LOW FREQUENCY LOW INTENSITY PULSE ELECTROMAGNETIC FILED *IN VIVO* INFLUENCE ON BLOOD CELLS PERMEABILITY IN RAT

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Keywords: pulse electromagnetic field (PEMF), blood cells, Na^+,K^+-ATPase activity, in vivo, rat.
Abstract: The *in vivo* influence of a low frequency and intensity (50 Hz, 2.7 mT) pulse electromagnetic field (PEMF) applied continuously and intermittently, 30 min for a period of 20 days, on the permeability of blood cells in rats was studied. For this aim, concentrations of Na^+, K^+ and Ca^{2+} in plasma (extracellular compartment) and blood cells (intracellular compartment) after their separation by centrifugation of blood collected from treated animals were determined by the flame photometry method. PEMF induced a decrease of the extracellular Na^+ concentration and an increase of the intracellular one, causing a reduction in the ratio Na^+_e/Na^+_i value as compared with the control, reflecting the increase of passive membrane permeability to this ion. Also, PEMF induce the reduction of extracellular K^+ level and increase the intracellular K^+ level and a consequent K^+_e/K^+_i ratio reducing, which indicates an increase in transmembrane K^+ active transport, correlated with dynamic of Na^+,K^+-ATPase activity. PEMF effect on Ca^{2+} is more diverse, manifesting a general trend of increasing of its intracellular concentration. These findings also indicate a possible increase in the membrane resting potential.

INTRODUCTION

In the past twenty years, researches on biological effects of electromagnetic fields, not only have expanded, but they became a subject of public concern and debate throughout the world. Generally, electromagnetic fields and waves interaction with biological systems is closely related to the frequency, mechanisms of interaction of low frequency fields are very different from those of high frequency. At low frequencies, the electric and magnetic components of an electro-magnetic field are independent, which means that it isn't a true electro-magnetic field as it happens at much higher frequencies (Chang & Wait, 1974; Olsen & Wong, 1992). In practice, extremely low frequency electromagnetic fields (ELF-EMF) are considered as part of 0-300Hz frequency domain (Lambrozo, 2001).
While significant progress is made on several fronts, a special emphasis is increasingly directed toward understanding the most comprehensive details of the effects of electromagnetic fields of low and very low frequency. This led to some new applications in the clinical treatment of traumatized tissues by exposure to pulsed electromagnetic fields (PEMF) of extremely low frequency and therapeutic treatment of skeletal-muscle system, such as repair of bone fractures or soft tissue injuries (Bassett et al., 1974, 1977, 1981, 1982, 1989, 1994; Markov 2002; Rosch & Markov, 2004). However, these very low frequency fields interact very differently in relation to the tissues diversity and to the physiological processes occurring in living systems. Also, it must bear in mind the negative effect of the electromagnetic field impact on the living structures and cellular processes with highly sensitivity to this physical phenomenon. Therefore, the positive effects of some kind of radiation with a certain frequency and intensity that occurs strictly localized, is not a guarantee of a general positive biocompatibility, fact which requires a greater and larger study of biological effects. Given these issues, it was considered useful to investigate, in every way possible, the influence of a low frequency and intensity electromagnetic fields with the normal (healthy) organisms often facing in nature, as well as highlighting the potential biological effects of biomedical applications. Therefore, in the experiments was used a device that is used for medical purposes.

MATERIALS AND METHODS

A MAGNETODIAFLUX device that generates a pulse electromagnetic field (PEMF) was used for treatment. Irradiation is accomplished by means of two Helmholtz coils connected to the device. If using two Helmholtz coils, the ideal solution to have a uniform electromagnetic field is their arrangement at a distance equal to their radius (Crowell, 2010). MAGNETODIAFLUX device feeds the two coils with a pulsating direct current (PDC) obtained by converting and rectifying the 220V/50Hz AC power. The PDC has a frequency of 50Hz or 100Hz, the PDC peak voltage variation indicated on the oscilloscope is 42V with 0.7A at 50Hz, respectively 1.4A at 100 Hz. The coils are 29cm in diameter with approximately 630 turns. To obtain a uniform field, two coils were placed at a distance of 14.5 cm. Based on the known physical values it was calculated the magnetic flux density on the central axis. Resulting values are 2.7 mT for

50Hz and 5.5 mT for 100Hz PDC. The two values were also verified by a direct measurement of the magnetic flux density using a digital tesla-meter for safety and accuracy of the experiment. As experimental animals were used white Wistar rats divided into homogeneous groups as sex, age and weight. It was organized two experimental variants (Table 1). From this point of view were held two different types of PEMF treatments.

