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Original article

Stimulatory effect of apigenin-6-C-β-L-fucopyranoside on insulin secretion and glycogen synthesis

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ABSTRACT

In vivo and in vitro treatments were carried out to investigate the effects of apigenin-6-C-β-L-fucopyranoside (**1**), isolated from *Averrhoa carambola* L. (Oxalidaceae), on serum glucose and insulin levels in hyperglycemic rats as well as its effect on glycogen synthesis in normal rat soleus muscle. Apigenin-6-C-β-L-fucopyranoside showed an acute effect on blood glucose lowering in hyperglycemic rats and stimulated glucose-induced insulin secretion. A stimulatory effect of **1** on glycogen synthesis was observed when muscles were incubated with this flavonoid and also its effect was completely nullified by pre-treatment with insulin signal transduction inhibitors. Taking this into account, the MAPK–PP1 and PI3K–GSK3 pathways are involved in the apigenin-6-C-β-L-fucopyranoside-induced increase in glycogen synthesis in muscle. This study provides evidence for dual effects of apigenin-6-C-β-L-fucopyranoside as an anti-hyperglycemic (insulin secretion) as well as an insulinomimetic (glycogen synthesis) agent.

1. Introduction

Diabetes mellitus is a complex metabolic disorder in the endocrine system characterized by the abnormalities in insulin secretion and/or insulin action that lead to progressive failure of glucose tolerance and cause chronic hyperglycemia [1]. In recent years there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Medicinal plants used in folk medicine for diabetes treatment may provide an alternative to the classical drugs used (insulin and oral hypoglycemic agents), with few side effects and low cost for the maintenance of treatment [2,3].

Flavonoids are naturally occurring phenolic compounds that are widely distributed in plants. They have a broad range of biological activities and numerous studies have been carried out on their potential role in the treatment of diabetes and other diseases [4–6]. They can exert effects on glucose transport and metabolism in peripheral tissues and ameliorate diabetic status [7–11]. We have previously reported the acute hypoglycemic effect of flavonoid and flavonoid-enriched fractions in diabetic rats, as well as the chalcone analogues in hyperglycemic rats [8,9,12–14]. Recently, it was demonstrated that crude extracts, flavonoid-enriched fractions from leaves of different species of *Averrhoa* are able to diminish serum glucose levels in hyperglycemic rats as efficiently as insulin [15–17].

Insulin is the most important hormone in the regulation of blood glucose concentrations. It mediates a wide spectrum of biological responses including synthesis and storage of carbohydrates, lipids and proteins, activation of specific gene transcription, and modulation of cellular growth and differentiation [18,19]. This hormone is synthesized and stored in pancreatic β-cells. Glucose is the primary stimulus for insulin secretion and when blood sugar concentrations rise, insulin is secreted into the blood stream. Initially, glucose enters β-cells through the high capacity glucose transporter type 2 (GLUT 2) and is phosphorylated by glucokinase. The generation of ATP from glycolysis increases the intracellular ATP/ADP ratio. ATP binds to ATP-dependent K^+ channels on the β-cell membranes closing these channels and depolarizing the cells. The depolarization activates voltage-sensitive calcium channels causing a calcium influx triggering insulin secretion [20,21]. Among the oral antihyperglycemic agents currently used in diabetes therapy, sulphonylureas, for example glipizide, are known to stimulate insulin secretion from β-cells by inducing ATP-dependent K^+ channels to close, which activates downstream events that lead

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to the release of insulin-containing vesicles [22]. Insulin released into the blood promotes glucose uptake by tissues such as fat and muscle. The binding of insulin to its receptor activates the intrinsic receptor tyrosine kinase, which results in autophosphorylation and recruitment of substrates, such as insulin receptor substrate (IRS) proteins. Specific tyrosine residues on the IRS proteins serve as docking sites for proteins that contain SH2 domains, such as the p85 regulatory subunit of phosphatidylinositol-3-kinase (PI3K) [18,19]. PI3K catalyzes the formation of phosphatidylinositol-3,4,5-trisphosphate (PIP3), an allosteric activator of phosphoinositide-dependent kinase (PDK). Targets of PDK include protein kinase B (PKB) and the atypical protein kinase C (aPKC) isoforms, which, when activated via phosphorylation, stimulate the translocation of GLUT4-containing vesicles to the plasma membrane [18,19]. Most of the glucose that enters cells, especially in muscle fibers through GLUT4 in response to insulin, is converted into glycogen. This hormonal effect involves the activation of glycogen synthase (GS), the enzyme that catalyzes the rate-limiting step in the conversion of intracellular glucose into glycogen [23,24]. Insulin activates GS by promoting dephosphorylation of several sites of the enzyme through the inhibition or stimulation of protein kinases and phosphatases [23,25]. Moreover, insulin also regulates GS activation by controlling the uptake and transport by GLUT4 of glucose and by regulating the phosphorylation and activation states of enzymes involved in the synthesis and degradation of glycogen [23,24].

