

LUPINALBUS SEED GLOBULINS INDUCE HYPOGLYCAEMIA AND HYPOTRIGLYCERIDEMIA IN WISTAR RATS

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ABSTRACT

Oral administration of *Lupinus albus* seed has been reported to decrease glycaemia similarly to that which occurs when either spartein or lupanin is administered to rats as a precursor to an oral glucose overload tolerance test (OGTT). Also, in OGTT rats, conglutin- γ has been shown to decreased glucose levels. Thus, we tested purified globulins and conglutin- γ from *L. albus* seed, to ascertain their hypoglycaemia and hypotriglyceridemia inducing effects in normal and alloxan-induced diabetic rats. Five groups of female adult Wistar rats were orally fed by metallic cannulae with: 1000 mg/kg globulins (I), 10 mg/kg glybenclamide (II), 100 mg/kg conglutin- γ (III) and 0.5 mL DMSO (IV) respectively, 30 minutes before an OGTT. Groups V and VI of rats were made diabetic after a single intraperitoneal injection of 120 mg/kg of alloxan, then orally received either 1000 mg/kg globulins divided into three doses for 3 days or 10 mg/kg glybenclamide, respectively when glycaemia reached between 200 to 600 mg/dl and displayed diabetic clinical manifestations. Diabetic animals receiving 1000 mg/kg globulins (V) showed a significant blood glucose decrease ($p < 0.05$), while triglyceride blood values, although lowered, displayed more variability than glycaemia values. It seems that *L. albus* globulins containing conglutin- γ and the conglutin- γ itself not only decreased glycaemia in normal animals under OGTT but also affected glycaemia and triglyceride levels in diabetic rats through an unknown mechanism.

KEYWORDS

Lupinus albus, hypoglycaemia, hypocholesterolemia, diabetes mellitus, globulins, conglutin- γ

INTRODUCTION

During the last decade, interest on *Lupinus* protein isolates has been focused on *L. albus*, *L. luteus*, *L. mutabilis* and *L. angustifolius* because they are protein-rich domesticated legumes species that can be customized for specific applications (Gueguen, J. and Cerletti, P., 1994). These protein isolates may also

represent an alternative for individuals wishing to substitute animal with plant proteins for the prevention of cardiovascular disease prevention due to a still unelucidated action mechanism (Arnoldi *et al.* 2007). Albumins (15%) and globulins (85%) are the two main groups of storage proteins found in Lupin seeds (Osborne, T.B., 1924). Although solubilisation of seed globulins is still an unresolved problem for large scale conglutin- γ scale preparation, albumins are easily extracted with cold water from deoiled flour. Conglutin- γ is partially solubilised from whole globulins by either 65°C water (Sgarbieri, C. *et al.* 1978), 1.0 M NaOH followed by precipitation at their Ip (Joubert, F.J., 1955; Garzon-de la Mora *et al.* 2008), 10% NaCl extraction followed by $(\text{NH}_4)_2\text{SO}_4$ precipitation and dialysis (Blagrove *et al.* 1980; Garzon-de la Mora *et al.* 2008) or other available pilot plant fractionation procedures (D'Agostina, A. *et al.* 2006). These procedures maintain the native protein properties, being always the RPHPLC conglutin- γ separation retention times identical and same Mt after MALDITOF-MS (Garzon-de la Mora *et al.* 2008). *L. albus* conglutin- γ has been reported to decrease blood glucose levels, under overloading conditions, and its effect has been compared with Metformin whose mechanism of action is not insulin secretagogue (Magni, Ch., 2004). Therefore we decided to investigate the blood glucose, cholesterol and triglyceride levels lowering properties of *L. albus* globulins and conglutin- γ , under the same glucose overloading conditions, in addition to alloxanised animals using the insulin secretagogue glybenclamide as control.

