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INFLUENCE OF EXTRACT OF PEACH ORDINARY (PERSICA VULGARIS) LEAVES ON THE STATE OF THYMIC-LYMPHATIC ELEMENT OF THE IMMUNE SYSTEM OF RATS IN CONDITIONS OF CHRONIC IMMOBILIZATION STRESS

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Мета – *вивчення впливу густого екстракту з листя персика звичайного (ГЕЛП) на стан органів тиміколімфатичної ланки імунної системи щурів за умов хронічного іммобілізаційного стресу.*
Матеріали та методи. *Модель хронічного іммобілізаційного стресу (ХІС) відтворювали протягом 18 діб шляхом щоденної чотирьохгодинної іммобілізації щурів у тісних пеналах. Досліджуваний ГЕЛП, що був отриманий на кафедрі хімії природних сполук НФаУ, з листя персика сорту «Сальве», заготовленого в Таджикистані, вводили внутрішньошлунково в умовноефективній дозі 100 мг/кг. Як препарат порівняння використовували сироп «Імуно-Тон» у дозі 3 мл/кг внутрішньошлунково. Стан тиміколімфатичної ланки імунної системи в умовах ХІС визначали після проведення евтаназії тварин під легким інгаляційним наркозом за результатами дослідження коефіцієнтів маси тимуса і селезінки, а також гістологічного дослідження їх структури на мікропрепаратах, що були приготовлені за загальноприйнятою методикою. Для оцінки характеру впливу ГЕЛП на стан органів тиміко-лімфатичної системи щурів в умовах ХІС проводили порівняння з інтактним контролем та контрольною патологією.*
Результати дослідження. *Встановлено протективний вплив ГЕЛП на стан тиміко-лімфатичної ланки імунної системи в умовах ХІС. У селезінці відмічали збільшення ширини маргінальної зони лімфоїдних фолікулів і муфт та кількості периваскулярних лімфатичних муфт у 1,2 разу ($p<0,05$) порівняно з контрольною патологією, зниження ознак стресогенної гіпоксії – кількості структур білої пульпи зі спазмом центральних артерій. У тимусі встановлено зниження ступеня змін з третьої-четвертої до першої фази акцидентальної трансформації, що підтверджується підвищенням коефіцієнту маси тимуса на 2,7 % порівняно з контрольною патологією.*
Висновки. *Доведена здатність ГЕЛП відновлювати порушену в умовах стресу активність органів тиміко-лімфатичної ланки імунної системи. Ймовірно стверджувати, що поліфенольні сполуки ГЕЛП виявляють антиоксидантні властивості, посилюючи активність антиоксидантної системи, а полісахариди – імуностимулювальні, результатом виявлення яких є стреспротекторна дія. За ефективністю ГЕЛП не поступався препарату порівняння «Імуно-Тон»*
Ключові слова: *густий екстракт, листя персика звичайного, хронічний іммобілізаційний стрес, тимус, селезінка, стреспротекторна дія*

1. Introduction

Stress is a universal physiological response to rather strong effects, which is aimed at mobilizing compensatory mechanisms that can be manifested by the transition from activation of the physiological apparatus to exhaustion as a result of overstrain of the reserve capacity of the organism. Stress can cause factors such as injury, loss of blood, surgical intervention, high and low

temperatures, excessive physical activity, ionizing radiation, emotional stress, infection, intoxication, as well as environmental and social factors [1].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Stress is manifested by multiple changes in the body. Regardless of the nature of the stress factor or other conditions, the manifestations of stress-reaction are universal and can lead to the development of various serious diseases of the nervous, cardiovascular systems, metabolism or increase the risk of their occurrence. With long-term effects, stress-inducing factors cause the development of a thymico-lymphatic involution, that is, it reduces the activity of the immune system. In these conditions, the organism becomes less resistant to infectious diseases, it becomes possible to increase the risk of oncopathology [2].

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Herbal medicinal products with a diverse therapeutic and prophylactic effect, for example, affecting the normalization of all or most of the homeostasis parameters disturbed by stress, are effective for the correction of stress-related disorders. Particularly important are phytomedicines containing the complex BAS: polyphenolic compounds that exhibit mild effect, mainly enhancing the activity of peripheral stress-limiting systems, in particular antioxidant [3, 4], and polysaccharides, the immunostimulatory effect of which is highlighted in the scientific literature [4].

