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EXOGENOUS MELATONIN ENHANCES IMMUNE SYSTEM ACTIVITY UNDER CONDITIONS OF CHRONIC STRESS

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It is well known that chronic stress can have serious health consequences, leading to depression, anxiety, hypertension, obesity, diabetes, and so on. Additionally, the stress-induced impact results in a decrease in immune system functions, rendering the body more susceptible to infections and other illnesses. In addition to regulating sleep, melatonin possesses pleiotropic properties: it inhibits oxidative stress, exhibits anti-aging effects, and demonstrates documented anti-cancer activity. Melatonin is an effective antioxidant that stabilizes mitochondria. It remains incompletely understood how melatonin affects immune defence under conditions of chronic stress.

This study aimed to determine the influence of melatonin on certain indicators of the immune system under conditions of stress exposure. Three groups of laboratory mice of the BALB/c strain were formed. The first group was kept under a 12-hour light-dark cycle. The second and third groups were subjected to chronic stress through constant illumination. The third group of animals additionally received exogenous melatonin in their drinking water. On the fifth, fifteenth, and thirtieth days of the experiment, indicators of leukocyte levels, phagocytic activity using the latex particle uptake method, and the Th1-dependent immune response were determined by the intensity of delayed-type hypersensitivity reactions.

Comparison of the indicators revealed that in the second group on the 5th day of the experiment, there was an increase in the total number of leukocytes, an elevation in the level of neutrophils, and a decrease in the level of monocytes. Animals receiving melatonin demonstrated similar results. At the end of the experiment, in the group receiving melatonin, the level of neutrophils decreased against an elevated level of lymphocytes and monocytes.

At the end of the experiment, the group subjected to chronic stress demonstrated a decrease in the number of colony-forming units. However, in the melatonin-treated group, the opposite was observed – an increase occurred. A similar trend was observed in the determination of hypersensitivity activity. In the melatonin-treated group, hypersensitivity indicators were higher compared to the group not receiving melatonin. Phagocytic activity in the third group was already higher on the fifth day of the experiment.

The results of the study indicate that the administration of melatonin has a positive impact on certain indicators of the immune system under conditions of chronic stress. Specifically, phagocytic activity increased, the level of lymphocytes was elevated, antibody production intensified, and delayed hypersensitivity reaction was enhanced.

Key words: *immune system, stress, melatonin, circadian rhythms, phagocytosis.*

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ЕКЗОГЕННИЙ МЕЛАТОНІН ПОСИЛЮЄ АКТИВНІСТЬ ІМУННОЇ СИСТЕМИ В УМОВАХ ХРОНІЧНОГО СТРЕСУ

Загальновідомо, що хронічний стрес може мати серйозні наслідки для здоров'я, спричиняє депресію, тривожність, зумовлюючи гіпертонію, ожиріння, діабет тощо.

Разом з тим, стресорний вплив призводить до зниження функцій імунної системи, зробивши організм більш вразливим до інфекцій та інших захворювань. Окрім регуляції сну, мелатонін має плейотропні властивості: інгібують окисний стрес, противікові, зафіксована протиракова активність. Мелатонін являє собою ефективний антиоксидант, стабілізує мітохондрії. Залишається не повністю розкритим, яким чином мелатонін впливає на імунний захист в умовах хронічного стресу.

У цьому дослідженні визначали вплив препарату мелатоніну на деякі показники імунної системи в умовах впливу стресу. Було сформовано три групи лабораторних мишей лінії BALB/c. Перша група утримувалася за умов освітлення 12 годин світла та 12 годин темряви. Друга та третя групи піддавалися впливу хронічного стресу у вигляді цілодобового освітлення. Третя група тварин додатково отримувала екзогенний мелатонін у складі питної води. На п'ятий, п'ятнадцятий та тридцятий день експерименту визначали показники рівня лейкоцитів, активність фагоцитозу методом індукованого НСТ-тесту та поглинання дисперсного латексу, Th2-залежну імунну відповідь визначали методом підрахунку колонієутворюючих клітин у селезінці після імунізації еритроцитами барана. Th1-заледжну ланку імунної відповіді досліджували за інтенсивністю реакції гіперчутливості сповільненого типу.

