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Hypericum caprifoliatum and *Hypericum connatum* affect human trophoblast-like cells differentiation and Ca^{2+} influx

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PEER REVIEW

Peer reviewer

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Comments

The investigators use a number of key techniques to evaluate the effects of *Hypericum* extracts on trophoblast function. They found various effects dependent on (1) type of *Hypericum* species, (2) type of extract (methanol versus hexane), (3) type of cell (fusiogenic versus proliferative). In most cases, observed effects were concentration-dependent allowing for the investigators to determine in which range *Hypericum* extracts become toxic to the trophoblast.

Details on Page 372

ABSTRACT

Objective: To study the effect of crude methanol and *n*-hexane extracts of *Hypericum connatum* (*H. connatum*) and *Hypericum caprifoliatum* on trophoblast-like cells.

Methods: BeWo and JEG-3 trophoblast-like cells were submitted to different extract concentrations (1, 5, 10 and 15 μg/mL) and evaluated in relation to cell viability and *in vitro* trophoblast differentiation and function. Cell viability was evaluated using WST-1 reagent. Differentiation was measured by luciferase production, hCG production/release, and mitogen-activated protein kinase signaling pathway activation. The function of the trophoblast-like cells was measured by $^{45}Ca^{2+}$ influx evaluation.

Results: The results showed a decrease in cell viability/proliferation. Both plants and different extracts induced a significant decrease in hCG production/release and luciferase production. *H. connatum* did not cause mitogen-activated protein kinase signaling pathway disturbance; however, *Hypericum caprifoliatum n*-hexane extract at 15 μg/mL inhibited extracellular signal-regulated kinase 1/2 activation. The significant increase in Ca^{2+} influx by JEG-3 cells was seen after short and long incubation times with *H. connatum* methanolic extract at 15 μg/mL.

Conclusions: The results indicated that these two *Hypericum* species extracts can interfere on trophoblast differentiation and Ca^{2+} influx, according to their molecular diversity. Although *in vivo* experiments are necessary to establish their action on placental formation and function, this study suggests that attention must be paid to the potential toxic effect of these plants.

KEYWORDS

BeWo, JEG-3, Hypericaceae, ERK1/2, p38, Ca^{2+} influx

1. Introduction

The genus *Hypericum*, a member of the Hypericaceae family, has received attention due to the antidepressant activity of *Hypericum perforatum* L. (*H. perforatum*), known as St. John's Wort. *H. perforatum* is widely used in human therapeutics for wound healing and to treat mild to moderate depression[1-3]. From this plant, several active compounds have been identified

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and studies have confirmed the antidepressant activity of the total extract and its constituents[4,5]. Especially regarding the hyperforin, studies have shown that this phloroglucinol derivative is related to significant effects on the voltage-dependent Ca^{2+} neuron channels[6,7].

Hypericum native Southern Brazil, are promising plants for therapeutic usage. As they produce neither hypericin nor pseudohypericin, they offer low phototoxic risk[8], one of the therapeutic limits of *H. perforatum*. Although Brazilian species do not present the naphthodianthrones found in *H. perforatum*[9], several other compounds are found, and dimeric acylphloroglucinols - such as uliginosin B, japonicin A, and hyperbrasilol B - are considered their chemotaxonomic markers[10]. A wide variety of compounds with different biological activities[9,11], including antidepressant[12,13], and anti-herpes virus activities were described for such markers/dimeric acylphloroglucinols[14].

The mammalian placenta is crucial to pregnancy. This transient organ allows the fetus to develop and maintain the pregnancy, in addition to being responsible for maternal-fetal exchange[15-17]. The function of the human placenta depends upon the successful formation and expansion of syncytiotrophoblast, which originates from trophoblast cell differentiation[17]. The trophoblast differentiation is regulated by multiple interacting signaling pathways including mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases 1/2 (ERK1/2) and the p38 enzymes[18,19]. The main functions of syncytiotrophoblasts are absorption, exchanges, and specific hormonal secretion[17]. Syncytiotrophoblasts are the primary site of fetal Ca^{2+} homeostasis regulation, which includes mechanisms characterized by Ca^{2+} entry, cytosolic diffusion and extrusion[16].

