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# Astragalin augments basal calcium influx and insulin secretion in rat pancreatic islets

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ABSTRACT

Astragalin is a flavonol glycoside with several biological activities, including antidiabetic properties. The objective of this study was to investigate the effects of astragalin on glycaemia and insulin secretion, in vivo, and on calcium influx and insulin secretion in isolated rat pancreatic islets, ex vivo. Astragalin (1 and 10 mg / kg) was administered by oral gavage to fasted Wistar rats and serum glucose and plasma insulin were measured. Isolated pancreatic islets were used to measure basal insulin secretion and calcium influx. Astragalin (10 mg/ kg) decreased glycaemia and increased insulin secretion significantly at 15–180 min, respectively, in the glucose tolerance test. In isolated pancreatic cells, astragalin (100 μM) stimulated calcium influx through a mechanism involving ATP-dependent potassium channels, L-type voltage-dependent calcium channels, the sarcoendoplasmic reticulum calcium transport ATPase (SERCA), PKC and PKA. These findings highlight the dietary coadjuvant, astragalin, as a potential insulin secretagogue that may contribute to glucose homeostasis.

## 1. Introduction

Diabetes is a complex disease, characterized by chronic hyperglycemia, caused by inadequate insulin secretion and/or decreased sensitivity in the insulin-sensitive cells [1,2]. Diabetes mellitus type 2 is the most common type of diabetes and is diagnosed in 90–95% of patients; it is estimated that the prevalence of this type of diabetes will continue to increase. Medicinal plants formed the basis of traditional medicine systems and approximately 80% of the world's population from developing countries use for the treatment of different diseases including diabetes [3], although the efficacy and safety of phytoconstituents and the molecular mechanisms of the activities of medicinal plants are unknown [4,5].

Flavonoids are a class of secondary metabolites and bioactive compounds that are widespread in the plant kingdom. These compounds are important components of the human diet and several studies have demonstrated a broad range of beneficial effects of flavonoids, such as the balancing of glucose metabolism [6–8].Astragalin (kaempferol-3-O-ß-glucoside) is a flavonoid that has been described as having antibacterial, antifungal, anti-inflammatory, antioxidant, neuro and cardioprotective, antiulcer, antitumor and antidiabetic activities, among others [9]. With regard to glucose homeostasis, astragalin is reported to be one of the main compounds of Gynura procumbens leaf extract, which presents antidiabetic effects via a mechanism similar to that exerted by metformin [10,11]. Astragalin has also been reported to be one of the components responsible for the antidiabetic activity of the extracts of roots of Astragalus membranaceus [12] and of Morus Alba L. [13]. Furthermore, astragalin has been shown to decrease diabetic retinopathy [14] and inhibit the activity of intestinal alpha glucosidase and alpha amylase [15,16]. As such, the aim of the present study was to investigate the acute in vivo effects of astragalin on glycaemia and insulin secretion. Additionally, role for calcium influx and insulin secretion in the mechanism of action of astragalin in isolated rat pancreatic islets was studied by proof-of-concept studies for scientific validation.

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## 2. Materials and methods

### 2.1. Chemicals

Kaempferol-3-O-β-glucopyranoside (astragalin), 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis, (acetoxymethyl ester) (BAPTA-AM), (bisindoylmaleimidine IX, 2-{1- [3-(amidinothio)propyl]-1H-indol-3-yl}-3-(1-methylindol-3-yl), maleimidemethanesulfonate salt) RO-318220, N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89), nifedipine, diazoxide, glibenclamide, thapsigargin, dantrolene and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). [$^{45}Ca^{2+}$] $CaCl_2$ (sp. act. 321 K Bq/mg $Ca^{2+}$) and OptiphaseHisafe III biodegradable scintillation liquid were purchased from Perkin-Elmer (Boston, MA, USA). All other chemicals (salts for the buffer) were of analytical grade and purchased from Diprolab (Florianópolis, SC, Brazil). The enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of human insulin (product code 2425-300) was purchased from Monobind Inc. (Lake Forest, CA 92630, USA).

### 2.2. Animals

Male Wistar rats (50–55 days old; 180–210 g), were kept in cages with controlled temperature (approximately 21 ± 2 °C) and light / dark cycles of 12 h (lights on between 6 and 18 h). The animals received food (Nuvital, Curitiba, PR, Brazil) and water ad libitum. All the animals were monitored and maintained in accordance with the ethical recommendations of the Brazilian Veterinary Medicine Council and the Brazilian College of Animal Experimentation. All procedures were performed in accordance with the Ethics Committee on Animal Use CEUA-UFSC (PP2119/17 Protocol). Euthanasia was carried out using an isoflurane protocol and subsequent guillotine decapitation.

