



ELSEVIER

Contents lists available at ScienceDirect

# Toxicology Reports

journal homepage: [www.elsevier.com/locate/toxrep](http://www.elsevier.com/locate/toxrep)

## Tolerability assessment of a lectin fraction from Tepary bean seeds (*Phaseolus acutifolius*) orally administered to rats



Roberto Ferriz-Martínez<sup>a</sup>, Karina García-García<sup>a</sup>, Iovanna Torres-Arteaga<sup>a</sup>,  
Adriana Jheny Rodríguez-Mendez<sup>b</sup>, María de Jesús Guerrero-Carrillo<sup>a</sup>,  
Ulisses Moreno-Celis<sup>a</sup>, Marco Vinicio Ángeles-Zaragoza<sup>a</sup>,  
Alejandro Blanco-Labra<sup>c</sup>, Marco Alonso Gallegos-Corona<sup>b</sup>,  
Juan Pablo Robles-Álvarez<sup>a</sup>, Elizabeth Mendiola-Olaya<sup>c</sup>,  
Héctor Mario Andrade-Montemayor<sup>a</sup>, Olga Patricia García<sup>a</sup>,  
Teresa García-Gasca<sup>a,\*</sup>

<sup>a</sup> Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, P.O. Box 184, Querétaro 76010, Qro., C.P. 76230, Mexico

<sup>b</sup> Facultad de Medicina, Universidad Autónoma de Querétaro, P.O. Box 184, Querétaro 76010, Qro., Mexico

<sup>c</sup> Departamento de Biotecnología y Bioquímica, Centro de Investigación y Estudios Avanzados del IPN, P.O. Box 629, Irapuato, Gto., C.P. 36821, Mexico

### ARTICLE INFO

#### Article history:

Received 1 June 2014

Received in revised form 19 October 2014

Accepted 20 October 2014

Available online 8 November 2014

#### Keywords:

Antinutritional factors

Lectins toxicity

*Phaseolus acutifolius*

Tepary bean

### ABSTRACT

Our previous studies have shown that a lectin rich fraction (TBLF) extracted from Tepary bean seeds differentially inhibits cancer cells proliferation *in vitro*. Before testing the *in vivo* anticancer effect, the acute and subchronic toxicological assays in rats were conducted, where an oral dose of 50 mg/body weight kg was determined as the NOAEL. This study evaluated the resistance to digestion and complete blood count (CBC) after 24 h of the orally administered 50 mg/kg TBLF. The digestion resistance test showed lectins activity retention after 72 h and the CBC study showed a high level of eosinophils, suggesting an allergic-like response. Tolerability was assayed after 6 weeks of treatment by dosing with an intragastric cannula every third day per week. It was observed a transient reduction in food intake and body weight in the first weeks, resulting in body weight gain reduction of 10% respect to the control group at the end of the study. Additionally, organs weight, histopathological analysis and blood markers for nutritional status and for liver, pancreas and renal function were not affected. Our results suggest that 50 mg/kg TBLF administered by oral route, exhibit no toxicity in rats and it was well tolerated. Further studies will focus on long-term studies.

© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-SA license (<http://creativecommons.org/licenses/by-nc-sa/3.0/>).

\* Corresponding author at: Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Av de las Ciencias S/N, Juriquilla, Querétaro, C.P. 76230, Mexico. Tel.: +52 4421921200 ext 5308; fax: +52 442 2342958.

E-mail address: [tggasca@uaq.edu.mx](mailto:tggasca@uaq.edu.mx) (T. García-Gasca).

<http://dx.doi.org/10.1016/j.toxrep.2014.10.015>

2214-7500/© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-SA license (<http://creativecommons.org/licenses/by-nc-sa/3.0/>).

## 1. Introduction

Lectins include a group of proteins from non-immune origin that share the property of binding specifically and reversibly to carbohydrates [1]. Many plant lectins have attracted the attention due to their effects on proliferation and differentiation of animal cells, including lymphocytes and cancer cells. The *in vitro* and the *in vivo* antitumor effects of plant lectins are apparently associated with their ability to modulate growth, differentiation, proliferation and apoptosis [2–4].

Toxicity of lectins must be considered before used as medical tools, mainly because they are considered antinutritional factors. It has been shown that binding lectins to intestinal epithelium can interfere with nutrient absorption, reduction of nitrogen retention, increased urine nitrogen excretion and reduction of insulin production in rats [5–8]. Antinutritional and negative effects on digestion and absorption have been described for lectins from different sources [9–12]. Studies with common bean (*Phaseolus vulgaris* L.) lectins show that they can interfere with bowel function, causing changes in systemic metabolism and affecting the growth in rats, decrease in glucose, lipids, vitamin B12 and nitrogen uptake [13,14]. Adverse effects in organs are produced by some diet lectins, which included *Phaseolus vulgaris*. Rats fed with navy beans showed morphological changes that include increased weight of kidney and heart, pancreatic acinar atrophy, fatty liver and multiple histological lesions as thymus atrophy respect to control healthy rats. Death by lectin administration can be produced by different mechanisms including the reduced availability of nitrogen in the body, the induction of bacterial overgrowth in the small intestine, the effects on blood cells or acting as ribosome inactivating proteins [15,16].