4. The field of research considering the general problem, which is described in the article

One of the promising plants is peach (Persica vulgaris), the leaves of which contain biologically active substances (organic acids, polysaccharides, tannins, carotenoids, flavonoids, etc.) [5], which exhibit a wide range of pharmacological activity: stress-resistant [6], the ability to increase the production of secretory immunoglobulin A [7]. Therefore, it was advisable to evaluate the stress-protective effect of the thick extract obtained from peach leaves on the model of chronic immobilization stress.

5. Formulation of goals (tasks) of article

The aim of the work was to study the effect of a thick extract of peach ordinary leaves (TEPL) on the state of organs of the thymico-lymphatic part of the immune system of rats under conditions of chronic immobilization stress (CIS).

6. Presentation of the main research material (methods and objects) with the justification of the results

A 30 % alcohol extract from peach leaves of the Salve variety harvested in Tajikistan in August 2014 after fruit harvest was investigated. The final extract was evaporated to a dense extract with a moisture content of 10.93 %. The extract was obtained at the Department of Chemistry of Natural Compounds of the National Pharmaceutical University under the direction of prof. V. S. Kislichenko.

To simulate the chronic stress of nonlinear males rats weighing 180-200 g, they were placed in close boxes for four hours for 18 days [8]. The study used 4 groups of animals of 6 individuals in each. The first group consisted of intact animals that were not exposed to stress and did not receive anything. The second group - animals of control pathology receiving distilled water in volume equivalent to the volume of the test substance. Animals of the third group received TEPL in a conditionally effective dose of 100 mg/kg, which was established earlier by the results of the study of activity of activity on the static load model [9]. Animals of the fourth group had administered the comparison drug (CD) – an analogue for the pharmacological action - the syrup “Immuno-Ton” containing the extract of the liquid eleutherococcus 16.6 g/100 ml, tincture of rhizomes with the roots of echinacea purpurea 8.0 g/100 ml, tincture St. John's wort 8.3 g/100 ml, at a dose of 3 ml/kg, which was calculated from the therapeutic dose for a person, taking into account the coefficient of species sensitivity [10]. The test substances and distilled water were administered intragastrally prophylactically for 5 days before the start of the experiment and every day for 40 minutes before exposure to stress.

After conducting laboratory tests, euthanasia of animals under an easy inhalation anesthesia was carried out. The collection of biomaterials for the study was carried out on the 18th day after the exposure of the stress corresponding to the depletion stage [8].

The state of organs (spleen and thymus) of the thymico-lymphatic chain of the immune system was determined by their weight factors and histological examination on microslide prepared according to the generally accepted method [11]. In the photographs (eyepiece 10, lens 20) using the Toupcam Granum program, the width of marginal zone (WMZ) of lymphatic follicles were determined in the spleen. On the microslides of the organ, the number of formed lymphatic follicles (LF) and periarterial lymphatic muffs (PLM) was calculated, which was proportional to the optical density [11].

In the course of research, the "General Ethical Principles of Animal Experiments" (Kyiv, 2001) were in line with the European Union Directives of 2010/10/63 on the Protection of Animals used for scientific purposes [12].

Statistical processing of the results was carried out using the methods of variation statistics using the standard statistical program package "Statistica, V. 6.0" [13, 14].

To assess the nature TEPL impact on the state of the thymico-lymphatic system, rats conducted under conditions of CIS compared with intact control (IC) and control pathology (CP).

Analysis of the integrated performance of state of thymico-lymphatic system in rats suggests that animals from the group CP on 18 day of CIS were likely to lower the mass coefficient (MC) of the central organ of the immune system thymus compared with animals with IC group (Table. 1). The weight of the spleen was somewhat reduced, but not reliably. It is known that the spleen of rats

is only a secondary body of all secondary organs of the immune system, in which all populations of cells found in lymphoid and hematopoietic organs are collected in one place. The variety of its cellular structure probably determines a wide range of functionality of the lymphoid organs under normal conditions, and especially after stress morphological changes in the thymus and spleen, likely reflecting their different roles in stress responses [1, 2].