Thus, taking into consideration the potential biological properties of *Hypericum* species and their phytochemical diversity, we have chosen two species with antiviral and antidepressant properties that could be used as alternative to mild depression or infections by pregnant women, and verified their effect on trophoblast viability/proliferation, differentiation, and Ca^{2+} influx. To study the effect of *Hypericum connatum* (*H. connatum*) and *Hypericum caprifoliatum* (*H. caprifoliatum*) on placental development and function, we used two *in vitro* models: the non-fusogenic JEG-3 cells and the fusogenic BeWo cells[20].

2. Materials and methods

2.1. Plant material

H. connatum and *H. caprifoliatum* (Guttiferae) were collected through botanical field work in the state of Rio Grande do Sul, Brazil, in December 2006. The voucher specimens were deposited in the herbarium of the Federal University of Rio Grande do Sul (ICN/UFRGS). The plant collection was authorized by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) (n. 003/2008; Protocol 02000.001717/2008 - 60).

2.2. Preparation of extracts

The dried and powdered plant materials were thoroughly extracted with *n*-hexane (3×24 h) at room temperature. The fractions were pooled, evaporated to dryness under reduced pressure and then stored at -20 °C until biological evaluation and high performance liquid chromatography analyses. The plant materials were also extracted with methanol using the same procedure.

2.3. Chemical analysis

The *n*-hexane extracts were analyzed by thin layer chromatography and high performance liquid chromatography, as described elsewhere[10,11].

2.4. Preparation of extracts for biological tests

For biological tests, the methanolic and *n*-hexane extracts were initially solubilized at 100 mg/mL in 100% sterile dimethyl sulfoxide (DMSO). Thus, a stock solution of 5 mg/mL in Ham's-F12 medium (Gibco®, Montréal, CA) or modified Eagle's medium (MEM-Gibco®, Montréal, CA) was prepared, distributed in aliquots, and stored at -20 °C until use. Immediately before biological tests, different concentrations of 5, 10, 15, and 30 μg/mL were prepared using Ham's F-12 medium or MEM supplemented with 10% fetal calf serum.

2.5. Cell lines

The human placental choriocarcinoma cell line, JEG-3 (ATCC - HTB-36 Manassas, VA, USA) and BeWo (ATCC - CCL-98 Manassas, VA) cells were used to perform the biological assays. Cells were maintained in Ham's F-12 or MEM, respectively, supplemented with 10% FBS in 5% CO_2 at 37 °C. For all tests, control consisted of cells treated with DMSO (<0.01%; v/v). Cells were used for different purposes: JEG-3 cells were used to investigate the events related to Ca^{2+} transport by trophoblast cells, while BeWo cells were used to evaluate the effect of plant extract on cell differentiation. To induce the differentiation state in BeWo cells, 50 μmol/L of forskolin α-(methylamino) isobutyric acid (Sigma-Aldrich, Canada) were used. Tests were carried out after 48 h of incubation. In order to quantify the cell fusion, 24 h seeded BeWo cells - stable transfected with pNL4.3ΔBstAsLuc and hpNL4.3ΔBstAsTat plasmids - were treated with the different concentrations of plant extracts and controls. After 24 h incubation at 37 °C, the supernatants were discharged and cells were submitted to disruption using lyses buffer (25 mmol/L Tris phosphate, pH 7.8, 2 mmol/L DTT, 1% Triton X-100, 10% glycerol). The luciferase activity were measured with Dynex MLX microplate luminometer (MLX Dynex Technologies, Chantilly, VA) with one single injection of luciferase buffer (20 mmol/L tricine,

1.07 mmol/L $(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$, 2.67 mmol/L $MgSO_4$, 0.1 mmol/L EDTA, 220 μmol/L coenzyme A, 4.7 μmol/L D-Luciferin potassium salt, 530 μmol/L ATP, 33.3 mmol/L DTT).