Some studies showed that intraperitoneal administration of Tepary bean (*Phaseolus acutifolius*) crude extract presented toxic effects as weight loss, negative efficiency on protein ratio, negative net protein utilization, poor digestion of proteins and death of rats and mice after 10 days treatment, however, after autoclaving the crude extract, the toxic effects were lost [17]. Studies on the toxicity of semipure lectins from Tepary bean intraperitoneally administered in CD-1 mice, found a lethal dose (LD<sub>50</sub>) of 1100 and 1120 mg/kg body weight for males and females, respectively [18].

A semipure lectin fraction from Tepary bean seeds (TBLF) obtained by a molecular weight exclusion chromatography protocol exhibits *in vitro* antiproliferative differential effect on cancer and normal cells [19]. Before testing the *in vivo* anticancer effect, we studied the acute toxicity of TBLF using intragastric doses from 5 to 2000 mg/body weight kg suggesting a secure dose of 50 mg/kg. The intragastric 50 mg/kg TBLF dose was assayed for subchronic toxicity (daily dosing for 28 days) where no toxic or adverse effects were observed, therefore 50 mg/kg TBLF was determined as the NOAEL [20]. Here we present a short-term assay in order to know the digestion resistance of lectins and the effect on complete blood count (CBC) after 24 h of 50 mg/kg TBLF single-dose administration. The anti-nutritional effects and toxic parameters of a

6-week schedule study (intragastric administration every third day) were studied; where food intake, body weight, biochemical blood markers and histopathological analysis were included.

## 2. Materials and methods

### 2.1. Experimental animals

Sprague Dawley (SD) rats were purchased from Institute of Neurobiology, Universidad Nacional Autonoma de Mexico (INB-UNAM) and placed in individual cages with *ad libitum* water and rodent chow food (Rodent Laboratory Chow 5001, Saint Louis, MO, USA). The animals remained 1 week for acclimatization where the circadian cycle was adjusted to 12 h light/12 h darkness, at 22 °C and a relative humidity of 30%. The animals were sacrificed by decapitation at the end of the experiments. The experimental protocol was based on the Mexican official standard [21] and approved by the INB-UNAM ethics committee.

### 2.2. Tepary bean lectin fraction (TBLF)

We have performed a standardized method for TBLF obtaining [19]. Some modifications were done in order to improve the lectin enrichment. Briefly, Tepary bean seeds were grinded (A-10 Analytical Tekmar mill) and degreased with chloroform-methanol 2:1 in a 4:1 w/v proportion, stirring for 15 min and then vacuum filter; this process was repeated 2 more times and flour was dried at room temperature in a fume hood. In order to obtain the crude extract, 100 g of degreased flour were dissolved in 500 mL of 50 mM Tris-HCl pH 8 with stirring for 12 h at 4 °C and centrifuging at 39,200 × g for 60 min (Bekman JA-20 centrifuge). A sequential precipitation was done using 40% ammonium sulphate saturation with slow stirring for 30 min, equilibrating for 30 min at 4 °C and centrifuging at 39,200 × g for 45 min. The supernatant was precipitated with 60% ammonium sulfate saturation and treated as previously described, but in this case the precipitate was recovered and the supernatant was discarded. The fraction was resuspended in a minimum volume of deionizer water, dialyzed through a 3-kDa pore size membrane and centrifuged, loading 4 mL aliquots on a Sephadex G-75 gel filtration 167 cm × 1.7 cm column (Pharmacia Biotech, Uppsala, Switzerland). The column was equilibrated with 0.01 M ammonium bicarbonate buffer pH 7.8. The experiment was performed at 4 °C collecting 0.3 mL/min fractions with the same buffer. Protein was monitored at 280 nm in a Beckman DU-65 spectrophotometer. Agglutination activity [22] was determined by microscopic counting using glutaraldehyde-fixed type A<sup>+</sup> human erythrocytes [23]. Specific activity was determined using protein concentration [24]. Electrophoretic profile was obtained by 10% polyacrylamide SDS-PAGE [25]. Glycoproteins were confirmed by periodic acid-Schiff staining (PASS) [26]. Additionally, lectins were observed by western blot using an anti-phytohemagglutinin antibody from *Phaseolus vulgaris* (Vector Laboratories Inc. Burlingame, CA, USA. cat.

No. AS-2300). The fraction was dialyzed against deionized water, lyophilized and stored at  $-20^{\circ}\text{C}$  until use.