The regulation of these cellular processes involves the activation of the PI3K–PDK–PKB pathway, similarly to the stimulation of glucose uptake. Additionally, activated PKB phosphorylates and inhibits glycogen synthase kinase 3 (GSK3). GSK3 is one of the several kinases that phosphorylates glycogen synthase providing the enzyme is in an inactive state. The inhibition of GSK3 activity results in the dephosphorylation and activation of glycogen synthase [23,26,27]. Additional mechanisms that contribute to glycogen synthesis involve dephosphorylation of glycogen synthase by phosphatases such as protein phosphatase 1 (PP1) that is activated by phosphorylation at specific sites by insulin [23,25,28]. Moreover, it has been suggested that the MAPK/p90 ribosomal S6 kinase ($p90^{rsk}$) insulin-stimulated pathway could also be involved in insulin-induced PP1G and GSK3 phosphorylation as well as glycogen synthase activation, besides the classical metabolic PI3K pathway [29,30].

Many studies have demonstrated the hypoglycemic effects of flavonoids, as well as their action on insulin secretion [8,12,31–33], glucose uptake [7,9,34] and glycogen metabolism [11,35,36]. Based on the efficient antihyperglycemic effect of apigenin-6-C-β-L-fucopyranoside (**1**), compared with exogenous insulin action in hyperglycemic rats, the aim of this study was to investigate the acute effect of **1** on insulin secretion and on glycogen synthesis as well as the mechanism of action of this flavonoid in a target tissue of insulin, soleus muscle.

2. Results and discussion

2.1. Effect of apigenin-6-C-β-L-fucopyranoside (*1*) on serum glucose and insulin levels in hyperglycemic rats

Table 1 shows the in vivo effect of apigenin-6-C-β-L-fucopyranoside (**1**) (50 mg/kg) and glipizide (10 mg/kg) on serum glucose levels in hyperglycemic normal rats following the treatment. As expected, after starting the glucose tolerance test serum glucose concentration was significantly increased when compared with zero time of the hyperglycemic group. Glipizide was able to reduce the glycemia significantly from 15 to 60 min after oral treatment and reached basal levels after that. The oral administration of **1** reduced significantly the serum glucose levels in hyperglycemic rats. This efficient and acute effect of **1** (50 mg/kg) reproduced up to 50% of the maximum glipizide action on the glucose serum lowering. Flavonoids are a large group of phenolic plant constituents and their bioactive potential as antihyperglycemic and/or hypoglycemic agents has been described [6]. We have previously demonstrated the effect of flavonoids and chalcones on serum glucose lowering in hyperglycemic and diabetic rats [8,12,14]. Furthermore, Hsu et al. [7] have demonstrated the antihyperglycemic effect of puerarin, an isoflavone, in normal rats, hyperglycemic normal rats and diabetic rats. Puerarin reduced glycemia in normal and diabetic rats in a dose-dependent manner and it was also able to attenuate the increase of plasma glucose induced by an intravenous glucose challenge in normal rats.

Table 1
Effect of apigenin-6-C-β-L-fucopyranoside (**1**) (50 mg/kg) and glipizide (10 mg/kg) on serum glucose levels in oral glucose tolerance curve.[a]

Serum glucose levels (mg/dL)				
Time (min)	Group I euglycemic vehicle (1% EtOH–H_2O)	Group II hyperglycemic glucose (4 g/kg)	Group III hyperglycemic plus **1** (50 mg/kg)	Group IV hyperglycemic plus glipizide (10 mg/kg)
0	115.76 ± 2.8	120.4 ± 6.5	121.97 ± 1.77	120.0 ± 2.10
15	114.73 ± 6.8	191.21 ± 11.91	158.62 ± 4.58*	160.0 ± 6.60*
30	125.53 ± 3.8	205.12 ± 9.75	166.39 ± 7.98*	143.0 ± 7.00*
60	128.25 ± 3.8	169.45 ± 10.21	140.98 ± 12.75*	123.0 ± 7.85*

[a] Values expressed as mean ± S.E.M.; $n = 6$ in triplicate for each treatment. Significant at $^*p \leq 0.05$ in relation to the corresponding hyperglycemic group.

Serum insulin levels were determined in fasted rats after an oral glucose loading (4 g/kg) as shown in Fig. 1. The glucose-induced insulin secretion was increased 71% at 15 min compared with zero time and returned to the basal levels after 60 min. Biphasic insulin secretion is the normal response of β-cells to a rapid and sustained increase in glucose concentration. The first phase corresponds to a prompt and marked, but transient (4–7 min), increase in the secretory rate. It is followed by a decrease to a minimum and sustained second phase that lasts as long as the glucose stimulation is maintained [21,37]. The rapid increase in insulin levels observed at 15 min after the oral glucose loading confirms, in this model, the classical profile of insulin secretion. It was observed that **1** stimulated insulin secretion at 15 min compared with the basal group and also potentiated the glucose effect after 30 and 60 min when compared to the hyperglycemic group. These results are in agreement with those reported for isoorientin, a C-glycosylflavone that significantly reduced serum glucose levels in hyperglycemic and diabetic rats due to the protection of β-cells from oxidative damage and also by restoring plasma insulin levels [31]. Furthermore, genistein and daidzein have been observed to potentiate glucose-induced insulin secretion as demonstrated by an in vitro direct action on pancreatic β-cells [33,38]. Anthocyanins, anthocyanidins, quercetin and (−)epicatechin have also been described as insulin secretagogues [32,39]. The results here presented suggest that **1** is a novel flavonoid with strong antihyperglycemic characteristics.

