Influence of the Preparation "Nicavet-1000" on a Morphofunctional Condition of some Organs of Rats at Experimental Aluminium Intoxication

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Abstract: During the conducted research, it is established that aluminium chloride intoxication leads to the considerable changes of composition of red blood, development of a hypercalcemia and change of a bone structure of animals. A number of significant morphofunctional changes in the hippocampus of the examined animals are also noted. The use of the tissue preparation "Nicavet-1000" leads to the normalization of both hematocrit and other parameters of red blood, as well as to the intensification of erythropoiesis. Use of the preparation "Nicavet-1000", judging from the results of research, prevents pathological changes in a bone tissue, caused by aluminium chloride. "Nicavet-1000" leads to the expressed normalization of both the morphological, and micromorphometric parameters characterizing a hippocampus of rats.

Keywords: Aluminium, intoxication, hippocampus, bone tissue, tissue preparation.

INTRODUCTION

Aluminium is the most widespread metal in a lithosphere and its extending use demands studying of its influence on an organism of mammals. There is an opinion that aluminium belongs to the category of eco-toxicants influencing, in particular, at human in living conditions as it arrives in a human body with food, (much of it contains in soy milk and tea, salts of aluminium are present at drinking water), and it arrives additionally from packing, ware, nutritional supplements and drugs. Preparation, storage and the use of foodstuff are also often integrated to use of aluminium wares.

Opinions on influence of aluminium on an organism of mammals are ambiguous. While there are as yet, no unequivocal answers to this problem, there are procedures to follow to ascertain the nature of human exposure to aluminium. It is also important to recognise critical factors in exposure regimes and specifically that not all forms of aluminium are toxicologically equivalent and not all routes of exposure are equivalent in their delivery of aluminium to target sites [1].

From the chemical point of view aluminium is the very active metal showing amphoteric properties that causes its high reactivity. Almost all salts of aluminium (except phosphate) are highly soluble in water that allows it to migrate easily in aqueous solutions of various nature [2].

Getting to an organism with water and food, aluminium is absorbed in a GIT, and besides aluminium ions bound to organic compounds are absorbed much easier [3]. The greatest quantities of the free ions of this metal at influence of low doses are absorbed in a large intestine, and similar doses of aluminium in the form of citrate are absorbed in initial department of small intestine [4]. It is established that the aluminium absorption in intestines in the form of citrate goes 50 times more effectively, than when using hydroxide or chloride of this metal [5].

About 40-50% of the microelement remain in an organism after absorption. If to speak about distribution of aluminium in blood and its binding with proteins, then it should be noted that 80% of this metal are localized in a blood plasma and are bound to proteinaceous macromolecules while the other 20% are not localized, being in a type of the colloid complexes [6-7].

Aluminium excretion at healthy people generally happens through intestines (89-94%), and also through kidneys (6-16%) [6]. Excess intake of aluminium in a

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human body leads to substitution of calcium and iron with it in bones, liver, brain and parathyroid, and to developing of osteomalacia. Aluminium enters in competitive interaction with these metals, binding with a transferrin and 1,25-dihydroxycholecalciferol, and it was shown that aluminium forms a complex with citrate which serves as a potential inhibitor of bone mineralization and of growth of crystals of calcium phosphate *in vitro*. Besides, the ATP level decreases. Also the maintenance of erythrocytes in blood decreases as there is a hemoglobin synthesis suppression [7-11]. The main organs on which aluminium makes the impact in a human body are nervous tissue, blood, bone system, kidneys, lungs, a reproductive system, marrow and mammary glands [12].

Excess intake of ions of aluminium to an organism leads to its accumulation in brain tissues where under their influence violations in the oxidation-reduction balance in nervous cells develop that provokes development of an oxidizing stress [10,13]. In view of a hypothesis that oxidizing stress is the process starting mechanisms of development of the pathologies observed at Alzheimer's disease the aluminium role in an etiology of this disease does not leave doubts as presence at cells of a brain of excess of such reactive metals as Al, Fe and Hg stimulates formation of the free radicals [14]. In tissues and cells where there is an aluminium accumulation, ratios of Al/Cu and Al/Zn change that can be also associated to development of an oxidizing stress [15]. It was shown that an aging raises a susceptibility of nervous tissues to aluminium as mechanisms of excretion of aluminium from cells of a brain weaken with age [16].

The aluminium role in a course of a disease of Alzheimer is confirmed also by the fact that at patients treated with chelating agents to excretion of aluminium from an organism the disease proceeded less intensively [17]. Aluminium makes impact on structure of neurones of a hippocampus, causing a degeneration of cholinergic fibers, and the area CA1 of a hippocampus is the most vulnerable to injuries, as well as at AD [18,19]; and also aluminium promotes development of inflammatory processes in a cerebral cortex and a hippocampus [20].