2.6. Cell viability

The cell viability procedure was carried out by seeding 10^3 cells/well in 96-well plates. After 24 h of the seeding, cells were treated with different extract dilutions and/or controls for 48 h. Controls consisted of cells treated with DMSO only and hydrogen peroxide (H_2O_2, 30% solution; Sigma-Aldrich, Canada) at 50 μmol/L, an oxidative stress inducer[21]. Cell viability was determined by the WST-1 based colorimetric assay (Roche Applied Science, Laval, Canada). For that, WST-1 reagent was added to the cells treated for 48 h and incubated at 37 °C and 5% CO_2. After 4 h of incubation, the absorbance was measured with a wavelength of 450 nm using a reference wavelength of 655 nm. Also, microscopic analysis was done every day by inversed light microscope to observe cell morphological appearance and confluence.

2.7. hCG production

For hCG release detection, BeWo cells were seeded in 24-well plates at a density of 4.5×10^4/well. After 24 h of the seeding, cells were treated with 5 and 15 μg/mL of the methanolic and *n*-hexane extracts. After 48 h of treatment, the influence of plant extract on hCG production in the supernatant was measured using ELISA kit (DRG®, Germany) which included the detection of the β chain of the hCG molecule. For β-hCG dosage, 50 and 200 fold diluted samples were used according to the manufacturer's specifications. Conditioned media were harvested, centrifuged and frozen at − 20 °C. The optical density was determined using 570 nm wave length and samples' values were established from a standard curve.

2.8. Trophoblast differentiation/proliferation cell signaling pathway

ERK1/2 and p38 phosphorylations were used to analyze the effect of *Hypericum* extracts on trophoblast cell differentiation pathway. For this purpose, cells were seeded in 9.2 cm^2 Petri dishes (1.8×10^5 cell/dish) and incubated for 24 h in 5% CO_2 and 95% humidity. Then, cells were treated with plant extract at 5 and 15 μg/mL. After 48 h of incubation, the cells were harvested for the western blot technique to be performed. The protein preparation and electrophoresis conditions were based on materials and methods described elsewhere[22]. Membranes were then incubated overnight at 4 °C with primary antibodies: antiphospho-ERK1/2 (1/1 000, Cell Signaling, Beverly, MA, USA) and antiphospho-p38 (1/1 000, Cell Signaling, Beverly, MA, USA) in TBS-T/5% BSA. Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Rabbit mAb detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when dually phosphorylated at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2), and singly phosphorylated at Tyr204. After overnight incubation, membranes were washed three times with TBS-T, and probed with secondary antibodies (1/2 500 for antirabbit-IgG, Cell Signaling, Beverly, MA, USA) for 2 h at room temperature. Blots were washed three times with TBS-T and the detection was performed using the BM chemiluminescence system (Roche Applied Scientific, Laval, Canada) and visualized by autoradiography. After stripping with 0.2 mol/L glycine, 0.5 mol/L NaCl, pH 2.5, membranes were similarly stained with anti-ERK1/2 (1/1 000, Cell Signaling, Beverly, MA, USA) and anti-p38 (1/1 000, Cell Signaling, Beverly, MA, USA); p38 MAPKinase antibody detects endogenous levels of total p38α, -β or -γ MAPK protein. After overnight incubation, membranes were washed three times with TBS-T, and probed with secondary antibodies (1/2 500 for antirabbit-IgG) for 2 h at room temperature. Mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH - 36 KDa, 1:5 000 in TBST-5% BSA, Chemicon, Tenecula, CA) and goat anti-mouse-IgG HRP-conjugated (1:10 000 in TBST-5% milk, Chemicon, Tenecula, CA) as second antibody were used to normalize the protein amount. Quantification was carried out by digitizing the images with a computer-based image analysis system and measuring the band intensities in grey-scale pixel values (Image J 1.38x, National Institutes of Health, USA).