### 2.3. Oral glucose tolerance test (OGTT)

Fasted rats were divided into 3 groups of six animals for each treatment. Group I: hyperglycemic control group that received glucose (4 g/kg; 8.9 M); Group II: hyperglycemic + astragalin (1 mg/kg); and Group III: hyperglycemic + astragalin (10 mg/kg). All treatments were administered orally by gavage. Serum glucose and plasma insulin levels were measured before the administration of treatment (zero time). Glucose overload was administered 30 min after the treatment serum glucose and plasma insulin were then determined at 15, 30, 60 and 180 min.

### 2.4. Plasma insulin measurements

Insulin was measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions with some modifications, since a kit to measure human insulin was used. All insulin concentrations were estimated by means of colorimetric measurements at 450 nm with an ELISA plate reader (OrganonTeknika, Roseland, NJ, USA) by interpolation from a standard curve. Samples were analyzed in quadruplicate and results were expressed as μIU/mL of plasma insulin.

### 2.5. Rat pancreatic islet isolation

Pancreatic islet isolation was carried out as described by Frederico et al. (2012). The rat pancreas was visualized by way of a central abdominal incision. The bile duct was clamped at the tip of the duodenum and cannulated at a point sufficiently proximal to the liver. Krebs Ringer-bicarbonate (KRb) supplemented with HEPES (8 mM) and glucose (3 mM) (KRb-HEPES) medium was introduced slowly into the bile duct by syringe until the pancreas was clearly distended. The pancreas was then gently removed and kept in a Petri dish with KRb-HEPES medium. The pancreatic tissue was cut into small pieces (2 x 2 mm) and incubated in plastic tubes in KRb-HEPES medium supplemented with collagenase (3 mg/mL). After incubation, the mixture was transferred to a conical tube (110 x 15 mm), resuspended in 10 mL with collagenase-free medium, and centrifuged at room temperature for 3 min at 4,500*g* in an Excelsa Baby centrifuge (model 206), FANEM, São Paulo, SP, Brazil. The supernatant was discarded, and the sediment resuspended in fresh KRb-HEPES medium. This washing procedure was repeated fivefold and, during the last two washes, the islets were allowed to settle without centrifugation. Aliquots (100 μL) of the final sediment with the isolated islets were transferred to Eppendorf tubes with the incubation medium, KRb-HEPES [17,18].

### 2.6. Basal insulin measurement

Studies of in vitro insulin secretion were carried out with pancreatic islets isolated from euglycemic rats. The islets (0.5 mL aliquot) were preincubated (60 min) and incubated (treatment time) at 37 °C in KRb, pH 7.4. The incubation was carried out in the absence or presence 100 μM of astragalin for 10 min. At the end of the incubation, an aliquot of 50 μL was used for insulin quantification by ELISA [19,20].The total amounts of protein in the incubation medium and the islets were determined by Lowry's Method [21]. Results are expressed as μIU of insulin/μg of protein.

### 2.7. Calcium influx

The isolated islets were pre-incubated in a Dubnoff metabolic incubator for 60 min to equilibrate 0.1 μCi/mL $^{45}Ca^{2+}$ contained in KRb-HEPES buffer at 37 °C, pH 7.4 and gassed with $O_2$:$CO_2$ (95:5; v/v). The islets were then incubated for 10 min in KRb-HEPES without (control) or with astragalin. In some experiments, channel blockers or kinase inhibitors were added during the last 15 min before the treatment and maintained during the entire incubation period (see figure legends). The following drugs were used: diazoxide (250 μM), glibenclamide (20 μM), nifedipine (1 μM), BAPTA-AM (50 μM), RO 31-8240 (20 μM) [17], H89 (10μM), thapsigargin (1 μM) or dantrolene (50 μM) [22]. One mL of cold buffer with lanthanum chloride (10 mM) at 2 °C was added to the samples to inhibit calcium flux. The tubes were centrifuged for 1 min at 1500*g*. The supernatant was preserved and the islets were washed twice in cold lanthanum chloride solution. The presence of $La^{3+}$ during the washing stage was found to be essential to prevent release of the intracellular $^{45}Ca^{2+}$ [23]. After $La^{3+}$ tissue washing, islets were homogenized with 300 μL of 0.5 M NaOH solution and boiled at 100 °C for 5 min. Aliquots of 50 μL were taken from each sample for measurement of radioactivity in scintillation liquid using a LKB rack beta liquid scintillation spectrometer (model LS 6500; Multi- Purpose Scintillation Counter-Beckman Coulter, Boston, USA). Aliquots of 5 μL were used for protein quantification by the Lowry method [21]. After 60 min of incubation with $^{45}Ca^{2+}$ the equilibrium of calcium in cytosolic, intracellular stores and extracellular calcium occur. It means that the amount of calcium in the medium is equivalent between the intra-extracellular medium. After 60 min with the addition of exogenous substances (drugs, solutes, inhibitors), the incubation medium can be modified, an experimental strategy to study the mechanism of influx of calcium in isolated cells, tissue fragments and / or cell culture [23], so the studies on calcium influx conducted below were made taking into account the above.