Порівняння показників показало, що у другій групі на 5-й день експерименту підвищувалася загальна кількість лейкоцитів, підвищувався рівень нейтрофілів, а рівень моноцитів знижується. Тварини, які отримували мелатонін демонстрували подібні результати. Наприкінці експерименту в групі яка отримувала мелатонін рівень нейтрофілів знижувався на тлі підвищеного рівня лімфоцитів та моноцитів.

Наприкінці експерименту група під дією хронічного стресу продемонструвала зниження кількості колонієутворюючих колоній, проте у групі яка отримувала мелатонін навпаки – підвищувалася. Подібна тенденція спостерігалася під час визначення активності гіперчутливості. У групі, яка отримувала мелатонін показники гіперчутливості були більш високими у порівнянні із групою яка його не отримувала. Активність фагоцитозу у 3-й групі була більш високою вже на п'ятий день експерименту.

Отримані результати дослідження вказують на те, що уведення мелатоніну позитивно впливає на деякі показники імунної системи в умовах хронічного стресу. Зокрема активність фагоцитозу посилювалася, підвищувався рівень лімфоцитів, посилювалося антитілоутворення, реакція сповільненої гіперчутливості була підвищеною.

Ключові слова: *імунна система, стрес, мелатонін, циркадні ритми, фагоцитоз.*

It is well known that sleep is one of the key aspects of human health. Regulating this process is aided by the hormone melatonin, which is known to be produced during sleep [16]. Its synthesis occurs in the pineal gland. Light can both inhibit and synchronize melatonin production, while darkness is the optimal period for hormone concentration (accumulation) [4]. The transformation of melatonin occurs in two stages: the first stage of transformation involves the conversion of tryptophan to serotonin followed by acetylation with serotonin N-acetyltransferase. The second stage is the production of melatonin, which occurs with the help of the enzyme hydroxyindole-O-methyltransferase [16].

The first reports on melatonin were obtained as early as 1958. American physician and prof. Aaron B. Lerner and his colleagues isolated a hormone from the pineal tissue of cattle. At that time, they identified it as a molecule that lightened the skin chromatofores. Scientists didn't even realize the significance of their discovery and what it would entail for scientific breakthroughs. Despite the hormone being

discovered as far back as the mid-20th century, its effects on the body remain insufficiently understood and require further research [11].

Modern endocrinology provides us with a clear understanding that melatonin is not only a sleep-regulating hormone but also performs anti-aging, antioxidant, immunomodulatory, and anti-cancer actions [3].

The anti-cancer action of melatonin is the most important, as oncological diseases currently rank second in causes of human mortality. A large body of research has confirmed the anti-cancer effects of melatonin. However, there is an assertion about the dual action of this hormone. Such effects can be observed both in the initiation and inhibition of oxidative stress. The influence of reactive oxygen species on the development of oncological diseases has also been thoroughly investigated. In particular, it has been demonstrated that an increase in mutation frequency, activation of growth receptors, enhanced oncogenic signalling, and promotion of angiogenesis were observed in cells exposed to reactive oxygen species. On the other hand, oxidative stress can inhibit the survival of cancer cells by inducing DNA damage, telomere shortening, and oxidation of biological molecules [14].

The production of active metabolites, enhancement of antioxidant enzyme expression, reduction of free radicals, and stabilization of mitochondria are all functions performed by melatonin as an effective antioxidant. However, the anti-tumour action of this indolamine is not always linked to its antioxidant activity. Recent research has shown that the anti-tumour action of melatonin can be achieved by stimulating the production of reactive oxygen species. On the other hand, many studies have confirmed melatonin's induction of apoptosis against various types of cancer, including gastric cancer and cervical cancer [3].

Thus, it can be asserted that while the anti-cancer function of melatonin is evident, the specificities of its influence on inhibiting the development of oncological diseases require further investigation [10].

