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Plant extracts effect on sICAM-1 release form human corneal cells

Wpływ ekstraktów roślinnych na poziom sICAM-1 po inkubacji ludzkich komórek rogówki

SUMMARY

Intercellular adhesion molecule-1 (ICAM-1), a cell surface glycoprotein, has been found to be associated with immunopathological processes within the eye. Expression of ICAM-1 is up-regulated during inflammatory diseases and its soluble form (sICAM-1) was shown to be elevated in patients with ocular inflammatory and infectious disorders.

The purpose of the present study was to analyse sICAM-1 level after human corneal cells (10.014 pRSV-T cell line) *in vitro* treatment with ethanol, ethyl acetate and heptane extracts of three herbs (*Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves). Ethanol and ethyl acetate extracts altered sICAM-1 level depending on herb, solvent and extract concentration applied. Heptane extract of each plant caused only concentration-dependent decrease of sICAM-1 level when added to the corneal cell culture.

Concluding, the analysed plant extracts, after further tests, could be considered as supplementary compounds of eye drops, in order to attain reduction of inflammatory responses during ocular diseases.

Keywords: *Lamium album* flos, *Euphrasia officinalis* herba, *Aloe vera* leaves, human corneal cells, sICAM-1

STRESZCZENIE

Cząstka adhezyjna (ICAM-1) jest powierzchniową glikoproteiną, która może być wiązana z procesami patologicznymi zachodzącymi w oku. Ilość ICAM-1 wzrasta podczas chorób o podłożu

zapalnym, a ilość rozpuszczalnej formy ICAM-1 (sICAM-1) jest podwyższona u pacjentów z chorobami zapalnymi oraz infekcjami oczu.

Celem pracy była analiza poziomu sICAM-1 po inkubacji ludzkich komórek rogówki (linia 10.014 pRSV-T) z ekstraktami etanolowym, octanowym lub heptanowym z trzech roślin (*Lamium album* kwiaty, *Euphrasia officinalis* ziele i *Aloe vera* liście). Ekstrakty: etanolowy i octanowy zmieniają poziom sICAM-1 w zależności od rośliny z której je uzyskano, rozpuszczalnika i stężenia ekstraktu. Ekstrakty heptanowe uzyskane z testowanych roślin, po dodaniu do hodowli komórek rogówki prowadziły tylko do spadku poziomu sICAM-1 w sposób zależny od stężenia.

Podsumowując, analizowane ekstrakty roślinne, po kolejnych testach, mogłyby zostać uznane za suplement kropli do oczu, dodawany w celu ograniczenia reakcji zapalnej w czasie chorób tego narządu.

Słowa kluczowe: *Lamium album* flos, *Euphrasia officinalis* herba, *Aloe vera* leaves, ludzkie komórki rogówki oka, sICAM-1

INTRODUCTION

Recent studies indicate a vital role of intercellular adhesion molecule-1 (ICAM-1) in ocular tissues and its up-regulation in a variety of inflammatory eye diseases (5). ICAM-1 is a member of cell surface glycoprotein of the immunoglobulin (IgG) superfamily which is a ligand for lymphocyte function-associated antigen-1 (LFA-1) and Mac-1 expressed on lymphocytes and macrophages, respectively (1, 3, 5). It has been also demonstrated that soluble ICAM-1 (sICAM-1) which results from enzymatic cleavage and shedding of the membrane-bound ICAM-1 (mICAM-1) is closely associated with the development of local ocular inflammation or allergic reactions (2, 5, 7). In a traditional, folk medicine, herbal remedies are used to suppress the inflammatory processes and heal ocular disorders. Therefore, the aim of this study was to analyse sICAM-1 level after human corneal cells (10.014 pRSV-T cell line) *in vitro* treatment with extracts of three herbs: *Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves.

MATERIALS AND METHODS

Plant material

A voucher specimens of plants (*Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves) are deposited at the Department of Pharmaceutical Botany, Medical University, Lublin, Poland and was identified by professor T. Krzaczek (Medical University in Lublin).

Extracts preparation

The materials for testing were: ethanol ethyl acetate and heptane extracts, derived from the *Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves.

