

FEDERAL STATE BUDGETARY INSTITUTION OF SCIENCE
PAVLOV INSTITUTE OF PHYSIOLOGY
OF THE RUSSIAN ACADEMY OF SCIENCES

"APPROVED"

Director of the Pavlov Institute
of Physiology

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Pavlov of the Russian Academy of Sciences



REPORT

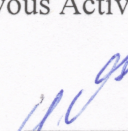
UNDER THE SCIENTIFIC COOPERATION AGREEMENT

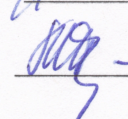
WITH THE AUTONOMOUS NON-COMMERCIAL ORGANIZATION "SFERA"

Topic: Study of the effect of non-ionizing electromagnetic radiation from the Archer AX73 Wi-Fi6 router and Lifetune Zone Max resonators on the behavior and blood parameters of rats with different nervous system excitability

Responsible executors

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INTRODUCTION

Technogenic sources of electromagnetic radiation (EMR), characterized by increased power and high radiation frequencies, have a significant impact on the functions of the human and animal body at various structural levels, which can pose a threat to the health and environmental safety of living organisms. In recent years, the mechanisms of high-frequency EMR exposure on biological objects and their effects have been actively studied (Jangid et al., 2025, Saliev et al., 2019, Karthick et al. 2017; Lai 2005, et al.). The nervous system is sensitive to the effects of EMR, and this effect is characterized by changes in innate behavioral patterns, conditioned reflex activity, and physiological processes (Schneider et al. 2014; Spivak et al. 2016, Shiryayeva et al., 2020), which is an important indicator of the adverse effects of natural and technogenic modifications of EMR. The body's response to these effects may be individual and related to the functional state of the nervous system. The role of hereditary characteristics of the nervous system in determining sensitivity/resistance to the effects of electromagnetic fields on the body has hardly been studied (Dyuzhikova et al. 2018; 2019), but this is extremely important for the prognostic assessment of their impact on humans and the development of methods and means of electromagnetic protection (Lai, 2005).

Protection systems against this type of radiation are being developed in various directions, for example, by reducing the power and changing various characteristics of EMR sources. Thus, devices (resonators, converters) have been developed that affect the reactions of living systems to non-ionizing EMR and have a protective effect (Zhabrev et al., 2005; Jasitis et al., 2018).

Currently, as a continuation of the work begun under the scientific and technical cooperation agreement with the Human Genome Research Foundation "Aires" (2018-2020), it is important to assess the effect of the latest generation of routers and new generation resonators on the behavior and general blood parameters of rats with contrasting nervous system excitability, which was the aim of the study.

Objectives of the study:

- 1). To investigate the effects of EMR from a standard WiFi router (provided by the customer) (operating mode: 4 days, 12 hours in active mode with a working computer) and resonators (provided by the customer) against this background with the weakening of the external magnetic field in a Faraday cage on behavior in the "open field", "elevated puls maze" and "dark-light chamber"
- 2). To investigate the effects of EMR from a standard WiFi router (provided by the customer) (operating mode: 4 days, 12 hours per day in active mode with a working computer) and resonators (provided by the customer) against this background when the external magnetic field in the

Faraday cage is weakened on hematological parameters (before and after exposure) in rats of two strains, high-excitability (LT, low-threshold) and low-excitability (HT, high-threshold).

MATERIALS AND METHODS

The study was conducted on 5-month-old male rats of the HT and LT strains selected in the Laboratory of Genetics of Higher Nervous Activity (Vaido, 2000, Vaido et al., 2018) and included in the Biocollection of the I.P. Pavlov Institute of Physiology, Russian Academy of Sciences (No. GZ 0134-2018-0003, patents for selection achievements No. 10769 and 10768, issued by the State Commission of the Russian Federation for the Testing and Protection of Selection Inventions, registered in the State Register of Protected Selection Achievements on January 15, 2020). All animals were kept under standard environmental conditions (23 ± 2 °C; 12 h/12 h dark/light cycle) with *ad libitum* water and food in the vivarium. Males were kept in groups of 6 in standard cages.

To create conditions for shielding external electromagnetic radiation, the experiments were conducted in a Faraday cage.

The study used the AX5400 dual-band Gigabit Wi-Fi6 router Archer AX73 (TP-link) provided by the customer with the following technical characteristics: wireless communication carrier frequency: 1.5 GHz, number and type of antennas: 6 high-performance antennas and Beamforming, gain coefficient of the standard antenna (antennas), dBi: 5dBi. Powerful 1.5 GHz 3-core processor.

The experiments used Lifetun Zone Max resonators provided by the customer. To assess their effect under the influence of router EMI, 6 resonators were used, as in previous experiments on rats. They were placed inside a Faraday cage, in the center of each side.

To study the effect of the router and resonators under the influence of the router's EMR, the "home" cage with animals was placed in a shielding chamber with the router located on a tray in the center of the top cover of the cage. The experimental groups were exposed for 4 consecutive days for 12 hours daily (from 8 p.m. to 8 a.m.). The control groups were groups of rats kept in the vivarium and not exposed.

Assessment of rat behavior

Animal behavior was assessed using standard tests: the Open Field (OF), Elevated Plus Maze (EPM), and Light-Dark Chamber (LDC). Behavior in the first test was assessed one hour after the animals were removed from the chambers. The second and third tests were performed on subsequent days, respectively.

The OF test is designed to study the exploratory and emotional behavior of rodents in a new environment; It allows for a comprehensive assessment of natural controlled behavior and its changes under the influence of various factors (Amiksheeva et al., 2003): to assess the level of emotionality (urination, defecation), motor activity (horizontal – number of sectors crossed, and vertical – posts reflecting exploratory activity), as well as the severity of the anxiety-depressive component (activity in the central, most open and illuminated sector of the field), level of fear based on freezing reactions, stereotypical behavior (grooming) (Kaluev, 1998, Buresh et al., 1991).

The EPM test is a device with two open and two closed arms, raised 1 meter above the floor. Studying rodent behavior in this test is a standard method for studying rodent anxiety-like behavior. It is believed that the choice of a closed arm is due to fear, while an open arm is chosen when anxiety levels are low. The level of anxiety is determined by the preference for open or close space, fear of heights, behavioral dynamics, and the "peeking" symptom. This test is included in the list of basic studies when testing various drugs.

The LDC is designed to study the behavior of rodents under conditions of variable stress (with free choice of comfortable conditions) and allows for the assessment of: preference for darkness and light; severity and dynamics of "peeking" behavior; habituation. The assessment of the behavior of laboratory rodents in a light-dark chamber is included in the list of studies to determine the anxiolytic activity of pharmacological drugs.

Complete blood count

Blood was collected from the tail vein of rats in all experimental and control groups before exposure (with a router-R or router and additional resonators-R+D placed in a Faraday cage) and after exposure for 4 days before behavioral testing.

Clinical blood analysis was performed using an automated hematology analyzer BC-20 Vet (Shenzhen Mindray Bio-medical Electronics Co.Ltd, PRC) in the pre-separation mode, for which whole blood with EDTA was added to a special diluent immediately before placing the sample in the hematology analyzer in two parallel samples. The analysis contained data on 21 parameters of rat blood: red blood cell count, mean corpuscular volume, hemoglobin concentration, mean hemoglobin content and concentration in red blood cells, coefficient of variation and standard deviation of red blood cell distribution width, hematocrit, platelet count, large platelets, their relative count and mean volume, platelet distribution width, white blood cell count, lymphocytes, monocytes, granulocytes, and their percentage content.

Statistical processing

Checking the normality of samples (Shapiro-Wilk test), ANOVA methods or Karskel-Wallis test, post hoc, Tukey, Mann-Whitney test. Correction for multiple comparisons – Bonferroni.

The graphs presenting the results in the figures show: median, maximum and minimum values, spread of values, interquartile ranges; *,** - significance of differences at different levels of significance (indicated on the graphs).

RESULTS AND DISCUSSION

RESULTS OF THE ANALYSIS OF THE BEHAVIOR OF HT RATS AFTER EXPOSURE TO A ROUTER (R) AND A ROUTER WITH RESONATORS (R+D)

OPEN FIELD

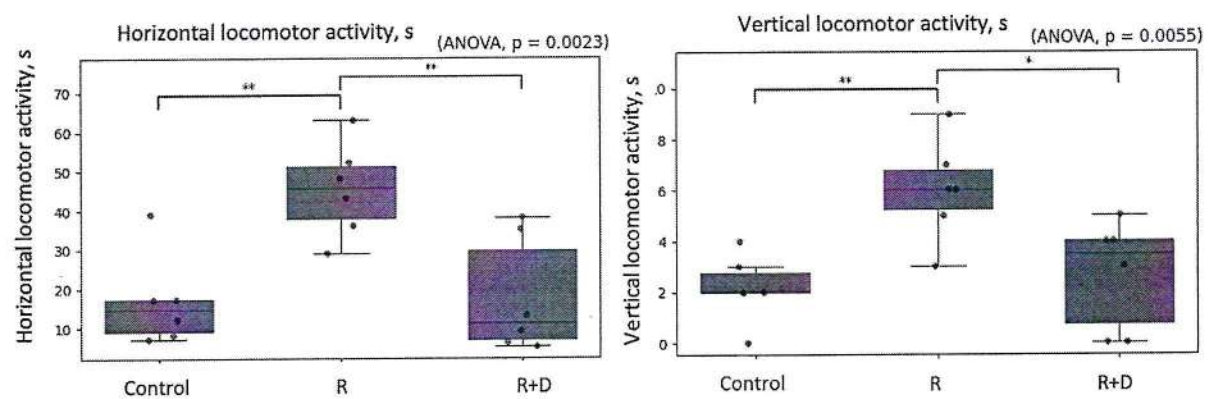


Fig. 1.1

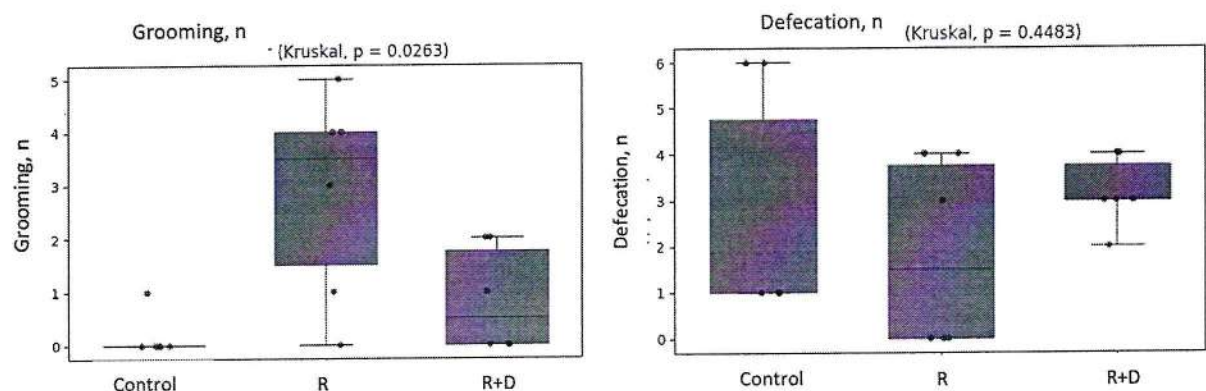
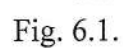
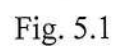
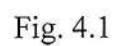


Fig. 2.1



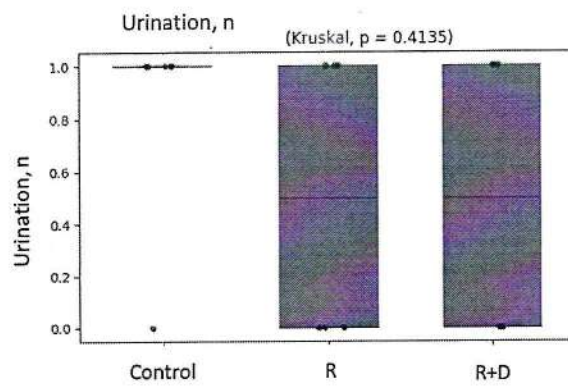


Fig. 7.1.

DARK-LIGHT CHAMBER

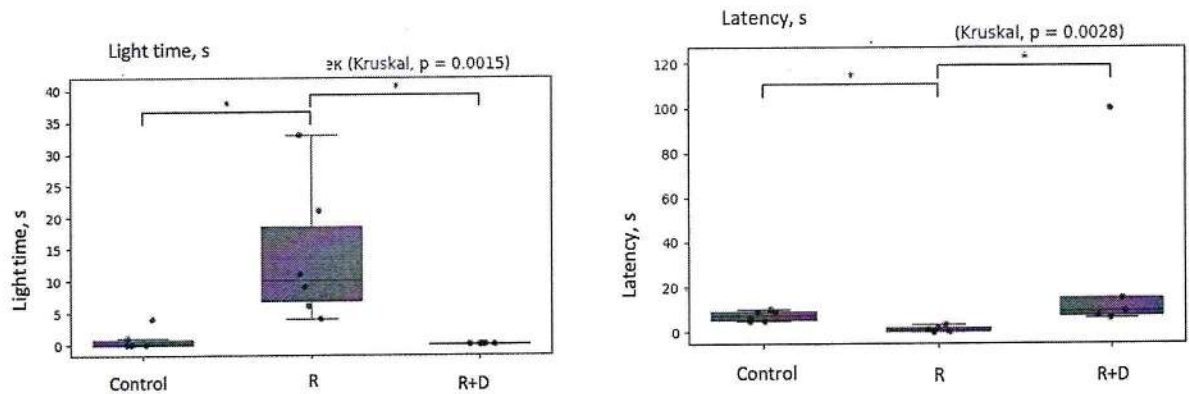


Fig. 8.1.