Table 1

TELP impact on integrated indicators of lymphatic organs thymico-lymphatic immune rats under CIS condition (n = 6)

Group of animals	Mass coefficient	
	thymus	spleen
Intact control (IC)	0.104±0.010	0.49±0.05
Control pathology (CIS)	0.077±0.006*	0.46±0.02
CIS + TELP, 100 mg/kg	0.098±0.006**	0.49±0.03
CIS + "Immuno-Ton", (1:100) 15 ml/kg	0.081±0.006*	0.44±0.04

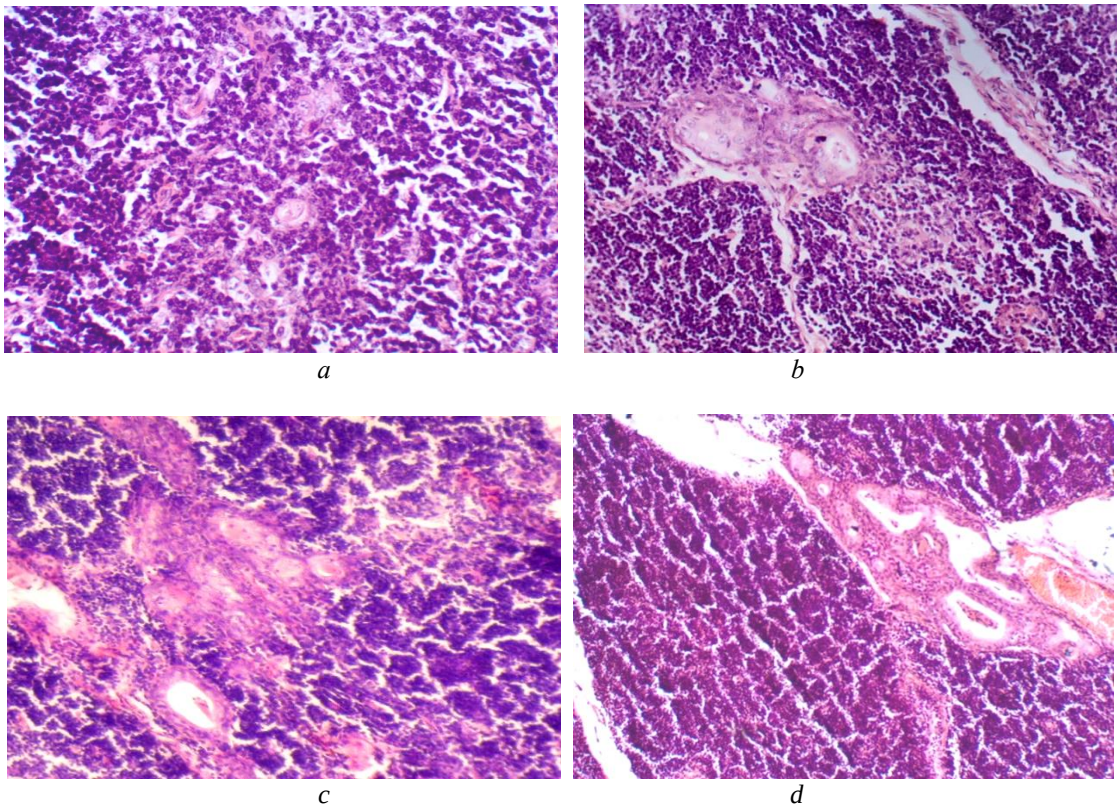
*Note: * – deviations are reliable for indicators of intact control, p <0.05; ** – deviations are reliable in relation to the parameters of the control group, p <0.05; n – the number of animals in the group*

Under the influence of the investigated preparation TELP there was a decrease in the degree of involution of the thymus. Against the background of the TELP, the thymus mass factor was 27 % higher (p <0.05) for this in the CP group and reliably did not differ from that in animals from the group of IC.

Compared with "Immuno-Ton", unlike TELP, the thymus state showed less pronounced protective effect, since the MC of thymus was significantly lower than that of intact animals (p <0.05) and did not differ from that of the CP group.

Both drugs did not have a likely effect on the weight of the spleen, but in the background of the effect of TELP its value was the same as in intact control.

