

Investigation of the Acute Effects of Dry Extract of *Glycine Max* on Postprandial Glycemia in Rats

Patrícia Batista Travassos¹, Vanessa Rodrigues Vilela¹, Marina Masetto Antunes¹, Roberto Barbosa Bazotte^{1*}.

¹ Universidade Estadual de Maringá, Departamento de Farmacologia e Terapêuticos, Maringá, Paraná, Brasil.

ABSTRACT

The acute effects of Glycine max (GM) on post prandial glycemia (PPG) in male Wistar rats were investigated. All substances were orally administered by gavage in overnight fasted animals. The elevation of PPG promoted by starch (1g/kg) was prevented by GM (2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg, 10.0 mg/kg, and 100.0 mg/kg). In conclusion GM showed potential antidiabetic effect.

Key Words: Glycine max, soy, diabetes, acarbose, phytotherapy, post prandial glycemia.

*Authors for correspondence: rbbazotte@uem.br

INTRODUCTION

The well-established beneficial effects on metabolism of extracts from soy have been attributed to the isoflavones¹⁻⁵. In agreement with this affirmation we previously demonstrated⁶ activation of peroxisome proliferator-activated receptors α by using a methanolic fraction from soybean seeds rich in isoflavones (daidzin, glycitin, genistin, daidzein, malonylglycitin, malonylgenistin, genistein, glycitein, and malonyldaidzin).

In addition, several studies reported antidiabetic properties of isoflavones from soy, not only in pre-clinical models^{7,8} but also in humans^{9,10}.

The mechanisms by which isoflavones from soy produce antidiabetic effects include: antioxidant and anti-inflammatory properties⁸ protection of beta cells¹¹, and stimulation of insulin release¹².

However, there are few studies supporting that this herbal preparations could show acute beneficial effect in diabetes¹³. Moreover, considering that genistein, the main isoflavone present in the soy¹⁴ inhibits alpha-glucosidase¹⁵, the possibility of an acute effect of extracts of GM on post prandial glycemia should be investigated. Furthermore, there is no dearth of reports evaluating if the inhibition of alpha-glucosidase has pharmacological significance *in vivo* conditions.

Thus, the present study was carried out to verify if the isoflavones from soy would show acute effects on postprandial glycemia.

MATERIALS AND METHODS

PLANT MATERIALS: Capsules (Soyfemme® from Aché Laboratories - Guarulhos, SP, Brazil) containing Isoflavones (40%) from dried extracts of *Glycine max (L.) Merr* (GM) were used. Immediately before the administration, the contents of the capsules were removed and dissolved in water.

EXPERIMENTAL PROCEDURES: One hundred and thirty male Wistar adult rats (*Rattus norvegicus*) weighing 250-300g were used. The rats were maintained under controlled temperature (23°C) and photoperiod (12 h light/12 h dark). All animals received free access to standard commercial laboratory diet (Nuvilab®, Curitiba, PR, Brazil). The manipulation of the animals followed the Brazilian animal protection law. All substances were orally administered through a gastric tube (gavage) in overnight (15-h) fasted rats and blood was collected by decapitation for glucose evaluation¹⁶.

Acute effect of soluble starch (1000 mg/kg) on glycemia. Glycemia was measured at 0 (baseline values), 5, 10, 15, 20, 30 and 60 min after the administration of soluble starch. We chose the dose of soluble starch on the basis of a previous study¹⁷. The results are presented in the Figure 1.

Acute effect of acarbose (0.1 mg/kg, 1.0 mg/kg or 10.0 mg/kg) on the elevation of glycemia after the administration of soluble starch (1000 mg/kg). Glycemia was measured 30 min after the simultaneous administration of soluble starch plus acarbose (0.1 mg/kg, 1.0 mg/kg or 10.0 mg/kg). The control group received only vehicle. Furthermore, an additional control group which received simultaneous administration of vehicle plus soluble starch was included. The results are showed in the Figure 2.

Acute effect of GM (2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg or 10.0 mg/kg) on the elevation of glycemia after the administration of soluble starch (1000 mg/kg). Glycemia was measured 30 min after the simultaneous administration of soluble starch plus GM (2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg or 10.0 mg/kg). The control group received only vehicle. The positive control group received simultaneous administration of soluble starch plus acarbose (10 mg/kg). Furthermore, an additional control group which received simultaneous administration of vehicle plus soluble starch was included. The results are showed in the Figure 3A.