Accumulation of aluminium in a brain leads to deterioration a hippocampus-dependent memory that is accompanied by accumulation of a β-amyloid in brain [21], and accumulation of tau protein and an apoptosis of neurons [22]. In addition, deposition of aluminium in bones leads to substitution of calcium with it [23], to inhibition of their mineralization [24] and to development of a hypercalcemia that at the moment is also associated with development of neurodegenerative diseases [25]. Thus, it is possible to assume synergetic and cumulative participation of the pathological processes caused by aluminium (an oxidative stress of neurones, violation of a homeostasis of calcium, glutamate toxicity) in development of the above mentioned violations and pathologies [26]. Also the aggravating influence of aluminium toxicity on a neurogenesis in a hippocampus at type II diabetes was shown [27].

Proceeding from it, we considered actual to make an aluminium chloride influence research on a morphofunctional condition of an organism of mammals and a possibility of correction of the arising deviations with use of the tissue preparation "Nicavet-1000".

MATERIALS AND METODS

Animals

Male Wistar Albino rats of body weights ranging from 200 to 220 g were used in the study. Age of the animals was 6 months old. The animals were fed with standard pellet diet and water *ad libitum*. They were maintained in controlled environment (12:12 h light/dark cycle) and temperature (23±2ºC). All the animal experiments were performed according to the compliance with the EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Treatment Design

The research is conducted on 60 male Wistar Albino rats divided into 3 equal groups:

I group: intact animals (n=20);

II group (control): animals with a model of experimental aluminium intoxication (n=20);

III group (experimental): animals with model of the experimental aluminium intoxication, received hypodermic injections of "Nicavet-1000" for the 80 and 85 day of the research in a dosage of 4,5 mg/kg.bw.

For 91 days of the research animals were sacrificed in carbon dioxide chamber, at then the whole blood and the blood serum, a femur (beforehand cleared of the soft tissues) and the brain were taken.

Model of Aluminium Intoxication

Aluminium intoxication model was achieved in rats by the oral administration of $AlCl_3$ in a dose of aluminium 100 mg/kg Bw daily for 90 days.

Preparation of "Nicavet-1000"

For preparation of acid hydrolysate **(AH)** 500 ml of distilled water brought in 20 g of protein-bearing powder, mixed and put in a shaker-thermostat ES 20/60 (Biosan, Latvia) for exposure at 50ºC and shaking at 100 rpm within 30 min. Then added 33% of HCl to its concentration to solution of 0,5% and exposured mix in a shaker thermostat at the similar mode within 60 min. Then the produced mass was autoclaved at 125ºC within 60 min. in a steam sterilizer SPVA-75-1-NN (Trans-Signal, Russia). The produced hydrolysate was neutralized by 1 M of NaOH to pH 6.8-7.2 (pH meter S400-B (Mettler Toledo, Spain) and centrifuged (the centrifuge with cooling SL40R (Thermo fisher scientific, USA)) at 4800 g within 120 minutes at a temperature 2-4ºC. The liquid received after a centrifugation was filtered sequentially by means of filtrational system Vivaflow 50 (Sartorius, France) with application of a polyethersulfone membrane of 0.2 microns and 30 kDa, 10 kDa MWCO for removal of not hydrolyzed proteins.

Characteristics of substance are presented in Table **1**.

Definition of Electrolyte Composition of a Blood Plasma

The electrolyte composition of the blood plasma and its pH were determined using an electrolyte analyzer E-Lyte-5 (Netherlands). Concentrations of K^+, Na^+, Cl^-, iCa^{2+}, nCa^{2+} and TCa^{2+} in the blood plasma were defined.

Hematological Analysis

Hematological analysis was performed using the hematological analyzer Abacus Junior Vet (Diatron, Austria). The examined parameters included red blood cells (RBC), hematocrit (HCT), hemoglobin, white blood cells (WBC), lymphocytes, neutrophils, monocytes, eosinophils and basophiles.

Histological Studies

Brain tissues previously fixed in formalin buffer (10%) for 24 h were washed under tap water for 20 min. Then, the serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4-mm-thick using sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stains for histological examination through the light microscope.

