Original Research Article

Hypoglycemic effect of Berberis microphylla G Forst root extract

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Abstract

Purpose: To evaluate the effect of the root extract of Berberis microphylla on glucose uptake and AMPK-activated protein kinase (AMPK) activity in non-resistant and insulin-resistant HepG2 cells.
Methods: B. microphylla root was extracted with absolute ethanol, filtered, concentrated and lyophilized. Subsequently, liver cells, HepG2 (resistant and non-insulin resistant), were exposed for 24 h to different concentrations of the extract (10, 5, 2.5 and 1.25×10^{-3} μg/μL) to determine the stimulation of glucose uptake and phosphorylation of AMPK.
Results: In HepG2 cells without resistance exposed to B. microphylla root extract, glucose uptake varied from 34 to 59 % of the available glucose while AMPK phosphorylation was 1.9 to 3.6 times the phosphorylation of the control. In insulin-resistant HepG2 cells, glucose uptake varied from 68 to 95 % of available glucose while AMPK phosphorylation was 1.8 to 3.3 times the phosphorylation of the control.
Conclusion: The root extract of B. microphylla possesses hypoglycemic effects and stimulates glucose uptake in HepG2 cells with and without resistance by activating AMPK protein.

Keywords: Calafate, Diabetes, Antihyperglycemic effect, Phytomedicine, Berberis, Insulin resistance

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a highly prevalent pathology, which has become a major public health problem worldwide [1]. Currently, it is considered a global epidemic since its prevalence has tripled during the last 30 years [2]. T2DM is a metabolic disorder characterized by high levels of blood glucose due to a deficiency in the action and secretion of insulin [3]. In the last years, AMP-activated protein kinase (AMPK) has been shown to be involved in regulating the energy balance by controlling the metabolism of glucose and lipids [4]. At present, several drugs that target AMPK are available for the treatment of T2DM [5], foremost among them metformin (first-line drug for the treatment of T2DM) [6]. Lamentably, 30 % of patients do not respond favorably to this treatment because they develop digestive disorders (diarrhea and vomiting), which can cause the discontinuation of the treatment with this drug [7]. Thus, the search for new alternative medicines for the treatment of this pathology is necessary. The *Berberis* genus has emerged as a phytotherapeutic alternative as several species of this genus are described as

having hypoglycemic potential, such as *B. lycium*, *B. aristata*, *B. asiatica*, *B. vulgaris*, *B. integerrima*, *B. ceratophylla*, *B. moranensis* and *B. crataegina* [8].

B. microphylla G. Forst is a South American species that has been utilized in ethno medicine for the treatment of febrile states, gastric pain and cold, among others [9]. At present, it is used as an alternative medicine for the treatment of T2DM. However, no scientific study assessing the antidiabetic activity of this plant has been reported. Therefore, the objective of this study is to evaluate the effect of *B. microphylla* root extract on glucose uptake and AMPK activity in non-resistant and insulin-resistant HepG2 cells.

EXPERIMENTAL

Extract preparation

The roots of *B. microphylla* were collected in the settlement of Bahía Mansa (53° 36' 39, 38'' S and 70° 55' 50, 56'' O) near the city of Punta Arenas, Chile. A sample of the species was identified by Dr Juan Marcos Henríquez, botanist and taxonomist at the Instituto de la Patagonia, Universidad de Magallanes, Chile (voucher no. 012837). The collected roots were cut up and dried at room temperature for 30 days; subsequently, the pieces were ground and 100 g of the dried root were extracted with 1000 mL absolute ethanol for 72 h at room temperature. The extract was filtered and concentrated in a rotary evaporator at 40 °C. Finally, it was lyophilized and stored at 4 °C until use.

HepG2 cell line

HepG2 cells were purchased from the American Type Culture Collection (ATCC). Cells were maintained at 37 °C in a 5 % CO_2 atmosphere in low glucose DMEM medium (1 mg/mL glucose), supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL). Prior to each experiment, the cells were plated in 96-well plates at a density of 10^4 cells/well. The growth medium was replaced with medium supplemented with 1 % FBS; and different concentrations of the root extract of *B. microphylla*, Metformin® (Mt) and Berberine® (Bb) were incubated for 24 h.

MTT assay

HepG2 cells were seeded into 96-well multi-plates under the conditions previously described. Subsequently, the medium was removed, and 20 µL of (4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazolium (MTT, 5 mg/mL) was added to each well and incubated for 4 h. The resultant formazan crystals were dissolved in 200 µL of dimethylsulfoxide (DMSO). Optical density (OD) was measured at 570 nm in a microplate reader.