### 2.8. Statistical analysis

Data are expressed as means ± S.E.M. We use one way analysis of variance (ANOVA) followed by Bonferroni post hoc test when comparing more than two groups (Figs. 1B, 5–7 and 8 ) or when we compared only 2 groups we used Student's *t*-test (Figs. 2–4 and 9). Differences were considered significant at $p \leq 0.05$. The homogeneity of the data was determined by the software GraphPad prism 6® by the

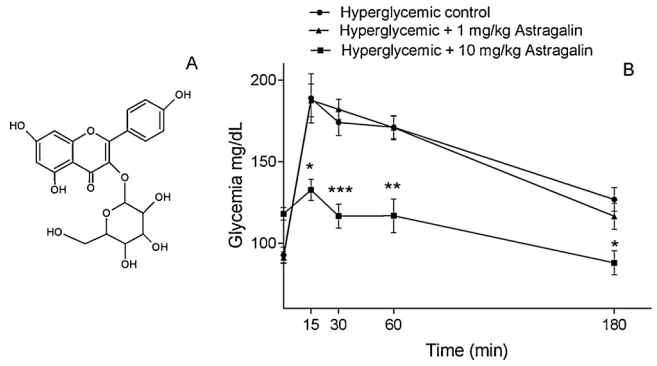


**Fig. 1.** Structure of astragalin (A), Effect of astragalin (1 mg/kg and 10 mg/kg) on the oral glucose tolerance curve in rats (B). Values are expressed as means ± S.E.M., n = 6. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001, when compared to the respective value in the hyperglycemic control group and using one-way ANOVA followed by Bonferroni post hoc test for each of the times in which the samples were taken.

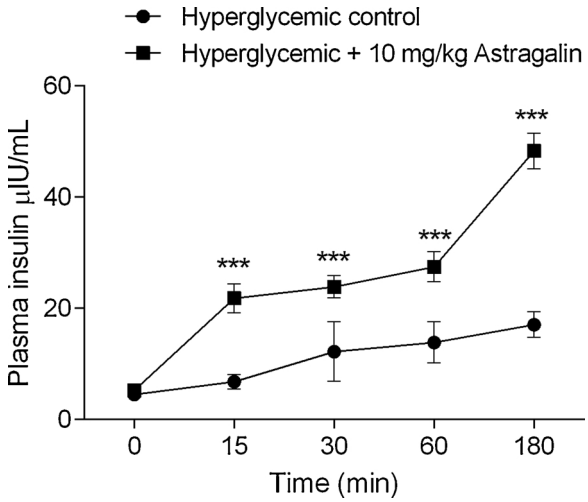


**Fig. 2.** Effect of astragalin on plasma insulin secretion in hyperglycemic rats. Values are expressed as means ± S.E.M.; n = 4. **p ≤ 0.01, *** p < 0.001 compared to the respective value of the hyperglycemic control group and using Student's *t*-test for each of the times in which the samples were taken.