The sections were successively rehydrated with 100% alcohol, 95% alcohol, and distilled water. Subsequently, the sections were stained in 0.1%

Table1: Characteristics of Preparation "Nicavet-1000"

Main indexes of quality	"Nicavet-1000"	Main indexes of quality	"Nicavet-1000"
Density (20ºC), g/cm^3	1.005-1.006	Amount of protein (polypeptides) by Coomassie, mg%	35
Optical density, X=460 (color intensity)	0.365	Total amount (amino acids and peptides), mg% (g/l)	1185 (11.85)
Amount of nonvolatile solid, g/l	23	Amount of carbohydrates (recalculation on glucose), %	0.23
Concentration of Sodium chloridum, %	0.84	Salicylsulphonic acid reaction for protein	positive
pH	6.97	Peptides molecular mass, kDa	<4.5
Total nitrogen, %	0.2	Antioxidatic indexes: - inhibition percent, % - antioxidatic activity, mmol/l	 88.3 1.55
Amine nitrogen, mg%	75.9	Total characteristic on expenses of raw materials, reactants and time	1+
Amount of amino acids, mg% (g/l)	475 (4.75)	Characteristic of technological effectiveness of process	There are no problems when receiving, from 1 l of water and 40 g of sublimatum 700 ml of a hydrolysate turn out
Tryptophan, mg%	36	Coefficient of the relation of amino acids to peptides	1:1.5
Number of peptide-containing compounds, mg% (g/l)	710 (7.1)		

Cresyl violet (Sigma-Aldrich) solution. The sections were then differentiated in 95% ethyl alcohol, dehydrated in 100% alcohol, and rinsed in xylene. Finally, the sections were mounted and observed under a light microscope Nikon ECLIPSE 80i (Japan). The average quantity of neurons was calculated by randomly selecting five Nissl-stained sections at the same site from each rat.

We determined the relative number of neurons in multiple fields of view on the total area of the pyramidal layer of CA1 and CA3 regions (further recalculated per 10.000 μm), areas of neuron's bodies, areas of nuclei, nuclear-cytoplasmic ratio (NCR). The average quantity of neurons was calculated by randomly selecting five Nissl-stained sections at the same site from each rat. The distribution of individual neuronal sizes for each group is presented in histograms.

All measurements were taken with use of image analyzer "AxioVision" (ZEISS, Germany).

Study of Structure of a Bone Tissue

For a study of structure of a bone tissue at white rats the X-ray Skyscan 1176 (Bruker) microtomograph with the main software was used.

Statistical Methods

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software v.5 (GraphPad Software, Inc., La Jolla, CA, USA). The intergroup variation was measured by one-way analysis of variance (ANOVA) followed by Dunnett's posttest. A p value of <0.05 was considered statistically significant.

RESULTS

Influence of the Preparation "Nicavet-1000" on some Hematological Parameters

It is established treating with aluminium chloride within three months leads to change of some hematological parameters of rats. So, in blood of these animals we noted decrease in a hematocrit (HCT) to 38.14±2.57% against 45.60±4.26 at intact animals. Also the quantity of erythrocytes (red blood cell count, RBC) decreases with $9.38\pm0.93\times10^{12}$ to $7.88\pm0.42\times10^{12}$ in monitoring, also the hemoglobin content (HGB) similarly changes, decreasing from 154.00±5.96 g/l to 140.90±5.11 g/l. It occurs against the background of increase in value of average content of a hemoglobin in an erythrocyte (MCH) of 18.01±0.57 pg and average concentration of a hemoglobin in an erythrocyte (MCHC) of 369.50±9.32 g/l concerning parameters of intact rats – 16.55±0.47 pg and 340.60±13.42 g/l respectively (Figure **1**).

At the same time, under influence of "Nicavet-1000" decrease in HCT to 46.88±1.26%, RBC to 8.51±1.13 $\times10^{12}$, HGB to 144.80±10.72 g/l is noted. MCH in this group practically does not differ from parameters of intact animals and makes 17.09±0.92 pg, the same is fair also concerning MCHC making 324.25±17.80 g/l.

