GLUTELINS HYDROLYSATES OF AMARANTH GRAIN HAVING HYPOGLYCEMIC ACTIVITY: IN VIVO MODEL

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Introduction. Diabetes mellitus (DM) is the first cause of death in Mexico (1). Type 2 of this disease affects 90% of those individuals that course with DM. Owing to the impact of this disease, a large number of hypoglycemic drugs have been developed that, although regulating glucose levels in blood, they present adverse secondary effects (for example, hypoglycemia, weight gain, etc.). Last generation drugs to control diabetes are based on inhibiting the enzyme dipeptidyl-peptidase-IV (DPP-IV), responsible for the degradation of incretin hormones (glucagon-like peptide-1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP]), which induce insulin synthesis (2).
The objective of this study was to assess whether glutelin hydrolyzates of the amaranth grain are able to inhibit DPP-IV.

Methods. Albumin and globulin were extracted with Na_2SO_4 (5% w/v) (3), glutelins were extracted with Tris-HCl (0.1 M, pH 8.0) after removing prolamins. Hydrolysis was performed with alcalase (0.8 AU/g protein) at pH 7.4 and 50ºC. The degree of hydrolysis was determined by quantifying the free amino groups with TNBS. Hydrolysis along time was analyzed by SDS-PAGE and Tricine-SDS-PAGE. DPP-IV activity was determined using Gly-Pro-pNa (Km 0.25 mM) as substrate (4). The hydrolyzates with the highest percentage of DPP-IV inhibition were characterized and purified through gel filtration (Sephadex G-200 and G-15). We assessed IC_{50} expressed with the concentration of the peptide in milligrams per milliliter (mg/mL) capable of inhibiting DPP-IV activity. Biological activity was evaluated in healthy and diabetic mice through intragastric administration of hydrolyzates (10–300 mg/kg), in an acute study administering anhydrous dextrose (2 g/kg) and measuring blood glucose every 30 min (5).

Results. The highest inhibitory activity on DPP-IV was of 84, 63, and 46%, for hydrolyzates of 24, and 48 h for glutelins, globulins, and albumins, respectively. The lower molecular weight peptidic fractions (0.45, 0.65, and 0.86 kDa) showed IC_{50} values of 0.12, 0.25, and 1.98 mg/mL for glutelins, globulins, and albumins, respectively. Glutelin hydrolyzates were the only ones to show in vivo biological activity with area under the curve values of 308 and 418 for glutelin and controls, respectively (Figure 1). The hydrolysates showed biological activity on reducing the hyperglycemic peak are low molecular weight peptides that is similar to that reported by Silva-Sanchez *et al*. 2008.

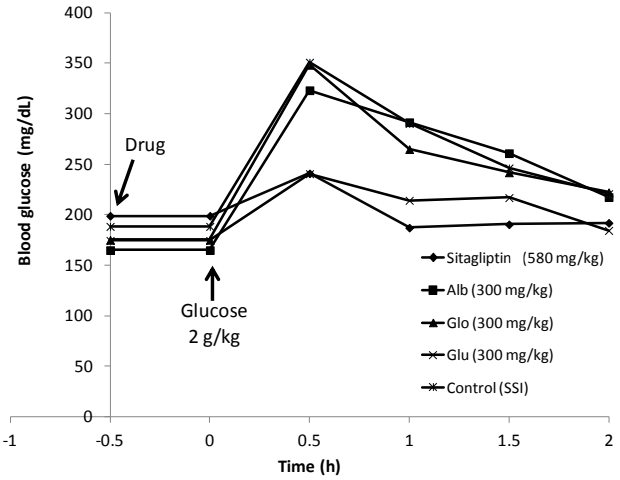

Fig. 1 Effects of hydrolysates of Albumin (Alb), Globulin (Glo) Glutelin (Glu), Sitagliptin (+; positive control); Control (-, negative control) on blood glucose, during the oral glucose tolerance test (OGTT) in diabetic mice.

Conclusions. Hydrolysates grain amaranth glutelin obtained with alcalase could be a potential source of peptide inhibitors of DPP-IV. The peptides obtained from the glutelin exhibit greater inhibition of DPP-IV in vivo and could be used for the development of nutritional supplements aimed at people with type 2 diabetes mellitus.

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