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Study of α-Amylase Inhibitors among Different Bean Cultivars and Evaluation of their Effectiveness Compared with a Commercial Product using *In Vitro/In Vivo* Experimental Systems

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ABSTRACT

Plant alpha-amylase inhibitors (αAIs) are great potential tools to manipulate resistance of crop plants against pests. They can be also considered as drug-design aims for remedy of diabetes and digestion disorder. In this study, extract of Phaseolusvulgaris L. (Red, Pinto, White and Cowpea Beans) was investigated. The rich part of αAI was almost purified by ethanol fractionation, and then studied in reaction with pancreatic α-amylase in vitro. The study of thermal stability, hemagglutination and measurement of the inhibitory activity of extracts of Red, Pinto, white and Cowpea beans were investigated. Also, an in vivo assay (acute and chronic) over 21 days performed (acute and chronic). Among the αAIs extracted from the beans that were loaded at the same concentration, the α-amylase extracted from White beans (lane C) had the highest match with peptide fractions 14 and 18 kDa and two fractions around 27 to 32 kDa. All fractions of common bean extract showed inhibitory activity. The White beans, Red beans, and Pinto beans extract revealed high inhibitory activity, similar to each other against pancreatic α-amylase. After 21 days of treatment, group 3 showed significant decreases in blood glucose level, compared to positive control. Length of the small intestine in group 1, over 21 days, significantly reduced relative to the negative control, but in group 4, the reduction was lower relative to negative control. The high inhibitory activity of fraction 3 from the beans showed that the ethanol fractionation is the proper techniques to (partially) purify the αAI from Pharsalus vulgarised. The results of this study have reported that the fraction 3, extracted from White beans, can be considered as raw material for pharmaceutical preparation of αAI to control the levels of glycemia, after successful optimizing formulation conditions to increase the half-life of the product.

Key words: α-Amylase Inhibitor, *Phaseolus vulgaris* , Carbo Blocker, Blood Glucose Level, Body Weight

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INTRODUCTION

Among grain legumes in the world, familiar beans (*Phaseolus vulgaris* L.) use widely for direct human consumption [1] (Figure 1). There are three common isoforms of alpha-amylase inhibitor (αIA) including αA1, αA12, and αAIL that the first isoform has anti-amylase activity in humans. They are not active against plant α-amylases and have classified as proteins of antifeedant or seed defense [2]. Recently, investigators have focused their attention on common bean proteins with particular functions, such as anti-obesity activities [3]. The kidney bean (*Phaseolus vulgaris)* has particularly high levels of the inhibitor [4]. The αAIs have known as starch

blockers because they prevent digestion and absorption of dietary starches by the body [5]. A starch blocker act as an interference material through the decomposition of complex carbohydrates, it reduces digestion or prolongs digestion, so that energy from carbohydrates is reduced or the body absorbs the energy via glucose [6]. The reaction mechanisms for inhibiting α-amylase by plant proteins are not clearly understood [7]. The reduction of sugars that are attached separately to the polypeptide inhibitor chain may play an important role in the mechanism, or that the inhibitor may cause focal changes in the enzyme molecule [8]. Long-term administration of amylase inhibitors reduces serum glucose level and body weight gain in nondiabetic (ND) Wistar rats [9]. Carbohydrate digestion has been targeted as a tool can control both postprandial increases of weight gain and serum glucose [10]. Carbohydrate-digesting enzymes inhibitors such as α-amylase and α-glucosidase, now are being actively searched, because they could eventually make effective drugs for obesity and diabetes [11, 12]. Among the plant resources, αAI of *Phaseolus vulgaris* L. has been presented to have relatively large potential as extensive anti-obesity and anti-diabetes therapy because it has not been connected with deleterious agents such as asthma and dermatitis which some cereal αAIs have been related [13].

Phaseolus vulgaris has been indicated has an α-amylase inhibitory activity and is believed to reduce weight through promoting the mobilization of fat reserves of the body, as a result of energy constraints [6]. Studies of a growing body of laboratory animal show that acute or chronic administration of the derivatives, extracts, and ingredients of *Phaseolus vulgaris* significantly decrease appetite, food intake, carbohydrate absorption, metabolism, lipid accumulation, body weight gain, glycaemia, and glucose absorption in lean and obese animals [14]. Several companies have provided αAI extract from common beans to control appetite and energy intake [15]. Therefore, only the αAIs, isolated from White common beans are used in commercially-produced αAI products [6, 15]. *Phaseolus vulgaris* are named as Phaseolamin and phase 2 as a single compound supplement or in combination with other dietary components. Animal studies have aimed that *Phaseolus vulgaris* reduce the weight [16], and a number of clinical trials have been conducted to evaluate its efficacy in human subjects [17]. Salting out, gel filtration column chromatography, and ion exchange chromatography are the methods that to purify αAI from different plant species [18, 19] that these are commonly high-priced and time-consuming. However, searching for efficient and cheaper protocols of αAI preparation is of interest. Moreover, to the best of our knowledge, no post-marketing evaluation has been done on commercially available αAIs products in Iran.

