

IMMUNOMODULATORY POTENTIAL OF *INULA HELENIUM* L.

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Abstract: *Inula helenium* L. (Asteraceae) is a widely occurring perennial herb in Europe and East Asia traditionally used as anti-inflammatory, anti-microbial, and anti-cancer agent. Our investigations refer to the immunomodulatory potential of *Inula helenium* hydroalcoholic root extract. HPLC analysis confirmed the presence of phenolic compounds (polyphenolcarboxylic acids such as caffeic, chlorogenic, rosmarinic, galic, protocatechuic and gentisic acids and flavonoids such as rutin, quercetin, kaempferol) and also of alantolactone as the main sesquiterpene lactone in the hydroalcoholic extract studied. The *in vitro* results highlighted the immunostimulatory and scavenger potential of the extract. The extract exhibited a scavenger effect on DPPH radical of over 80% at 50-100μg/mL, probably due to high content of phenolic acids. Smaller doses of extract (25-50μg/mL) enhanced the proliferation rate of alveolar macrophages better than levamisole. Simultaneous administration of *Inula* extract and LPS and exposure of cells for 21 hours to this combination maintained cellular viability to over 50%. Antitumoral potential of *Inula* extract on breast cancer cell line BT-20 was not confirmed in our study; only in very high dose (500μg/mL) the extract induces a cytotoxic effect of 50%. The studies should be continued in order to investigate the compound(s) responsible for pharmacological activity and to elucidate the mechanism of action.

Keywords: HPLC, polyphenols, flavonoids, alantolactone, immunostimulation.

Introduction

Inula helenium L. (Asteraceae) is a widely occurring perennial herb in Europe and East Asia. Its active components belong mostly to sesquiterpene lactones class - eudesmanolides (alantolactone, isoalantolactone, 4α,5α-Epoxyalantolactone, diplophyllin) and germacranolides (isocostunolide) (Seca et al., 2014) being used as anti-inflammatory, anti-microbial, and anti-cancer agents. The root and the rhizome are the most used plant parts for various preparations - infusion, powder, wine, syrup having the following therapeutic indications: (i) antiseptic in exanthema, bacterial and fungal dermatitis; (ii) antipruritic in dry patches; (iii) to facilitate urinary and digestive functions; (iv) to treat symptomatic cough and bronchitis; (v) to treat failure by the dyspeptic hepatobiliary or biliary dyskinesia; (vi) as an adjunct in the fight against hyperglycemia and obesity (Ghedira et al., 2011).

As the immunomodulatory activity of this herb has not been investigated, the aim of this study is to highlight the potential of a hydroalcoholic extract obtained from roots of *Inula helenium* to exert a stimulatory/suppressive action *in vitro*.

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Material and methods

Plant material

The plant material was obtained from SC Dacia Plant SRL (Braşov county) which cultivates this species.

Chemicals

Analytical grade solvents, lypopolysaccharide and cell culture media were purchased from Sigma-Aldrich, Germany. CellTiter 96® Aqueous Non-Radioactive Cell proliferation Assay kit was purchased from Promega, USA and levamisole from Romvac, Romania.

Preparation of the herbal extract

Amount of 100 g of dried and milled *Inulae radix* in 1000 mL of 50% ethyl alcohol was soaked for 10 days at room temperature in a dark place and then filtrated. The crude extract was concentrated under reduced pressure (72-74 mmHg), dissolved in 20% propylenglycol and used for further investigations.

HPLC analysis

Chromatographic separation was performed on a HPLC ELITE – LaChrom system, with DAD detector and a Inertsil ODS 3 column (250 x 4.6 mm, 5µm) at 25°C. Separation of polyphenols was performed using a mobile phase consisting of an A solution (water acidified with phosphoric acid, pH = 2.5) and a B solution (methanol) at an initial flow rate of 1 mL/min; with an injection of 20µL. Separation of alantolactone was performed using a mobile phase consisting of water/methanol solution (40/60 v/v) at an initial flow rate of 1 mL/min; with an injection of 20µL.

