of gut wall and pancreas and mobilize body energy stores (7, 12).

Plant materials containing lectins have frequently been observed to suppress eating in laboratory animals. In the present study we examined the effect of a panel of purified lectins on voluntary food consumption of rats. In addition, we attempted to locate the origin of the satiety signal.

Material and methods

Chemicals

Kunitz-type trypsin inhibitor (STI), concanavalin A (ConA) and bovine serum albumin (BSA) were purchased from Sigma. The rest of the lectins were purified by column chromatography, desalted by dialysis, freeze-dried, then checked by haemagglutination and SDS-PAGE.

Kidney bean (*Phaseolus vulgaris*) phytohaemagglutinin (PHA) was purified by the combination of ion-exchange and affinity chromatography (1). One of the preparations contained L_4 isolectin and the other one a mixture of EL_3, E_2L_2 and E_3L isolectins. The rest of the lectins was purified by affinity chromatography: Snowdrop bulb (*Galanthus nivalis*) agglutinin (GNA) on agarose-bound mannose (10), soyabean (*Glycine max*) agglutinin (SBA) on epichlorohydrine crosslinked guar-gum (9), wheat germ (*Triticum vulgare*) agglutinin (WGA) on N-acetyl-glucosamine-Sepharose (13), asparagus pea (*Tetragonolobus purpureas*) agglutinin (TPA) on L-fucose-Sepharose (2), black locust (*Robinia pseudoacacia*) bark lectin (RPA-I) (11) and black elder (*Sambucus nigra*) bark lectin (SNA-I) on fetuin-Sepharose column (3), mistletoe (*Viscum album*) lectin (ML-1), in form of an ammonium sulfate precipitate, on lactosyl-Sepharose (4), and fava bean (*Vicia faba*) agglutinin (VFA) on Sephadex G-100 (14). This latter lectin was dialyzed against 10^{-3} M $CaCl_2$ containing 10^{-5} $MnCl_2$ before liophilization.

Animal experiments

In the experiments involving the comparison of PHA and RPA-I male hooded Lister rats with 175 g mean weight were used. In all other experiments female 110 to 140 g Harlan-Wistar rats were applied, group means were between 120 and 130 g. Carbohydrate specificities of the lectins are shown in Table I. Nine lectins were tested in oral experiments (PHA, RPA-I, ConA, SBA, WGA, SNA-I, TPA, GNA and ML-1) and seven lectins intraperitoneally (PHA, RPA-I, ConA, WGA, SNA-I, VFA and GNA).

In the food consumption experiments the rats were allotted randomly into groups of 4 to 6. A BSA-treated control group was included into each experiment. After 24 h fast the animals were transferred to individual cages and had free access to water throughout. Due to the feeding habits of the rats the experiments were started at dusk and finished in the morning. All manipulations were performed in dim light. The lectin or the control BSA was administered by gastric intubation or intestinal infusion, dissolved in 0.4 ml

physiological saline, i.p. lectin was injected in 0.2 ml saline. Immediately after the treatment intact, pre-weighed cylinders of granulated chow were laid onto the feeding grid and removed periodically for weighing to calculate cumulative food consumption for 12, 18 or 24 hours. During the experiment the animals consumed the same commercial, granulated chow they were used to, i.e. we did not need to comprise the lectin into food granules (partial inactivation) or to feed it in form of powder (scatter by the rats). At the end of one of the experiments the rats were killed with an overdose of ether, decapitated, bled and eviscerated. Gastric and small intestinal contents were flushed with PBS, the washings collected, centrifuged, decanted and the wet pellet weighed. Cecal and colon contents were weighed together with the gut wall.

Table I

The lectins used in the experiments

Abbreviation	Latin name	Source	Carbohydrate specificity
VFA	*Vicia faba*	broad bean	glucose/mannose
ConA	*Canavalia ensiformis*	jack bean	glucose/mannose
GNA	*Galanthus nivalis*	snowdrop bulb	mannose-(1,3)mannose
WGA	*Triticum vulgare*	wheat germ	GlcNAc-β(1,6)Gal-sialic acid
SBA	*Glycine max*	soybean	galactose/GalNAc
ML-1	*Viscum album*	mistletoe	galactose
TPA	*Tetragonolobus purpureas*	asparagus pea	L-fucose
SNA-I	*Sambucus nigra*	bark of elderberry	sialic acid-α(2,6)galactose
PHA	*Phaseolus vulgaris*	kidney bean	complex specificity*
RPA-I	*Robinia pseudoacacia*	false acacia bark	complex specificity*

*Not inhibited by simple sugars.
With the exception of fucose, the sugars are in "D" configuration.

