PACS: 61.72.U-

STUDY OF SOME BIOPHYSICAL AND BIOCHEMICAL PARAMETERS IN STRESS – EXPOSED LABORATORY RATS (*Wistar albino*)

A.N. Nasibova[1,2,3], R.I. Khalilov[1,2,3], M.A. Bayramov[1], M.F. Bayramova[1], L.T. Kazimli[1], R.S. Qasimov[1]

[1]*Institute of Radiation Problems of ANAS*
[2]*Baku State University, Department of Biophysics and Biochemistry*
[3]*Joint Ukraine - Azerbaijan International Research and Education Center of Nanobiotechnology and Functional Nanosystems*
aygun.nasibova@mail.ru; aygunnasibova21@gmail.com

Abstract: The effect of different doses of ionizing gamma radiation was studied on laboratory rats by electron paramagnetic resonance (EPR) spectroscopy. Paramagnetic centers were studied in the internal organs of control and irradiated rats. A stress factor, ionizing gamma radiation, has been determined to cause the generation of broad-spectrum EPR signals which characterize iron oxide magnetic nanoparticles in the liver organs of laboratory rats. At the same time, blood samples of control and irradiated rats were analyzed. Their clotting time was determined, the rate and number of erythrocyte sedimentation were determined.

Keywords: EPR spectra, stress factor, gamma radiation, laboratory rats, blood samples.

1. Introduction

In our previous work, the effects of stress factors (ionizing gamma radiation, radioactive contamination, etc.) on plant systems were studied. Studies using the EPR method have shown that the stress factor causes the formation of crystalline iron oxide magnetic nanoparticles. The results can be used in environmental assessment and biomonitoring, as well as in the formation of functional magnetic nanoparticles [1, 2, 3].

At the same time, the effect of various stress factors on animal organisms was studied. During the initial research, pelvic snails (*Helix Pomatia*) were the object of research. In studies conducted by the EPR method, paramagnetic centers were studied in the pelvis and body parts of snails exposed to different doses of ionizing gamma radiation (100 Gy, 300 Gy, 500 Gy, 800 Gy). Free radical EPR signals ($g = 2{,}023$; $\Delta H = 10$ G) was recorded in them. In the spectra taken from the pelvic parts of snails, a linear increase in the amplitude of the free radical signal was observed depending on the amount of radiation dose. This result can be used as a dosimetric tool in the future. Thus, the pelvic parts of grape snails can be used as a bioindication parameter to measure the radiation background of the environment [4, 5].

As a continuation of the experiments, in subsequent studies, the effect of stress factors on laboratory rats of animal organisms as a living system was studied by the EPR method. In experiments with animal organisms, the selection of laboratory rats as the object of study is considered very convenient. It is known from the literature that, in research, the most commonly used laboratory animals are rodents [6, 7, 8]. Among laboratory animals, 80% are rats and mice. Thus, they can easily adapt to laboratory conditions. Rodents are nocturnal organisms (active at night, passive during the day). Due to their small size, their physiological parameters are very high (heart rate, respiratory rate, etc.). Paramagnetic centers have been studied in laboratory rats exposed to different doses of ionizing gamma radiation by the EPR method. The EPR spectra of their internal organs were identified and blood tests in control (non-irradiated) and irradiated rats were compared.

2. Materials and methods.

In the experiments, the rats were divided into four groups, each with two rats. 1) control; 2) 3 Gy irradiated; 3) 6 Gy irradiated; 4) 8 Gy irradiated. The rats were irradiated on a ^{60}Co source RHUND 20000 device (P = 0,286 rad / sec). It should be noted that newborns in rats and mice are more resistant to radiation. Newly weaned from milk (20-30 days old) rats are the most sensitive to radiation. Adolescents (3-4 months) are the most resistant to stress, and then when they age increases (after 6 months) their resistance to any stress factor, including radiation, decreases.

Rats, which were irradiated at different doses were kept in the laboratory (30 days) at room temperature (fig. 1). 37-day-old rats were used in the experiments.