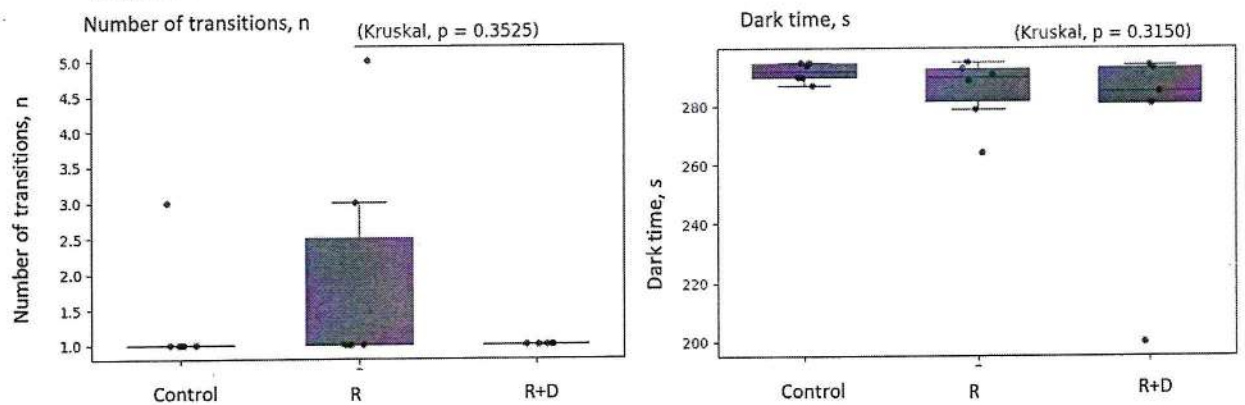


Fig. 9.1.

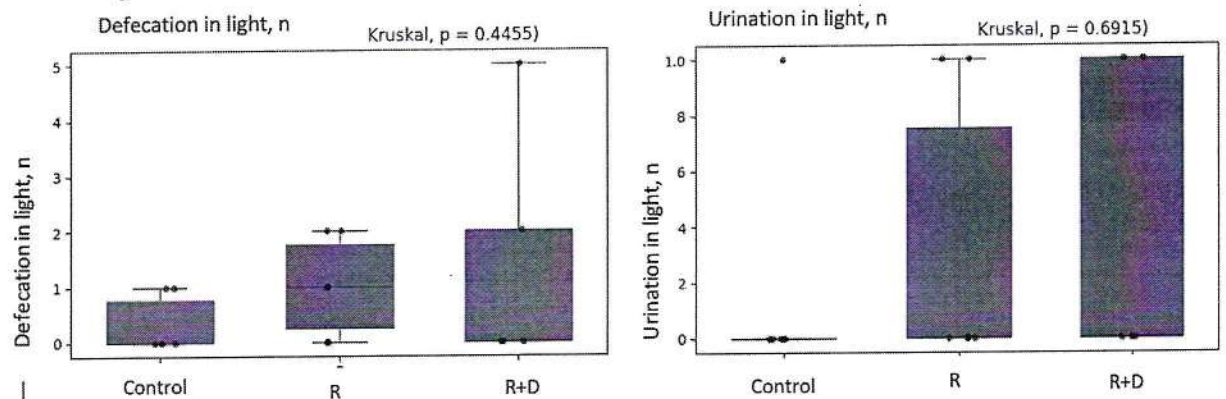


Fig. 10.1.

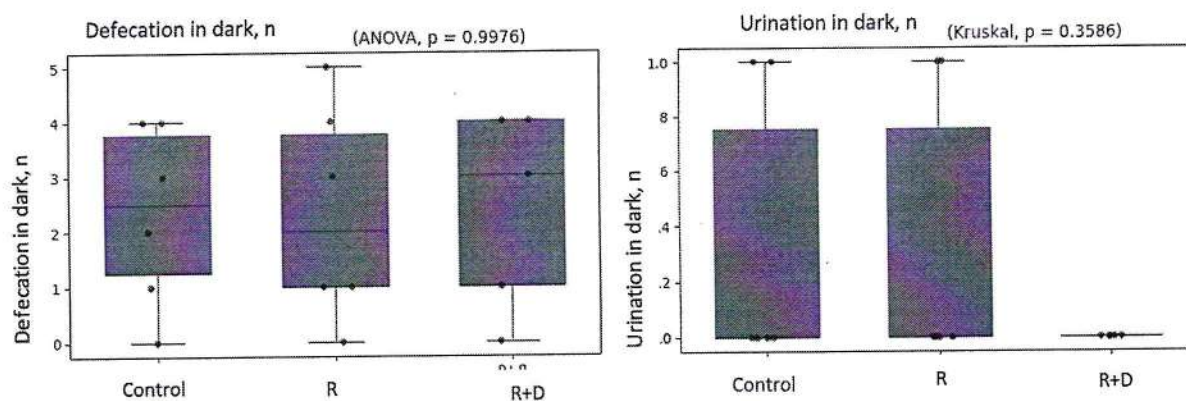


Fig. 11.1.

In rats of the HT strain, after exposure to the router (R) in the open field test, overall motor activity (horizontal and vertical) increased (Fig. 1.1.). In the presence of additional resonators (R+D), motor activity indicators returned to the control level (Fig. 1.1.). No changes were observed in the other analyzed indicators (Fig. 2.1.-4.1.).

In the dark-light chamber, after exposure to the router (R), the latent period of animals entering the dark chamber decreased and the time spent in the light chamber increased (Fig. 8.1). In the presence of resonators (R+D), the duration of the latent period and the time spent in the light chamber were comparable to the control (Fig. 8.1). No changes were observed in the other parameters.

In the elevated cross maze, no changes in behavior were observed under the influence of the router (R) and resonators (R+D) for any of the parameters (Fig. 5.1.-7.1.).

RESULTS OF THE ANALYSIS OF THE BEHAVIOR OF LT RATS AFTER EXPOSURE TO THE ROUTER (R) AND THE ROUTER WITH RESONATORS (R+D)

OPEN FIELD

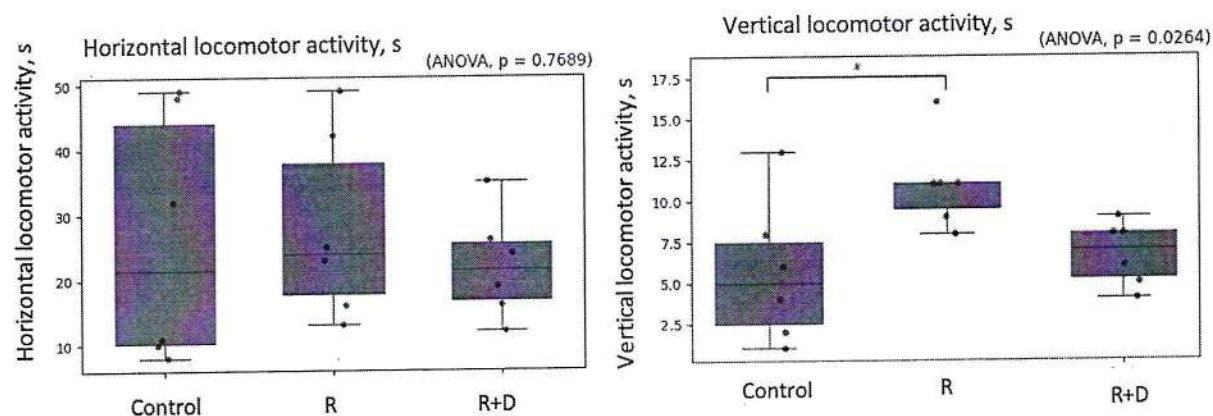


Fig. 1.2.

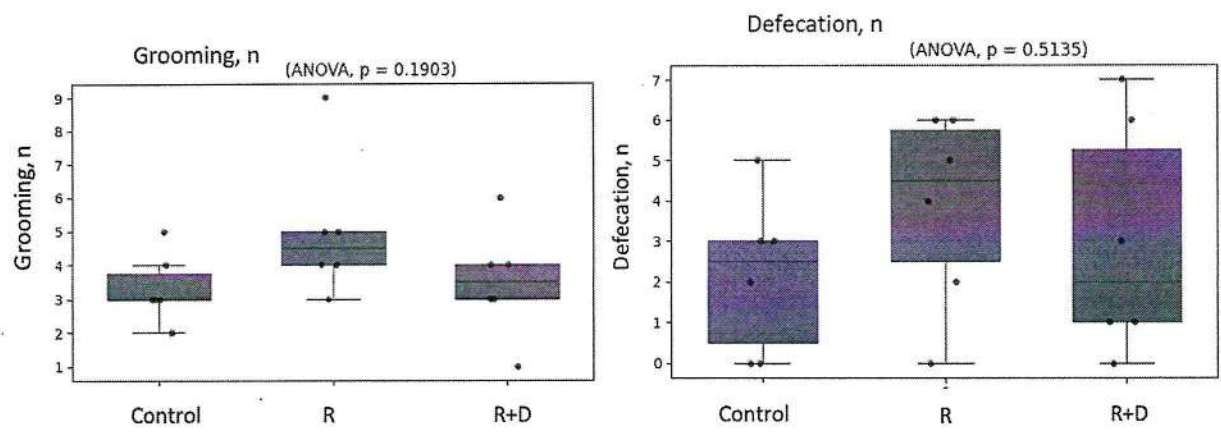


Fig. 2.2.

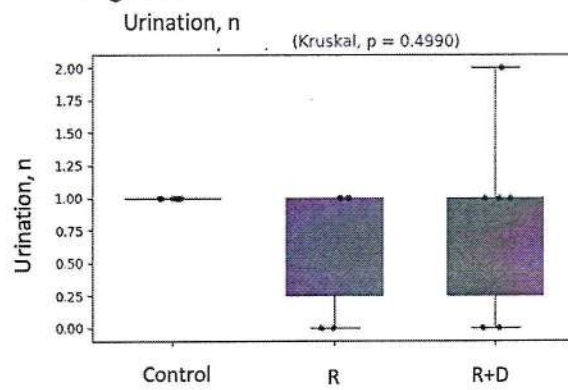


Fig. 3.2.

ELEVATED PLUS MAZE

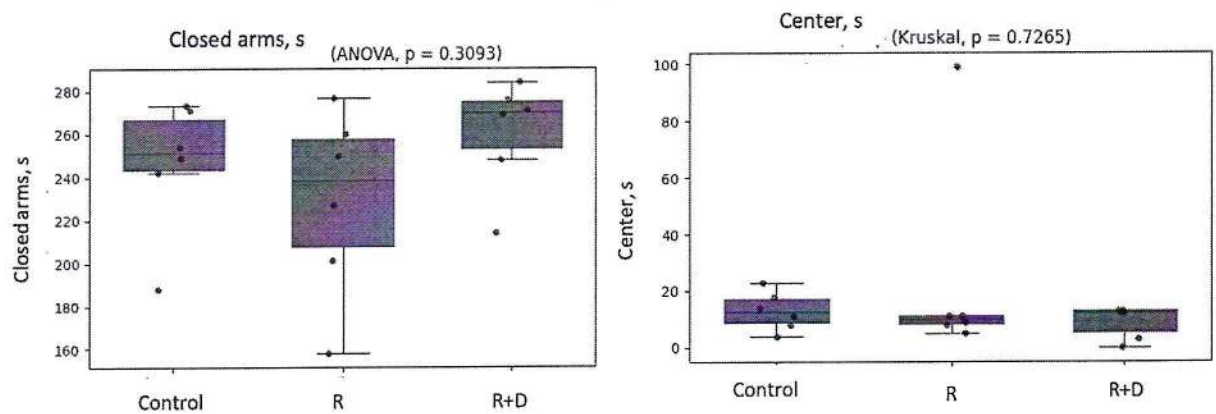


Fig. 4.2.

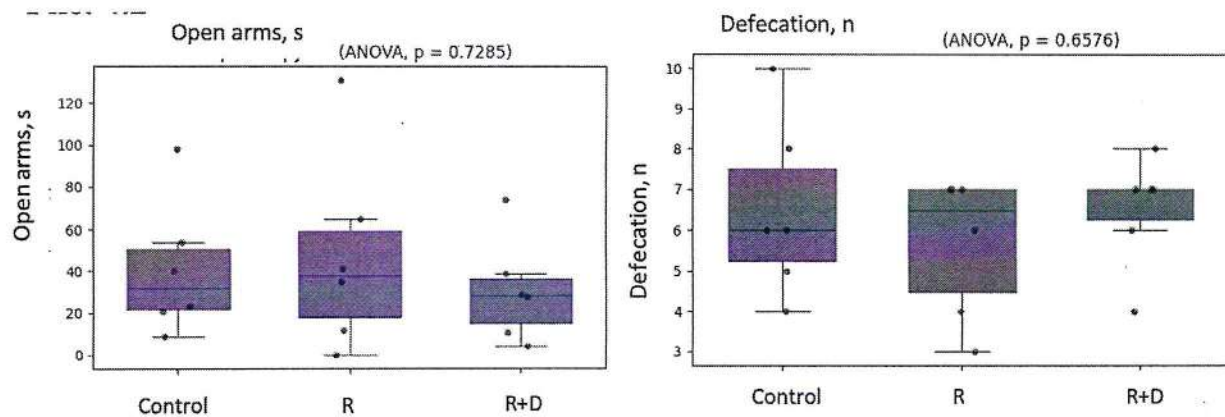


Fig. 5.2.