Acute effect of GM (0.1 mg/kg, 1.0 mg/kg or 100.0 mg/kg) on the elevation of glycemia after the administration of soluble starch (1000 mg/kg). Glycemia was measured 30 min after the simultaneous administration of soluble starch plus GM (0.1 mg/kg, 1.0 mg/kg, or 100.0 mg/kg). The control group received only vehicle. The positive control group received simultaneous administration of soluble starch plus acarbose (10 mg/kg). Furthermore, an additional control group that received simultaneous administration of vehicle plus soluble starch was included. The results are presented in the Figure 3B.

STATISTICAL ANALYSIS: The results are reported as means \pm standard error of the means (SEM). Significance of differences between the groups was evaluated by Newman-Keuls multiple comparison test. A 95% level of confidence ($P < 0.05$) was accepted for all comparisons.

RESULTS

In the first set of experiments, the acute effect of oral administration of soluble starch on glycemia was investigated. As shown in Figure 1, elevation ($P < 0.05$) of glycemia was observed from 15 min and this difference was maintained 60 min and 120 min (not showed) later. Because increased ($P < 0.05$) blood glucose was well established 30 min after the administration of soluble starch this time was used in the following experiments.

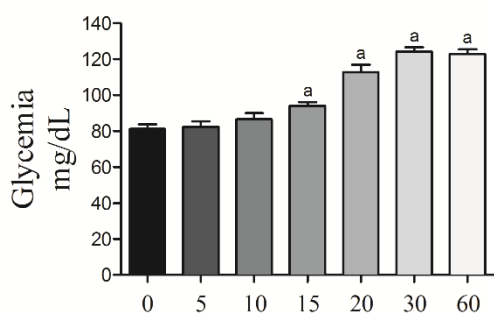


Figure 1. Effect of the acute administration (oral gavage) of soluble starch 1000 mg/kg (SS) on glycemia in 15-h fasted rats. The blood glucose concentrations were evaluated at 0, 5, 10, 15, 20, 30 and 60 min after SS administration. The results are presented as mean \pm standard error of the mean. $n=5$ for each time. Newman-Keuls multiple comparison test. ^a $p < 0.05$ for comparisons between 0 min vs. 10 min, 15 min, 20 min, 30 min and 60 min.

In the second set of experiments, the acute effect of increasing doses of acarbose on the elevation of glycemia promoted by the administration of soluble starch was evaluated. As shown in Figure 2, the dose of 1.0 mg/kg and 10 mg/kg impair ($P < 0.05$) the elevation of glycemia promoted by soluble starch. Considering that acarbose (10 mg/kg) impaired the elevation of glycemia promoted by the administration of soluble starch this dose was used in the following experiments.

In the third set of experiments, the acute effect of increasing doses of GM on the elevation of glycemia promoted by the administration of soluble starch was evaluated. As shown in Figure 3A, the doses of 2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg, and 10.0 mg/kg decrease ($P < 0.05$) the intensity of elevation of glycemia promoted by soluble starch.

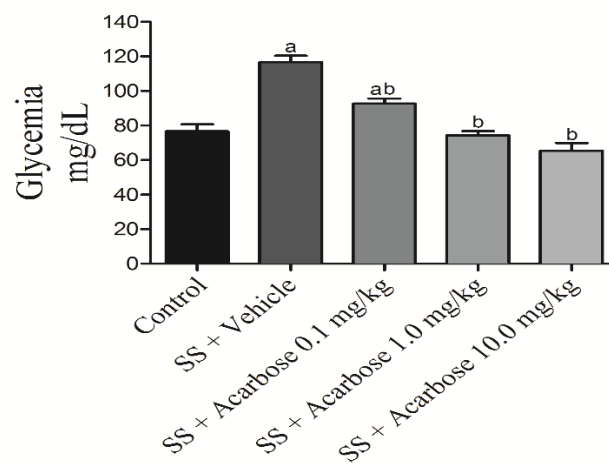


Figure 2. Effect of the acute administration (oral gavage) of soluble starch 1000 mg/kg (SS) plus acarbose on glycemia in 15-h fasted rats. The blood glucose concentrations were evaluated at 30 min after the administration of SS plus vehicle or SS plus acarbose (0.1, 1.0 and 10.0 mg/kg). The control group received oral (gavage) vehicle. The results are presented as mean \pm standard error of the mean. $n=6$ for each group. Newman-Keuls multiple comparison test. ^a $p < 0.05$ when compared with control group; ^b $p < 0.05$ when compared with SS + vehicle group.