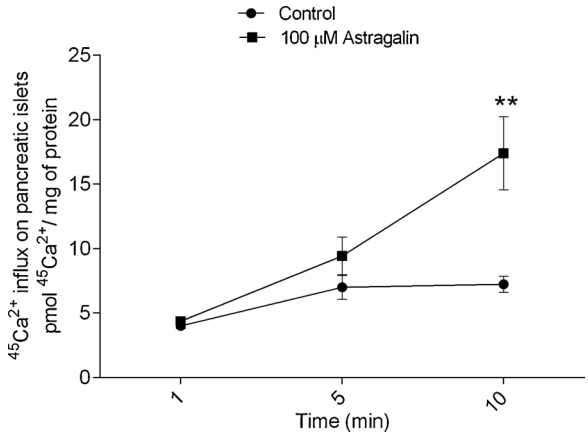


**Fig. 3.** Time-course of the effect of astragalin on $^{45}Ca^{2+}$ influx on isolated rat pancreatic islets. The pancreatic islets were pre-incubated for 60 min with 0.1 µCi/mL of $^{45}Ca^{2+}$. After they were incubated with/without 100 µM of astragalin for 1, 5 and 10 min. Values are means ± S.E.M.; n = 5. **p ≤ 0.01 when compared to the control group and using Student's *t*-test for each of the times in which the samples were taken.

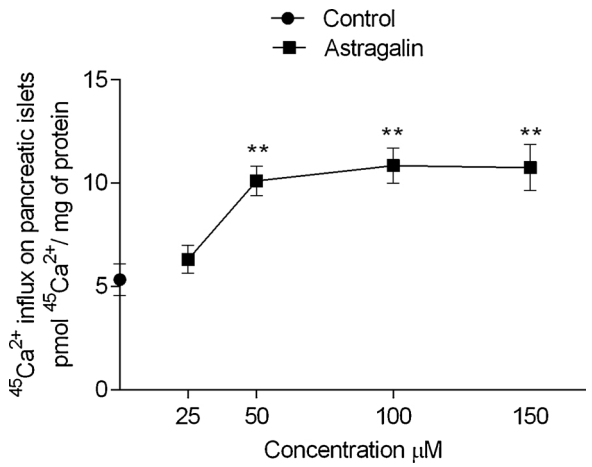


**Fig. 4.** Dose-response curve of the effect of astragalin on $^{45}Ca^{2+}$ influx on pancreatic islets of 50 day-old rats. Preincubation = 60 min; Incubation = 10 min. Values are expressed as means ± S.E.M.; n = 5. Significant for ** p ≤ 0.01, compared to the control group and using Student's *t*-test for each of the concentration.

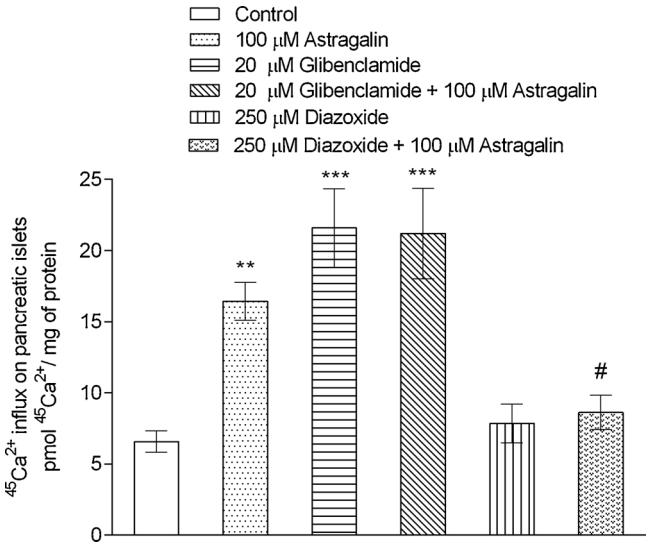


**Fig. 5.** Involvement of potassium channels in the stimulatory effect of astragalin on $^{45}Ca^{2+}$ influx on pancreatic islets. Islets were incubated with glibenclamide (20 µM) or diazoxide (250 µM) during the last 15 min of the pre-incubation and during incubation. Preincubation = 60 min; Incubation = 10 min. Values are expressed as the means ± S.E.M.; n = 5. ** p < 0.01; *** p ≤ 0.001; compared to the control group; # p < 0.05; compared to the astragalin group and using one way analysis of variance (ANOVA) followed by Bonferroni post hoc test.

Bartlett*t*-test for ANOVA or by a test of equality of variance for Student's *t* test

## 3. Results

### 3.1. Oral glucose tolerance test and plasma insulin measurements

Astragalin is a flavonol glycoside of kaempferol, whose chemical structure is shown in Fig. 1A. As show in Fig. 1B, serum glucose was significantly reduced after oral treatment with 10 mg/ kg astragalin in rats during the period studied. Percentile, this dose of astragalin decreases glycaemia in 30%, 33%, 32% and 31% compared to control at 15, 30, 60 and 180 min.

