

Immunomodulating activity of leaf decoctions from four *Staphylea* L. species

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Summary

Immunomodulating activity of leaf decoctions from four *Staphylea* species (*Staphylea colchica* Stev., *S. elegans* Zab., *S. holocarpa* Hemsl. and *S. pinnata* L.) was studied on human mononuclear cells. Phagocytic activity of mononuclear cells was highest after incubation with the extract of *S. holocarpa* Hemsl. *S. colchica* Stev. possesses the highest lysozyme activity, a little lower values were determined by *S. holocarpa* Hemsl. and *S. pinnata* L. The inhibition of the peroxidase activity of mononuclear cells was most significant in *S. holocarpa* Hemsl., in opposition on *S. pinnata* L., the only species that showed the stimulating effect on the immune cells again. All the studied species showed the immunomodulating activity, *S. pinnata* L. seems to be immunostimulating only. *S. colchica* Stev. and *S. holocarpa* Hemsl. are the most prospective species from the view of immunomodulating activity.

Key words: *Staphylea colchica* Stev., *Staphylea elegans* Zab., *Staphylea holocarpa* Hemsl., *Staphylea pinnata* L., *Staphyleaceae*, immunomodulating activity

Presently, the immunological research is devoted to screening of new potential drugs, especially from plants containing the immunological active compounds. This study reports the immunomodulating effects of the *Staphylea* L. water extracts for the first time.

Staphylea L., bladdernut (Staphyleaceae), belongs to ornamental shrubs growing throughout the northern genial area. It is used in traditional Chinese medicine – *S. bumalda* DC. as a cough remedy and as a folk anti-diarrhoeal medicine, as it is meant to possess a blood refreshing effect after delivery. Traditional medicine of Indians uses *S. trifolia* L. with antirheumatic, dermatological, sedative and gynecological indications [1]. In the year 2000 Jantová et al. have found out that its extracts possess significant cytotoxic and antibacterial activity [2, 3]. Other paper describes the antioxidant activity correlating with the total phenolic content in ethylacetate and chloroformic extracts of *Staphylea colchica* Stev. (SC), *S. elegans* Zab. (SE), *S. holocarpa* Hemsl. (SH) and *S. pinnata* L. [4]. By now, the foundation of these biological effects and elucidated chemical structures in its extracts is not known. That is why the main aim of this study is another test of its biological activity, very close to those already reported. In this paper we describe the phagocytic activity and phagocytic index, lysozyme and peroxidase activity of human mononuclear cells after incubation with decoction lyophilizates from four *Staphylea* L. species (SC, SE, SH and SP).

MATERIAL AND METHODS

Microorganisms

Micrococcus luteus ATCC. The strain of *Enterococcus faecalis* was taken from collection of microorganisms of Department of Molecular and Cellular Biology of Drugs, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia.

Plant material

The leaves of four *Staphylea* L. species were collected at Dendrobiology Institute, Slovak Academy of Sciences – Arboretum Mlyňany, in June 2005. The fresh leaves were dried at room temperature (22°C) for 3 weeks and then processed at laboratory mill (Fritsch, Germany). The dried, minced leaves were used for preparation of the water decoctions according to the Czecho-Slovak Pharmacopoea IV (PhBs IV, 1987) and lyophilized after cooling.

Isolation of mononuclear cells and their cultivation with decoctions

Human mononuclear cells (MO) taken from healthy volunteers were isolated on Histopaque®-1077 (Sigma) according to manufacturer's method, transferred to RPMI 1640 (SEVAPHARMA) and enriched with 10% fetal calf serum (GIBCO). They were stored at 4°C. Decoctions of the leaf drug and MO (0.1 mg/mL) were

cultivated for 48 h at 37°C in a 5% CO₂ environment. MO were washed three times in physiological saline and diluted to 2 x 10⁶ cells/mL

Determination of phagocytic activity and phagocytic index

A suspension of 100 µL MO after cultivation with decoctions was incubated with 50 µL of suspension of *E. faecalis* for 1 h at 37°C. Then the suspension was applied to a microscopic slide. The slides were dried at room temperature and stained according to Wright (eosin methylene-blue, CAS Nr. 68988-92-1). Phagocytic activity was calculated as the percentage of phagocytosing cells with three or more particles. The index of phagocytosis is the average number of particles ingested by one cell.