Dried and pulverized material was weighed in portions of 5 g each, and then exhaustive extraction was carried out in an ultrasonic bath (Bandelin, Sonorex, Germany) (U–230, V ~ 50/50Hz; and – 1.2 A, P – 120/480 W; *f* – 35 kHz; temp. 20–80^{o}C)

Raw material underwent fourfold extraction under the following conditions:

I – 15 min extraction using 100 ml of 90% ethanol in temp. 45^{o}C

II – 15 min extraction using 100 ml of 90% ethanol

III – 15 min extraction with 70 ml of 90% ethanol

IV – 15 min extraction with 70 ml of 90% ethanol

Each time the extract was decanted from the above raw material. After the extraction process, all four solutions were combined, then evaporated to dryness in a rotary vacuum evaporator IKA RV

05-ST 1 (IKA-Werke, Staufen, Germany) (at 35°C). The resulting dry residue was used in biological research tests.

The process of preparing two other extracts: ethyl acetate and heptane with *Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves proceeded similarly but using 100% solutions.

Part of the dry residue was dissolved in dimethylsulfoxide (DMSO) to obtain a concentration of 100 mg/ml (stock solution). The final quantity of DMSO in the highest of applied plant extract concentration did not exceed 0.1%. Such concentration of DMSO in culture medium had no influence on 10.014 pRSV-T cell viability, as was shown in our previous tests.

Cell culture

Human normal corneal cell line 10.014 pRSV-T (ATCC No. CRL-11515) was used (Fig. 1). The cells were cultured as monolayers in 25 cm^2 culture flasks (Nunc. Roskilde, Denmark) coated with PureCol™ ultrapure collagen (INAMED Biomaterials, Fremont, CA, USA) at 3.1 mg/ml concentration. Cell line was maintained in Defined K-SFM (keratinocyte-serum free medium) (Gibco) supplemented with 75 µg/ml endothelial cell growth factor (ECGF) (Sigma), 0.05 mg/ml bovine pituitary extract (BPE) (Gibco), 500 ng/ml hydrocortisone (Sigma) and 0.0005 mg/ml bovine insulin (Gibco) and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin) (Sigma, St Louis, MO) at 37°C in a humidified atmosphere with 5% CO_2.

Incubation of cells with plant extracts

For the purpose of the current experiments, the total number of cells was estimated by counting in haemocytometer. A dose of 1.5 ml of cell suspension ($1x10^5$ cells/ml) was added to appropriate wells of 24-well flat-bottomed plates. After 24 h of incubation, the medium was discarded and a new one was added, with appropriate concentrations of extracts (8 µg/ml and 20 µg/ml). As controls, cells cultured in 1.5 ml of medium without extracts presence were used. The total cell number was equivalent to these in the sample wells.

The incubation was performed for further 24 h and then supernatants were collected and stored at –80°C (not longer than three months) until ELISA analysis.

ELISA assay

The levels of human sICAM-1 were tested immunoenzymatically (ELISA) using commercially available kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instruction. The optical density at 450 nm with the correction wavelength of 570 nm of each ELISA sample was determined using a microplate reader. The sICAM-1 concentrations were calculated on the basis of a standard curve (6). The detection limit was 0.049 ng/ml.

Statistical analysis

Results are presented as means ± SD from three experiments. Data were analysed using one-way ANOVA analysis of variance with Dunnett's post-hoc test. Differences of $p \leq 0.01$ were considered significant.

RESULTS

Ethanol, ethyl acetate and heptane extracts of three herbs: *Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves in two concentrations: 8 µg/ml and 20 µg/ml were used. Their activity on human corneal cells (10.014 pRSV-T cell line) (Fig. 1) were tested as culture supernatant sICAM-1 level, analysed by ELISA.

