Effect of supplementation with extract of white bean flour in murine model

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ABSTRACT

Common bean supplementation (*Phaseolus vulgaris*) *"in natura"* causes loss of body weight associated with a deficiency in nutrient absorption and histopathological changes. This effect has been attributed to phytohemagglutinin (PHA) present in high concentrations in red and white beans. The main objective of this work was to evaluate the safety of white bean flour as a dietary supplement. Animals were treated for 14 days with extract of white bean flour (WBFE) at doses of 2.65g/kg and 5.30g/kg. A significant reduction in body weight was observed, accompanied by the reduction of mean values of glycemia, in both groups in relation to the control group. Significant structural changes were also observed in the intestinal epithelium. Additionally, mice treated with WBFE 5.30g/kg presented mononuclear inflammatory infiltrate in the lamina propria of the intestinal mucosa accompanied by a dose-dependent increase in the dosage of chemokine MCP-1 and nitric oxide, although without causing intestinal and hepatic oxidative and oxidative damage. The deleterious effects resulting from the use of the WBFE are not permanent since the treated animals after 14 days without WBFE stimuli. In conclusion, commercial bean flour did not prove to be safe as oral dietary supplementation at the dosages used because of the antinutritional and immunomodulatory effects.

Keywords: White bean flour; Weight loss; safety; Immunomodulation

1 INTRODUCTION

White beans (*Phaseolus vulgaris*) belongs to the family Fabaceae. Brazil is the world's largest producer of common beans (*Phaseolus vulgaris* L.), which are one of the most widely consumed grain legumes in the world (Rezende *et al.*, 2017). White bean flour (WBF) contains phaseolamine, an inhibitor of the alpha-amylase digestive enzyme capable of inhibiting carbohydrate absorption, that was used as a natural dietary supplement against obesity (Udani *et al.*, 2004; Celleno et al., 2007; Hayat *et al.*, 2014). According to the Ministry of Health, obesity increased 60% between 2006 and 2016 in Brazil (Ministry of Health, 2017).

In humans, consumption of raw or undercooked beans has been associated with severe, but temporary, gastrointestinal discomfort (Nasi *et al.*, 2009). Although beans contain a variety of antinutritional factors, this discomfort is attributed, in large part, to the high concentration of phytohemagglutinins (PHA) found mainly in red and white bean varieties, common bean varieties (Phaseolus vulgaris) (Kumar *et al.*, 2013).

Studies have shown that PHA and other lectins show a toxic and antinutritional effect when administered to rats. This occurs due to its resistant to degradation by proteases, which allows them to bind and be extensively endocytosed by epithelial cells of the small intestine, causing damage to the microvilli, with histomorphometric and histopathological changes in the small intestine mucosa (Vasconcelos and Oliveira, 2004; Chokshi, 2007). Some authors have shown that after removal of toxic lectin, the intestinal epithelium appears normal, suggesting that epithelial damage is reversible in rat intestinal mucosa (Weinman *et al.*, 1989; Dignass *et al.*, 1996).

In response to intestinal mucosal injury, the recruitment of leukocytes plays a role in mediating the host's inflammatory response through the release of inflammatory mediators that coordinate the resolution of inflammation and tissue repair (Leoni *et al.*, 2015). In the acute phase of inflammation, inflammatory

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monocytes and resident macrophages are stimulated by lectins to secrete effector molecules such as nitric oxide (NO), reactive oxygen species (ROS) and chemokines MCP-1 (Kesherwani and Sodhi, 2007).

In some cases, continuous exposure to the aggressive antigen generates oxidative stress, amplifying the inflammatory response, which can become chronic, generating tissue damage and inflammatory bowel diseases (Kumar et al. 2010). Oxidative stress is the imbalance between antioxidants and reactive oxygen (ROS) and nitrogen (RNS) species that cause damage to cell membranes through a process called lipid peroxidation (Agrawal and Sharma, 2010). To avoid oxidative damage caused by ROS and RNS, the cells have an antioxidant enzymatic system such as superoxide dismutase (SOD) and catalase (CAT) (Hermes-Lima, 2004).

In relation to current legislation, commercial phaseolamine (white bean protein) cannot be marketed as an herbal medicine. According to Resolution-RE nº 1.992, of May 3, 2010, it ordered the suspension of advertising and marketing of the unregistered products containing phaseolamine (extract of Phaseolus vulgaris) in all communication vehicles throughout the country with indications for fat burning, appetite reduction and weight loss, among other properties not approved by the National Sanitary Surveillance Agency.

Thus, the present study aimed to evaluate the safety of commercial white bean flour as a dietary supplement, in a murine model, through antinutritional and pro-inflammatory effects, mainly due to the high concentration of lectin present in the extracts administered orally.