Glucose consumption

HepG2 cell models without resistance were seeded into 96-well plates for 24 h under the conditions previously described. *B. microphylla* root extract (10, 5, 2.5 y 1.25 x 10^{-3} µg/µL), Mt (0.25 x 10^{-3} µg/µL) and Bb (0.25 x 10^{-3} µg/µL) were added to the fresh medium at different concentrations in respective wells. The control consisted of cells without treatment.

The glucose consumption was quantified using the enzymatic-colorimetric method GOD-PAP (Glucose Liquicolor, Germany). Quantification of glucose uptake was calculated by obtaining the difference between the initial glucose content (t = 0 h) and the final glucose content (t = 24 h) in the medium.

Insulin resistance in HepG2 cells was induced following the method previously described by Xie *et al* [10], with modifications. The cells were seeded into 96-well -plates with fresh medium, which contained 2 % FBS, 1 % antibiotic and 100 nM bovine insulin for 24 h. The resistance was confirmed by control cells without treatment (without insulin). Subsequently, the medium was replaced with a medium containing 2 % FBS, 1 % antibiotic and *B. microphylla* root extract at different concentrations (10, 5, 2.5 y 1.25 x 10^{-3} µg/µL), Mt (0.25 x 10^{-3} µg/µL) and Bb (0.25 x 10^{-3} µg/µL) for 24 h.

AMPK phosphorylation

HepG2 cell model without and with resistance were exposed to different concentrations of *B. microphylla* root extract (10, 5, 2.5 y 1.25 x 10^{-3} µg/µL), Mt (0.25 x 10^{-3} µg/µL) and Bb (0.25 x 10^{-3} µg/µL). Subsequently, glucose consumption was quantified. To determine the degree of AMPK phosphorylation in the cells, the Enzy FluoTM AMPK phosphorylation assay kit (EAMPK-100) was used according to the protocol indicated by the manufacturer.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range method. Values were considered statistically significant when $p < 0.05$.

RESULTS

HepG2 cells viability *vs.* exposure to extract of *B. microphylla* root, Mt and Bb

The percentage of viability of HepG2 cells, which had been exposed to different concentrations of *B. microphylla* root extract, Mt and Bb, was determined through the use of the MTT test, using cells without treatment as control. Table 1 shows that 100 % viability of HepG2 cells exposed to *B. microphylla* root extract occurs at a 10 x 10^{-3} µg/µL concentration. Starting from this concentration, the following tests were carried out using lower concentrations. On the other hand, as shown in Table 2, 100 % cell viability after exposure to Mt occurs at a concentration of 4 x 10^{-3} µg/µL, and with Bb occurs at a concentration 0.25 x 10^{-3} µg/µl. Therefore, for comparative purposes in the following assays, this latter concentration will be chosen for both compounds.

Table 1: Cell viability of HepG2 cells treated with *B. microphylla* root extract

Concentration (x10^{-3} µg/µL)	Cell viability (treated with *B. microphylla*) (%*, mean ± SD)
Control	100 ± 0
2.5	100 ± 9.1
5	100 ± 8.6
10	100 ± 2.4
20	91.3 ± 6.5
40	65.2 ± 9
80	48.0 ± 1.0
160	35.5 ± 1.3

Data expressed as % viability compared to control cells (100% survival); data are mean ± SD (n = 3)

Table 2: Cell viability of HepG2 cells treated with Mt and Bb

Concentration (x 10^{-3} µg/µL)	Cell viability (treated with Mt) (%*, mean ± SD)	Cell viability (treated with Bb) (%*, mean ± SD)
Control	100 ± 0	100 ± 0
0.25	100 ± 4.6	100 ± 2.2
0.5	100 ± 5.6	83.9 ± 8.3
1	100 ± 7.3	82.89 ± 2.7
2	100 ± 5.1	61.09 ± 2.2
4	100 ± 6.3	57.6 ± 2.4
8	97.9 ± 3.2	42.2 ± 4.2
16	93.3 ± 2.3	38.2 ± 2.9

Data expressed as % viability compared to control cells (100% survival); data are mean ± SD (n = 3)

Glucose consumption stimulation

The percentage of glucose consumption in non-insulin-resistant and insulin-resistant HepG2 cells stimulated with *B. microphylla* root extract, Bb and Mt, was determined for a period of 24 h. It was observed that *B. microphylla* root extract, Bb and Mt significantly stimulated glucose consumption at all concentrations tested with respect to the control for both cells models. This finding determines a positive correlation between the dose of *B. microphylla* root extract and glucose uptake.

