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Strus Oksana
Department of Drug Technology and Biopharmaceutics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Polovko Nataliia
Department of Drug Technology, National University of Pharmacy, Kharkiv, Ukraine

Correspondence
Strus Oksana
Department of Drug Technology and Biopharmaceutics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Study of the sapropel extract influence on the main biochemical and hematological blood indices of rats

Strus Oksana and Polovko Nataliia

Abstract
Humic substances are a specific group of macromolecular compounds formed in peat and sapropels in the process of decomposition of dead plant and animal tissues. It was set out that products based on humic substances possess a wide range of biological properties that are already widely used in veterinary medicine and medicine. In particular, preparations containing humic substances affect the nonspecific and specific resistance of the organism, have antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, membrane and hepatoprotective properties, the ability to exacerbate the activity of metabolic processes in the body. The sapropel extract was obtained from Prybych deposit, located in Volyn region, Ukraine. The technology of sapropel extract production using the cavitation method provides better extraction of humic and fulvic acids, since their content exceeds 20%. The objective of our work was to determine the effect of sapropel extract on the basic biochemical and hematological blood indices of clinically healthy rats during oral administration over 30 days. According to the results of the study, the absence of chronic toxic effects of sapropel extract on the basic biochemical and hematological indices in rats blood was established.

Keywords: Sapropel extract, hematological and biochemical indices, chronic toxicity

1. Introduction
Sapropels - is a natural organic and mineral formations, deposits of freshwater reservoirs, which are formed from dead plant and animal organisms, mineral substances of biochemical and chemical origin and mineral components. Humic substances are the main biologically active components of sapropel. Humic substances are a specific group of macromolecular compounds formed in peat and sapropels in the process of decomposition of dead plant and animal tissues. [Allard B., 2006] [1].

It was set out that products based on humic substances possess a wide range of biological properties that are already widely used in veterinary medicine and medicine. In particular, preparations containing humic substances affect the nonspecific and specific resistance of the organism, have antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, membrane and hepatoprotective properties, the ability to exacerbate the activity of metabolic processes in the body. [Keeler C. *et al.*, 2006; Pena-Mendez E. *et al.*, 2005; Klocking R and Helbig B., 2005; Kala K. Jacob *et al.*, 2019] [2-5].

Prybych deposit, located in Volyn region, occupies the area of 39 ha and yield 212 thousand tonnes of organic type sapropel (Strus O.Y., 2019; Zander-Ukraine - two facets of one life) [6, 7]. The experimental research has shown the presence of significant amounts of carboxylic acids, amino acids, micro- and macroelements, humic acids in the sapropel composition, extracted from Prybych Lake (Strus O.Y., 2015a; Strus O.Y., 2015b) [8, 9]. The presence of anti-inflammatory and reparative activity of the extract has been confirmed (Klocking R. and Helbig B., 2005; Kala K. Jacob *et al.*, 2019; Strus O.Y. *et al.*, 2014a; Strus O.Y. *et al.*, 2014b] [4, 5, 10, 11]. The study of antibacterial characteristics of aqueous extract of sapropel in Prybych natural deposits revealed its antibacterial effectiveness against *Staphylococcus aureus*, *Escherichia coli*, *Basillus subtilis*, *Candida aibicans*; and minor antibacterial effect against *Proteus vulgaris* and *Pseudomonas aeruginosa* (Strus O.Y., 2015c) [12].

The technology of sapropel extract production using the cavitation method provides better extraction of humic and fulvic acids, since their content exceeds 20% (Strus O.Y. and Polovko N.P., 2016) [13]. The presence of large amounts of sapropel in Ukraine and the results of the research suggest that sapropel is a promising raw material for preparing effective products for application in medicine, pharmacy and veterinary medicine.

An important stage in pharmaceutical development is the study of the possible toxic effects of

medicinal substances and products. One of the parameters is the identification of influence on main blood indices as a stage of the research of chronic toxicity of substances.
The objective of our work was to determine the effect of sapropel extract on the basic biochemical and hematological blood indices of clinically healthy rats during oral administration over 30 days.