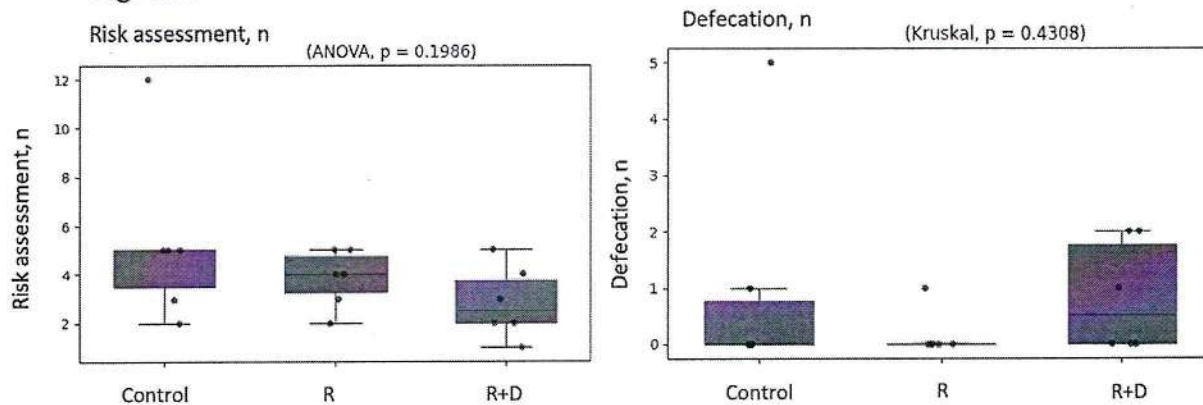


Fig. 6.2.

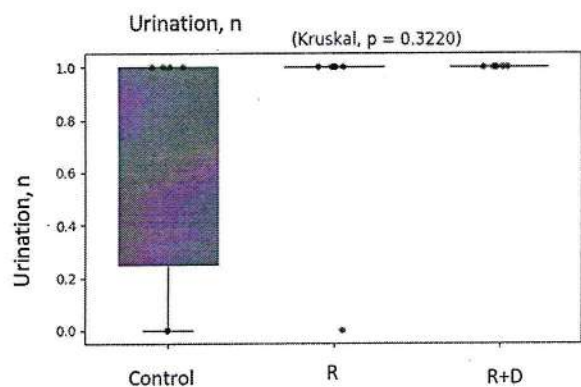


Fig. 7.2.

DARK-LIGHT CHAMBER

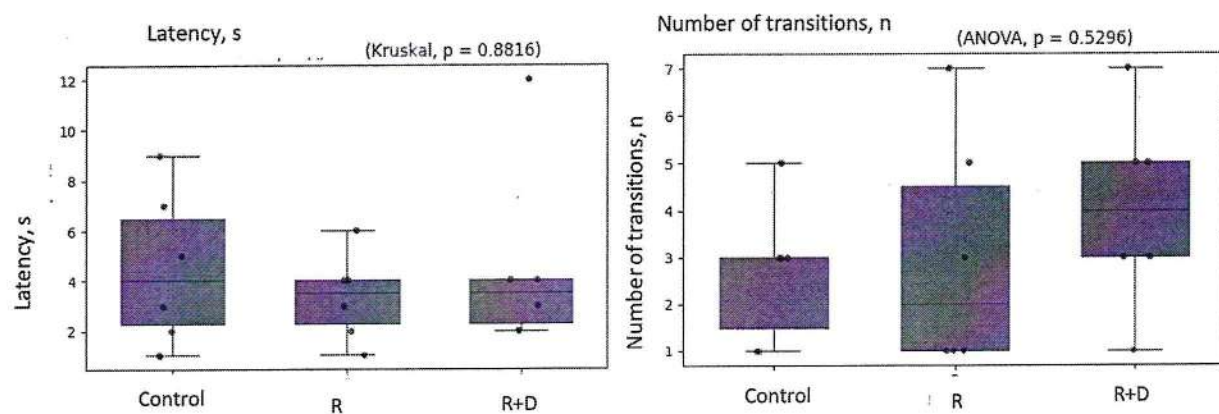


Fig. 8.2.

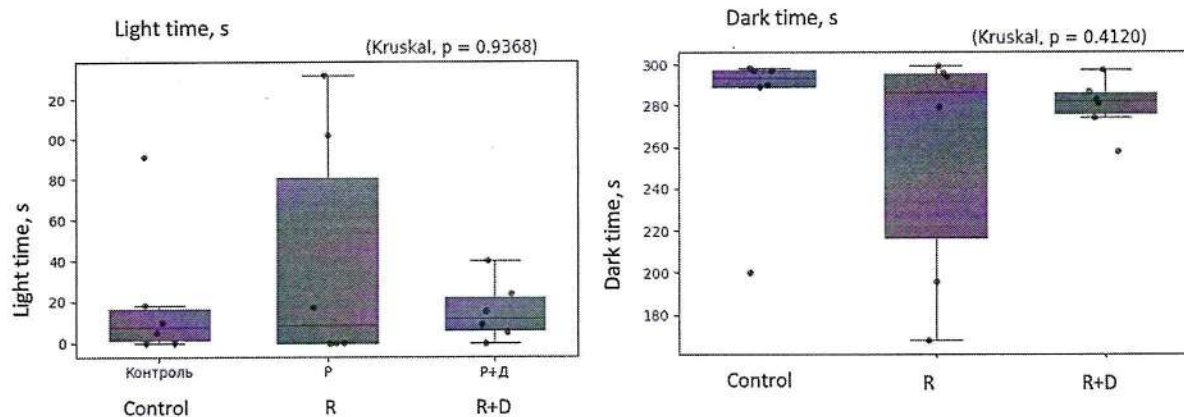


Fig. 9.2.

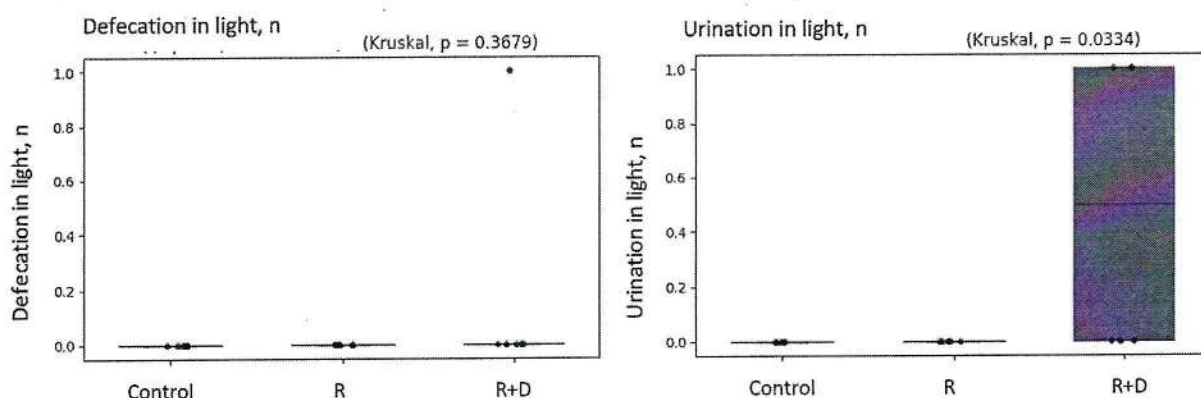


Fig. 10.2.

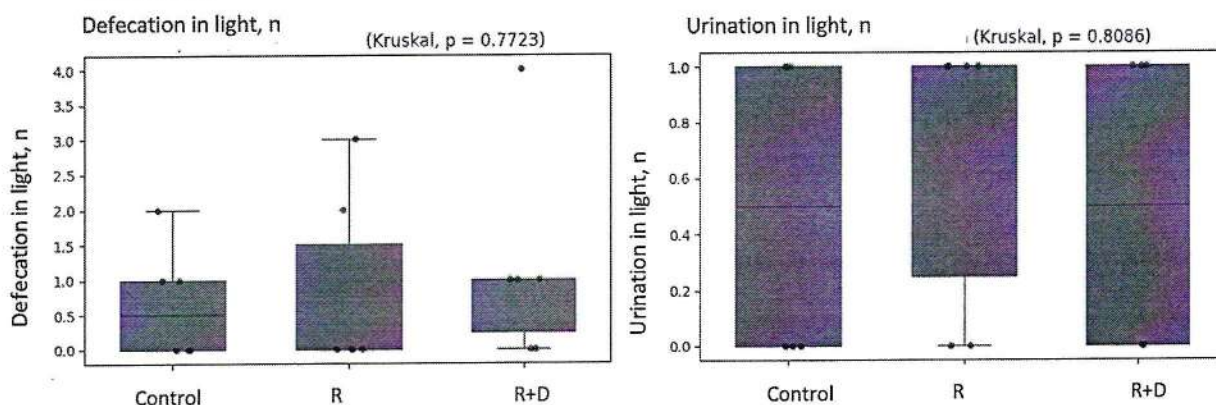


Fig. 11.2.

In rats of the LT strain, vertical motor activity increased after exposure to the router (R) in the open field test (Fig. 1.2.). In the presence of resonators (R+D), motor activity indicators returned to the control level (Fig. 1.2.). No changes were observed in the other analyzed parameters (Fig. 2.1., 3.1.).

In the elevated plus maze and the dark-light chamber, no changes in behavior were observed under the influence of the router (R) and resonators (R+D) for any of the indicators (Fig. 4.2.-7.2., Fig. 8.2.- 11.2.).

RESULTS OF CHANGES IN THE BLOOD PARAMETERS OF HT RATS AFTER EXPOSURE TO A ROUTER (R) AND A ROUTER WITH RESONATORS (R+D)

HEMATOCRIT

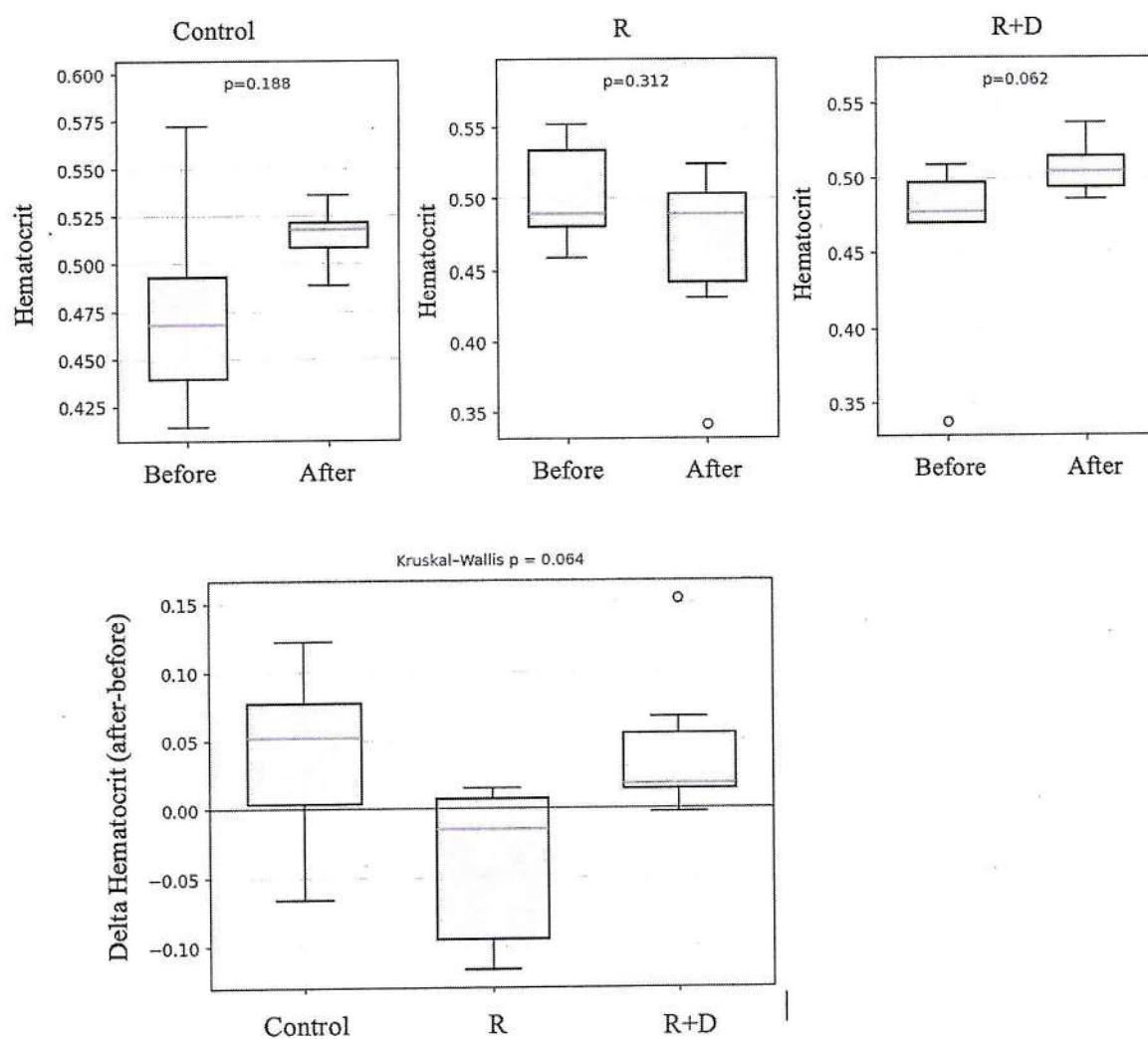


Fig. 12.1.