2 MATERIAL AND METHODS

2.1 Experimental animals

BALB/c isogenic mice, with 6 to 8 weeks of age, male (25 to 30 grams), were obtained from the Central Bioterium of the Federal University of Viçosa (UFV) and

kept in the bioterium of the Immunology and Virology group of the Department of General Biology - DBG/UFV. During the tests, the animals were kept under controlled conditions of temperature (21 ± 2 °C) and relative humidity (60 to 70%) in light/dark (12/12) cycles per day. The animals received food and water ad *libitum*. The animal experimentation was carried out respecting ethical principles of the Veterinary Practitioner's Code of Professional Ethics, with an experimental protocol approved by the Animal Ethics Commission of the UFV (60/2014).

2.2 Food Product

A sample of commercial white bean flour (WBF) with an indication for use as a human food supplement (CaCaLia Comercial Ltda) was obtained from local commerce in the city of Ubá, MG, Brazil.

2.3 Preparation of crude protein extracts (PE) of commercial white bean flour (WBF)

The concentrations of crude protein extracts (PE) of commercial white bean flour (WBF) were calculated from the manufacturer's recommended human dose (17.5 g/day). To commercial WBF was added 0.15M PBS buffer pH 7.4 at the following concentrations: 160mg/mL and 320mg/mL. After homogenization, the blends were maintained at room temperature, remaining under gentle shaking for approximately 16 hours. Subsequently, each suspension was filtered using filter paper and then centrifuged (4000 g for 10 min) until total removal of solid particles. The supernatant of each concentration, called white bean flour extract (WBFE), was labeled and stored in a refrigerator (4 °C) for further analysis.

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2.4 Treatment of animals

To evaluate the efficacy and toxicity of WBFE in healthy mice, the animals were randomly divided into three experimental groups (n = 10 animals/group), one being a control group and two exposed to WBFE. Controls received PBS, while the other animals received WBFE in the following concentrations: 2.65 g/kg (T1) and 5.30 g/kg (T2). During the days of treatment, doses of WBFE were administered daily orally with the aid of a gavage cannula, with a volume of 0.5 mL per animal. The mice were weighed individually on a calibrated balance, daily, for a period of 14 days. Thus, the mean weight (g) was made for each animal group. Five animals per group were euthanized with 14 days of treatment to assess acute exposure. The other animals received only commercial ration for another 14 days, being euthanized with 28 days to evaluate recovery of the possible deleterious effects caused by WBFE. For euthanasia the animals were fasted for 12 hours and anesthetized with thiopental sodium, euthanasia was due to total cardiac puncture. Blood was centrifuged at 3,000 g for 10 minutes to obtain serum. The small intestine was collected for histological analysis, cytokine dosage, nitric oxide and oxidative stress.

2.5 Biochemical Evaluation

Plasma levels of glucose, cholesterol, and triglycerides were determined by the enzymatic colorimetric glucose oxidase method (Gold Analisa®). The results were expressed as mg dL^{-1}.

2.6 Histological processing

In histological processing, the small intestine was kept immersed in Karnovsky solution for 24 hours and then transferred to 70% ethanol. Subsequently, fragments of the proximal portion of the jejunum were dehydrated

in increasing ethanol solution and included in methacrylate (Historesin, Leica Microsystems, Nussloch, Germany). Semi-serial sections of 2μm were obtained in a rotating microtome (RM 2255, Leica Biosystems, Nussloch, Germany), with a minimum of 40 μm between the cuts. The obtained sections were then stained with hematoxylin-eosin (HE) and mounted on glass slides. Photomicroscope (Olympus BX-53, Tokyo, Japan) equipped with a digital camera (Olympus DP73, Tokyo, Japan) were used for the images morphometric analysis. Histopathological analyzes were performed at the Laboratory of Pathology of the Department of Medicine and Nursing of UFV, in light microscope CX31 with a camera coupled U-CMAD3 (Olympus, Tokyo, Japan). All images were analyzed using the Image J® program.

2.7 Intestinal histomorphometry

Ten villi and twenty crypts were measured from 10 images captured from histological slides at 10x magnification using the photomicroscope (Olympus BX-53, Tokyo, Japan), a CX31 light microscope with U-CMAD3 coupled camera (Olympus, Tokyo, Japan) of the Laboratory of Pathology of the Department of Medicine and Nursing of UFV. The height of each villus was represented by the distance between the apex of the villi to the basal region, which corresponds to the opening portion of the crypts in the intestinal lumen. The villus width was measured, taking the average of three points of the same villi used for height measurement, in the apical, middle and basal regions. The depth of each crypt was determined from the base of the crypt to the opening region thereof which represents the transition between the crypt and the villi. Mitotic activity was expressed as the number of mitoses per 100 cell crypts. The result was the mean obtained from the measurements of each histological slide.

