Revista Brasileira de Farmacognosia 25 (2015) 258–263

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

Hypoglycemic and hypolipidemic effects of *Solidago chilensis* in rats

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ARTICLE INFO

Article history:
Received 18 December 2014
Accepted 16 May 2015
Available online 10 June 2015

Keywords:
Antihyperlipidemic activity
Arnica-do-brasil
Asteraceae
Hypoglycemic

ABSTRACT

Solidago chilensis Meyen, Asteraceae, is traditionally used to treat inflammation. However, phytochemical and pharmacology investigations are lacking. This study evaluated the hypoglycemic and hypolipidemic effects of hydroalcoholic extract from *S. chilensis* aerial parts in rats. In oral glucose tolerance tests the rats received saline (0.5 ml/100 g) in control group (C), hydroalcoholic extract (125, 250 or 500 mg/kg *p.o.*; $n = 6$) or glibenclamide (10 mg/kg *p.o.*; $n = 6$). After 30 min, glucose (4 g/kg) was administered. Rats treated with hydroalcoholic extract 500 demonstrated decreased glucose levels at 180 min (−22.1%), when compared with group C, similar to glibenclamide. Moreover, treatment with hydroalcoholic extract 500 significantly increased the glycogen content in the liver and soleus muscle, and hydroalcoholic extract 250 specifically inhibited the enzyme maltase when compared with group C. Furthermore, all hyperglycemic rats treated with hydroalcoholic extract (125, 250 and 500) exhibited an accentuated decrease in total cholesterol levels (−36.8%, −36.7% and −41.3%, respectively). Our results suggest that hypoglycemic and hypolipidemic effects of hydroalcoholic extract could be associated with increased production and release of insulin as well as with insulinotropic and antioxidant effects.

Introduction

Diabetes mellitus (DM) comprises a group of disorders involving distinct pathogenic mechanisms with hyperglycemia as the common denominator (Teixeira et al., 2000). Hyperglycemia in diabetes may be related to numerous physiological events, such as decreased glucose in cells, reduced glucose utilization by various tissues, and increased hepatic production of glucose (gluconeogenesis) (Prabhakar et al., 2013). Complications experienced by patients with diabetes are often related to chronic hyperglycemia, including retinopathy, peripheral vascular disease, renal failure, neuropathy, and cardiovascular diseases that cause both morbidity and premature mortality (Hirany et al., 2000; Piaulino et al., 2013).

It is well established that patients with type 2 DM frequently have abnormal serum lipid profiles comprising elevated low density lipoproteins (LDL) and triglycerides levels along with moderately decreased high density lipoproteins (HDL) level (Zimmet, 2000), all of which are associated with an increased risk of cardiovascular diseases (Dai et al., 2013). Many studies have shown that elevated serum cholesterol concentrations can cause coronary atherosclerosis (Park and Velasquez, 2012) that is associated with heart disease, stroke, and death in both developed and developing countries (Raida et al., 2008).

Medicinal plants have been used for many years by different cultures worldwide to treat DM (Modak et al., 2007). Investigating herbal medicines has become progressively important in the search for a new, effective, and safe therapeutic agent to combat DM. More than 200 pure bioactive principles isolated from plants have been shown to lower serum glucose levels (Grover et al., 2002; Warjeet, 2011), including phenolics and flavonoids (Negri, 2005).

Solidago chilensis Meyen, Asteraceae, is a species native to the southern region of South America. It is widely distributed in south and southeast Brazil, where it is popularly known as arnica-do-brasil and is used to relieve inflammation (Lorenzi and Matos, 2002). Its main chemical constituents are acetophenone, carotenes, diterpenoids with labdanic and clerodanic skeletons (Soares-Valverde et al., 2009), flavonoids, glycosides, 3-methoxybenzaldehyde, essential oils, and saponins (Silva et al., 2010), with quercetrin being the major constituent (Torres et al., 1987).

