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Effect of a sorbent based on aluminum oxide and polydimethylsiloxane on thymus cellular composition in mice kept under 24-hour lighting

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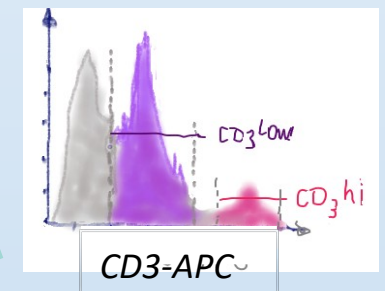
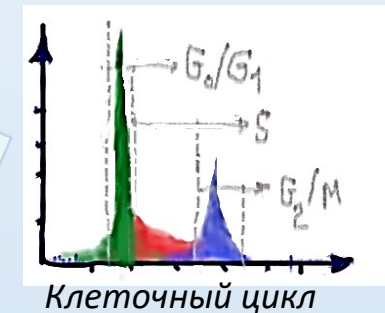
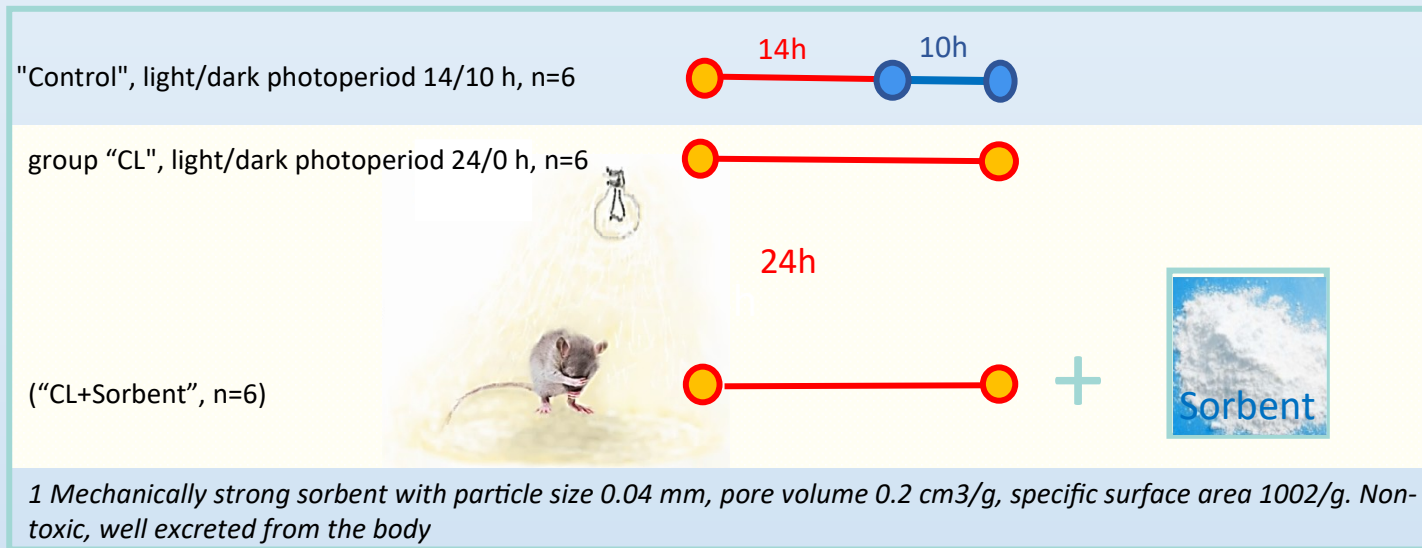
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Experiment design

Motivation and purpose. Prolonged stress can cause a decrease in immunity, excessive accumulation of metabolites and lead to pathological processes with the development of infectious, cardiovascular, neurodegenerative diseases and cancer. Round-the-clock lighting, being a factor in the violation of natural daily biorhythms, has a stressful effect on the body and causes an imbalance in the secretion of melatonin. Violation of melatonin synthesis affects the neuroendocrine system and immune reactivity, increasing stress pressure. Unbalanced response and/or long-term exposure alters biochemical parameters, composition and amount of metabolites and contributes to their accumulation. Sorbents can become one of the factors for correcting the effects of stress.

The aim of the study was to study the effect of the sorbent on the T-cell composition of the thymus of C57Bl/6J mice kept under 24-hour illumination for 14 days.