LEUKOCYTES

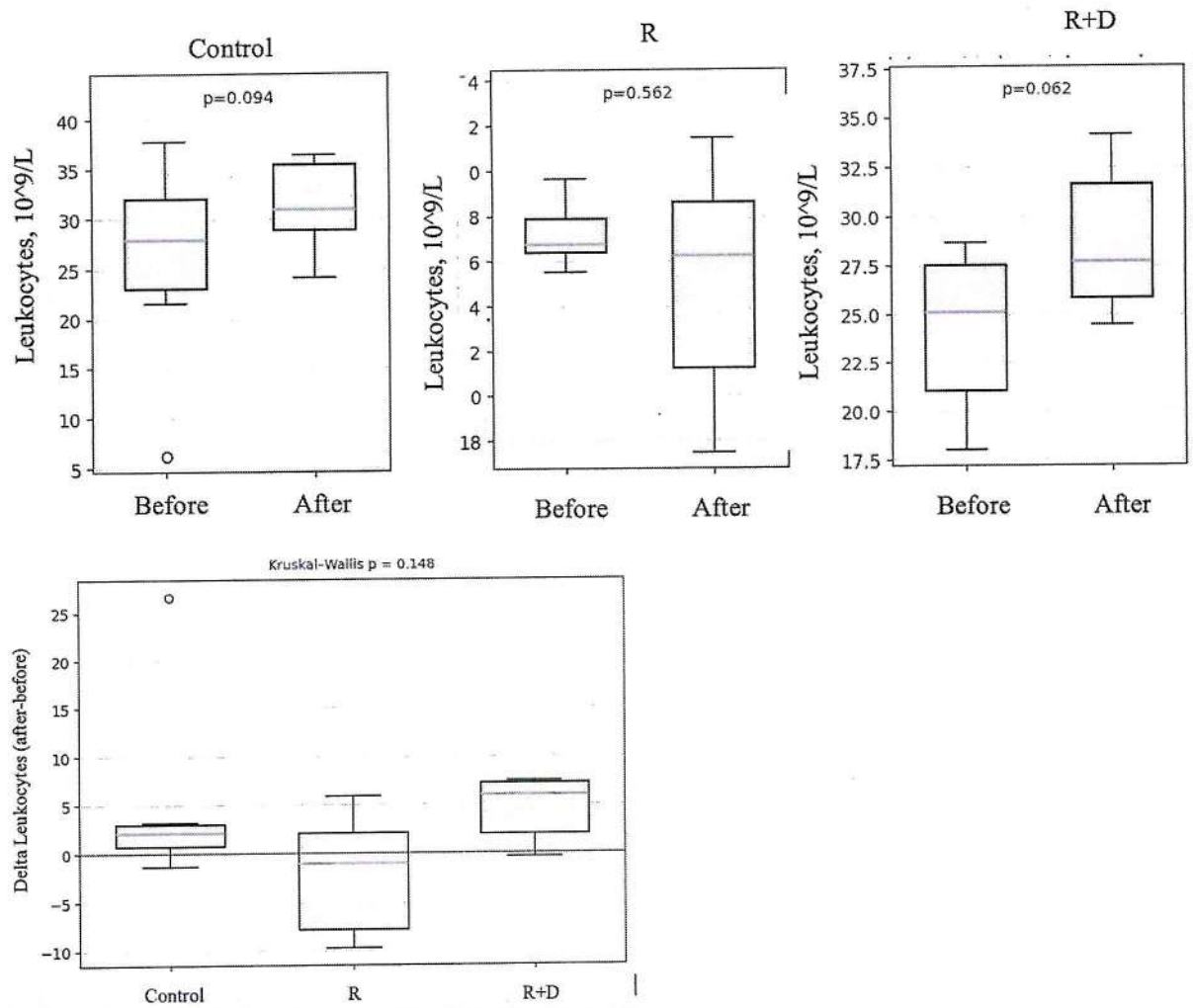
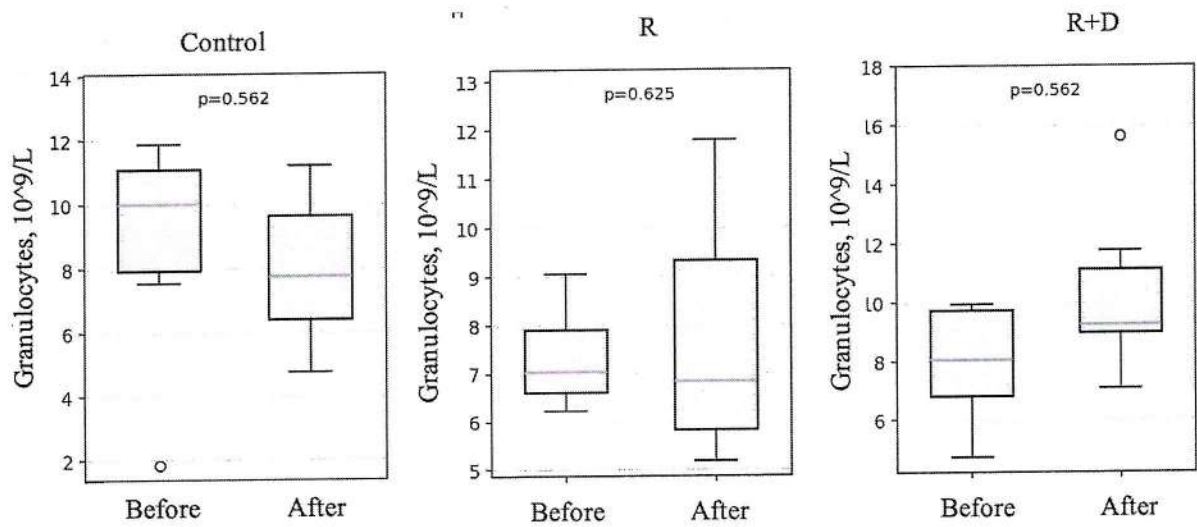


Fig. 13.1.

GRANULOCYTES, $10^9/L$



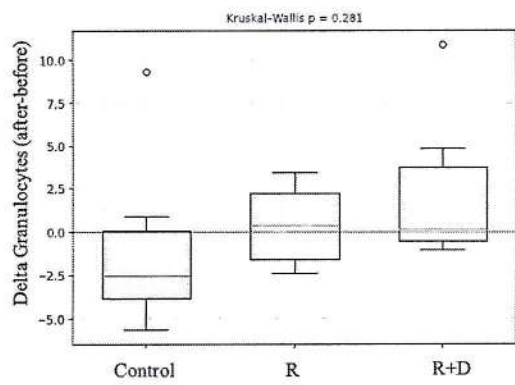


Fig. 14.1.

LYMPHOCYTES, %

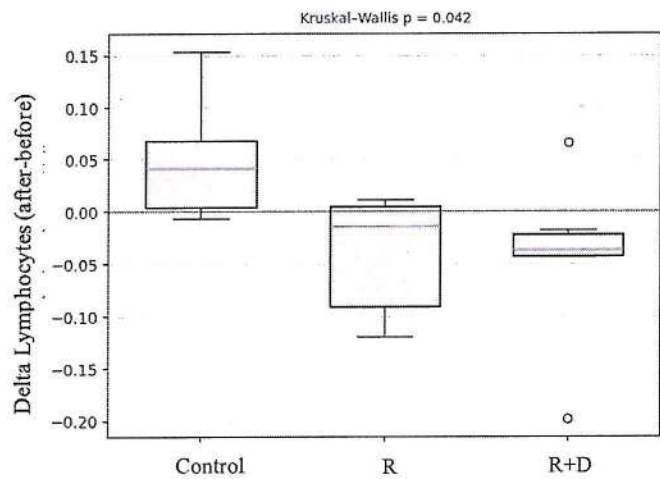
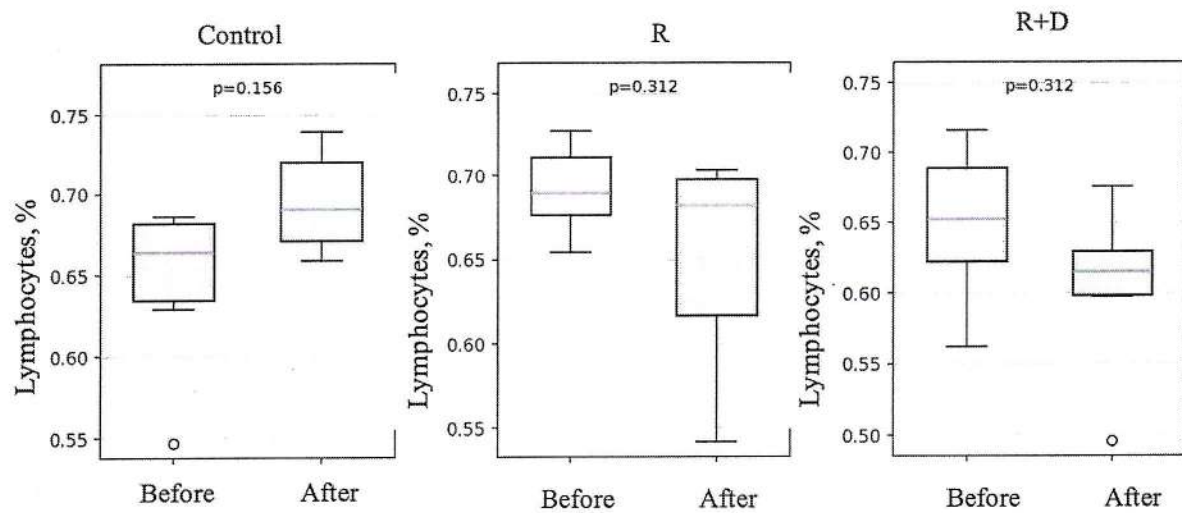


Fig. 15.1.