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http://dx.doi.org/10.1016/j.bjp.2015.05.001
0102-695X/

Ethnopharmacological investigations have found this species to have antispasmodic, antihemorrhagic (Alonso, 1998), wound-healing (Facury-Neto et al., 2004), and anti-inflammatory effects (Tamura et al., 2009). Recently, there has been considerable progress in the investigation of *S. chilensis* and gastric protection (Bucciarelli et al., 2010) as well as a better understanding of the effect of *S. chilensis* on insulin resistance in obese mice (Melo et al., 2011). However, the hypoglycemic and hypolipidemic effects of *S. chilensis* on the glucose tolerance curve have not yet been studied.

Therefore, the objective of this study was to investigate the hypoglycemic and hypolipidemic effects of hydroalcoholic extract (HE) from *S. chilensis* in rats. This study evaluated the glucose tolerance curve along with, liver and soleus muscle glycogen levels, disaccharidase activity, total cholesterol (TC), and alanine aminotransferase (ALT) levels. Moreover, the *in vitro* free radical scavenging properties of *S. chilensis* were evaluated.

Materials and methods

Plant materials

Aerial parts of *Solidago chilensis* Meyen, Asteraceae, were collected in Chapecó, SC, Brazil (S 27°06′38.83″/W 52°34′26.52″). The voucher specimen was identified by Osmar dos Santos Ribas and is deposited in the herbarium of the Botanical Museum of Curitiba (MBM number 356792).

Preparation of hydroalcoholic extract

Dried aerial parts of *S. chilensis* (50 g) of the same particle size (300 μm; 48 Tyler/Mesch) were macerated in 80% methanol (1000 ml) for six days. Hydroalcoholic extract (HE) from *S. chilensis* was concentrated to dryness under reduced pressure at 40 °C and then freeze-dried and stored at −20 °C.

High-performance liquid chromatography analysis

Chromatography analysis was performed using a Varian® ProStar HPLC system consisting of an automatic injector, ternary gradient detectors, pumps, and a UV/Vis Kromasil® C18 reversed-phase ODS column (5 μm; 25 mm × 4.5 mm). The mobile phase consisted of two solvents: H_2O:acetic acid (40:1, v/v; solvent A) and CH_3CN (solvent B) that were filtered through 0.45 μm Millipore polytetrafluoroethylene membranes. Separations were performed with a linear gradient: 86% solvent A and 14% solvent B for 15 min, 35% solvent B for 20 min and 100% solvent B for 2 min. UV absorbance at 360 nm was measured, and the results were compared with the retention times of an authentic external standard followed by a UV spectrum analysis. The flow rate of the mobile phase was 1 ml/min^{-1}, and the injection volume was 20 μl. The chromatographic runs were performed at 22 °C. UV absorbance at 360 nm was measured (Apáti et al., 2006). Quercetrin (12.5, 25, 50, 100 and 200 μg/ml; Sigma–Aldrich®) was analyzed in triplicate, and a calibration curve was generated. HE was dissolved in MeOH (1 mg/ml) and filtered through a micropore filter (0.45 μm) before the chromatographic profile was generated. The results are expressed as the concentration of quercetrin (%) in the dried plant material.

In vitro *2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay*

The free radical scavenging activity of HE was measured using the method described by Brand-Williams et al. (1995) with some modifications. HE (1 ml; 5–200 μg/ml) was added to 2 ml of a solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals in ethanol (0.004%). The mixture was vigorously shaken and allowed to stand for 30 min at room temperature (RT). The absorbance (Abs_{sample}) of the resulting solution was measured at 517 nm, and the antioxidant activity (AA) percentage was calculated using the following formula:

$$AA\% = 100 - \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}}$$

A solution of ethanol (2 ml) and HE (1 ml) was used as the blank (Abs_{blank}). A solution of DPPH (2 ml) and ethanol (1 ml) was used as the control ($Abs_{control}$). Ascorbic and gallic acids were used as standards. Free radical scavenging activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC_{50}). The IC_{50} value was determined by interpolation from the nonlinear regression of the plot of percentage of inhibition against the concentration of HE, which is defined as the amount of HE needed to scavenge 50% of DPPH radicals.

