

# INFLUENCE OF PHYTOECDYSTEROIDS TO ERYTHROPOIESIS, LEUKOPOIESIS AND TITER OF ANTIBODIES IN EXPERIMENTAL ANIMALS

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DOI: 10.29322/IJSRP.8.6.2018.p7809  
<http://dx.doi.org/10.29322/IJSRP.8.6.2018.p7809>

**Abstract:** Individual phytoecdysteroids and total ecdysteroid-containing preparations, which studied by us have ability to increase the number of cells in the peripheral organs of immunity and the titer of antibodies to ER (erythrocyte of ram (sheep)) in the blood of mice. This confirms the high immune stimulating activity of some animals and helps to better understand their action mechanism to immune system of the organism, as a whole.

**Key words:** individual phytoecdysteroids, total phytoecdysteroids, immunal, T-activin, erythrocytes, leukocytes, antibody titer, immunity, mice

We studied the effect of phytoecdysteroids to erythropoiesis, leukopoiesis and antibody titer in experimental animals in this work.

The experiment was performed in white mongrel mice; the weight is 18-22 grams. Each test group consisted of 10 animals. The animals were kept on a standard ration of the vivarium.

The thymus-dependent antigen of erythrocyte of ram (EB) was used in the experiments. Before immunization, the ER was centrifuged twice at 1000 turnover for 10 minutes in medium of 199 or in physiological solution. ER was introduced once inside peritoneum at a dose of  $2 \times 10^8$  cells per mouse.

We used the phytoecdysteroids in the work, isolated from plant raw materials by researches of Laboratory of Chemistry of Glycosides of the Institute of Chemistry of Plant Substances of the Academy of Sciences of Uzbekistan named after S.Yu. Yunusov. Individual compounds were represented by a-ecdysone, 2-deoxy- $\alpha$ -ecdysone, integristeron A, sileneozides A and B, ecdysterone and turkesterone, isolated from *Rhaponticum integrifolium* C. Wink.; *Rhaponticum carthamoides* (Willd) Iljin; *Silene praemixta* M. Pop.; *Silene brahuica* Boiss.; *Ajuga turkestanica* (Rgl.) Brig.

Total ecdysteroid – containing preparations, so confirmed to study were received from the same plants, but contained multiple biologically active compounds of this rate. By Mamadaliyeva N.Z. and coauthors (2003) was isolated total ecdysteroid preparation from *Silene viridiflora*, the composition involves the followings: ecdysterone, sileneozides A, D, polipodin B and others [1]. Saatov Z. (1993) received a total ecdysteroid preparation from *Silene brahuica*, the composition involves the ecdysterone, sileneozides A, B, C, D, E and etc. B.Z. Usmanov with coauthors (1975) isolated a total ecdysteroid containing preparation from *Ajuga turkestanica*, in which the composition identified with ecdysterone, turkesteron, siasteron and others. These total t ecdysteroid –containing preparations were conventionally called by TEP-1, TEP-2 and TEP-3, respectively.

The phytoecdysteroids and total ecdysteroid-containing preparations were input to animals at a dose of 5 mg / kg (in preliminary experiments, these doses were installed as the most effective) in the form of an aqueous emulsion with apricot gum

(due to poor solubility) by the atraumatic probe - peros in the work. In those cases, if other way of introduction has been used, an indication is given on the index of the material further on the text.

Control animals received (by similar ways) an adequate amount, only in water emulsion by apricot gum (it was installed in previous experiments that the apricot gum itself in using amount to the immunogenesis of animals doesn't influence). The multiplicity of the introduction of the investigated substances was described in each case separately.

T-actinin and immunal from the producing firm of Lek d.d. (Slovenia) were used as reference - preparations for determining the immunotropic actions of phytoecdysteroids, which were introduced as well as the test substances from the calculation of 0,5 and 50 mg / kg, respectively.

The amount of erythrocytes and leukocytes in peripheral blood was counted in the immunized animals, the antibody titer was determined in the hemagglutination reaction [2].

Statistical processing of the received data was carried out on a personal computer using the t-criteria of Styudent and the standard package of Microsoft Excel. The medium arithmetical (M), the average standard deviation ( $\sigma$ ), the standard error (m), the criteria of Styudent (t) with the calculation of probability error (p) were calculated. Differences of average dimension were considered positive at a significance level of  $p < 0.05$ .