Table 1. Variants used for the treatment of experimental animals.

	Frequency of the PDC [Hz]	**Magnetic flux density [mT]**	**Application mode**	**Irradiation time [min./day]**	**Period of the experiment [days]**
Variant (VA1)	50 Hz	2,7 mT	Continuum	30 min/day	20 days
Variant (VA2)	50 Hz	2,7 mT	Intermittent	30 min/day	20 days

For each experimental variant were used 100 rats divided into 10 uniform lots in terms of age and weight. Before being placed in the experiment, rats have been handled for three weeks to adjust to human presence. Both before and during the experiment were guaranteed a constant temperature and a food with a complex composition (grasses, meat and poultry bones, carrots, flavored fat, minerals, vitamins, micro elements. 15%-crude protein, 6%-crude fat, 2%-crude fiber, vitamin A = 5500Ui/Kg, vitamin E = 50Ui/Kg, Vitamin B1 2mg/Kg). Maintaining the same type of food was essential to not induce changes in the animal metabolism, so any metabolic changes to be due to the influence factor (PEMF). The first batch consisting of 20 rats (10+10) was used as a reference control group, killed, on the first day of the experiment. The other remaining eight lots were four pairs, each a control/probe, that have been irradiated 30 min/day for a period of 20 days and were sacrificed at each 5 days interval during the experiment. Irradiation was performed by placing the animal in a plastic tube with many holes to not suffer from lack of air. In the plastic cylinder the animal movements were very limited, forcing the animal to stand in the uniformity zone of the electromagnetic field generated by the two coils. Both control and irradiated groups were placed in the tube and held 30 min. with the difference that the sample groups were irradiated, and the control was not. So it was tried to eliminate the potential differences caused by the tube stress immobilization from the control group of animals, the only factor that differentiated groups was the presence/absence of PEMF. Every five days, the animals were killed and the blood collected for analysis. In all cases it was intended to conduct experiments on bio-ethical compliance in laboratory animals.

To highlight the possible variations that can occur in changing concentrations of the blood ions (Na^+, K^+, Ca^{2+}), the blood was collected on anticoagulant and was centrifuged in order to separate the plasma (external environment) from the blood cells (internal environment). From the obtained plasma appropriate dilutions were made in order to be analyzed by flame photometer, and thus can be precisely determined the Na^+, K^+, Ca^{2+} concentrations. Also, the blood cells deposited by centrifugation, most of them been erythrocytes (98% of sediment) were hemolyzed in order to reveal the concentrations of the three ion track. To ensure the most accurate quantitative identification of the abovementioned ions in blood plasma and blood cells, the flame photometer has undergone a very rigorous calibration operation. Although, Na^+, K^+, Ca^{2+} blood concentrations are controlled by multiple pathways, analyzed them comparatively, those in the external compartment (blood plasma) with those of internal compartment (blood cells) during the 20 days of treatment and corroborate them with total Na^+,K^+-ATPase activity determined from centrifuged blood cells, may indirectly assess the possible influence of PEMF on the control mechanisms of these ions in general and in particularly their permeability.

RESULTS AND DISCUSSIONS

Dynamics of Na^+ ion values show an evident influence of treatment with the PEMF both in terms of its level in plasma and blood cells throughout the experiment.

In the first experimental variant (VA1), the exposure of animals to a continuously 50Hz PEMF applied 30min/day, values of plasma concentrations in the control group are between 2.9674 and 3.2546 mg/mL, with very weak variations throughout the experiment (Fig.1). Compared with these values of the control group, there is a significant change at treated group only after 5 days of treatment (2.1273 mg/mL) after which levels returned to normal by the end of the experiment. From this Na^+ plasma concentration dynamic is clear that the PEMF action occurs only after the first 5 days of treatment with a significant reduction in Na^+ blood plasma (Na^+_e),

this effect being, however transient, because during the later course of experiment, levels returned and maintained at the control group values.