Animals

The experimental protocol was approved by the Ethics Committee on Animal Use of the Community University in the Region of Chapecó, Brazil (CEUA No. 020/2013). Male *Rattus norvegicus*, Wistar ($n = 30$) weighing 250–275 g were used in the study. The animals were housed in wire-bottomed 17 cm × 33.5 cm × 40.5 cm cages in a controlled environment at 22 ± 2 °C with a 12 h light–dark cycle and minimal noise. The rats had *ad libitum* access to water and commercially prepared rodent chow pellets (Nuvilab® CR-1).

Oral glucose tolerance curve

Animals were fasted overnight and divided into groups containing six rats each. The control group (C), received saline (0.5 ml/100 g); the HE group received HE (125, 250 or 500 mg/kg) (Patil et al., 2011); and the glibenclamide group received glibenclamide (10 mg/kg) (Zhao et al., 2011). All drugs were diluted with saline (0.9%) in established doses and administered orally by gavage in a volume of 0.5 ml/100 g body weight (Trovato et al., 1996; Diehl et al., 2001). Glucose levels were measured before the rats received the treatment (zero time) and 30 min after glucose was administrated (4 g/kg) (Alam et al., 2011; Pereira et al., 2012). Blood samples were collected from the tail vein just prior to and 30, 60 and 180 min after glucose loading, and the glucose level (mg/dl) was assayed by a glucometer (Accu-Chek® Performa). At the end of the experimental period, the animals were anesthetized with a mixture of lidocaine and sodium thiopental (10 and 150 mg/kg, respectively). Blood aliquots were collected for biochemical analyses via cardiac puncture, and the animals were then euthanized by exsanguination (Concea, 2012). The liver and soleus were collected for later analysis, as was a segment of the small intestine.

Glycogen measurements

The harvested liver and soleus were assessed for glycogen content 3 h after treatment. Glycogen was isolated from these tissues as described by Krisman (1962). The tissue was weighed, homogenized in 33% KOH, and boiled at 100 °C for 30 min, with occasional stirring. After cooling, 96% ethanol was added to the samples, which were then heated to boiling and cooled in an ice bath to aid glycogen precipitation. The homogenate was centrifuged (1300 × *g*) for 15 min, the supernatant was discarded, and the resulting pellet was washed and resolubilized in water. Glycogen content was determined by treatment with an iodine reagent, and the absorbance was measured at 460 nm. The results were expressed as milligrams of glycogen per gram of tissue.

Disaccharidase extraction and assays

The extracted small intestine segment was washed in 0.9% NaCl solution, dried on filter paper, weighed, trimmed, and homogenized (300 × *g*) with 0.9% NaCl (400 mg of duodenum per 1.0 ml of 0.9% NaCl) for 1 min at 4 °C. The resulting extract was centrifuged at (1300 × *g*) for 8 min. The supernatant was assessed to measure *in vivo* maltase, sucrase, and lactase activity as well as protein determination. The activity of maltase (EC 3.2.1.20), lactase (EC 3.2.1.23), and sucrase (EC 3.2.1.48) was determined using a glucose diagnosis kit based on the reagent glucose oxidase. To determined isaccharidase activity, duodenum homogenates (10 μl) were incubated at 37 °C for 60 min with 10 μl of the substrate (equivalent to 0.056 μM of maltase, sucrase, or lactase) (Dahlqvist, 1984; Pereira et al., 2011). One enzyme unit (U) was defined as the amount of enzyme that catalyzed the release of 1 μmol of glucose per minute under the assay conditions. The specific activity was defined as enzyme activity (U) per milligram of protein. Protein concentration was determined by the method described by Lowry et al. (1951), using bovine serum albumin as the standard. The assays were performed in duplicate along with appropriate controls.

Biochemical analysis of serum samples

Upon collection, serum samples were immediately centrifuged (3000 × *g*) for 15 min. Serum TC and ALT levels were determined by enzymatic colorimetric methods (UV/vis) using commercial Labtest® kits according to the manufacturer's instructions. A semi-automated analyzer (BioSystems®, model BTS 310) was used for all analysis (Li et al., 2012).

Statistics

All results shown are presented as mean values ± SEM. The data were evaluated by one-way ANOVA followed by Tukey's test and correlation analyses using SPSS 20.0. A *p*-value of <0.05 was considered statistically significant.

