Oral Toxicity of Kintoki Bean
(Phaseolus vulgaris) Lectin

Tomoko HARA,¹ Ikuyo TSUKAMOTO,¹ and Masamitsu MIYOSHI²

¹Graduate Division of Human Culture
(Doctoral Degree Program),
Nara Women’s University, Nara 630, Japan
²Laboratory of Nutritional Chemistry,
Department of Food Science and Nutrition,
Nara Women’s University, Nara 630, Japan

(Received March 14, 1983)

Summary There are several antinutritive factors in the Kintoki bean such as lectin, trypsin inhibitor, lack of methionine etc. The present experiment has revealed that lectin is mainly responsible for the growth impairment of experimental animals orally fed raw Kintoki bean. Mice fed raw Kintoki bean as the only protein source lost their body weight and died in 8 days, while mice fed the heated bean grew normally. When mice on a 10% albumin diet ingested 20mg or 40mg or 60mg Kintoki bean lectin by daily stomach-feeding, their body weights were reduced to 84%, 74%, 71% of the control group after 5 days respectively and some of them could not live to complete the experiment. The apparent rates of the intestinal absorption of carbohydrate, lipid, and protein were considerably reduced, when rats were fed a diet containing 0.4% lectin. Especially, the rate of protein absorption was decreased to 26.3% from 55.5% of the control rate. The main tissues of mice that had ingested Kintoki bean lectin by stomach-feeding were subjected to microscopic observation. No changes were observed in the liver, kidney, spleen and pancreas. But in the small intestine, the epithelial cells lining the villi were considerably disordered and conspicuously disrupted. These results indicate that the Kintoki bean lectin is one of the most promoting factors for growth impairment in experimental animals and that the first target organ in the case of oral feeding is the small intestine.

Key Words toxic lectin, oral toxicity, Phaseolus vulgaris bean, growth impairment, disruption of the villi

Bean protein is expected to cover exceedingly the protein deficiency which people are now facing in the world (1, 2). It has been recognized, however, that the nutritive values of legumes are very poor unless they are subjected to cooking or

¹ 原 知子，塚本幾代，² 三好正満
some other forms of heat treatment. The depression in the protein value of raw beans has been generally attributed to the presence of several antinutritive factors in the beans (3, 4). For example, there are toxic substances or growth inhibitory factors in many species of beans such as trypsin inhibitor, lectin, goiterogenic factor, cyanogenetic glucoside, saponin and substances causing lathyrism and favaism (1, 5, 6). Particularly, lectin having hemagglutinating and cell-agglutinating activity has been found in not only beans but more than one thousand species of plants (7, 8). Still more, it has been found in microorganisms and animals including vertebrate tissues such as liver, brain, muscle, heart and lung (9). The wide distribution of lectin in foods necessarily results in our constant ingestion of this potentially toxic protein. Lectin is generally heat-labile. It has been, however, reported that lectin cannot be completely inactivated under some cooking conditions (10), and that tomatoes and seaweeds ingested raw also contain lectin (11).

Thinking of these facts, we are compelled to take many kinds of lectin in active forms. From another point of view, the extract of Phaseolus vulgaris beans was used for human weight control but toxic symptoms were seen from its use in America. It was also reported that children were poisoned by kidney bean in England (12). The toxic effects of the whole beans containing plural toxic substances have been studied for many years. But the specific function of each toxic substance has not been well characterized.

We already purified a lectin from Phaseolus vulgaris Kintoki bean and characterized it chemically and confirmed that the intraperitoneal or intravenous injection of the Kintoki bean lectin causes death of mice in a short period (13, 14). Therefore, it is important to elucidate the mechanisms of the toxic effects caused by lectin fed orally.

In this paper we investigated the growth inhibitory effects of the Kintoki bean lectin on mice and rats fed orally, and analyzed the toxic function from the nutritional and morphological points of view.

**EXPERIMENTAL**

*Preparation of Kintoki bean lectin.* Kintoki bean lectin was purified according to the method described previously by Hamaguchi et al. (13). Hemagglutinating activity was assayed with a microtiter plate by the serial double dilution method using 1% mouse red blood cells.

*Feeding experiments.* The following growth experiments were carried out.