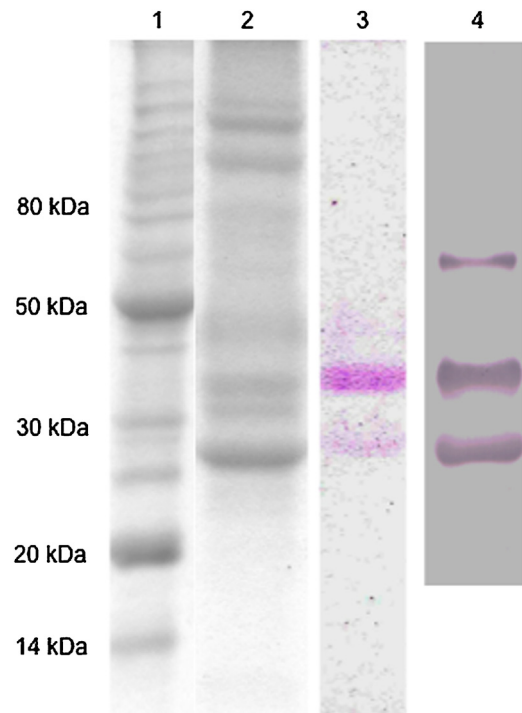
### 2.3. Digestion-resistance assay and complete blood count (CBC) determination after a single dose of TBLF

Five-week old male SD rats were divided into 2 groups ( $n=8$  per group). After fasting for 24 h, the treated group received a single dose of the lyophilized 50 mg/kg TBLF dissolved in standard saline solution (0.9% NaCl in deionized water) using an intragastric cannula [20], while the control group received saline solution. Autoclaving ( $121^{\circ}\text{C}$  for 15 min) was necessary for denature food lectins. Feeding was restarted with *ad libitum* water and autoclaved chow food (Rodent Laboratory Chow 5001, Saint Louis, MO, USA). Feces from 4 rats per group were collected at 0, 24, 48, 72, 96 and 120 h, fecal protein was extracted in PBS, filtered through a 0.22 mm membrane and agglutination specific activity was determined by microscopic counting [22]. Other 4 rats per group were sacrificed at 24 h in order to recover blood for CBC (CellDyn<sup>®</sup> 1600) and a commercial kit for differential blood cells staining was used for cell counting from blood smear (Hycel, Mexico; cat. number 548). Erythrocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes and platelets were counted using a  $100\times$  microscope objective. Results are expressed as absolute blood counts or percentage respect to control animals.

### 2.4. Tolerability study

Fifteen-week old male SD rats were randomly selected in 2 groups ( $n=12$  per group). Treated rats were dosed with 50 mg/kg TBLF dissolved in saline solution and control group was administered with saline solution by using an intragastric cannula. For CBC determination, 4 rats per group were daily administered for 30 days and sacrificed. For the tolerability assessment, treated group was administered with the 50 mg/kg TBLF in saline solution every third day for 6 weeks and control group administered with saline solution. This administration schedule was defined from the digestion resistance data and it will be used in further studies, *i.e.* against colon cancer. Food intake was determined twice a week and body weight weekly. After the 6-week administration schedule, rats were sacrificed by decapitation. Blood was collected in vacutainer tubes without anticoagulant and serum was recovered by centrifugation at  $5000\times g$  for 5 min and stored at  $-80^{\circ}\text{C}$  until use for clinical chemistry parameters determination as described below. Liver, kidney, stomach, pancreas, small intestine, colon, thymus and spleen were dissected, weighted and fixed in 10% buffered formalin. A veterinary pathologist conducted the histopathological analyses for liver, kidney, small intestine and colon using Hematoxylin-Eosin staining and analyzed by microscopy (Olympus, model BX51, Evolution MP).

Commercial kits (Diagnostic Chemicals Limited, Canada) were used for determination of liver function using aspartate aminotransferase (AST) (Catalog No. 319-10), alanine aminotransferase (ALT) (Catalog No. 318-10) and total bilirubin (Catalog No. 243-17) kits. Urea



**Fig. 1.** TBLF electrophoretic profile and lectins identification. TBLF was obtained by molecular weight exclusion chromatography. Monodimensional SDS-PAGE was performed. (1) Molecular weight markers, (2) TBLF, (3) glycoprotein PASS, (4) western blot using anti-phytohemagglutinin from *Phaseolus vulgaris*.

(Catalog No. 283-17) and  $\alpha$ -amylase (Catalog No. 341-10) were measured as renal and pancreas function markers, respectively. Serum creatinine (Catalog No. 221-30), total protein (Catalog No. 200-55), glucose (SL ELITech, Clinical Systems, France. Catalog No. B01-4509-01), and albumin (SL ELITech, Clinical Systems, France. Catalog No. ALBU-0600) were determined as nutritional status markers.