ERYTHROCYTES, 10¹²/L

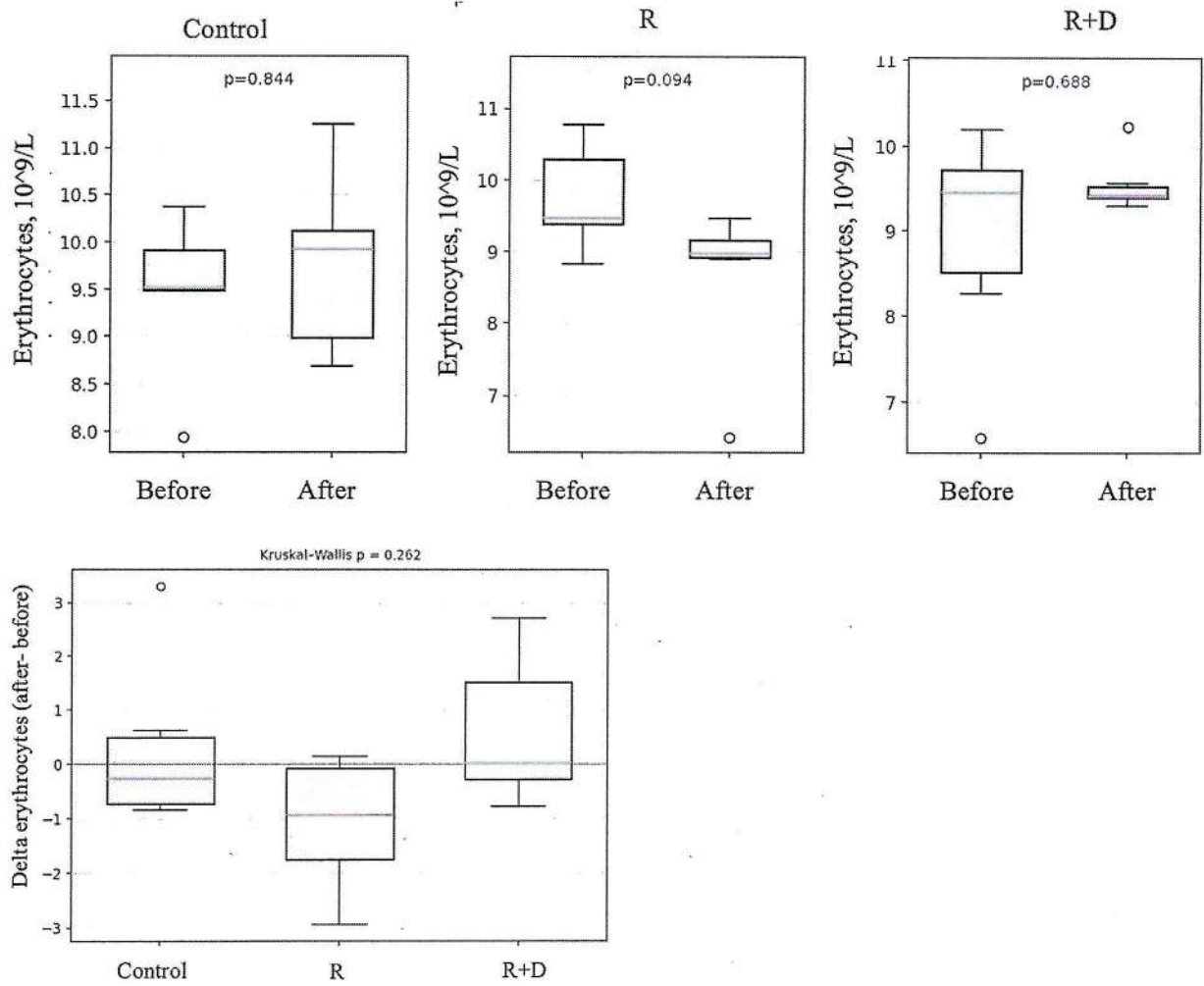
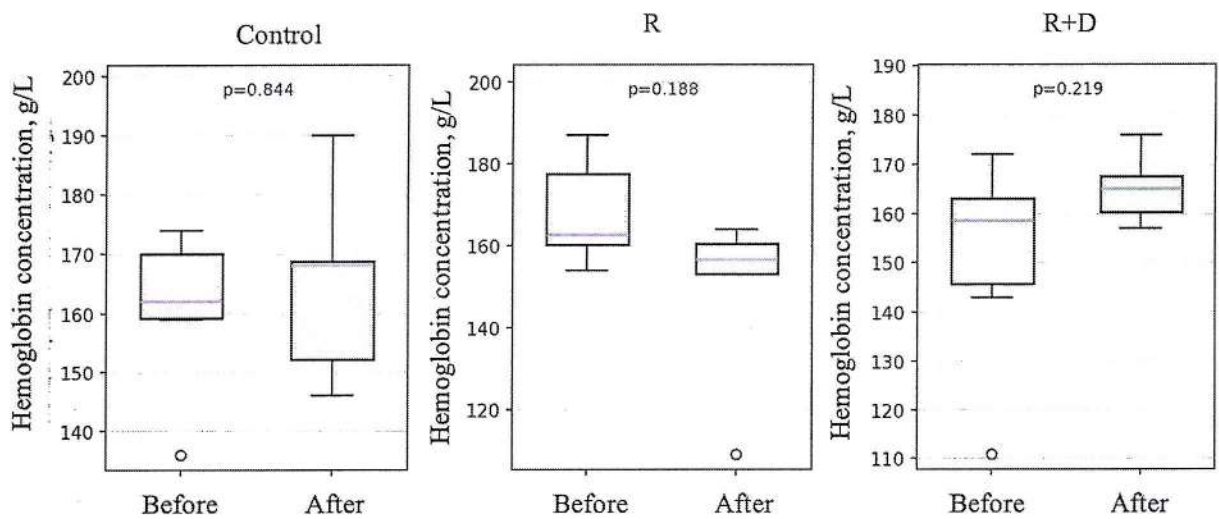


Fig. 16.1.

HEMOGLOBIN CONCENTRATION, g/L



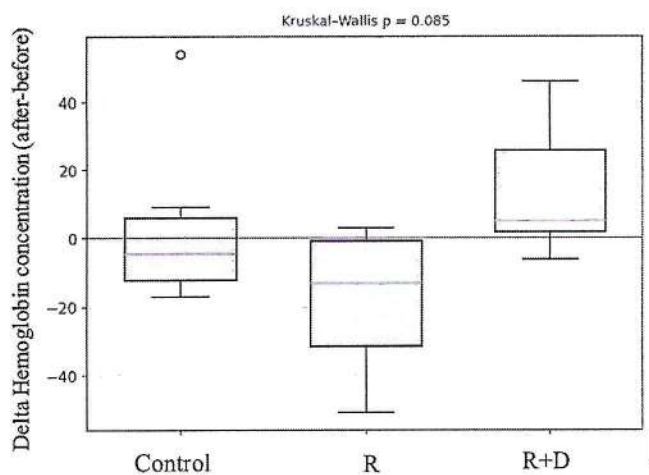


Fig. 17.1.

PLATELET VOLUME

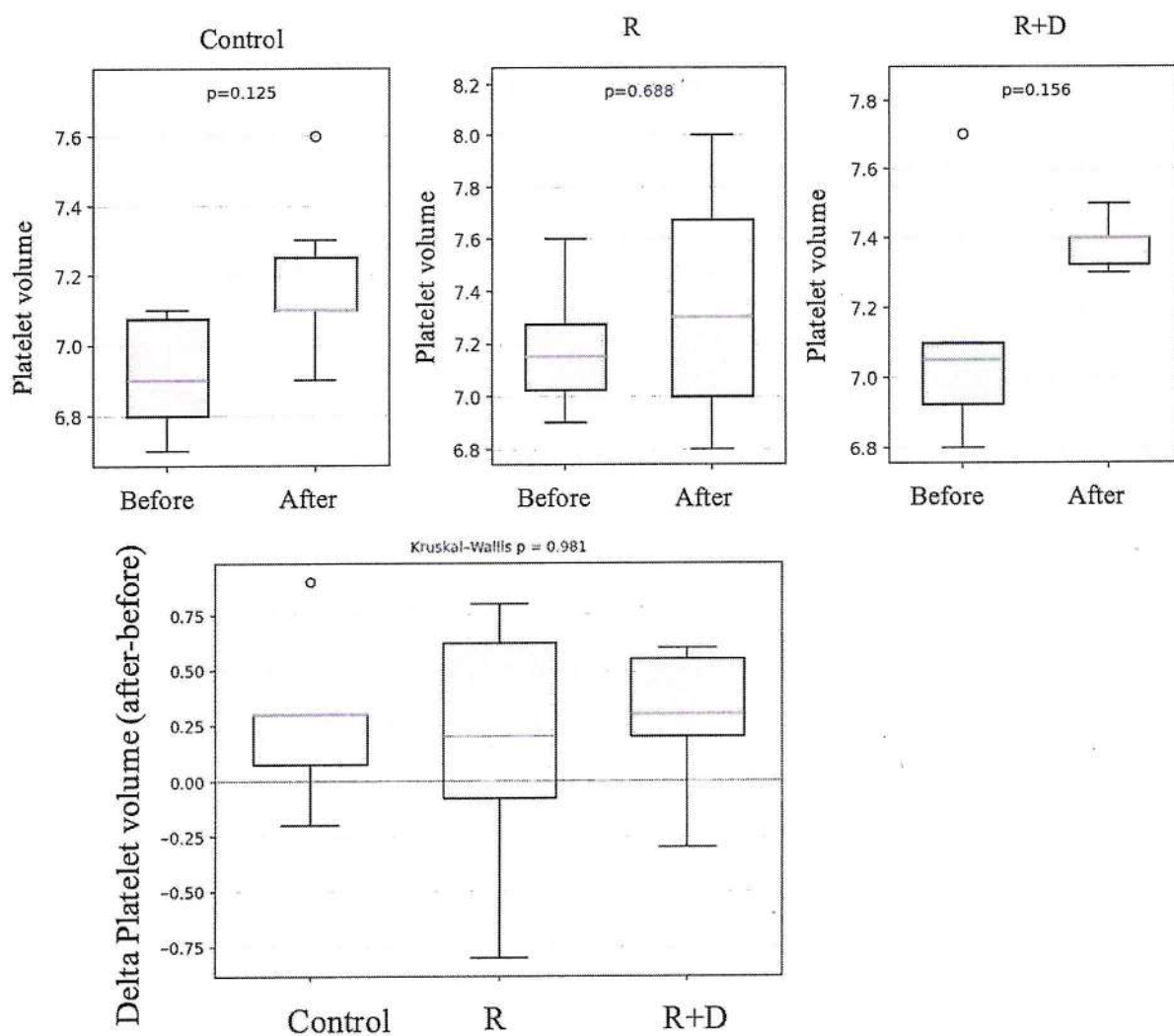


Fig. 18.1.

RATIO OF LARGE PLATELETS TO THEIR TOTAL NUMBER

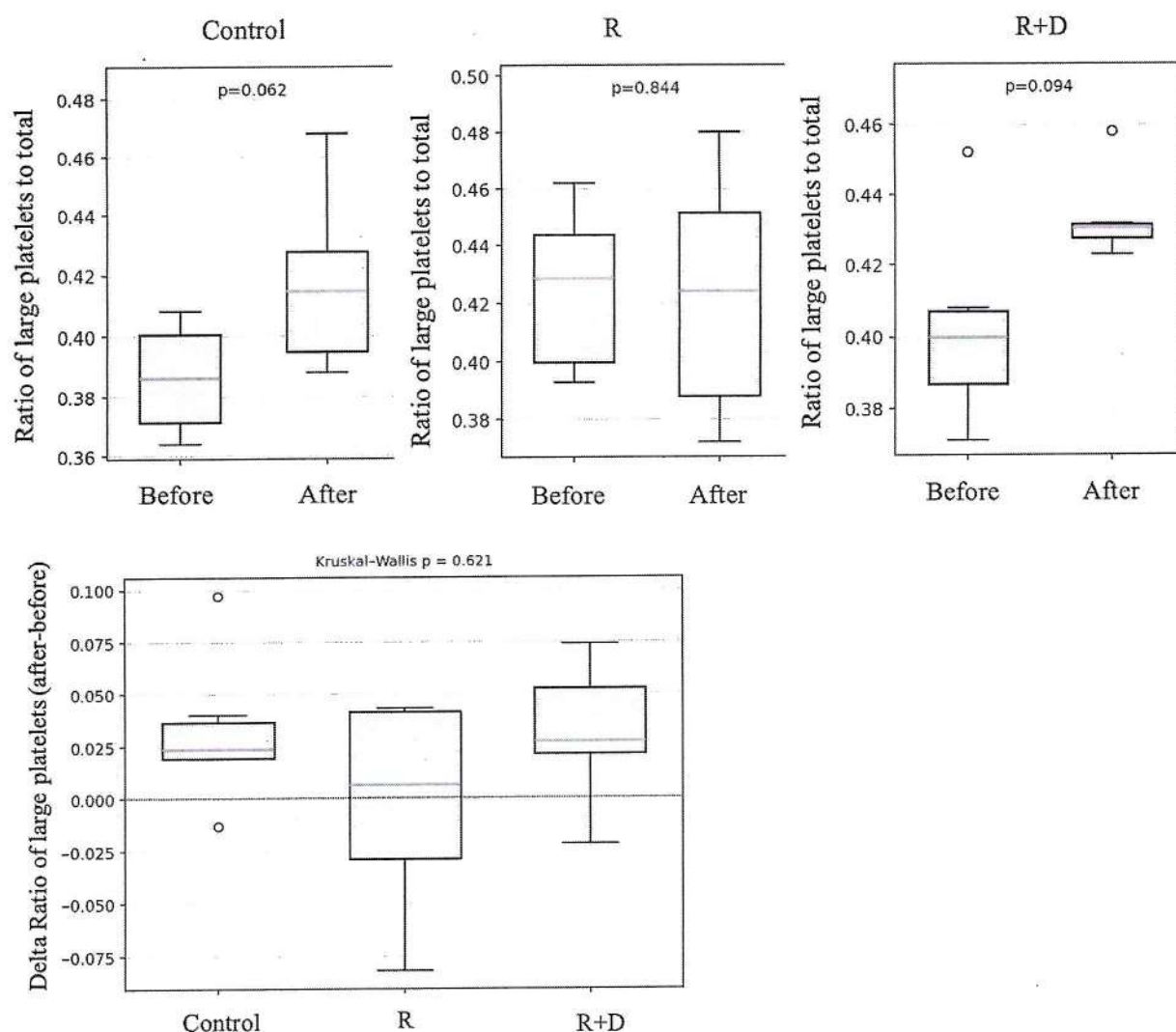


Fig. 19.1.

In rats of the HT strain:

1. a decrease in hemoglobin concentration after router exposure (R) and an increase in this parameter after exposure to resonators (R+D) (Fig. 17.1)
2. a decrease in lymphocytes in the presence of resonators (R+D) significantly greater than that under the action of the router (R) (Fig. 15.1)

No changes were observed in the other analyzed indicators (Figs. 12.1.-14.1., 16.1., 18.1., 19.1.)