In this same experiment, plasma insulin was determined in fasted

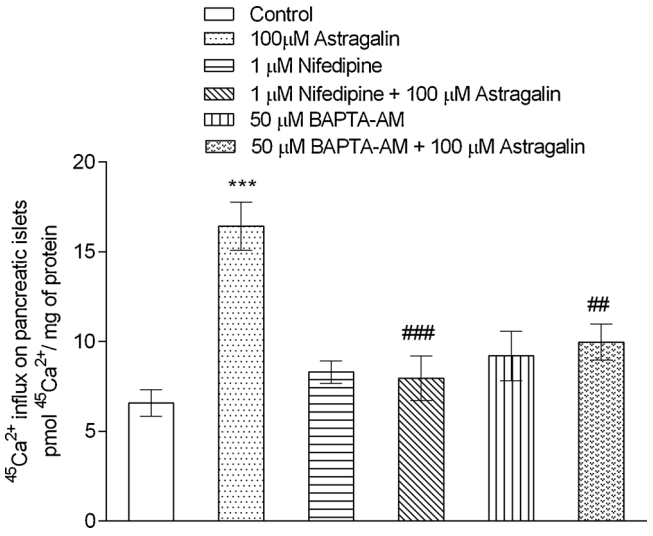


**Fig. 6.** Involvement of voltage-dependent calcium channels and intracellular calcium stocks in the effect of astragalin on $^{45}Ca^{2+}$ influx on pancreatic islets. Nifedipine (1 μM) and BAPTA-AM (50 μM) were present for the last 15 min of preincubation and during incubation. Preincubation = 60 min; Incubation = 10 min. Values are expressed as the mean ± S.E.M.; n = 5. *** $p \leq 0.001$, when compared to the control group;## $p \leq 0.01$ and ### $p \leq 0.001$, when compared to the astragalin group and using one way analysis of variance (ANOVA) followed by Bonferroni post hoc test.

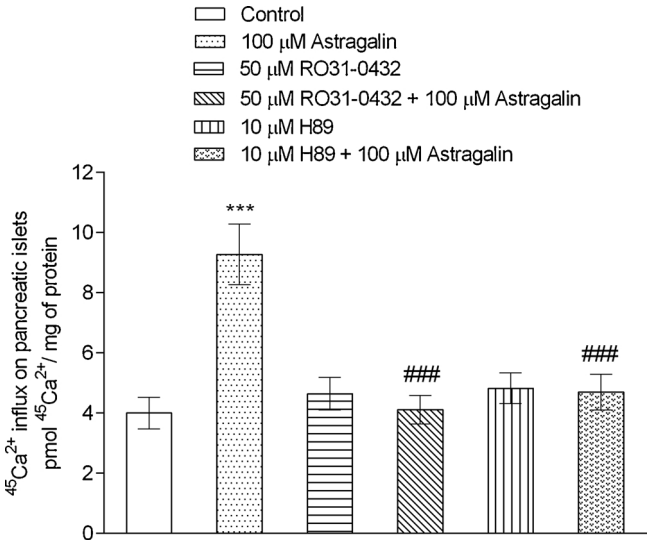


**Fig. 7.** Influence of protein kinases (PKC and PKA) on the stimulatory effect of astragalin on $^{45}Ca^{2+}$ influx on pancreatic islets. RO310432 (50 μM) and H89 (10 μM) were present during the last 15 min of preincubation and during incubation. Preincubation = 60 min; Incubation = 10 min. Values are expressed as mean ± S.E.M.; n = 5. Significant for *** $p \leq 0.001$; compared to the control group. Significant for ### $p \leq 0.001$; compared to the astragalin group and using one way analysis of variance (ANOVA) followed by Bonferroni post hoc test.

rats after the oral glucose loading (4 g/kg) at 15, 30 and 60 min after glucose loading, using the OGTT, as shown in Fig. 2. A significant and potent effect of astragalin on insulin secretion was detected over the period studied, with increases of approximately 220%, 95%, 98% and 183% observed related to values observed for the respective time points in the hyperglycemic control group.