Fig. 1. Laboratory rats, which irradiated in different doses and kept in cages

During this period, the vital activity and behavior of rats were monitored and the morpho-physiological changes caused by this effect were observed after irradiation. Thus, their vitality, activity decreased, nutrition deteriorated. They even failed to clear the secretion of the Harder gland (a brownish-red substance containing porphyrin inside the eye sockets). In general, this secretion is not seen in healthy animals, because they often clear this secretion. However, if this secretion is observed in animals, it means that they have a health problem.

No mortality was observed in the control and 3 Gy doses of irradiated samples within one month after irradiation. One of the rats irradiated at a dose of 6 Gy died on the 22nd day, and the rat irradiated at a dose of 8 Gy died on the 8th day.

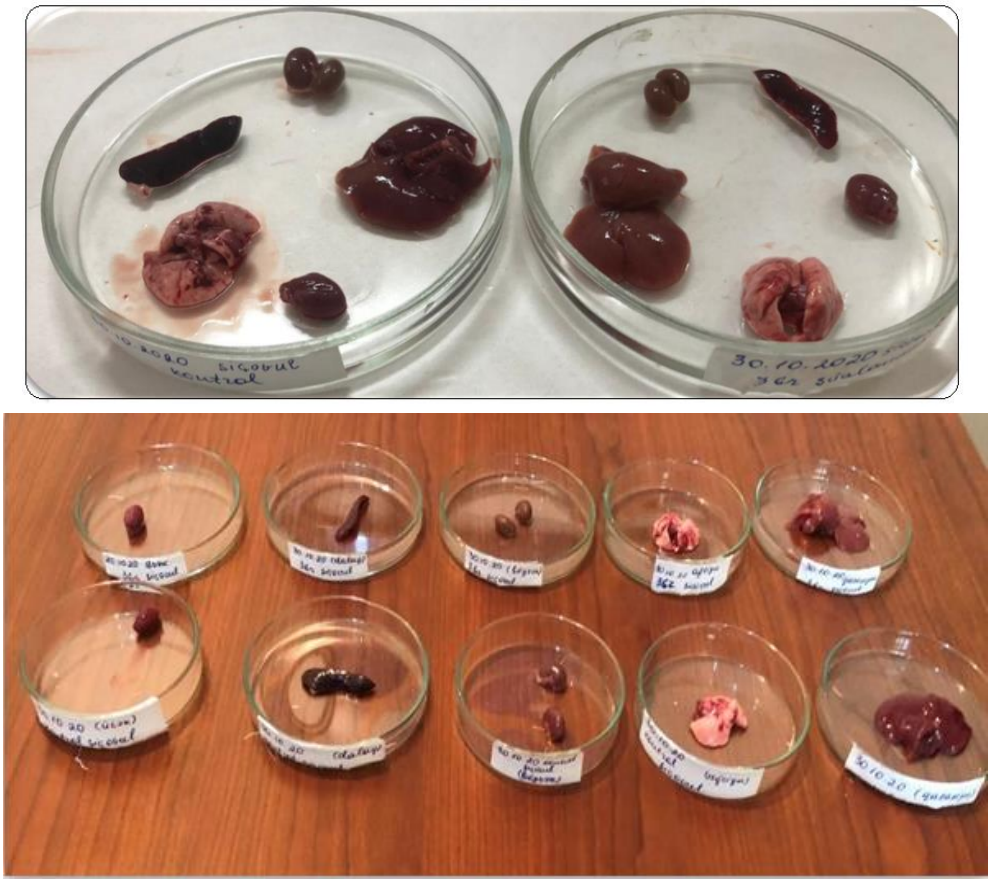

Fig.2. Internal organs of the rat (heart, spleen, kidneys, lungs, liver)

Their internal organs (heart, liver, lungs, spleen, and kidneys) were cut and collected separately in Petri dishes and dried under natural conditions at room temperature.

It should be noted that the rules of bioethics (anesthesia with 10% ketamine) were followed during the cutting. Morphological changes were observed in the internal organs of rats, which were irradiated at different doses. So, there were differences in their colors and sizes. After 7 days of drying, they were ground and prepared for EPR studies. EPR studies were performed at room temperature on 3 cm range BRUKER-EMX (Germany) EPR spectrometers. The samples were placed inside a special capillary ampoule and placed in a resonator located between the electromagnetic poles of the spectrometer.