MATERIALS AND METHODS

Animals. Female Wistar rats weighing 200-250 g obtained from the Bioterium of the CUCS, Universidad de Guadalajara, were housed at 25°C, 65-70% of relative humidity and placed within 40 x 24 x 15 cm individual polycarbonate cages under 12 h light-darkness cycles (08:00 a 20:00 h). Rats were deprived of food from 7 a.m. until the time of performing the glucose tolerance test otherwise they remained under free access to water and to standard rat chow. Weight measurements, food and water intakes were recorded daily in alloxanised animals.

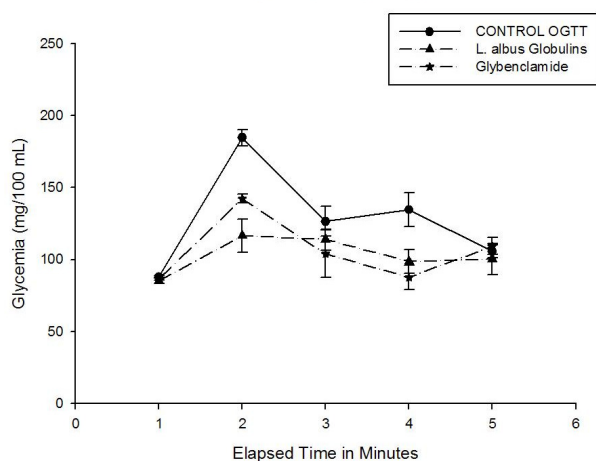


Fig. 1. *L. albus* globulins and OGTT.

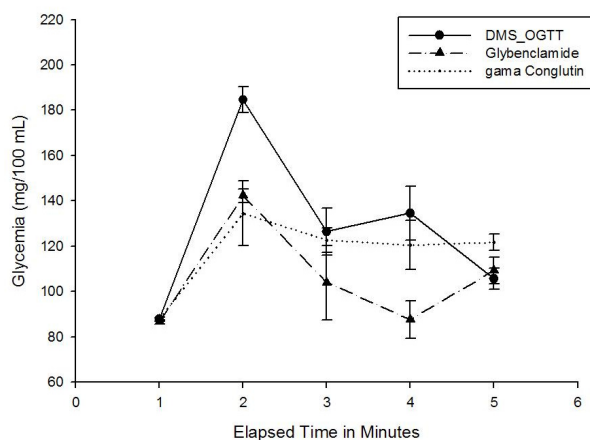


Fig. 2. *L. albus* gamma conglutins.

Reagents and chemicals. Dextrose, Alloxan, glybenclamide, N-hexane, sodium acetate and potassium acetate and other chemicals of analytical grade were obtained from Sigma-Aldrich (St Louis MI, USA). Acetonitrile (HPLC grade). THF (silylation grade) were obtained from Pierce (Rodgau, F.R.G.).

Plant material. *Lupinus albus* seeds were donated by E. van Santen (Auburn University, USA). The *L. albus* seeds were dehulled and the kernel ground to a fine flour which was then defatted with hexane using Soxhlet distillation equipment. The flour was then stored within a drying chamber at 4°C until use.

Preparation of protein isolates. The albumin fraction of the total proteins was removed by stirring with double distilled and demineralised water (water:flour ratio 10:1 g seed) at 4°C for 2 h. After centrifugation at 5000 rpm, 4°C, three times, and lyophilisation, albumins were stored in a desiccator chamber at 4°C. The resulting pellets were dissolved in 10% NaCl solution (3X). Pooled supernatants were mixed with (NH₄)₂SO₄ to reach 85% saturation. After centrifugation, globulins were dissolved in 0.2% sodium Benzoate in 0.15 M Na₂HPO₄/0.15 M NaH₂PO₄ buffer, pH 6.8 and dialysed against 0.2M acetate buffer, pH 4.8 to isolate α-conglutins. The supernatant was dialysed

against water during 48 hours, and centrifuged to obtain β-conglutins, and the crude conglutin-γ in the supernatant. The latter was suspended in 0.5 M Na₂HPO₄/NaH₂PO₄ buffer, pH 7 and (NH₄)₂SO₄ was added to 50% saturation to obtain conglutin-γ in the precipitate. Purified conglutin-γ was precipitated with 80% (NH₄)₂SO₄ and similarly processed like globulins. Each precipitate was freeze-dried at -50°C and 13 x 10⁻³ mbar, weighed and stored for further proteins studies. Homogeneity of the purified conglutin-γ was verified by SDS-PAGE, RPHPLC and MALDITOF-MS (Szepesi, G., 1992; Barth H.G. *et al.* 1994; Lin, D. *et al.* 2003).