2. Materials and methods

2.1. Materials

The objects of the study were whole blood, plasma and blood serum of rats after oral administration of sapropel extract for 30 days.
The sapropel extract was obtained from Prybych deposit, Volyn region, Ukraine [Zander-Ukraine two facets of one life] [7]. Sapropel treated with 0.1 N alkali solution and used Cavitation at a speed of 3000 rot/min for 60 minuts, at 50 - 60° C to obtain a homogeneous mixture. The resulting extract was evaporated to 1:10 of basal volume. Stressed and by lemon acid adjusted to pH 7.0. (Strus O.Y. and Polovko N.P., 2016; Strus O.Y. *et al.*, 2018; Strus O.Y. *et al.*, 2019) [13-15]. The extract was standardized according to the following indicators: appearance (colour, consistency), pH value, organic matter content, dry residue and quantitative carbon content of humus substances (Strus O.Y. and Polovko N.P., 2016) [13].
In the whole blood *hematological parameters* were determined: hemoglobin content (g/l), hematocrit (%), number of erythrocytes (1012 / l), leukocytes (109 / l) and platelets (109 / l); content (in %) of lymphocytes, MID (eosinophils, monocytes), reticulocytes, granulocytes and *superoxide dismutase* activity (SOD; IU/mg hemoglobin), *glutathione peroxidase* (GPX; μmol / min.× mg hemoglobin) and *catalase* (CAT; μmol / min.× mg hemoglobin).
The *blood plasma* was determined by the content of lipids hydroperoxides (IUE_{480} / ml), diene conjugates (nmol / ml) and thiobarbituric acid-active products (TBA- active products, nmol / ml); content of fractions of soluble proteins (%).
In the *serum*, the content of total protein (g/l) was determined; gamma-glutamyl transpeptidase activity (GGT), alkaline phosphatase (ALP) and creatine kinase (CK, IU/l); activity of enzymes of transamination: aspartate aminotransferase (AST) and alanine aminotransferase (ALAT, IU/l); creatinine content (mmol / l).

2.2. Methods

2.2.1. Sampling

Sampling was carried out taking into account the "General Ethical Principles of Animal Experiments" (Ukraine, 2001), the Law of Ukraine "On Protection of Animals from Cruel Treatment" of February 21, 2006, as confirmed by the protocol (No. 65 dated November 8, 2017) of the Commission from a bioethical examination and in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals (Stratsburg: Counsil of Europe 18.03.1986).

2.2.2. Determination of hematological parameters, of the activity of SOD, GPO and CAT

The hemoglobin content was determined by acetone-cyanadrin method, hematocrit, the number of red blood cells, leukocytes and platelets; content of lymphocytes, MID (Eozinophils, monocytes), reticulocytes, granulocytes - on a hematological analyzer "Mythic" (Switzerland). The activity of superoxide dismutase (SOD) was determined by reaction with nitrosin tetrazolium (Chevari S.H. *et al.*, 1991) [16], glutathione peroxidase (GPO) - with Elman reagent (Moim V.M., 1986) [17] and catalase (CAT) - reaction with ammonium molybdate (Korolyuk M.A. *et al.*,1991) [18].

2.2.3. Determination of the content of lipid hydroperoxides, diene conjugates and TBA -active products

Determination of the content of diene conjugates based on the properties of fatty acid molecules with two double conjugate bonds intensively absorb light at a wavelength λ = 233 nm. The optical density is measured on a spectrophotometer at a wavelength of 233 nm against n-heptane (Vlyzlo V.V. *et al*., 2012) [19].
Determination of the content of lipid hydroperoxides by the protein precipitation reaction with trichloroacetic acid solution (TCA) and extraction of lipids with ethanol followed by the interaction of the investigated extracts with ammonium thiocyanate. Measurement of optical density is carried out on a spectrophotometer at a wavelength of 480 nm for 10 minutes after the addition of ammonium thiocyanate (Vlyzlo V.V. *et al*., 2012) [19].
Determination of the content of TBA-active products (malonic dialdehyde-MDA) by the reaction between (MDA) and thiobarbituric acid (TBA), which, at high temperature and acidic medium, proceeds to form trimethine complex; the optical density is measured on a spectrophotometer at a wavelength of 535 and 580 nm in the centrifuge, to eliminate the absorption of painted complexes by TBA-substances of non-lipid nature (Vlyzlo V.V. *et al*., 2012) [19].