At the same time, blood samples from control and irradiated rats were studied comparatively (fig. 3). Their coagulation times were determined: control sample - 10-15 sec.; 3 Gy irradiated sample - 60 sec; 6 Gy irradiated sample - 60 sec.

Fig.3. Control (left), 3 Gy (center) and 6 Gy (right) irradiated rat blood samples

The experiment was carried out under a microscope in five stages, the average score was calculated. The average value obtained determines the number of erythrocytes.

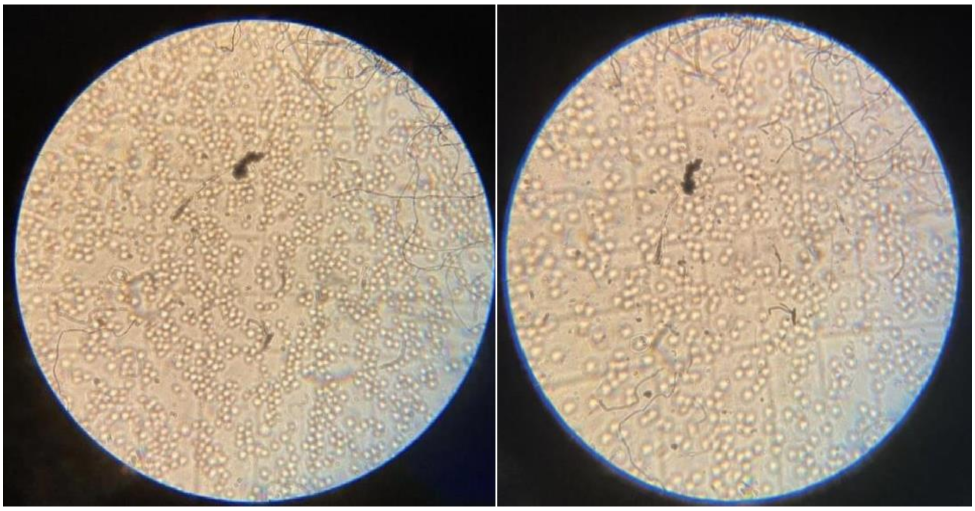

Fig.4. Microscopic view of erythrocytes of 3 Gy (right) and 6 Gy (left) irradiated rats

The rate of erythrocyte sedimentation rate was determined on a Panchinkov device, and hemoglobin was determined on a hemometer device. The results are given in Table 1.

Table 1

Hematological parameters of blood samples in control and irradiated rats

Hematological parameters	Control	3 Gy	6 Gy
Erythrocyte	$8,1\cdot 10^9$ l	$7,6\cdot 10^9$ l	$7,3\cdot 10^9$ l
Leukocytes	$7,6\cdot 10^9$ l	$3,8\cdot 10^9$ l	$3,3\cdot 10^9$ l
Hemoglobin	16 gr/l	15,2 gr/l	12 gr/l
Erythrocyte sedimentation rate	10 mm/hour	2 mm/hour	2 mm/hour

3. Conclusions and discussions

EPR spectra of internal organs dried at room temperature were recorded under natural conditions. These spectra are given in Figures 5 - 8. Signals of free radicals, manganese ions, and iron oxide magnetic nanoparticles in liver samples were observed in the internal organs of control and irradiated rats.