In the previous study, we indicated that Carboblocker, as a commercially prepared phase 2, shows a low activity in porcine pancreatic α-amylase inhibition. Accordingly, in the previous study, a new isolation procedure was investigated by ethanol 48% that improved the inhibitory potency of αAI *in vitro.* Therefore, the objectives of the present study are the purification of pancreatic αAI from various types of beans by ethanol 48% and investigation of their inhibitory activity. As well as, since acarbose can inhibit both amylase and glycosidase activities, thus, αAIs may probably inhibit the glycosidase activity too. In an attempt, thermal inactivation at different temperatures and hemagglutination was studied. The present study investigates the efficacy of the acute and chronic administration of aαAI, isolated from White kidney beans, on the concentrations of glycemic, food intake, body weight, weight and length of the digestive tract in a rat, compared with carboblocker nutritional supplements.

Figure 1: Different types of beans, used in the current study, and a nutritional supplement from white beans

MATERIALS AND METHODS

The chemicals and reagents were analytical grade. The 3,5-dinitrosalicylic acid and soluble starch were bought from Sigma-Aldrich (St. Louis, MO, USA). Phase 2 (with commercial name of carbo blocker) α-amylase inhibitor was purchased from

a local market and other used reagents were the highest grade commercially available. Each experimental point indicated in the Figures is average of at least two or three independent measurements with standard errors < 5%. Whenever the data were analyzed, P-values less than 0.05 were considered statistically significant.

Purification of αAI

Common beans (Pinto beans, White beans, Red beans and Cowpea beans) were obtained from a local supermarket. Five-hundred grams of bean meal was extracted by 5. Vol. of 10 mM sodium phosphate buffer (pH 7) with overhead stirring for 24 hours at 4 °C. This extract was centrifuged (8500×rpm for 30 min). After 30 min, the mixture was centrifuged at 5000× rpm for 1 hours) (fraction 1), and more ethanol was added to the supernatant to a concentration of approx, 33% (v/v). The mixture was stirred for a further 30 min, and centrifuged (5000× rpm for 1 h) (fraction 2). At last, ethanol level in the supernatant was increased to 48% (v/v) and the mixture was centrifuged like before (fraction 3).

The αAI activity

The α-amylase inhibitory activity was evaluated by measuring the residual α-amylase activity. The assay was performed by adding 50, 75, 60 and 55 µl of sample extract of White beans, Red beans, Pinto beans and Cowpea beans (0/3 mg/ml) to 20 µl of porcine pancreatic α-amylase solution. The mixtures were brought to a total volume of 200 µl with 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride. Then, samples were pre-incubated at 37 °C for 30 min [20]. After addition of 200 µl of substrate solution (1% soluble starch) and incubation for 5 min, the reaction was stopped by adding 400µl of 3, 5 dinitrosalicylic acid reagent, followed by boiling for 5 min in a water bath. Afterward, 5 ml of water was added and the solution was mixed and it stand at room temperature for 15 min. Absorbance was measured at 545 nm[20].

Thermal inactivation

Irreversible thermo-inactivation of the αAI was investigated at different temperatures ranging from 30 to 70 °C, for 5-30 min. At regular intervals, the sample was removed and immediately cooled on ice. Thereafter, the residual inhibitory activity was measured by adding 20 µl of the enzyme (20 mg/ml) to 50, 75, 60 and 55 µl of extract sample of White beans, Red beans, Pinto beans and Cowpea beans (0/3 mg/ml) and pre-incubated at 37 °C for 30 min, and then the substrate was added to the mixture. The activity of the enzyme solution in the absence of αAI was considered as the control (100%) [21].

Hemagglutination assay

This assay was done based on study of [22]. The glucose concentration, in capillary blood samples, was determined by Bionime advantage system, Roche.

Animals

The study included adult male Wistar rats with body weight of 363 g and standard error (SEM) of 11 g. They had been maintained on a high carbohydrate diet [23]. The rats were taken care in accordance with the principles of [4] for the experimental animals. The study was approved by the Animal Ethics Committee of the Kermanshah University of Medical Sciences.

Study of αAI acute effects

The animals were randomly divided into 4 groups (seven rats in each group) with the following treatments: group of rats in an untreated negative control received 1mL of 50% in NaCl (9 g/l) (group 1: NC); group of rats with a positive control received starch (2 g/kg body weight, soluble potato starch; Sigma, Alcobendas, Madrid, Spain) dissolved at 50% in NaCl (9 g/l) (group 2: PC); group of rats treated with fraction 3, extracted from White beans (57/7 mg/kg) and starch (2 g/kg body weight) dissolved at 50% in NaCl (9 g/l) (group 3: FW) and rats treated with a commercial product, carbo blocker (70 mg/kg) and starch (2 g/kg body weight) dissolved at 50% in NaCl (9 g/l) (group 4: CB). The commercial product carbo blocker, fraction 3; extracted from White beans and starch was administered by gavage. Blood glucose was determined at 9.00 o'clock (time 0) after 18 h fasting. Measurements of blood glucose and the extracting of blood was based on study of [4].