The effect of phytoecdysteroids to the amount of erythrocytes and leukocytes in peripheral blood of mice was considered in the research (table).

**Table 1:** The influence of substances to the amount of erythrocytes and leukocytes in peripheral blood of white mongrel mice (M $\pm$ m)

Condition of experiment	Erythrocytes $\times 10^9$ /ml	IS	leukocytes $\times 10^6$ /ml	IS
Control (n=10)	7,8 $\pm$ 0,8	-	7,6 $\pm$ 0,5	-
2-deoxy- $\alpha$ -ecdysone (n=10)	8,3 $\pm$ 0,4	+1,06	8,1 $\pm$ 0,3	+1,07
$\alpha$ - Ecdysone (n=10)	9,3 $\pm$ 0,5	+1,19	9,3 $\pm$ 0,2*	+1,22
Integristerone A (n=10)	9,3 $\pm$ 0,5	+1,19	9,4 $\pm$ 0,3	+1,24
Sileneozide A (n=10)	9,6 $\pm$ 0,4	+1,23	9,7 $\pm$ 0,2*	+1,28
Sileneozide B (n=10)	9,9 $\pm$ 0,4*	+1,27	9,9 $\pm$ 0,3*	+1,30
Ecdysterone (n=10)	11,6 $\pm$ 0,7*	+1,49	10,6 $\pm$ 0,7*	+1,39
Turkesteron (n=10)	11,8 $\pm$ 0,6	+1,51	11,2 $\pm$ 0,5*	+1,47
TEP-1 (n=10)	12,2 $\pm$ 0,4	+1,56	11,7 $\pm$ 0,7*	+1,54
TEP-2 (n=10)	12,9 $\pm$ 0,4*	+1,65	13,7 $\pm$ 0,2*	+1,80
TEP-3 (n=10)	13,6 $\pm$ 0,2*	+1,74	15,9 $\pm$ 1,1*	+2,09
T-activin (n=10)	8,5 $\pm$ 0,3	+1,09	8,5 $\pm$ 0,3	+1,12
Immunal (n=10)	11,2 $\pm$ 0,4	+1,44	14,1 $\pm$ 0,3*	+1,86

Note. n- Number of animals, \* - reliable with respect to control ( $p < 0,05$ )

In the control group, the number of blood erythrocytes is equal to  $7,8 \pm 0,8 \times 10^9$ / ml. It was installed, as in other experiments of this series, that 2-deoxy- $\alpha$ -ecdysone proved to be the weakest stimulator of erythropoiesis. Then, in the rate of  $\alpha$ -ecdysone, integristeron A, sileneozides A and B, ecdysterone and turkesteron, the activity gradually increased ( by 1,19, 1,19, 1,23, 1,27, 1,49 and 1,51 times, respectively). And significant differences with the control were recorded, beginning with sileneozide B.