2.2. Effect of apigenin-6-C-β-L-fucopyranoside (*1*) on glycogen synthesis

Insulin is the most important hormone that regulates glycogen synthesis in skeletal muscle [18,23]. This hormone induces its effects by phosphorylating/dephosphorylating several intracellular enzymes activating or deactivating them and subsequently stimulating GS activity [23,29,40].

It was studied the effect of apigenin-6-C-β-L-fucopyranoside (**1**) on glycogen synthesis in rat soleus muscle. As shown in Fig. 2,

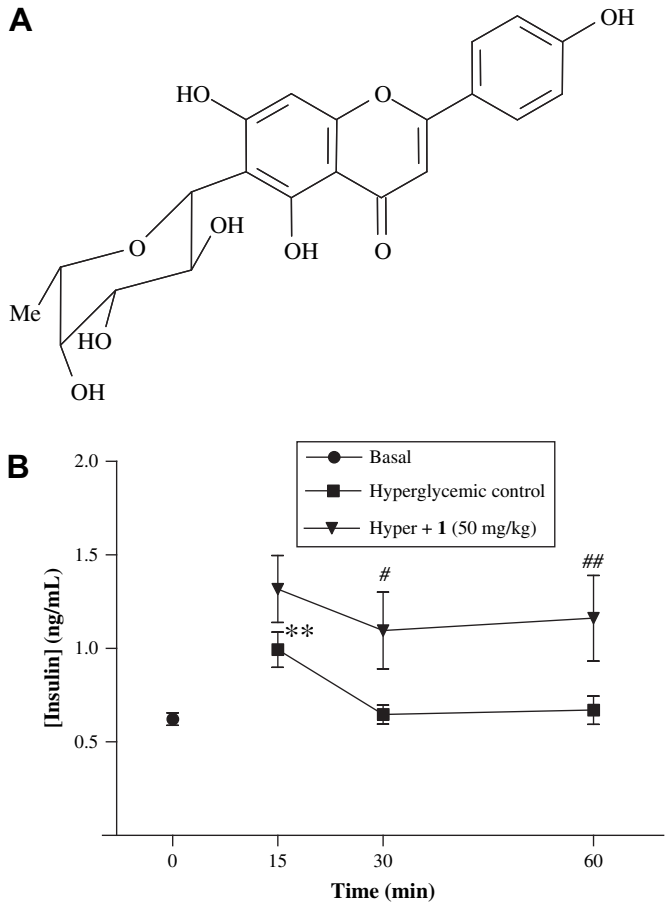

Fig. 1. Apigenin-6-C-β-L-fucopyranoside (**1**) structure (A) and effect of 50 mg/kg apigenin-6-C-β-L-fucopyranoside (**1**) on serum insulin levels in hyperglycemic rats (B). Values are expressed as mean ± S.E.M.; $n = 8$ in duplicate for each group. Significant at $^{**}p \leq 0.01$ in relation to zero time; $^{\#}p \leq 0.05$ and $^{\#\#}p \leq 0.01$ in relation to hyperglycemic group.

apigenin-6-C-β-L-fucopyranoside (**1**) at 1 and 100 μM caused a significant increase in glycogen synthesis (around 50%) in soleus muscle compared with the basal group. The stimulatory effect of apigenin-6-C-β-L-fucopyranoside (**1**) is comparable with those reported to insulin (100 nM; 113% of stimulatory effect compared with the control group) [41] although the dose of **1** was higher than that used to insulin.

Flavonoids have been demonstrated to act on insulin signaling pathways that regulate glucose uptake and glycogen synthesis [6]. We have demonstrated the hypoglycemic effect of kaempferitrin in diabetic rats and the stimulatory effect of this flavonoid on glucose uptake in rat soleus muscle [8,9]. Also, it has been demonstrated that kaempferol 3-neohesperidoside, structurally very similar to kaempferitrin, was able to decrease blood glucose levels in diabetic rats. Additionally, it was shown to increase glucose uptake and glycogen content in soleus muscle more efficiently than kaempferitrin [9,12,42]. Catechin, myricetin and procyanidin extracts derived from grape seeds have also been shown to increase glycogen synthesis and glucose uptake in insulin responsive tissues and cells [34–36]. These findings of glycogen synthesis in soleus muscle support the insulinomimetic effect of apigenin-6-C-β-L-fucopyranoside.