2.2.4. Determination of the content of soluble protein fractions and total protein, of the activity of GGT, ALP and the activity of transamination enzymes

The content of soluble protein fractions was determined by the vertical electrophoresis method in the plates of 7.5% polyacrylamide gel (PAAG) (Vlizlo V.V. *et al*., 2012) [19].
For determination of the content of total protein, of the activity of GGT, ALP and the activity of transamination enzymes, the biochemical analyzer "Humulyzer 2000" (Germany) was applied. (Vlizlo V.V. *et al.*, 2012) [19].
The content of total protein was determined by a biuret reagent; the activity of gamma-glutamyltranspeptidase (GGT) - kinetically with glycylglycine and L-gamma-glutamyl-3-carboxy-4-nitroanilide; the activity of alkaline phosphatase (ALP) with sodium beta-glycerophosphate on the content of inorganic phosphorus with a solution of molybdenate and ammonium vanadate; the activity of transamination enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALAT) - with dinitrophenylhydrazine; the content of creatinine by the Jaffe reaction; the activity of CK - kinetically at the rate of NADPH formation. (nicotinamide-β-adenine dinucleotide phosphate Dihydronicotinamide-adenine dinucleotide phosphate TPNH NADPH) (Vlizlo V.V. *et al*., 2012] [19].

2.2.5. Statistical analysis of the results

Statistical analysis of the recieved results was carried out by the method of variation statistics using Student's t-criterion (Plokhinsky N.A., 1970)[20] and with the use of a personal computer and software Excel. The difference between the arithmetic mean values was considered statistically significant at: * P<0,05; ** P<0,01; *** P<0,001.

3. Results and Discussion

In preclinical studies, experimental animals were used which were grown in vivarium of the Laboratory of Molecular Biology and Clinical Biochemistry of the Institute of Animal Biology of the National Academy of Agrarian Sciences, which is equipped in accordance with sanitary and hygienic standards. Experimental animals were kept under standard sanitary conditions: during the experiment, the animals were in vivariance at a temperature of 19-24 ° C, a moisture content of no more than 50%, a natural daylight and night mode, in plastic cages, on a balanced diet (Stefanov O.V., 2001) [21]. Before the experiment, the animals were acclimatized in the room for testing within 7 days.

The studies were conducted on clinically healthy male rats (Wistar line), aged 6-8 months, weighing 200-230 g, which were kept under standard vivarium conditions in a generally accepted diet. The animals were divided into two groups (n = 7 in each): 1 - control, which received water and 2 - experimental, which instead of water were given out the sapropel extract at a dose of 2 ml / kg body weight for 30 days. Whole blood was obtained by decapitation of animals under a slight chloroform anesthetic set into sterile test tubes with heparin or with K_2EDTA (for hematological study). To get of plasma, blood was immediately centrifuged at 3,000 rev. /min. within 10 minutes.

3.1. Investigation of protein and energy metabolism indices

As a result of a study of the total serum protein content, it was found that administration of sapropel extract by clinically healthy rats during thirty days did not cause hyper-and hypoproteinemia (Table 1). The value of the content remained within the limits of the physiological norm (61.3-79.5 g / l). However, in the experimental group of animals, it was 11% higher than in control group.

Table 1: Content of total protein, fractions of proteins in blood serum of rats; n=7

Index	Protein concentration, g/l		
	Control group	Experimental group	Physiological norm
M±m	69.3±6.4	77.2±2.0	-
lim	61.3-75.1	70.5-79.5	60-80

However, the study of the protein fractions in blood serum in rats showed that the use of sapropel extract led to possible changes in the protein spectrum (Table 2).