Operation

For the infusion experiments polyethylene cannula was inserted into the lumen of the duodenum. After a 12-hour fast 120 g female Wistar rats were anaesthetised by xylazine and ketamine, injected separately i.p. After laparatomy the cannula was inserted 0.5–1.0 cm long into the lumen of the small intestine and fixed by a purse string suture and Histoacryl Blau (Braun GmbH) tissue glue. The other end of the cannula was lead across the abdominal muscles and under the skin up to the neck. When crossing the skin the cannula was fixed with suture and glue, and used for infusing lectin or control solution into the gut. The abdominal wound was closed by sutures and protected by Plastubol spray. Tardomyocel, a retard mixture of antibiotics was administered locally to support reconvalescence. After 7 to 10 days the animals were used for the experiment. BSA-treated control rats were prepared comparably.

Statistical analysis

The results were subjected to one-way analysis of variance (ANOVA).

Results

Orally administered lectins

Nine lectins with different carbohydrate binding specificities were administered by gastric gavage and BSA control was applied at each experiment. Only PHA and RPA-I (Figs 2–4) were able to suppress food consumption markedly in a single oral dose of 100 mg/kg b.w. as compared to the control. ConA had marginal effect (Fig. 1), whereas SBA, WGA, SNA-I, TPA, GNA and ML-1 was ineffective. In an additional control experiment soybean Kunitz inhibitor (STI) was tested at the level of 100 and 200 mg/kg b.w., but no suppression could be demonstrated.

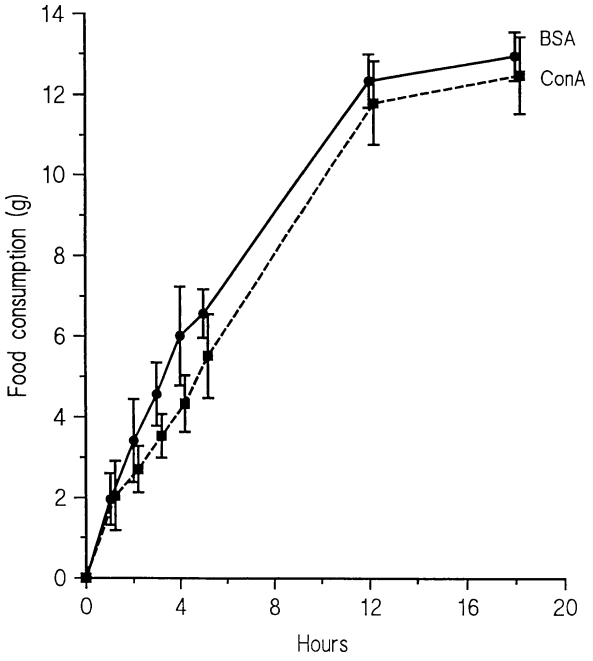

Fig. 1. Effect of ConA on food consumption of growing rats (n = 4). The lectin tended to suppress consumption, the difference reached the level of significance ($p<0.05$) only at 4 h, if compared to the BSA-treated control. Administration by gastric intubation, 100 mg/kg b.w.

When PHA L_4 isolectin was administered by gastric gavage, food consumption tended to decrease within the first hour of the experiment, the difference (compared to BSA) reached the level of significance at 5 hour (Fig. 2). The two curves showed a V-like divergence during the nocturnal feeding period (up to 12 h from the start of the experiment) then they were running mostly parallel to each other during the next daylight period.

