

# Results Of The Study Of The Immunotropic Action Of Cyclosiversioside A In Comparison With Immunal In Normal Conditions And In Secondary Immunodeficiency Conditions

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## Abstract

Cycloartan glycoside cyclosiversioside A, isolated from *Astragalus schahrudensis* Bunge, when administered orally to mice (10 mg / kg) simultaneously with their immunization with thymus-dependent antigen – sheep erythrocytes (intraperitoneal at a dose of  $2 \times 10^7$  / mouse) significantly stimulates the process of forming the primary antibody cells, secreting IgM in the spleen of mice.

Along with this, cyclosiversioside A increases the total cellularity of the central and peripheral organs of immunity, stimulates erythro- and leukopoiesis. All these changes are reliably expressed not only in normal mice, but also in mice with secondary immunodeficiency states, caused by damage to the liver by carbon tetrachloride, or by their total radioactive irradiation. Cyclosiversioside A has a stimulating effect on immunogenesis to a much greater extent than a preparation with immunostimulating activity – immunal.

**Keywords:** cycloartan glycosides, cyclosiversioside A, immunal, stimulation of immunogenesis

## INTRODUCTION

Many plant compounds with metabolic activity also have a pronounced immune stimulating influence, especially in secondary immunodeficiency states. In this respect, flavonoids [7], pectin substances [5], phytoecdysteroids [14], polyphenols [17,18] are distinguished. Another group of substances that have a beneficial effect on the course of metabolic processes in the organism, as established in recent years, the groups are cycloartan glycosides [3, 8, 12]. However, the possible role of cycloartan glycosides in the regulation of the immune status has not been adequately studied, although some reports indicate that their research is promising in the corresponding context [15]. In this work, we have analyzed the potential immunomodulatory activity of one of the cycloartan glycosides, cyclosiversioside A, contained in plants of the genus *Astragalus*, widespread in the Central Asian region. The experiments were carried out in comparison with the known immunomodulatory agent of plant origin immunal.

### The aim of the research.

To study the presence and severity of immunostimulating activity in cycloartan glycoside – cyclosiversioside A (compared to immunal) in normal conditions and in secondary immunodeficiency states, developing in acute toxic hepatitis and radiation exposure of animals.

### Research methods and materials.

Cyclosiversioside A used in this work was isolated from *Astragalus schahrudensis* Bunge [2]. Its effect to the immune system was examined on white outbred mice weighing 19-20 g – intact and with developed secondary immunodeficiency states.

Acute toxic hepatitis was caused by subcutaneous injection of carbon tetrachloride (CCl<sub>4</sub>) in the form of a 20 % oil solution, 0.2 ml each for 3 days [4].

The animals were irradiated at a sublethal dose of 5 Gray on a Theratron  $\gamma$ -medical irradiator with a power of 1.2 Gy / min. The irradiation time is 20 minutes. The distance from the tube to the surface of the body of mice is 65 cm; the source is <sup>60</sup>Co. In the first case, immunization of mice with thymus-dependent antigen - sheep erythrocytes (intra-abdominal at a dose of 2x10<sup>7</sup> / mouse) and administration of cyclosiversioside A (oral – 10 mg / kg) were carried out on the last day of CCl<sub>4</sub> administration, in the second, sheep erythrocytes and cyclosiversioside A were injected in the same way 5 days later after irradiation. In both cases (as in intact animals), on the 5<sup>th</sup> day after the introduction of cyclosiversioside A and the antigenic stimulus, mice were taken into the experiment. To judge the effect of cyclosiversioside A on the primary immune response, the number of antibody-producing cells in the spleen was determined [16]. In addition, the total quantity of cells in the central (thymus, bone marrow – the femur was used) and peripheral (mesenteric lymph nodes, spleen) organs of immunity were calculated using the Goryaev camera. Additionally, the effect of cyclosiversioside A on some hematological parameters was assessed, for which the quantity of erythrocytes and leukocytes was counted in the peripheral blood. The reference – preparation in the experiments was immunal (dried juice from a freshly harvested herb of flowering *Echinacea purpurea* L., containing flavonoids, derivatives of coffees and chicoric acids, saponins, polysaccharides, a number of phytosterols, etc.) [1] from the manufacturer “Lek d.d.” (Slovenia), administered in a similar way at the rate of 50 mg / kg. When conducting experiments on mice, we adhered to the general principles of ethics in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986). The obtained data were processed statistically by using the Student’s t-test.