Experimental studies were carried out on C57Bl/6 mice, which were kept for 14 days in normal lighting conditions (control) and in conditions of continuous lighting (CL).. A cell suspension was prepared from the thymus and the resulting lymphocytes were analyzed on a CytoFlexS-100 flow cytometer, determining the cell cycle of lymphocyte and CD3hi and CD3low thymus T-lymphocyte subpopulations .



The sorbent increases the relative number of immature CD3^{low} T-lymphocytes in 24-hour lighting conditions

- Determination of CD3^{low} and CD3^{hi} lymphocytes of the thymus
- Continuous exposure of mice to light for 14 days led to a decrease in the content of CD3^{low} lymphocytes to 38.7% [35.35; 39.5] compared with the control 43.4% [41.25; 45.46], as well as a decrease in the number of CD3^{hi} T cells to 7.74% [7.5; 7.9] compared with the control - 8.05% [7.85; 8.58]. Accordingly, this caused a decrease in the ratio of CD3^{low} T-lymphocytes to mature CD3^{hi} lymphocytes. The use of the sorbent increased the relative number of young CD3^{low} T-lymphocytes up to 47.0% [46.08; 47.25]. The content of mature CD3^{hi} lymphocytes after the introduction of the sorbent did not differ from the other groups, since there was a wide range of data. The ratio of CD3^{low}/CD3^{hi} lymphocytes significantly exceeded the values of the "KO" group and was slightly higher than the control.