Study of αAI chronic effects

In the chronic effect, the animals were randomly divided into 4 groups (seven rats in each group) with the following treatments: rats in an untreated negative control consumed normal diet (group 1: NC); group of rats with positive control received

1mL of NaCl (9 g/l) and consumed high carbohydrate diets (group 2: PC); group of rats treated with fraction 3, extracted from White beans(57/7 mg/kg) dissolved in NaCl (9 g/l) and consumed high carbohydrate diets (group 3: FW) and rats treated with a commercial product, carbo blocker (70 mg/kg) dissolved in NaCl (9 g/l) and consumed high carbohydrate diets (group 4: CB). The commercial product carbo blocker, fraction 3; extracted from White beans and NaCl (9 g/l) was administered by gavage at 20:30 pm over a 21-day period to the 2.5-month-old Wistar rats. Every day at 9.00 o'clock during this period, food and water intake were measured. Body weight and blood glucose were measured once in every two days. After 21 days of treatment, the rats were killed for to measure digestive organs similar to study of [4].

Histopathology analyses

Dissected pancreas and livers from negative control, positive control, FW, and CB were fixed in 10% formaldehyde, and processed and used for histopathological analyses. Tissue processing was carried out using an auto Technicon and the prepared 5 µm thick sections for hematoxylin and eosin test [24].

Statistical analyses

Results were expressed in mean ± SEM. The data were statistically analyzed using SPSS software, V. 16, one way ANOVA and repeated measure with multiple comparisons versus the control group. P-values of less than 0.05 were considered as significant..

RESULTS

Aqueous extract preparation/α-amylase inhibitor purification

The first step in the present study was the purification of α-amylase inhibitor from common beans (Pinto beans, White beans, Red beans and Cowpea beans). A crude extract preparation from common bean seeds was obtained by aqueous extraction and a three-step fractionation by ethanol. This gave a partially purified αAI that inhibits porcine pancreatic α-amylase. Then, the extracts were enriched from the last ethanol fractionation (fraction 3). According to the literature, the αAIs are tetramer ($\alpha_2\beta_2$) glycoproteins with molecular weight of 36 to 56 kDa [16] which are composed of 15 to 18 kDa subunits [18]. Thus, the αAI might be dissociated into relatively smaller peptides during electrophoresis. In this study, the isolated αAI (fraction 3 of common beans) contained two peptide fractions with the molecular weight of 14 to 18 kDa [25] together with other two fractions around 27 to 32 kDa. The peptide fractions ranging between 14 and 18 kD a corresponded to α and β subunits. The larger proteins (between 27 and 32 kDa) probably corresponded to the unprocessed αAI proportions, as suggested by Pueyo *et al.,* (1993) or undissociated aggregates of the smaller polypeptides (Bernfeld, 1955) [26]. This profile is also similar to the αAI profile of a White common bean variety that reported by Tormo *et al.,* (2006) and Wang *et al*., (2011) [9, 25, 27]. Figure 2 showed among the αAIs extracted from the beans that were loaded at the same concentration, the α-amylase extracted from White beans (lane C) had the highest match with peptide fractions 14 and 18 kDa and two fractions around 27 to 32 kDa.

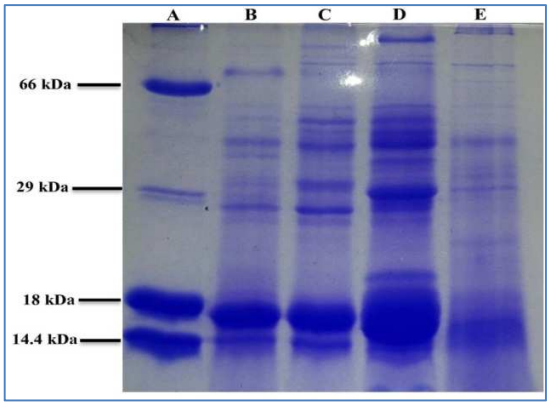

Figure 2: Polypeptide pattern of "α-amylase inhibitor" extracts under SDS-PAGE separation (12.5% slab gel) and Coomassie brilliant blue staining: Lane A: molecular weight of markers. Lane B: αAI extracted of the Red beans. Lane C: αAI extracted of the White beans. Lane D: αAI extracted of Pinto beans. Lane E: αAI extracted of Cowpea beans. (two peptide fractions with the molecular weight around 16 and 18 kDa, with other two peptide fractions around 27 and 32 kDa) purified after three-step fractionation using ethanol[4]

Inhibitory activity of fractions/purified αAI

The inhibitory activities of three isolated fractions of common beans extract (15%, 33% and 48% ethanol) against porcine pancreatic α-amylase were determined *in vitro.* The α-amylase inhibitory activity was assayed by measuring the

residual α-amylase activity after the enzyme and inhibitor were pre-incubated for 30 min at 37 °C. As reported previously [28], no immediate inhibition was observed when the substrate, α-amylase and fractions of common bean extract (fractions 1, 2 and 3) were mixed together. Figure 3 illustrates that all fractions showed inhibitory activity. It was shown that the α-amylase inhibitory activity rose in the order fraction 3 > fraction 2 > fraction 1 of White beans, Pinto beans, Red beans and Cowpea beans. Regarding the higher amylase inhibitory activity, fraction 3 was selected for further investigations.