2.9. Ca^{2+} uptake

Radiolabeled Ca^{2+} ($^{45}CaCl_2$, MP/ICN Biochemicals - Irvine, CA, USA) was used to evaluate the total cell Ca^{2+} incorporation. JEG-3 cells were seeded at 2.5×10^5 of density/well in 24 well plates. After 24 h of seeding, cells were treated with *H. connatum* and *H. caprifoliatum* (5 and 15 μg/mL) and vehicle (DMSO). Subsequently, after 24 h of treatment, cells were washed twice with 500 μL of Hank's balanced salt solution buffer containing 1.26 mmol/L of $CaCl_2$ and 0.1% BSA (HBSS 1.26 mmol/L-BSA) and incubated at 37 °C with 250 μL of this same solution for 10 min to stabilize. Then, HBSS 1.26 mmol/L-BSA containing $^{45}CaCl_2$ (5 μCi/well) was applied to each well. After different time points varying from 0 to 15 min, the incubation was stopped and cells were washed three times with 1 mL of cold phosphate buffer saline (4 °C) containing 4 mmol/L ethylene glycol tetraacetic acid. Cells were then solubilized in 500 μL of 0.5 mol/L NaOH, and $^{45}Ca^{2+}$ cell-associated radioactivity was measured by a β-scintillation 1.400TM counter (PerkinElmer Inc., USA). The cellular protein content of each well was evaluated by spectrophotometric quantification using BCA reagent (Pierce, Rockford, IL, USA) with BSA as standard.

Also, in order to verify the effect of *H. connatum* and *H. caprifoliatum* extracts on channels of the cell surface membrane, confluent JEG-3 cells cultures (48 h) were washed and let to stabilize as described above. Then, cells were treated with HBSS 1.26 mmol/L-BSA (CTL) or the same HBSS containing $CoCl_2.6H_2O$ (200 μmol/L), KCl at 25 mmol/L and 50 mmol/L, or *H. connatum* and *H. caprifoliatum* extracts at 5 and 15 μ/mL for 10 min, where the Ca^{2+} uptake is likely to depend mainly on membrane transfer[22]. Then, cells were treated with HBSS 1.26 mmol/L-BSA-$^{45}CaCl_2$ (5 μCi/well) for 0 and 1 min. The incubation was stopped and proteins were harvested as described above.

2.10. Statistical analysis

Paired *t*-test, and One-way ANOVA followed by Tukey's test were performed. Statistical analyses were performed using free trial GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

3. Results

The effect of two Brazilian *Hypericum* species, *H. connatum* and *H. caprifoliatum*, and the relationship with their compounds were studied. The chromatographic analysis of both *H. caprifoliatum* and *H. connatum* presented dimeric acylphloroglucinols, uliginosin B and hyperbrasilol B. Nevertheless, the main component of the *n*-hexane extract of *H. caprifoliatum* was the HC1, a tautomeric mixture of unresolved acylphloroglucinols. On the other hand, the methanolic extracts of the plants showed the presence of phenolic acid and flavonoids as main compounds. Both extracts presented chlorogenic acid, hyperoside, isoquercitrin, guaijaverin and quercitrin.

To study the effect of *H. connatum* and *H. caprifoliatum* extracts on placental development and function, we used two *in vitro* models: JEG-3 and BeWo cell lines. First, the results of the WST-1 solution technique and microscopic examination showed differences between plant species and respective extracts (Figure 1). The JEG-3 cells (Figure 1 A and B), the most proliferative trophoblast derived cell line, showed they were more sensitive to the effect of both extracts and plants than the fusogenic trophoblast derived cell line (BeWo cells) in relation to cytotoxic/antiproliferative property (Figure 1 C and D). In the case of 5 and 15 μg/mL *H. caprifoliatum n*-hexane extract, cells maintained morphologically unchanged as observed by microscopy, leading us to continue differentiation and Ca^{2+} transport experiments.