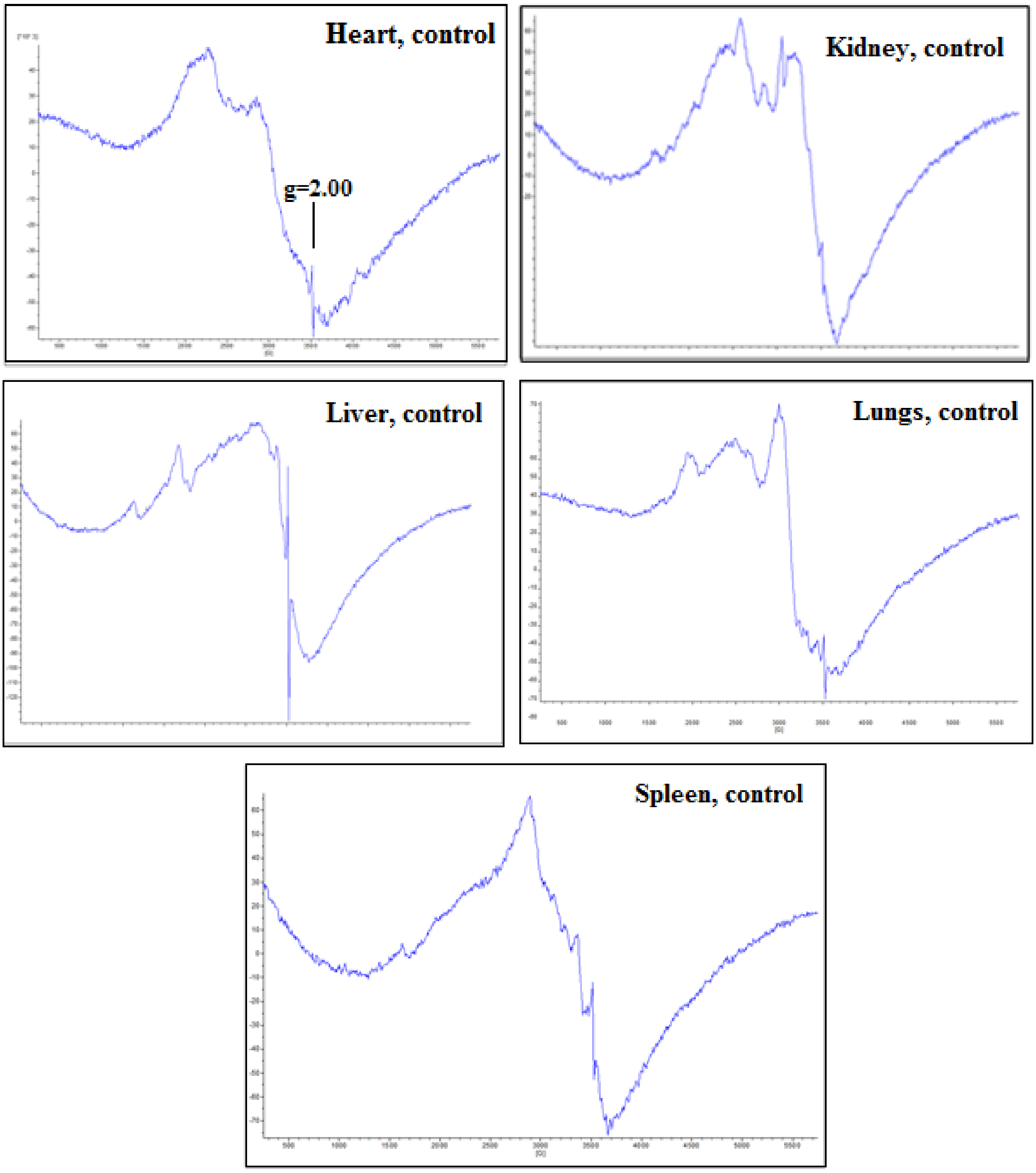

Fig.5. EPR spectra of the internal organs of a non-irradiated (control) rat.

