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Blood parameter changes in lead acetate-exposed mice as a result of leonardite and lignite exposure

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Abstract

The study aimed to research *in vivo* changes in the hematological and biochemical parameters of laboratory rats' blood when they were continuously exposed to a moderate dose of lead acetate while receiving humic feed additives made of leonardite and lignite. Two-month-old white rats were used to assess the detoxifying properties of the humic compounds leonardite and lignite. The analog pair approach was used to create four groups of eight animals from 32 male laboratory rats for the study. Rats received a pre-meal injection of lead acetate at a dose of7 mg/100 g of animal weight (1/110 LD50) using a veterinary feeding needle. By creating solutions from lignite and leonardite at a dosage of 18 and 25 mg/kg depending on the active ingredient, humic feed additives were supplied to animals. It has been demonstrated that lignite- and leonardite-based feed additives may affect the morphological parameters of lead acetate-treated rats' blood, including hemoglobin, hematocrit, erythrocyte, and platelet count. These parameters were very near to the levels of the intact rats, which suggests that these humic feed additives may have an anti-anemic impact. The effects of feed additives from leonardite and lignite on labora-tory rats in groups that were also exposed to a toxic agent for all 21 days of the experiment led to the normaliza- tion of markers of the state of protein metabolism in the group of intact animals (serum protein, albumins, urea, creatinine), particularly activity of the enzyme's aspartate aminotransferase and alanine aminotransferase. As one of the most crucial indicators of lipid metabolism together with cholesterol, the concentration of triglycerides was slightly lower in the humic substances treated groups than in the intact animals. It was discovered that using a feed supplement made from leonardite in a dosage of 18 mg/kg, which contained more fulvic acids than lignite, led to more favorable blood test results in the research group.

Keywords: humic substances; lead; lead intoxication; laboratory rats; feed additives.

1. Introduction

Lead compounds are among the most common environ- mental pollutants among all other substances formed by heavy metals. Water-soluble compounds, such as lead ace- tate, are incredibly poisonous. Environmental pollution often occurs as a result of manufactured emissions fromenterprises and fuel combustion. While water bodies, pas- tures, and places where farm animals and their feed are grown get polluted. All this leads to the poisoning of ani- mals (Kalia & Flora, 2005).Lead is a thiol poison; in the body, it interacts with the SH groups of various enzymes. This cellular protoplasmic poison has astringent, irritating, and cauterizing effects (Nemsadze et al., 2009). It can coagulate proteins of the protoplasm of cells, forms albuminates; lower the resistance of erythrocytes, and increase the permeability of cell mem- branes, which leads to the loss of 80% of potassium and water (Soltaninejad et al., 2003; Garcia & Corredor, 2004;Bello & Idris, 2018). Then hemolysis of erythrocytes isnoted (Fukumoto et al., 1983). Typical is the inhibition of enzyme activity and an increase in urea concentration (Okediran et al., 2017).Lead can freely accumulate in the body and be excreted naturally. However, general intoxication occurs with con- centration increases to critical levels (Stohs & Bagchi, 1995;Zralý et al., 2008). Therefore, preventive measures should be taken in unfavorable biogeochemical zones where the pollutant is known. A convenient form of pharmacological prophylaxis, where pollution is insignificant, could be feed additives (Dewanjee et al., 2013; Annaç et al., 2022; Akkoyun et al., 2023).

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Humates are possible active substances of the feed addi- tive that could prevent the toxic effect and its effects on the animal organism. Often humates include three components: humic acids, fulvic acids, and mineral humic residue. It is already known about the positive effect of humic acids onthe redistribution of lead in the organs of affected rats, its excretion from the body, and the synthesis of the antioxidantGlutathione (Žatko et al., 2014; Vašková et al., 2019).The last studies on piglets show a high antioxidant effi- cacy of humic feed additives based on leonardite and lignite in diarrhea (Trckova et al., 2018). The authors note that were mixed with feed. Table 2 depicts the experiment's layout.
form chelate complexes with heavy metal ions that take a neutral charge, which does not allow them to be absorbed in the gastrointestinal tract (Glynn, 1995).

Thus, **this study aimed** to show changes in the hemato- logical and biochemical parameters of the blood of laborato- ry rats under the constant influence of a moderate dose of lead acetate with the administration of humic feed additives based on leonardite and lignite.