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2.8 Intestinal histopathology

A qualitative analysis was carried out under optical microscopy in order to ascertain the presence or absence of alterations in the tissues of the animals treated acutely with the crude extracts (EB) of the white bean flour (WBF) compared to the tissues of the animals of the control group.

2.9 Dosing of cytokines

Fragments of approximately 1g of the small intestine were homogenized in the presence of a protease inhibitor (protease inhibitor cocktails; Sigma-Aldrich, St. Louis, MO, USA) in a portable tissue homogenizer (YO-04727-09; LabGEN) and centrifuged at 3000 g for 10 min. The supernatant was collected for cytokine analysis, which was performed using BD Cytometric Bead Array (CBA)/Mouse Inflammation Kit. The cytokines measured were: Tumor Necrosis Factor (TNF), Interferon-ɣ (IFN-ɣ), Interleukin-12 (IL-12), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10) and monocyte-1 chemotactic protein (MCP-1). Standard curves were determined for each of the cytokines from a range of 20 to 5000 pg/mL. According to the manufacturer, the lower limit of cytokine detection for CBA is 2.5 pg/mL. Data were collected using the BD FACSVerse of the Nucleus of Microscopy and Microanalysis at the Federal University of Viçosa (UFV) and analyzed in FCAP 3.0 software.

2.10 Dosing of nitric oxide

NO levels were indirectly estimated based on the determination of NO, nitrate and nitrite metabolites by the Griess reaction. The intestinal homogenate was centrifuged at 3000 g for 10 min and the supernatant from the samples collected. In a 96-well plate, 50 µL of the samples were deposited. A standard curve was prepared by the addition of 50 µL of 250 µM sodium nitrite and 50 µL of PBS,

successive 1: 2 dilutions were performed in addition to the blank well containing only 50 µL of PBS. Then, 50 µl of 1: 1 mixture of sulfanilamide solutions (1% in 2.5% H_3PO_4) and N-naphthylethylenediamine dichloride (0.1% in 2.5% H_3PO_4) were added in each well. After 10 minutes of incubation in the absence of light and at room temperature, readings were made for all wells (standard curve and samples). Optical density (OD) was determined using a 570 nm filter from the Thermo Scientific-Multiskan ™ GO plate reader. Results were expressed as µM.

2.11 Determination of catalase (CAT)

The enzymatic activity of Catalase present in the intestinal homogenates of the mice treated with WBFE was evaluated by measuring the rate of decomposition of H_2O_2 over time, according to Aebi (1984). To this end, 6 µl of sample together with 600 µl of phosphate buffer (0.1 M and pH 7.0) was used as blank for each sample while for reading the phosphate buffer was added H_2O_2 (30%). Thus, in a Quartz cuvette, the samples were subjected to the reading at 240 nm in a spectrophotometer, during 60 seconds, in order to follow the enzymatic kinetics. The enzymatic activity was expressed in Catalase Units per mg of protein.

2.12 Determination of Superoxide Dismutase (SOD)

The determination of the catalytic activity of SOD was determined by the pyrogallol method based on the enzyme's ability to catalyze the reaction of superoxide (O^{-2}) and hydrogen peroxide, according to Sarban et al. (2005). To this end, 30 µL of sample was pipetted together with 99 µL of phosphate buffer (0.1M pH 7.0), 6 µL MTT (1.25 mM) and 15 µL pyrogallol (100 µM) in 96-well plate and incubated for 5 minutes at 37 ° C. The standard and blank were made in the same manner, but for both there was no sample and 129 µL and 144 µL of buffer, respectively, were used, whereas in the blank no pyrogallol was added. Finally, after the incubation time, the reaction was stopped with 150 µl of DMSO (1.25 mM) and

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the samples were read at 570 nm in a plate reader (Thermo Scientific-Multiskan ™ GO). The enzymatic activity was expressed in SOD units per mg protein.

2.13 Determination of Maldaloaldehyde (MDA)

The supernatants of the intestinal homogenates collected were submitted to the reaction with thiobarbituric acid (TBARS), according to the methodology of Buege e Aust (1978). Thus, 200 µL of each sample was added to 400 µL of TBARS solution (15% TCA, 0.375% TBA and 0.25M HCl), vortexed for 10 seconds and placed in a water bath at 90 °C for 40 minutes. Then, after cooling, thiobarbituric acid reactive substances were extracted with the addition of 600 µL of n-butanol followed by centrifugation at 3500 g for 5 minutes. Finally, after centrifugation, 200 µL of the supernatants were carefully removed and subjected to reading at 535 nm in a plate reader (Thermo Scientific-Multiskan ™ GO). The values of TBARS were expressed in malondialdehyde (MDA) ηmols per mg protein.

