

Immunostimulating activity of triterpene glycosides in *Saponaria roots officinalis* L.

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#### SUMMARY

The effect of triterpene glycosides of *Saponaria officinalis* L. roots on the synthesis of cytokines INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4 by human mononuclear cells in vitro was studied. Their dose-dependent immunomodulatory effect was established, aimed at stimulating cellular immunity, which suggests the possibility of their use in the production of functional food products for the prevention of immune diseases and prevention of food allergic reactions.

Key words: triterpene glycosides, saponins, functional foodstuffs, immunological reactions, immunomodulatory dose-dependent activity, *S. officinalis* L., cytokines, whole blood of healthy donors.

#### RESUME

The effect of triterpene glycosides of the roots of *Saponaria officinalis* L. on synthesis of cytokines INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4 by human mononuclear cells in vitro was researched. We found dose-dependent immunomodulatory effect aimed at stimulating cellular immunity, which suggests the possibility of their use in the production of functional foods for prevention of immune diseases and prevention of food allergies.

Keywords: plant saponins, triterpene glycosides, functional foods, immunological reactions, dose-related immunomodulating activity, *S. officinalis* L., cytokines, whole blood from healthy donors.

Plant saponins - triterpene and steroidal glycosides with a wide spectrum of biological action [1, 2], belong to the class of low molecular weight bioregulators and are widely used in medicine and pharmaceuticals. Glycosides of the roots of ginseng (*Panax* genus), Manchurian aralia (*Aralia mandshurica*), licorice (*Glycyrrhiza glabra*), etc. are known as medicinal

preparations are the active principle of many herbal tea and herbal teas. However, at present, triterpene glycosides are becoming especially attractive as biologically active components in the production of functional food products that contribute to the prevention and treatment of various diseases [3, 4]. This area is especially actively developing in Japan - the founder of the Food for Specified Health Use (FOSHU) functional nutrition program. In Russia, little attention is paid to the study of the biological properties of triterpene glycosides and their use in various industries, as evidenced by the limited number of domestic publications of a fragmentary nature [5, 6, 7, 8, 9, 10, 11].

Foreign studies in recent years have shown that some triterpene glycosides (bark of the tree *Quillaja saponaria* M., roots of plantation ginseng *Panax* genus, roots of *Platycodon grandiflorum*, etc.) have immunostimulating activity, since they are able to simultaneously stimulate a balanced immune response of the cell and humoral types as a result of secretion cytokines [12, 13]. At the same time, they can shift the balance towards the cellular immune response and reduce the synthesis of allergenic IgE antibodies, thereby participating in the regulation of allergic reactions [14]. The proven immunomodulatory effect of triterpene glycosides on the mammalian immunity system served as the basis for the development of a range of preventive food products against diseases of the immune system:

This work is devoted to the study of the possibility of using triterpene glycosides of the roots of *Saponaria officinalis* L. as an immunomodulatory agent in the production of functional food. For this purpose, the effect of glycosides on the synthesis of cytokines by human mononuclear cells in vitro was investigated.

The roots of *S. officinalis* L., which have the trade name red soap root, are included in the pharmacopoeias of a number of Western European countries and are widely used in homeopathy [17, 18].

#### Materials and methods

The object of the study was the triterpene glycosides of the roots of *S. officinalis* L. (double form) with an established chemical structure [19]. The study of the effect of saponins on spontaneous and mitogen-induced cytokine production in whole blood of healthy donors was carried out according to the method [20]. Fresh heparinized blood (20 IU / ml) from 10 donors was diluted 3-fold with sterile RPMI 1640 medium (Serva) containing 3% L-glutamine and 100 µg / ml gentamicin. To 1.35 ml of diluted blood was added 0.15 ml of RPMI 1640 medium (culture of intact cells) or standard mitogen phytohemagglutinin (PHA) at a final concentration of 10 µg / ml (culture of mitogen-stimulated cells). In order to study the effect of saponins on the production of cytokines, solutions of drugs in a volume of 0.15 ml at a concentration of 0.1 were added to the test tubes; 1.0 and 10.0 µg / ml. The tubes were placed in a thermostat and incubated at 37 ° C in an atmosphere of 5% CO<sub>2</sub> 24 hours, after which they were centrifuged for 5 minutes, the supernatants were stored at -20 ° C.