Because acarbose prevent 100% the elevation of glycemia we also express the results as percent of effect in comparison with acarbose. Thus, the percent of reduction in the elevation of glycemia after the administration of soluble starch in the groups SS + GM 2.5 mg/kg, SS + GM 5.0 mg/kg, SS + GM 7.5 mg/kg, SS + GM 10.0 mg/kg were 21.8%, 28.8%, 25.4%, and 20%, respectively.

Since GM (2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg, and 10.0 mg/kg) decrease ($P < 0.05$) the intensity of elevation of glycemia promoted by soluble starch, the experiments described in the Figure 3A were repeated again with lower (0.1 mg/kg and 1.0 mg/kg) and higher (100.0 mg/kg) doses of GM.

As shown in Figure 3B, the dose of 100.0 mg/kg, but not the doses of 1.0 mg/kg, and 0.1 mg/kg, decrease ($P < 0.05$) the elevation of glycemia promoted by soluble starch. The percent of reduction in the elevation of glycemia after the administration of soluble starch in the groups SS + GM 0.1 mg/kg, SS + GM 1.0 mg/kg, and SS + GM 100.0 mg/kg in comparison with SS + acarbose (100%) were 0%, 0%, and 29.90%, respectively.

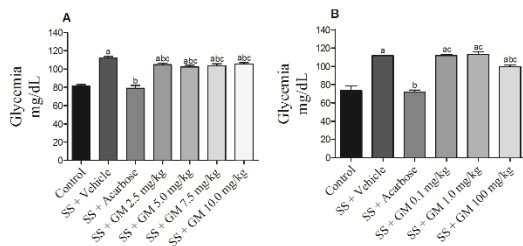


Figure 3. Effect of the acute administration (gavage) of vehicle (Control), soluble starch 1000 mg/kg (SS) plus vehicle, SS plus acarbose 10.0 mg/kg, and SS plus *Glycine max* (GM) in the doses of 2.5, 5.0, 7.5 and 10.0 mg/kg (**Fig. 3A**) or GM 0.1, 1.0 and 100 mg/kg (**Fig. 3B**) in 15-h fasted rats. The glycemia was evaluated at 30 min after the administration of SS. The results are presented as mean \pm standard error of the mean. $n=5$ for each group. Newman-Keuls multiple comparison test. ^a $p < 0.05$ when compared with control group; ^b $p < 0.05$ when compared with SS + vehicle group; ^c $p < 0.05$ when compared with SS + acarbose group.

DISCUSSION

An effective strategy for pre diabetes and type 2 diabetes treatments is the inhibition of intestinal α -glucosidase. In this context, acarbose inhibits α -glucosidase from brush border of the small intestine and slows carbohydrate digestion retarding intestinal absorption and thereby reducing postprandial hyperglycemia¹⁸. However, the undigested carbohydrates are fermented by colonic bacteria causing abdominal distention, flatulence, meteorism and diarrhea. Due to this side effect it is common the abandonment of treatment¹⁹.

Considering that in Brazil there is not another antidiabetic drug with ability to slow intestinal absorption, the possibility of using new compounds instead acarbose has been investigated.

In fact there are many *in vitro* studies showing inhibition of α -glucosidase by isoflavones from plants²⁰⁻²². However, there is absence of *in vivo* studies demonstrating antihyperglycemic properties of these compounds.

Furthermore, as we previously demonstrated²³⁻²⁵ non-diabetic rats represents a suitable pre-clinical model to investigate the impact of oral carbohydrates on post prandial glycemia.

Thus, by using this rat model we investigate if dried extracts of GM could reduce the elevation of glycemia promoted by oral ingestion of soluble starch.

To the best of our knowledge we demonstrate for the first time the antihyperglycemic properties of

GM from *in vivo* experiments, *i.e.*, the oral administration of GM (2.5 mg/kg, 5.0 mg/kg, 7.5 mg/kg, 10.0 mg/kg and 100.0 mg/kg) decrease ($P < 0.05$) the intensity of elevation of glycemia promoted by soluble starch (Fig. 3A and 3B).

Thus, in agreement with several studies that demonstrated antidiabetic properties to GM⁷⁻¹³ we can conclude that isoflavones from dried extracts of GM show acute antihyperglycemic effect.

The difference from our results and several studies showing antidiabetic properties for GM is the fact that those evaluations were done after chronic treatment and our evaluation involve acute effects of GM on post prandial glycemia elevation after an oral overload of soluble starch.

Thus we concluded that the antidiabetic potential of GM also include acute effects preventing the elevation of post prandial glycemia.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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