2.14 Statistical analysis

The results were expressed as the mean ± standard deviation of the mean. They were submitted to analysis of variance one way (ANOVA) followed by the post-test (Tukey) for multiple comparisons. Statistical significance was considered when $p < 0.05$. Analyzes were performed using the GraphPad Prism 5.01 program (GraphPad Software, Inc. (San Diego, CA).

3 RESULTS AND DISCUSSION

3.1 Effect of WBFE on body weight, glycemia and serum biochemical parameters

There were no cases of death or symptoms of gastroenteritis in any of the experimental groups during the 14 days of treatment. The results showed that oral

administration of WBFE induced progressive loss of body weight (Figure1A) and dose-dependent reduction in serum glucose levels (Figure1B) in groups T1 and T2, compared to control (p <0.05). On the other hand, there was no difference in triglyceride and total cholesterol levels between the control groups, T1 and T2 (data not shown).

Figure 1 – Systemic effects of extract of white bean flour. Weight gain percentage (A) and fasting blood glycose (B). The results represent the mean ± SD of five mice per group obtained using the ANOVA one-way com teste post-hoc de Tukey, * p<0.05; **p<0.01; ***p<0.005 in two experiments with similar results
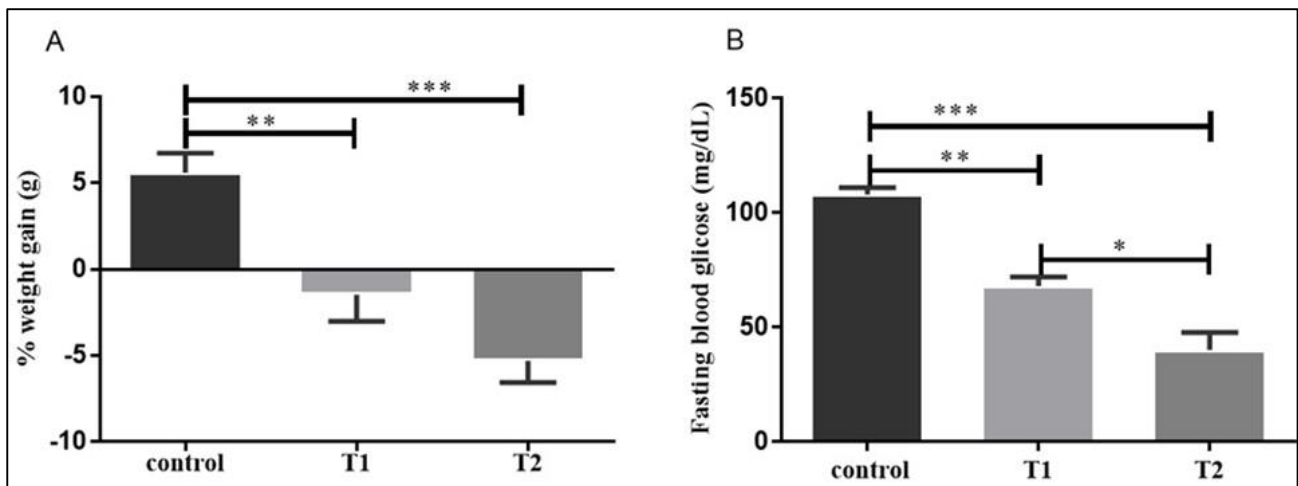
Oral supplementation of WBFE induced daily loss of body weight in T1 and T2 groups in relation to control, suggesting that WBFE reduced the nutritional and caloric viability required for body weight gain, mainly in animals in the growth phase. This effect of reducing body weight was accompanied by a dose-dependent reduction of glycemia at the end of treatment, showing that WBFE interfered in glucose uptake, contributing to the loss of body weight of treated animals. These results corroborated with studies in which supplementation of raw common bean as well as its purified phytohemagglutinin (PHA) reduced the absorptive activity of the intestinal mucosa, resulting in inhibition of growth and progressive loss of weight (Kik *et al.* nineteen ninety; Zucoloto *et al.*, 1991; Ramadass *et al.* 2010;

Gislason. 2011). Similar to our analysis, studies have shown that the administration of bean flour (*Phaseolus acutifolius*) and the purified trypsin inhibitor caused body weight lossdue to pancreatic hypertrophy/hyperplasia. However, this antinutritional factor was not primarily responsible for toxicity (Osman *et al.* 2003). This data was confirmed by another study in which the weight loss observed in rats was due to the presence of the lectin in the extract, since the extract was absent from trypsin inhibitor (Martínez *et al.*2015).

