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New research highlights: Impact of chronic ingestion of white kidney beans (*Phaseolus vulgaris* L. var. Beldia) on small-intestinal disaccharidase activity in Wistar rats

Nader Nciri[a,b,c], Namjun Cho[c,*]

[a] Intestinal Immunophysiology-Research Unit (02/RU/09-02), Faculty of Medicine of Tunis, University of Tunis El Manar, 15 Street Djebel Akhdar, Bab Saâdoun, 1007, Tunis, Tunisia

[b] Department of Animal Resources, Fisheries, and Food Technology, National Institute of Agronomy of Tunisia, 43 Charles Nicolle Avenue, El Mahrajène, 1082, Tunis, Tunisia

[c] Department of Energy, Materials, and Chemical Engineering, Korea University of Technology and Education, 1600 Chungjeol-ro, Byeongcheon-myeon, Dongnam-gu, Cheonan-City, Chungnam-Province 330-708, 31253, Republic of Korea

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ABSTRACT

The chronic ingestion of raw or undercooked kidney beans (*Phaseolus vulgaris* L.) is involved in the pathogenesis of multiple organ dysfunction; the underlying mechanisms are still poorly understood. The objective of this study was to assess the gavage effects of a raw Beldia bean variety on the brush border disaccharidase activities in the jejunal mucosa of Wistar rats. Twenty young adult male rats were randomly assigned into 2 groups of 10 rats each: Control, rats were gavaged with 300 mg of a rodent pellet flour suspension (RPFS); Experimental, rats were orogastrically fed a dose of 300 mg Beldia bean flour suspension (BBFS). Prior to determining the disaccharidase activity by Dahlqvist method, the blood and stool specimens were collected on day 10. The sera and feces were screened for the presence of lectins by serologic and hemagglutination assays. The results showed that the brush border maltase and sucrase activities were significantly diminished but lactase activity did not undergo any change in BBFS-gavaged animals as compared with control. Preliminary immunobiochemical assays revealed the absence of lectins in the systemic circulation and feces of rats, but further work is required to prove this. Overall, the dietary administration of BBFS caused depression of the activity of the small intestinal enzymes maltase and sucrase.

1. Introduction

It is well established that the incorporation of raw kidney bean (*Phaseolus vulgaris* L.) in animal diets reduces food intake, depresses growth, and may cause death [1–4]. Similarly, improperly-prepared beans are known to be toxic for human beings [5,6]. Earlier, toxic effects were also shown to occur in insects [7], birds [8], and also ruminants [9].

This was attributed to the presence in the seeds of potentially bioactive compounds usually referred as phytohemagglutinins (PHAs); known also as *Phaseolus vulgaris* lectins. PHAs are carbohydrate-binding proteins and can interact with most differentiated mammalian cells which express membrane glycoconjugates containing complex oligosaccharide chains. They have been reported from different species of kidney beans and have been found to be responsible for most of the toxicological manifestations. Among beans varieties, red kidney beans and white kidney beans (i.e., also known as cannellini) in particular are rich in phytohemagglutinins [10].

PHA is resistant to breakdown by both digestive enzymes and bacteria in the consumer's gut and therefore most phytohemagglutinins survive, at least in part, the passage through the digestive tract in immunologically and functionally intact form. This allows the PHAs to bind specifically to the glycoconjugates located on the luminal surface of the gut and to partially endocytose into the circulation system [11]. Once bound to digestive tract, *P. vulgaris* lectin can cause dramatic changes in the cellular morphology and metabolism of the small intestine and activate a cascade of signals which alters the intermediary metabolism. Thus, PHAs may induce changes in some, or all, of the digestive, absorptive, protective or secretory functions of the digestive system and affect cellular proliferation and turnover [12–14]. It has been demonstrated that high intakes of PHA can seriously damage the intestinal wall, result in an overgrowth of coliform bacteria in the gut

* Corresponding author at: Department of Energy, Materials, and Chemical Engineering, Korea University of Technology and Education, 1600 Chungjeol-ro, Byeongcheon-myeon, Dongnam-gu, Cheonan-City, Chungnam-Province 330-708, 31253 Republic of Korea. Tel.: +82-41-560-1342, fax: +82-41-560-1224.
E-mail addresses: nader.nciri@koreatech.ac.kr (N. Nciri), njuncho@koreatech.ac.kr (N. Cho).

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lumen [15], and can significantly affect the internal organs, such as the small intestine, pancreas, liver, and thymus [16]. It is also evident that PHA can affect the immune system since a powerful humoral anti-lectin IgG-antibody response has been shown to occur after inclusion of the lectin in the diet [17].

Considering the vast and varied biological activities that phytohemagglutinins can cause, it is surprising that there exists a paucity of studies related to the nutritional implications of dietary lectins in Tunisia. The reasons for the experimental gap are unclear, in part, be due to a lack of awareness within the health and medical communities of which variety of beans in our diet contain lectin activity and the extent of exposure.

Incidents of foodborne illness associated with undercooked red kidney beans have been reported in Canada [18], Australia [19], and in the United Kingdom [6]. Recently, the importation of dry red kidney beans (a variety of the species *Phaseolus vulgaris* L.) for cultivation or consumption in South Africa is prohibited because of their potential toxicity to humans [20]. It has been established that the hemagglutinating lectins (e.g., phytohemagglutinin, PHA) in kidney beans are responsible for this toxicity [20].