1. Ten mice (ddY strain, about 20 g body weight, male) were fed *ad libitum* a bean diet containing raw or autoclaved (120°C, 20 min) Kintoki bean at a 10% protein level. The composition of the bean diet was as follows; raw or autoclaved Kintoki bean powder 52.2 g, starch 9.8 g, sugar 30.0 g McCollum mineral mixture 5 g, soy bean oil 1 g and cellulose powder 1 g. A vitamin mixture (Harper’s, Oriental Co.) was added to the diet of each animal (0.04 g/day). Mice were weighed at 9:00 every morning and caged in a room kept at 23–25°C with lighting automatically.
regulated to provide a 12h light period and a 12h dark period.

2. Nine mice (ddY stain, about 20g body weight, male) were divided into 3 experimental groups. They were fed \textit{ad libitum} a basal albumin diet and each group was given 20 mg or 40 mg or 60 mg Kintoki bean lectin dissolved in 1 ml water by stomach-feeding daily. As the control experiment, five mice from two groups were fed 1 ml water or 40 mg albumin dissolved in 1 ml water by stomach-feeding daily. Five mice from the other control group were not treated with stomach-feeding at all. Before starting the experiment, it was confirmed that the stomach-feeding of 1 ml water did not affect the normal growth of mice. The composition of the basal albumin diet was as follows; albumin 10 g, wheat starch 63 g, cellulose powder 8 g, soy bean oil 6 g, McCollum mineral mixture 6 g, sugar 5 g and vitamin mixture (Harper's, Oriental Co.) 2 g. During the breeding period, the mice were fastened from 9:00 to 13:00. At the end of the fasting time, they were weighed and given lectin or control substances by stomach-feeding. From 14:00 until 9:00 of the following day the mice were fed the basal albumin diet \textit{ad libitum}. The daily food intake was recorded. Other breeding conditions were the same as those of experiment 1. On the seventh day, the mice were decapitated, and the liver, kidney, spleen, pancreas, stomach and intestine were excised and weighed. These tissues were fixed in formalin and imbedded in paraffine. Their sections were subjected to hematoxylin-eosin staining and observed under a microscope.

3. Six rats (Wister strain, about 60g body weight, male) were fed \textit{ad libitum} a control bean diet containing autoclaved Kintoki bean powder. Another six rats were fed \textit{ad libitum} an experimental bean diet consisting of the control diet supplemented with intact Kintoki bean lectin at 0.4\% level. Body weight was measured daily and food intake were recorded at 9:00 on the morning of the fourth and fifth days. Based on the analyses of the feces and food intake on the fourth and fifth days, the apparent rates of intestinal absorption/digestion of protein, carbohydrate, lipid were calculated according to the following formula;

\[
\frac{\text{Nutrient intake} - \text{Nutrient excreted in feces}}{\text{Nutrient intake}} \times 100 (\%)
\]

Total nitrogen was estimated by the semi-micro Kjeldahl method (15), carbohydrate by the Bertrand method (16), and lipid according to the Van de Karmer's method (17).

RESULTS AND DISCUSSION

As presumed from our previous data (13, 14) and other investigators' data (4, 18, 19) indicating the presence of antinutritive factors in raw beans, whole Kintoki bean when orally ingested was proved to have a toxic effect on mice and rats. Mice fed a diet containing autoclaved Kintoki bean at a 10\% protein level increased their body weight 10\% in nine days, and their motion was also normal in experiment 1. On the other hand, mice fed raw Kintoki bean lost their body weight
conspicuously as shown in Fig. 1, and a bad coat of hair, great reduction of motion and aerenterectasia were noticeably found. All of them died in 8 days. The Kintoki bean contains two main physiologically active substances; trypsin inhibitor and lectin. In addition to these substances, lack of methionine supposedly causes an acute lethal effect, or acts as a promotor of the toxicity. It was reported, however, that a bean diet supplemented with the sulfur containing amino acids did not ameliorate the growth of animals (6). In our control experiment using autoclaved bean, the diet was lacking in the sulfur-containing amino acids, but the mice did not die. The lack of methionine is not conclusively the main lethal factor. However, other toxic factors present in the bean cannot be separately discussed, and the lectin dose cannot be kept constant by the ad libitum feeding in the experiment conducted using the whole beans.