On the other hand, different from the present study, the oral administration of different commercial extracts based on white beans induced a reduction effect in hyperglycemia in healthy and diabetic rats, contributing to the reduction of body weight gain due to the presence of phaseolamine. It acts inhibiting the activity of pancreatic amylase in the intestinal lumen without causing histopathological changes and malnutrition (Cholski *et al.* 2007; Fantini *et al.*2009; Gouveia *et al.*2014).

The evaluated treatments did not interfere in lipid metabolism. Recent studies have shown similar results, where the authors observed that the consumption of a home preparation of white bean flour in increasing concentrations (0.5, 0.75, 1 g / kg) did not significantly alter the concentration of triglycerides and total cholesterol of adult mice compared to the control (Molz and Cordeiro. 2014).

3.2 Effect of WBFE on morphometry and histopathology of the intestinal jejunal mucosa

In the intestinal epithelium, significant morphometric changes were observed in the T1 and T2 groups. The villus height decreased (Figure 2A), while the depth of the crypts (Figure 2B) was increased in comparison to control ($p < 0.05$). Although the results indicated a trend of dose-dependent morphometric changes, there was no significant difference between the treated groups ($p > 0.05$). The
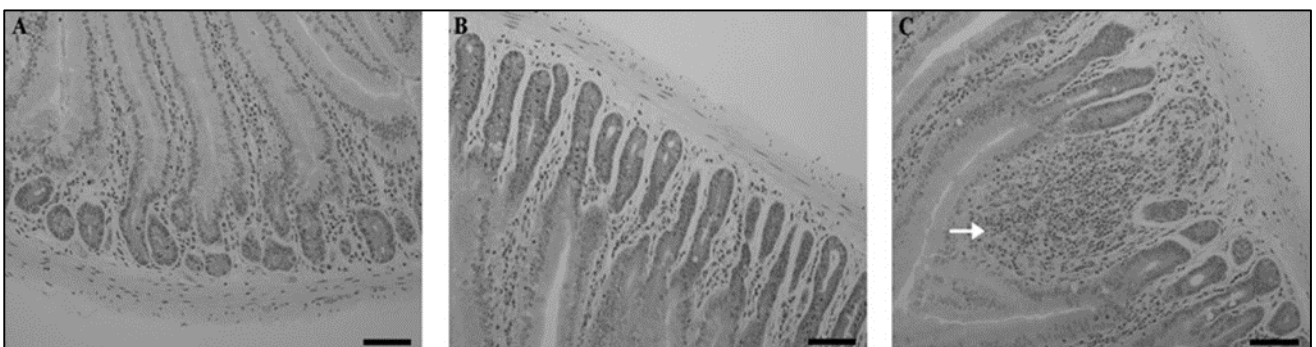
mitotic index in the crypts was significantly increased (Figure 2C) in relation to the control, in a dose-dependent manner ($p < 0.05$).

Figure 2 – Morphometric analysis of intestinal mucosa after WBFE oral administration (T1 e T2). Villus height (A), Cript depth (B) and mitotic index (C). The results represent the mean ± SD of five mice per group obtained using the ANOVA one-way com teste post-hoc de Tukey, * $p<0.05$; **$p<0.01$; ***$p<0.005$ in two experiments with similar results.
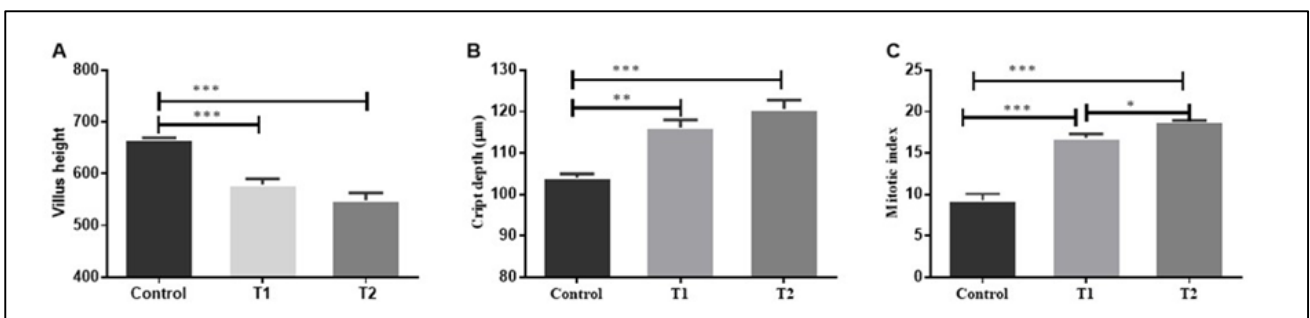
In the lamina propria of the T2-intestinal mucosa of the inflammatory infiltrate composed of mononuclear cells was observed (Figure 3). Both the control group and the T1 group had no apparent inflammatory foci on the lamina propria.