In the non-resistant HepG2 cells, *B. microphylla* root extract increased glucose uptake statistically significantly (different letters) by 59, 53, 45 and 34 % in the concentrations of 10, 5, 2.5 and 1.25 (x10^{-3}) µg/µl, respectively, as shown in Figure 1. The response to Bb and Mt, equally, was significant with respect to the control.

In resistant HepG2 cells, the percentage of glucose uptake increased significantly (different letters) by 95, 91, 84 and 68 % for the concentrations of 10, 5, 2.5 y 1.25 x 10^{-3} µg/µl, (Figure 1), respectively. Bb and Mt increased the percentage of glucose uptake by 73 and 81 %, which is likewise statistically significant.

AMPK activation

To determine the stimulation pathway of *B. microphylla* root extract, the activation of AMPK in HepG2 cells (non-insulin-resistant and with insulin-resistant) was assessed. As shown in Figure 2, the stimulation of AMPK by *B. microphylla* root extract in both cell models is statistically significant in all tested concentrations (different letters) as opposed to the control (untreated cells). A dose-response relationship was generated; that is to say, as the concentration of *B. microphylla* root extract increases, the stimulation of AMPK grows as well. In non-resistant HepG2 cells, *B. microphylla* root extract significantly increases AMPK phosphorylation to 3.6, 2.7, 2.3 and 1.9 times more than the phosphorylation in the control at all doses tested, 10, 5, 2.5 y 1.25 x 10^{-3} µg/µl, respectively. Bb and Mt also significantly stimulate the phosphorylation of AMPK to 2.9 and 1.7 times more than the phosphorylation in the control.

In insulin-resistant HepG2 cells exposed to the *B. microphylla* root extract, the stimulation of AMPK at the doses 10, 5, 2.5 y 1.25 x 10^{-3} µg/µl was 3.3, 2.5, 2.2 and 1.8 times greater than that of the control, respectively. The AMPK response stimulated by Bb and Mt was also 2.8 and 1.7 times greater than that of the control. All the responses differ significantly from the control.
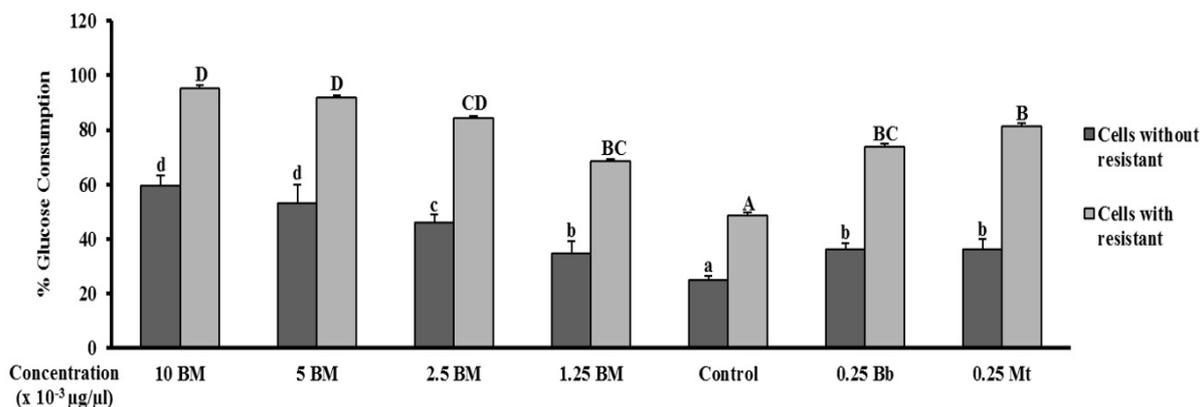
Figure 1: Glucose uptake in insulin-resistant and non-resistant HepG2 cells exposed to different concentrations of *B. microphylla* root extract, Mt and Bb**.** Different letters (**A, B, C, D** for insulin-resistant cell and **a, b, c, d** non-insulin-resistant cell) have significant difference by Duncan's test ($p < 0.05$).
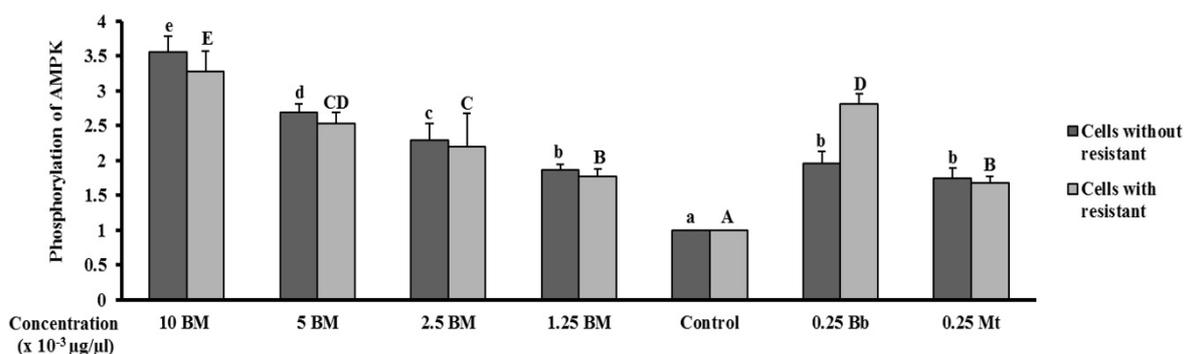