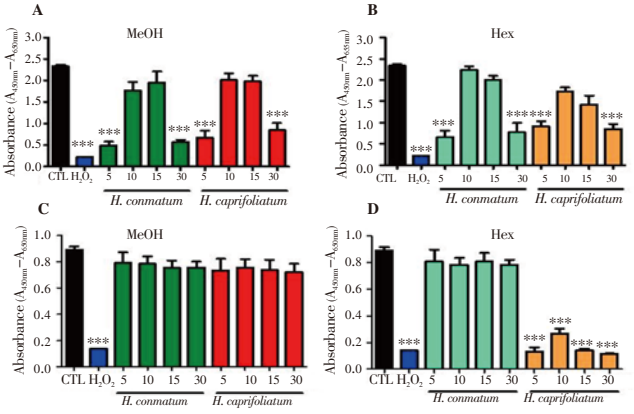

Figure 1. Cell viability of trophoblast-like cells treated with different doses of methanolic and hexane extract from the aerial parts of *H. connatum* and *H. caprifoliatum*. Cell viability was measured by WST-1 colourimetric assay. JEG-3 (A and B) and BeWo (C and D) cells were treated with either different concentrations (5, 10, 15, and 30 μg/mL) of *H. connatum* or *H. caprifoliatum* methanolic and hexane extracts. Controls consisted of cells treated with DMSO only (CTL) (< 0.5%) and hydroxide peroxide (H_2O_2) (30% solution). Statistical analysis were carried out using One way ANOVA followed by Tukey's multiple comparison tests and paired *t*-test (n=5).

The biochemical (hormone production) and morphological (cell fusion) differentiation of trophoblast-like cells (Figure 2) were also evaluated. For that purpose, we used the fusogenic BeWo cell line from which we measured the biochemical differentiation marker hCG hormone after forskolin or extracts stimulation. Also, we used two stable transfected cell lines derived from BeWo cells, $BeWo_{TAT}$ and $BeWo_{LUC}$. Results showed a statistically significant decrease in the hCG production by BeWo cells for all concentrations and plant extracts (Figure 2 A). However, the inhibition of cell fusion detected by luciferase transactivation gene expression was remarked only for *H. caprifoliatum n*-hexane extract at 15 μg/mL.

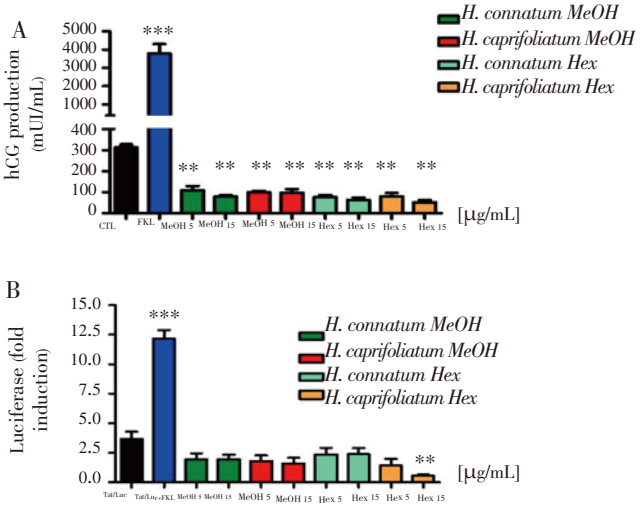

Figure 2. Effect of *H. connatum* and *H. caprifoliatum* on trophoblast-like biochemical differentiation (A) and fusion (B). Biochemical differentiation was verified by measuring the production of the differentiation marker, the human chorionic gonadotropin (hCG) hormone. For hCG release detection, BeWo cells were seeded in 24 well plates at a density of 4.5×10^4/well. After 24 h of seeding, cells were treated with extracts. After 48 h of treatment, the influence of plant extract on hCG production in the supernatant was measured using ELISA kit (DRG®, Germany), which included the detection of the β chain of the hCG molecule. For β-hCG dosage, 50 and 200 fold diluted samples were used following the manufacturer's specifications. Conditioned media were harvested, centrifuged and frozen at −20 °C. The optical density was determined using 570 nm wave length and sample's values were established from a standard curve. The BeWo cell fusion was assessed by luciferase enzyme expression. Control consisted of cells treated with DMSO only (CTL) or 50 μmol/L of isobutyric acid Forskolin α-(methylamino) (FKL) only. Data represent mean±SEM of three individual experiments. Statistical analysis were carried out using One way ANOVA followed by Tukey's multiple comparison test. $^{**}P<0.001$; $^{***}P<0.0001$.

