The phagocyte-stimulatory properties of plant extracts from *Compositae* family

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S u m m a r y

The phagocyte-stimulatory properties of plants from *Compositae* family were studied. The extracts of *Compositae* family exhibit the stimulatory effect on the reticuloendothelial system. These properties depend on dose as well as the solubility of extract.

Key words: *Compositae, plant extracts, solubility of extract, phagocyte-stimulatory properties*

INTRODUCTION

The herbal extracts contain various plant substances which have several biological activities, including immunostimulatory properties.

The study was initiated due to the increasing number of diseases caused by immunodeficiency and scarcity of available drugs stimulating production of antibodies and enhancing phagocytosis. Due to the fact that a considerable number of immunostimulatory drugs are of plant origin and plants of *Compositae* family have become particularly appreciated, the subject of our study was to evaluate the immunostimulatory properties of selected plants from the *Compositae* family.

Echinacea purpurea is the most widespread plant from *Compositae* family which has immunostimulatory properties. The active substances from *Echinacea purpurea* (echinacein, essential oils, betaine, glycoproteins) have immunostimulatory properties such as: enhancement of granulocytes and macrophages activity as well as lymphocyte B stimulation to produce cytokines [1, 2]. In addition, these substances have antiviral, antiprotozoan and antifungal properties [3]. Anti-inflammatory properties of *Echinacea purpurea* result from its ability to inhibit cyclooxygenase and lipooxygenase. *Echinacea purpurea* has been used in treatment of numerous immunodeficiency diseases and chronic inflammatory processes. The aim of the study was to evaluate the phagocyte-stimulatory activity of extracts from 10 plants from *Compositae* family: *Achillea millefolium, Anthemis nobilis, Arnica montana, Centaurea cyanus, Eupatorium cannabinum, Matricaria chamomilla, Tagetes erecta, Taraxacum mongolicum, Taraxacum officinalis.*

EXPERIMENTAL

The preparation of extracts

From each plant 3 extracts were prepared:
1. hydrosoluble
2. partially hydrosoluble
3. liposoluble.

Extracts were prepared using initial maceration and then methyl alcohol percolation.

Maceration: The material was chopped to desired size, suspended in methanol at 30-40% (w/v) and left for 2 h in a tightly closed vessel.

Percolation: The soaked material was transferred to a percolator, covered with methanol to 10% (w/v) and left for 24 h. Next, the extract was collected at a flow rate of 1-1.2 ml/min.

Extraction: The solvent was evaporated completely under reduced pressure and at temperature not exceeding 40° C.

Hexane extract: the components were washed out with n-hexane: 90% methanol (1:1) solution using a dilution factor of 1:20 (dry extract: solvent w/v) and transferred to a separator. The hexane phase was collected and the aqueosus-alcoholic phase was washed twice with n-hexane at 1:1 (v/v). The combined hexane phases were dehydrated with anhydrous sodium sulfate, filtered, and dried under reduced pressure at temperature not exceeding 40°C.

Water and acetate extracts: components remaining in the aqueosus:alcoholic phase were washed out with ethyl acetate in water (1:1) using a dilution factor of 1:20 (dry extract: solvent w/v) and transferred to a separator. The acetate phase was

collected and aqueosus phase was washed twice with ethyl acetate in water (1:1 v/v). The combined acetate phases were dehydrated with anhydrous sodium sulfate, filtered, and dried under reduced pressure at temperature no exceeding 40°C.

Evaluation of phagocyte – stimulatory properties of extracts

To determine phagocyte-stimulatory properties of plant extracts Carbon Clearance Test by Wagner et al. [4] modified by Institute of Natural Fibres and Medicinal Plants in Poznań has been used. This test is used to study the activity of reticuloendothelial system *in vivo* and is based on the measurement of the intensity of elimination of Indian ink suspension particles from blood. The modifications of the above mentioned method concerned the use of rats instead of mice and blood sampling from the tail rather than from retrobulbar venous plexus. The extracts were dissolved in olive oil (extract 3), in a mixture of 1 ml hexane + 9 ml olive oil or 1 ml ethanol + 9 ml olive oil (extract 2) and in physiological saline (extract 1) and administered intravenously in two doses: 0.001 mg/100 g solution at a concentration of 0.01 mg/ml at a volume of 1 ml/kg, and 0.01 mg/100 g solution at a concentration o 0.1 mg/ml at the volume of 1 ml/kg 24 h before study to male Wistar rats. The rats weighed 200–300 g and were fed with standard diet + drinking water *ad libitum*. The control group received the extract of *Echinacea purpurea* 0.001 mg/100 g at a concentration of 0.01mg/ml at the volume of 1 ml/kg. After drug administration the animals received water (*ad libitum*). The control group and the studied groups consisted of 6 animals. Each extract was examined in 18 rats. Phagocyte activity was tested in 3 rats from each group at one time. The Rotring Werke black ink solution heated in water bath to the temperature of 37°C, in 1% gelatin solution in 0.9% NaCl at a volume of 0.5 ml/100g body mass was slowly administered into caudal vein. Twenty four hours after the study, the rats were put to sleep with 1 ml/kg sodium thiopental at the volume of 0.1 ml/100 mg body mass.