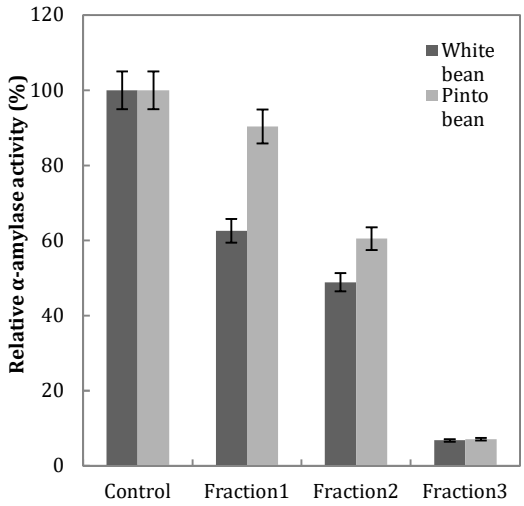

Figure 3: Alpha-amylase inhibitory activities of three isolated fractions of White bean extract and pinto bean extract (fractions 1, 2 and 3 correspond to 15%, 33% and 48% ethanol, respectively) at the same concentration against porcine pancreatic α-amylase. Both inhibitor and enzyme were pre-incubated for 30 min at 37 °C. The activity of the enzyme solution in the absence of αAI was considered as the control (100%)

Inhibition of the α-amylase by fraction 3 of common beans was concentration-dependent. As the level of αAI protein increased, the residual α-amylase activity decreased. That Le Berre-Anton et al. (28) agreed with these results. The results of inhibitory activity of common beans extracts (Pinto beans, White beans, Red beans, and Cowpea beans) on α-amylase are illustrated in Figure 4. As shown, the White beans, Red beans, and Pinto beans extract revealed high inhibitory activity, similar to each other against pancreatic α-amylase. On the other hand, Cowpea beans extract revealed low inhibitory action, similar to nutritional supplements carboblocker.

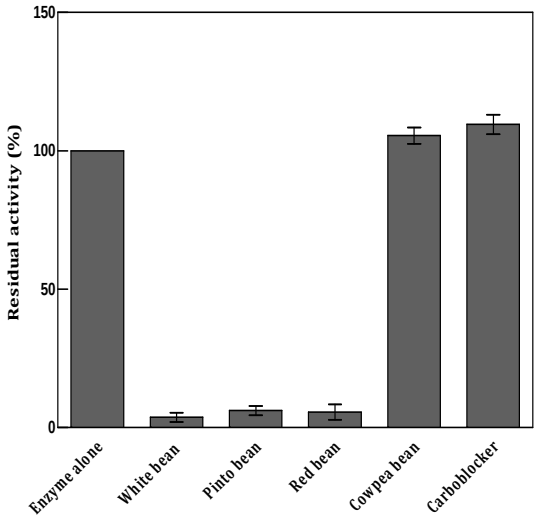

Figure 4: The inhibitory action of fraction 3 of common beans extracts against porcine pancreatic alpha-amylase. Alpha-amylase was pre-incubated with the inhibitor for 30 min and then with a starch solution for 5 min, at 25 ◦C. Absorbance of the mixture was measured at 540 nm.

The enzyme inhibition strength is usually indicated as the IC_{50} value, which is the required level of an inhibitor for inhibiting 50% of the enzyme activity. Additionally, we used a commercially available product, (nutritional supplements carbo blocker) α-amylase inhibitor to serve as a comparative reference. To ensure the dissolution of the protein content of nutritional supplements carboblocker, several buffer systems were tested. Surprisingly, the nutritional supplements carboblocker showed no detectable amylase inhibitory activity in the protein level range from 0 to 0.35 mg/ml. All examined extracts inhibited enzyme activity in a dose-dependent manner (from 0 to 0.35 mg/ml). Among all, fraction 3 of Pinto beans and fraction 3 of White beans have shown the highest enzyme inhibitory activity with an IC_{50} value 0.059 mg/ml and 0.107 mg/ml. Also, fraction 3 of Red beans showed the lowest inhibitory activity (IC_{50} =0.177 mg/ml) and fraction 3 of Cowpea beans showed no inhibitory activity, as shown in Figure 5. Our results showed that the fraction 3 from Pinto beans, is very active against porcine pancreatic α-amylase, and completely inhibited starch hydrolysis with the addition of 0.3 mg/ml of extract sample.