Figure 3 – Histopathological analysis of proximal jejunum mucosa. Normal lamina propria in control (A) and T1group (B). Inflammatory inflitrate (arrow) in proximal jejunum mucosa of group T2 (C). Hematoxylin and eosin staining sections (HE). Scale: 40µm

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Oral supplementation of WBFE reduced the villus height in the T1 and T2 groups, indicating that WBFE caused toxicity to the epithelial absorptive cells and consequently reduced the intestinal absorption area, leading to the observed glucose malabsorption in the animals of these groups. This result was consistent with studies that have shown the toxic effect of agglutinins isolated from wheat (WGA) and red and white bean (PHA) supplementation on the morphology and absorptive function of intestinal mucosa in rats (Lorenzsonn and Olsen. 1982; Lorenz-Meyer *et al.* 1985; Zucoloto *et al.* 1991). According to these studies and others in the literature, the interaction of high concentrations of leguminous lectins with glycosylated sites of the enterocyte membrane led to damage at the base of the villi and increased permeability of the epithelial barrier, reducing nutrient absorption, and consequent weight loss (Ramadass *et al.* 2010; Ferreti *et al.* 2012; Kumar *et al.* 2013; Neiri *et al.* 2015).

After compromising the integrity of the intestinal mucosa, the T1 and T2 groups presented increased crypt depth due to hyperplasia, a result of the high mitotic index observed in the study. This indicates an adaptive response to WBFE toxicity in an attempt to restore the integrity of the epithelial layer and consequently the absorption of nutrients (Neiri *et al.* 2015). However, the intestinal mucosa was unable to self-regenerate. Probably due to the long exposure period of the WBFE.

Simultaneously with the toxic effect on the intestinal epithelium, a mononuclear inflammatory infiltrate was observed on the lamina propria of the T2 intestinal mucosa, indicating that the WBFE is able to induce inflammation. A similar result was observed in in vitro and in vivo studies, which showed that wheat, as well as soybean and common bean lectins, induced an increase in intestinal permeability that allowed the passage of dietary lectins into the bloodstream and consequent activation of phagocytes (Sjolander *et al.* 1984, 1986; Pusztai. 1993; Benjamin *et al.*1997; Sarv and Hrak. 2009; Punder and Pruimboom. 2013). Based

on these studies, the proinflammatory effect of WBFE appears to be involved with the ability of PHA lectin to increase cell permeability and facilitate its transcytosis into the bloodstream. This inflammatory response may be related to the attempt to repair the integrity of the enterocyte membrane and eliminating WBFE antigens, including lectin (Leoni *et al.* 2015).

3.3 Effect of WBFE on the inflammatory profile of the intestinal jejunal mucosa

The concentration of MCP-1 increased in the T1 and T2 groups in relation to the control, and this increase was dose-dependent ($p < 0.05$) (Figure 4A). The concentration of nitric oxide (NO) increased significantly in the T2 group when compared to the control group ($p < 0.05$), in a dose-dependent increase ($p < 0.01$) (Figure 4B). There was no detectable increase of Tumor Necrosis Factor (TNF), Interferon-ɣ (IFN-ɣ), Interleukin-12 (IL-12), Interleukin-4 (IL-4), Interleukin-6 -10 (IL-10) after challenge with WBFE at any of the examined doses (data not shown).