2.3. Effect of various inhibitors on the stimulatory action of apigenin-6-C-β-L-fucopyranoside (1) on glycogen synthesis in rat soleus muscle

To determine the mechanism by which **1** induced glycogen synthesis in the soleus muscle, we performed the glycogen synthesis assay with 5 nM calyculin A, a specific inhibitor of PP1 activity, 50 μM PD98059, a specific inhibitor of MEK, 100 nM wortmannin, a specific inhibitor of PI3K, or 50 mM LiCl, a known inhibitor of GSK3. The inhibitor concentrations used were those previously reported in the literature [29,40,42]. Fig. 3 shows that the glycogen synthesis stimulated by **1** was inhibited by calyculin A and PD98059 pre-treatment. When only calyculin A or PD98059 was added to the muscle samples, no significant change resulted compared with the basal glycogen synthesis. Thus, the stimulatory effect of **1** on glycogen synthesis seems to be mediated, at least in part, through insulin signaling involving the MAPK and PP1 pathways. These results are in agreement with the literature concerning the role of MAPK and PP1 activity on glycogen synthesis regulated by insulin. Insulin has been demonstrated to increase PP1 activity and glycogen synthase activity in HepG2 cells. The same result has been reported with regard to L6 rat skeletal muscle cells and

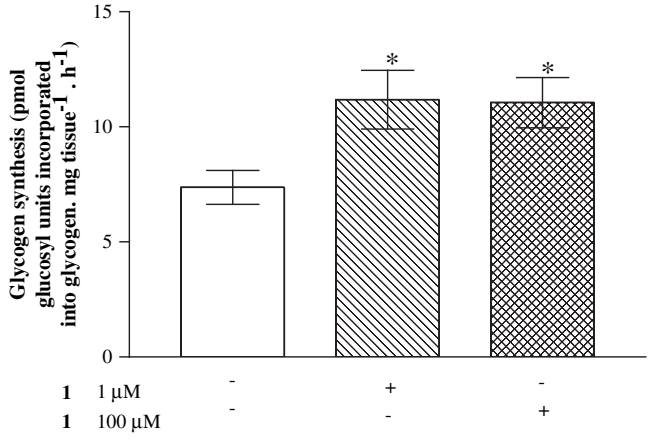

Fig. 2. Dose–response curve of apigenin-6-C-β-L-fucopyranoside (**1**) on glycogen synthesis in rat soleus muscle. Preincubation time = 30 min; incubation time = 60 min. Values are expressed as mean ± S.E.M.; $n = 6$ in duplicate for each group. Significant at $^{*}p \leq 0.05$ in relation to basal group.

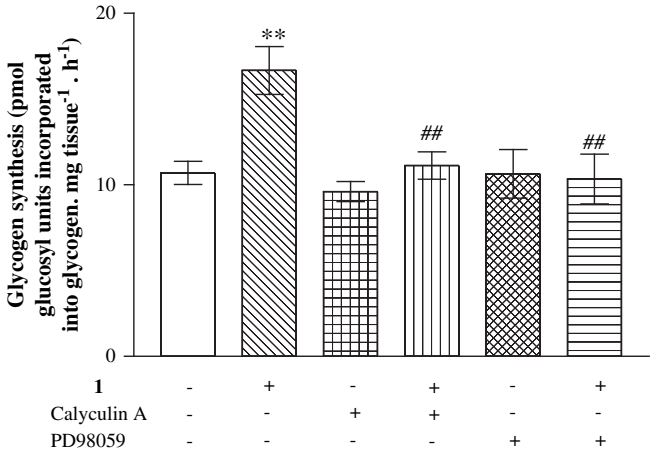

Fig. 3. Effect of 5 nM calyculin A and 50 μM PD98059 on the stimulatory action of 1 μM apigenin-6-C-β-L-fucopyranoside (**1**) on glycogen synthesis in rat soleus muscle. Basal group = no treatment. Signals (+) and (−) indicate presence and absence, respectively, of each substance in the incubation medium. Preincubation time = 30 min; incubation time = 60 min. Values are expressed as mean ± S.E.M.; $n = 6$ in duplicate for each group. Significant at $^{**}p \leq 0.01$ in relation to basal group; $^{\#\#}p \leq 0.01$ in relation to apigenin-6-C-fucopyranoside (**1**) group.

adipocytes [40,43]. The activation of PP1 by insulin is mediated by increasing its phosphorylation state at site 1 [23,43]. This site is readily phosphorylated in vitro by $p90^{rsk}$, a kinase that is phosphorylated and activated by MAPK when cells or tissues are incubated with insulin [30,44]. The MAPK pathway has been proposed to counteract increased glycogen synthesis stimulated by insulin in hepatocytes since PD98059 totally inhibited insulin-stimulated glucose incorporation into glycogen [29]. Furthermore, Dent et al. have suggested that the phosphorylation and activation of $p90^{rsk}$ by MAPK increase its activity toward site 1 on PP1 in rabbit skeletal muscle [30].