Results

Chemical constituents of S. chilensis

The amount of quercetrin in HE was quantified by HPLC using an analytical curve ($r = 0.999$; $y = 0.735x = 2.6971$) with a retention time of 10.02 min. The HPLC analysis revealed the quercetrin concentration to be 2.4% in the aerial parts of *S. chilensis* (Fig. 1).

Determination of DPPH radical scavenging activity

The DPPH assay showed that HE exhibits antioxidant properties *in vitro* (Fig. 2). The highest scavenging effect was observed for HE, with an IC_{50} of 59.12 ± 3.14 μg/ml, although it showed lower scavenging abilities than ascorbic and gallic acids, which were used as standards (16.32 ± 2.94 and 2.14 ± 1.58 μg/ml, respectively).

Effect of HE on the oral glucose tolerance curve

Table 1 shows that HE500 had a significant antihyperglycemic effect when compared to the C group ($F_{(4, 21)} = 12.0$; $p < 0.05$). Lower serum glucose (approx. 22% lower) was detected 180 min after treatment; glibenclamide showed similar results.

Effect of HE on hepatic and soleus glycogen content

Fig. 3 shows that HE and glibenclamide did not affect hepatic and soleus glycogen content compared with other treatment groups.

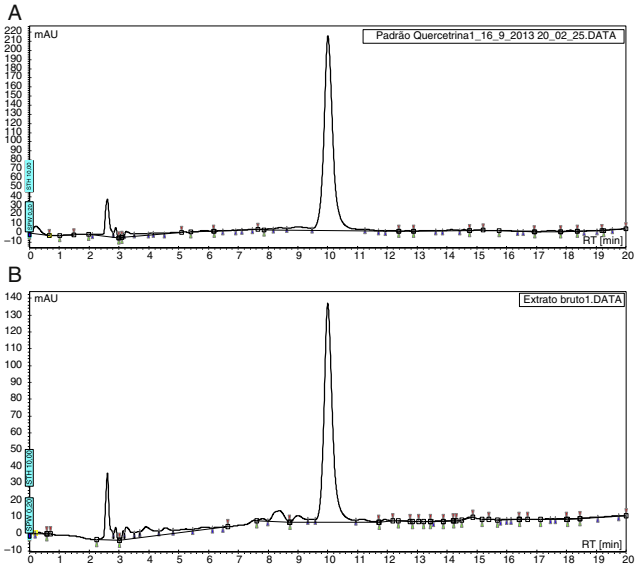

Fig. 1. Analysis by high performance liquid chromatography (HPLC): A. quercetrin (200 μg/ml); B. hydroalcoholic extract from aerial parts of *Solidago chilensis* (1 mg/ml in MeOH) (RT: 10.02 min). HPLC Varian®, Kromasil® ODS column (5 μM) reversed phase C-18 (25 mm × 4.5 mm) at 24 ± 2 °C. Two solvent systems used for analysis; H_2O:acetic acid (40:1, v/v) (solvent A) and CH_3CN (solvent B). The flow was 1 ml/min, and the gradient used had 86% of A for 15 min, 65% of A for 20 min, and 100% of B for 2 min. The detection by UV was realized at 360 nm.

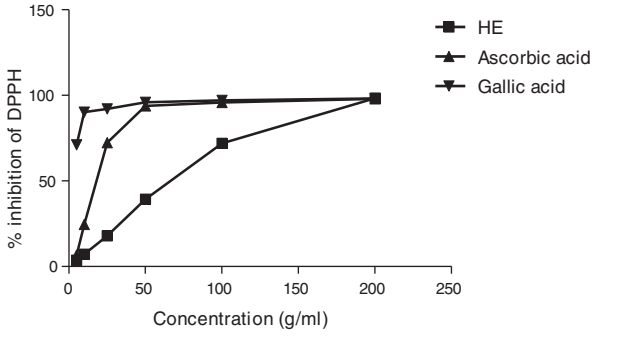

Fig. 2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenger activity of hydroalcoholic extract from *Solidago chilensis* (HE) compared with standards ascorbic and gallic acids. Results are expressed as means ± SEM ($n = 3$).

However, HE 500 significantly increased hepatic ($F_{(4, 25)} = 6.6$; $p < 0.05$) and soleus ($F_{(4, 23)} = 3.9$; $p < 0.05$) glycogen content compared with group C.