Regarding the Na^+ blood cells concentration (Na^+_i), the normal values at the control group are between 0.1519 and 0.1604 mg/mL. Influence of treatment with this type of PEMF was also evident during the first five days (Fig.1), as in blood plasma, but the recorded values are significantly higher this time than the control group, unlike the effect found for plasma. In the following period, the effect was also transient, returning to normal values by the end of experiment, as in blood plasma.

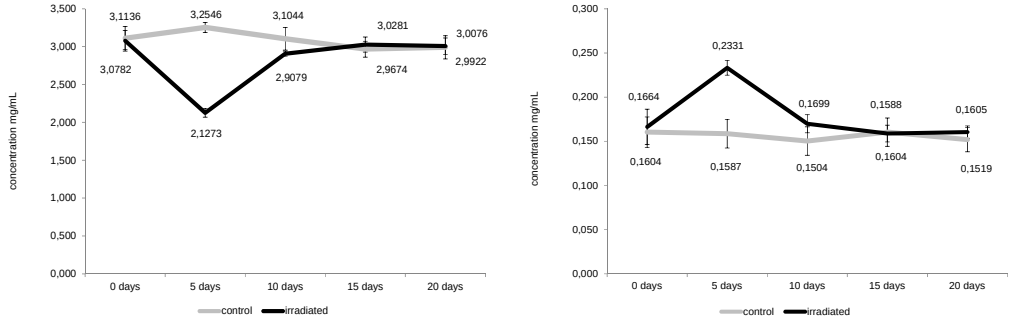

Fig. 1. The Na^+ concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied continuously for 30min/day from the beginning of the experiment.

In the second experimental variant (VA2), 50Hz PEMF applied intermittently for 30 min/day, a strong treatment effect is manifested, particularly in the cellular compartment (Fig.2). Thus the values recorded in plasma shows a significantly weaker effect of decreased Na^+, only after 5 days of treatment, in other phases of the experiment values lays in the level of normal (control). Na^+ blood cells concentration was recorded a significant increase in values throughout the experiment compared with control, with the effect gradually increased towards the end.

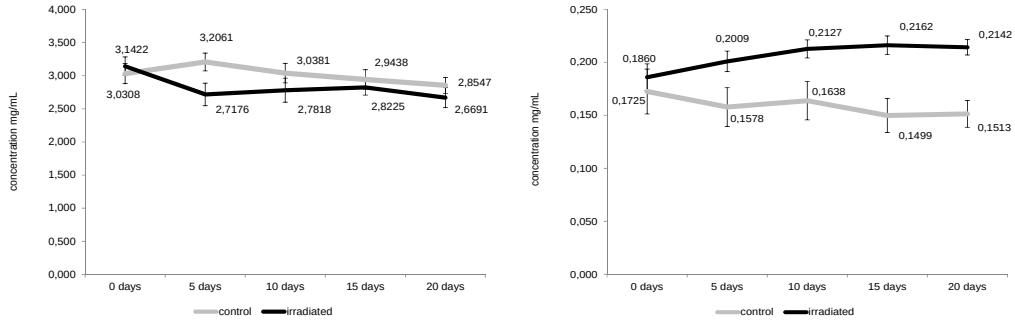

Fig. 2. The Na^+ concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied intermittently for 30min/day from the beginning of the experiment

Potassium is one of the most important intracellular ions existing in cell environment, where, unlike the sodium, its concentration is very high. From the records it appears that the influence of treatment with the PEMF is obvious, as with Na^+, leads to a series of changes in the concentration both in plasma (K^+_e) and blood cells (K^+_i).

For the first experimental versions (VA1) it was observed a significant decrease at 5 days of treatment, followed by a recovery during the next 10 days, but recorded a significant fluctuation

to the value found in control towards the end of 20 days experiment (Fig.3). Values recorded in plasma concentrations fall in the range of values considered physiologically normal, the lowest being 0.1083 mg/mL and the highest 0.1979 mg/mL. In blood cells, the concentration of K^+ undergoes a major increase after 5 days of treatment, followed by a downward trend in the next 10 days, the values, however, remain significantly higher compared with the control, the value of the concentrations reestablishing only at the end the 20 days of experiment.