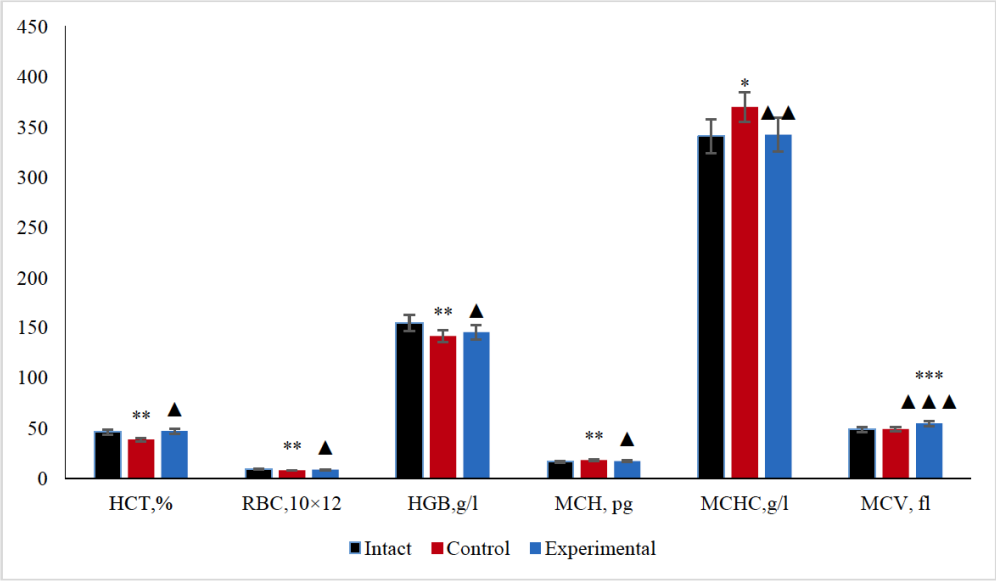

Figure 1: Some hematological parameters of rats at aluminum intoxication and its correction with the preparation "Nicavet-1000". Hereinafter: Reliability of differences from parameters of intact animals: * ≤ 0.05, ** ≤0.005, *** ≤0.0005. Reliability of differences of parameters of the experimental group from control: ▲ ≤0.05, ▲▲ ≤0.005, ▲▲▲ ≤0.0005.

Table 2: Content of Electrolytes in Plasma of Rats

Group	K^+, mmol/L	Na^+, mmol/L	Cl^-, mmol/L	iCa^{2+}, mmol/L	nCa^{2+}, mmol/L	TCa^{2+}, mmol/L
Intact (n=20)	5.37+0.43	144.83±1.89	108.14±1.58	1.23±0.04	1.32±0.01	2.51±0.49
Control (n=20)	5.91±0.38 ***	147.12±2.91 ***	109.84±2.91	1.27±0.04 *	1.33±0.03	2.69±0.06 ***
Experi-mental (n=20)	5.50±0.40 ▲▲	142.92±1.56 * ▲▲▲	106.88±2.14	1.22±0.03 ▲▲	1.31±0.03 ▲▲▲	2.54±0.04 *** ▲▲▲

Besides, under the influence of "Nicavet-1000" the value of average volume of erythrocytes (MCV) significantly increases, making 54.40±3.20 fl against 48.67±2.09 fl at intact animals and 48.75±2.86 fl at control group.

Influence of the Preparation "Nicavet-1000" on Electrolytic Composition of Rats Blood

In the analysis of electrolytic composition of blood of rats it is established that under the influence of $AlCl_3$ in comparison with control group there is an increase in contents of K, Na, ionized Ca (iCa) and total Ca (TCa) in a blood plasma.

Use of the preparation "Nicavet-1000" reduces levels of K, iCa and TCa to the level of intact animals, and the content of ions of Na appears below, than at intact rats (Table **2**).

Influence of the Preparation "Nicavet-1000" on a Morphofunctional Condition of a Hippocampus of Rats

At a microscopic research at intact rats the CA1 and CA3 areas are clearly differentiated as topographically, and on specific structure of neuronic layers and correspond to normal cytoarchitectonics of a hippocampus. The CA1 area is formed by densely located medium neurons for which the homogeneous light spherical centraly located nucleus with basophilic nucleolus is typical. The CA3 area consists of widely spaced large nervous cells in which the light nucleus with an individual large nucleolus is noted (Figures **2**, **3**). Herewith, at intact animals the number of cells in CA1 averages 52.81±5.20 on 10000 μm^2, and in CA3 – 18.21±3.12 per same area.

At animals of control group both qualitative and quantitative changes of neurons of the CA1 and CA3 areas are noted. Morphologically they are shown in disorganization of neuronic layers, the chromatolysis phenomenon, swelling and pycnomorphic changes of nuclei, deformations of apical dendrites of pyramidal neurons in the CA3 area, emergence of binucleate cells (Figures **4**, **5**). The specific quantity of cells in the area CA1 decreases to 34.58±3.68, in the CA3 area – to 13.82±2.80 cells on 10000 μm^2 (Tables **3, 4**).

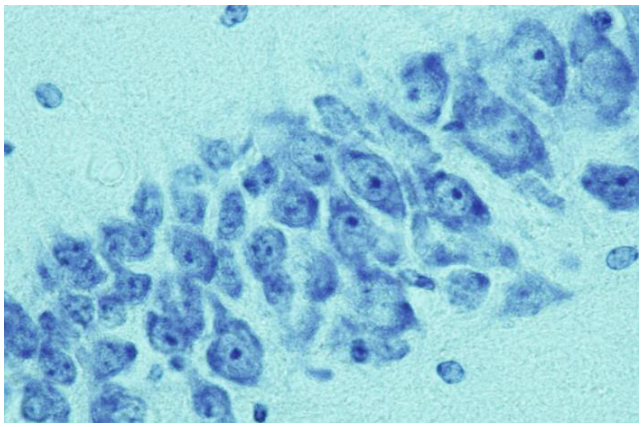

Figure 2: Hippocampus of an intact rat. CA1 area. ×1000, Nissl staining.