Table 2: The content of protein fractions in blood serum of rats; n=7

Index	Content of protein fractions, %			
	Albumin	Globulins		
		α-	β-	γ-
Control group				
M±m	57.2±3.2	10.9±3,6	11.9±2.9	20.6±1.4
lim	47.4– 60.1	7.8 – 13.5	10.7– 13.3	18.4–22.0
Experimental group				
M±m	55.0±4.7	9.5±2.1	7.5±2.3*	25.1±1.6*
lim	51.9–59.8	7.1–11.4	5.3–9.8	23.2 – 27.2

Thus, in animals which used the test extract, the content of gamma globulins was higher by 4.5% (p<0.05), while the content of beta-globulins, on the contrary, decreased by 4.4% (p<0.05) compared to control. Thus, given that the content of total protein and albumin has not changed we can assume that the test extract has immunostimulant action.

The key enzymes that characterize the protein metabolism in the body and the condition of organs functioning, associated with it include ALAT, AST and GGT (Friedman R.B. and Young D.S.,1997; Tietz N.W., 1994) [22, 24].

Therefore, to establish the functional condition of the internal organs, in particular the liver, and also to detect the effect of sapropel extract, the activity of AST, ALAT and GGT was studied. (Table. 3).

Table 3: The activity of serum enzymes in rats; n=7

Group	Index	Activity of enzymes, Iu / l		
		AST	ALT	GGT
Control	M±m	80.3±4.7	73.1±2.4	1.5±0.1
	lim	66.3-85.0	70.0-75.1	1.1-1.6
Experimental	M±m	63.0±5.2*	62.8±1.3**	1.2±0.1*
	lim	56.9-69.1	61.1-65.8	1.0-1.4
Physiological norm	lim	55-89	63-75	1.0-1.6

It was established that after 30-day administration of sapropel extract, the activity of AST, ALAT and GGT in blood serum of the experimental rats remained within the limits of the physiological norm. Thus, the values of the above-mentioned indices were significantly lower compared to the control group of animals by 21.5% (P<0.05), 14.0% (P<0.01) and 20.0% (P<0.05) respectively. It should be noted that in all animals in the control group the activity of the investigated enzymes was on the upper limit of physiological oscillations. Therefore, we can conclude that the tested extract not only does not cause cholestatic phenomena, but also has a membrane-protective effect, which may indicate his possible hepatoprotective activity.

An important indicator of the kidneys functioning is the content of creatinine in serum or blood plasma. Creatinine is the ultimate protein metabolite product in the body of animals and humans. The level of creatinine in the blood is determined by its production and elimination through the filtration barrier of the kidneys, i.e. creatinine clearance. Creatinine is involved in the energy metabolism of muscle and other tissues, and is excreted by the kidney from the urine, so the concentration of creatinine in plasma or serum is a key indicator of kidney function. In addition, low levels of creatinine may indicate a disturbed of energy metabolism in muscle tissue (Friedman R.B. and Young D.S.,1997; Tietz

N.W., 1999) [22, 23].

Another important indicator of the functional condition of the liver and, respectively the protein metabolism is ALP. In addition, the activity of the enzyme is evident in the disturbed mineral metabolism, in particular calcium and associated with it bone tissue pathogenesis. [Friedman R.B. and Young D.S., 1997; Tietz N.W., 1994) [22, 24]. Thus, in order to find out the influence of the sapropel extract on the process of osteosynthesis, in particular calcification, the activity of ALP in blood serum of rats was determined.

CK is an enzyme that characterizes the condition of energy metabolism in muscle tissue. The activity of CK in serum of patients with certain diseases of the skeletal muscle (muscular dystrophy, myositis, polymyositis, malignant hyperthermia, trauma, acute rhabdomyolysis), central nervous system (acute cerebrovascular disease, cerebral ischemia, Raynaud's syndrome) and thyroid gland disease is significantly increased (Friedman R.B., Young D.S., 1997; Tietz N.W., 1999) [22, 23]. After myocardial infarction, an increase in the activity of the CK is observed after 3-6 hours and reaches its maximum after 24-36 hours. An enzyme is rapidly eliminated from the plasma, usually its activity returns to normal level after 3-4 days (Friedman R.B. and Young D.S., 1997; Tietz N.W., 1994; Young D.S., 1991) [22, 24, 25].