Determination of peroxidase activity

Supernatant (150 µL) from pelleted sonicated cells was added to 50 µL of the peroxidase substrate (1,2-phenylenediamine and H₂O₂). After 20 min incubation in darkness the reaction was stopped by adding 4 mol. L⁻¹ of sulphuric acid. The changes in absorbance were spectrophotometrically registered at 409 nm with comparison to physiological solution [5].

Determination of lysozyme activity

Supernatant (150 µL) from pelleted sonicated cells was added to 50 µL of *Micrococcus luteus* in the phosphate buffer and the absorbance was spectrophotometrically measured at 405 nm at 0 and 20 min periods with comparison to physiological solution [5].

Statistical analysis

The unpaired Student's test was used.

RESULTS AND DISCUSSION

Phagocytic activity was measured microscopically as a number of ingested or touching cells of *Enterococcus faecalis* by mononuclear cells. All the samples increased this activity gently, but none of the tested decoctions influenced it significantly (fig. 1). Unlike the phagocytic activity, SE, SH and SP showed significant activating

effect on phagocytic index (Fi) at a level of significance (SE $p < 0.05$; SH $p < 0.001$; SP $p < 0.01$). SC did not reach the level of statistical significance (fig. 1). The results of lysozyme activity show that in this case all of the tested decoctions were active and increased the activity, compared to the control, but only three of them were statistically significant (SC $p < 0.001$; SH $p < 0.001$; SP $p < 0.01$, see fig. 2). Finally, the peroxidase activity was determined. All of the tested species were active and reached the statistical significance ($p < 0.001$). SC, SE and SH decreased the activity and possess a suppressive activity on the natural human immune cells. Unlike these findings, SP increased the activity of cells and acted immunostimulatingly.

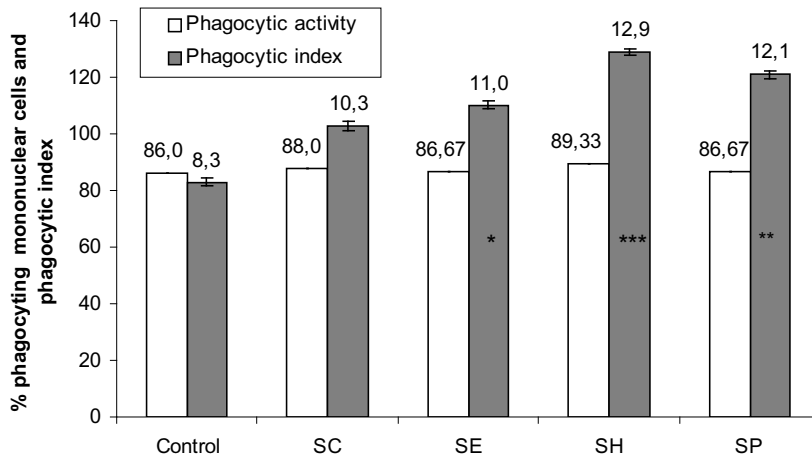


Figure 1. Phagocytic activity and phagocytic index of human mononuclear cells treated by desintegrated suspension of *Enterococcus faecalis* and decoctions from leaves of SC, SE (* $p < 0.5$), SH (***) $p < 0.001$), SP (**) $p < 0.01$)

Based on our results, we do suggest that the decoction from *S. colchica* Stev., *S. elegans* Zab. and *S. holocarpa* Hemsl. possess immunomodulating activity. *S. pinnata* L. showed immunostimulating effect on phagocyte activity of human mononuclear cells. By the determination of the peroxidase activity it showed again its immunostimulating effect. *S. colchica* Stev. and *S. holocarpa* Hemsl. are the most prospective potential sources for further investigations and possible usage in immunological diseases.