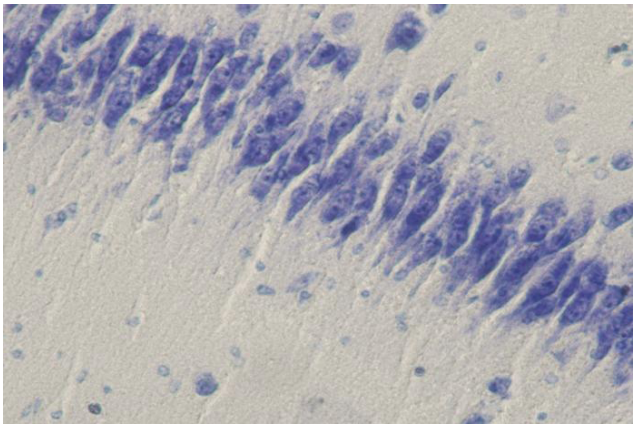

Figure 3: Hippocampus of an intact rat. CA3 area. ×400, Nissl staining.

The morphological picture of the explored areas of a hippocampus of rats of the experimental group shows that changes in neurons of the CA1 and CA3 areas of a hippocampus have considerably less expressed character, than in similar structures of a brain of animals of control group. In particular, we noted disorganization of neuronic layers of organ, and the

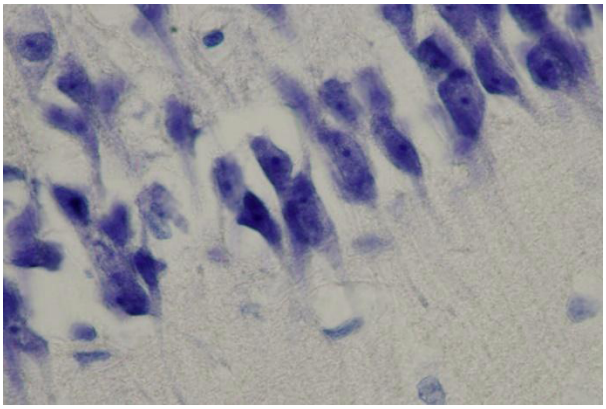

Figure 4: Hippocampus of rat of control group. CA1 area. ×1000, Nissl staining.

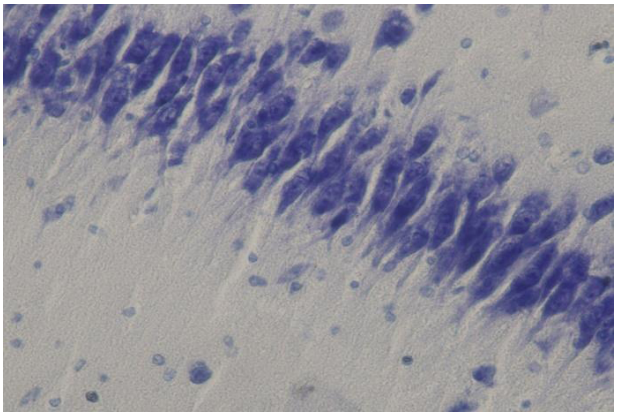

Figure 5: Hippocampus of rat of control group. CA3 area. ×400, Nissl staining.

changed neurons meet simplely, without forming clusters, as in control group. Besides, decrease of specific quantity of cells has doubtful character, making 48.84±6.51 in the CA1 and 15.05±3.26 in area CA3 (Figures **6**, **7**).

It is also established that in a hippocampus of animals of control group both in the CA1 area, and in CA3 there is the significant increase in a fraction of chromatolyzed and hyperchromatic neurons, and also the wrinkled cells at essential decrease in a fraction of unaltered neurons of a hippocampus. At the same time in a hippocampus of the experimental animals the same parameters do not differ from parameters of intact control.

By results of micromorphometric researches it is established that in the area CA1 of control animals there is an essential decrease of the area of a perikaryon with the invariable area of a nucleus. Respectively, there is an increase in nucleo-cytoplasmic ratio (NCR). In the CA3 area both decrease in sizes of a nucleus and perikaryon, and in nucleo-cytoplasmic ratio is noted.

At the same time, similar parameters in group of the experimental animals do not differ from parameters of intact rats.