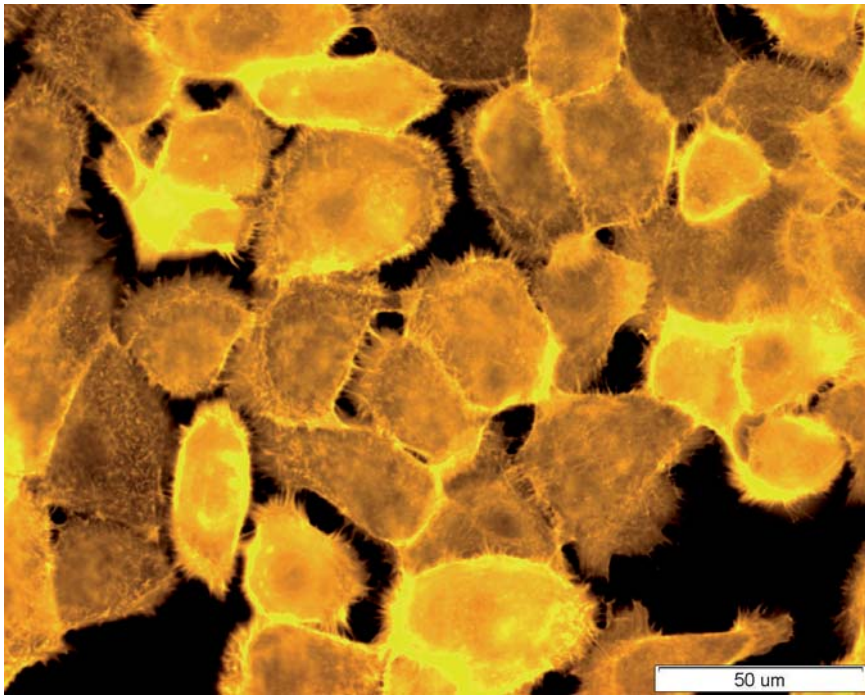
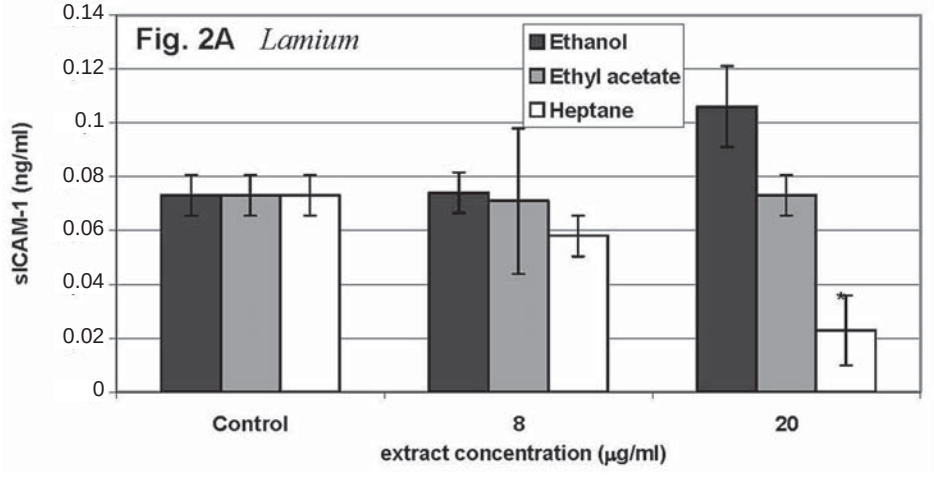
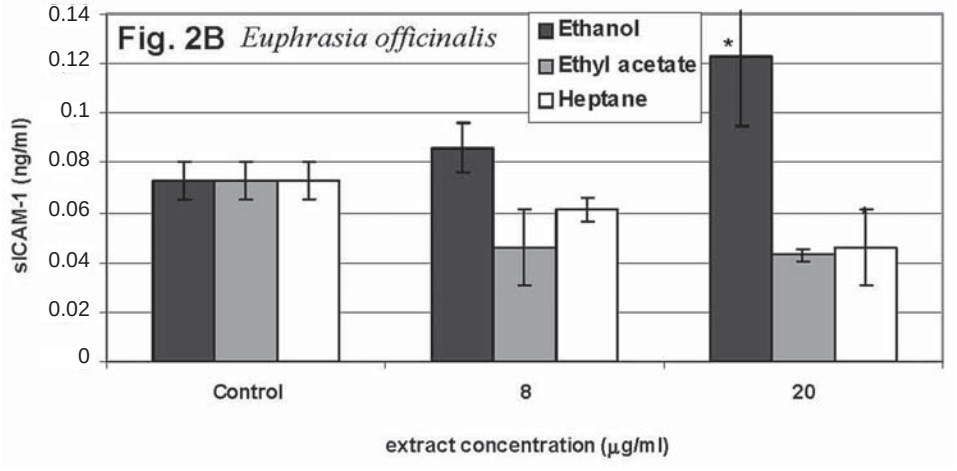


Fig. 1. Human corneal cells (10.014 pRSV-T cell line). TRITC-phalloidin fluorescent staining. Magn. 400x. Bar 50 µm