As a result of the conducted studies, it was found that in the control and experimental groups, the concentration of creatinine in serum was within the limits of physiological oscillations – 69,8 - 87,3 mmol / l, and the quantity of the value of the indicator probably did not differ between the groups (Table 4), which indicates that the use of sapropel extract does not affect the kidneys functional condition and energy metabolism in muscle tissue.

Table 4: Creatinine content, activity of ALP and CK in blood serum of rats; n=7

Index	Control group	Experimental group	Physiological norm
	Concentration of creatinine, mmol / l		
M±m	76.5±7.2	79.6±6.3	-
lim	69.8-85.0	71.4-87.3	68-104
Activity of ALP, IU / l			
M±m	321.7±10.7	349.8±8.9*	-
lim	311.8-333.0	341.0-358.0	320-365
Activity of CK, IU / l			
M±m	171.7±10.7	199.8±8.9	-
lim	167.8-183.0	180.0-208.0	170-220

It was found that in blood serum of animals, which was administered sapropel extract for thirty days, the activity of enzyme was 8.7% (P<0.05) higher than in control group (Table 4). However, the value of the index did not exceed the physiological norm in all animals without exception. Consequently, the use of the sapropel extract does not cause osteosynthesis. Moreover, the obtained results confirm the absence of hepatotoxic effect of the latter.

Investigation of the CK activity in blood serum of rats which were administered the sapropel extract during thirty days revealed, that the value of the indicator has tended to increase (by 16%) compared with the control, however, did not exceed the upper limit of the physiological norm in all experimental animals (180.0-208.0 IU/L), which confirms the absence of toxic effects of sapropel extract on energy metabolism in muscle tissue (Table 4).

3.2. Studying the of the enzymatic level of antioxidant protection and the intensity of oxidative processes

It is known that SOD belongs to the class of oxidoreductase, and is a key enzyme of the antioxidant protective system, which is localized both in cells and in the extracellular space and catalyzes the dismutation of the highly active superoxideonium radical into Hydrogen peroxide and Oxygen. Reducing the activity of SOD under the influence of various factors causing oxidative stress can lead to an increase in the content of peroxide oxidation products of unsaturated fatty acids, of membrane structures lipids as a result of the activation of the generation of Oxygen active forms in the conditions of deviation in oxidative processes (Houston-Ludlam G., 2003) [26].

GPX is the key enzyme of the glutathione chain of antioxidant protection. The activity of GPX in erythrocytes is a highly informative indicator in the study of the antioxidant system condition and may serve as a marker of the effectiveness of medicinal products and their toxicity for the organism [Herbette S. *et al.*, 2007; Delaunay A. *et al.*, 2002; Loginov A.S. *et al.*, 1997) [27-29].

CAT is another enzyme that destroys H_2O_2. It is known that CAT, unlike GPX, restores only Hydrogens peroxide and is activated when the cell concentration of Hydrogen peroxide significantly exceeds the physiological levels, which occurs during oxidative stress when free radical processes are activated (Bai J., 2001) [30]. Such non-physiological situations of oxidative damage arise under various stress conditions, and catalase is the main inhibitor of stress response [Scibior D. and Czeczot H., 2006) [31].

As a result of the research carried out, it was found that the whole blood of clinically healthy rats is characterized by SOD activity - 2.71 ± 0.21 IU / mg hemoglobin (Table 5). In rats, which were administered the sapropel extract for 30 days, a tendency towards a decrease (by 5%) of the activity of the enzyme was revealed. At the same time, in three out of seven experimental animals, it did not differ from the control.

Table 5: Activity of superoxide dismutase, glutathione peroxidase and catalase in whole rats blood; n=7

Index	Control group	Experimental group
	SOD activity, IU/ mg hemoglobin	
M±m	2.71 ± 0.21	2.59 ± 0.07
lim	2.50– 2.99	2.40 – 2.62
	GPX activity, μmol / min × mg hemoglobin	
M±m	0.45 ± 0.028	0.60 ± 0.023**
lim	0.41 – 0.48	0.58 – 0.62
	CAT activity, μmol / min × mg hemoglobin	
M±m	1.82 ± 0.07	1.90 ± 0.09
lim	1.72 – 1.92	1.70 – 1.99

Thus, the GPX enzyme activity in the whole blood on the control group of rats was 0.45 ± 0.028 μmol / min × mg hemoglobin (Table 5). In the experimental group, after the thirty days administration of sapropel extract, the value of the index was significantly increased by 33% (P<0.01) in all animals which were under experiment. In the study of catalase activity, similar results were obtained (Table 5). The activity of the CAT enzyme is lower in the control group of animals (1.82 ± 0.07 mmol / min × mg hemoglobin) compared with the experimental (1.90 ± 0.09 μmol / min × mg hemoglobin), although no significant difference was found.