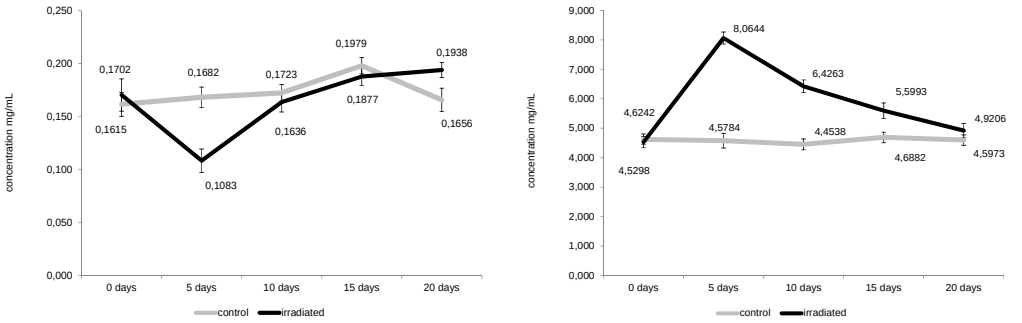

Fig. 3. The K^+ concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied continuously for 30min/day from the beginning of the experiment

In VA2 experimental variant, the PEMF type used, causing a significant change in the concentration of K^+ to the control, only in blood cells. And in this case is found concentration increases, but they appeared after 10 days of treatment with a significant maximum at 15 days, while plasma concentration remains unchanged throughout the period of the experiment (Fig.4).

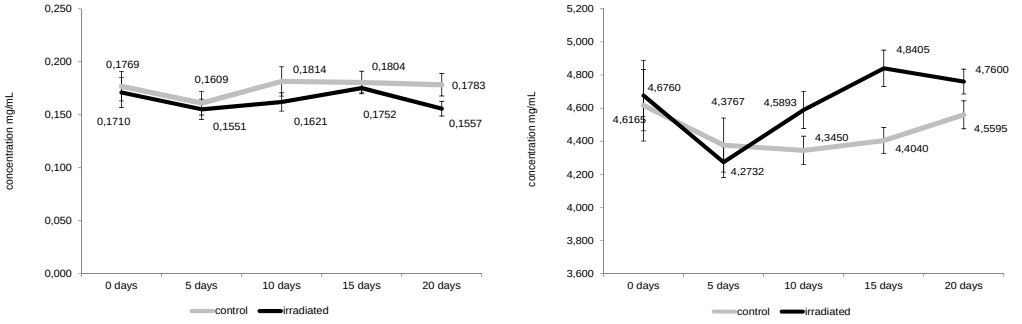

Fig. 4. The K^+ concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied intermittently for 30min/day from the beginning of the experiment

Calcium is the third major cation in cell physiology in particular, and in the body in general, especially due to his involvement in cellular signaling mechanisms. Unlike Na^+ and K^+, Ca^{2+} is an element whose physiological control mechanisms are more complex. For this reason, the ion concentration changes in both the extracellular fluid (Ca^{2+}_e) and blood cells (Ca^{2+}_i) may alter the cells directly, but mostly indirectly on many parameters that can influence the overall reactivity of the organism.

The first experimental variant (VA1) is characterized by a variation of concentration, similar in terms of dynamics, with the Na^+, both in terms of plasma and blood cells (Fig.5). Thus there is a significant decrease in Ca^{2+} concentration in parallel with a significant increase at 5 days after the beginning of the experiment, followed by a rapid return within 5 days and is keeping

unchanged until the end of experiment compared with the concentrations recorded in the control group. This confirms once again that 50Hz/2.7mT PEMF applied 30min/day continuously show their influence very quickly, causing significant changes to 5 days after onset of the experiment, then its influence is, apparently, effectively countered by the mechanisms involved in controlling the concentration of these ion.

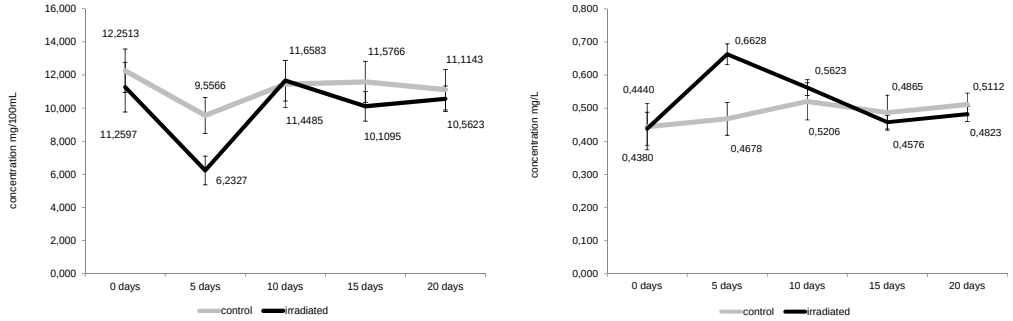

Fig. 5. The Ca^{2+} concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied continuously for 30min/day from the beginning of the experiment