Moreover, to evaluate the interference on trophoblast-like cells differentiation, MAPKs activation was evaluated through phosphorylated and total form protein expression. Activation (phosphorylation) of ERK1/2 and p38 MAPK was measured and the results showed a strong decrease only by *H. caprifoliatum n*-hexane extract at 15 μg/mL on the phosphorylated form of ERK but not p38 protein after 48 h of incubation (Figure 3).

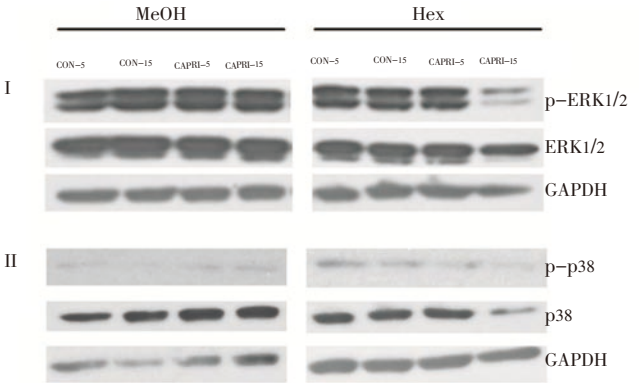

Figure 3. Effect of *H. connatum* and *H. caprifoliatum* on differentiation cell signaling pathways. Phosphorylation state (activation) of ERK1/2 (I) and p38 MAPK pathways (II) after 48 h of treatment were analyzed. The phosphorylation of ERK1/2 and p38 and total ERK1/2 and p38 were detected by western blot analysis. Treatment consisted of *H. connatum* (CON) and *H. caprifoliatum* (CAPRI) methanolic (MeOH) and *n*-hexane (Hex) extracts at 5 and 15 μg/mL. Mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was used as a control to measure cell protein expression.

To achieve the effect on trophoblast Ca^{2+} influx, a time dependent curve after 48 h treatment with plant extracts was performed (Figure 4). Results showed a significant increase in Ca^{2+} influx at plateau time (15 min) only for *H. connatum* at 15 μg/mL though all extracts showed a slight increase in Ca^{2+} internal concentration ($[Ca^{2+}]i$). The Ca^{2+} uptake due to the direct effect on cell surface channels (Figure 5) was verified using confluent JEG-3 cells that were treated for 10 min. The JEG-3 cells submitted to 200 μmol/L of $CoCl_2$ had a significant decrease in $[Ca^{2+}]i$ which confirmed the blocking of functionality of voltage-gated channels and store-operated Ca^{2+} channels in trophoblast cells. Conversely, cells treated with *H. connatum* at 15 μg/mL showed a significant increase on $[Ca^{2+}]i$.