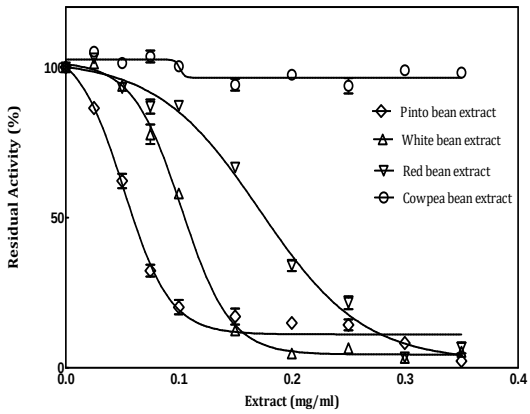

Figure 5: Comparison of the inhibitory effect of fraction 3, extracted from the Pinto beans, White bean, Red bean and Cowpea bean on the activity of porcine pancreatic α-amylase (PPA). The extract of Cowpea bean shows no inhibitory activity against PPA at the concentration range from 0 to 0.35 mg/ml, but fraction 3 extract of Pinto beans, White beans and Red beans showed inhibitory activity against PPA, respectively IC50=0.059 mg/ml, IC50=0.107 mg/ml, IC50=0.177 mg/ml [4].

Thermal stability of the αAI

Clinical efficacy and Biological activity of a remedial protein are conditional upon the bioavailability, structural stability, and clearance rates of the protein [29]. Temperature is one of the most important parameters that can effect on protein stability (half-life). Ali et al., [5] reported that αAIs are fairly heat-stable. The irreversible thermo-inactivation of the fraction 3, extracted from the common beans (Pinto beans, White beans, Cowpea beans and Red beans) were investigated at different temperatures between 30˚C to 70˚C. The thermo-stability profile indicated that the inhibitor was almost stable in a temperature range from 30 °C to 50 °C but the amylase inhibitory property declines sharply at 70 °C. Figure 6 shows the influence of temperature on the biological activity of the Pinto beans, Red beans, and White beans extracts. As indicated by the irreversible thermo-inactivation analyses, α-amylase inhibitors within extracts of Pinto beans, Red beans and White beans retained ~36%, ~43.5% and ~3.5of their initial activities, respectively, after 30 min of incubation at 70 °C.

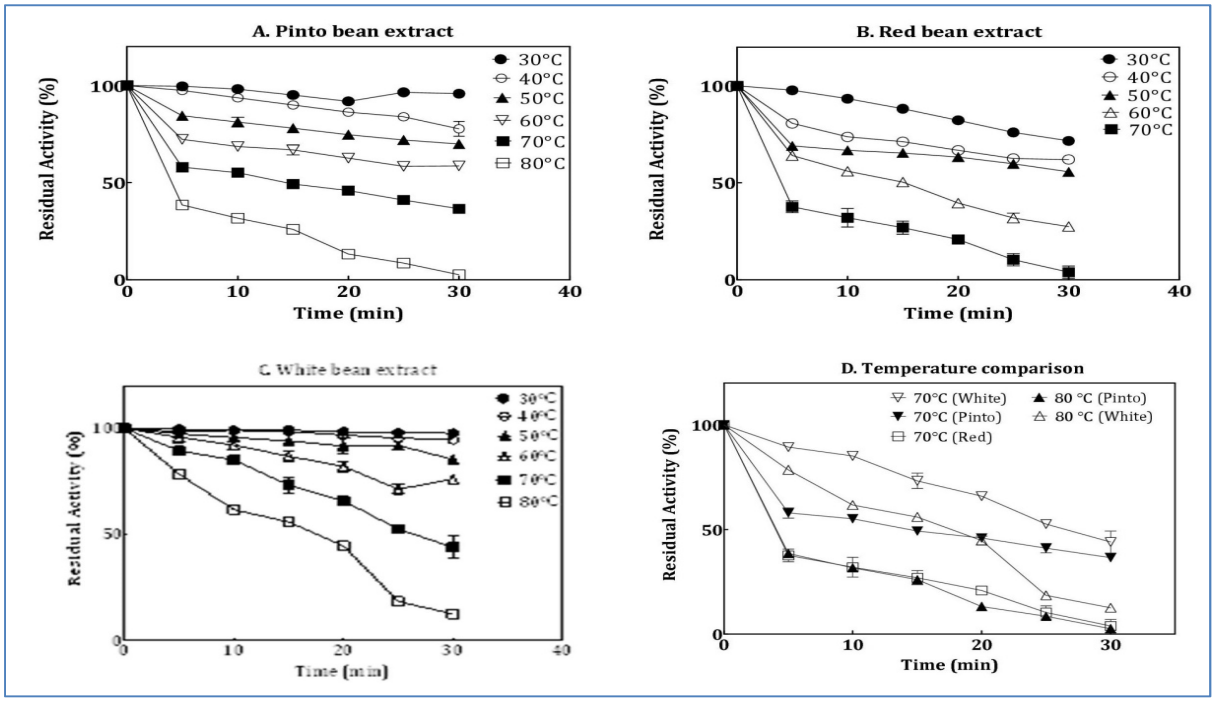

Figure 6: Irreversible thermal inactivation of extract of Pinto beans, Red beans and White beans at 30 °C(•), 40 °C (○), 50 °C (▲), 60 °C (Δ), 70 °C (■) and 80 °C (□) is presented in Figures A,B, and C. The activity of the enzyme solution in the absence of extracts was considered as the control (100%). D). Comparison of thermal inactivation in the extract of white beans, pinto beans and red beans at 70 °C and 80 °C [4].