At the next stage histological studies of thymus and spleen were performed. In Fig. 1 shows the structure of thymus rats on the 18th day of the CIS.



a *b* *c* *d*

Fig. 1. Structure of the thymus of rats on the 18th day of the IC: a – thymus of rats IR; b – thymus of rats of CP; c – the thymus of rats against the background of the effect of TEPL at a dose of 100 mg/kg: d – thymus of rats against the background of the action of the preparation "Immuno-Ton" in a dose of 3 ml/kg

Histologically, it was found that the lumbar structure of thymus intact animals is well expressed. In the brain substance among the lymphocytes, there are visible cells of the reticulo-epithelium with a large light nucleus, small and few thymus cells (Fig. 1, a). In fig. 2 there are the structure of the spleen of rats on the 18th day of the CIS.

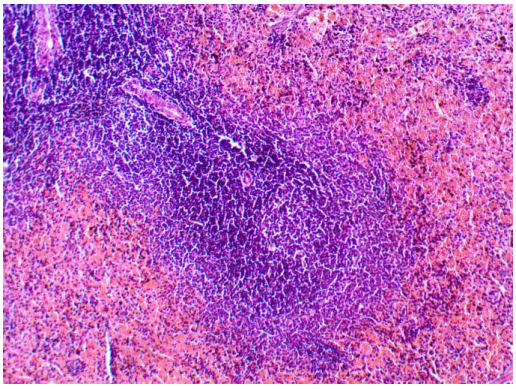
In the spleen of intact animals, the hematopoietic field was clearly divided into red and white pulp. The

white pulp is fed by lymphoid follicles and perivascular lymphatic arteries and arterioles that go to the lymphoid follicle. The lymphoid follicles themselves were well-formed, varied in size, had a ball-like or long-oval shape. The boundary between white and red pulp is quite expressive. The red pulp contained a large number of cells that had nuclei, red blood cells. The sinuses are not visible (Fig. 2, a). Such a state of the spleen reflects the level of immunogenesis in normal conditions.

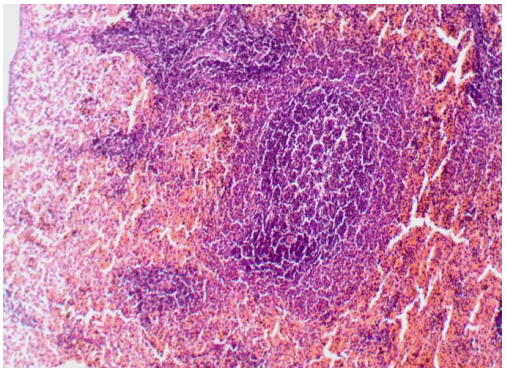
It was established that on the thymico-lymphatic link of the immune system of the CIS, it had an immunosuppressive effect. In the lymphoid follicles of the spleen of animals from the CP group, the central artery was in a state of spasm, which is a morphological imprint of the manifestation of hypoxia of stress genesis; the marginal zone was narrowed 1.4 times (Fig. 2, b, 3, a). It is known that the marginal white pulp region is involved in the immune response, because it is a place of "capture" of immune complexes, altered blood cells, and the like. Actually in marginal zones of lymphoid follicles and cooperation of deterministic B-lymphocytes with antigen occurs. When deterministic B-lymphocytes are activated by an appropriate antigen, they are proliferated and differentiated into antibody-forming cells that accumulate in red pulp [15]. The narrowing of these zones is a reflection of a decrease in the immune status of the body. Morphometrically, a decrease in the width of the marginal zone and a decrease in the number of lymphoid follicles and perivascular lymphatic clusters (reduction of the optical density units) was detected 1.5 times (Fig. 3, a, b). At the same time, in the lymphoid follicles, the number of reactive centres increased, their activity was an indicator of the development of immune responses in the humoral type. Probably this is a compensatory reaction of the body to stress.

In the thymus of stressed animals, signs of inhibition of lymphopoiesis have been detected. Against the background of normal migration of T-lymphocytes in T-dependent periorterial zones of lymphoid follicles and coupling of the spleen, delymphatization of the cortical substance of the thymus was observed. The reactive changes that arose as a result of stressful effects were classified as 2-4 phases of the incidental transformation (Fig. 1, b).