 sorbent -  young CD3^{low} T lymphocytes

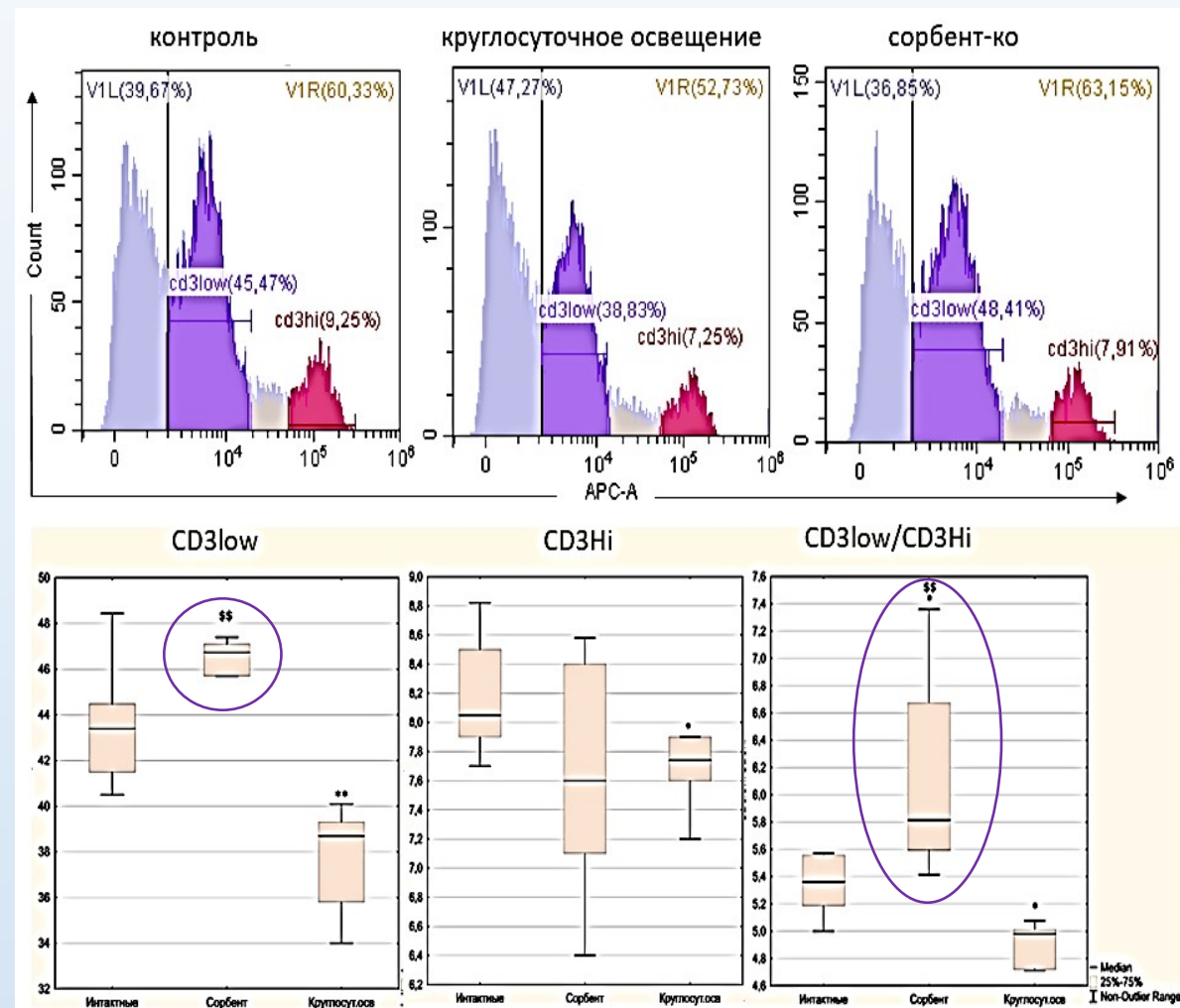


Figure 1. Cellular composition: **A** - Histograms of flow cytometry of CD3^{hi} and CD3^{low} distribution of T-lymphocytes in the "Control", «Sorbent+CL» and under continuous illumination ("CL" group). On the abscissa axis - CD3-APC fluorescence, on the ordinate axis - the number of cells. **B** - Cellular composition of thymus T-lymphocytes, median and quarterly range of the percentage of CD3^{hi} and CD3^{low} T-lymphocytes. Statistically significant differences: * - p<0.05; ** - p<0.01; Mann-Whitney test.

The effect of the sorbent on the cell cycle of thymus cells in 24-hour lighting conditions

- During the study of the cell cycle, 3 regions of cell populations were identified: gate P1Lym - the smallest cells, P2Lym - the main pool of cells and P3 - larger cells. Round-the-clock illumination inhibits the proliferation of P1Lym cells, reducing the S phase to 4% and increasing cell death by up to 12%. Sorbent intake under 24-hour lighting conditions promotes an increase in the S and G2 / M phases of the cell cycle to 8.8% [7.15; 13.12] and 1,54 % [1.15; 2.8] accordingly with a simultaneous decrease in the number of apoptotic cells to 5% compared with round-the-clock illumination .
- Thus, the increase in the percentage of CD3low T-cells is due to the greater survival and higher level of proliferation of T-lymphocytes under conditions of sorbent intake.
- Proliferation and differentiation of thymus lymphocytes depends on the microenvironment, the main cells of which are epithelial cells. Thymus epithelial cells have a high renewal rate. Continuous illumination for 14 days reduces the S but increases the G2/M phase of P3 large cells that have a high proliferation rate and are stromal cells. In the conditions of taking the sorbent, the cell cycle of these cells is restored.