Figure 4 – Inflammatory response elicite by WBFE oral administration. MCP1 quantification (A) and nítric oxid (B) in intestinal homogenate. The results represent the mean ± SD of five mice per group obtained using the ANOVA one-way com teste post-hoc de Tukey, * p<0.05; **p<0.01; ***p<0.005 in two experiments with similar results
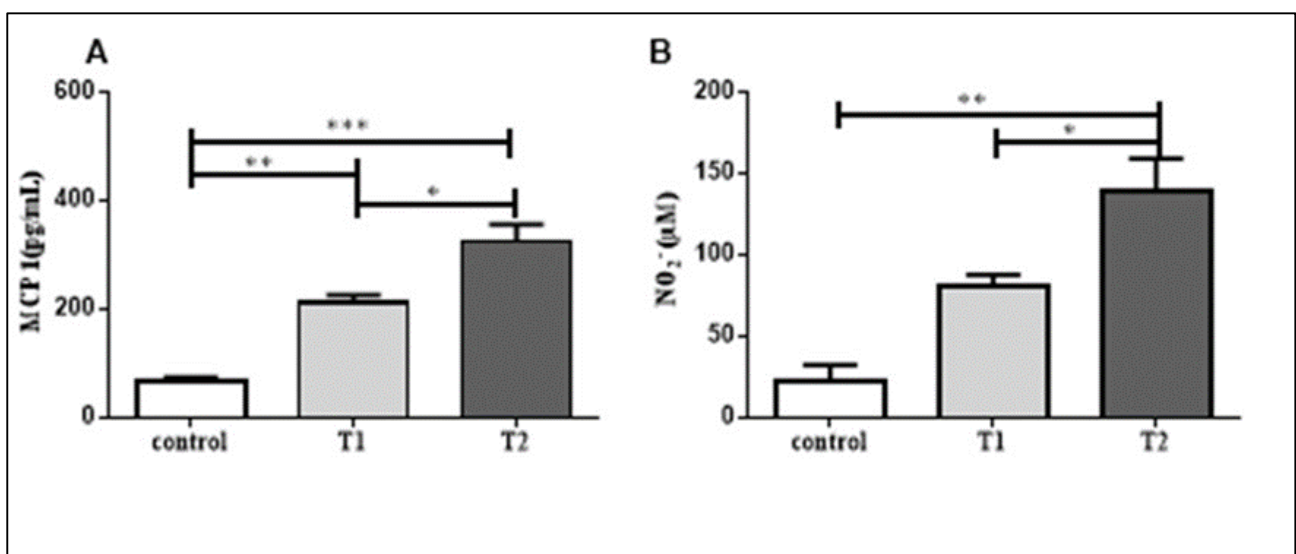
MCP-1, a member of the CC chemokine family, is implicated in the mediation of acute and chronic inflammation in various tissues, including intestine. Supplementation of WBFE induced a dose-dependent increase of MCP-1 in intestinal homogenate, indicating the immunostimulatory potential of WBFE. A similar effect was observed after exposure of wheat and bean "castor", in which proteolysis resistant antigens stimulated monocytes and macrophages of the immune system with a consequent increase in MCP-1 production in rat intestine (Yoder *et al.*, 2007; Junker *et al*., 2012). Research in vitro also showed MCP-1 secretion in mononuclear leukocytes stimulated by common bean PHA lectin (Yoshimura *et al.* 1989; 1990). Based on these findings, inflammatory cells from the intestines of the T1 and T2 groups were responsible for the initiation of intestinal inflammation by MCP-1 secretion.

MCP-1, also called CCL2, plays an important role in the recruitment of circulating inflammatory monocytes and additional macrophages to the inflamed sites (Takada *et al.*, 2010). Studies have shown that MCP-1 plays a key role in the pathogenesis of a variety of intestinal diseases that are characterized by mononuclear cell infiltration (Reinecker *et al.*, 1995; Grimm *et al.*, 1996). After exposure to WBFE, the dose-dependent increase of MCP-1 coincided with the inflammatory infiltrate in the T2 group, indicating that an inflammatory monocytes influx into the inflamed area correlates with MCP-1 levels.

Simultaneously with the production of MCP-1, the nitric oxide (NO) dosage increased significantly in the T2 group coinciding with areas of inflammatory infiltrate, indicating that WBFE stimulated inflammatory cells to synthesizes nitric oxide in a dose-dependent manner. An in vitro study showed macrophages activated by PHA lectin from common bean. (Kesherwani and Sodhi, 2007).

Some immune responses may be mediated by the production and release of nitric oxide by activated inflammatory cells. NO shows immunoregulatory function on the production of several chemokines, including MCP-1 and the chemotactic

response to leukocytes (Bogdan C., 2001). The increase of NO induced by WBFE coincided with the increase of MCP-1, thus suggesting the immunoregulatory role of nitric oxide during the influx of mononuclear cells to the inflammatory infiltrate observed in the T2 group. In another study was suggested that NO could be involved in the leukocyte migration induced by a lectin from Dioclea rostrate (DrosL) (Figueiredo *et al.* (2009).

3.4 Evaluation of oxidative stress in intestinal jejunal mucosa after administration of WBFE

The SOD activity, although apparently increased in the T1 and T2 groups, did not present a significant difference ($p > 0.05$) in relation to the control group (Figure 5A). CAT activity significantly decreased in the T2 group ($p < 0.05$) with respect to the control group and the T1 group ($p < 0.01$) (Figure 5B). The concentration of MDA, although numerically higher in the groups treated with WBFE than in control, did not present a significant difference ($p > 0.05$) (Figure 5C).