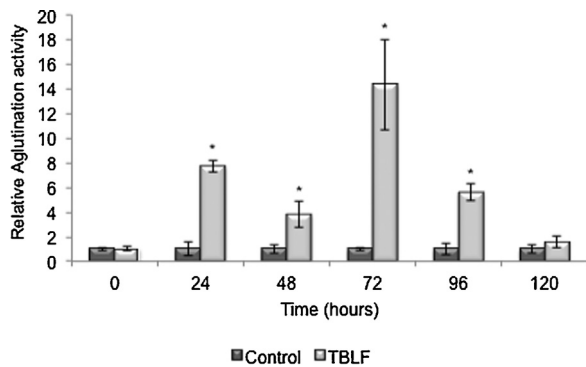
### 2.5. Statistical analysis

Differences between TBLF treated rats against control rats were calculated by the *t*-student test ( $p \leq 0.05$ ) using the SPSS 17 software.

## 3. Results and discussion

### 3.1. TBLF profile

The molecular weight exclusion chromatography protocol shows a reproducible profile for TBLF obtainment. The method allows observing the two main lectins (Fig. 1), similar than the observed profile previously obtained [19]. The presence of lectins was confirmed by PASS and western blot. The specific agglutination activity for the TBLF was 5566 AU/protein mg.



**Fig. 2.** Agglutination activity from rat feces administrated with TBLF. Rats were administrated with a single dose of TBLF (50 mg/kg) and feces were recollected every 24 h for 5 days. Agglutination activity was determined using A\* human erythrocytes. Asterisks show statistical significant difference ( $t$  test  $p \leq 0.05$ ).

### 3.2. Lectins from TBLF resist digestion up to 72 h

Some lectins exhibit high resistance to digestion by proteolytic enzymes in mammals, allowing them to effectively bind to intestinal epithelial cells. Lectins can also resist bacterial degradation and can remain in their biological and immunological intact forms [5–7]. It has been reported that this kind of proteins can be recovered with their intact biological activity after passing through the digestive tract of mice over a period of 24 h as *Pisum sativum* and Kintoki bean lectins [27–29]. In order to establish the resistance to gastric digestion of TBLF, agglutination activity was monitored through 120 h in feces after a 50 mg/kg TBLF single dose (Fig. 2). Agglutination activity in TBLF-treated rats was 8 and 4 times higher than in control rats after 24 and 48 h, respectively. However, instead of diminishing, it increased 15 times after 72 h and then gradually diminished until basal levels at 120 h. This result suggests that no retained lectin was excreted within the first 48 h and retained lectin releases after 72 h maintaining its biological activity.

### 3.3. TBLF exhibits an allergic-like response after 24 h

CBC is shown in Table 1 where only granulocytes count showed difference ( $p = 0.001$ ) with an increase of 3.86 times in TBLF-treated animals respect to control rats. The proportion of granulocytes and lymphocytes was different respect to control animals, mainly due to an increment of

granulocytes (Fig. 3A). Blood smears were used to differential counting of cells (Fig. 3B). Lymphocytes decreased 20% while neutrophils and eosinophils increased 2.4 and 20 times, respectively. Basophils, monocytes, erythrocytes, and platelets did not show significant changes (data not shown). This result suggests an allergic-like response, mainly indicated by the eosinophils increase.

### 3.4. Six weeks TBLF administration provoked partial reduction of food intake and decrease in body weight gain

Fifty mg/kg TBLF dose was administrated via intra-gastric cannula every third day for 6 weeks. Significant decrease in food consumption was observed from the first week of administration until the fourth week respect to control group ( $p \leq 0.05$ ). However, on the fifth week, food consumption was the same than the control group (Fig. 4A), maybe as the result of compensatory mechanisms where the treated animals overcame the negative effects of the lectins administration. Rats body weight also showed significant changes ( $p \leq 0.05$ ) between the two groups (Fig. 4B). Treated animals presented a transient decrease of body weight in the first weeks (5.25% respect to the start of dosing) however; at the end of the study, a recovery of weight was observed resulting in a reduction in body weight gain of 10% respect to the control group. It is known that lectins can provoke nonspecific interference with nutrient absorption, causing changes in animal nutrition status. Our results show that TBLF administration causes antinutritional effects at the beginning of the experiment with a final recovery, which resulted in a reduction in body weight gain.