In the analysis of the frequency distribution of the area of nuclei of neurons of the CA1 area it is established that at intact animals the maximum quantity of nuclei (35%) accounts for the range of 20-25 μm^2. At the same time in control group this category is presented by 20% of nuclei, and the number of nuclei with sizes in range of 15-20 μm^2 increases, and the

Table 3: Characteristic of Population of Neurons of a Hippocampus of Rats

Area CA1						
Group	**Unaltered neurons, %**	**Chromato-lyzed neurons, %**	**Hyperchro-matic neurons, %**	**Ghost nerons,%**	**Wrinkled cells, %**	**Apoptotic cells, %**
Intact (n=20)	74.0±2.66	7.06±1.12	10.81±1.41	4.69±0.97	2.38±0.91	1.03±0.49
Control (n=20)	41.79±3.60 **	19.21±3.76 ***	28.44±3.39 ***	4.89±1.05	4.50±0.60 **	1.15±0.44
Experi-mental (n=20)	74.29±2.89▲▲	6.09±0.89▲▲	11.06±0.89▲▲	3.46±0.89	2.87±0.47▲▲	2.23±1.22
Area CA3						
Group	Unaltered neurons, %	Chromato-lyzed neurons, %	Hyperchro-matic neurons, %	Ghost nerons,%	Wrinkled cells, %	Apoptotic cells, %
Intact (n=20)	76.82±2.66	7.14±1.10	9.50±1.44	4.32±0.88	2.0±0.0.80	1.13±0.45
Control (n=20)	43.98±5.90 **	17.88±3.76 ***	28.44±3.39 ***	4.0±0.73	4.50±0.60 **	1.20±0.40
Experi-mental (n=20)	74.80±4.66 ▲▲	6.9±1.0 ▲▲	10.50±0.90 ▲▲	3.88±0.52	2.44±0.40 ▲▲	1.48±0.35

Table 4: Morphological Parameters of Neurons of a Hippocampus of Rats

Group	Morphometric parameters of neurons in area					
	CA1			CA3		
	S of nucleus, μm²	S of perikaryon, μm²	NCR	S of nucleus, μm²	S of perikaryon, μm²	NCR
Intact (n=20)	45.84±2.70	143.29±3.29	0.32±0.10	68.22±3.58	204.88±6.55	0.33±0.09
Control (n=20)	48.58±3.81	131.22±6.81 **	0.35±0.15 *	54.88±2.52 ***	182.80±5.92 ***	0.30±0.10 *
Experi-mental (n=20)	46.44±4.00	145.82±4.85 ▲▲	0.32±0.12	64.57±4.66 ▲▲	201.99±6.88	0.32±0.08 ▲

maximum quantity of nuclei has sizes in the range of 30-35 μm². The distribution curve of the sizes of nuclei of neurons of rats of the experimental group practically coincides with that at intact group in a form, but the small peak in the range of 15-20 μm² appears (Figure **8**).

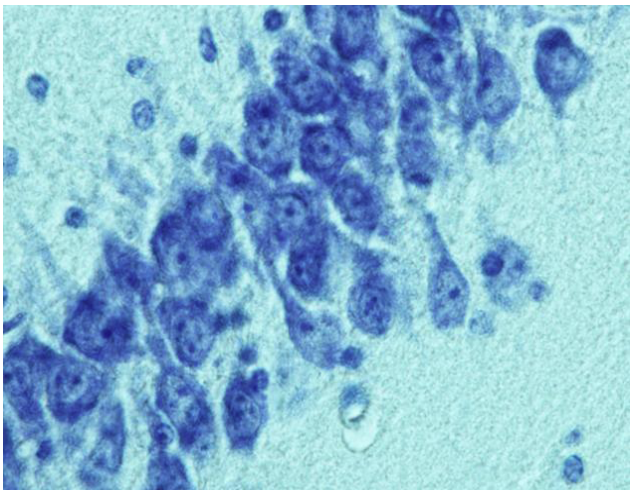

Figure 6: Hippocampus of rat of experimental group. CA1 area. ×1000. Nissl staining.

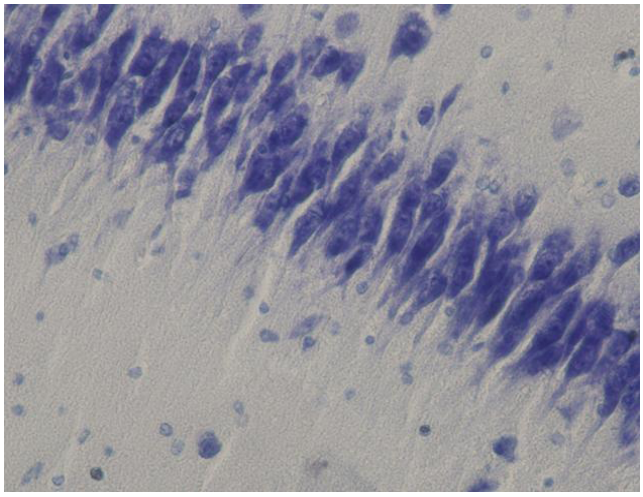

Figure 7: Hippocampus of rat of experimental group. CA3 area. ×400, Nissl staining.