Figure 5 – Oxidative stress profile. Activity of superoxide dismutase (A), catalase (B) and malondialdehyde levels (C) in intestinal homogenate. The results represent the mean ± SD of five mice per group obtained using the ANOVA one-way com teste post-hoc de Tukey, ***$p<0.005$ in two experiments with similar results
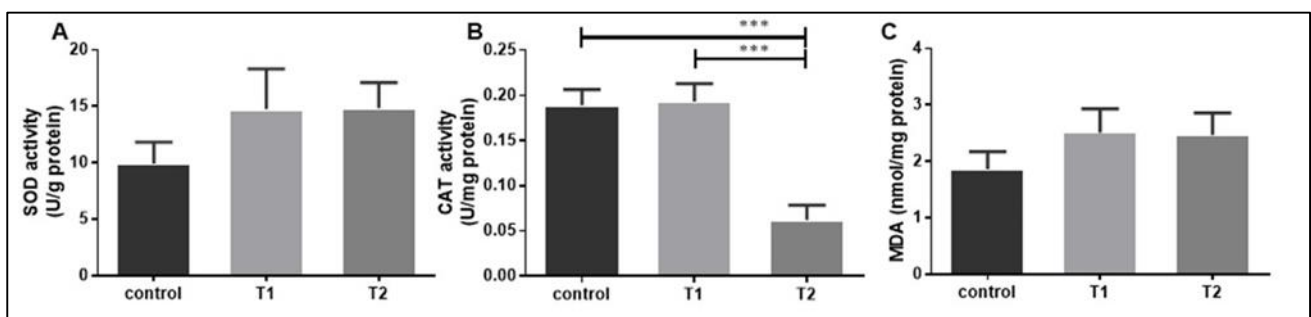
The activity of SOD and CAT are important defense mechanisms against the toxic effects of ROS, among them, superoxide radicals (O_2^-) and hydrogen peroxide

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(H2O2). SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, which is then degraded by CAT in water and oxygen (Droge, 2002).

In this study, the activity of the SOD enzyme had an expressive increase in the intestinal mucosa of the treated groups, suggesting that the phagocytosis of WBFE peptides by the inflammatory cells induced great production of superoxide radical, initiating ROS generation. The activity of CAT showed a significant decrease in the T2 group, leading to the exhaustion of this enzyme due to the high production of hydrogen peroxide. These results corroborate with studies that previously showed a decrease in antioxidant enzymes activity, such as catalase, due to its excessive use in the removal of free radicals, generating an enzymatic depletion (Ighodaro, OM, and Akinloye, OA, 2017).

Malondialdehyde (MDA), a byproduct of lipid peroxidation, indicates tissue damage following oxidative stress. In the groups treated with WBFE, a non-significant increase in the level of MDA in the intestine was observed, indicating that changes in SOD and CAT activity protected cells through the elimination of ROS excess, avoiding oxidative stress at the end of WBFE treatment. This result was concomitant with the increase in nitric oxide, confirming its antioxidant effect, preventing damage to the intestine. At higher concentrations, antioxidant effects of NO occur through their direct action in decomposing primary lipid radicals from lipid peroxidation, attenuating tissue damage (D'ischia *et al.* 2000). Thus, phagocytosis in inflammatory cells of the intestinal mucosa did not generate oxidative damage, evidencing that WBFE triggered a controlled immune response.

3.5 Evaluation reversal of intestinal damage after treatment with SFFB

After removal of WBFE supplementation from the diet of the treated groups, after 28 days of experimentation, there was no significant difference ($p > 0.05$) for the variables percentage of mean body weight gain, serum glucose levels, and epithelium histomorphometry intestinal infection between the control groups, T1

and T2 (data not shown). These data indicated that 14 days after FFB removal, there was a repair of the intestinal epithelium histology, with the consequent re-establishment of glycemia and weight gain in the treated mice. This data corroborated with studies in which epithelium damage induced by high PHA doses was reversible (Weinman *et al.*, 1989; Dignass *et al.*, 1996).

4 CONCLUSION

The commercial white bean flour evaluated in the present study proved not to be safe because of the oral extract dose-dependent toxic effects, characterized by changes in serum glucose levels and histomorphometry alterations of mucosal epithelium in the proximal jejunum of the small intestine. These alterations contributed to the reduction in body weight of mice exposed to WBF doses for 14 days of treatment. After removal of supplementation with WBFE, the observed changes were reversed, with the consequent reestablishment of the body weight in the treated mice.

In addition, the higher dose of WBFE stimulated an acute inflammatory response, characterized by histopathological alterations in the lamina propria of the intestinal mucosa and an increase in the dosage of inflammatory mediators without causing oxidative stress, in order to eliminate antigens, present in the WBFE, mainly lectins.

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