2. Materials and methods

The testing of lignite and leonardite detox features, two humic substances (Table 1), was performed on white rats aged two months. The experiment was conducted in theconditions of the vivarium of the Dnipro State Agrarian and Economic University. In order to carry out the research, four groups of eight animals were established from 32 male la- boratory rats using the analog pair method. Animals of each group were placed in separate cells with the same condi- tions, free access to water, and a balanced diet. The study lasted 21 days.

Table 1

Leonardite and lignite's (dry matter's) humic composition and specific mineral content

Parameter (wt%)	Leonardite	Lignite
Dry matter	87.14	93.25
Humic substances	69.22	42.98
Humic acids	58.24	34.12
Low molecular substances include fulvic acids	10.98	8.86
Ca	14.11	9.94
Mg	1.02	3.13
P	0.13	0.81
Fe	13.56	12.09

The administration of factors that influence groups of an-imals occurred *per os* every day of the study. Dietary treat- ments of rats were as follows: Group I – intact animals with standard vivarium diet; Group II – standard vivarium diet together with the toxic agent – lead acetate ($Pb(C_2H_3O_2)_2$); Group III – standard vivarium diet together with toxic agent and leonardite based humic feed additive; Group IV – stand-ard vivarium diet together with toxic agent and lignite based humic feed additive. Lead acetate was injected with a veter- inary feeding needle (UNO Life Science Solutions, Nether- lands) for rats prior to feeding at a dose of 7 mg/100 g of animal weight (1/110 LD_{50}) (Tkachenko & Melnikova, 2008). Feed additives of humic nature were fed by forming solutions from leonardite and lignite at dosages based on theactive substance of 18 and 25 mg/kg. The resulting solutions All animals were evaluated once daily for clinical symp- toms of toxicity and twice daily for mortality. Blood for research was collected once toward the end of the in-life phase of the study from the right ventricle of the heart under thiopental narcosis (60 μg/kg).

The following indicators were determined in stabilized EDTA blood to find out the morphological profile: RBC (red blood count), HGB (hemoglobin amount), HCT (hema- tocrit, erythrocyte count), MCV (mean cell volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), WBC (white blood count), PLT (platelet count). Hematological profile analysis was carried out using the PCE-90Vet hematological analyzer (High Technology Inc., USA).

During the biochemical study, the following quantitative indicators of blood serum were determined: serum protein, albumin, globulin, albumin/globulin ratio, urea, creatinine, glucose, ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), total choles-terol, triglycerides, calcium, inorganic phosphorus. Bio- chemical analysis of blood serum was carried out using an automatic biochemical analyzer BioChem 200 (High Tech- nology Inc.,

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USA).

All manipulations and scientific investigations involving animals were conducted following the "European Conven- tion for the Protection of Vertebrate Animals used for Ex- perimental or other Scientific Purposes" (Strasbourg, France, March 18, 1986, ETS No. 123) and the Law of Ukraine "On Protection of Animals from Cruel Treatment" (Kyiv, February 21, 2006, No. 3447-IV).

The experimental results were statistically processed us- ing Student's t-test utilizing MS Office Excel 2019. Theresults were deemed significant at $P < 0.05$. The data pre- sented in the tables as mean values and standard errors (m ± SE).

3. Results and discussion

No treatment-related deaths or overt clinical symptoms such as hair loss, scabbing, soft or mucoid excrements, de- creased defecation or feces that are smaller than usual, and vocalization upon handling were noted in any of the treated groups during the study. At the end of the study, the animalsin all treatment groups did not appear to have significant disorders.

The results of the hematological analysis are shown in Table 3. There are parameters of the hematological profileof the rats of group II that changed significantly in compari- son to intact animals of group I: hemoglobin less by 9.8 %($P < 0,05$), hematocrit less by 14.7 % ($P < 0,001$), MCV lessby 12.1 % ($P < 0,001$), MCHC more on 9,7 % ($P < 0,05$),
platelet count less by 4.2 % ($P < 0,001$), white blood countmore on 28.0 % ($P < 0,01$). Changes in the listed parametersare typical for the poisoning of lead compounds (Yuan et al.,2014).

In group III, an increase in RBC by 6.4 % ($P < 0.05$) wasnoted compared with animals from group II. It may indicate a successful correction of anemic phenomena caused by the constant toxic effect of lead acetate.