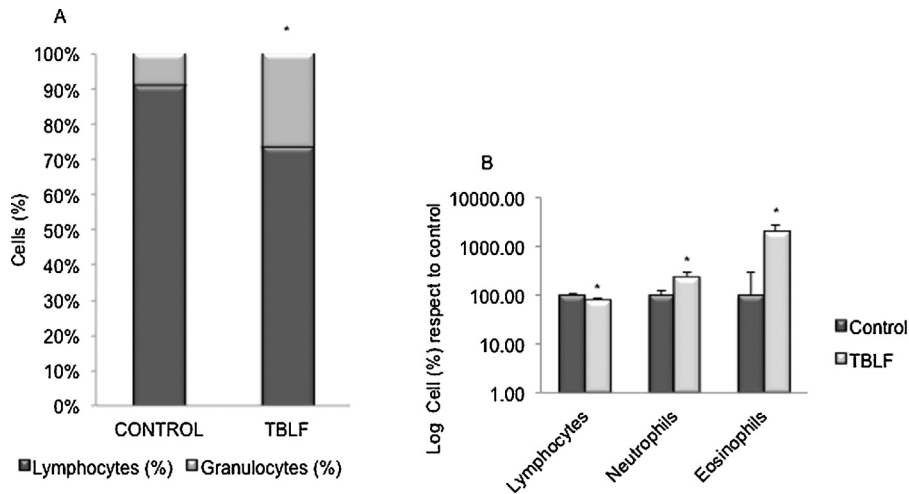
### 3.5. Six weeks TBLF administration did not cause organs or blood markers alterations

The effect of TBLF on organs and blood markers is shown in Table 2. No significant differences were observed in spleen, heart, liver, kidney, stomach, thymus, pancreas, small intestine and colon weight. Small intestine and colon length were also determined and no significant differences were found with respect to the control group. No histopathological alterations were observed in colon, small intestine, liver and kidney (Fig. 5). A strong association between changes in the morphology and structure of the intestine and the ingestion of lectins have been observed,

**Table 1**  
CBC after 24 h of a single-dose TBLF oral administration.

Blood parameter	Control	TBLF
White blood cells ( $10^3/\mu\text{L}$ )	5.16 ± 1.35	6.78 ± 1.56
Lymphocytes ( $10^3/\mu\text{L}$ )	4.70 ± 1.27	5.00 ± 1.44
Granulocytes ( $10^3/\mu\text{L}$ )	0.46 ± 0.21	1.78 ± 0.53*
Red blood cells ( $10^6/\mu\text{L}$ )	6.73 ± 0.20	6.55 ± 1.59
Platelets ( $10^3/\mu\text{L}$ )	874.40 ± 84.97	737.75 ± 216.71
Hemoglobin (g/dL)	12.50 ± 0.37	12.24 ± 2.88
Hematocrit (%)	42.20 ± 1.06	40.76 ± 9.52
Mean corpuscular volume (fl)	62.80 ± 2.77	62.40 ± 1.14
Mean corpuscular hemoglobin (pg)	18.60 ± 0.99	18.74 ± 0.33
Mean corpuscular hemoglobin concentration (g/dL)	29.62 ± 0.77	30.00 ± 0.48

\* Statistically significant difference ( $t$  test,  $p = 0.001$ ).

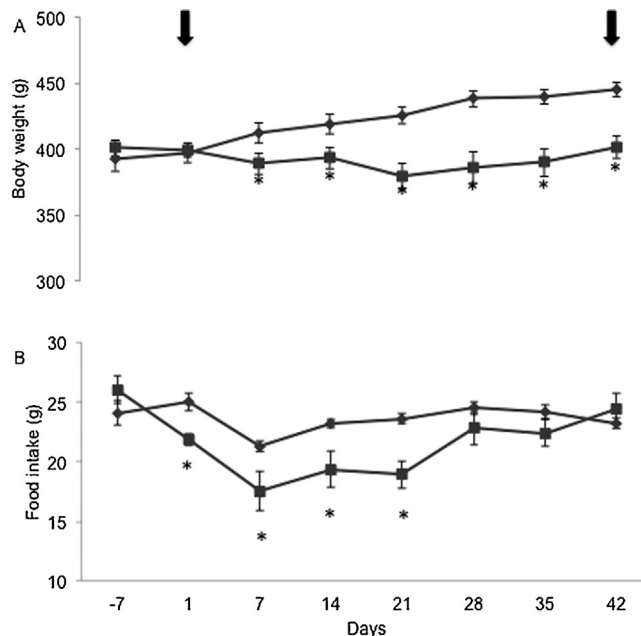


**Fig. 3.** White blood cells counting after 24 h of a single dose of TBLF. CBC was determined and blood cells were counted from blood smears by differential staining. (A) Percentage of granulocytes and lymphocytes were determined by automated counting. (B) White cells count was determined by microscopic counting. Results are expressed as percentage respect to control animals in a logarithmic scale. Asterisks show statistical significant difference ( $t$  test  $p \leq 0.05$ ).