RESULTS OF CHANGES IN BLOOD PARAMETERS OF LT LINE RATS AFTER EXPOSURE TO A ROUTER (R) AND A ROUTER WITH RESONATORS (R+D)

HEMATOCRIT

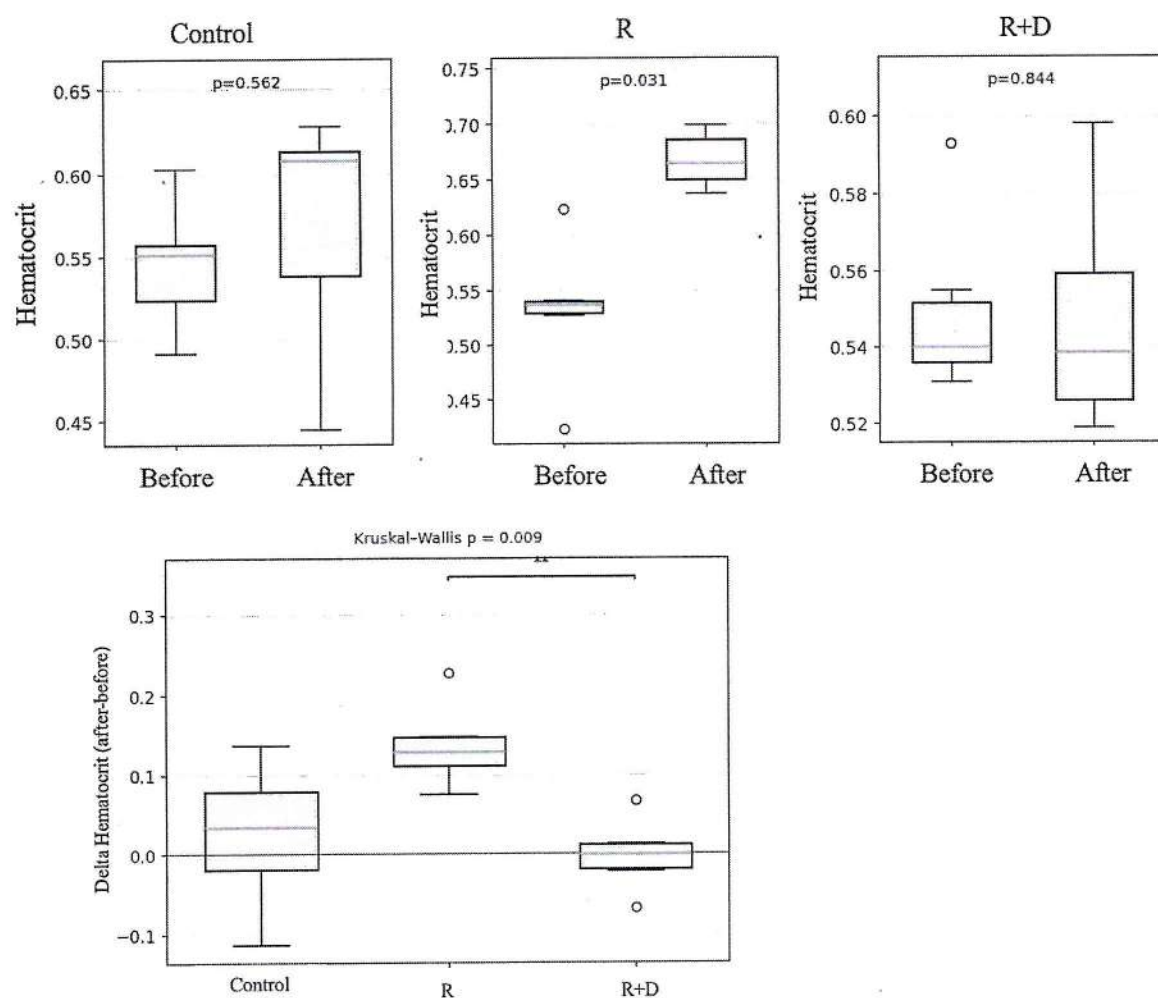
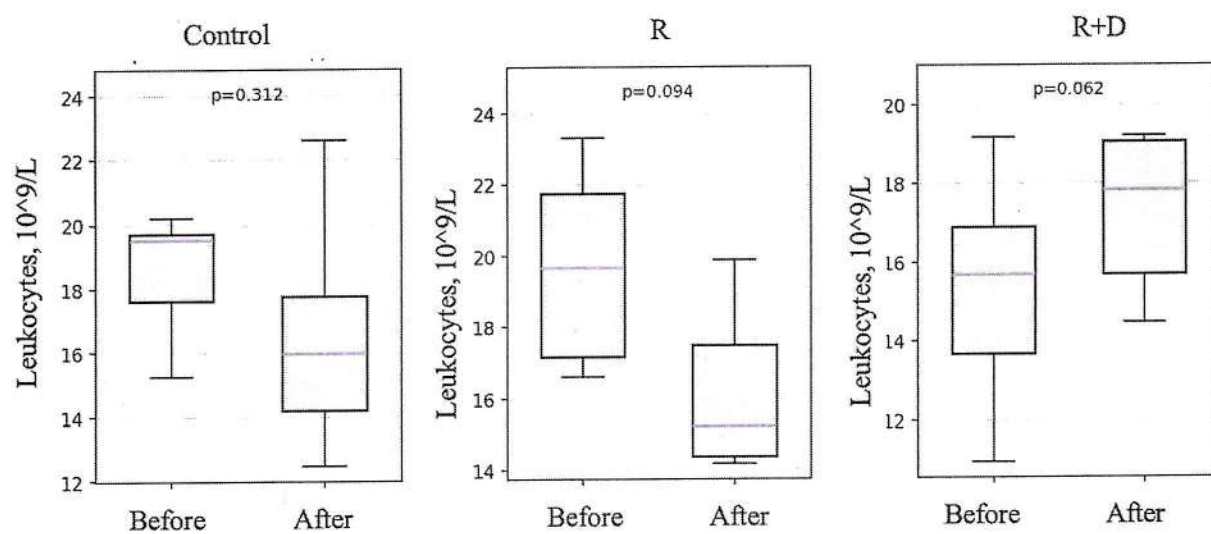


Fig. 12.2.

LEUKOCYTES



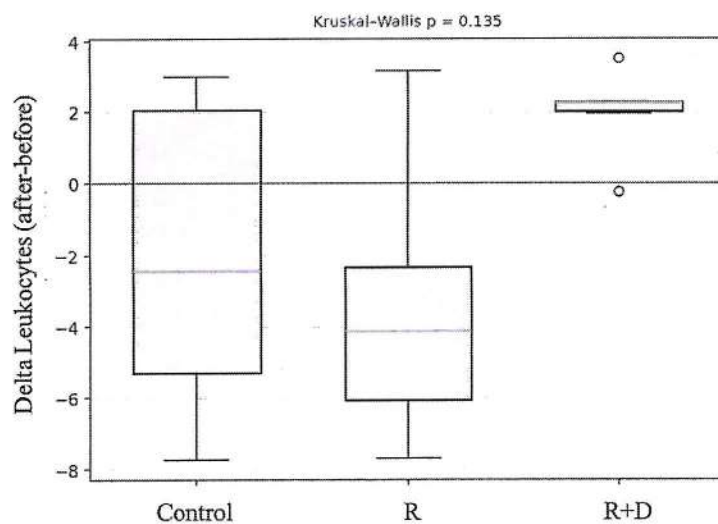


Fig. 13.2.

GRANULOCYTES, $10^9/L$ AND IN %

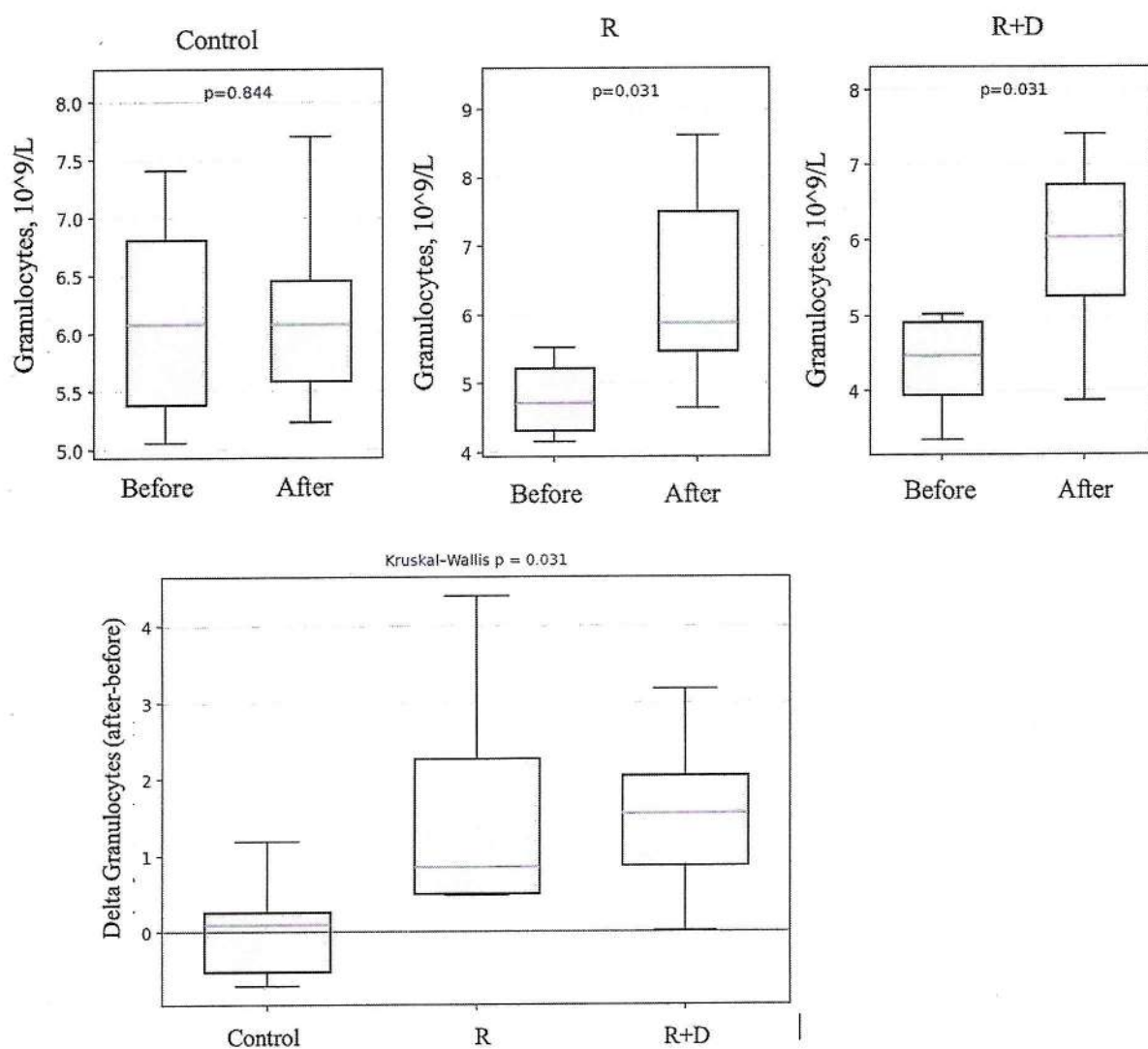


Fig. 14.2.1.