Figure 2: Activation of AMPK protein phosphorylation by exposure to the extract of *B. microphylla* root, Mt and Bb in insulin-resistant and non-resistant HepG2 cells**.** Different letters (**A, B, C, D** for insulin-resistant cell and **a, b, c, d** non-insulin-resistant cell) have significant difference by Duncan's test ($p < 0.05$)

DISCUSSION

Berberis genus has been used as an alternative therapy for the treatment of diabetes or as a hypoglycemic agent by several indigenous peoples around the world. From here, the genus potentially emerges as a drug to combat this pathology [8]. In the literature, there are several reports, developed mainly in animal models, which highlight the hypoglycemic activity of the *Berberis* genus. These works show that this plant species generates a decrease in blood glucose levels in different murine species [11–19]. Berberine, the active compound of this species, is also described as a glucose uptake stimulator in HepG2 cells [20]. Similarly, the root extract of *B. microphylla* stimulates the uptake of glucose in HepG2 cells, beginning with the tested concentration of 1.25 x 10-3 µg/µL; the root extract also exerts this same effect on resistant hepatic cells at the sameextract also exerts this same effect on resistant hepatic cells HepG2 at the same concentration and in both cases generates a dose-dependent response.

With respect to possible mechanisms of action, *B. julianae* extract has been shown to increase translocation and expression of glucose transporter GLUT4 in muscle cells L6, causing increased glucose uptake as well as increasing the phosphorylation of AMPK in the hepatic and muscular tissue of mice [21]. In the same manner, it has been demonstrated in mouse muscle cells that berberine is involved in the activation of AMPK and p38 MAPK [22].

When exposing HepG2 cells with and without insulin resistance to different concentrations of *B. microphylla* root extract, an increasing AMPK phosphorylation in both experiments could be determined, which generated a positive correlation; that is to say, as the extract concentration increased, the phosphorylation of AMPK also grew.

This may happen because the increase in the activity of AMPK in hepatic cell lines leads to a decrease in the expression of glucose-6-

phosphatase (G6Pase) mediated by post-translational silencing of its transcription factor, FOXO1a [23,24]. However, further studies are still needed to clarify this mechanism of repression.

AMPK acts as a master metabolic switch in response to alterations in the cellular energy load and plays an important role in energy homeostasis through the coordination of adaptive responses in low energy metabolic states [25]. In the literature, there have been a number of reports on AMPK activators, such as AICAR, metformin, rosiglitazone and leptin, as well as natural products including berberine, caffeic acid phenethyl ester (CAPE), epigallocatechin-3-gallate (EGCG), nicotine, β-sitosterol, and corosolic acid, which have been used as potential drugs in the treatment of type 2 diabetes [26].

CONCLUSION

We can affirm that the root extract of *B. microphylla* would have beneficial therapeutic effects against diabetes because it is capable of increasing glucose consumption under conditions of no resistance and insulin resistance. The possible mechanism of action would be the stimulation of AMPK. This mechanism explains, in part, why *B. microphylla* would show hypoglycemic activity and may be used in the treatment of type 2 diabetes mellitus. However, further research is needed for a complete understanding of the underlying actions of its different phytochemical components.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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