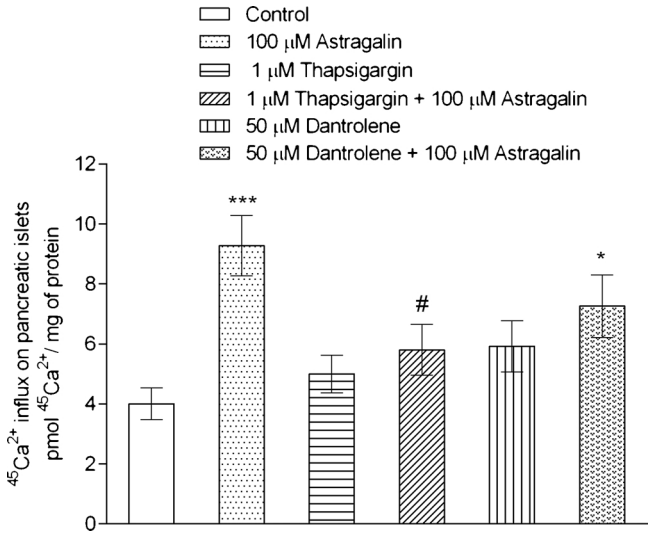


**Fig. 8.** Involvement of intracellular calcium mobilization in the stimulatory effect astragalin on $^{45}Ca^{2+}$ influx on pancreatic islets. Thapsigargin (1 μM) and dantrolene (50 μM) were present during the last 15 min of preincubation and during incubation. Preincubation = 60 min; Incubation = 10 min. Values are expressed as mean ± S.E.M., n = 5. *$p \leq 0.05$ and ***$p \leq 0.001$, when compared to the control group; #$p \leq 0.05$, when compared to the astragalin group.

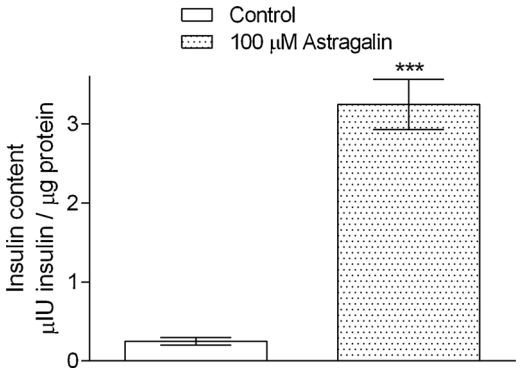


**Fig. 9.** Effect of astragalin on basal insulin secretion in rat pancreatic islets. Preincubation Time = 60 min; incubation time = 10 min. Values are expressed as mean ± S.E.M., n = 5. *** $p \leq 0.001$, when compared to the control group and using Student's *t*-test for each of the concentration.

### *3.2. Time-course of the effect of astragalin on calcium influx on pancreatic islets*

The effect of 100 μM astragalin on calcium influx on isolated rat pancreatic islets was determined at different times (1, 5 and 10 min). When compared with the control group, astragalin significantly increased the calcium influx (1.4 fold) at 10 min in isolated pancreatic islets (Fig. 3).

### *3.3. Dose-response curve of the effect of astragalin on calcium influx on pancreatic islets*

The effects of different concentrations of astragalin on calcium influx were studied. As shown in Fig. 4, 25 μM astragalin did not significantly affect calcium influx; however significant increases in calcium influx were seen with 76.5%, and 89% and 88% for the concentrations of 50 μM,100 μM and 150 μM astragalin, respectively. For subsequent experiments with astragalin, a concentration of 100 μM was used with 10 min of incubation.

### 3.4. Involvement of $K^+_{ATP}$ channels in the stimulatory effect of astragalin on calcium influx on pancreatic islets

To investigate whether the astragalin-induced increase in calcium influx occurs via $K^+_{ATP}$ channels, glibenclamide (20 μM) and diazoxide (250 μM) were used. As observed in Fig. 5, in the presence of glibenclamide, the $^{45}Ca^{2+}$influx increased 2.3-fold, compared to the control, in the presence of astragalin. However, when astragalin was used together with glibenclamide, no additional stimulatory effect was observed. On the other hand, as expected, diazoxide significantly decreased the influx of $^{45}Ca^{2+}$ and, in the presence of this compound, the stimulatory effect of astragalin decreased by 47.5%.