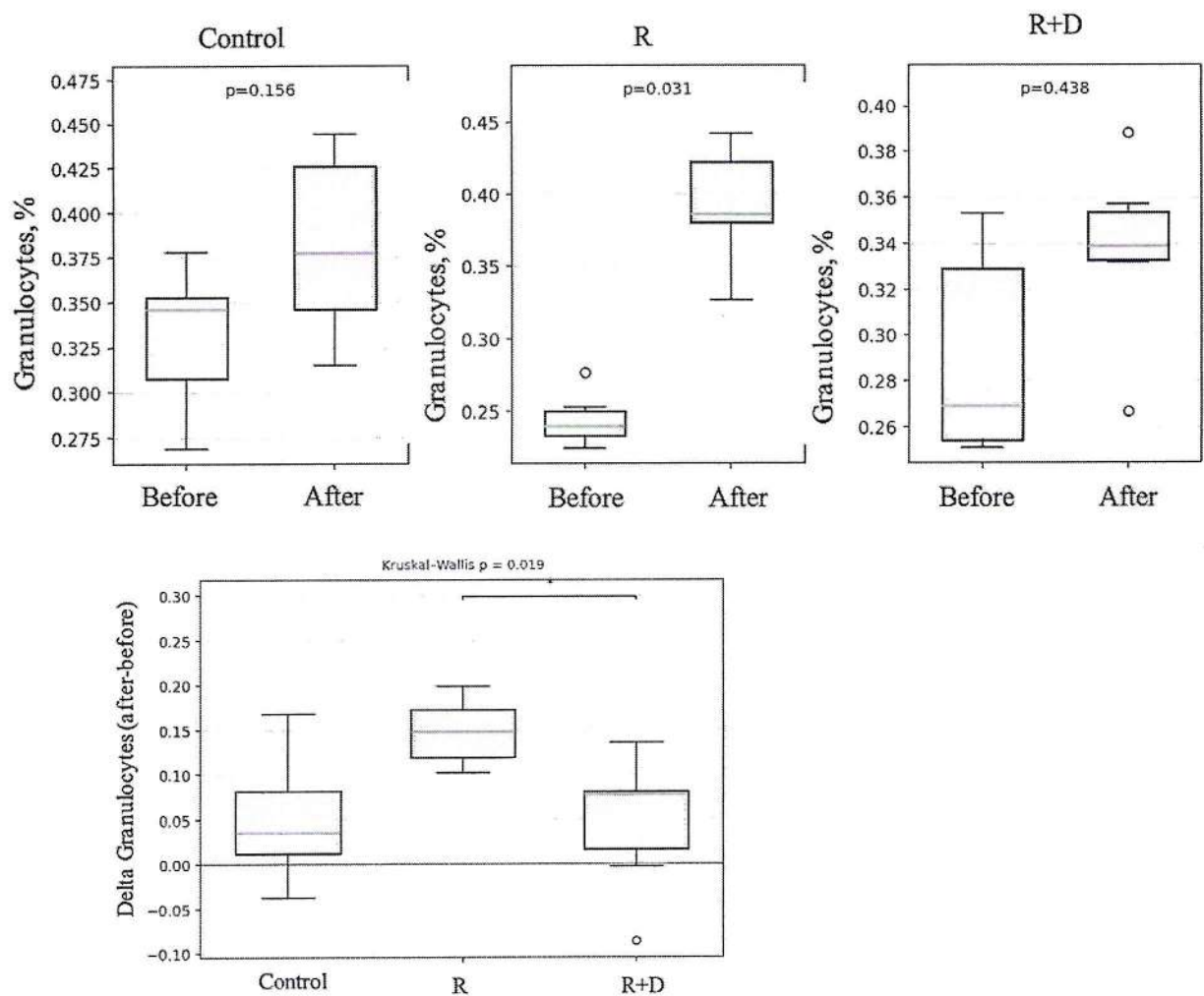
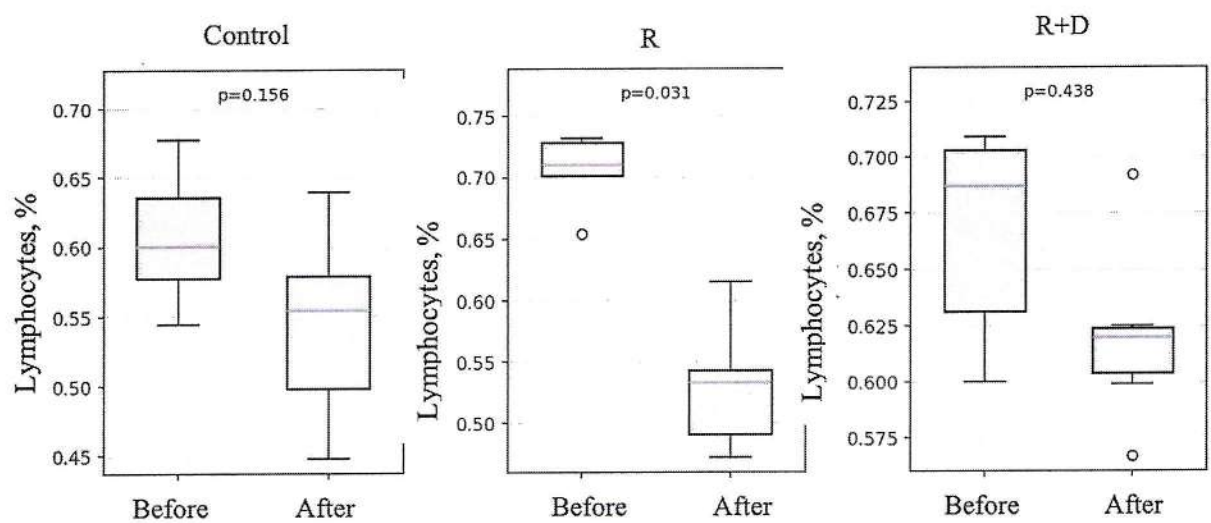


Fig. 14.2.2.

LYMPHOCYTES, %



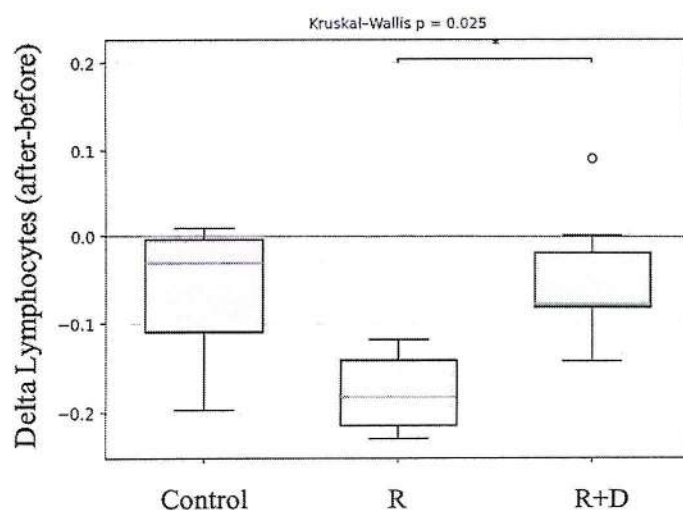


Fig. 15.2.

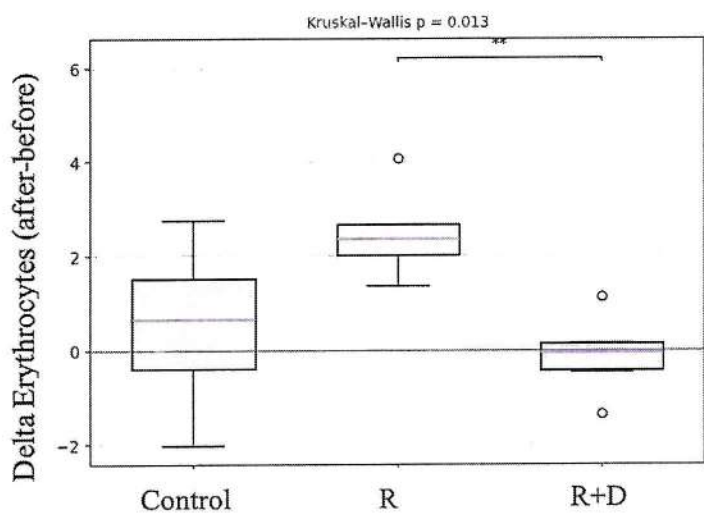
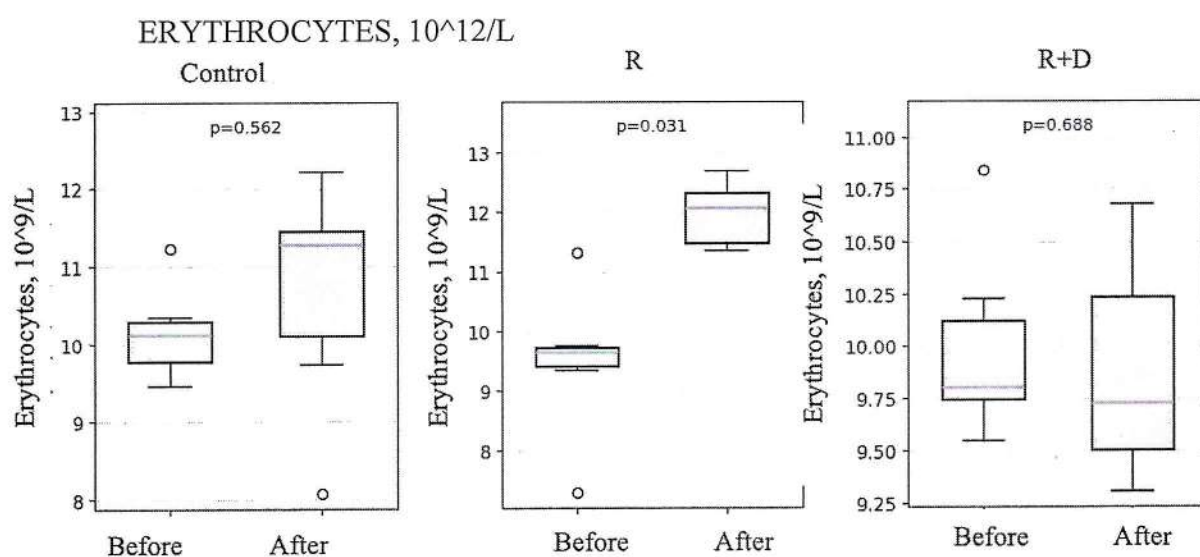


Fig. 16.2.

HEMOGLOBIN CONCENTRATION, G/L

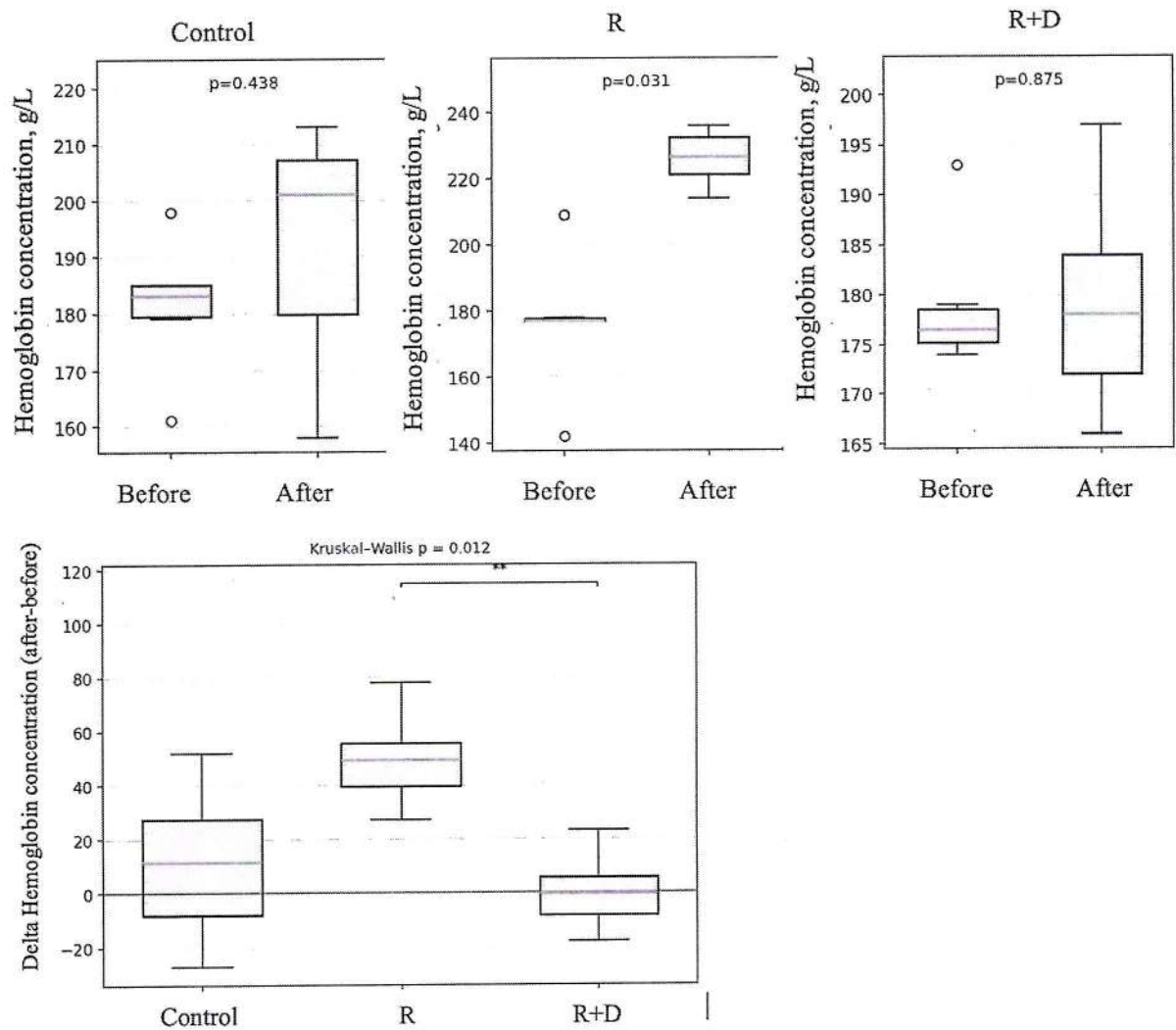
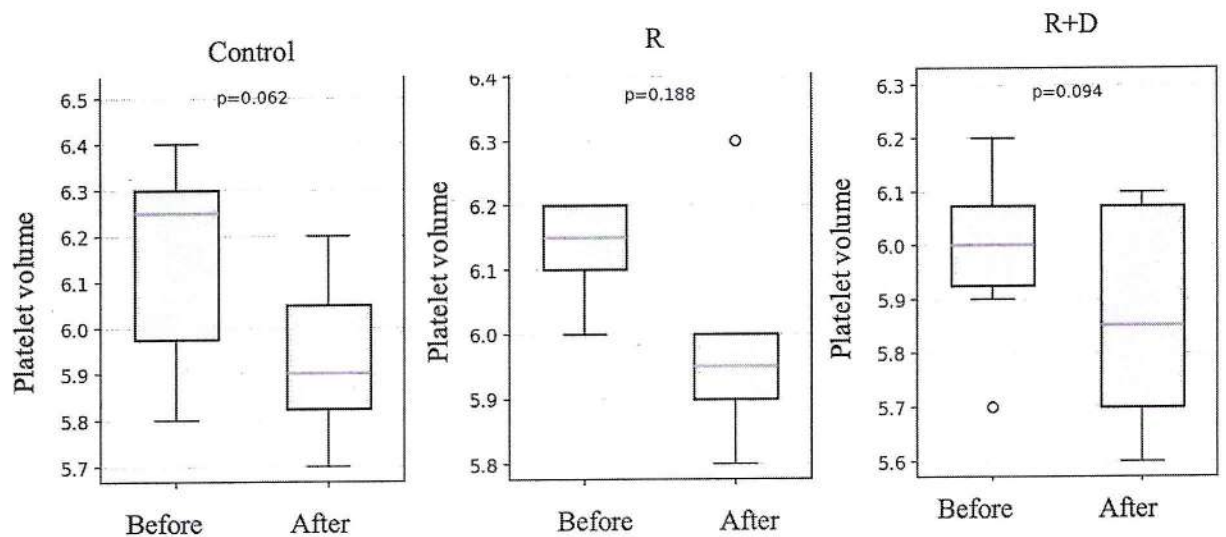


Fig. 17.2.