In addition to the aforementioned properties, melatonin also plays a key role in reproductive medicine: it controls the production of gonadotropins and steroid hormones. Moreover, it participates in sexual maturation, follicular genesis, pregnancy, and menopause. During pregnancy, the maximum concentration of melatonin is secreted in the third trimester, and then returns to optimal levels after delivery [9].

Supporting cellular redox homeostasis and exerting «control» over the aging process is another important function of melatonin. Elevated levels of free radicals and the formation of non-radical oxygen species with subsequent accumulation in cells can disrupt cellular redox balance (oxidative stress) [12, 13].

Melatonin is a universal antioxidant that is highly popular among athletes. It is known that antioxidant supplements are common practice among athletes who need to enhance their endurance and physical performance, which is significantly achievable by minimizing the detrimental effects of oxidative stress on the body [7]. As stress is a universal trigger that stimulates the onset of diseases and contributes

to the development of oncological conditions. The aim of this study was to determine the effect of melatonin supplementation on certain indicators of the immune system under conditions of stress exposure.

MATERIALS AND METHODS

B the experiment, male BALB/c mice aged 4 months, weighing 26–30 g, were used. The experiment was conducted in the spring (March). The mice were kept at a constant temperature (22±2oC) on a standard laboratory diet with free access to food and water. The animals were divided into 3 groups. The first group served as the control (n = 20) and were kept for 30 days under a 12-hour light:12-hour dark cycle. The second group (n = 20) was kept for 30 days under continuous illumination. The third group (n = 20) was also kept for 30 days under continuous illumination, but the animals in this group received melatonin. This supplement was added to the drinking water at a dose of 0.01 mg per 1 g of body weight.

On the fifth, fifteenth, and thirtieth days of the experiment, the animals were weighed and euthanized by decapitation under thiopental anaesthesia in the morning (from 10:00 to 12:00). Blood was collected from the decapitation wound, as well as lymphoid organs (thymus and spleen). To study innate immunity, the number of leukocytes in the blood was determined by standard methods. The Th1-dependent immune response was assessed by the intensity of delayed-type hypersensitivity reaction, and the Th2-dependent immune response was evaluated by the number of antibody-producing cells in the spleen after immunization with allogeneic erythrocytes. The functional activity of phagocytes was determined by spontaneous and induced nitroblue tetrazolium tests and by phagocytosis of monodisperse polystyrene latex particles [5]. All experimental procedures were conducted in accordance with the principles of humanity outlined in the directives of the European Community (86/609/EEC) and the Helsinki Declaration on the protection of vertebrate animals used for laboratory and other scientific purposes [15].

Statistical analysis of the results was carried out using the «Statistica 10» program. The validity of the differences was determined according to the Man-Whitney and Wilcoxon criteria. Changes were considered likely under $P<0.05$. The research has been carried out under Directive 2010/63/EU on the protection of animals used for scientific purposes.

RESULTS AND DISCUSSION

Comparison of the experiment's results on the fifth day showed that in the second group (exposed to stress), there was an increase in the total number of leukocytes. The level of neutrophils was elevated, while the level of monocytes was decreased. In the group receiving melatonin, there was also an increase in the level of leukocytes, specifically neutrophils. The level of monocytes was decreased.

On the fifteenth day after exposure to stress, in the second group (stress only), compared to the control, the level of leukocytes was elevated, specifically due to neutrophils. In the stress group receiving melatonin, an increase in the level of

leukocytes also occurred, but due to an increase in the level of monocytes. The level of neutrophils did not increase.

At the end of the experiment, in the stress group, there was also an elevated level of neutrophils. In the stress group receiving melatonin, the level of neutrophils decreased against a background of higher levels of lymphocytes and monocytes (Fig. 1).