The revealed peculiarities of the activity of the antioxidant enzymes studied in rat blood confirm the study of the content of intermediate (diene conjugates and hydroperoxides) and terminal (TBA-active) products of lipids peroxide oxidation, which include MDA and other aldehydes.

The final link of lipids peroxide oxidation is the aldehydes formation, among which in the quantitative aspect dominated MDA (TBA-active products) (Marnett L.J., 1999; Buddi R. *et al*., 2002) [32, 33].

It was established that the content of primary products of lipid peroxidation of lipids in animals of the experimental group was at the same level as the control one (34.7 ± 2.72) (Table 6).

Table 6: The content of diene conjugates, lipid hydroperoxides and TBA-active products in blood plasma of rats; n=7

Index	Content of diene conjugates, nmol / ml	
	Control group	*Experimental group*
M±m	34.7±2.72	36.5±3.11
lim	30.1-39.4	33.4-39.7
	The content of hydroperoxides of lipids, IUE $_{480}$ / ml	
M±m	6.08±0.99	6.02±0.71
lim	5.87 – 7.11	5.32 – 6.73
	The content of TBA-active products, nmol/ml	
M± m	5.11±0.78	5.72±0.21
lim	4.74-6.04	5.50-5.98

Similar changes were found in the content of lipid hydroperoxides in rats blood plasma after administration of sapropel extract for 30 days (Table 6). The value of the indicator did not increase in all experimental animals.

The concentration of TBA-active products in the blood plasma of rats in both the control and experimental groups did not differ (Table 3.13). The value of the indicator varied within the range - 4.74-6.04 nmol / ml.

Thus, the results of the study of the activity of the enzymatic level of antioxidant protection and the intensity of peroxide oxidation of lipids indicate the absence of toxic effects of sapropel extract.

After its administration, the activation of glutathione peroxidase and catalase was recorded, which in turn optimizes the intensity of oxidative processes, as the level of lipoperoxidation products remains unchanged.

3.3. Study of hematological parameters of rats blood

The results of the study of the main indices of erythropoiesis revealed that in rats, which were administered the sapropel extract within thirty days, the values of hemoglobin, hematocrit and the number of erythrocyte were within the limits of physiological oscillations (Table 7). In this case, hemoglobin content increased significantly by 23.3% (P<0.05) compared to the control group of animals, and the hematocrit and the number of erythrocytes remained unchanged.

Table 7: Hematological indices of rats blood; n=7

Index		Control group	Experimental group	Physiological norm
Hemoglobin, g / l	M±m	121.5±11.2	149.8±6.8*	-
	lim	111.9-132.8	140.4-155.1	117-155
Hematocrit, %	M±m	45.8±0.7	46.5±0.4	-
	lim	45.1-46.6	46.0-48.0	43-48
Erythrocytes, 10^{12} / l	M±m	7.9±0.4	8.1±0.3	-
	lim	7.4-8.3	7.8-8.5	7.9-8.3
Leukocytes, 10^9/l	M±m	6.5±0.3	6.6±0.5	-
	lim	6.0-6.9	6.1-7.1	6.0-7.3
Lymphocytes,%	M±m	59.2±0.9	60.8±0.4	-
	lim	58.1-62.0	60.0-62.3	54-63
MID (Eozinophils, monocytes), %	M±m	4.6±0.2	4.7±0.3	-
	lim	4.4-4.9	4.4-5.0	4.5-5.3
Reticulocytes,%	M±m	24.5±0.7	24.9±0.5	
	lim	23.1-25.2	24.2-25.4	23-25
Granulocytes,%	M±m	37.5±1.4	37.7±2.3	-
	lim	35.0-38.7	35.3-39.9	33-41
Platelets, 10^9/l	M±m	532.5±10.6	539.7±12.1	-
	lim	522.0-549.3	527.3-551.9	520-550

Studies on the amount of leukocytes and the content of their individual fractions has shown that thirty days use of sapropel extract in clinically healthy rats did not lead to probable or biased changes in the values of these indices. The content of cellular elements of blood remained within the limits of physiological norms and did not differ from the control.