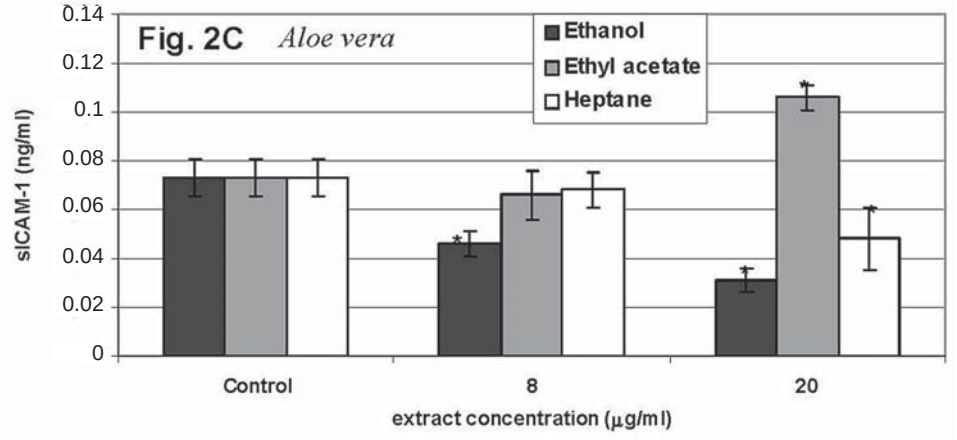


Fig. 2. sICAM-1 level in supernatants of culture of 10.014 pRSV-T human normal corneal cells during 24 h incubation with ethanol, ethyl acetate and heptane extracts of *Lamium album* flos (A), *Euphrasia officinalis* herba (B) and *Aloe vera* leaves (C). Two extracts concentrations were used: 8 µg/ml and 20 µg/ml. ELISA test.

* $p \leq 0.01$ – a culture of corneal cells treated with plant extracts compared to a non-treated control culture

Ethanol extracts of *Lamium album* flos and *Euphrasia officinalis* herba increased sICAM-1 level in a concentration-dependent manner, while from *Aloe vera* leaves significantly decreased the soluble molecule amount. Compounds which came from ethyl acetate extract of *Lamium album* flos had no influence

on sICAM-1 level but from *Euphrasia officinalis* herba decreased the soluble molecule amount. On the contrary, ethyl acetate extract of *Aloe vera* leaves at a concentration 20 μg/ml significantly increased ICAM-1 release from corneal cells. When heptane extracts of each plant were added to the corneal cell culture only concentration-dependent decrease of sICAM-1 level was observed (Fig. 2).

DISCUSSION

In the recent years special attention has been focused on herbal preparations as modulators of cellular adhesion molecules expression. One of the most important molecules responsible for ocular tissue homeostasis and immune responses within the eye is ICAM-1. It is synthesized by epithelial cells and serves as a signalling molecule playing a vital role in a variety of inflammatory and allergic eye diseases but also may take part in antigen presentation by ocular epithelium (2, 5, 7). It has been proposed that sICAM-1 may represent a link between local and systemic inflammation and thus its increased general level may indicate a development of, e.g. local ocular inflammatory state (2). Therefore, it became evident that substances which may alter the expression of ICAM-1 may simultaneously regulate inflammatory responses. Among others, components of medicinal plants have been shown to influence the expression of adhesion molecules. Mo et al. (4) have revealed that purified extract from a mixture of 13 oriental herbs (CML-1) demonstrates anti-inflammatory activity and down-regulates ICAM-1 expression. Similarly, sasanquasaponin (SQS) from *Camellia oleifera* markedly inhibited the over-expression of serum sICAM-1 and thus decreased inflammation induced by burns (3). The mechanism of herbal compounds action on ICAM-1 expression appeared to act at the transcriptional level by inhibiting the IκB/NF-κB signalling pathway. In consequence, NF-κB factor which is required for the adhesion molecules expression and regulation of inflammatory conditions remains not activated (4).

In our study we showed that selected extracts of *Lamium album* flos, *Euphrasia officinalis* herba and *Aloe vera* leaves down-regulate sICAM-1 expression. However, it was difficult to establish which one was the most potent because their activity strongly depended on the kind of the solvent used, their concentration and the plant they were isolated from. Nevertheless, it may be generally concluded that these plant extracts, mainly ethanol extract of *Aloe vera* leaves or ethyl acetate of *Euphrasia officinalis* herba, after further tests, could be considered as supplementary compounds of eye drops, in order to attain reduction of inflammatory responses during ocular diseases.

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