PLATELET VOLUME



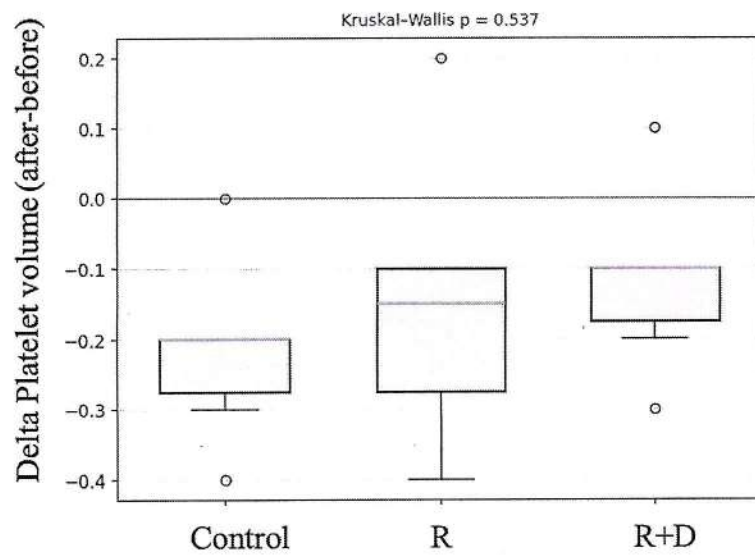


Fig. 18.2.

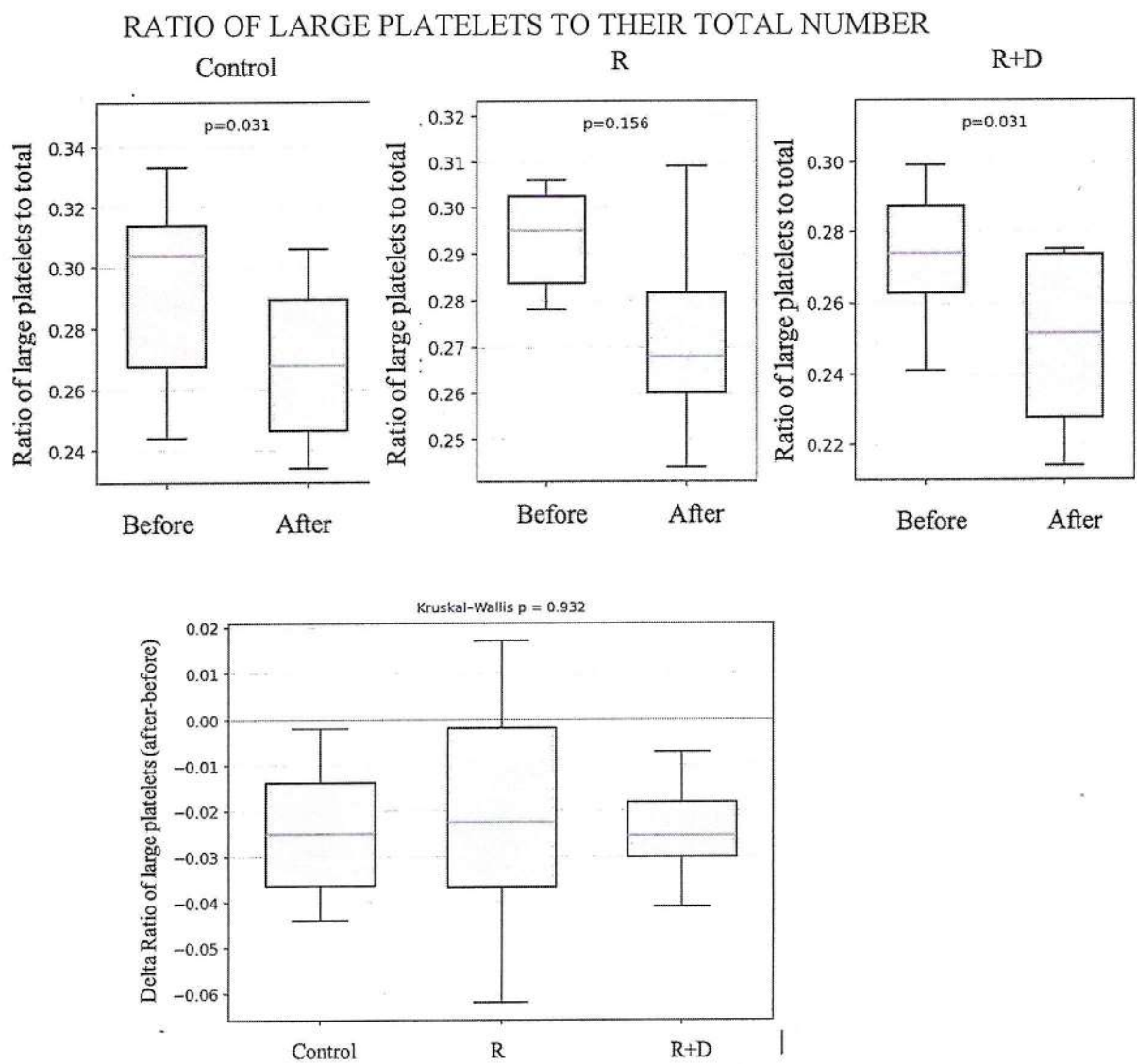


Fig. 19.2.

In rats of the LT strain:

1. The effect of the router (R) leads to an increase in hematocrit (Fig. 12.2), the level of which normalizes in the presence of resonators (R+D) (Fig. 12.2).
2. The influence of the router (R) leads to an increase in granulocytes (absolute value (Fig. 14.2.1) and in % (Fig. 14.2.2)), while resonators (R+D) normalize this indicator to the control level (% , Fig. 14.2.2.).
3. The influence of the router (R) leads to a decrease in the level of lymphocytes (Fig. 15.2), in the presence of resonators (R+D) this indicator is normalized (Fig. 15.2).
4. The influence of the router (R) leads to an increase in the red blood cell count (Fig. 16.2), which normalizes in the presence of resonators (R+D) (Fig. 16.2).
5. The influence of resonators (R+D) leads to a decrease in hemoglobin concentration to the control level compared to the influence of the router (R), which increases hemoglobin concentration (Fig. 17.2).

It is important to note that in both strains, the action of the router leads to a decrease in lymphocyte content. At the same time, the change in hemoglobin concentration is different: in the low-excitability HT strain, there is a decrease, and in the high-excitability LT strain, there is an increase, along with an increase in red blood cell content. At the same time, an increase in hematocrit and granulocyte content (associated with the development of inflammation) under the influence of router radiation is characteristic only for the high-excitability LT strain.

Elevated hemoglobin levels observed in the LT group are associated with increased homocysteine in the blood and the risk of adverse cardiovascular outcomes (Zhang et al., 2022). In contrast, decreased hemoglobin and red blood cell parameters were observed in patients with major depressive disorder (Yildiz et al., 2024). It should be noted that it is rats of the HT strain that are prone to developing symptoms of post-traumatic stress disorder after chronic psychoemotional stress.

CONCLUSIONS

1. The results of this study confirmed the greater susceptibility of high-excitability rats of the LT strain to the influence of router EMR, as evidenced by greater changes in parameters compared to the alternative strain.
2. For blood parameters, genotype-dependent changes after exposure to router radiation have been demonstrated for the first time.
3. A recovery in behavioral activity and blood parameters in both strains was demonstrated through the use of resonators under router operating conditions.

Previously (2018-2020), based on the results of behavioral analysis in five standard tests (OF, EPM, DLC, Conditional passive avoidance reflex, Morris maze) in rats of two strains with high and low nervous system excitability after exposure in a Faraday cage, with and without additional exposure factors such as EMR from a standard WiFi router (LinkSys E1200-EE/RU wireless router with the following technical characteristics: wireless carrier frequency: 2.4 GHz, number and type of antennas: 2 internal antennas, gain coefficient of standard antenna (antennas), dBi: 4 dBi) and Aires Defender Pro resonators, established:

1. The experimental conditions influenced the behavior of rats of two strains with contrasting nervous system excitability when studying research, locomotor activity, emotionality, anxiety, and cognitive abilities.
2. The genetically determined level of nervous system excitability mediated the nature of the influence of the experimental factors under study on various components of rat behavior in the test series used.
3. The Aires Defender Pro resonators themselves had a significant effect on the behavior of rats. In rats of the high-excitability LT strain, their effect, compared to the router's EMR under conditions of exposure in a Faraday cage, mainly affected the parameters of emotionality, reducing it to control values, and also caused a decrease in the values of some elements of general activity.
4. In rats of the low-excitability HT strain, the introduction of Aires Defender Pro resonators into the experimental setup mainly led to a decrease in locomotor activity, followed by increased anxiety, and caused some improvement in cognitive abilities.
5. Impaired memory consolidation during training in passive avoidance conditioned reflex due to the weakening of the electric field in the Faraday cage and in combination with the additional influence of the router's EMR is pronounced in cases of hereditary high-excitability of the nervous system (LT), but not low (HT), and is leveled out after the use of resonators (Shiryayeva et al., 2020).
6. The chromosomal apparatus of highly excitable rats of the LT strain is more susceptible to the damaging effects of high-frequency EMR compared to the HT. The high excitability of the nervous system determines the greater severity of the reduction in mitotic disorders in the presence of Aires Defender Pro resonators, which indicates that the effectiveness of the protective effect of resonators depends on the functional state of the nervous system of animals (Dyuzhikova et al., 2019).

The mechanisms of non-ionizing EMR exposure on the body are currently being actively researched, with numerous discussions regarding the presence/absence of pathogenic effects, as data from different studies are contradictory. Possible ways in which EMR can negatively affect

the body are also being discussed (Karthick et al., 2017; Pall, 2016; Terzi et al., 2016, Saliev et al., 2019, etc.). A recent study by Spandole-Dinu et al., 2023 demonstrated that prolonged exposure to radiofrequency radiation at a frequency of 2.45 GHz also leads to an increase in motor activity in mice. At the same time, there were no significant structural or morphological changes in the brain, except for abnormalities in ependymal cells. A study by Obajuluwa et al., 2017 showed that exposure to 2.5 GHz radiofrequency waves from a Wi-Fi device for 4, 6, and 8 weeks caused a significant increase in anxiety levels and affected the locomotor function of rats, with a significant decrease in acetylcholinesterase (AChE) activity and an increase in AChE mRNA expression in the brains of rats. The data showed that prolonged exposure to Wi-Fi can lead to the development of neurodegenerative diseases associated with significant changes in AChE gene expression and certain neurobehavioral parameters related to brain damage.

The effect of an alternating, but not static, electric field (35 kV/m) on learning ability and spatial memory has been demonstrated in mice (Di et al., 2019). The effect of high-frequency electromagnetic fields on the memory of adult male rats has been demonstrated in social discrimination tests (Schneider et al., 2014). It has been shown that ultra-high frequency EMF causes metabolic reprogramming of brain cell mitochondria in Wistar rats, which increases the rate of superoxide radical and nitric oxide formation, which can initiate the development of neurodegenerative diseases and cancer (Burlaka et al., 2016).

There is growing evidence that chronic exposure to radiofrequency electromagnetic fields (RF-EMF) can cause non-thermal biological effects with significant consequences for male reproductive health, including reduced sperm count and motility, changes in testicular architecture, disruption of the blood-testis barrier, and suppression of testosterone synthesis. They identify the pathways through which RF-EMF can impair fertility—through oxidative stress, mitochondrial dysfunction, DNA damage, apoptosis, autophagy dysregulation, and hormonal disorders based on redox imbalance, mitochondrial collapse, and steroidogenic disorders, and there is emerging evidence of epigenetic and transgenerational effects (Jangid et al., 2025).

CONCLUSION

The results of this study confirm the negative impact on the behavior and hematological parameters of rats of UHF EMF from a standard latest-generation router and demonstrate the positive effect of new-generation resonators on the restoration of a number of behavioral elements and blood parameters affected by non-ionizing electromagnetic radiation. They also indicate the need to take into account the typological characteristics of the nervous system of the organism when determining the degree of sensitivity to the effects of EMF, when developing means of protection against the negative effects of EMF, and when correcting the effects of various radiation sources.

This approach is important for understanding the causes of individual variability in sensitivity to EMF and, on this basis, determining ways to correct EMF-induced pathogenic processes in humans.

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