Additionally, the stimulatory effect of **1** on glycogen synthesis was totally inhibited in the presence of wortmannin, as shown in Fig. 4. The involvement of the PI3K pathway in glycogen synthesis regulated by insulin has previously been demonstrated in 3T3-L1 adipocytes, hepatocytes and L6 myotubes [29,45,46]. It has been demonstrated that PI3K phosphorylates and activates PDK 1 which acts on PKB increasing its activity [26,27]. One important target of PKB is GSK3. Under basal conditions, GSK3 is constitutively active and phosphorylates and inactivates GS. The inactivation of GSK3 isoforms GSK3α and GSK3β by insulin is achieved through phosphorylation of Ser^{21} and Ser^{9}, respectively, resulting in the activation of GS and an increase in glycogen synthesis [27,40,47].

To study the involvement of GSK3 in the mechanism of action of **1** in relation to glycogen synthesis, lithium chloride (LiCl) was used. It was observed that **1** and LiCl caused similar increases in glycogen in soleus muscle (around 69% and 78%, respectively). These results suggest that, although the stimulatory effect of **1** and LiCl is very close, they may act on the same site of GSK3, since no additive effect on glycogen synthesis was observed (Fig. 4).

In hepatocytes from normal and diabetic rats lithium and insulin induced a substantial increase in glycogen accumulation as well as in GS activity. Also, lithium potentiated the effect of insulin in these events [48,49]. Additionally, in L6 myotubes and 3T3-L1 adipocytes, LiCl and insulin induced an increased inhibition of GSK3 activity, confirming the key role of GSK3 on GS dephosphorylation and activation [50]. In agreement with this finding, it has been demonstrated that in L6 myocytes, insulin stimulates glycogen synthase and glycogen synthesis through the inactivation of GSK3 by a phosphorylation-dependent mechanism (involving the PI3K/PKB pathway) while LiCl, unlike insulin, exerts its effect through a phosphorylation-independent mechanism (involving a direct inhibitory action on GSK3) [51,52]. Since no additive effects of **1** and LiCl on glycogen synthesis were observed we can suppose that the action of the flavonoid may occur at a distinct GSK3 site of insulin action.

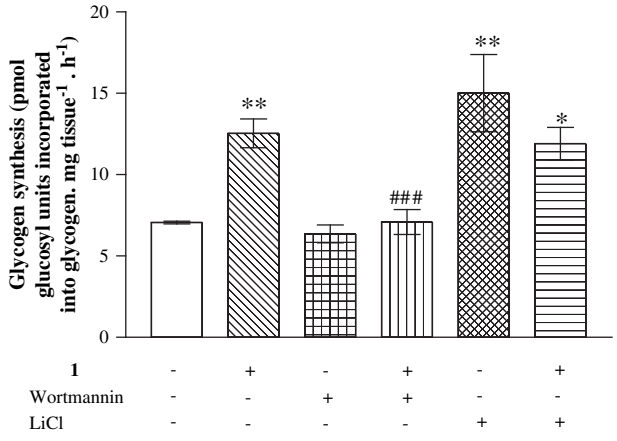

Fig. 4. Effect of 100 nM wortmannin and 50 mM LiCl on the stimulatory action of 1 μM apigenin-6-C-β-L-fucopyranoside (**1**) in glycogen synthesis in rat soleus muscle. Basal group = no treatment. Signals (+) and (−) indicate presence and absence, respectively, of each substance in the incubation medium. Preincubation time = 30 min; incubation time = 60 min. Values are expressed as mean ± S.E.M.; $n = 6$ in duplicate for each group. Significant at $^{**}p \leq 0.01$ and $^{*}p \leq 0.05$ in relation to basal group. Significant at $^{###}p \leq 0.001$ in relation to apigenin-6-C-fucopyranoside (**1**) group.

3. Conclusions

We showed that apigenin-6-C-β-L-fucopyranoside stimulated insulin secretion and potentiated glucose-induced insulin secretion in hyperglycemic rats. In addition, this flavonoid stimulated glycogen synthesis in rat soleus muscle through mechanisms well known to insulin signal transduction. These results constitute the first evidence indicating that lowering blood glucose levels may occur as a consequence of the insulin secretagogue and insulinomimetic effects of apigenin-6-C-β-L-fucopyranoside.

4. Experimental

4.1. General experimental procedures

The inhibitors of PI3K, wortmannin, PP1, calyculin A, mitogen-activated protein kinase (MEK), PD98059, GSK3, and lithium chloride were purchased from Sigma–Aldrich Co (St. Louis, MO). Glipizide was purchased from Akros Ltd (USA). D–[^{14}C (U)]–glucose (^{14}C–G), specific activity 9.25 GBq/mmol and biodegradable liquid scintillation were obtained from Perkin–Elmer Life and Analytical Sciences (Boston, MA, USA). Enzyme-linked immunosorbent assay (ELISA) for quantitative determination of rat insulin (catalogue no. EZRMI-13K) was purchased from Millipore (St. Charles, MO, USA). Salts and solvents were purchased from Merck AG (Darmstadt, Germany).

4.2. Plant material

The leaves of *Averrhoa carambola* were collected in Santo Amaro da Imperatriz, Santa Catarina, Brazil and identified by Prof. Daniel de Barcellos Falkenberg. A voucher specimen was deposited at the herbarium of the Botany Department at the Universidade Federal de Santa Catarina, Florianópolis, under the number FLOR-24.144.