It is reported that the toxicity of various species of kidney bean is proportional to the lectin contents and that lectin is the principle toxin (20). Therefore, we investigated the oral toxic effects of lectin itself using a purified lectin. Similar attempts have been recently carried out in several laboratories (19). Our experiment focused on the quantitative ingestion of pure Kintoki bean lectin to mice, and aimed to reveal the direct single effect of the lectin on mice. Experimental mice on a basal albumin diet were fed 20mg or 40mg or 60mg lectin dissolved in 1.0 ml distilled water by stomach-feeding daily. The body weight of the mice given the lectin decreased considerably, and the degree of the weight reduction is roughly proportional to the lectin dose as shown in Fig. 2. One third or two thirds of the mice in the groups of 40mg and 60mg lectin-feeding died in a few days. On the
other hand, mice given albumin and water by stomach-feeding grew as satisfactorily as mice without stomach-feeding. Comparing the control mice fed 40 mg albumin by stomach-feeding, their body weights are reduced, on the last day, to 84%, 76% and 71%, in the order of 20 mg, 40 mg and 60 mg lectin injection, respectively. In experiment 1 of the raw Kintoki bean feeding (Fig. 1), lectin intake per capita calculated from its contents in the bean is only about 10–20 mg per day, while the body weight reduction is more serious than in experiment 2 of stomach-feeding where larger amounts of lectin, 20–60 mg, were given to mice. The difference between the two cases can be attributed to 1) the difference in the basal diets; the autoclaved bean diet in experiment 1 being inferior in amino acid balance to the albumin diet in experiment 2, 2) raw beans fed to the experimental groups in experiment 1 contain another toxicant, trypsin inhibitor, which should amplify the growth reduction of experimental animals in experiment 1, 3) the obvious decrease of the food intake in experiment 1 (from 3.9 g to 1.8 g per day per capita). When lectin was ingested by stomach-feeding, the intake of the basal albumin diet did not change in experiment 2. The PER were $-1.04$, $-2.73$ and $-3.02$ in the order of 20 mg, 40 mg and 60 mg groups of lectin ingestion in contrast to the control PER, 2.89. In other words, the protein efficiency is obviously lowered, when only a small amount of lectin was added to the basal diet.

Table 1 shows the apparent rates of intestinal absorption/digestion of carbohydrate, lipid and protein in rats (experiment 3). Using Kintoki bean as the single source of protein, the rate of absorption of protein is considerably low, 55.5%, even in the control group of the autoclaved bean diet. Addition of the lectin, however, further lowered the absorption rate to 26.3%. Although to much less extents, the rates of carbohydrate and lipid were also lowered from 99.6% to 94.4% and from

---

**Fig. 2.** Growth of mice fed various amounts of lectin by stomach-feeding.
Table 1. Effects of the addition of Kintoki bean lectin to the diet on the absorption of major nutrients by rats.

|                  | Autoclaved bean + lectin | Autoclaved bean    |
|------------------|--------------------------|-------------------|
| Body weight change (g/day) | -1.3                     | +1.2              |
| Food intake (g/day)         | 4.8                      | 7.8               |
| N absorbed (%)            | 26.3                     | 55.5              |
| Carbohydrate absorbed (%)  | 94.4                     | 99.6              |
| Lipid absorbed (%)        | 83.3                     | 92.6              |
| PER                       | -0.37                    | +0.65             |

Table 2. Effects of the addition of Kintoki bean lectin to the diet on the tissue weight of mouse and rat.

| Animal | Diet                  | Liver (g/100 g b.w.) | Pancreas (g/100 g b.w.) | Spleen (g/100 g b.w.) | Kidney (g/100 g b.w.) |
|--------|-----------------------|----------------------|-------------------------|-----------------------|-----------------------|
| Mouse  | Albumin diet          | 6.75 ± 0.24          | 1.20 ± 0.18             | 0.32 ± 0.01           | 1.52 ± 0.33           |
|        | Albumin diet + lectin | 5.41 ± 0.69          | 1.04 ± 0.38             | 0.21 ± 0.00           | 1.57 ± 0.22           |
| Rat    | Autoclaved bean diet  | 3.85 ± 0.19          | 0.48 ± 0.05             | 0.29 ± 0.03           | 1.16 ± 0.05           |
|        | Autoclaved bean diet + lectin | 3.79 ± 0.33          | 0.58 ± 0.11             | 0.25 ± 0.22           | 1.32 ± 0.12           |

Means ± SE.