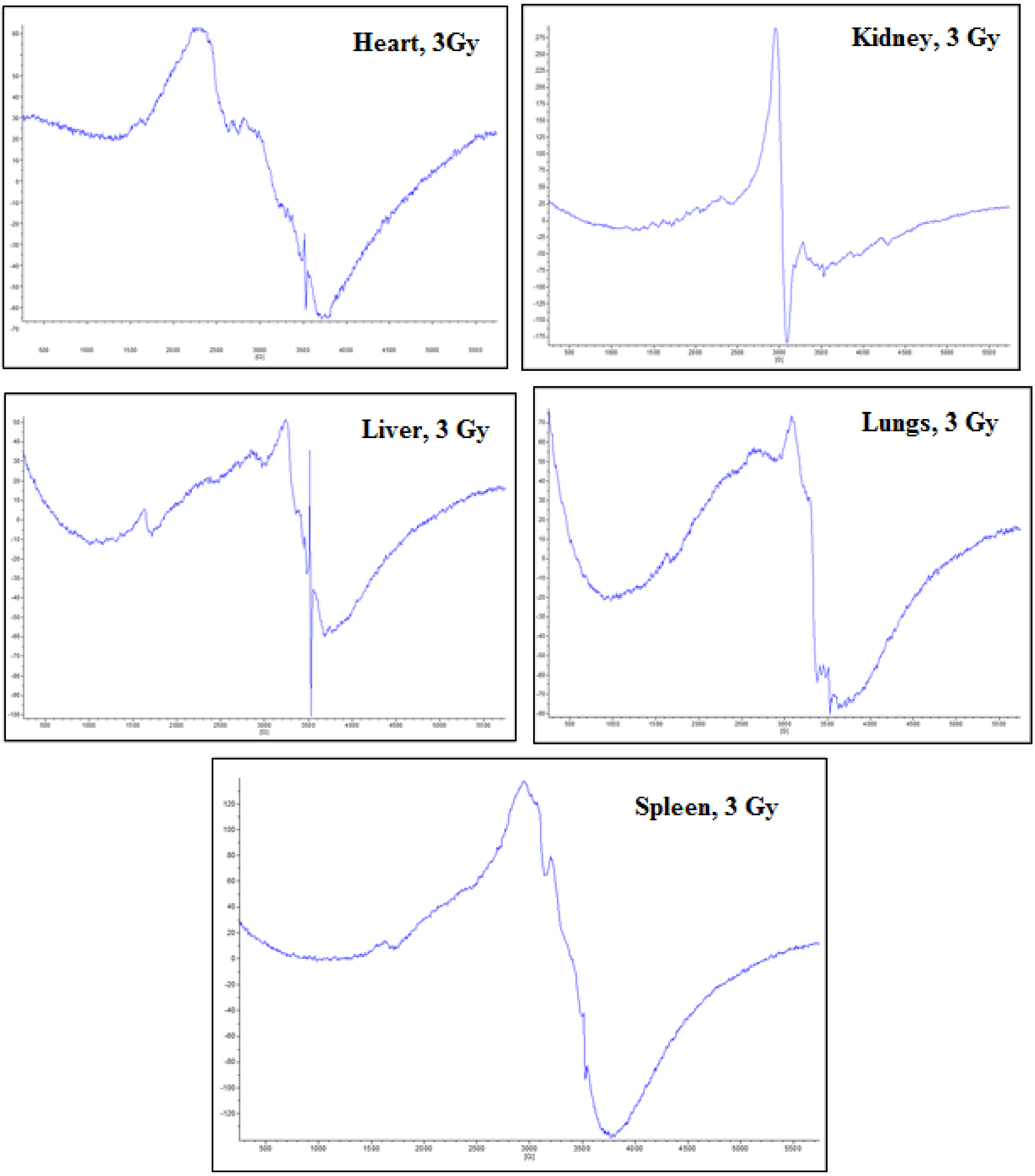

Fig. 6. EPR spectra of the internal organs of a rat irradiated at a dose of 3 Gy.