The first sampling was initiated in the third minute of study. Blood was sampled in 3-minute intervals into a test-tube with 2 ml distilled water for haemolysis. The experiment lasted 15 minutes and included 5 samplings in 3-minute intervals using Eppendorf micropipette.

Immediately after the study, absorption measurement at 691 wave length with Semco S91E spectrophotometer was performed. The extinction values were changed into their logarithms and subjected to linear regression analysis. The coefficients were subjected to Q Dixon and t-Student tests for significance level $p<0.01$. The values of regression coefficients were expressed as mean values. The result determining immunostimulation made up a quotient of mean value of studied extracts in examined group (RCtr) to the mean value in the control group (RCc). The extracts were classified as having phagocyte-stimulatory properties when the ratio of regression coefficients RCtr/RCc was greater than 1.0. The study was approved by the local ethics committee (Nr 23/2001).

RESULTS AND DISCUSSION

The ratios of RCtr/RCc coefficients for extracts of 10 plants from *Compositae* family are shown in table 1.

Table 1.

The ratios of RCtr/RCc coefficients for extracts of plants from *Compositae* family

plant	hydrosoluble extracts RCtr/RCc D1	D2	hartially hydrosoluble extracts RCtr/RCc D1	D2	liposoluble extracts RCtr/RCc D1	D2
Eupatorium cannabinum (herba)	0.52	0.73	1.46	1.06	0.74	0.80
Anthemis nobilis (anthodium)	0.95	0.57	0.7	0.77	0.85	1.0
Tagetes erecta (flos)	1.12	0.83	0.62	0.92	0.80	0.83
Tagetes erecta (herba)	0.76	0.7	1.01	1.08	0.53	0.69
Achillea millefolium (herba)	0.84	0.72	1.01	1.08	0.98	0.83
Achillea millefolium (flos)	0.89	0.93	0.89	0.82	0.84	0.58
Centaurea cyanus (flos)	0.97	0.67	0.60	1.15	1.12	1.15
Taraxacum mongolicum (radix)	0.65	0.78	0.79	0.87	1.09	0.95
Taraxacum officinalis (radix)	0.83	0.74	1.07	1.05	0.85	0.87
Arnica montana (flos)	0.81	0.66	0.81	0.62	0.55	0.80

D1 – dose 0.01 mg/kg body weight
D2 – dose 0.1 mg/kg body weight

The herbal extracts contain numerous active substances shoving various properties. Several substances have been shown to posses reticuloendothelial system potentiating activity [5]. In order to investigate the immunological activity of extracts from *Compositae* family, the *in vivo* Carbon Clearance test was used. As shown in table 1, some extracts demonstrated phagocytosis enhancement suggesting immunostimulatory properties. The phagocyte stimulatory properties were different depending on studied plants, dose used as well as the solubility of extract.

Numerous studies have shown the relationship between the solubility of plant extract and their biological activity [6]. The immunopharmacologic activities of herbal extracts are complex and are still not completely understood. Findings made *in vitro* not always agree with *in vivo* observations. Moreover, the effects of different compounds of herbal extracts may be antagonistic, in some cases they are immunosupressive, in others immunostimulating [7]. The effector cells of the immune response include T and B lymphocytes mononuclear phagocytes and granulocytes. These cells carry out specific effectors activities by acting in an integrated system which is regulated on multiple levels by a network of membrane

signals and soluble mediators that make possible communication between the immune system and others [8]. The altered immune system regulation can determine the response to chronic inflammatory diseases.

Numerous compounds of herbal extracts have been seen to influence the function of enzyme system that is involved in the immune response. The metabolic activation of phagocytes during phagocytosis causes the activation of NADPH oxidase: a complex enzymatic system that catalyses NADPH oxidation to produce a superoxide radical and other reactive products of oxygen [9]. An alternative pathway in macrophages and granulocytes leads to the production of hypochlorous acid that has a potent antimicrobial activity. Considering the results of the present study, it can be confirmed that some herbal extracts from *Compositae* family exhibit the stimulatory effect on the reticuloendothelial system. These properties depend on dose as well as the solubility of extract.

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BADANIE EKSTRAKTÓW ROŚLIN Z RODZINY *COMPOSITAE* STYMULUJĄCYCH FAGOCYTOZĘ

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S t r e s z c z e n i e

Wykazano, że ekstrakty z roślin należących do rodziny *Compositae* odznaczały się działaniem stymulujacym układ siateczkowo-śródbłonkowy. Właściwości te zależne były od dawki ekstraktu i jego rozpuszczalności.

Słowa kluczowe: *Compositae, ekstrakty ziołowe, rozpuszczalność ekstraktu, własności stymulujące fagocytozę*