## Results and discussion

Conducted experiments have shown that cyclosiversioside A, as well as immunal, when administered to intact mice, increases the process of primary antibody production, increasing the number of antibody-forming cells in the spleen that secrete IgM in response to immunization with sheep erythrocytes. This is clearly seen when calculating the number of antibody-forming cells for the entire spleen and for 1 million splenocytes, the level of which also had a clear tendency to increase (Table 1).

**Table 1** The influence of cyclosiversioside A and immunal on the immune response of normal mice after administration of sheep erythrocytes (M ± m, n = 10)

Experimental conditions	The number of nucleated spleen cells, x10 <sup>6</sup>	Number of antibody- forming cells		The number of Thymus cell, x10 <sup>6</sup>	The number of bone marrow cell, x10 <sup>6</sup>	The number of Mesenteric lymph node cell, x10 <sup>6</sup>
		on the spleen	per 1 million splenocytes			
Intact animals	176.0±12.8	5300.0±480.0	30.1±3.6	26.2±2.4	13.2±1.2	16.4±1.4
Cyclosiversioside A	252.0±18.8*	15640.0±796.4*. <sup>1</sup>	62.1±5.2*	50.4±5.2*. <sup>1</sup>	28.6±1.8*. <sup>1</sup>	38.2±3.2*. <sup>1</sup>
Immunal	226.0±15.8*	12084.0±656,6*	53.5±4.4*	37.2±2.8*	19,2±1,4*	28.6±1.8*

Note. \* -Reliable to the indicators of intact animals, <sup>1</sup>-significant between the experimental groups (p <0.05)

**Table 2** The influence of cyclosporin A and immunal on the immune response of mice with developed acute toxic hepatitis upon administration of sheep erythrocytes (M ± m, n = 10)