Effect of HE on disaccharidase activity

Disaccharidase activity was significantly affected by HE only at a dose of 250 mg/kg, as it inhibited maltase activity ($F_{(4, 24)} = 3.4$; $p < 0.05$) compared with group C (Fig. 4).

Table 1
Effects of hydroalcoholic extract from *Solidago chilensis* on a glucose tolerance curve (mean ± SEM) ($n = 6$).

Groups	Glucose level (mg/dl)			
	Initial (time zero)	30 min	60 min	180 min
C	93.6 ± 2.0	128.5 ± 4.6	136.0 ± 1.1	126.0 ± 6.3
HE 125	95.5 ± 2.6	144.6 ± 4.9	150.1 ± 12.7	114.2 ± 5.8
HE 250	100.8 ± 3.2	151.6 ± 20.2	150.1 ± 6.1	114.2 ± 2.6
HE 500	91.0 ± 4.0	148.2 ± 10.1	142.2 ± 1.6	98.2 ± 2.3*
GLIB	93.3 ± 1.1	149.8 ± 5.5	149.3 ± 3.5	78.5 ± 8.9*

C, control; HE, hydroalcoholic extract from *S. chilensis* (125, 250 or 500 mg/kg); GLIB, glibenclamide (10 mg/kg).

* $p < 0.05$. One-way ANOVA, compared to group C in the respective time.

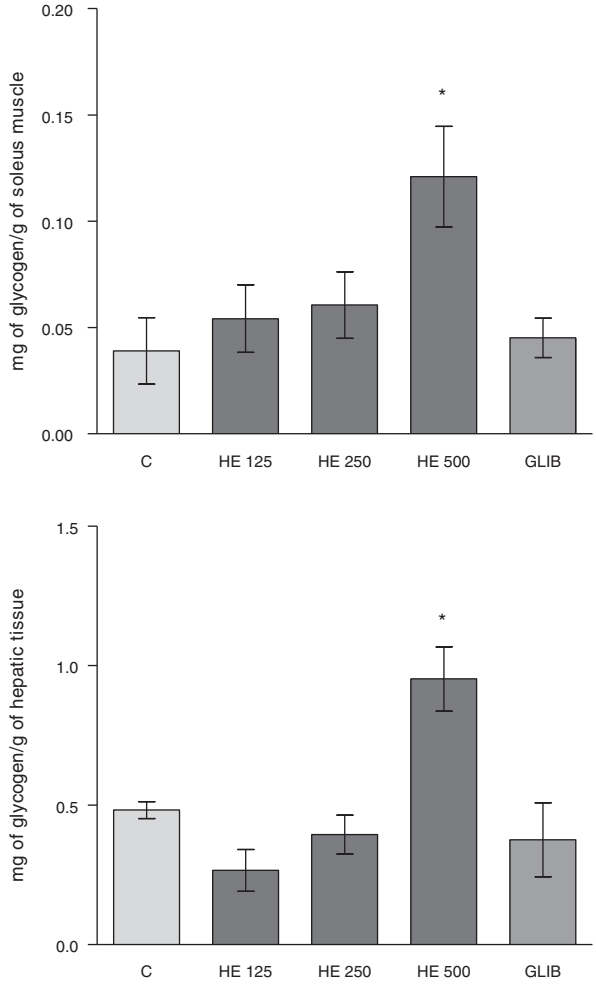

Fig. 3. Effect of hydroalcoholic extract from *Solidago chilensis* (HE; 125, 250 and 500 mg/kg) and glibenclamide (GLIB; 10 mg/kg) on hepatic and soleus glycogen content in hyperglycemic rats. Values are expressed as mean ± SEM ($n = 6$). $^{*}p < 0.05$ one-way ANOVA compared to the control group (C).