The concentration of cytokines (INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4) was determined by the method of enzyme-linked immunosorbent assay using commercial test systems of OOO Cytokin (Russia) in accordance with the instructions attached to the kits.

Statistical processing of the data obtained was carried out using the Statistica-7 software package.

#### Results and discussion

The results of the study showed that the baseline level of cytokines in the blood of healthy donors fluctuated significantly and is consistent with the data of many other sources. Despite this, we have revealed a differentiated effect of triterpene glycosides on the spontaneous induction of the cytokines INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4 by mononuclear cells. Thus, the introduction of glycosides into an intact cell culture caused a decrease in INF $\gamma$  production at all studied concentrations. However, a statistically significant inhibitory effect was observed only in donors with an initially relatively increased level of INF $\gamma$  at a glycoside concentration of 1.0  $\mu\text{g} / \text{ml}$ . Glycosides at a concentration of 0.1 and 10  $\mu\text{g} / \text{ml}$  did not show statistically significant changes in INF $\gamma$  production (Table 1, Fig. 1). Also, no significant response of donor cells with an initially relatively low INF $\gamma$  level ( $10.34 \pm 5$ ,

Table 1

Cytokine levels (INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4) in cell supernatants healthy donors (n = 10)

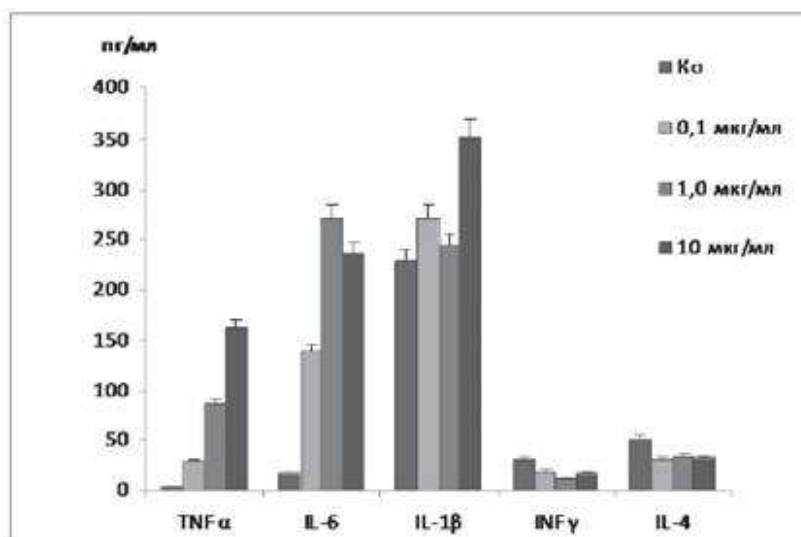
Уровень цитокинов (пг/мл)	M $\pm$ m	Минимум-максимум
INF $\gamma$	30,19 $\pm$ 7,07	9,87–59,82
IL-4	51,27 $\pm$ 5,83	35,93–77,86
IL-6	16,03 $\pm$ 5,82	2,36–45,12
IL-1 $\beta$	229,36 $\pm$ 28,25	130,44–407,38
TNF $\alpha$	3,35 $\pm$ 0,30	2,60–5,02

Under the influence of glycosides, a statistically significant increase in TNF $\alpha$  production by intact cells was observed. At the same time, with an increase in concentration, the effect intensified.

A significant increase in the level of IL-1 $\beta$  by intact cells was detected only at a glycoside concentration of 10  $\mu\text{g} / \text{ml}$ , while concentrations of 0.1 and 1  $\mu\text{g} / \text{ml}$  did not cause significant changes in their production.

Noteworthy is the multidirectional response of intact cells to the production of IL-6 and IL-4 under the action of glycosides. Thus, at an initially low level of IL-6 ( $16.03 \pm 5.82$   $\text{pg} / \text{ml}$ ), a significant increase in its amount was noted, while the maximum effect was observed at a glycoside concentration of 1 and 10  $\mu\text{g} / \text{ml}$ . At the same time, in 2 donors with an initially high level of IL-6 (496.59 and 80.67  $\text{pg} / \text{ml}$ ), its concentration sharply decreased (113.41 and 15.76  $\text{pg} / \text{ml}$ , respectively). The opposite pattern was observed upon induction of IL-4 by cells; a significant decrease in its level was noted in donors with a relatively high content ( $51.27 \pm 5.83$

pg / ml), while at a relatively low level (13.56 pg / ml), an increase in its production was found under the action of all doses of glycosides. However, due to the small sample size (2–3 donors for each cytokine), it is not possible to judge the reliability of the results.