VA2 occur in Ca^{2+}_e and Ca^{2+}_i, as in the past, a pattern different from that encountered in the evolution of Na^+ and K^+ concentrations (Fig.6). In plasma concentrations, there was a slight decrease which achieve a significantly threshold values only at the end of the experiment. In Ca^{2+}_i, there is a fluctuation from the fifth day where shows a slight increase, followed by five days of a return to normal levels (compared with control values), and at 15 days to show another increase, which is maintained larger to the end of the experiment.

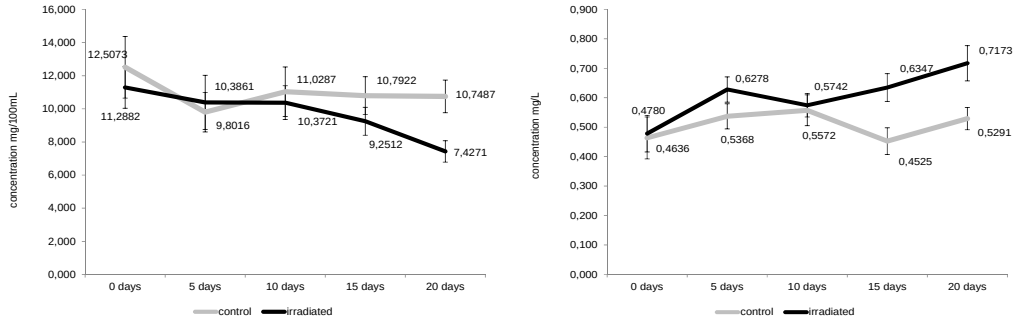

Fig. 6. The Ca^{2+} concentrations, determined in blood plasma (left) and blood cells (right) from rats treated with 50 Hz, 2.7 mT PEMF, applied intermittently for 30min/day from the beginning of the experiment

In all living cells, ATP is a readily available and renewable energy source. Coupling between biosynthesis and hydrolysis of ATP is crucial for living cells. Release of energy from ATP is achieved by ATPases activity which is accompanied by a flow of ions across membranes. These enzymes are called ionophore and ATPases blood cell membrane are a P-type ATPase category providing ion exchange of Na^+ and K^+, one side of the membrane (Cojocaru et al. 2007).

In the first experimental variant (VA1) it was determined a fluctuation during the 20 days of experiment. After 5 days of treatment is was a slight but significant decrease compared to the control, following by a constant increase over the next 10 days and a backing to the normal values (compared with control) at the end of the experiment. 50Hz/2.7 mT PEMF applied

continuously for 30 min/day, acting on the enzymatic activity through its inhibition, especially after five days, where it was observed a distinct change in ions concentration. Increased enzymatic activity at 15 days of treatment in conjunction with the restoration of Na^+_e, Na^+_i, K^+_e, K^+_i, Ca^{2+}_e, Ca^{2+}_i could be interpreted as a compensation of the ratio ionic changes occurring within five days. In the VA2 variant the PEMF was applied intermittently. There are significant decreases from 10 and 15 days after the applicable treatment and emphasizing to the end of the experiment (Fig.7).

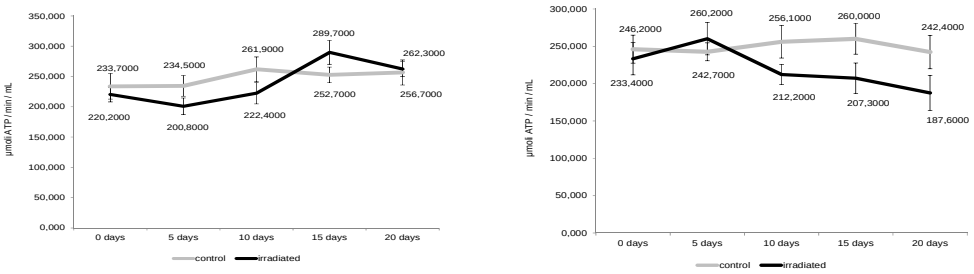

Fig.7. Dynamics of Na^+,K^+-ATPase activity in blood cells in rats treated with 50Hz, 2.7 mT PEMF, applied continuously (right) and intermittently (left) for 30min/day.