Hemagglutinating activity of extracts and the nutritional supplement; carbo blocker

In this study, an initial evaluation of hemagglutinating activity content, in all extract samples and nutritional supplements carbo blocker, was carried out by using the blood cells (human RBC). Figure 7 shows that a strong hemagglutination activity was detected in nutritional supplements carbo blocker (The highest lectin).The results indicated lowest hemagglutinating activities (The lowest lectin) respectively in extracts of White beans, Cowpea beans, Pinto beans and Red beans, compared with positive control.

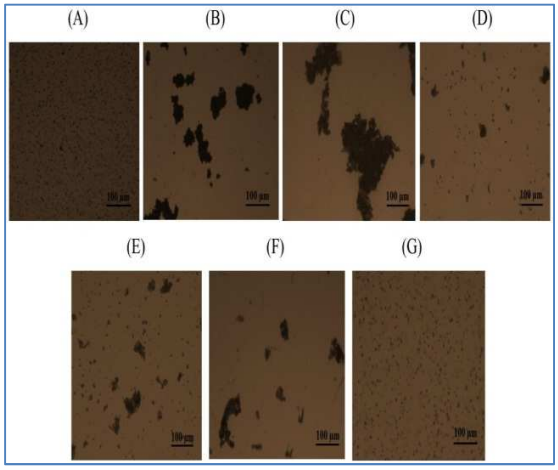

Figure 7: Hemagglutination activity of fraction 3 of common beans (Pinto beans, White beans, Cowpea beans, and Red beans) and nutritional supplements carboblocker.(A) Negative control contained 100 µl of human RBC and 100 µl of PBS. (B) Positive control contained 100 µl of normalized sample (Phytohemagglutinin) and 100 µl of human RBC. (C) Nutritional supplements carbo blocker: contained 100 µl of human RBC, 100 µl of PBS and 100 µl of nutritional supplements carbo blocker. (D) Extract of Cowpea beans(E) Extract of Pinto beans(F) Extract of Red beans(G) Extract of White beans[22]. D, E, F, G were performed by same protocol as described in experiment C. Abbreviations: RBC, Red blood cell; PBS, Phosphate buffered saline [4].

Acute effect on fasting blood glucose

Figure 8 shows the remedial efficacy of acute anti-hyperglycemic on serum glucose concentration measured at various time intervals [4]. The oral administration of the starch overload (2 g/kg body weight) can increase glycemia that sixty minutes after administration, there was a maximum value. The simultaneous administration of starch and fraction 3, extracted from White beans (57.7 mg/kg body weight), can reduce glycemic. The blood glucose level changes, at 180 min after from gavage, were observed with fraction 3, extracted from White beans, 95.8 mg/dl (SEM 0.9), with nutritional supplements carbo blocker, 113.4 mg/dl (SEM 3), with starch, 118.4 (SEM 6) and with 50% in NaCl (9 g/l) 89 mg/dl (SEM 1.1).According to the repeated measure test, serum glucose level between different groups of rats had a significant difference (P<0.001). Two groups comparison showed that between groups positive control and negative control, CB and negative control, FW and negative control, CB and positive control, CB and FW with a significant difference (P<0.05) and between groups positive control and CB without significant difference (P<0.928).

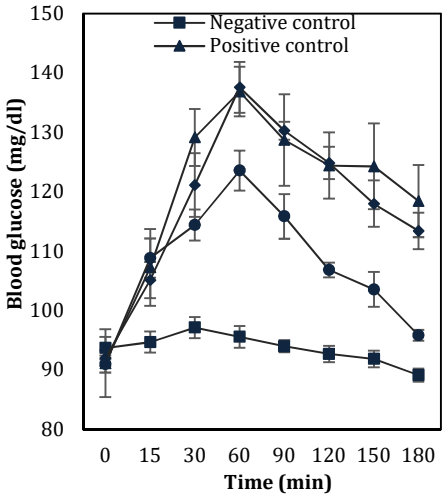

Figure 8: Serum glucose values (mg/dl) obtained after the oral administration to 2.5-month-old Wistar rats of starch (2 g/kg body weight) alone (Positive control) or with 57.7mg α-amylase inhibitor/kg body weight (FW) or with 70 mg nutritional supplements carbo blocker/kg body weight (CB). Also, serum glucose values obtained after the oral administration (Negative control). Standard errors of the mean=vertical bars [].