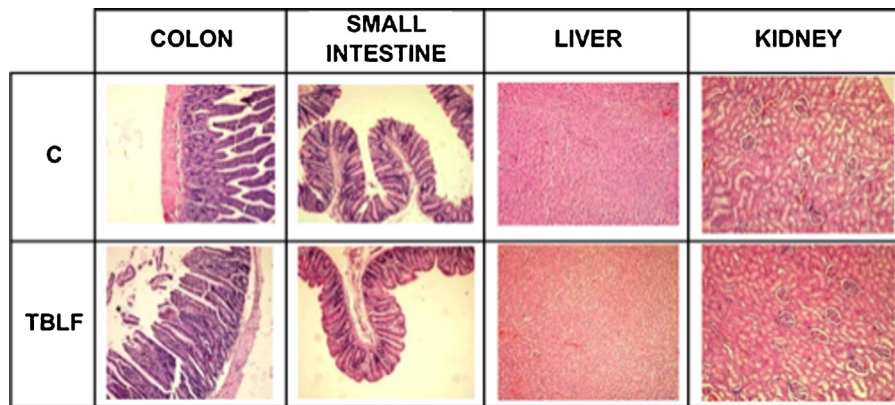
such changes may result from the decrease in intestinal permeability as shown with Con A, wheat agglutinin and navy bean lectin. The intestinal growth is maybe the result of compensatory process in order to maintain nutrient uptake, since some lectins directly stimulate intestinal cells causing hyperplasia and hypertrophy [9]. However, TBLF at the tested dose did not provoked macroscopic effects or histological changes on the intestinal tract.

Different blood markers were determined in order to study hepatotoxicity (AST and ALT), renal injury (urea, creatinine), pancreatic damage ( $\alpha$ -amylase), and nutritional

status (albumin, total protein, creatinine and glucose). No differences between groups were found, suggesting that the oral TBLF administration exhibit no toxicity at the end of the treatment (Table 3). Urea levels were found out of range, according to reference values for SD rats [30] but the high values applied to both, control and treated animals. To find out if blood parameters could change during the treatment, in a separated experiment total protein, albumin, creatinine and ALT were measured every 2 weeks and CBC was determined after 4 weeks. No significant differences between groups were found, suggesting



**Fig. 4.** Food intake and body weight changes after TBLF oral administration. Rats were orally administrated with TBLF (50 mg/kg) every third day for 6 weeks after an adaptation period (7 days). Dosing was done to complete 6 weeks, from day 1 to day 42 (arrows indicate the start and end of treatment). (A) Net food intake, (B) Net body weight changes. Asterisks show statistical significant difference ( $t$  test  $p \leq 0.05$ ).



**Fig. 5.** Histopathological analysis after TBLF oral administration. Rats were orally administrated with TBLF (50 mg/kg) every third day for 6 weeks. Colon, small intestine, liver and kidney were collected after sacrifice, stained and observed (10 $\times$ ).

no adverse effects after long-term treatment (data not shown).

Other studies have observed that intragastric administration of saline extract of *P. acutifolius* to rats caused intestinal cells microvilli destruction, as well as breaking of endoplasmic reticulum outline [31]. These authors attributed the toxicity and poor nutritional value of *P. acutifolius* to high concentrations of phytohemagglutinin, however, it is known that Tepary bean protease inhibitor is also present in crude protein extracts [17]. TBLF does not contain the protease inhibitor form Tepary bean as a result of the chromatographic procedure [19]. Therefore,

the results obtained in the present work could be attributed to the lectins contained in TBLF.

#### 4. Conclusions

Here we report that after subchronic oral administration, TBLF provoked antinutritional effects in rats resulting in a transient decrease of food intake and body weight in the first weeks. The final result was observed as a reduction in body weight gain respect to the control group. The digestion assay suggests that lectins from Tepary bean can remain intact into the digestive tract up to 72 h. CBC at 24 h post-administration showed an allergic-like response that disappeared after 4 weeks of treatment. Blood markers suggest no toxic effects and no alterations in the evaluated organs. Taking together, our results showed that TBLF provoke a reduction in body weight gain with no other remaining effects, suggesting compensatory mechanisms and good tolerability. More studies are needed to determine effects on nutrient availability and intestinal integrity after TBLF administration, especially in long-term assays and in different development stages.

**Table 2**

Organs weight or length after TBLF subchronic oral administration.

Organs	Control	TBLF
Colon weight (g)	2.94 $\pm$ 0.40	2.68 $\pm$ 0.40
Colon length (cm)	19.38 $\pm$ 1.14	19.60 $\pm$ 0.81
SI weight (g)	8.06 $\pm$ 0.23	8.28 $\pm$ 0.52
SI length (cm)	125.96 $\pm$ 2.40	122.02 $\pm$ 5.08
Spleen (g)	0.84 $\pm$ 0.03	0.83 $\pm$ 0.05
Heart (g)	1.65 $\pm$ 0.06	1.52 $\pm$ 0.06
Liver (g)	11.98 $\pm$ 0.42	10.57 $\pm$ 0.37
Kidneys (g)	3.31 $\pm$ 0.12	3.18 $\pm$ 0.15
Stomach (g)	2.21 $\pm$ 0.08	2.14 $\pm$ 0.07
Thymus (g)	0.45 $\pm$ 0.04	0.48 $\pm$ 0.05
Pancreas (g)	1.46 $\pm$ 0.27	1.73 $\pm$ 0.12

No statistically significant difference was founded (*t* test,  $p \geq 0.05$ ).