### 3.5. Involvement of the voltage-dependent $Ca^{2+}$ channels in the stimulatory effect of astragalin on calcium influx on pancreatic islets

The role of L-type voltage-dependent calcium channels (L-VDCC) and intracellular calcium in the stimulatory effect of astragalin on$^{45}Ca^{2+}$ influx was studied. As expected (Fig. 6), in the presence of nifedipine and BAPTA-AM alone, calcium influx did not increase and, in the presence of these two inhibitors, the stimulatory effect of astragalin decreased by 52% and 39%, respectively.

### 3.6. Involvement of PKC and PKA in the stimulatory effect of astragalin on calcium influx on pancreatic islets

The islets were incubated with RO31-0432 (50 μM), or in the presence of H89 (10 μM), to study the role of protein kinases in the stimulatory effect of astragalin on the influx of calcium in isolated pancreatic cells. When astragalin was incubated in the presence of these inhibitors, significant decreases of 56% and 43%, respectively, were observed in the stimulatory effect of astragalin on $^{45}Ca^{2+}$ influx (Fig. 7).

### 3.7. Involvement of calcium from stores on the stimulatory effect of astragalin on calcium influx on pancreatic islets

The effect of astragalin in the mobilization of intracellular calcium from stores was studied. The islets were incubated in the presence of thapsigargin (1 μM) and dantrolene (50 mM). As seen in Fig. 8 only in the presence of thapsigargin the astragalin stimulatory effect was significantly reduced by 37%.

### 3.8. Effect of astragalin on basal insulin secretion

Another glucose homeostasis parameter was investigated after ex vivo treatment of isolated pancreatic islets with astragalin. Fig. 9 shows the effect of astragalin on basal insulin secretion from the islets. The stimulatory effect of 100 μM astragalin on insulin release was approximately 12-fold greater than that observed in the control group, after10 min of incubation.

## 4. Discussion

In the present study, we demonstrate for the first that astragalin causes hypoglycemic and insulin secretagogue activities. Previous studies have shown that some isolated flavonoids such as kaempferitin, kaempferol-3-neohepheridoside [24], isovitexin and swertisin [25] showed hypoglycemic effects. Additionally, the mechanism of action of some flavonoids on insulin secretion have been reported; these include rutin [22], quercetin and kaempferol (astragalin aglycone) [26].

The abrupt increase in postprandial glucose promotes insulin secretion, which is characterized by a biphasic pattern [27]. Our results clearly demonstrated that astragalin potentiates the first (between 4–10 min) and second (gradual maintenance of increase and extended durability) phase of the insulin secretion.

The calcium signals regulate both the first and second phases of insulin secretion, and calcium acts as a second messenger in insulin secretion in pancreatic β-cells. Astragalin, like other flavonoids such as quercetin and rutin, releases insulin and increases calcium influx from rat isolated pancreatic islets [22]. It should be noted that the concentrations of astragalin used for these tests had already been previously reported in other in vitro tests by other authors, demonstrating the range of concentrations of its biological activity [28–30].

Glibenclamide, similarly to other sulfonylureas, is a $K^+_{ATP}$ channel blocker. This compound was used to block these channels, causing depolarization, which is essential for insulin secretion [31,32,33]. Diazoxide exerts the opposite effect, binding with the SUR1 domain of the $K^+_{ATP}$ channels, and allowing their activation, preventing depolarization of the membrane [20,32]. Although, the presence of glibenclamide and astragalin did not change the stimulatory effect of the compound on calcium influx, we may suggest that the effect of astragalin on calcium influx in pancreatic islets is dependent upon plasma membrane $K^+_{ATP}$ channels. This suggestion derives from the fact that the presence of astragalin exerted a similar effect on potassium channels to that of glibenclamide and other flavonoids, such as myricetin [34], kaempferol [35] and quercetin [36],which have been reported to increase depolarization and insulin secretion. Depolarization mediated by $K^+_{ATP}$ channels occurs during the first phase of insulin secretion and is mediated by easily releasable vesicles, explaining the effect of astragalin during the first phase of insulin release from pancreatic beta-cells.

Glucose metabolism in pancreatic β-cells activates several intracellular pathways, including intracellular $Ca^{2+}$ signaling, which target several proteins, such as L-VDCC [37]. Glucose metabolism leads to the closure of $K^+_{ATP}$ channels, plasma membrane depolarization and, subsequently, $Ca^{2+}$ influx through L-VDCC, followed by exocytosis of insulin granules. Dysfunctional β-cell L- VDCC have been observed in diabetic patients and also in diabetic animal models [38]. From our studies, in the presence of nifedipine (L-VDCC blocker), the stimulatory effect of astragalin on calcium influx was completely blocked. Some studies have demonstrated that other flavonoids, such as rutin [22], as quercetin [39] and as kaempferol [40],exhibit similar intracellular signaling pathways.