In the CA3 area of a hippocampus of intact rats the maximum quantity of nuclei of pyramidal neurons measures in the range of 25-30 μm² (22%), in a hippocampus of control animals there is a shift of a curve to the left, and in the experimental group the frequency distribution of the sizes of nuclei of neurons differs from the control group insignificantly (Figure **9**).

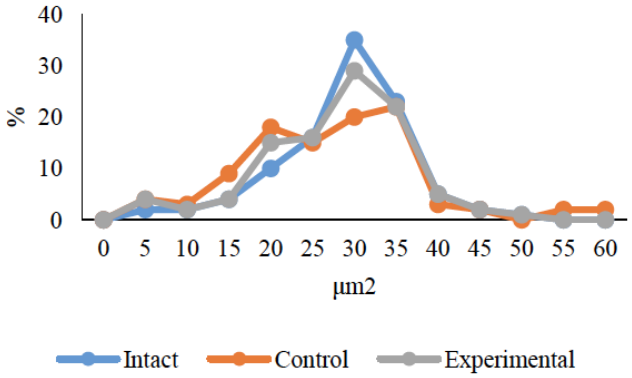

Figure 8: The frequency distribution of the sizes of nuclei in the areas CA1 of a hippocampus of white rats.

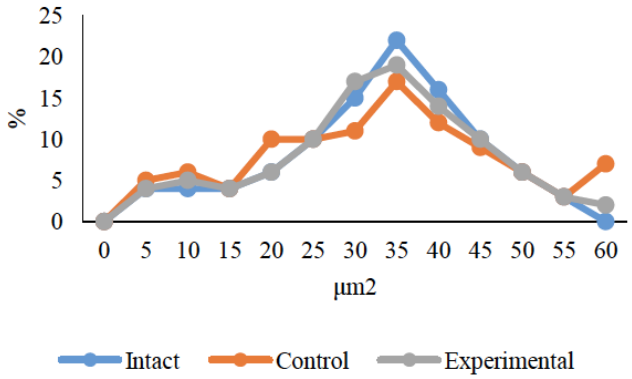

Figure 9: The frequency distribution of the sizes of nuclei in the areas CA3 of a hippocampus of white rats.

When analyzing the size of the soma of neurons, it was established that in the CA1 area the maximum number of neurons has dimensions in the range of 110-120 μm² (37%), in the control the curve assumes a

flattened appearance with the peak retained in the range of 110-120 μm^2 with a certain increase in the number of cells with dimensions, lying on the right side of the curve, and the curve of distribution of cell sizes in the hippocampus of experimental animals does not differ from control (Figure **10**).

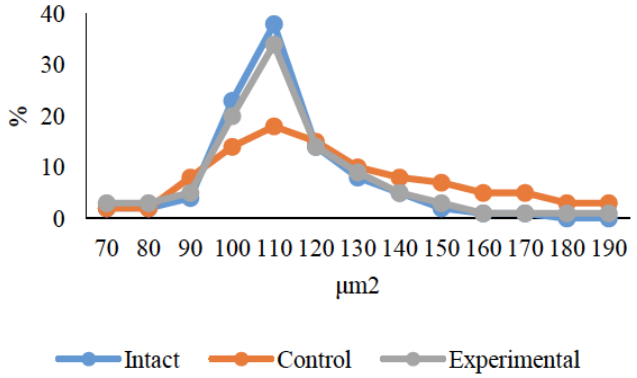

Figure 10: The frequency distribution of the sizes of cells in the areas CA1 of a hippocampus of white rats.

In the CA3 area at intact rats we allocated 2 peaks – 140-150 μm^2 and 170-180 μm^2 (19% and 20% respectively). In control group at flattening of the curve the second peak (170-180 μm^2) remains, and the first peak lies in the range of 120-130 μm^2. In group of the experimental rats the first peak is a little flattened, but in general the curve repeats distribution of the sizes of a soma of neurons in control group (Figure **11**).

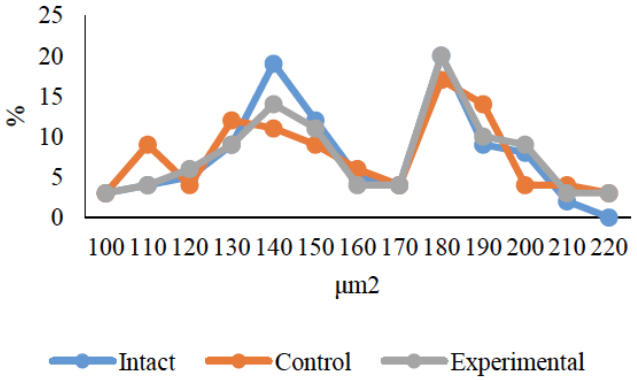

Figure 11: The frequency distribution of the sizes of cells in the areas CA3 of a hippocampus of white rats.