In the thymus of rats, the general morphological manifestations of the reactive stress reaction were observed on the background of the introduction of TEPL. Although the frequencies were smaller in size than intact controls, no noticeable loss of lymphocytes, similar to those in the CP group, was detected. The image of the "starry sky" was moderate. However, there was an expansion of the brain layer, observed focal proliferation of reticulo-epithelial cells, increased and cystic expansion of the thymus cells (Fig. 1, c) – signs of increased synthesis of hormones in the thymus and activation of cellular immune responses did not change with respect to CP. In general, the microscopic picture corresponded to the first (rarely second) phase of the accident transformation [9].



a



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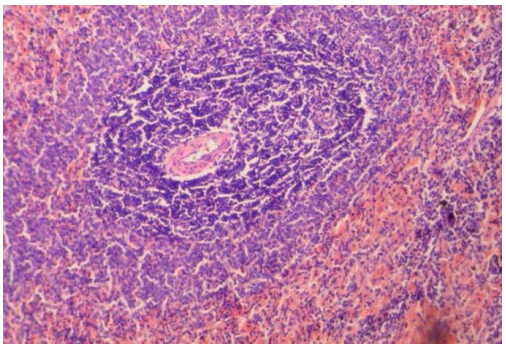

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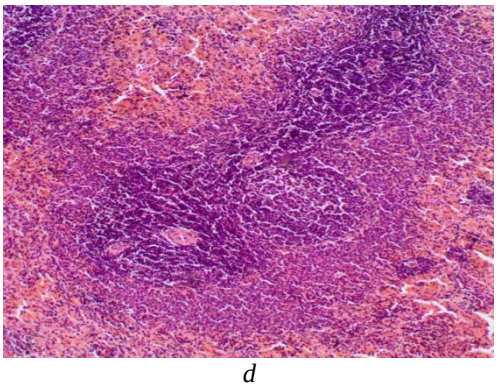
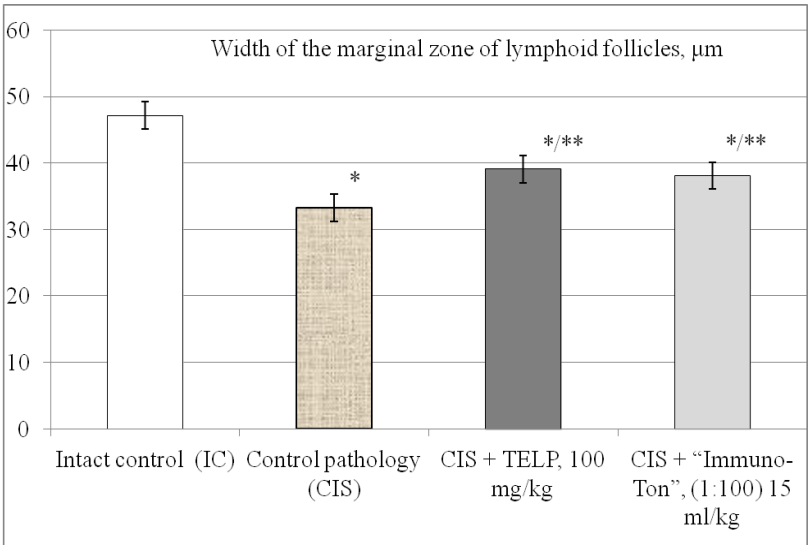


d

Fig. 2. Structure of the spleen of rats on the 18th day of the CIS: a – spleen of the rat IC; b – spleen of rats CP; c – spleen of rats in the background of TEPL in a dose of 100 ml/kg; g - a spleen against the background of the action of CD "Immuno-Ton" in a dose of 3 ml/kg

In the spleen of rats, which were administered TEPL before each immobilization session, a part of the lymph nodes showed an active reactive centre, the marginal zone remained slightly narrowed, the border with the red pulp fuzzy. In others, the marginal zone became moderately wide; the reactive centre was not activated. In general, with the TEPL, the width of the marginal zone of follicles and the number of lymphoid follicles and clusters was significantly higher in 1.2 times ($p<0.05$) compared with that in animals from the CP group (Fig. 3). Spasm of the central arteries in a significant number of lymph nodes was less pronounced compared with those in the animals of the CP group (Fig. 2, c). There were fewer macrophages and blast cells in red pulp.