Chronic effect on level blood glucose and weight

Figure 9 shows the oral administration of fraction 3, extracted from the White beans and nutritional supplements carboblocker over 21 days. According to the repeated measure test, blood glucose level between different groups of rats had a significant difference (P<0.001). Two groups comparison showed that between the positive control and the negative control, CB and the

negative control, FW and the positive control, CB and FW, there was a significant difference (P<0.05), but there was no significant difference between the positive control and CB (P<0.824) or the negative control and FW (P<0.718).

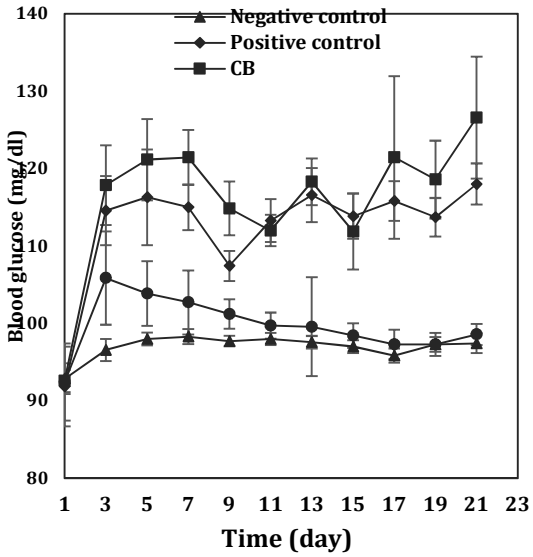

Figure 9: Evaluation of the blood glucose values (mg/dl) of Wistar rats over the 21 days (Positive control) or containing 57.7 mg alpha-amylase inhibitor/kg body weight (FW) or with 70 mg nutritional supplements Carbo blocker/kg body weight (CB). (They had been maintained on high carbohydrate diets) Also, blood glucose values obtained from negative control. Standard errors of the mean = vertical bars [4].

Table 1 shows the efficacy of treatment on blood glucose level of the rats on the first and twenty-first days. After 21 days of treatment, FW group showed significant decreases in blood glucose level, compared to positive control.

Table 1: Chronic effect of the treatment on blood glucose level during the first and 21 days to a treatment with an inhibitor of pancreatic amylase extracted from White kidney beans

Groups	Blood glucose level (mg/dl)	
	Day 1	Day 21
Negative control	92.8±1.9	97.4±1.2
Positive control	91.8±5.1	118±2.6
CB	92.5±1.4	126±7.8
FW	92.4±0.8	98.5±1.3

Each value represents mean ± standard error (SEM); n = 7, each group

The result showed that after 21 days of treatment, according to repeated measure test, there was no significant difference in body weight between different groups of rats.

Chronic effect on intakes of food and water

Table 2 displays the effects of 21 days of treatment on intakes of food and water on the rats. Water intake between the experimental groups didn't have significant difference, but there was a significant increase in food intake for FW groups compared to the negative control.

Table 2: Average of intake water and food on the day during the experimental period, from 2.5-month-old Wistar rats subjected for 21 days to a remedy with an inhibitor of pancreatic amylase isolated from White kidney beans (*Phaseolus vulgaris*)

	Negative control	Positive control	FW	CB
Water(ml/d)	43.1±0.5	41.9±0.8	44.8±0.9	44.3±0.7
Food(g/d)	28.4±0.7	29.9±0.6	40.8±0.5	31.7±0.5

Each value represents mean ± standard error of the mean (SEM); n = 7, each group

In FW group, over 21 days, there was a significant decreasing in the weight of the small intestine, the large intestine, liver and also a slight decreasing in the weight of the pancreas. Also in CB group, there was a decreasing in the weight of the small intestine and the large intestine and also a slight increase in the weight of the liver and pancreas. Length of the small intestine in FW group, over 21 days, significantly reduced relative to the negative control, but in CB group the reduction was lower relative to negative control (Table 3).

Table 3: The small intestine weight and length and also the liver, pancreas, and large intestine weights of Wistar rats, which had been subjected to 21 days of treatment with an inhibitor of pancreatic amylase isolated from kidney beans (*Phaseolus vulgaris*)

	Negative control	Positive control	FW	CB
Length of small intestine (mm)	118.6±3.6	115.5±3.6	105.5±2.3	111±3.4
Weight of small intestine(g)	9.6±0.4	9.2±0.4	6.3±0.2	8±0.1
Liver (g)	14.6±0.3	14.9±0.3	12.7±0.3	14.7±0.1
Pancreas (g)	1.6±0.1	1.5±0.1	1.2±0.1	2.2±0.1
Large intestine (g)	3.1±0.08	2±0.0	1.5±0.2	2.3±0.1

Each value indicates mean ± standard error (SEM); n = 7, each group

Chronic effect on pancreas and liver tissue

Histopathological studies of the sections of pancreas and liver revealed the normal

morphological features. Figure 10 illustrates that the morphologies of these two tissues were similar to those rats in the negative control groups.