The recorded data on the dynamics of plasma and intracellular values reveals a number of specific issues depending on ion type and the PEMF nature applied for the treatment of experimental animals. It is noted, where some aspects characteristic for each ion, both in terms of dynamics in a cellular compartment throughout the experiment, as well as the relationships between extracellular and intracellular values.

Regarding the Na^+ there is an evident influence of all treatment alternatives, variations occurring extracellular correlated statistically with the intracellular concentration. Another important aspect is that was found in all experimental variants, after 5 days of treatment was recorded the largest variation in the concentration of Na^+ compared with control. This effect, however, was transient and it wasn't obvious in the following stages of treatment. Another important aspect is clear from the values of ionic ratios (extra/intracellular) that showing the distribution of one side of the blood cells membrane. It is thus generally a reduction in the values of this ratio compared with control (stronger at 5 days of treatment). Given that Na^+ has a much higher concentration in the extracellular than the intracellular compartment, these results reflect a possible increase in passive membrane permeability to this ion under the action of PEMF.

For K^+ there is a value dynamics broadly similar to that recorded at Na^+. Also, in this case, the largest variation recorded was after 5 days of treatment, particularly the intracellular concentration compared with other stages of treatment. Since K^+ is distributed normally, mostly in the intracellular compartment, from the data obtained on the ion concentrations and in particular extra and intracellular concentration ratio values, which generally have lower values than those of control, it could estimate that PEMF treatment was induced some enhancement of the active transmembrane ion transport. In fact, this is statistically correlated with data on the PEMF effect on the Na^+,K^+-ATPase activity.

PEMF influence is manifesting more diverse on the Ca^{2+} dynamics compared to Na^+ and K^+. Thus there is a statistical correlation of extra- and intracellular values of the experimental variants it was observed in general a slight increase in the intracellular concentration values correlated statistically with a certain reduction of the plasma values. Given the complexity of

the Ca^{2+} ion distribution and its diverse role at cellular level, there may be difficult to assess the transmembrane flow and the cellular forms (ionic or bonded) under the action of this PEMF. Moreover, similar results were obtained in other papers (Maniu at al., 2004; Neacşu & Maniu, 2003, 2005; Neacşu et al., 2005, 2008). However, it can see a general trend of increasing intracellular Ca^{2+} concentration. It is important to follow PEMF effect on the dynamics of Ca^{2+} values because this ion is involved in cell signaling, PEMF action having more complex influences on the cellular level. Furthermore, there are theoretical models that support this experimental evidence (Eichwalde & Kaiser, 1993, 1995; Eichwalde et al., 1994; Grundler et al., 1992; Monteith & Roufogalis, 1995; Walleczek, 1992, 1994, 1995).

CONCLUSIONS

In general, the treatment with 50Hz/2.7 mT PEMF applied continuously and intermittently determined statistically correlated variations for intra- and extracellular concentrations of those three ions. From the dynamics of the three ion concentration values it can be appreciated, however, that the PEMF action occurs on cell membranes ion channels activity and probably on the degree of ions hydration and their mobility, as seen in other works (Blank at al., 1995; Blank, 1995, 2005, Loginov et al., 1992).
PEMF treatment effects on Na^{+}, K^{+} and Ca^{2+} concentration is also reflected in the total (add up) values of three ions in the plasma and blood cells, which involves a ratio change in the extracellular and intracellular amounts under the action of treatment. The amount of the three analyze ions is their contribution to intra or extracellular osmotic pressure. The data obtained shows that, generally, all PEMF variants treatment causes a reduction in values of this ratio, which reflects a decrease in osmotic pressure contribution of the amount of the three ions in the blood cells, compared with the situation in plasma. One such issue can be correlated with the proportion of intracellular water compared with the extracellular water as well as water cell membranes permeability under the action of PEMF. It can be considered as a PEMF influence on cellular electrolyte balance.

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