Effects of HE on TC and ALT

Following treatment, group C rats had higher serum TC than rats in the other groups. All hyperglycemic rats treated with HE (125, 250 or 500) exhibited an accentuated decrease in TC (−36.8%, −36.7% and −41.3%, respectively), compared with group C

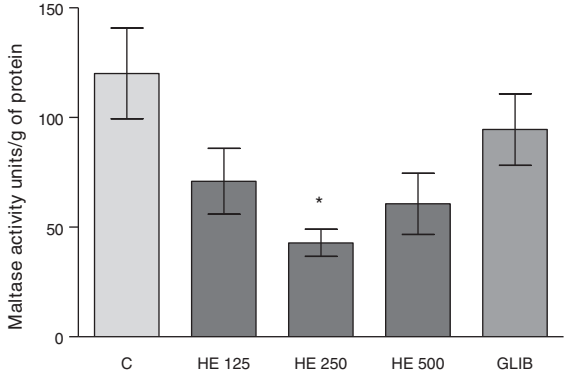

Fig. 4. Effect of hydroalcoholic extract from *Solidago chilensis* (HE; 125, 250 and 500 mg/kg) and glibenclamide (GLIB; 10 mg/kg) on the specific activity of maltase, in a segment of the small intestine. Values are expressed as mean ± SEM ($n = 6$). $^{*}p < 0.05$ one-way ANOVA compared to control group saline (C).

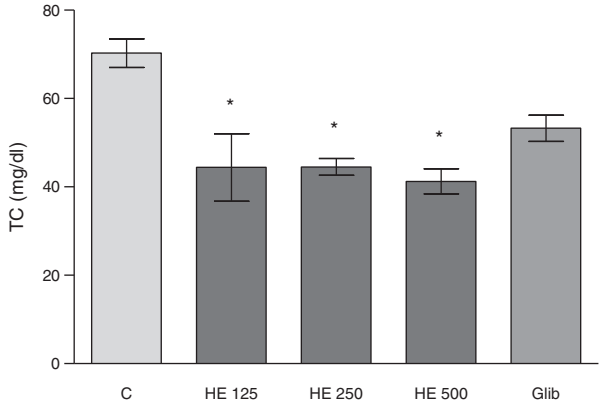

Fig. 5. The effects of treatments on total cholesterol (TC) values (mean ± SEM; $n = 6$). Hyperglycemic rats were given saline (control group; C) or the following treatments: hydroalcoholic extract from *Solidago chilensis* (HE; 125, 250 or 500 mg/kg); glibenclamide (GLIB; 10 mg/kg). $^{*}p < 0.05$ one-way ANOVA compared to control group saline (C).

($F_{(4, 23)} = 5.7$; $p < 0.05$; Fig. 5). There was no difference in serum ALT activity between the groups (data not shown).

Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia. It is associated with alterations in carbohydrate, protein, and lipid metabolism (Pereira et al., 2011). Plants exert antihyperglycemic and hypoglycemic activity primarily via their ability to restore pancreatic tissue function by increasing insulin output, inhibit intestinal absorption of glucose, or facilitate metabolites in insulin-dependent processes (Patel et al., 2012).

The present study showed that a glucose dose of 4 g/kg can considerably increase rats serum glucose levels, which were mitigated by a single oral dose of HE at 500 mg/kg for 180 min following glucose administration. Recently, it was demonstrated that rutin reduces serum glucose levels and potentiates *in vivo* insulin secretion (Kappel et al., 2013); rutin mechanism of action can also be explained by mammals synthesizing glycogen to maintain appropriate glucose levels. Glycogen is how mammals store glucose for future use, mainly in skeletal muscles and the liver (Jensen et al., 2011). Insulin and glucagon regulate glycogen metabolism by activating and inhibiting several enzymes and proteins (Ferrer et al., 2003); the healthy organism removes serum glucose rapidly when glucose is in excess, but insulin-stimulated glucose disposal is reduced inorganisms with insulin resistance and type 2 DM (Jensen et al., 2011). In the present study, rats administered HE 500 had significantly increased glycogen content in the liver and soleus compared with rats in group C, which helped lead to the HE 500 rats lower serum glucose levels. In agreement with Torres et al. (1987), the phytochemical analysis by HPLC confirmed that the quercetrin flavonoid is the major bioactive substance of *S. chilensis*. Flavonoids may exert beneficial effects in DM by enhancing insulin secretion; reducing apoptosis and promoting proliferation of pancreatic β-cells; improving hyperglycemia through regulating hepatocyte glucose metabolism; reducing insulin resistance, inflammation, and oxidative stress in muscle and fat; and increasing glucose uptake in skeletal muscle and white adipose tissue (Babu et al., 2013). This finding is in agreement with Prasath and Subramanian (2011), who reported the antidiabetic effect of the flavonoid fisetin (tetrahydroxyflavone) in a study conducted with diabetic rats. Oral administration reduced serum glucose and increased serum insulin concentrations. Further, treatment with HE 500 increased glycogen levels and the activity of glycogen synthase, whereas it suppressed glycogen phosphorylase, suggesting that flavonols may improve

