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THE IMMUNOMODULATORY EFFECT OF *ASPERULA ODORATA* L. AND *ASPERULA HUMIFUSA* M. BIEB. BESSER DRY EXTRACTS

N.V.Kashpur, N.S.Yurchenko*, T.V.Ilyina*, A.M.Kovalyova*, O.V.Goryacha*, M.V.Smilyanska, S.D.Peremot

State Institution "Institute of Microbiology and Immunology named after I. I. Mechnikov, National Academy of Medical Sciences of Ukraine
National University of Pharmacy*

Key words: Asperula odorata; Asperula humifusa; phagocytic index; phagocytic number

The research presents results of in vitro studies of the effect of dry extracts of Asperula odorata and Asperula humifusa on the functional activity of macrophages and their hematogenous precursors. It has been found that the extracts studied reveal a dose-dependent stimulatory effect on the transformation and phagocytic activities of macrophages and their mononuclear precursors. The Asperula humifusa dry extract exhibits a more expressed immunomodulatory effect in the dose range from 5 to 100 µg/ml. When using the extracts studied in the dose of 100 µg/ml the maximal stimulation of the functional activity of immune competent cells has been observed. Therefore, the extract of Asperula odorata increases the transformation activity of mononuclear cells by 57%, the phagocytic index is 46% and the phagocytic number is 54% compared to the control. The extract of Asperula humifusa increases the transformation activity by 64%, the phagocytic index is 44% and the phagocytic number is 56%. The research results confirm opportunities of using the given dry extracts with the purpose of immunopathological states correction.

Due to deterioration of the environment and living conditions of the population in recent years there is a noticeable negative impact of a variety of factors on human health, and the body's adaptive systems in particular. This leads to decrease in the functional activity of immune competent cells of the system of mononuclear phagocytes, T- and B-lymphocytes; and it causes the change in the body's immunological reactivity and development of secondary immunodeficiency [4]. The problem of searching medicines with a positive immunomodulatory effect is important today [9, 12].

Immunomodulatory drugs of the plant origin are of particular interest. They are known not only to enhance the body's adaptive capacity, but also to restore its functional competence disrupted by regular maladies. Herbal immunostimulants act physiologically, do not accumulate, have a significant difference between therapeutic and

toxic doses [1, 15]. Thanks to combination of active components plants are able to provide a multi-faceted effect on the body (immunostimulating, anti-inflammatory, antimicrobial, antiviral, antitoxic, etc.) [2, 3, 8, 10, 14]. This creates the conditions for a complex and multifunctional impact of therapeutic and preventive purposes both on the affected organ and the whole body.

Detoxification reactions in the body require a large amount of vitamins and minerals that are insufficient in a daily diet. Constant stress exhausts their reserves. An important feature of medicinal plants is that they contain vitamins and minerals in a chelated form, which is available to be digested, and therefore, the use of herbal remedies for treatment and rehabilitation is more attractive.

The aim of the research was to study the immunomodulatory effect of dry extracts of sweet woodruff – *Asperula odorata* L. and spreading bedstraw – *Asperula hu-*

mifusa M. Bieb. Besser on the transformation and functional activity of macrophages and their hematogenic precursors.

Materials and Methods

The objects of the study were dry extracts of *Asperula odorata* and *Asperula humifusa* obtained by extraction of the exhausted herbal material with heated water remaining after sequential obtaining from the corresponding raw material of lipophilic and phenolic complexes.

The immunomodulatory effect was studied *in vitro* in the reaction of macrophage transformation of mononuclear cells of the peripheral blood [5, 7, 11, 13].

Primary cultures of immune competent cells from blood donors were treated with heparin and obtained by sedimentation at 4-8°C. Blood mononuclear cells were cultured in the nutrient medium 199 with 10% fetal serum. Sodium salt of benzyl penicillin in the concentration of 100 IU/cm, streptomycin and amphotericin B were added to the nutrient medium.