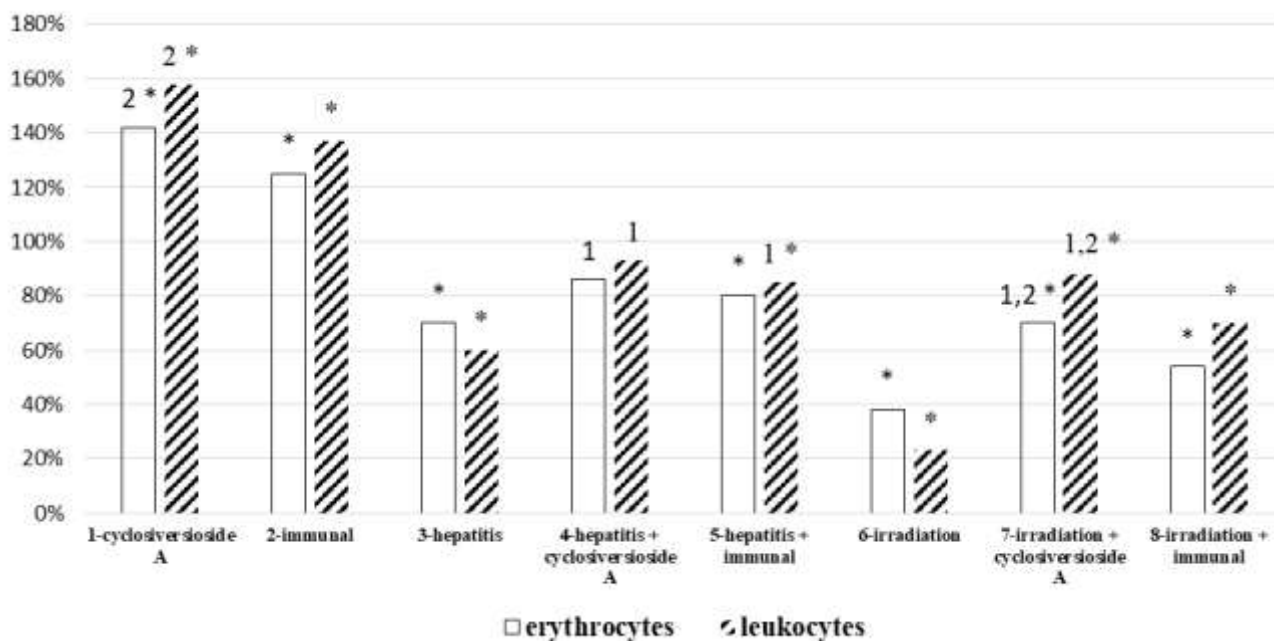
Experimental conditions	Number of nucleated spleen cells, x10 <sup>6</sup>	Number of antibody-forming cells		The number of Thymus cell, x10 <sup>6</sup>	The number of bone marrow cell, x10 <sup>6</sup>	The number of Mesenteric lymph node cell, x10 <sup>6</sup>
		on the spleen	per 1 million splenocytes			
Intact animals	180.0±11.6	5520.0±296.2	30.6±2.4	30.2±2.4	11.8±1.2	15.8±1.6
Control (hepatitis)	110.0±10.2*	1950.0±162.4*	17.7±1.2*	14.2±1.2*	5.2±0.4*	7.4±0.6*
Hepatitis + cyclosporin A	170.0±11.2 <sup>1</sup>	4750.0±280.4 <sup>1,2</sup>	27.9±2.6 <sup>1,2</sup>	26.6±1.8 <sup>1,2</sup>	9.8±0.9 <sup>1,2</sup>	12.6±1.2 <sup>1</sup>
Hepatitis + immunal	162.0±13.4 <sup>1</sup>	3510.0±210.8* <sup>1</sup>	21.6±12.0* <sup>1</sup>	19.2±1.4* <sup>1</sup>	7.2±0.8* <sup>1</sup>	10.2±0.8* <sup>1</sup>

Note. Here, in table 3 and in the figure \* -reliable to the indicators of intact animals; <sup>1</sup>-reliably to control; <sup>2</sup>-significantly between experimental groups (p<0.05)

Among other revealed facts of assessing the immunobiological properties of cyclosporin A, it should be noted that an increase under its influence, the general cellularity of the central and peripheral organs of immunity, as well as a noticeable stimulation of erythro- and leukopoiesis (Table 1, Fig.1.) on all the indicators under consideration, in the carried out experiments, cyclosporin A looked like a more active immunostimulating property than the well-known preparation – immunal.

The immunostimulating effect of cyclosporin A was quite clearly manifested in reproduced secondary immunodeficiency states, in which profound disturbances in the immunological reactivity of the organism are revealed. Thus, with acute toxic hepatitis and with irradiation of animals, the number of antibody-forming cells in the spleen decreased by 64.7 and 88.5%, respectively, the number of nucleated spleen cells decreased by 38.9 and 81.3% (when calculating the number of antibody-forming cells per 1 million the decrease in splenocytes was 42.2 and 38.4%). Extremely negative changes were also noted in the general cellularity of the central and peripheral organs of immunity, as well as on the part of erythro- and leukopoiesis. It was under these conditions that cyclosporin A showed a pronounced effect aimed at restoring the studied parameters. At the same time, as in