92.6% to 83.3%, respectively. As endogenous nitrogen was not taken into consideration in calculating the absorption rate, the real difference is assumed not so strikingly large. On the other hand, when the mouse fed lectin were dissected, the small intestine was observed expanded and the muscle on the serosal side appeared very thin and fragile. In the liver, kidney, spleen and pancreas, no changes were found histologically. Considering the binding properties of lectin to the epithelial cells (21) and the decrease of the rates of intestinal absorption/digestion of main nutrients in rats, the intestine is possibly regarded as the first and probably the main target tissue of orally fed lectin. When mice were injected the Kintoki bean lectin intraperitoneally, congestion occurred in the liver, lung and spleen (14). Table 2 shows the tissue weight of mice fed 40 mg lectin by stomach-feeding on an albumin diet in experiment 2 and of rats in experiment 3. When the same lectin was orally fed, no congestion was found and only the weight of the spleen decreased (Table 2). Any significant change in the liver and pancreas weight which often accompany the effects of trypsin inhibitor was not found in the present experiment. But in the small intestine, the disorder of the epithelial cell lining on the villi, and their conspicuous disruption were observed. Many of the disruptions occurred at the side and top of
Fig. 3. Section of small intestine 10 cm distal from pylorus (×1,000, HE-stain), (a) from control mouse fed 40 mg albumin per day, (b) from experimental mouse fed 40 mg lectin per day.
Fig. 4.

*J. Nutr. Sci. Vitaminol.*
the villi, but few at the crypts. Figure 3a shows the normal lining of the epithelial cells on the villi of the mice fed 40 mg albumin by stomach-feeding, while Fig. 3b shows the abnormality of the cell lining on the villi of the mice administered Kintoki bean lectin. In addition, the villi themselves are shortened and sparsely located as often found in malabsorption syndrome (Fig. 4b). Obviously, the Kintoki bean lectin exhibits more varied toxic functions by different mechanisms in the oral administration than in the intraperitoneal injection. The conspicuous disruption of the epithelial cells on the villi suggests that the binding of the lectin to the cell surface receptors should bring about the nonspecific inhibition of nutrient absorption in the small intestine. The rare density of the villi also suggests that the lectin binding should result in the serious detachment of the epithelial cells from the villi as Jaffe proposed (3).

For the lectin to exhibit toxicity to its full extent, it must be stable in the digestive tract. According to our preliminary experiment, the Kintoki bean lectin is not easily inactivated by intraluminal digestive enzymes either *in vitro* or *in vivo*. Consequently, it is concluded that the Kintoki bean lectin administered by stomach-feeding can afford to attack the intestine as the first target of its toxic action and cause the abnormality of intestinal nutrient absorption. However, such an inhibition of nutrient absorption is brought about by not only the physical disruption in the intestinal tissue but other reasons. For example, it has been reported that the winged bean diet lowered the intraluminal sucrase activity (18), and that raw field bean diet accelerated the guanidinoacetate methyltransferase activity and thereby the protein catabolism in the liver (22). The binding of the intrinsic factor-vitamin B12 complex to brush border membranes is also reported to be interfered by a lectin (23). Furthermore, considering such a conspicuous disruption of the intestinal cells as found in this experiment, a small but significant amount of lectin possibly penetrates into the blood stream and reveals a more direct toxic action as Pusztai reported in a paper about the presence of the immunologically intact lectin in the blood of rat orally fed kidney bean lectin (19, 24). In this paper, we concluded that the growth retardation of mice and rats observed with a Kintoki bean diet is largely due to the lectin which causes serious damage to the intestinal cells, possibly resulting in the decrease of nutrient absorption. Mechanisms of the lectin toxicity, however, seem not to be so simple. Especially, the behavior of lectin in the digestive organs should be investigated in more details. Studies are being undertaken in our laboratory focusing on this point and, further, on the absorption of nutrient by everted small intestine.

We are indebted to the late Prof. Yoichi Hamaguchi for advice and encouragement. Thanks are also due to Akiko Nishino and Noriko Yagi.

---

**Fig. 4.** Section of small intestine 10 cm distal from pylorus (×100, HE stain). (a) from control mouse fed 40 mg albumin per day, (b) from experimental mouse fed 40 mg lectin per day.
REFERENCES