Dry extracts were applied to primary cultures of immune competent cells in the doses of 5 µg/ml, 50 µg/ml and 100 µg/ml, and in-

N.V.Kashpur – senior researcher of the Immunorehabilitation laboratory at the State Institution "Institute of Microbiology and Immunology named after I.I.Mechnikov of the National Academy of Medical Sciences of Ukraine" (Kharkiv)

N.S.Yurchenko – post-graduate student of the Department of Pharmacognosy, National University of Pharmacy (Kharkiv)

The effect of dry extracts of *Asperula odorata* and *Asperula humifusa* on indexes of macrophage transformation and the phagocytic activity of macrophages and their hematogenic precursors

Dry extract	Concentration, µg/ml	IMTM, %	Phagocytic index, %	Phagocytic number
<i>Asperula odorata</i>	5	45.0±4.3	56.6±2.6	10.3±0.6
	50	46.6±3.4	59.4±3.3	10.8±1.1*
	100	51.2±2.4*	67.7±2.6*	11.7±1.1*
<i>Asperula humifusa</i>	5	45.6±3.3	54.8±2.3	10.9±1.2
	50	48.2±2.2	61.3±2.4*	11.3±0.8*
	100	53.5±4.3*	66.8±3.4*	11.8±1.2*
Control	-	32.6±3.4	46.4±2.8	7.6±0.6

Note. * – P < 0.05 compared to the intact control.

cubated at 37°C for 23 hours. To assess the phagocytic activity of macrophages and their precursors in 23 hours of cultivation the reference strain of *Staphylococcus aureus*-209P inactivated by heating was introduced into the culture.

The immunomodulatory effect of dry extracts on immune competent cells was assessed according to the following criteria:

- IMTM – index of macrophage transformation of mononuclear cells;
- phagocytic index (FI);
- phagocytic number.

The control included the setting of the reaction of macrophage transformation of the peripheral blood mononuclear cells without addition of the extracts studied. The indexes obtained were processed statistically using the "Microsoft Excel" software [6].

Results and Discussion

No difference in the mechanism of action of dry extracts of *Asperula odorata* and *Asperula humifusa* on immune competent cells in the peripheral blood was observed (Table).

Dry extracts greatly stimulate the transformation and phagocytic activity of mononuclear cells in the peripheral blood.

Introduction of sweet woodruff's extract to the primary cultures of immune competent cells in the dose of 5 µg/ml caused the increase in transformation activity of peripheral blood mononuclear cells by 38%, the phagocytic index – by 22%, the phagocytic number – by 35% compared to the intact control. The dry extract of spreading bedstraw used in the same dose increased the transformation activity of mononuclear cells by 40%, the phagocytic index – by 18% and the phagocytic number – by 43%.

Increasing the dose of dry extracts to 50 µg/ml resulted in a pronounced stimulation of the transformation and phagocytic activity of macrophages and their precursors. Thus, the extract of *Asperula odorata* increased the transformation activity of mononuclear cells by 43%, the phagocytic index – by 28%, the phagocytic number – by 42% compared to the intact control. Using the dry extract of *Asperula humifusa* IMTM increased by 48%, the phagocytic index – by 32%, the phagocytic number – by 49%.

When using the extracts studied in the dose of 100 µg/ml the maximal stimulation of the func-

Table

tional activity of immune competent cells was observed. Thus, the extract of *Asperula odorata* increased the transformation activity of mononuclear cells by 57%, the phagocytic index – by 46% and the phagocytic number – by 54% compared to the control. The extract of *Asperula humifusa* increased the transformation activity in 64%, the phagocytic index – by 44% and the phagocytic number – by 56%.

Therefore, it has been found that the extracts studied show a dose-dependent stimulatory effect on the transformation and phagocytic activity of macrophages and their mononuclear precursors.

The comparative evaluation of the activity of dry extracts of *Asperula odorata* and *Asperula humifusa* has shown that the extract of *Asperula humifusa* possesses a more expressed immunomodulatory effect in the dose range from 5 to 100 µg/ml.

Indirect data were obtained indicating the absence of toxicity of the extracts under research in relation to mononuclear macrophages and their precursors in the dose range of 5-100 µg/ml, confirming our findings while studying their toxicity.

The data concerning the positive immunomodulatory activity of dry extracts of *Asperula odorata* and *Asperula humifusa* obtained confirm the advisability of further research in finding new ways and principles of correction of immunodeficiency.