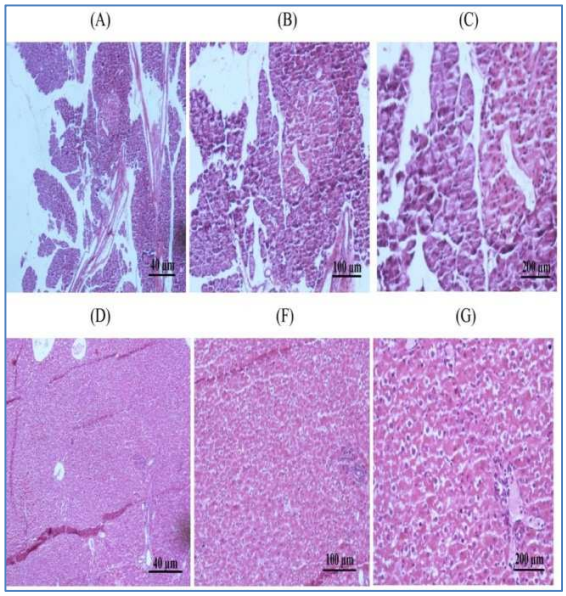

Figure 10: Histopathological effect of remedy with an pancreatic amylase inhibitor separated from White kidney beans (*Phaseolus vulgaris*) and nutritional supplements carbo blocker on the pancreas tissues (A, B, C) and liver tissues (D, F, G) of rats from different experimental groups (Magnification 40×, 100×, 200×). Studies showed that by consumption of pancreatic amylase inhibitor, isolated from kidney beans (*Phaseolus vulgaris*) and nutritional supplements carbo blocker, there is no tissue damage of the liver and pancreas in different groups of rats

DISCUSSION

The results indicated that the fraction 3 of Pinto beans extract had considerably greater amylase inhibitory potency (IC_{50}=0.059 mg/ml) than other extracts. On the other hand, fraction 3 of White beans extract was stable up to 60 °C and also showed that it has the lowest amount of lectins. Therefore, fraction 3, extracted from White kidney beans, was recognized as a more effective inhibitor, to compare with nutritional supplements carbo blocker *in vivo*.

The results showed that the fraction 3 of White beans extract decreased glycaemia levels in FW group rats, compared to the positive control and CB groups of rats followed by acute period (reduction of postprandial glycaemia), and the results also showed significant decreases of glycemia levels in the chronic period (reduction of basal glycaemia). One study [4] described similar results for growing non-diabetic Wistar rats, and for healthy and type-2 diabetic subjects, respectively. These previous studies were all carried out under acute conditions, and that is why the present study was designed to investigate the effect of the prolonged daily administration of the fraction 3 of White beans extract compared to nutritional supplements carbo blocker.

The results confirmed that over 21-days chronic period, there was no change in water intake in different groups but the food intake in the FW group increased compared to other groups, and despite the increase in food intake, the body weight in this group did not differ significantly from other groups. As did those previous studies and the present study, no signs of malabsorption were observed, such as diarrhea or increasing stools, although the dose used by that researcher was larger than the one in the present case.

The chronic administration of fraction 3 of White beans extract led to changes of weight and length in the digestive tract, but a decrease was observed in the absolute weight of the liver, pancreas, small and large intestines. The reasons for the weight loss of the small intestine, large intestine, liver and pancreas in the FW group, are the decrease in the body lipid content in the rats, although this may have the result of altered body lipid metabolism. Accordingly, the inclusion of αAI in the diet, seriously affected the proper functioning of the digestive tract, significantly reducing the apparent digestibilities and absorption of dietary starch and proteins, but not for lipids. The efficacy of the α-amylase inhibitor was the most pronounced on the rat's intestines, particularly the cecum. This was clearly the consequence of the poor breakdown of the dietary starch in the small intestine and its accumulation in the cecum.

CONCLUSION

Overweight/obesity as an excess accumulation of body fat and a chronic disequilibrium between food consumption and energy expenditure is noticeably more prevalent [30]. The high inhibitory activity of fraction 3, extracted from Pinto beans, White beans, Red beans showed that the ethanol fractionation is the proper techniques to (partially) purify the αAI from Pharsalus vulgarised. In this study, there were no signs of malabsorption such as diarrhea or increasing stools. The results of this study have reported that the fraction 3, extracted from White beans, can be

considered as raw material for pharmaceutical preparation of αAI to control the levels of glycemia, after successful optimizing formulation conditions to increase the half-life of the product.

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Abbreviations

αAIs: Alpha-amylase inhibitors
ND: Nondiabetic
PBS: Phosphate buffered saline
SEM: Standard error

Author Contributions

Fatemeh Ghorbani, Cyrus Jalili & Reza Khodarahmi designed the study and contributed to analysis, interpretation of data, and drafting of manuscript. Fatemeh Ghorbani collected data. Abbas Aghaie analyzed data. Arezou Ghahghaei reviewed and edited the manuscript for intellectual content. Masoud Sadeghi did the final revision of the manuscript. All authors gave final approval of the version to be published.

Conflict of interest

The authors have declared that there was no conflict of interest.

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