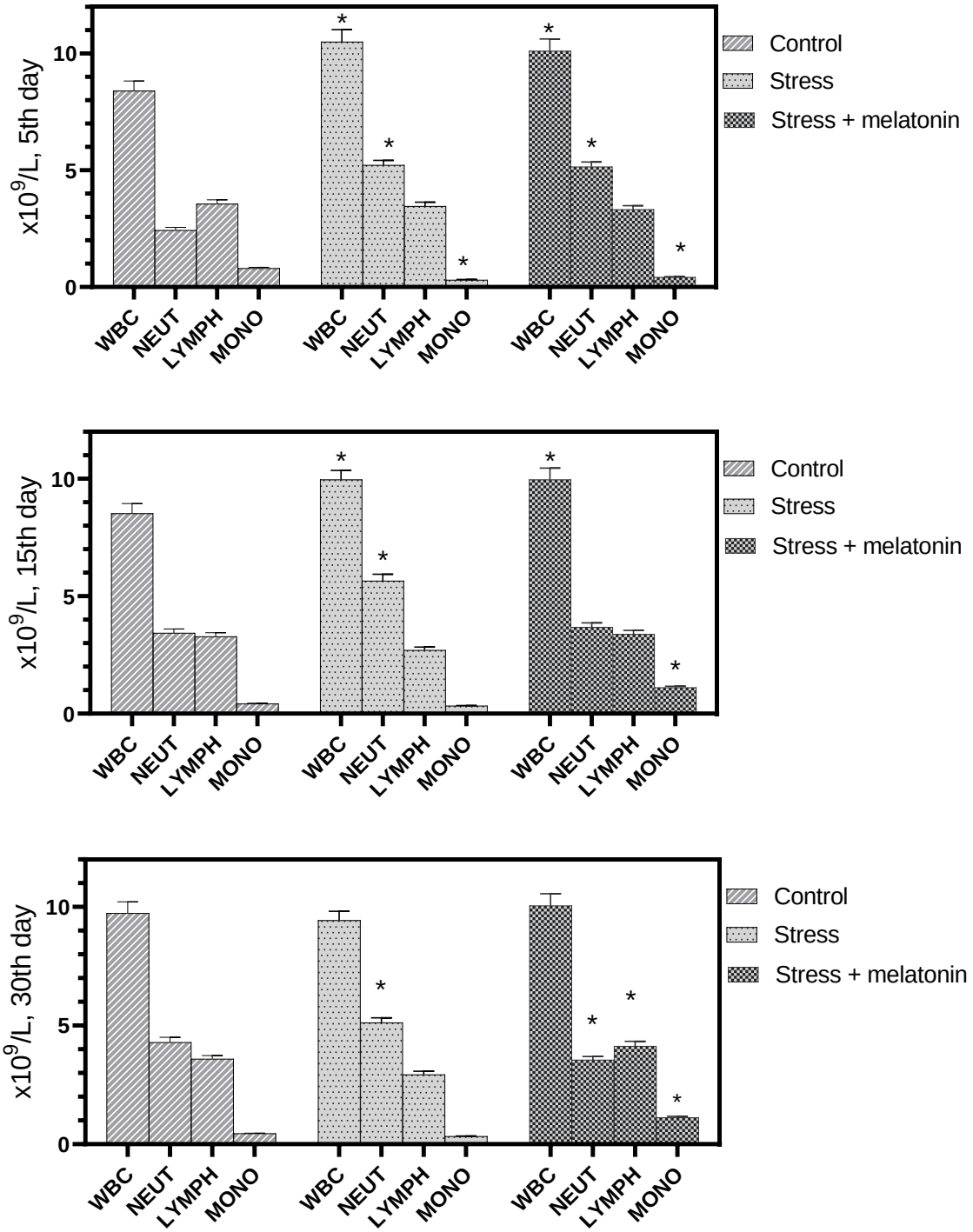

Fig. 1. Dynamics of leukocyte levels in peripheral blood on the 5th, 15th, and 30th days of the experiment

Notes: * P<0.05 compared to control values

On the fifth day after the start of the experiment, the number of antibody-producing colonies was slightly decreased in both the stress group and the group receiving melatonin. On the fifteenth day, in the stress group (Group 2), there was a significant decrease ($p < 0.05$) compared to the control. On the thirtieth day, in Group 2, the number of antibody-producing colonies continued to decrease, while in the group receiving melatonin (Group 3), it increased (Fig. 2).