glucose homeostasis by modulating enzymes that regulate carbohydrate metabolism. It was also demonstrated that kaempferitrin, the major flavonoid found in *Bauhinia forficata* Link., leaves, is able to diminish serum glucose levels and increase glucose uptake in the rat soleus as efficiently as insulin (Jorge et al., 2004). This effect could be related to the increased muscle glycogen content seen in the present study with the HE 500 treatment.

In the present study, we demonstrated that HE 250 reduced maltase activity. Several plants exert antihyperglycemic activity via inhibiting enzymes that hydrolyze carbohydrates in the small intestine, and the effect appears to involve interactions with polyphenolic compounds (Mai and Chuyen, 2007). Although HE 500 more significantly lowered rats serum glucose levels, it did not significantly inhibit maltase activity. This finding is in agreement with Pereira et al. (2011, 2012), who found that higher doses of extracts and substances, did not affect disacharidase activity. These findings reinforce the idea that HE affects serum glucose by increasing glucose storage (as glycogen) in the liver and muscle.

Rats treated with HE (125, 250 or 500 mg/kg) showed decreased serum TC. A previous study using other plants suggested that these effects can be attributed to the restoration of triacylglyceride catabolism by stimulating lipolytic pathways involving plasma lipoprotein lipase (Xie et al., 2007). In the present study, HE could have stimulated similar effects. Intracellular glucose and lipid metabolic disorders are the basis of a variety of metabolic diseases. Glucose and lipid metabolic disorders are closely related to the occurrence and progression of DM, obesity, hepatic steatosis, and cardiovascular disease (Meng et al., 2013).

We cannot discount the possibility that HE also interferes with cholesterol's metabolic cycle at other points, such as intestinal uptake, endogenous metabolism, and transport by lipoproteins (Bei et al., 2012; Roman-Junior et al., 2015), which were not assessed in this study.

Free radical scavenging properties of *S. chilensis* were observed in the DPPH assay. Thus, we propose that the plants hydroalcoholic extract may have contributed to the hyperglycemic rats improved lipid metabolism and oxidative stress. This is characteristic of polyphenols (Liu et al., 2014); however, further studies are required to confirm the *in vivo* antioxidant effects of *S. chilensis* and its benefits in hypoglycemic and hypercholesterolemic animal models. Levels of ALT did not differ between treatment groups, indicating the absence of HE toxicity at the doses tested.

In summary, our results showed that HE exerted marked hypoglycemic effects via increasing the production and release of insulin as well as via increasing insulinotropic activity. The hypolipidemic effect of HE in rats possibly involved reduced levels of lipoproteins as well as antioxidant activity. Furthermore, there was strong evidence that quercetrin, the major constituent of *S. chilensis* extracts, is largely responsible for the observed biological activities. However, the underlying mechanisms of these effects need to be elucidated by further studies.

Conclusions

Hydroalcoholic extract of *S. chilensis* may be effective in maintaining glucose homeostasis by reducing serum glucose levels and TC.

Conflicts of interest

The authors declare no conflicts of interest.

Authors contribution

MS, APS and MG contributed in all steps of this study. EI, FB, GDS, AS, RC and RM contributed to biological studies. EF, GP and SMW contributed to biochemical analyses. LZ and WARJ have guided the laboratory work and contributed to design of the study. All the authors have read the final manuscript and approved the submission.

Acknowledgements

This work was supported by the Unochapecó [modality Art. 171 – FUMDES], CNPq-PIBIC (edital N° 228/Reitoria/2014), PIBIC-FAPE (edital N° 121/Reitoria/2013) and FAPESC.

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