The hemoglobin concentration in groups III and IV was higher by 6.3 % ($P < 0.01$) and 6.0 % ($P < 0.05$). These indicators were close to those of intact animals from group I.The mean for group III rats, in which the feed additive basedon leonardite fed into the diet, had a lower standard error.

The percentage of hematocrit was significantly increasedfor rats of groups III and IV by 6.5 % ($P < 0.01$) and 5.4 % ($P < 0.05$) compared with animals of group II. An increasein the standard error for these groups was noted, which may indicate a heterogeneous volume of red blood cells in the blood of the studied animals of groups III and IV.

The calculated MCV parameter increased significantly inanimal groups III and IV by 9.4 % ($P < 0.05$) and 8.3 % ($P < 0.05$), respectively, compared with group II. The observed changes indicate that feed additives humic substances can positively influence the average size of erythrocytes.

In group III of rats, a decrease in the MCHC parameter by 8.7 % ($P < 0.05$) was observed compared to group II animals. The observed changes indicate a decrease in hemo- globin saturation of the erythrocyte, while the numerical value is close to that of the intact animals of the group I.

Despite the pronounced thrombocytopenia in animals of group II, animals of groups III and IV that received feed additives of humic nature they have had indicators of 3.1 % ($P < 0.001$) and 3.8 % ($P < 0.001$) higher.

Table 3

Effect of Lead acetate on hematological parameters in laboratory rats under the influence of humic feed additives on the 21st day of the study (m ± SE, n = 8)

Parameter	Group I (Intact)	Group II (Lead acetate)	Group III (Leonardite)	Group IV (Lignite)
RBC, 10^{-12}/L	7.08 ± 0.15	6.89 ± 0.14	7.33 ± 0.09 $^{\$}$	7.21 ± 0.11
HGB, g/L	157.15 ± 2.34	147.30 ± 2.36 *	156.65 ± 1.93 $^{\$\$}$	156.14 ± 2.95 $^{\$}$
HCT, %	46.27 ± 1.36	39.48 ± 0.79 ***	46.03 ± 1.41 $^{\$\$}$	44.90 ± 1.76 $^{\$}$
MCV, fL	65.30 ± 0.96	57.40 ± 1.18 ***	62.80 ± 1.73 $^{\$}$	62.18 ± 1.83 $^{\$}$
MCH, pg	22.25 ± 0.48	21.43 ± 0.49	21.39 ± 0.27	21.69 ± 0.42
MCHC, g/L	341.25 ± 8.68	374.54 ± 11.81 *	341.96 ± 8.06 $^{\$}$	351.47 ± 14.55
PLT, 10^{-9}/L	868.84 ± 6.16	832.38 ± 4.77 ***	858.59 ± 5.76 $^{\$\$}$	864,09 ± 5.95 $^{\$\$\$}$
WBC, 10^{-9}/L	5.93 ± 0.22	7.59 ± 0.39 **	7.47 ± 0.09 ***	7.22 ± 0.20 ***

Note: Probability of the difference of experiment groups to the intact group: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$

Probability of the difference of experiment groups to the control group: $^{\$}$ – $P < 0.05$; $^{\$\$}$ – $P < 0.01$; $^{\$\$\$}$ – $P < 0.001$

The effect of lead acetate on clinical chemistry parame- ters is presented in Table 4. As in the results of hematologi- cal analysis, a significant change in biochemical parameters in rats of group II compared with the intact group I, charac- teristic of lead poisoning was noted (Suradkar et al., 2009): serum

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protein less by 3.7 % (P < 0.01), albumin less by 8.6 % (P < 0.001), albumin/globulin ratio less by 11.9 %(P < 0.001), urea more on 10.7 % (P < 0.05), creatinine more on 7.9 % (P < 0.01), ALT more on 35.2 % (P < 0.05), AST more on 39.9 % (P < 0.01), ALP less by 18.9 % (P < 0.01), total cholesterol less by 17.6 % (P < 0.05), tri- glycerides more on 1.2 mmol/L (P < 0.001), calcium less by 23.4 % (P < 0.05), inorganic phosphorus less by 9.0 % (P < 0.05).

Changes in serum protein for rats in groups III and IV were statistically significant compared to group II animalsby 2.9 % (P < 0.05) and 3.86 % (P < 0.01). At the sametime, a significant increase in albumin concentration was also noted for comparable groups of animals by 3.43 % (P < 0.05) and 6.54 % (P < 0.01). It should be noted that the standard error of the index in animals of group III was sig- nificantly lower than in group IV. Serum protein and albu- min parameters for groups III and IV were close to the intactrats.