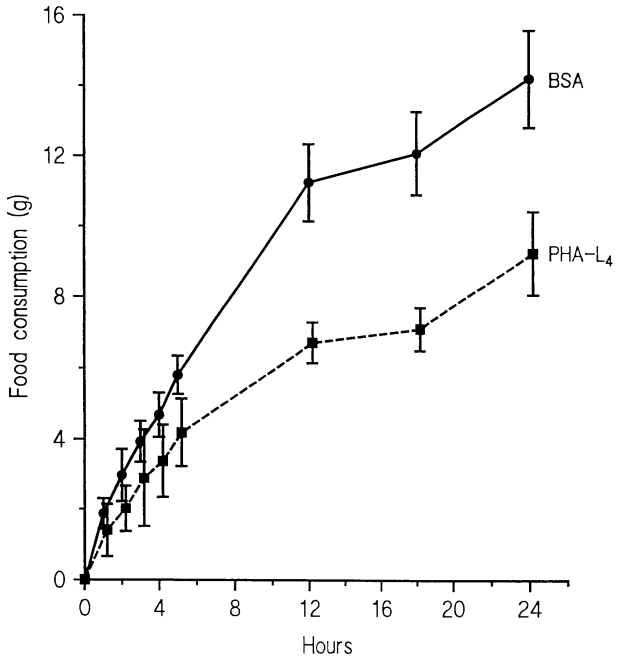

Fig. 2. Effect of PHA L_4 isolectin (100 mg/kg b.w.) administered by gastric gavage on food consumption of growing rats (n = 4). The difference was significant ($p<0.05$) at 5 h the first time and highly significant ($p<0.01$) afterwards

Experiments with cannulated rats

In another experiment the proximal duodenum of rats was chronically cannulated and PHA-L_4 or control BSA was infused. Food consumption was measured as before. The difference in food consumption became significant already within the first hour (Fig. 3) and further increased during the dark period.

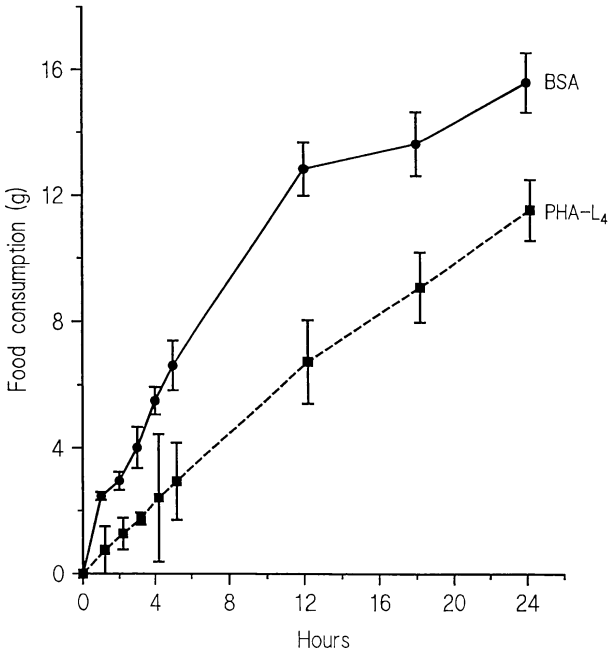

Fig. 3. Effect of PHA L_4 isolectin (100 mg/kg b.w.) infused into the proximal duodenum on food consumption of growing rats (n = 4). The difference was significant ($p<0.05$) at 1 and 4 h, and highly significant ($p<0.01$) at other time points

Comparison of the oral effects of PHA and RPA-I

In another experiment the effect of three lectins administered by gastric gavage was compared. Both PHA (mixed E and L type isolectins) and RPA-I suppressed food consumption, the difference was not significant between them (Fig. 4). As compared to the BSA-treated control group, the difference reached significance at 2 h ($p<0.05$). The curve of the SNA-I-treated group (not shown) did not differ significantly from that of BSA.