The abrupt increase in intracellular calcium, both from the extracellular milieu and from internal stores of the endoplasmic reticulum (ER) [41],is determinant for insulin secretion by beta-cells. As such, BAPTA-AM was used as an intracellular calcium chelator. Thapsigargin is a non-competitive inhibitor of SERCA, an ATPase dependent calcium pump located at the ER membrane and responsible for calcium reuptake into the ER when the concentration is low within the ER. The inhibition of this pump leads to decreased calcium levels in the ER and increased cytosolic calcium, maintaining the uptake of basal extracellular calcium [42]. A role for RyR in the effects of astragalin was also studied; RyR is activated by small elevations in intracellular calcium concentration ($[Ca^{2+}]_i$), causing the effect known as calcium-mediated calcium release [43,44]. This effect amplifies the calcium signal produced by other mechanisms, such as the opening of calcium channels present at the plasma membrane or through the inhibition of the SERCA pump [43]. To study the RyR, dantrolene, a RyR blocker, was used.

Since the stimulatory effect of astragalin on calcium influx was inhibited in presence of both BAPTA and thapsigargin, these data indicate that astragalin may increase the release of ($[Ca^{2+}]_i$), as well as stimulate the entry of extracellular calcium through the L-VDCC. The increased calcium influx may also stimulate the release of calcium from the ER and consequently increase insulin secretion in the pancreatic β-cells. Several flavonoids have been reported to inhibit the SERCA pump, including myricetin [45], quercetin, galangin, fisetin and kaempferol, among others [46].Knowing that SERCA pump inhibition increases the magnitude of depolarization-triggered $Ca^{2+}$ entry and subsequent insulin exocytosis in rat pancreatic β-cells [47], a possible mechanism by which astragalin increases intracellular calcium may be via SERCA inhibition.

The activation of PKC and PKA, involved in several pathways of signal transduction, can lead to a slight increase on $Ca^{2+}$ influx [48]. Therefore, the involvement of these protein kinases in the stimulatory effect of astragalin on calcium influx was studied. Two inhibitors were used;RO31-0432, an inhibitor of PKC, activated by an increase in diacylglycerol (DAG) or calcium ions [49] and H89, a PKA AMP-cyclic-dependent protein inhibitor [50].After the islets were incubated with these inhibitors, the stimulatory effect of astragalin on calcium influx was inhibited, indicating that such stimulation is mediated through the action of these protein kinases. Previous studies show that PKC activation modulates L-VDCC and insulin secretion in β-cells [51–53]. Other flavonoids that activate PKC and PKA also influence glucose homeostasis. Quercetin has been shown to induce ERK1/2 phosphorylation in pancreatic β-cells, potentiating insulin secretion [54], and stimulates glucose uptake through the AMP-activated protein kinase pathway in muscle [55]. Additionally, kaempferitrin is able to activate PKC, promoting glucose uptake in skeletal muscle [56]. Also, kaempferol can activate hepatic akt, suppressing hepatic gluconeogenesis and enhancing hepatic insulin sensitivity [57], while rutin has been shown to increase skeletal muscle glucose uptake, by activating PKA, and stimulates calcium influx into pancreatic beta islets by activating PKC [22].

Finally, our study demonstrates that astragalin increases insulin secretion in a mechanism mediated by different intracellular targets, leading to a decrease in glycaemia in vivo. Although several flavonoids have been shown to act as insulin secretagogues [58], few studies have described their intracellular pathways.

## 5. Conclusion

In conclusion, astragalin performs a hypoglycemic effect that is mediated by insulin secretion in hyperglycemic rats. In pancreatic β-cells isolated from rats, astragalin stimulates calcium influx through $K^{+}_{ATP}$ channels, L-VDCC, mobilization of calcium from intracellular stores and activation of the PKC and PKA protein kinases, which may eventually lead to insulin secretion. To our knowledge, this is the first time that the mechanism of action of astragalin on insulin secretion has been reported. Our data suggests that the dietary co-adjuvant, astragalin, exhibits an insulin secretagogue potential that may contribute to glucose homeostasis.

## Conflict of interest statement

The authors declare that they have no conflicts of interest.

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