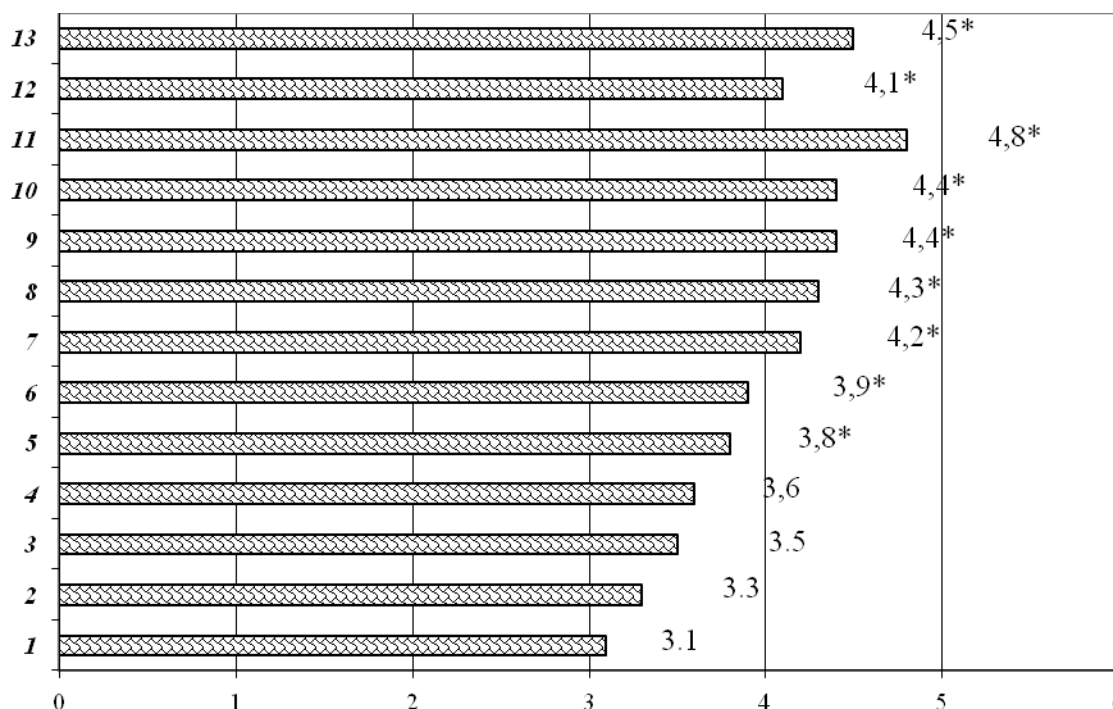
The total ecdysteroid-containing preparations and in the counting of erythrocytes were stood out by their more pronounced activity. Under the influence of TEP-1, TEP-2, TEP-3, the number of erythrocytes in the blood was increased by 1,56, 1,65 and 1,74 times. T-activin and immunal increased the number of erythrocytes in the blood by 1,09-1,44 times (table).

The amount of leukocytes in the blood in the control group of animals was  $7,6 \pm 0,5 \times 10^6$ / ml. in our experiments. It was installed that the preparation of 2-deoxy- $\alpha$ -ecdysone improperly increases the amount of blood leukocytes. The following preparations:  $\alpha$ -Ecdysone, integristerone A, sileneozides A and B significantly and approximately increase the number of blood leukocytes by 1,22, 1,24, 1,28 and 1,30 times in the same degree. Ecdysterone and turkesteron increased the amount of leukocytes in the blood by 1,39 and 1,47 times.

A large stimulating effect on the white germ of hematopoiesis is summarized by preparations such as: TEP-1 increases the number of blood leukocytes by 1,54 times, TEP-2 by 1,80 and TEP-3 by 2,09 times. T-activin and immunal increase the number of blood leukocytes by 1,12 and 1,86 times (these data are also reflected in the table).

At the end of this part of the work, the data were presented, received in the study of the influence of phytoecdysteroids to antibody titer ( $\log_2$ ) in peripheral blood in white mongrel mice (Fig.). 2-deoxy- $\alpha$ -ecdysone and  $\alpha$ -ecdysone unreliable increase the titer of antibodies to ER in the blood of mice by 1.06 and 1.13 times, respectively.

Other of the drugs significantly increase the titer of antibodies to ER in the peripheral blood of mice reliably in various degrees of severity: integristerone – by 1,16, sileneozide A - by 1,23, sileneozide B – by 1,26, ecdysterone – by 1,35, and turkesterone – by 1.39 times. More pronounced stimulating effect in regard to the titer of antibodies to ER in the blood of mice has TEP-1, TEP-2 and TEP-3: this index is increased respectively by 1,42, 1,42 and 1,55 times. T-activin increases the antibody titer to ER in the blood of mice by 1,32 times, and immunal – by 1,45 times.



**Fig 1. The influence of phytoecdysteroids to the titer of antibodies to ER in peripheral blood in white mongrel mice:**

1- control; 2-2-deoxy-  $\alpha$  -ecdysone; 3- $\alpha$ -ecdysone; 4 integristerone A; 5-silenioside A; 6-silenioside B; 7-ecdysterone; 8-turkesteron; 9-TEP-1; 10-TEP-2; 11-TEP-3; 12-T-activin; 13-immunal. The ordinate titer of antibody on axis ( $\log_2$ ). \* -Suitable with reference to control data (p <0.05).

Thus, individual phytoecdysteroids and total ecdysteroid-containing preparations have the ability to increase the number of cells in the peripheral organs of immunity and the titer of antibodies to ER in the blood of mice.

The received result of the experiment confirms the high immune stimulating activity of some phytoecdysteroids and helps to better understand their mechanism of action to the immune system of the organism, as a whole.

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