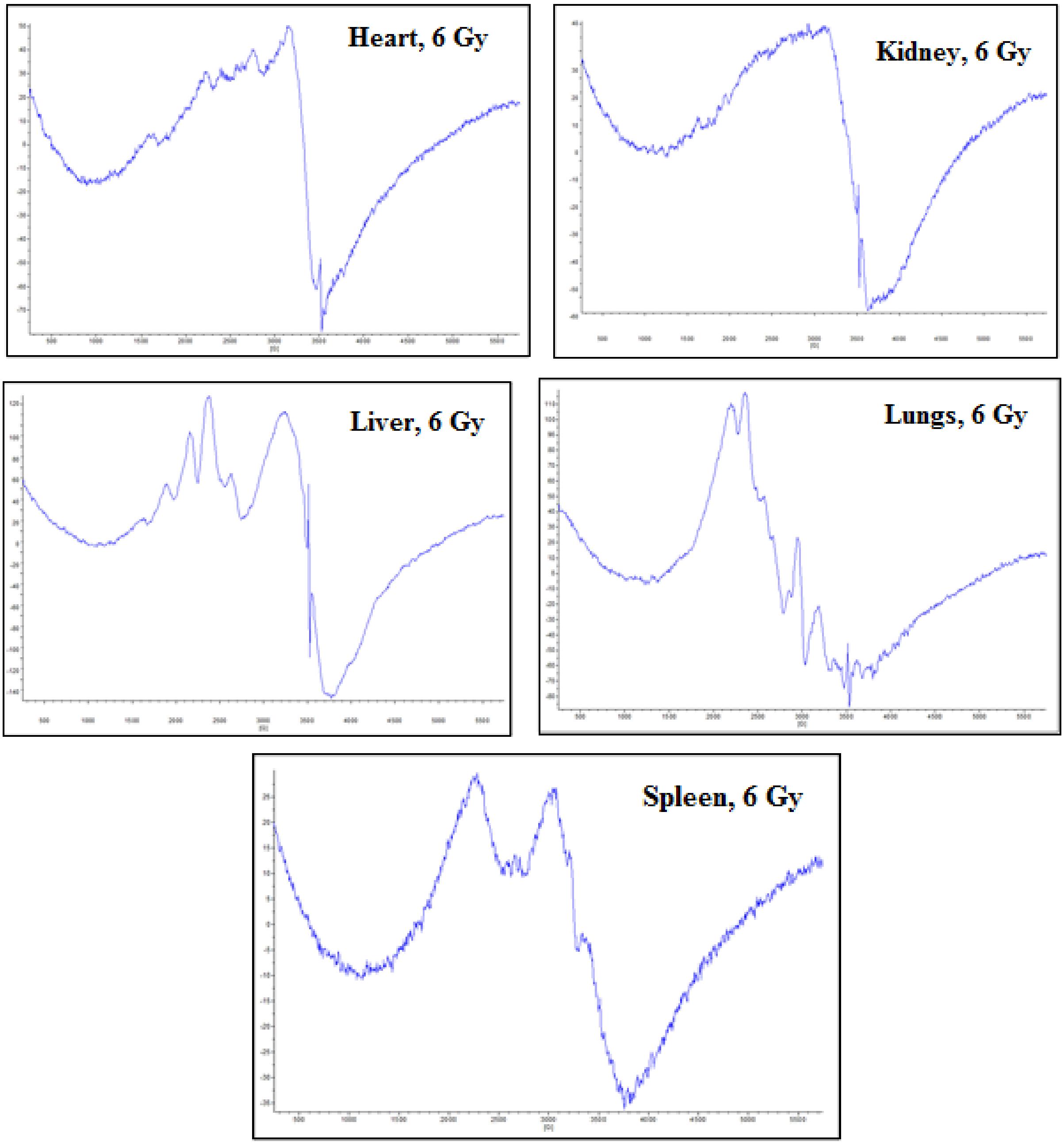

Fig.7. EPR spectra of the internal organs of a rat irradiated at a dose of 6 Gy.