Evaluation of scavenger potential

Free radical (DPPH) scavenger activity was assayed according to Oms-Oliu et al. (2009).

Immunomodulatory potential – *in vitro* test

The viability of NR8383 alveolar macrophages incubated with different concentrations of extracts in the presence or absence of stimuli that trigger immune response (LPS) was carried out by a colorimetric method using kit CellTiter 96® Aqueous Non-Radioactive Cell proliferation Assay. Lypopolysaccharide (LPS) was used due to its ability to induce a rapid dose-dependent response in the host - secretion of cytokines (TNF, IL-1, IL-6) mediated by TLR-4. This activity is similar to Gram-negative bacterial endotoxin. Alveolar macrophages NR8383 were cultivated in 96 well-plates 10⁴ cells/well in DMEM F12 medium, supplemented with 15% fetal bovine serum, 1% antibiotic, kept at 37°C with 5%CO₂. The extract was dissolved in culture media without serum in concentrations of 25-150µg/ mL and incubated with cells for 21 hours with 10 µg/mL LPS. All samples were tested in duplicate and levamisole 100µg/mL was used as reference substance.

A potential antitumoral effect of the *Inula* extract was tested on BT-20 cell line of breast cancer. Cells were cultivated in 96 well-plates 5x10³ cells/ well in EMEM medium, supplemented with 10% fetal bovine serum, 1% antibiotic, kept at 37°C with 5%CO₂. The extract was dissolved in culture media without serum in concentrations of 31.2-500µg/ mL and incubated with cells for 20 hours. All samples were tested in duplicate.

Results and discussions

Chemical composition of *Inula* extract (I5) as resulted by HPLC is presented in Table 1 and reflect the existence of both phenolic compounds and sesquiterpene lactones.

Table 1. Chemical composition of the *Inula helenium* extract

Compound	mg% by HPLC	Compound	mg% by HPLC
Alantolactone	2.765	Quercetin	0.674
Chlorogenic acid	27.78	Kaempferol	0.557
Caffeic acid	3.177	Gallic acid	9.327
Rutin	1.595	3,4-dihydrobenzoic acid (protocatechuic acid)	0.768
Rosmarinic acid	1.05	2,5-dihydrobenzoic acid (gentisic acid)	97.081

The extract exhibits a scavenger effect on DPPH radical of over 80% at 50-100µg/mL, probably due to high content of phenolic acids.

As is it presented in Fig. 1, smaller doses of extract (25-50µg/mL) enhance the cells proliferation rate better than levamisole. The results are comparable to studies of Park et al. (2013) which show an approximately 80% survival rate of macrophages at a dose of 50µg/mL.

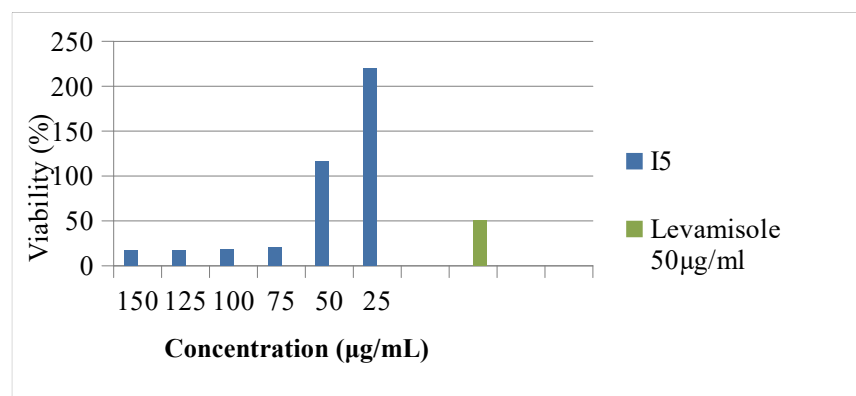


Figure 1. Viability of rat alveolar macrophages exposed to *Inula* extract

Simultaneous administration of *Inula* extract and LPS and exposure of rat alveolar macrophages for 21 hours to this combination maintain cellular viability to over 50% (Fig. 2). At 50µg/mL the effect of *Inula* extract is comparable to levamisole, the reference substance but a certain stimulatory effect on cells proliferation is observed at minimal concentration (25µg/mL).