**Table 3**

Blood markers after TBLF subchronic oral administration.

Blood markers	Control	TBLF
Glucose (mg/dL, RV: 50–160)	93.5 $\pm$ 7.10	96.9 $\pm$ 6.69
TP (mg/dL, RV: 5.9–7.9)	8.3 $\pm$ 0.30	8.4 $\pm$ 0.44
ALB (mg/dL, RV: 3.8–4.8)	3.2 $\pm$ 0.02	2.9 $\pm$ 0.13
AST (U/L, RV: 39–262)	186.7 $\pm$ 21.50	160.1 $\pm$ 10.90
ALT (U/L, RV: 35–80)	65.5 $\pm$ 3.10	66.8 $\pm$ 4.93
Urea (mmol/L, RV: 9.28–22.13)	29.5 $\pm$ 4.03	30.8 $\pm$ 5.07
Serum creatine (mg/dL, RV: 0.5–1.5)	0.9 $\pm$ 0.02	0.9 $\pm$ 0.02
$\alpha$ -Amylase (U/L, RV: NA)	2.0 $\pm$ 0.04	1.9 $\pm$ 0.07

No statistically significant difference was founded (*t* test,  $p \geq 0.05$ ).

TP: Total protein, ALB: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, RV: Reference values (16), NA: Data not available.

#### Conflict of interest

The authors declare that they do not have any conflict of interest.

#### Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

#### Acknowledgments

We would like to acknowledge to Veronica Andrade-Portillo, Josue Lopez-Martinez, Evelyn Flores, Omar Perez-Segura, Miguel Angel Ortiz-Aguilar and Adin Meraz-Perez for their technical assistance. This work was supported by CONACYT, Ciencia Basica under grant number 82349; CONCYTEQ, FOMIX under grants numbers

QRO-2007-C01-78221 and QRO-2011-C02-175340.  
Publication was possible with the founding of PIFI.