intact animals, its activity in the considered aspects exceeded the corresponding effect of the immunal (Table 2, 3; Fig.1.). Thus, in mice with acute toxic hepatitis under the influence of Cyclosiversioside A and immunal after immunization with sheep erythrocytes, the number of nucleated spleen cells increased relative to the control by 54.5 and 47.35, the number of antibody-forming cells for the entire spleen increased by 143.6 and 80 % (for 1 million splenocytes, respectively, by 57.6 and 22.0%). The total cellularity of the thymus, bone marrow and mesenteric lymph nodes increased under the influence of cyclosivioside A and immunal increased by 87.3 – 35.2; 88.5 – 38.5 and 70.3 – 37.8%. In the case of radioactive irradiation under the influence of both preparations, the total cellularity of the spleen increased by 89.4 and 58.8%, the number of antibody-forming cells for the entire spleen increased by 181.5 and 91.0% (by 1 million splenocytes, respectively by 48.3 and 20.4%) (Table 3.). The total cellularity of the thymus, bone marrow and mesenteric lymph nodes increased by 95.7 – 51.1; 62.9 – 37.0 and 120.8 – 54.2%. A similar pattern was observed at calculating the content of erythrocytes and leukocytes in the blood (Fig.1.).



**Picture 1.** The influence of cyclosiversioside A and immunal on the content of erythrocytes (light bars) and leukocytes (shaded bars) in blood in mice immunized with normal ram erythrocytes with developed secondary immunodeficiency under conditions of acute toxic hepatitis and radiation (% of the corresponding indicators for intact animals, accepted as 100 %).

Thus, the obtained data show that Cyclosiversioside A is an immunoactive agent that has certain advantages in terms of the severity of the effect over the known immunotropic agent immunal. These results are important in themselves; nevertheless, they are also interesting from the point of view of expanding knowledge about the biological activity of cycloartan glycosides. We have previously shown that Cyclosiversioside A and structurally related compounds of this series optimize carbohydrate, energy and lipid metabolism in the myocardium, especially under conditions of myocarditis and atherosclerosis, they will show a normalizing effect on the disturbed antioxidant and NO-ergic systems, and have interferon – inducing activity, similar to phytoecdysteroids, reduce blood coagulability, have a stress-protective effect [6, 9, 10, 11, 12, 13].

**Table 3** The influence of Cyclosiversioside A and immunal to the immune response of irradiated mice after administration of sheep erythrocytes (M ± m, n = 10)

Experimental conditions	Number of nucleated spleen cells,	Number of antibody-forming cells	The number of thymus cell, x10 <sup>6</sup>	The number of bone	The number of Mesenteric lymph node
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	x10 <sup>6</sup>	on the spleen	per 1 million splenocytes		marrow cell, x10 <sup>6</sup>	cell, x10 <sup>6</sup>
Intact animals	174.6±34.2	5820.0±360,2	33.3±2.6	25.6±1.8	12.2±1.2	16.2±1.4
Control (irradiation)	45.6±4.2*	670.0±22.4*	14.7±0.6*	9.4±0.8*	5.4±0.6*	4.8±0.4*
Irradiation + cyclosiversioside A	86.4±6.2*,1	1886.0±170.6* .1,2	21.8±2.2*,1	18.4±1.4*1,2	8.8±0.5*1,2	10.6±0.8*1,2
Irradiation + immunal	72.4±5.8*,1	1280.0±152.8* <sup>.1</sup>	17,7±1,2* <sup>.1</sup>	14.2±1.2* <sup>.1</sup>	7.4±0.4* <sup>.1</sup>	7.4±0,6* <sup>.1</sup>

All of this opens up the prospect of developing on the basis of cycloartan glycosides and Cyclosiversioside A, in particular, effective immunomodulatory agents with a fairly wide range of beneficial effects on metabolic processes in the organism.

### Conclusions:

1. Cyclosiversioside A has a pronounced stimulating effect to immunogenesis both in normal animals and, especially, in animals with secondary immunodeficiency developing against the background of toxic CCl<sub>4</sub> – hepatitis and radiation exposure.

2. By its ability to increase the number of antibody-forming cells in the spleen of animals in response to their immunization with sheep erythrocytes, to increase the total cellularity of the central and peripheral organs of immunity, to activate erythro- and leukopoiesis, Cyclosiversioside A surpasses the known preparation of immunal.

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