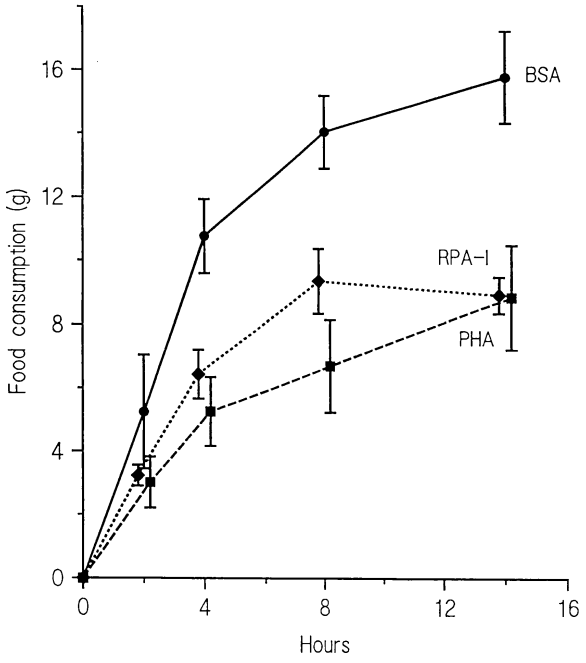

Fig. 4. Effect of PHA (mixed isolectins) and RPA-I on food consumption of growing rats (n = 5). The difference, compared to BSA, was already significant at 2 h ($p<0.05$) and highly significant afterwards ($p<0.01$). The two lectins differed significantly ($p<0.05$) at 8 h only. The lectins were administered by gastric gavage, 100 mg/kg b.w.

Three hours after the end of the experiment the rats were killed and the content of different portions of the gastrointestinal tract were weighed. In the RPA-treated group the stomach content was significantly higher ($p<0.001$), than in the other groups (Fig. 5). Accordingly, there were significantly less small intestinal and caecal contents in the RPA-I group, than in most of the other groups. In the colon little inter-group difference was found.

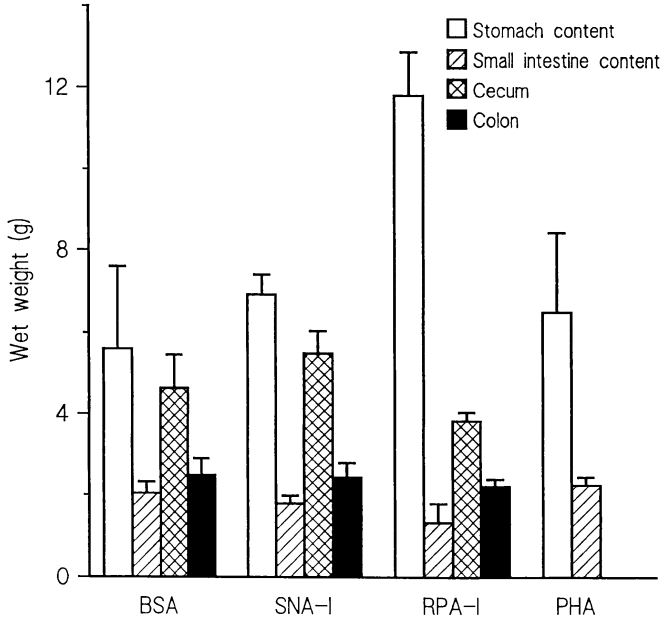

Fig. 5. Effect of lectins (100 mg/kg b.w.) on the weight of wet gastrointestinal contents 17 hours after the administration of the lectin by gastric gavage (n = 5). The wall of the cecum and colon was weighed together with the contents

Intraperitoneal lectins

In the experiment with mixed PHA isolectins a dose-dependent suppression of food consumption was found (Fig. 6). If used in the same dose as orally (100 mg/kg b.w.) most of the lectins (PHA, RPA-I, ConA, WGA, SNA-I) induced an almost total refusal of food. Little or no effect was found with GNA, if compared with control BSA or STI. The effect of VFA was intermediate.

Discussion

Many observations indicate that raw legume meals containing antinutritional proteins suppress food consumption by laboratory and farm animals. We started, therefore, a systematic study of the effect of isolated lectins (Table I) on voluntary food consumption in rats. The oral dose of the lectins (100 mg/kg b.w.) used in the experiments was sufficient to exert pronounced biological effects as shown in previous experiments (1).

Soybean trypsin inhibitor (Kunitz type), a non-lectin antinutritional protein did not affect food consumption.

Larue-Achagiotis et al. (6) showed that rejection of food containing ConA was not due to taste aversion and the effect was not lessened if the lectin was administered by gastric gavage. The lectins used in the present experiments had no taste or astringent effect and were administered through gastric intubation.