4.3. Extraction and isolation

The powdered, dried leaves (281 g) were extracted with EtOH–H_2O (4:1). The extract was concentrated to dryness by rotatory vaporization at 60 °C under reduced pressure (41.3 g; crude extract–dry leaves ratio = 14.7%). The concentrated extract was then suspended in EtOH–H_2O (4:1) and successively extracted with *n*-hexane, EtOAc and *n*-BuOH. The ethyl acetate soluble fraction (EtOAc) of 6.9 g was subjected to silica gel (100–200 mesh) CC and eluted with an ethyl acetate/ethanol mixture gradient to afford 36 fractions. Fractions 6–8 (150 mg) and 10–12 (200 mg) were purified by recrystallization from MeOH to give pure (HPTLC in ethyl acetate/methanol/acetic acid 80:14:6 and NMR) compound **1**. This compound was identified by NMR analysis (^{1}H, ^{13}C, DEPT, COSY, HMQC and HMBC) and comparison with literature data [53].

Apigenin-6-C-β-L-fucopyranoside (compound **1**): Yellow amorphous powder (drug–extract ratio = 0.36%). ^{1}H NMR (400 MHz, CD_3OD): aglycone moiety δ: 6.61 (s, H-3), 6.53 (s, H-8), 7.84 (d, $J = 8.0$ Hz, H-2′ and H-6′), 6.92 (d, $J = 8.0$ Hz, H-3′ and H-6′); sugar moiety: 4.64 (d, $J = 8.4$ Hz, H-1″), 4.03 (t, $J = 8.7$ Hz, H-2″), 3.97 (dd, $J = 3.0$ and 6.5 Hz, H-3″), 3.50 (d, $J = 3.0$ Hz, H-4″), 3.17 (m, H-5″), 1.44 (d, $J = 6.2$ Hz, H-6″). ^{13}C NMR (400 MHz, CD_3OD) aglycone moiety δ: 165.1 (C-2), 99.6 (C-3), 183.1 (C-4), 161.7 (C-5), 108.0 (C-6), 163.9 (C-7), 94.0 (C-8), 161.8 (C-9), 102.8 (C-10), 121.9 (C-1′), 128.3

(C-2′ and 6′), 115.9 (C-3′ and 5′), 157.8 (C-4′); sugar moiety: 71.9 (C-1″), 69.1 (C-2″), 78.5 (C-3″), 70.6 (C-4″), 70.8 (C-5″), 18.1 (C-6″).

4.4. Experimental animals

Male Wistar rats weighing 180–200 g from the Central Animal House – UFSC were used. The rats were housed in plastic cages in an air-conditioned animal room and fed on pellets with free access to tap water. Room temperature was controlled at 21 °C with a 12 h light:12 h dark cycle. Animals described as fasted had been deprived of food for 16 h but allowed free access to water. For all oral treatments, 0.5 mL of each respective substance was given by gavage. All the animals were monitored carefully and maintained in accordance with the ethical recommendations of the Brazilian Veterinary Medicine Council and the Brazilian College of Animal Experimentation (Protocol CEUA/PP007).

4.5. Apigenin-6-C-β-L-fucopyranoside (**1**) treatment

Fasted rats were divided into groups of six animals for each treatment: Group I, euglycemic, normal fasted rats that received the vehicle, 1% EtOH–H_2O, p.o.; Group II, hyperglycemic rats that received glucose (4 g/kg, p.o.); Group III, rats that received glucose (4 g/kg, p.o.) plus apigenin-6-C-β-L-fucopyranoside (**1**) (50 mg/kg, p.o.); Group IV, rats that received glucose (4 g/kg, p.o.) plus glipizide (10 mg/kg, p.o.); by oral gavage. Blood samples were collected just prior to and at 15, 30 and 60 min after the glucose loading. After centrifugation, serum samples were used either immediately to determine the blood glucose levels.

4.6. Determination of the serum insulin

Fasted rats were divided into groups of six animals for each treatment: Group I, hyperglycemic rats that received glucose (4 g/kg, p.o.); Group II, rats that received glucose (4 g/kg, p.o.) plus apigenin-6-C-β-L-fucopyranoside (**1**) (50 mg/kg, p.o.). Blood samples were collected just prior to and at 15, 30 and 60 min after the glucose loading. Blood samples from the tail vein were collected and prepared for the analysis of insulin. The insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The range of values detected by this assay was: 0.2–10 ng/mL. The intra- and inter-assay coefficients of variation for insulin were 3.22 and 6.95, respectively. All insulin levels were estimated by means of colorimetric measurement at 450 nm with an ELISA plate reader (Organon Teknika, Roseland, NJ, USA) by interpolation from a standard curve. Samples were analyzed in duplicate and results were expressed as nanograms of insulin serum per milliliter.