Consequently, the obtained results indicate that the studied sapropel extract does not have a toxic or allergenic effect, does not cause disturbances in the immune status and the system of hematopoiesis of the body.

4. Conclusions

It has been established that the thirty-day administration of sapropel extract in clinically healthy rats does not cause both hyper-and hypoproteinemia. However, in the plasma protein spectrum, the content of gamma globulins increases and the content of beta-globulins decreases in comparison with the control group, while remaining within the limits of physiological oscillations, which indicates the immune stimulating effect of the sapropel extract.

The use of sapropel extract does not affect the functional state of the kidneys and energy metabolism in the muscle tissue. It has been shown that in the control and experimental groups of animals, the concentration of serum creatinine was within the limits of physiological oscillations of 69.8-87.3 mmol / l.

It was shown that in rats blood serum, which for thirty days received sapropel extract, ALP activity was higher in comparison with the control group, but did not exceed the physiological norm in all animals, which confirms the absence of hepatotoxic effect of sapropel extract and indicates the absence of osteosynthesis disorders.

Investigation of activity of creatine kinase in rats blood serum

against the background of of sapropel extract administration showed that the value of the indicator tendentiously increased (by 16%) compared with the control, but did not exceed the upper limit of the physiological norm in all experimental animals (180 -208 IU / liter), which confirms the absence of toxic effects of sapropel extract on energy metabolism in muscle tissue.

The results of the study of the enzymatic level activity of antioxidant protection and the intensity of the peroxide oxidation of lipids are confirmed by the absence of the sapropel extract toxic effects in a dose of 2 ml / kg of body weight in rats.

Evaluation of the main indicators of erythropoiesis showed that, with the use of sapropel extract for thirty days, the hemoglobin content was significantly increased compared to the control group of animals by 23.3% (P<0.05), but did not exceed the physiological norms. The hematocrit and the number of red blood cells did not change.

Investigation of the amount of leukocytes and the content of their individual fractions did not reveal any probable or biased changes in the values of these indicators, which confirms the absence of toxic or allergenic action and violations in the immune status and system of hematopoiesis of the organism provided with the administration of sapropel extract.