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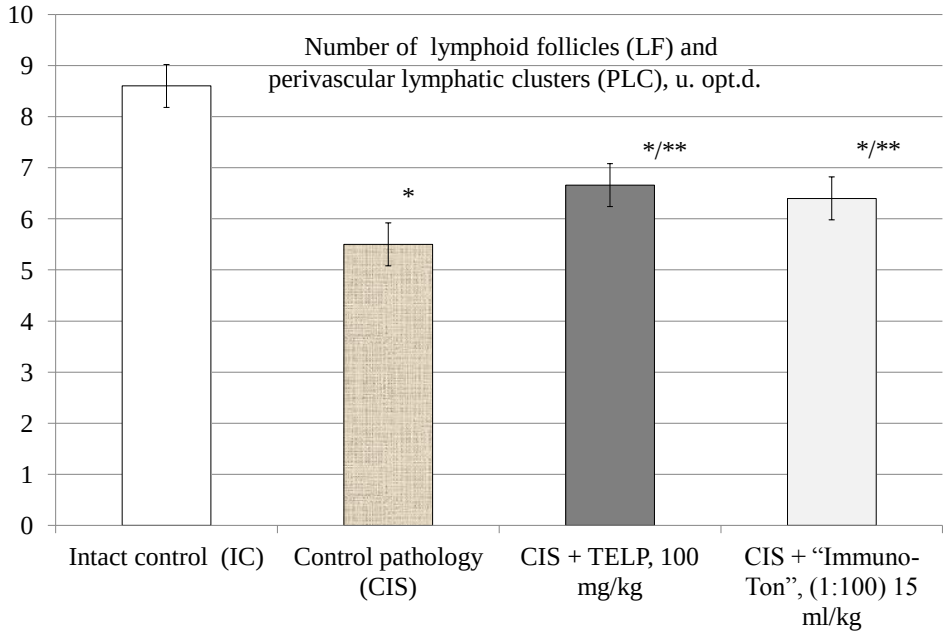

b

Fig. 3. Spleen of rats on the background of chronic immobilization stress under the influence of TEPL at a dose of 100 mg/kg and CD "Immuno-Ton" in a dose of 3 mg/kg: a – width of the marginal zone (WMZ); b – the number of lymphoid follicles (LF) and perivascular lymphatic clusters (PLC); * – statistical differences with respect to the indicators of animals in the group of infectious agents, $p<0.05$; ** – statistical differences with regard to the indicators of animals in the CP group, $p<0.05$

Thus, the protective effect of TEPL on the state of the thymico-lymphatic system of the immune system in the conditions of the CIS has been established. In the spleen, an increase in the width of the marginal zone of lymphoid follicles and muff and the number of perivascular lymphatic clusters was noted in 1.2 times ($p<0.05$) in comparison with the control pathology (Fig. 3), the decrease in the signs of stress hypoxia – the number of structures of white pulp with central spasm arteries In the thymus, a decrease in the degree of change from the third to fourth phase to the first phase of the accidental transformation is established, which is confirmed by an increase in the thymus mass factor by 2.7 % compared with the control pathology.

The comparison drug "Immuno-Ton" also to some extent corrected the expressiveness of the morpho-

logical signs of immunosuppression in the thymus and spleen of rats that arose as a result of CIS. By expressiveness of the stress-protective effect of the investigated TEPL was not inferior to the CD "Immuno-Ton".

7. Conclusions from the conducted research and prospects for further development of this field

1. The ability of TEPL to restore the activity of organs of the thymico-lymphatic system of the immune system, which has been disturbed under stress conditions, has been proved. Taking into account the data of previous studies [6, 7], it is likely to assert that polyphenolic compounds of TEPL exhibit antioxidant properties, increasing the activity of the antioxidant system, and polysaccharides - immunostimulants, the result of which is the stress-protective effect.

2. The effectiveness of TEPL was not inferior to the drug comparison "Immuno-Ton".

3. The obtained results substantiate the expediency of further investigation of the stress-protective and immunotropic action of TEPL.

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