4.7. Glycogen synthesis on rat soleus muscle

The assays of ^{14}C-glucose incorporation into glycogen were conducted as described by Cazarolli et al. [9,41]. Slices of soleus muscle from normal rats were distributed (alternately left and right) between basal and treated groups. The muscles were dissected, weighed, and preincubated and incubated at 37 °C in Krebs Ringer-bicarbonate (KRb) buffer of the composition 122 mM NaCl, 3 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, 1% BSA and 5 mM D-glucose and bubbled with O_2/CO_2 (95%:5%, v/v) until pH 7.4. Apigenin-6-C-β-L-fucopyranoside (**1**) (1 and 100 μM) was added to the preincubation (30 min) and incubation medium (60 min) in the presence or absence of 100 nM wortmannin, 5 nM calyculin A, 50 μM PD98059 or 50 mM lithium chloride. ^{14}C-glucose (0.15 μCi/mL) was added to each sample during the incubation period. After incubation, the muscles were removed, washed in cold KRb and dried on filter paper. The muscles were homogenized in 0.5 N KOH and boiled at 100 °C for 20 min, with occasional stirring. After cooling, 95% ethanol was added to the samples, which were heated to boiling followed by cooling in an ice bath for 20 min to aid the precipitation of glycogen. The homogenates were centrifuged at 664*g* for 15 min, the supernatant was discarded, and pellets resolubilized in water. 30 μL aliquots of the samples were placed in liquid scintillation vials on an LKB rack beta liquid scintillation spectrometer (model 1215; EG and G-Wallac, Turku, Finland), for the radioactivity measurements. The results were expressed as pmol glucosyl units incorporated into glycogen. mg tissue^{-1}. h^{-1}.

4.8. Statistical analysis

Data were expressed as mean ± S.E.M. One or two-way analysis of variance (ANOVA), followed by the Bonferroni *post-test* or non paired Student's *t*-test, was used to identify significantly different groups. Differences were considered to be significant at the $p \leq 0.05$ level.

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References

[1] R. Gadsby, Adv. Drug Deliv. Rev. 54 (2002) 1165–1172.
[2] J.K. Grover, S. Yadav, V. Vats, J. Ethnopharmacol. 81 (2002) 81–100.
[3] G. Zareba, N. Serradell, R. Castañer, S.L. Davies, J. Prous, N. Mealy, Drugs Future 30 (2005) 1253–1282.
[4] J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481–504.
[5] L.H. Cazarolli, L. Zanatta, E.H. Alberton, M.S.R.B. Figueiredo, P. Folador, R.G. Damazio, M.G. Pizzolatti, F.R.M.B. Silva, Mini Rev. Med. Chem. 8 (2008) 1429–1440.
[6] L.H. Cazarolli, L. Zanatta, E.H. Alberton, M.S.R.B. Figueiredo, P. Folador, R.G. Damazio, M.G. Pizzolatti, F.R.M.B. Silva, Mini Rev. Med. Chem. 8 (2008) 1032–1038.
[7] F.L. Hsu, I.M. Liu, D.H. Kuo, W.C. Chen, H.C. Su, J.T. Cheng, J. Nat. Prod. 66 (2003) 788–792.
[8] E. De Sousa, L. Zanatta, I. Seifriz, T.B. Creczynski-Pasa, M.G. Pizzolatti, B. Szpoganicz, F.R.M.B. Silva, J. Nat. Prod. 67 (2004) 829–832.
[9] A.P. Jorge, H. Horst, E. De Sousa, M.G. Pizzolatti, F.R.M.B. Silva, Chem. Biol. Interact. 149 (2004) 89–96.
[10] M. Pinent, M. Blay, M.C. Bladé, M.J. Salvadó, L. Arola, A. Ardévol, Endocrinology 14 (2004) 54985–54990.
[11] I.M. Liu, S.S. Liou, T.W. Lan, F.L. Hsu, J.T. Cheng, Planta Med. 71 (2005) 617–621.
[12] L.H. Cazarolli, L. Zanatta, A.P. Jorge, E. De Sousa, H. Horst, V.M. Woehl, M.G. Pizzolatti, B. Szpoganicz, F.R.M.B. Silva, Chem. Biol. Interact. 163 (2006) 177–191.
[13] L. Zanatta, E. De Sousa, L.H. Cazarolli, A.J. Cunha, M.G. Pizzolatti, B. Szpoganicz, F.R.M.B. Silva, J. Ethnopharmacol. 109 (2007) 151–155.
[14] E.H. Alberton, R.G. Damazio, L.H. Cazarolli, L.D. Chiaradia, P.C. Leal, R.J. Nunes, R.A. Yunes, F.R.M.B. Silva, Chem. Biol. Interact. 171 (2008) 355–362.
[15] M. Provasi, C.E. Oliveira, M.C. Martino, L.G. Pessini, R.B. Bazotte, D.A.G. Cortez, Acta Sci. Health Sci. 23 (2001) 665–669.
[16] M. Provasi, C.E. Oliveira, L.C. Fernandes, O. Tchaikovski, R.B. Bazotte, L.E.R. Cortez, D.A.G. Cortez, Acta Sci. Health Sci. 27 (2005) 45–48.
[17] P.N. Pushparaj, C.H. Tan, B.K.H. Tan, J. Ethnopharmacol. 72 (2000) 69–76.
[18] A. Taha, A. Klip, J. Membr. Biol. 169 (1999) 1–12.
[19] A.R. Saltiel, C.R. Kahn, Nature 414 (2001) 799–806.
[20] P. Detimary, J.C. Jonas, J.C. Henquin, J. Clin. Invest. 96 (1995) 1738–1745.
[21] P. Rorsman, Diabetologia 40 (1997) 487–495.
[22] A.Y.Y. Cheng, I.G. Fantus, CMAJ 172 (2005) 213–226.
[23] A.K. Srivastava, S.K. Pandey, Mol. Cell. Biochem. 182 (1998) 135–141.