Orally we tested a panel of nine different lectins. Two gut-binding lectins (PHA and RPA-I) suppressed food consumption markedly and significantly (Figs 2–4), while ConA had little or no effect (Fig. 1). Six other lectins were without effect. This latter group consisted of both gut-binding lectins (SBA, WGA, ML-1) and those which had few receptors on the brush border (TPA, GNA, SNA-I). Although the fucose-specific TPA is able to bind to the gut, this binding may be inhibited by the high affinity of this lectin to gastrointestinal mucus. Two of the lectins (SNA-I and ML-1) contained ribosome inactivating protein (RIP) subunits, but this did not appear to affect the results.

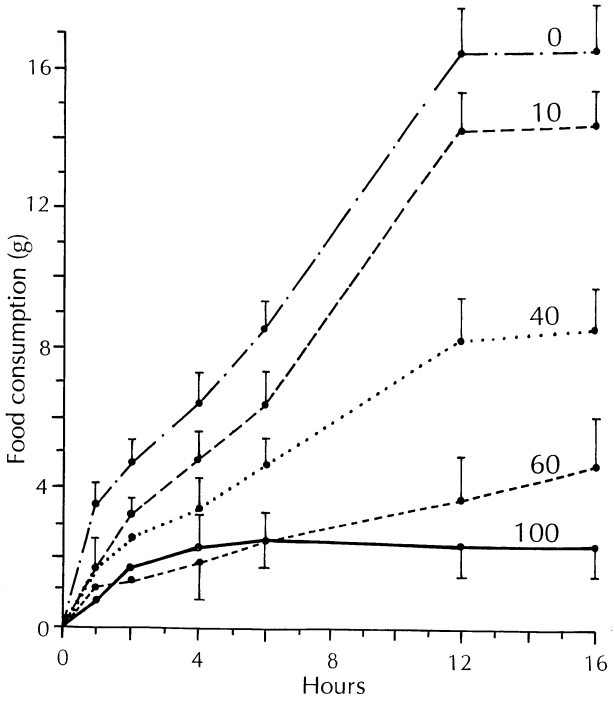

Fig. 6. Effect of different doses (up to 100 mg/kg b.w.) of intraperitoneally administered PHA (mixed isolectins) on food consumption of growing rats (n = 5)

Our findings with ConA are only in partial agreement with those of Larue-Achagiotis et al. (6), which may be due to the fact, that in comparable experimental situations they used 8.5-fold higher lectin doses than we did. ML-1 also suppresses food consumption, if fed in extremely high quantities (8).

Using PHA L_4 isolectin, we examined the effect of the place of administration on the suppression of food consumption. Gastric gavage (Fig. 2) and infusion of the lectin directly into the proximal duodenum (Fig. 3) had similar effects, except that in the latter case the difference to the BSA control became significant earlier. It appears, therefore, that the satiety signal arose beyond the pylorus and that the mouth and stomach were unnecessary for the suppression of food consumption.

Two different preparations of PHA were used in the present experiments, one of them was the L_4 isolectin and the other a mixture of isolectins consisting of the closely related E and L polypeptide chains. Both preparations exerted similar effects. The latter preparation was compared with RPA-I, a related lectin. Although both lectins had marked and similar depressive effects on food consumption (Fig. 4), their mediation did not appear to be quite the same. Although the rats were killed as late as 17 h after the administration of the lectin, maximal gastric fill was observed in the RPA-treated rats (Fig. 5) and less intestinal digesta was found than in the groups treated with PHA, SNA-I or BSA. These findings indicate a powerful and long-lasting inhibition of gastric emptying after administration of RPA.

The experiments always started in the evening, after a 30-hour fast. If the animals were fasted only from early morning to evening, i.e. during the inactive daylight period, the stomach was not fully emptied and the response to PHA was much lower.