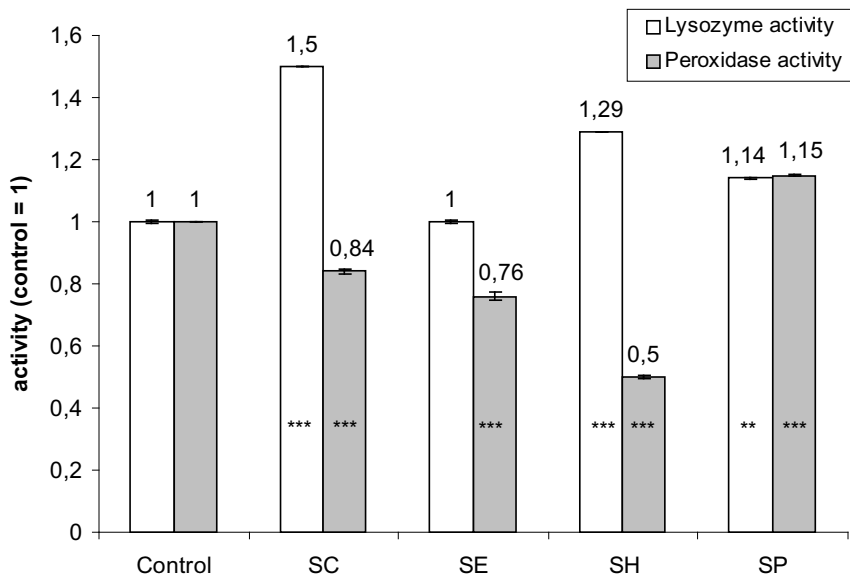


Figure 2. Lysozyme activity and peroxidase activities of human mononuclear cells treated by desintegrated suspensions of *Micrococcus luteus* and decoctions from leaves of SC (** $p < 0.001$), SE, SH (** $p < 0.001$), SP (** $p < 0.01$). Peroxidase activity of human macrophages treated by decoctions from leaves of SC (** $p < 0.001$), SE (** $p < 0.001$), SH (** $p < 0.001$), SP (** $p < 0.001$)

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REFERENCES

1. Dictionary of Chinese Traditional Medicine. Jiang Su New Medicinal College. Shang Hai People's Press, Shang Hai 1977.
2. Jantová S, Nagy M, Ružeková L, Grančai D. Antibacterial activity of plant extracts from the families Fabaceae, Oleaceae, Philadelphaceae, Rosaceae and Staphyleaceae. *Phytother Res* 2000; 14: 601-3.
3. Jantová S, Nagy M, Ružeková L, Grančai D. Cytotoxic effects of plant extracts from the families Fabaceae, Oleaceae, Philadelphaceae, Rosaceae and Staphyleaceae. *Phytother Res* 2001 15:22-5.
4. Laciková L, Muselík J, Mašterová I, Grančai D. Antioxidant activity and phenolic compounds in different extracts of four *Staphylea* L. species. *Molecules* 2007; 12:98-102.
5. Bukovský M, Mlynarčík D, Ondráčková V. Immunomodulatory activity of amphiphilic antimicrobials on mouse macrophages. *Int J Immunopharmac* 1996; 18: 423-6.

DZIAŁANIE IMMUNOMODULACYJNE WYWARU Z LIŚCI CZTERECH ODMIAN *STAPHYLEA* L.

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Streszczenie

Działanie immunomodulacyjne wywaru z liści czterech gatunków *Staphylea* (*Staphylea colchica* Stev., *S. elegans* Zab., *S. holocarpa* Hemsl. and *S. pinnata* L.) badano na ludzkich komórkach jednojądrzastych. Działanie fagocytowe komórek jednojądrzastych było najwyższe po inkubacji w ekstrakcie *S. holocarpa* Hemsl. *S. colchica* Stev. działała najbardziej na lizosomy, nieco niższe wartości notowano w przypadku *S. holocarpa* Hemsl. i *S. pinnata* L. Zahamowanie działania peroksydacyjnego komórek jednojądrzastych było najwyższe w przypadku *S. holocarpa* Hemsl., w przeciwieństwie do *S. pinnata* L., jedyne gatunku, u którego powtórnie stwierdzono stymulujące działanie na odporność komórek. Wszystkie badane gatunki wykazały działanie immunomodulacyjne, przy czym w przypadku *S. pinnata* L. immunostymulacja wydaje się być jedynym działaniem. *S. colchica* Stev. i *S. holocarpa* Hemsl. są najbardziej obiecującymi gatunkami immunomodulującymi.

Słowa kluczowe: *Staphylea colchica* Stev., *Staphylea elegans* Zab., *Staphylea holocarpa* Hemsl., *Staphylea pinnata* L., *Staphyleaceae*, działanie immunomodulacyjne