CONCLUSIONS

Dry extracts of *Asperula odorata* and *Asperula humifusa* have a pronounced stimulatory effect on the transformation activity of immune competent blood cells and increase their functional characteristics. It has been found that *Asperula humifusa* dry extract in the dose range from 5 to 100 µg/ml possesses a more expressed immunomodulatory effect.

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ІМУНОМОДУЛЮЮЧА ДІЯ СУХИХ ЕКСТРАКТІВ ASPERULA ODORATA L. ТА ASPERULA HUMIFUSA M. ВІБВ. BESSER
Н.В.Кашпур, Н.С.Юрченко*, Т.В.Льїна*, А.М.Ковальова*, О.В.Горяча*, М.В.Смілянська, С.Д.Перемот
ДУ «Інститут мікробіології та імунології ім. І.І.Мечникова НАМН України», Національний фармацевтичний університет*

Ключові слова: *Asperula odorata*; *Asperula humifusa*; фагоцитарний індекс; фагоцитарне число

Наведені результати вивчення *in vitro* впливу сухих екстрактів *Asperula odorata* та *Asperula humifusa* на функціональну активність макрофагів і їх гематогенних попередників. Встановлено, що досліджені екстракти виявляють дозозалежну стимулюючу дію на трансформаційну та фагоцитарну активність макрофагів і їх мононуклеарних попередників. Більш виражену імуномодулюючу дію має сухий екстракт *Asperula humifusa* в дозовому діапазоні концентрацій від 5 до 100 мкг/мл. При використанні досліджуваних екстрактів у дозі 100 мкг/мл відзначається максимальна стимуляція функціональної активності імунокомпетентних клітин: екстракт *Asperula odorata* збільшує трансформаційну активність мононуклеарів на 57%, фагоцитарний індекс – на 46% і фагоцитарне число – на 54% у порівнянні з контролем; екстракт *Asperula humifusa* підвищує трансформаційну активність на 64%, фагоцитарний індекс – на 44%, а фагоцитарне число – на 56%. Результати досліджень підтверджують перспективу використання сухих екстрактів видів маренки з метою корекції імунопатологічних станів.

ИММУНОМОДУЛИРУЮЩЕЕ ДЕЙСТВИЕ СУХИХ ЭКСТРАКТОВ ASPERULA ODORATA L. И ASPERULA HUMIFUSA M. ВІБВ. BESSER

Н.В.Кашпур, Н.С.Юрченко*, Т.В.Ильина*, А.М.Ковалева*, О.В.Горячая*, М.В.Смелянская, С.Д.Перемот
ГУ «Институт микробиологии и иммунологии им. И.И.Мечникова НАМН Украины», Национальный фармацевтический университет*

Ключевые слова: *Asperula odorata*; *Asperula humifusa*; фагоцитарный индекс; фагоцитарное число

Приведены результаты изучения *in vitro* влияния сухих экстрактов *Asperula odorata* и *Asperula humifusa* на функциональную активность макрофагов и их гематогенных предшественников. Установлено, что исследованные экстракты проявляют дозозависимое стимулирующее действие на трансформационную и фагоцитарную активность макрофагов и их мононуклеарных предшественников. Более выраженное иммуномодулирующее действие имеет сухой экстракт *Asperula humifusa* в дозовом диапазоне концентраций от 5 до 100 мкг/мл. При использовании исследуемых экстрактов в дозе 100 мкг/мл отмечается максимальная стимуляция функциональной активности иммунокомпетентных клеток: экстракт *Asperula odorata* увеличивает трансформационную активность мононуклеаров на 57%, фагоцитарный индекс – на 46% и фагоцитарное число – на 54% в сравнении с контролем; экстракт *Asperula humifusa* повышает трансформационную активность на 64%, фагоцитарный индекс – на 44%, а фагоцитарное число – на 56%. Результаты исследований подтверждают перспективу использования сухих экстрактов видов ясенников с целью коррекции иммунопатологических состояний.

Address for correspondence:

4, Blyukher str., Kharkov, 61168, Ukraine.

Tel. (572) 67-92-08. E-mail: n-yurchenko88@ukr.net.

National University of Pharmacy

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