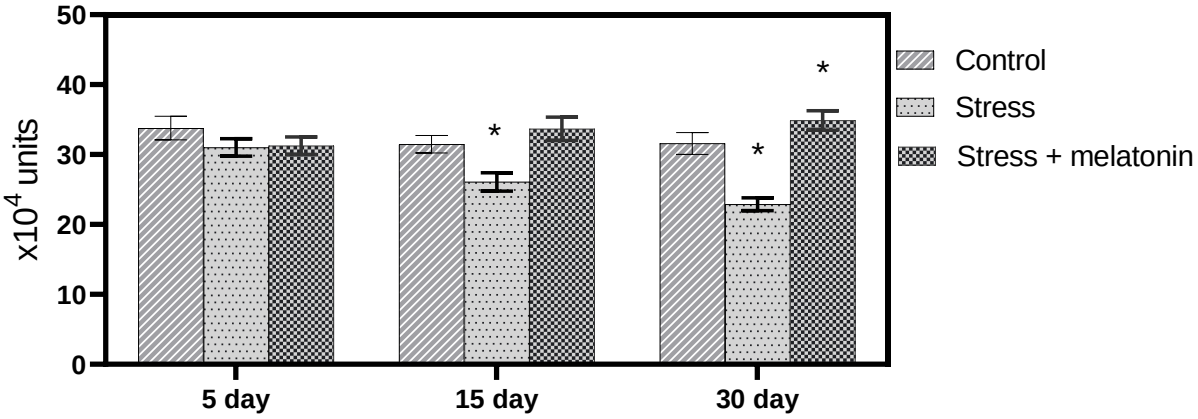

Fig. 2. Antibody-producing colonies
Notes: * P<0.05 compared to control values

On the fifth day, the hypersensitivity activity in the stress group was reduced. In the group receiving melatonin, the delayed hypersensitivity activity was also reduced, but to a lesser extent than in the stress group. On the fifteenth day, there was a sharp decrease in delayed hypersensitivity activity in the stress group without melatonin. In the group receiving melatonin, the delayed hypersensitivity activity on the fifteenth day was higher compared to the control. Particularly notable were the results in this group (Group 3) - the hypersensitivity indicators were higher (Fig. 3).

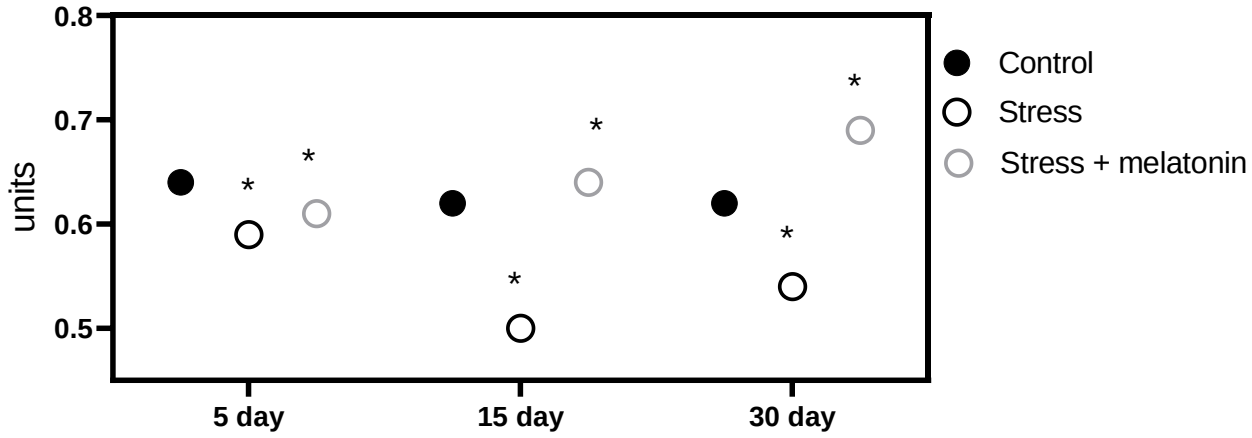

Fig. 3. Delayed-type hypersensitivity
Notes: * P<0.05 compared to control values

The phagocytosis indicators in the stress group and the group receiving melatonin (Fig. 4) were found to be intriguing. On the fifth day of the experiment, the phagocytosis activity was higher in Groups 2 and 3.