## References

- [1] N. Sharon, Lectins: past, present and future, *Biochem. Soc. Trans.* 36 (2008) 1457–1460.
- [2] E. González de Mejía, V. Prisecaru, Lectins as bioactive plant proteins: a potencial in cancer treatment, *Crit. Rev. Food Sci. Nutr.* 45 (2005) 425–445.
- [3] R.A. Ferriz-Martínez, I.C. Torres-Arteaga, A. Blanco-Labra, T. García-Gasca, The role of plant lectins in cancer treatment, in: *New Approaches in the Treatment of Cancer*, Nova Science Publishers, Inc., Hauppauge, NY, 2010, pp. 71–89.
- [4] Y.S. Chan, T.B.A. Ng, Lectin with highly potent inhibitory activity toward breast cancer cells from edible tubers of *Dioscorea opposita* cv. nagaimo, *PLOS ONE* 8 (1) (2013) e54212.
- [5] A. Pusztai, S. Bardocz, Biological effects of plant lectins on the gastrointestinal tract: metabolic consequences and applications, *Trends Glycosci. Glicotechnol.* 8 (1996) 149–165.
- [6] M. Rhodes, Beans means lectins, *Gut* 44 (1999) 593–594.
- [7] F. Lajolo, M. Genovese, Nutritional significance of lectins and enzyme inhibitors from legumes, *J. Agric. Food Chem.* 50 (2002) 6592–6598.
- [8] J. Czerwinski, M. Leontowicz, M. Gralak, Response of rats to a moderate intake of soybean lectin, *J. Anim. Feed Sci.* 14 (2005) 37–540.
- [9] M. Figueroa, J. Mancini, F. Lajolo, Antinutritional effect of phytohemagglutinins of *Phaseolus Vulgaris*, *Arch. Lat. Nutr.* 34 (1984) 488–499.
- [10] A. Pusztai, F. Greer, M. Silva, A. Prouvost-Danon, T. King, Effect of wheat germ agglutinin on the interleukin pathway of the human T lymphocyte activation, *J. Immunol.* 134 (1985) 314–323.
- [11] A. Pusztai, S.W.B. Ewen, G. Grant, D.S. Brown, J.C. Stewart, W.P. Peumans, E.J.M. Van Damme, S. Bardocz, Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins, *Br. J. Nutr.* 70 (1993) 313–321.
- [12] H. Sanae, O. Hideo, M. Setsuko, I. Yuki, S. Kumiko, S. Setsuko, H. Yukihiko, Y. Yoshio, Soybean protein isolate and soybean lectin inhibit iron absorption in rats, *J. Nutr.* 122 (1992) 1190–1196.
- [13] D. Donatucci, I. Liener, C. Gross, Binding of navy bean (*Phaseolus vulgaris*) lectin to the intestinal cells of the rat and its effect on the absorption of glucose, *J. Nutr.* 117 (1987) 2154–2160.
- [14] F. Marzo, R. Alonso, E. Urdaneta, F.J. Arricibita, F. Ibáñez, Nutritional quality of extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats, *J. Anim. Sci.* 80 (2002) 875–879.
- [15] F. Greer, A. Putzai, Toxicity of kidney bean (*Phaseolus vulgaris*) in rats: changes in intestinal permeability, *Digestion* 32 (1985) 42–46.
- [16] A. Pusztai, S. Bardocz, Lectins, biomedical perspectives, vol. 6, Taylor and Francis e-Library, London, 2005, pp. 93.
- [17] M. Osman, P. Reid, C. Weber, The effect of feeding Tepary bean (*Phaseolus acutifolius*) proteinase inhibitors on the growth on pancreas of young mice, *J. Nutr.* 2 (2003) 111–115.
- [18] R. Reynoso Camacho, E. González de Mejía, G. Loarca Piña, Purification and acute toxicity of a lectin extracted from tépari bean (*Phaseolus acutifolius*), *Food Chem. Toxicol.* 41 (1) (2003) 21–27.
- [19] T. García-Gasca, M. García-Cruz, E. Hernández-Rivera, J. Lopez-Martínez, A. Castañeda-Cuevas, L. Yllescas-Gasca, J. Rodríguez-Mendez, E. Mendiola-Olaya, J. Castro-Guillen, A. Blanco-Labra, Effects of Tepary beans (*Phaseolus acutifolius*) protease inhibitor and semipure lectin fraction on cancer cells, *Nutr. Cancer* 64 (8) (2012) 1269–1278.
- [20] C. Lopez-Sanchez, F.J. Lopez-Martínez, A. Castañeda-Cuevas, L. Yllescas-Gasca, R. Ferriz-Martínez, I. Torres-Arteaga, M.A. Gallegos-Corona, A.J. Rodríguez-Mendez, E. Mendiola-Olaya, A. Blanco-Labra, T. García-Gasca, Evaluación de la toxicidad in vitro e in vivo de lectinas de frijol Tépari, *Ciencia@UAQ* 3 (2010) 3–13, Available from: <http://www.uaq.mx/investigacion/revista.ciencia@uaq/ArchivosPDF/v3-n1/Evaluacion.pdf>
- [21] NOM-062-ZOO, Norma Oficial Mexicana. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio, 1999, Available from: <http://148.243.71.63/default.asp?doc=743>
- [22] W.G. Jaffe, Hemagglutinins, in: I.E. Liener (Ed.), *Toxic Constituents of Plant Foodstuffs*, Academic Press, New York, 1980, pp. 69–101.
- [23] R. Turner, I. Liener, The use of glutaraldehyde-treated erythrocytes for assaying the agglutinating activity of lectins, *Anal. Biochem.* 68 (1975) 651–653.
- [24] M. Bradford, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal. Biochem.* 72 (1976) 248–254.
- [25] H. Shagger, G. Von Jagow, Tricine-sodium dodecyl sulphate-polyacrilamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa, *Anal. Biochem.* 166 (1987) 368–379.
- [26] C. Gerard, Purification of glycoproteins, in: M.P. Deutscher (Ed.), *Guide to Protein Purification*, Academic Press, San Diego, CA, 1990, pp. 535–536.
- [27] T. Hara, Y. Mukunoki, I. Tsukamoto, M. Miyoshi, K. Hasegawa, Susceptibility of Kintoki bean lectin to digestive enzymes *in vitro* and its behavior in the digestive organs of mouse *in vivo*, *J. Nutr. Sci. Vitaminol.* 30 (1984) 381–394.
- [28] S. Nakata, T. Kimura, Effect to a moderate intake of ingested toxic bean lectins on the gastrointestinal tract in the rat, *J. Nutr.* 115 (1985) 1621–1629.
- [29] L. Le Gall, B. Quillien, J. Séve, J. Guéguen, J.P. Lallès, Weaned piglets display low gastrointestinal digestion of pea (*Pisum sativum* L.) lectin and pea albumin 2, *J. Anim. Sci.* 85 (2007) 2972–2981.
- [30] Canadian Council on Animal Care, *Guide to the Care and Use of Experimental Animals*, 1993, Available from: [http://www.cac.ca/Documents/Standards/Guidelines/Experimental\\_Animals.Vol1.pdf](http://www.cac.ca/Documents/Standards/Guidelines/Experimental_Animals.Vol1.pdf)
- [31] A.A. Sotelo, M.T. Gonzales, E. Gonzales, E. Velasco, A. Fieria, Ultrastructural change of epithelial intestinal cells induced by ingestion of raw *Phaseolus acutifolius*, *Nutr. Rep. Int.* 27 (1983) 329–337.