Most of the orally administered lectins bind to the intestinal brush border and partial absorption into the circulation of the rat was demonstrated (7). Bound PHA stimulates the release of cholecystokinin (5) and glucagon-like peptide (Glp-1), which hormones modulate food consumption, and decrease serum gastrin concentration (Jordinson M, Baintner K and Pusztai A., unpublished). The question arose, whether PHA bound to the intestinal epithelium would be sufficient to suppress food consumption by releasing gastrointestinal peptide hormones or the absorbed PHA was the functional agent by releasing cytokines or other mediators. In control experiments, therefore, we injected lectins intraperitoneally to rats in a dose comparable to that of the oral administration (100 mg/kg b.w.). The effect of i.p. lectins differed markedly from that of the oral ones, this will be dealt with in a separate paper. In respect to food consumption i.p. PHA and RPA-I had much higher effect than orally. Both these lectins and the orally ineffective ones (WGA, SNA-I, ConA) inhibited the intake almost completely. The smaller effect of VFA might be due to the instability of the molecule. GNA, a lectin that was applied in biotechnology as a transgenic insecticide, was ineffective orally and had little or no effect intraperitoneally, if compared to the control BSA or STI, possibly due to the lack of receptors.

Oral PHA did not work well, if the rats had a full stomach or if the dose was decreased from 100 to 50 mg/kg b.w., whereas intraperitoneally 10 mg/kg b.w. PHA was already effective (Fig. 6).

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REFERENCES

1. Bardocz S, Grant G, Ewen SWB, Duguid TJ, Brown DS, Englyst K, Pusztai A: Reversible effect of phytohaemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut 37, 353–360 (1995)
2. Blumberg S, Hildeheim J, Yariv J, Wilson KJ: The use of 1-amino-L-fucose bound to Septharose in the isolation of L-fucose-binding proteins. Biochim. Biophys. Acta 264, 171–176 (1972)
3. Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ: A lectin from elder (*Sambucus nigra* L.) bark. Biochem. J. 221, 163–169 (1984)
4. Göckeritz W, Körner IJ, Kopp J, Bergmann J, Pfüller K, Eifler R, Pfüller U: Mistletoe lectins: Comparative studies on cytotoxicity, receptor binding and their effect on the cytosolic calcium content in human lymphocytes. In: Van Driessche E: Lectins. Biology, Biochemistry, Clinical Biochemistry. Proc. of the 10th International Lectin Meeting (Szeged, Hungary), 10, 345–353 (1994)
5. Herzig KH, Bardocz S, Grant G, Nustede R, Fölsch UR, Pusztai A: Red kidney bean lectin is a potent CCK releasing stimulus inducing pancreatic growth. Gut 41, 333–338 (1997)
6. Larue-Achagiotis C, Picard M, Louis-Sylvestre J: Feeding behavior in rats on a complete diet containing Concanavalin A. Reprod. Nutr. Dev. 32, 343–350 (1992)
7. Pusztai A (1991): Plant Lectins. Cambridge Univ. Press, Cambridge
8. Pusztai A, Grant G, Gelencsér E, Ewen SWB, Pfüller U, Eifler R, Bardocz S: Effects of an orally administered mistletoe (type-2 RIP) lectin on growth, body composition, small intestinal structure, and insulin levels in young rats. Nutr. Biochem. 9, 31–36 (1997)
9. Pusztai A, Watt WB, Stewart JC: A comprehensive scheme for the isolation of trypsin inhibitors and the agglutinin from soybean seeds. J. Agric. Food Chem. 39, 862–866 (1991)
10. van Damme EJM, Allen AK, Peumans WJ.: Isolation and characterization of a lectin with exclusive specificity towards mannose from snowdrop (*Galanthus nivalis*) bulbs. FEBS Letters 215, 140–144 (1987)
11. van Damme EJM, Barre A, Smeets K, Torrekens S, van Leuven F, Rougé P, Peumans WJ: The bark of *Robinia pseudoacacia* contains a complex mixture of lectins. Plant Physiol. 107, 833–843 (1995)
12. van Damme EJM, Peumans WJ, Pusztai A, Bardocz S, (1997)*:* Handbook of Plant Lectins: Properties and Biomedical Applications. Wiley, Chichester
13. Vretblad P: Purification of lectins by biospecific affinity chromatography. Biochim. Biophys. Acta 434, 169–176 (1976)
14. Wang JL, Becker JW, Reeke GN jr, Edelman GM: Favin, a crystalline lectin from *Vicia faba*. J. Mol. Biol. 88, 259–262 (1974)