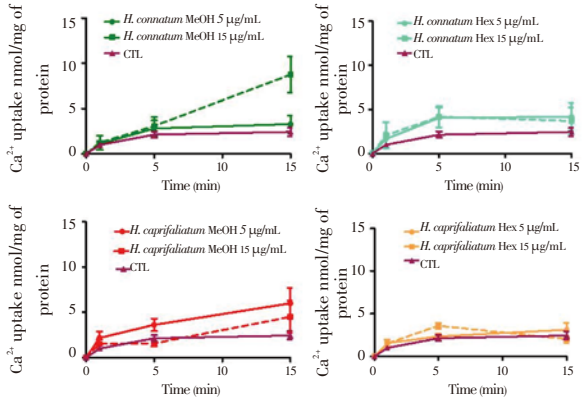

Figure 4. Effect of *H. connatum* and *H. caprifoliatum* on JEG-3 cell Ca^{2+} influx. Cells were pretreated for 24 h with *H. connatum* and *H. caprifoliatum* methanolic (MeOH) and *n*-hexane (Hex) extracts at 5 and 15 μg/mL, and control (DMSO only). The Ca^{2+} influx was carried out at different time points (0 to 15 min) using $^{45}Ca^{2+}$ and expressed as nmol of Ca^{2+} per mg of cellular proteins. Data represent mean±SEM of three individual experiments. Paired *t*-test (*P<0.05; **P<0.05; ***P<0.005) was used to analyze each time point.

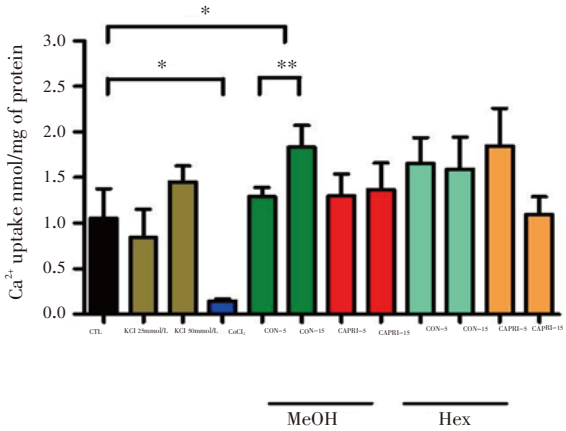

Figure 5. Effect of *H. connatum* and *H. caprifoliatum* on JEG-3 cell surface membrane controlled Ca^{2+} influx. Confluent JEG-3 cells were pretreated for 10 min with *H. connatum* [CON-5 (5 μg/mL) and CON-15 (15 μg/mL)], *H. caprifoliatum* [CAPRI-5 (5 μg/mL) and CAPRI-15 (15 μg/mL)], $CoCl_2.6H_2O$ ($CoCl_2$) (200 μmol/L), or KCl (25 mmol/L and 50 mmol/L) and analyzed for Ca^{2+} influx at 0 (zero) and 1 min using $^{45}Ca^{2+}$. The Ca^{2+} influx were expressed as nmol of Ca^{2+} per mg of cellular proteins. Data represent mean±SEM of three individual experiments. Paired *t*-test (*P<0.05) was used to analyze the difference among treatments.

4. Discussion

Results from our study point to an important interaction between human placental derived cells and plant secondary metabolic compounds of clinical interest. First, a clear decrease on cell amount was seen depending on plant extract, extract dose, or cell line used.

Differences in toxicity degree between the two kinds of cells presented in this study showed the trophoblast-like cells tended to respond differently in accordance to fusogenic capacity corroborating the phenomena discussed by other authors[20,23]. It was not possible to establish the difference between cell death, apoptosis and decreased proliferation by means of the protocol used in our study. However, results from previous studies with such plant extracts may point to an antiproliferative property of extracts obtained from plants with potential to antitumoral activity[9].

The analysis of the trophoblast-like cells biochemical differentiation showed a significant inhibition of hCG production/release for all extracts and doses. However, results from fusion assay through luciferase production did not reveal the same effect. Taking these two results into consideration, it seems that *Hypericum* extracts do not interfere in the cell fusion process but it may influence the hCG production through an extrinsic way such as G protein receptor and cell signaling activation. The difference between biochemical differentiation and fusion phenomena is described for trophoblast cells meaning that the signaling pathways for this two markers seems to be individually orchestrated[18,19].

Interestingly, the inhibition of cell fusion detected by

luciferase transactivation gene expression and the effect on inhibition of MAPK activation were remarked only for *H. caprifoliatum* *n*-hexane extract at 15 μg/mL. Thus, it seems that the inhibition of hCG production and BeWo cell fusion by *H. connatum* and *H. caprifoliatum* extracts is related to other mechanisms but MAPK pathways activation. Additionally, the strong action of *H. caprifoliatum* *n*-hexane extract at 15 μg/mL in hCG production and anti-fusion effect together may suggest a synergic action over both signaling pathways.