[24] P.J. Roach, Curr. Mol. Med. 02 (2002) 101–120.
[25] M.J. Brady, A.R. Saltiel, Recent Prog. Horm. Res. 56 (2001) 157–173.
[26] E. Hajduch, G.J. Litherland, H.S. Hundal, FEBS Lett. 492 (2001) 199–203.
[27] A. Mora, K. Sakamoto, E.J. McManus, D.R. Alessi, FEBS Lett. 579 (2005) 3632–3638.
[28] C.B. Newgard, M.J. Brady, R.M. O'Doherty, A.R. Saltiel, Diabetes 49 (2000) 1967–1977.
[29] J. Carlsen, K. Christiansen, J. Vinten, Cell. Signal. 09 (1997) 447–450.
[30] P. Dent, S.N. Lavoinne, F.B. Caudwell, P. Watt, P. Cohen, Nature 348 (1990) 302–308.
[31] E. Sezik, M. Aslan, E. Yesilada, S. Ito, Life Sci. 76 (2005) 1223–1238.
[32] B. Jayaprakasam, S.K. Vareed, L.K. Olson, M.G. Nair, J. Agric. Food Chem. 53 (2005) 28–31.
[33] D. Liu, W. Zhen, Z. Yang, J.D. Carter, H. Si, K.A. Reynolds, Diabetes 55 (2006) 1043–1050.
[34] K.C. Ong, H.E. Khoo, Life Sci. 67 (2000) 1695–1705.
[35] A.K. Valsa, S. Sudheesh, N.R. Vijayalakshmi, Indian J. Biochem. Biophys. 34 (1997) 406–408.
[36] M. Pinent, M.C. Bladé, M.J. Salvadó, L. Arola, A. Ardévol, J. Agric. Food Chem. 53 (2005) 5932–5935.
[37] J.C. Henquin, M. Nenquin, P. Stiernet, B. Ahren, Diabetes 55 (2006) 441–451.
[38] J.C. Jonas, T.D. Plant, P. Gilon, P. Detimary, M. Nenquin, J.C. Henquin, Br. J. Pharmacol. 114 (1995) 872–880.
[39] C.S. Hii, S.L. Howell, J. Endocrinol. 107 (1985) 1–8.
[40] N.A. Syed, R.L. Khandelwal, Mol. Cell. Biochem. 211 (2000) 123–136.
[41] L.H. Cazarolli, P. Folador, M.G. Pizzolatti, F.R.M.B. Silva, Biochimie 91 (2009) 843–849.
[42] L. Zanatta, A. Rosso, P. Folador, M.S.R.B. Figueiredo, M.G. Pizzolatti, L.D. Leite, F.R.M.B. Silva, J. Nat. Prod. 71 (2008) 532–535.
[43] L. Ragolia, N. Begum, Mol. Cell. Biochem. 182 (1998) 49–58.
[44] I. Azpiazu, A.R. Saltiel, A.A. DePaoli-Roach, J.C. Lawrence, J. Biol. Chem. 271 (1996) 5033–5039.
[45] D.A.E. Cross, D.R. Alessi, P. Cohen, M. Andjelkovich, B.A. Hemmings, Nature 378 (1995) 785–789.
[46] P.R. Shepherd, B.T. Navé, K. Siddle, Biochem. J. 305 (1995) 25–28.
[47] S.J. Oreña, A.J. Torchia, R.S. Garofalo, J. Biol. Chem. 275 (2000) 15765–15772.
[48] J.E. Rodríguez-Gil, J.J. Guinovart, F. Bosch, Arch. Biochem. Biophys. 301 (1993) 411–415.
[49] J.M. Fernández-Novell, J.E. Rodriguez-Gil, A. Barberà, J.J. Guinovart, Arch. Biochem. Biophys. 457 (2007) 29–34.
[50] K. MacAulay, E. Hajduch, A.S. Blair, M.P. Coghlan, S.A. Smith, H.S. Hundal, Eur. J. Biochem. 270 (2003) 3829–3838.
[51] W.S. Choi, C.K. Sung, Biochim. Biophys. Acta 1475 (2000) 225–230.
[52] W.J. Ryves, A.J. Harwood, Biochem. Biophys. Res. Commun. 280 (2001) 720–725.
[53] D. Araho, M. Masaazumi, C. Wen-hua, K. Toshimitsu, M. Kenji, I. Takao, Nat. Med. (Tokyo, Japan) 59 (2005) 113–116.