Protein measurement. A standard curve was generated using a known concentration of the protein bovine serum albumin (BSA). After binding Coomassie brilliant blue G-250 to each protein sample in an acidic solution their O.D. was recorded at 595 nm.

Alloxan treatment. After 18 hrs of food deprivation rats were i.p. injected with a single 120 mg/kg dose of monohydrated alloxan, and one hour later, received an oral over load of glucose. On the third day following the alloxan administration, and also after 18 hrs of food deprivation, conglutin-γ treatment was started and glycaemia, triglycerides and cholesterol were measured.

Glucose Overload Tolerance Test (GOTT). The hypoglycaemic effect of globulins and conglutin-γ was measured in female Wistar rats following an overload glucose tolerance test (GOTT). After 18 hours of food deprivation and 30 minutes before the oral glucose overload (OGO) through a metallic canulae, rats orally received either 1000 mg/kg globulins solubilised in carboxymethylcellulose (CMC) or 100 mg/kg of conglutin-γ or 10 mg/kg of glybenclamide, respectively. On time zero, each rat orally received 2 g/kg of glucose solution through a metallic cannulae, and were immediately anaesthetised through an IM injection of Trileptam-Zolazepam in order to collect 3 mL of blood from the retroocular plexus into 7.5 mmol/L EDTA containing tubes on times 30, 60, 90 and 120 min. after the OGO. All samples were centrifuged at 2000 rpm, 4°C during 10 min, and the supernatants were withdrawn by triplicate into Eppendorf tubes for glucose, triglycerides and cholesterol measurement.

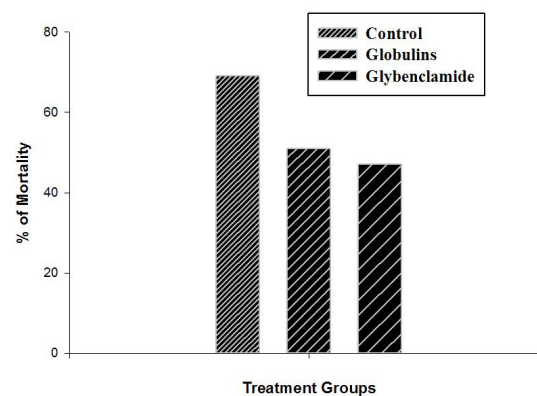


Fig. 3. Mortality of alloxanised Wister rats.

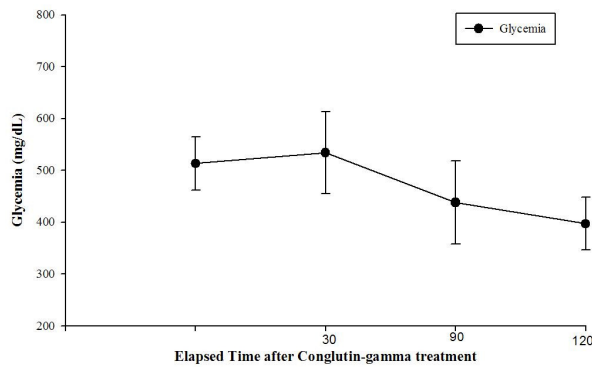


Fig. 4. Diabetic rats and conglutin gamma.

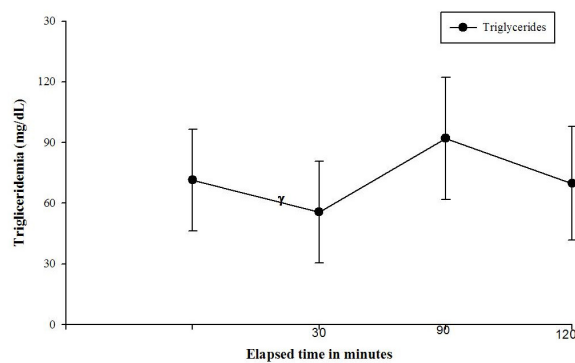


Fig. 5. Diabetic rats and conglutin gamma.