Results from Ca^{2+} influx assay were remarkably interesting. The specific *H. connatum* methanolic extract dosage (15 μg/mL) that caused an increase in [Ca^{2+}]i through Ca^{2+} membrane channels (Figure 5) was the same that increased the Ca^{2+} influx at plateau time (15 min) in the kinetic curve (Figure 4A). This phenomena may represent an effect on the sequestration of Ca^{2+} in internal stores by compounds present in this extract[23,24].

The experiments conducted herein showed the effect of the extracts of two Brazilian *Hypericum* species, *H. connatum* and *H. caprifoliatum*, on trophoblast proliferation/differentiation and Ca^{2+} influx. However, both plant extracts and plant species caused the reduction in β-hCG production; the results evidenced the differences between both species and among the different concentrations used in relation to toxicity and biological activity. It was verified that *H. caprifoliatum* interferes more intensely on proliferation/differentiation of trophoblast cells while *H. connatum* seemed to interfere on Ca^{2+} transport system. The chromatographic analysis of the *n*-hexane extracts showed the presence of the expected compounds previously found in these plants. Both *H. caprifoliatum* and *H. connatum* presented dimeric acylphloroglucinols, uliginosin B and hyperbrasilol B. Nevertheless, the *n*-hexane extract of *H. caprifoliatum* presented HC1 as the main component, a tautomeric mixture of unresolved acylphloroglucinols. The presence of HC1 could explain the differences observed in the activity of the extracts. On the other hand, the methanolic extracts of the plants showed the presence of phenolic acid and flavonoids as main compounds. Both extracts presented chlorogenic acid, hyperoside, isoquercitrin, guaijaverin and quercitrin, as previously reported[25]. Thus, the plants presented a similar qualitative chemical composition but the concentration of the compounds was different and could explain the results. Additionally, according to the literature, the methanolic extract of *H. connatum* also presents flavan-4-ol luteoforol, a compound with antiviral activity, and the biflavonoid amentoflavone[14]. Therefore, the variability of compounds found in these two *Hypericum* species may explain the different results found for trophoblast differentiation and function.

The several publications describing the Brazilian *Hypericum* species' biological activity or toxicity and the studies carried out herein demonstrated the potential of these plants to interact with the mammalian system. On one side, this interaction can be promising, leading to useful biological properties, but on the other side, it makes us ponder the threshold between the toxic and non-toxic effect and attention should be paid to the potential toxic effect of these plants to pregnant women.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Depression during pregnancy is, contrary to popular belief, not uncommon. Understandably, pregnant women are reluctant to take antidepressant medication during pregnancy, so natural sources with possible antidepressant activity, such as *Hypericum*, may offer acceptable alternatives if shown not to have toxic side-effects.

Research frontiers

The use of a luciferase reporter system to monitor cell fusion of BeWo cells together with the molecular biological techniques provides sensitive tools for the evaluation of the effects of *Hypericum* extracts.

Related reports

The researchers are among only a few who study the effects of *Hypericum* species on trophoblast function. Most other studies focus on the chemical identification of bioactive components or elucidating the mechaisms of antidepressant activity or photoxicity of *Hypericum* species.

Innovations and breakthroughs

The identification of differential effects of different *Hypericum* species and different extract fractions on trophoblast viability and function is novel and warrants further investigation.

Applications

The information presented in this study will contribute to the safe evaluation of *Hypericum* species when considered as alternative to anti-depressant therapy for women during pregnancy.

Peer review

The investigators use a number of key techniques to evaluate the effects of *Hypericum* extracts on trophoblast function. They found various effects dependent on (1) type of *Hypericum* species, (2) type of extract (methanol versus hexane), (3) type of cell (fusiogenic versus proliferative). In most cases, observed effects were concentration-dependent allowing for the investigators to determine in which range *Hypericum* extracts become toxic to the trophoblast.

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