Rice. 1. Influence of the concentration of triterpene glycosides *S. officinalis* L. for induction cytokines by intact cells of peripheral blood.

The inducing efficiency of glycosides was assessed by the activity of the standard PHA mitogen. As you can see from the table. 2, in the culture of cells stimulated by PHA, a significant increase in the production of all studied cytokines was revealed, which is many times higher than the effect of glycosides. Consequently, the effect of glycosides on the induction of the studied cytokines is significantly lower than that of PHA. The use of glycosides against the background of pretreatment of cells with PHA did not show significant changes in cytokine production.

table 2

Cytokine production in the culture of intact peripheral blood cells under the influence of triterpene glycosides *S. officinalis* L.

Цитокины, пг/мл	Контроль	Сапонины мг/мл		
		0,1	1,0	10
INFγ	30,19 ± 7,07	18,70 ± 5,18	11,91 ± 3,67+	17,02 ± 4,18
TNFα	3,35 ± 0,30	29,42 ± 10,58*	86,11 ± 18,85**	162,67 ± 53,46**
IL-1β	229,36 ± 28,25	271,82 ± 31,31	243,50 ± 30,51	351,80 ± 27,13*
IL-6	16,03 ± 5,82	138,16 ± 32,35**	271,41 ± 49,55**	235,72 ± 44,65**
IL-4	51,27 ± 5,83	31,04 ± 4,74*	33,07 ± 5,25*	31,51 ± 4,64*

Примечание:

статистическая достоверность по сравнению с контролем:

\* –  $p \leq 0,05$ ; \*\* –  $p \leq 0,01$

Table 3

Cytokine production in the culture of PHA-stimulated peripheral cells  
blood under the influence of triterpene glycosides *S. officinalis* L.

Цитокины, пг/мл	Контроль	Сапонины мкг/мл		
		0,1	1,0	10
INF $\gamma$	2632,7 $\pm$ 434,3	2814,5 $\pm$ 398,8	2963,0 $\pm$ 342,4	2672,2 $\pm$ 414,4
TNF $\alpha$	1348,5 $\pm$ 108,6	1429,5 $\pm$ 114,4	1438,1 $\pm$ 88,9	1402,3 $\pm$ 78,3
IL-1 $\beta$	401,19 $\pm$ 17,29	415,38 $\pm$ 9,01	427,62 $\pm$ 3,48	409,93 $\pm$ 3,50
IL-6	440,25 $\pm$ 7,06	429,41 $\pm$ 8,98	433,83 $\pm$ 7,73	435,85 $\pm$ 5,54
IL-4	140,73 $\pm$ 10,39	144,22 $\pm$ 10,83	146,77 $\pm$ 10,07	149,26 $\pm$ 10,83

So way, carried out study revealed undeniable immunomodulatory, dose-dependent effect of *S. officinalis* L. triterpene glycosides on the production of INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4. This effect was manifested only in the culture of intact cells and was expressed in the stimulation of the production of TNF $\alpha$  and IL-6, which correlates with an increase in the concentration of the glycoside in the culture, and in the inhibition of the production of INF $\gamma$  and IL-4, regardless of the concentration of the glycoside. The analysis of the results obtained allows us to judge about the predominant stimulation of the cellular type immune response by the studied glycosides, which, apparently, indicates their antiallergic potential.

The data obtained in vitro on the activity of glycosides in relation to the studied cytokines suggest a possible regulation of their properties in vivo, which indicates the prospects for further studies of the biological properties of triterpene glycosides of the roots of *S. officinalis* L.

#### conclusions

Triterpene glycosides of *S. officinalis* L. roots have undoubted immunomodulatory dose-dependent activity in relation to INF $\gamma$ , TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-4. The insignificant effect of glycosides in comparison with the action of PHA suggests the possibility of their use in the production of functional food products in order to prevent immune diseases. The low potential allergenicity of glycosides and the ability to shift the equilibrium towards the cellular immune response indicate their antiallergic activity.

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