Illness is usually related to the ingestion of raw (e.g., fresh, soaked, or sprouted form as a part of salad), flour (e.g., porridge for infant food), or little processed (e.g., steamed or prepared in dehydrators, slow cookers, or crockpots) beans [[26],21–25]. Symptoms generally appear within 1–3 h after consumption of improperly prepared beans. Onset is usually marked by extreme nausea, followed by vomiting and diarrhea. Some individuals also report abdominal pain. Those affected rarely require hospitalization or treatment, and adverse symptoms generally subside within a short period of time. Outbreaks of poisoning have been associated with cooking kidney beans in slow cookers. However, it is important to note that PHA can be deactivated by boiling beans for 10 min at 100 °C and the U.S. Food and Drug Administration recommends an initial soak of at least 5 h in water which should then be discarded. If the beans are cooked at a temperature below boiling (i.e., without a preliminary boil), as in a slow cooker, the toxic effect of hemagglutinin is increased. It was therefore found that beans cooked at 80 °C are five times as toxic as raw beans. In studies of casseroles cooked in slow cookers, internal temperatures often did not exceed 75 °C [26].

In Tunisia, dry bean production has rallied since 2011, reaching 170 tons in 2013 [27]. According to the National Agricultural Research Institute of Tunisia (INRAT, Tunis, Tunisia), Twila, Coco, and Beldia beans are, by far, the most commonly consumed white dry varieties. Bean consumption may be on the upswing due to increased public recognition of possible health benefits of beans and to increase in Tunisian population.

To ensure the beans safety, Tunisia government ordered that a Food Toxicology Department be set up in order to coordinate the work concerning beans safety and take effective measures to ensure beans safety for consumers. At the present, this food safety department composed of the Department of Animal Resources, Fisheries, and Food Technology, National Institute of Agronomy of Tunisia, and the Intestinal Immunophysiology-Research Unit, Faculty of Medicine of Tunis has started the work and commenced the supervision in an all-round way, and has subjected the phytohemagglutinin (PHA), present in white dry beans, to an extensive investigation in the fields of hematology, cytology, immunology, biotechnology, microbiology, and clinical use.

In a preliminary study, the phytohemagglutinin present in white kidney beans (*Phaseolus vulgaris* L.) variety Beldia, frequently consumed by Tunisian population (INRAT, Tunis, Tunisia), was characterized and some of its properties were described [28]. Biochemical and immunological evidence indicated that raw Beldia seeds contain elevated levels of bioactive lectins, approximately 9.20 g/kg [28]. The raw Beldia beans demonstrated when orally administered to growing rats for 10 days at dose of 300 mg induced substantial modification of the morphology and physiological functions of the small intestine (i.e., disruption of absorption process) [29]. Furthermore, serological data revealed effectively the presence of biologically active PHA in the rat jejunal lumen, 3 h and 30 min after oral challenge with 300 mg of Beldia bean flour suspension [30].

Kidney bean poisoning is known to affect animals as well as human beings. The mechanisms proposed for the toxicity of ingested *Phaseolus vulgaris* lectins always call for more answers. Therefore, this work was undertaken to highlight the impact of a raw Beldia variety gavage on the disaccharidases activities in the jejunal brush border of Wistar rats and to fill the gaps that exist currently.

2. Materials and methods

2.1. Plant material

The common white beans (*Phaseolus vulgaris* L. var. Beldia) were purchased in August 2016 from a local market in Ariana city, Tunis, Tunisia. The seeds were free of dust, foreign material, and broken and small kernels. The authentication of plant material was performed by the Department of Botany, National Agricultural Research Institute of Tunisia (INRAT), Ariana, Tunis, Tunisia. Bean seeds were aseptically ground using a mortar and pestle, and then passed through a U.S. No. 200 sieve. The powder was packed, sealed in polyethylene bags, and stored in a cold room at 4 °C until use.

2.2. Extraction of seed proteins

The extraction of proteins from seed flour was done following the method of Itoh et al. [31] with slight modifications. Briefly, one hundred grams (100 g) of white kidney beans (*Phaseolus vulgaris* L., var. Beldia) were soaked overnight in 1 L of distilled water at room temperature (ca. 25 °C), which will help in softening them. These soften seeds then crushed with Tris-NaCl (Tris-HCl 50 mM, 50 mM NaCl, at pH 8 and 4 °C) in a mortar and pestle. The prepared mixture then centrifuged at 3500 rpm at 4 °C for 30 min and filtered through Whatman No. 1 filter paper. Finally, the liquid supernatant (i.e., referred to as the total crude extract) was precipitated using ammonium sulfate. The protein fractions were collected by centrifugation at 3500 rpm for 30 min and stored frozen in aliquots.

2.3. Protein concentration determination

Protein concentration in the white Beldia bean extracts was determined following the Bradford method [32] using BSA (i.e., bovine serum albumin) as a standard. Absorbance was measured at 280 nm. Lectin specific activities were expressed as titer over milligrams of protein as hemagglutination units (HU) per mg of protein.

2.4. Animals, diets, and management

Twenty (20) young Wistar male rats (*Rattus norvegicus albinus*), weighting 60–80 g were initially acclimatized for 7 days, were used in this study. *Rattus albinus* were purchased from the Pasteur Institute of Tunis, Tunis, Tunisia, kept singly in polypropylene cages on chopped aspen wood bedding, and maintained in a well-ventilated, thermostatically controlled room (25 ± 1.4 °C) with 12:12 h light-dark cycle. They were fed with standard pellet diet from Cereal Office, Tunis, Tunisia, and water *ad libitum*. The proximate composition of the daily feed offered to rats was determined by the Food Technology Service (STA), National Institute of Nutrition and Food Technology (INNTA), Tunis, Tunisia, according to AOAC [33]. One hundred grams (100 g) dry rodent pellets, deprived of lectins, contained approximately 12.77% protein, 2.52% lipid, 69.18% carbohydrate, and 350 Kcal energy. Rats were handled daily by the same investigator and were fasted overnight (15 h) with free access to water before the experiment. Animals were