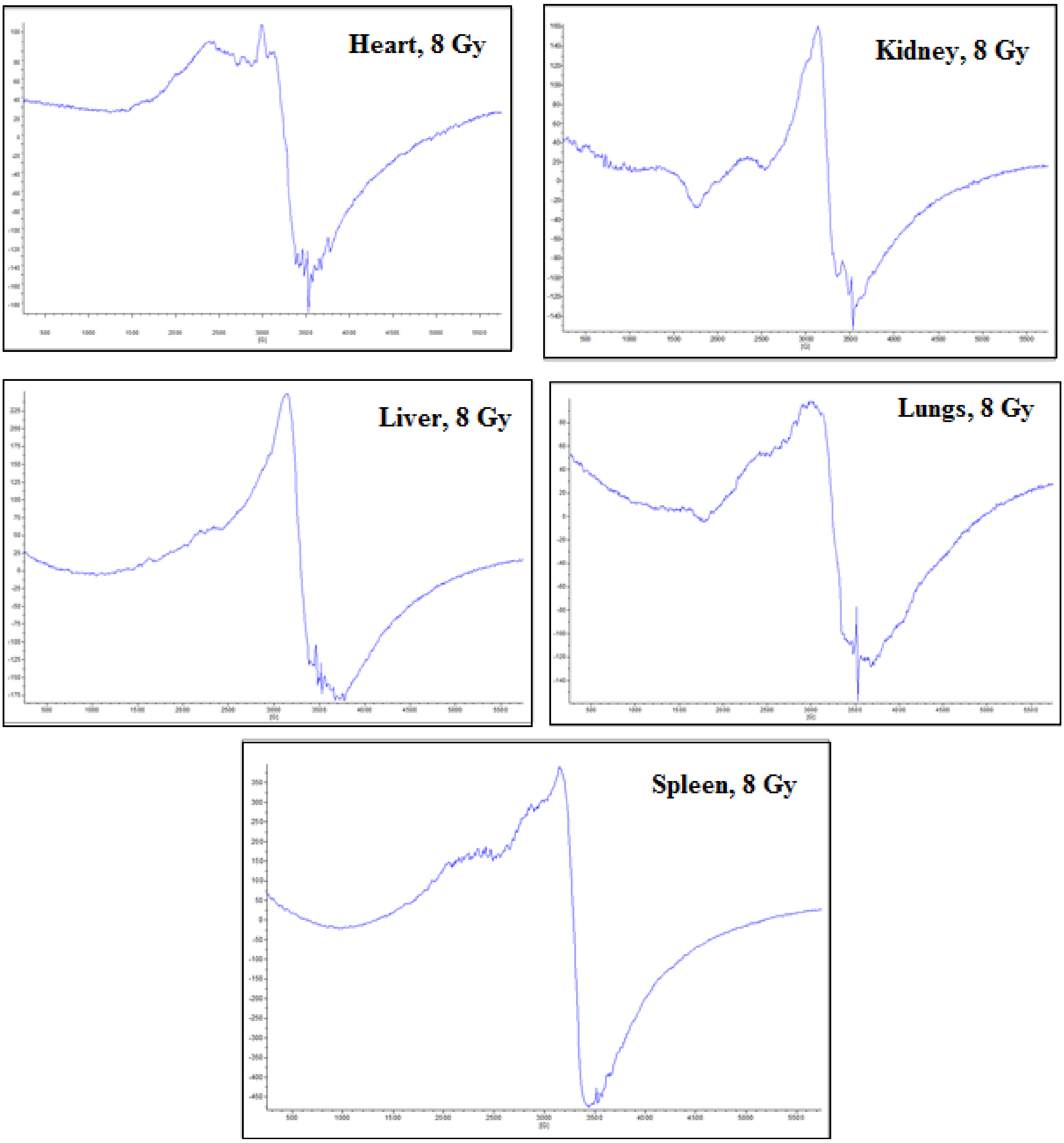

Fig.8. EPR spectra of the internal organs of a rat irradiated at a dose of 8 Gy

Examination of the spectra of control samples shows that signals of free radicals and six-component manganese ions are observed in lung and heart samples. Radiation at doses of 3 Gy and 6 Gy, along with a free radical signal in the spectra obtained from liver samples, reveals the formation of a broad EPR signal characterizing crystalline magnetic nanoparticles.

Summarizing the results, we can say that an increase in the radiation dose leads to an increase in the intensity of the free radical signal in the samples. In addition, the formation of new paramagnetic centers, signals of iron oxide nanoparticles as a result of the influence of radiation in liver samples was revealed. At a radiation dose of 8 Gy (lethal dose), this signal disappears. This indicates the formation of abnormal magnetic properties in the liver during stress.

The results obtained can be widely used and applied in biomedical research, treatment, and diagnostic purposes.