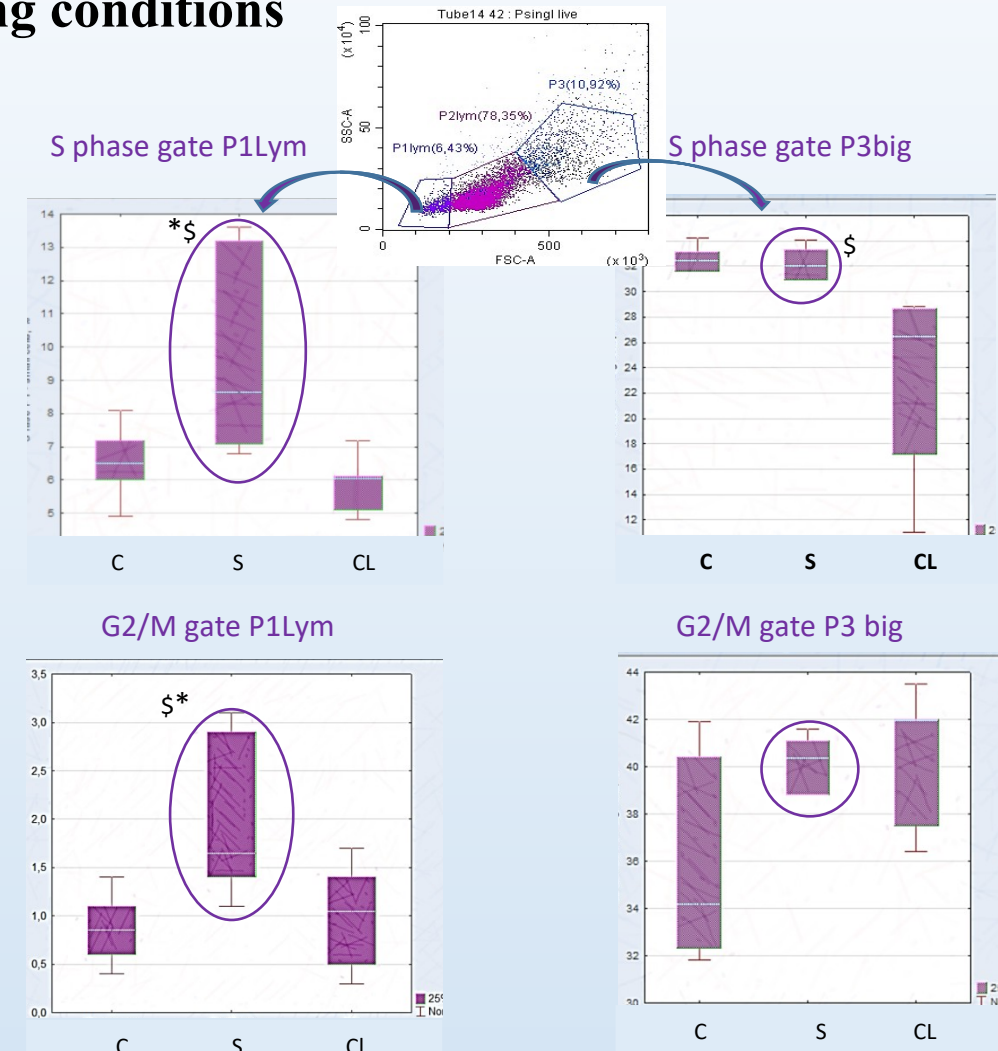
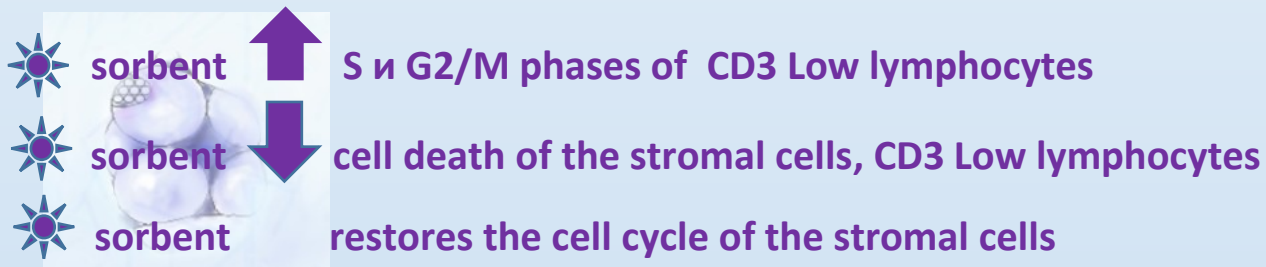


Figure 2. The cell cycle of all thymocytes. Values of the median, first and third quartiles of the thymocyte number at different stages of the cell cycle in «Control», «Sorbent» and «CL» mice

Statistically significant differences: *-from C; \$- from CL , * - p<0.01 Mann –Whitney test.

Discussion and conclusion

The hypothalamic-pituitary-adrenal (HPA) axis is the main physiological stress system producing cortisol. Cortisol is intensively synthesized under stress conditions and causes apoptosis of cortisol-sensitive young lymphocytes. The utilization of cortisol and many xenobiotic occurs under the action of the CYP3A enzyme synthesized by the cells of the liver and intestines. The microbiota and intestinal cells, releasing many active substances, influence many reactions of the HPA axis and at the same time are highly sensitive to stress themselves. (2). Previous studies (3) showed that the use of sorbents leads to an increase in the surface of microvilli of epithelial enterocytes by 30-60 %, which can have a significant effect on the processes of absorption and secretion. In this study, continuous illumination for 14 days has an inhibitory effect on the viability and proliferation of both young lymphocytes and thymus stromal cells. The sorbent in this case can absorb metabolites and possibly excess of cortisol, also by stimulating the formation of intestinal crypts, normalize the functions of intestinal microbiota helps reduce stress



The use of a sorbent-based aluminum oxide and polydimethylsiloxane alleviates the pressure of round-the-clock lighting stress, maintaining the viability of thymus cells, increasing the proliferation of young CD3low lymphocytes and normalizing the cell cycle of thymus stromal cells. The sorbent had no effect on the maturation and differentiation of T lymphocytes.

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² Ran Huo,^{1,2,3} et. al¹ Microbiota Modulate Anxiety-Like Behavior and Endocrine Abnormalities in Hypothalamic-Pituitary-Adrenal Axis Front Cell Infect Microbiol. 2017; 7: doi: 10.3389/fcimb.2017.00489

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