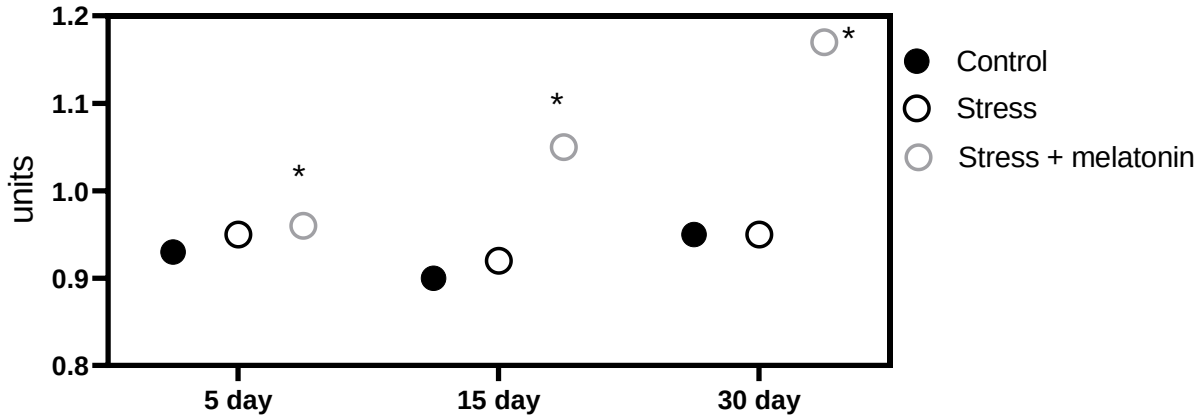

Fig. 4. Phagocytosis Activity
Notes: * P<0.05 compared to control values

On the fifteenth day, the group receiving melatonin showed a significant increase in phagocytosis activity. A similar situation was observed at the end of the experiment on the thirtieth day. In contrast, in Group 2, the phagocytosis activity was similar to that in the control group.

Melatonin plays a crucial role in regulating the response to stress and its impact on the body. During stressful situations, changes occur in the production and functioning of melatonin, which can exert both protective and adaptive effects on the body.

Firstly, melatonin possesses antioxidant properties that can help protect the body's cells from damage caused by stress and oxidative stress [1]. This is particularly important in conditions of increased free radical activity, which often accompany stress.

Secondly, melatonin contributes to the normalization of circadian rhythms and improves sleep quality, which is a key factor in the body's recovery after stressful situations. Regular sleep and normalization of circadian rhythms help reduce the impact of stress on the body and its ability to adapt.

Previous researchers have shown that plant-derived melatonin exhibits anti-tumour activity [8] and is a promising adaptogen [6]. Melatonin can directly influence the hormonal status of the body under stress conditions by participating in the regulation of cortisol and other stress hormones [2]. This helps the body maintain balance and adapt to stressful conditions.

Thus, melatonin plays an important role in regulating the response to stress, supporting the body's protective mechanisms, and promoting adaptation to adverse conditions.

The results obtained indicate that administering melatonin during stress leads to the activation of the immune system. We observed an increase in the level of

monocytes, corresponding to enhanced phagocytic activity. There was also an increase in lymphocyte levels and, consequently, antibody production. Thus, melatonin enhanced humoral immunity, increased the hypersensitivity reaction, and strengthened phagocytosis.

CONCLUSIONS

The experiment results demonstrate that melatonin during stress affects the immune system. Exogenous administration of melatonin under conditions of stress induced by continuous illumination affects the level of leukocytes. At the end of the experiment, the level of neutrophils decreases, but the level of lymphocytes and monocytes increases.

Furthermore, the influence of melatonin on the humoral immune response was identified: the number of antibody-producing colonies in the spleen increases under the influence of melatonin in stress conditions.

The activity of delayed hypersensitivity reaction increases under the influence of melatonin in stress conditions.

Melatonin affects the activity of phagocytosis under stress. In the middle and at the end of the experiment, the activity of phagocytosis in the group receiving melatonin increases.

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