Influence of Preparation "Nicavet-1000" on Structure of a Bone Tissue

In control group of animals, we noted different deformations of bones.

In the analysis of a condition of a bone tissue of rats it is revealed that both in control, and in the experimental group cortical mineral density (Cortical BMD) of the femur does not change. So, at intact animals this parameter makes 16.88±2.29 g/cm^3, in control groups it is equal to 17.38±1.34 and 17.34±1.33 g/cm^3.

Trabecular mineral density up to the growth zone of a bone at intact animals made 1.76±0.13 g/cm^3, in control group it decreases to 1.24±0.22 g/cm^3, and at the experimental animals – 1.59±0.12 g/cm^3.

More expressed changes are observed in value of trabecular BMD beyond of the growth zone. At intact animals, the BMD value makes 7.35±0.56 g/cm^3, at animals of control group it increases up to 8.83±0.67 g/cm^3, and in the experimental group decreases to 6.88±0.52 g/cm^3.

DISCUSSION AND CONCLUSION

The conducted research demonstrates that use of aluminium chloride within three months leads to a number of the considerable changes of composition of blood. Apparently, the main target is the system of red blood. Due to decrease in RBC and HGB there is both a decrease of a hematocrit, and compensatory increase of MCH and MCHC.

Significant increase in reticulocyte content is a compensatory reaction to hemolytic action of aluminium, but it does not compensate the erythrocyte destruction. In the opinion of Zaman *et al*. this is evidence of disturbances of genesis and development of red blood cells induced by aluminium. This hypothesis have been confirmed by investigations of bone marrow, which reveal a significant decrease in the contents of proerythroblasts and all types of erythroblasts. Disturbance of development of erythrocytes is associated with formation of a very stable complex by aluminium and ATP. The absence of sufficient ATP leads to violations in transformation of reticulocytes into mature erythrocytes, eliminating certain proteins from this process. The reduced level of hemoglobin is presumably associated with violations in heme biosynthesis because of inhibit linking of Fe with heme and decrease in activity of enzymes involved in heme biosynthesis, mainly dehydratase of delta-aminolevulonic acid (ALA-D) [28-31].

Use of preparation "Nicavet-1000" in the same conditions leads to a normalization of both a hematocrit, and other parameters of red blood; an increase in level of MCV demonstrates intensification of erythropoiesis.

Violations in the electrolytic composition of blood caused by aluminium chloride influence also are

smoothed over by effect of preparation "Nicavet-1000". It is remarkable that at a normalization of level of maintenance of ions of K, Na and the value of iCa^{2+}, level of the total calcium in the experimental group, though goes down in comparison with control, but nevertheless remains significantly higher, than at intact animals.

Considering results of the conducted research of femurs, it is possible to claim that we observe inclusion of aluminium to a bone tissue. Owing to aluminium intoxication, deposition of salts of calcium to an osteoid decreases. Expressiveness of an osteomalacia intimately correlates with the content of aluminium in a bone tissue. In places of deposition of the aluminium, which is forming structures from 20 to 100 nanometers, almost completely absent active osteoblasts, and in the cells covering the surface of a bone no endoplasmic reticulum is detected. The bone tissue at aluminium intoxication looks inactive, an acute inhibition of processes of remodeling (new growth) of a bone tissue is characteristic of it. Further accumulation of aluminium leads finally to development of an osteomalacia [32]. Organic acids significantly change biochemical influence of aluminium. The mechanism by means of which aluminum induces changes in a bone tissue is not finally determined. However, the conducted researches with culture of a bone tissue showed that aluminum an citrate form a metal-citrate complex through a hydroxyl group. This complex inhibits the growth of calcium phosphate crystals and the mineralization of osteoid. Further the poor calcification of big mass of an osteoid conducts to a softening of bones, development of deformations and pathological changes [33-34]. Use of the preparation "Nicavet-1000", judging by results of researches, prevents pathological changes in a bone tissue, caused by aluminium chloride.

The complex of changes in a hippocampus of rats of the experimental group in general corresponds to an Alzheimer's disease picture. Besides, it is possible to assume that the hypercalcemia can be the reason of neurodegenerative changes in a hippocampus though such assumption though is confirmed by literary data, but demands further studying [35-39].

Application of "Nicavet-1000" leads to the expressed normalization of both the morphological, and micromorphometric parameters characterizing a hippocampus of rats.

Thus, preparation "Nicavet-1000" in case of intoxication with aluminium or its chloride smooths over toxic effect of these compounds concerning red blood parameters and hematocrit, significantly weakens expressiveness of the developing hypercalcemia, and, as a result, leads to less expressed changes in a hippocampus of rats.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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