References

1. R.I. Khalilov, A.N. Nasibova. Endogenous EPR-detected ferriferous nanoparticles in vegetative objects. // News of Baku University. V.3, P.35-40. 2010.
2. Aygun Nasibova, Rovshan Khalilov, Uzeyir Qasumov, Boris Trubitsin, Alexander Tikhonov. EPR signals in plant systems and their informational content for environmental studies. // European Journal of Biotechnology and Bioscience. V.4, I.2, P.43-47. 2016.
3. T.S. Kavetskyy, R.I. Khalilov, O.O. Voloshanska, L.M. Kropyvnytska, T.M. Beyba, V.A. Serezhenkov, A.N. Nasibova, A. Akbarzadeh, S.Ya. Voloshanska. Self-Organized Magnetic Nanoparticles in Plant Systems: ESR Detection and Perspectives for Biomedical Applications. // Book: Advanced Nanotechnologies for Detection and Defence against CBRN Agents. P.487-492. 2018.
4. A.N. Nasibova. Formation of magnetic properties in biological systems under stress factors. // Journal of Radiation Researches. V.7, I.1, P.1-5. 2020.
5. A.N. Nasibova. The use of EPR signails of snails as bioindicative parameters in the study of environmental pollution.// Advances in Biology & Earth Sciences. V.4, I.3, P.196-205.2019.
6. Mahesh Kumar J, Reddy PL, Aparna V, Srinivas G, Nagarajan P, Venkatesan R, et al. Strobilocercus fasciolaris infection with hepatic sarcoma and gastroenteropathy in a Wistar colony.// Veterinary Parasitology. V.141. P.362–367. 2006
7. Aman Dev Moudgil, Lachhman Das Singla, Kuldip Gupta, Prashants Daundkar, Vemu Bhaskar. Histopathological and morphological studies on natural Cysticercus fasciolaris infection in liver of Wistar rats.//Journal of Parasitic Diseases. V.40, I.2. P.255-258. 2016.
8. Reshma K., Debjith C., Sudha K. Chiranth Rahul, Charu Y. Chronic effects of ionising radiation induced renal damage in albino Wistar rats. // Biomedicine. V.40, I.2. 2020.

ИЗУЧЕНИЕ НЕКОТОРЫХ БИОФИЗИЧЕСКИХ И БИОХИМИЧЕСКИХ ПАРАМЕТРОВ У ЛАБОРАТОРНЫХ КРЫС (*Wistar albino*) ПОДВЕРГЩИХСЯ СТРЕССУ

А.Н. Насибова, Р.И. Халилов, М.А. Байрамов, М.Ф. Байрамова, Л.Т. Казимли, Р.С. Касумов

Резюме: Методом ЭПР спектроскопии исследованы действия различных доз ионизирующей гамма-радиации на лабораторные крысы. Во внутренних органах контрольных и облученных крыс изучены парамагнитные центры. Установлено, что стрессовый фактор - ионизирующее гамма-излучение в органах печени лабораторных крыс вызывает генерацию сигналов ЭПР широкого диапазона, характеризующих магнитные наночастицы оксида железа. Одновременно были проанализированы образцы крови контрольных и облученных крыс. Определяли время их свертывания, скорость и количество оседания эритроцитов.

Ключевые слова: Спектры ЭПР, стрессовый фактор, гамма-излучение, лабораторные крысы, образцы крови.

STRESS AMİLLƏRƏ MƏRUZ QALMIŞ LABORATORİYA SİÇOVULLARINDA (*Wistar albino*) BƏZİ BİOFİZİKİ VƏ BİOKİMYƏVİ PARAMETRLƏRİN TƏDQİQİ

A.N. Nəsibova, R.İ. Xəlilov, M.A. Bayramov, M.F. Bayramova, L.T. Kazımlı, R.S. Qasımov

Xülasə: Elektron Paramaqnit Rezonans (EPR) spektroskopiyası metodu ilə ionlaşdırıcı qamma radiasiyanın müxtəlif dozalarının laboratoriya siçovullarına təsiri öyrənilmişdir. Kontrol və şüalanmış siçovulların daxili orqanlarında paramaqnit mərkəzlər tədqiq edilmişdir. Müəyyən edilmişdir ki, stress faktor olan ionlaşdırıcı qamma radiasiya laboratoriya siçovullarının qara ciyər orqanlarında dəmir oksidi maqnit nanohissəciklərini xarakterizə edən geniş EPR siqnallarının generasiyasına səbəb olur. Eyni zamanda kontrol və şüalanmış siçovulların qan nümunələrinin analizi aparılmışdır. Onların laxtalanma müddətləri müəyyən edilmiş, eritrositlərin çökmə sürəti və sayı təyin edilmişdir.

Açar sözlər: EPR spektrləri, stress faktor, qamma-radiasiya, laboratoriya siçovulları, qan nümunələri.