1) Jaffe, W. G. (1980): Hemagglutinins (Lectins), in Toxic Constituents of Plant Foodstuffs, ed. by Liener, I. E., Academic Press, New York and London, pp. 73–102.
2) Jaffe, W. G. (1973): Toxic proteins and peptides, in Toxicants Occurring Naturally in Food, ed. by Committee on Food Protection, Food and Nutrition Board, National Research Council of U.S.A., National Academy of Science, Washington, D.C., pp. 106–129.
3) Jaffe, W. G. (1960): Uber Phytotoxins aus Bohnene (Phaseolus vulgaris), Arzneim. Frosch, 12, 1012–1016.
4) Santidrian, S., Marzo, F., Lasheras, B., Cenarruzabeitia, M. N., and Larralde, J. (1980): Growth rate and composition of skeletal muscle of chickens fed different raw legume diets. Growth, 44, 343–354.
5) Haynes, R., and Feeny, R. E. (1967): Fractionation and properties of trypsin and chymotrypsin inhibitors from lima beans. J. Biol. Chem., 242, 5378–5385.
6) Liener, I. E. (1976): Legume toxins in relation to protein digestibility—A review. J. Food Sci., 41, 1076–1081.
7) Brady, P. G., Vanniner, A. M., and Banwell, J. G. (1978): Identification of the dietary lectin, wheat germ agglutinin, in human intestinal contents. Gastroenterology, 75, 236–239.
8) Lis, H., and Sharon, N. (1973): The biochemistry of plant lectins (phytohemagglutinins). Annu. Rev. Biochem., 42, 541–574.
9) Simpson, D. L., Thorne, D. R., and Loh, H. H. (1978): Lectins: Endogenous carbohydrate-binding proteins from vertebrate tissues: Functional role in recognition processes? Life Sci., 22, 727–748.
10) Korte, R. (1972): Heat resistance of phytohemagglutinins in weaning food mixtures containing beans (Phaseolus vulgaris). Ecol. Food Nutr., 1, 303–307.
11) Nachber, M. S., Oppenheim, J. D., and Thomas, J. O. (1980): Lectins in the U. S. diet. Isolation and characterization of a lectin from the tomato (Lycopersicon esculentum). J. Nutr. Sci. Vitaminol., 25, 2056–2061.
12) Anonymous (1976): Unusual outbreak of food poisoning. Br. Med. J., 2, 1268.
13) Hamaguchi, Y., Yagi, N., Nishino, A., Mochizuki, T., and Miyoshi, M. (1977): The isolation and characterization of a lethal protein from Kintoki beans (Phaseolus vulgaris). J. Nutr. Sci. Vitaminol., 23, 525–534.
14) Miyoshi, M., Nakabayashi, J., Hara, T., Yawata, T., Tsukamoto, I., and Hamaguchi, Y. (1982): The lethal protein from Kintoki beans (Phaseolus vulgaris) identified as a lectin. J. Nutr. Sci. Vitaminol., 28, 255–264.
15) Cole, J. D., and Parks, C. R. (1964): Semimicro Kjeldahl procedure for control laboratories. Ind. Engi. Chem. (Analytical ed.), 18, 61.
16) Nogeikagaku Zikkensho (in Japanese): ed. by Mitsui, T., Mitsuda, H., and Hata, T., Sangyotosho, Tokyo, pp. 525–527 (1976).
17) Eiyogaku Zikken (in Japanese): ed. by Inagaki, C., Sangyotosho, Tokyo, pp. 32–34 (1976).
18) Kimura, T., Satanchote, C., and Yoshida, A. (1982): Effect of feeding of raw winged bean seeds on gastrointestinal function in rats. J. Nutr. Sci. Vitaminol., 28, 27–33.
19) Putztai, A., Clarke, E. M. W., Grant, G., and Kin, T. P. (1981): The toxicity of Phaseolus vulgaris lectins. Nitrogen balance and immunochemical studies. J. Sci. Food Agric., 32, 1037–1046.
20) Pusztai, A., and Palmer, R. (1977): Nutritional evaluation of Kidney beans (Phaseolus vulgaris): the toxic principle. *J. Sci. Food Agric.*, **28**, 620–623.

21) Etzler, N. E., and Branstrator, M. L. (1974): Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. *J. Cell Biol.*, **62**, 329.

22) Santidrian, S., Sobrini, F. J., Billo, J., and Larralde, J. (1981): Guanidinoacetate methyltransferase activity in growing male rat fed on a raw field bean (Vicia faba L.) diet. *Enzyme*, **26**, 103–106.

23) Boedeker, E. C., and Boldt, D. H. (1975): Effect of plant lactins on the binding of human intrinsic factor—Vitamin B12 complex to isolated guinea pig brush border membranes. *Gastroenterology*, **68**, 866.

24) Pusztai, A., Vlarke, E. M. W., and King, T. P. (1979): The nutritional toxicity of Phaseolus vulgaris lectins. *Proc. Nutr. Soc.*, **38**, 115–120.