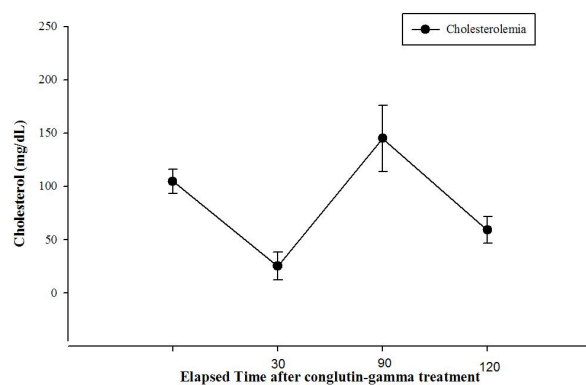


Fig. 6. Diabetic rates and conglutin-gamma.

Glucose, cholesterol and triglycerides. Glucose oxidase and peroxidase, cholesterol esterase and peroxidase and glicerolkinase and gliceraldehyde-3-phosphate-oxidase kits were used to measure glycaemia, triglycerides and cholesterol, respectively (Biosystems).

Statistical analyses. Differences in *L. albus* globulins, conglutin- γ and glybenclamide on glycaemia, cholesterolemia and triglyceridemia were carried out with Student's *t* test. Values were always expressed as means \pm SEM; *P*-values < 0.05 were considered significative.

RESULTS AND DISCUSSION

Evaluation of the glycaemia and cholesterol-lowering effect of *L. albus* globulins and conglutin- γ was carried out due to the availability of adequate amounts of these purified protein fractions for animal studies; similarly to those of isoflavone-depleted soy products as well as those of soy foods with highly variable isoflavone contents, claimed nowadays to reduce cholesterolemia. Although the mechanism of how *L. albus* globulins and conglutin- γ were able to induce hypoglycaemia during an OGTT in normal Wistar rats is not clearly understood the action appears almost like glybenclamide (Figs 1 and 2). Also, despite we neither know how the alloxanised rats mortality rate was reduced in rats receiving globulins and glybenclamide treatment (Fig. 3), at least we are authorised to think that glybenclamide mortality reduction might have occurred through its insulin secretagogue known effect (Luzi, L. and Pozza, G. 1997). Since glybenclamide is a commonly prescribed sulfonylurea (SU) against diabetes mellitus type 2, it was used as a positive control. After binding to the sulfonylurea receptor, glybenclamide inhibits ATP-sensitive K⁺ channels, leading to depolarisation of the β -cells releasing insulin into blood stream. On these grounds, *Lupin albus* conglutin- γ and the conglutin- γ containing globulins might have exerted their hypoglycemic effect through insulin release stimulation instead of inhibiting gluconeogenesis as some authors suggest when they use Metformin as a positive control. Under glybenclamide treatment, cholesterol and triglycerides levels decrease in presence of drug-released insulin in well controlled diabetes mellitus 2 suffering patients (Luzi, L. and Pozza, G. 1997). Therefore, we think that similar phenomena also occurred in alloxanised rats submitted to *Lupin albus* conglutin- γ and the conglutin- γ contained in globulins treatment (Figs 4-6). In alloxanised Wistar rats glycaemia, triglyceridemia and cholesterolemia displayed a tendency to remain at low levels at 30, 90 and 150 min. after having received an oral administration of 100 mg/kg of conglutin- γ . Obviously, more experiments in animals and the forthcoming experiments in human beings will allow us to know how useful globulins and conglutin- γ are the treatment of the metabolic syndrome, diabetes, atherosclerosis, cardiovascular risks, hormone dependent tumours treatment in comparison to isoflavone free soy products nowadays used for these purposes.

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