5. References

1. Allard B. A comparative study on the chemical composition of acids from forest soil, agricultural soil and lignite deposit Bound lipid, carbohydrate and amino acid distributions. Geoderma. 2006; 130:77-96
2. Keeler C, Kelly E, Maciel G. Chemical-structural information from solid-state 13CNMR studies of a suite of humic materials from a lower monate forest soil, Colorado, USA. Geoderma. 2006; 130:124-140.
3. Pena-Mendez E, Havel J, Patocka J. Humic substances — compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. Review.Appl. Biomed. 2005; 3:13-24.
4. Klocking R, Helbig B. Medical Aspects and Applications of Humic substances. 2005, 3-16. https://doi.org/10.1002/3527600035.bpol1013
5. Kala K, Jacob, Prashob Peter KJ. Chandramohana Kumar N. Humic substances as a potent biomaterials for therapeutic and drug delivery system-a review. International Journal of Applied Pharmaceutics. 2019; 11(3):1-4
6. Strus OY**,** Polovko N, Yezerska O. Justification of technological parameters of the cream production with sapropel extract. Pharmacia. 2019; 66(1):19-25
7. Zander-Ukraine - two facets of one life: Company history http://www.zander-ukraine.com/about/history/
8. Strus OY. The study of amino acid composition of sapropel by capillary electrophoresis method. News of Pharmacy. 2015a; 2(82):12-16.
9. Strus OY. Investigation of carboxylic acids composition of sapropel by a chromato-mass spectroscopy method. Coll. of Scien. Papers of Staff Memb. of P.L. Shupyk NMAPE. 2015b; 24(5):228-233. (in Ukr, English abstr)
10. Strus OY, Polovko NP, Maloshtan LM, Yatsenko EY. Study of anti-inflammatory and reparative properties of sapropel extracts from Prybych deposits. Coll. of Scien. Papers of Staff Members of P.L. Shupyk NMAPE (in Ukr, English abstr.). 2014; 23(4):392-398.
11. Strus OY, Polovko NP, Maloshtan LM, Solodchenko TP. The study of the properties of sapropel deposits of Pribich. Problems of Medical and Environmental Genetics and Clinical Immunology. 2014; 3(123):169-173. (in Ukr, English abstr.)
12. Strus OY. Study of sapropel extracts from Prybych natural deposits. J Chem. and Pharm. Res. 2015c; 7(6):133-137.
13. Strus OY, Polovko NP. Analysis of extraction methods and improvement of sapropel extract's technology Coll. of Scien. Papers of Staff Memb. of P.L. Shupyk NMAPE 2016; 26:266-271. (in Ukr, English abstr.).
14. Strus O, Polovko N, Plaskonis Y. The investigation of the development of a cream composition with the sapropel extract. Asian J Pharm Clin Res. 2018; 11(7):147-150.
15. Strus O, Polovko N, Silaeva L. Justification of composition of the cream with sapropel extract. Int. J of Green Pharmacy. 2019; 13(1):42-47
16. Chevari SH, Andian TA, Strenger JI. Determination of antioxidant blood parameters and their diagnostic value in the elderly. Laboratory work. 1991; 10:9-13.
17. Moim VM. Simple and specific method for the determination of glutathione peroxidase in erythrocytes Laboratory work. 1986; 12:16-19.
18. Korolyuk MA, Ivanova LI, Mayorova IG, Tokarev VE. Method for determining the activity of catalase. Laboratory work 1991; 12:9-10.
19. Laboratory methods of research in biology, livestock and veterinary medicine: Textbook / Vlyslo V.V., Fedoruk R. S., Ratic I. B. and others; Ed. V.V. Lviv: Spolom, 2012. 764.
20. Plokhinsky NA. Biometrics, Moscow: MSU, 1970, 53-60
21. Stefanov OV. Pre-clinical research of medicinal products. Guidelines, Kyiv: Avicenna, 2001, 528 .
22. Friedman RB, Young DS. Effects of disease on clinical laboratory tests, 3rd ed., AACC Press, Washington, DC, 1997
23. Tietz NW. Textbook of Clinical Chemistry, 3rd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1999
24. Tietz NW. Textbook of Clinical Chemistry, 2nd edition. Burtis CA, Ashwood ER. WB Saunders Co., 1994
25. Young DS. Effects of drugs on clinical laboratory tests, 3rd ed. Washington, D.C.: AACC Press, 1991
26. Houston-Ludlam G. Cu/Zn Superoxide Dismutase: A Key Player in the Antioxidant Defense System. Genetics 2003; BSCI: 230.
27. Herbette S, Roeckel-Drevet P, Drevet JR. Seleno-independent glutathione peroxidases. More than simple antioxidant scavengers. FEBS J. 2007; 274:2163-2180.
28. Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB. A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. Cell. 2002; 111:471-481.
29. Loginov AS, Matiushin BN, Tkachev VD. Clinical significance of liver glutathione system in chronic lesions. Therapeutic archive. 1997; 69(2):25-27.
30. Bai J. Mitochondrial catalase and oxidative injury. Biol. Signals. Recept 2001; 10(3, 4):189-199.
31. Scibior D, Czeczot H. Catalase: structure, properties, functions. Postepy. Hig. Med. Dosw. 2006; 60:170–180.
32. Marnett L.J. Lipid peroxidation-DNA damage by malondialdehyde. Mutat. Res. 1999; 424(1, 2):83-95.
33. Buddi R, Lin B, Atilano SR *et al*. Evidence of oxidative stress in human corneal diseases. Cytochem. 2002; 50(3):341-351.