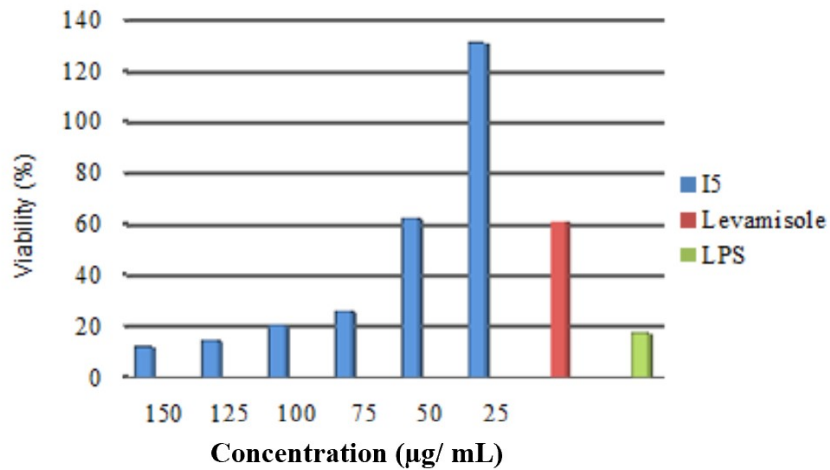


Figure 2. Viability of rat alveolar macrophages exposed to both *Inula* extract and LPS for 21 hours

However, antitumoral potential of *Inula* extract, was not confirmed on breast cancer cell line BT-20 in our study (Fig. 3). Only in very high dose (500µg/mL) the extract induces a cytotoxic effect of 50%. It was showed that alantolactone, the main constituent of *Inula helenium* herb inhibits the growth of triple-negative breast cancer MDA-MB-231 cells, which express constitutively activated signal transducer and activator of transcription 3 (STAT3) (Chun et al., 2015). In BT-20 cell line STAT 3 is expressed at low level (Li and Shaw, 2002) and this could be a reason for *Inula* extract to fail in inducing a cytotoxic effect.

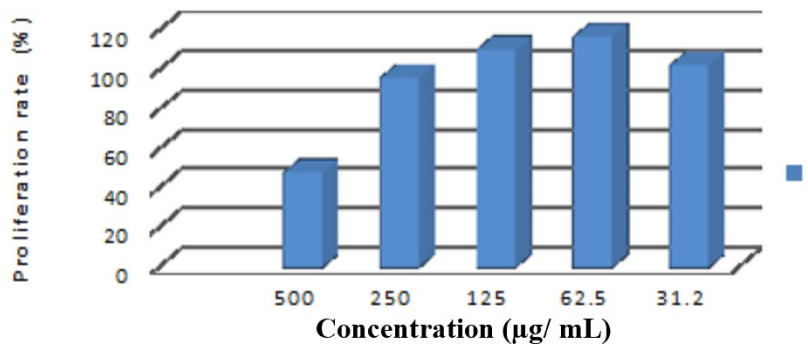


Figure 3. Viability of BT-20 breast cancer cells exposed to *Inula* extract

Conclusions

The analysis of *Inula helenium* hydroalcoholic extract highlighted the presence of phenolic compounds such as polyphenolcarboxylic acids (caffeic, rosmarinic, chlorogenic), flavonoids (rutin, quercetin, kaempferol) and also of a sesquiterpene lactone – alantolactone. *In vitro* assays carried out on macrophages of one of the most important cell type involved in the immune response confirmed the immunostimulatory potential of the extract. The studies should be continued in order to investigate the compound(s) responsible for this action and to elucidate the mechanism of action.

Acknowledgements

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