Another critical indicator of protein metabolism – urea, was significantly decreased in III group of animals by 6.0% (P < 0.05) compared to II group of rats. A decrease in creat- inine concentration in animals of groups III and IV relative to the group of lead-treated animals by 5.5 % (P < 0.05) and 5.0 % (P < 0.05). The parameters of creatinine in the groups of animals treated with humic feed additives were close to the intact laboratory rats, while the standard error was less than the mean in group I.

The concentration of the ALT enzyme in animals of groups III and IV was lower compared to the group of ani- mals, where treatment with lead acetate alone took place by 23.8 % (P < 0.05) and 20.8 % (P < 0.05). Also, for these compared groups of animals, a decrease in AST activity wasnoted by 24.4 % (P < 0.01) and 16.7 % (P < 0.05), respec- tively. Conversely, the ALP enzyme concentration increasedby 17.3 % (P < 0.01) and 13.4 % (P < 0.05), respectively. The indicator was close to the value in the intact animals.

For animals of group III, normalization of total choles- terol concentration was observed. This indicator was signifi-cantly increased by 17.5 % (P < 0.05) compared with group II. The concentration of triglycerides, one of the most cru- cial indication parameters of lipid metabolism along with cholesterol, was reduced compared with group II by 68.8% (P < 0.01) and 47.7 % (P < 0.01) for groups III and IV. These changes allow us to conclude that there is an effect of the feed additive on the lipid metabolism of rats treated with lead acetate.

Among macronutrients, an increase in this parameter was observed for rats of group III by 18.7 % (P < 0.05) compared with the group exposed only to lead acetate. Atthe same time, there was an increase in the concentration of this parameter by 6.3 % (P < 0.05) and 11.2 % (P < 0.01) forgroups III and IV compared with the second. At the same time, changes in the IV group of animals were close to the intact rats of the I group.

Table 4

Effect of lead acetate on clinical chemistry parameters in laboratory rats under the influence of humic feed additives (m ± SE, n = 8) on the 21st day of the study

Parameter	Group I (Intact)	Group II (Lead acetate)	Group III (Leonardite)	Group IV (Lignite)
Serum protein, g/L	69.91 ± 0.39	67.31 ± 0.78 **	69.26 ± 0.24 $	69.91 ± 0.39 $$
Albumin, g/L	42.66 ± 0.26	38.97 ± 0.30 ***	40.31 ± 0,35 ***$	41.52 ± 0.66 $$
Globulin, g/L	27.26 ± 0.15	28.34 ± 0.60	28.95 ± 0.22 ***	28.39 ± 0.86
Albumin/globulin ratio	1.56 ± 0.01	1.38 ± 0.03 ***	1.39 ± 0.02 ***	1.48 ± 0.06
Urea, mmol/L	4.69 ± 0.15	5.20 ± 0.14 *	4.89 ± 0.02 $	4.84 ± 0.11
Creatinine, μmol/L	31.27 ± 0.49	33.74 ± 0.66 **	31.90 ± 0.54 $	32.07 ± 0.41 $
Glucose, mmol/L	6.66 ± 0.38	5.86 ± 0.32	6.41 ± 0.57	6.16 ± 0.04
ALT, U/L	48.66 ± 5.98	65.83 ± 4.63 *	50.16 ± 5.63 $	52.13 ± 3.31 $
AST, U/L	133.11 ± 8.30	186.25 ± 12.62 **	140.80 ± 3.60 $$	155.11 ± 3.78 *$
ALP, U/L	227.19 ± 9.74	184.17 ± 8.50 **	216.00 ± 6.42 $$	209.88 ± 7.80 $
Total cholesterol, mmol/L	1.99 ± 0.13	1.63 ± 0.09 *	1.92 ± 0.07 $	1.86 ± 0.03 $
Triglycerides, mmol/L	0.36 ± 0.08	1.58 ± 0,23 ***	0.49 ± 0.14 $$	0.81 ± 0.09 **$$
Calcium, μg/mL	33.87 ± 2.14	25.94 ± 2.19 *	30.78 ± 0.51 $	27.36 ± 0.88 *
Inorganic phosphorus, μg/mL	417.48 ± 9.57	379.87 ± 9.82 *	403.73 ± 5.09 $	422.50 ± 6.19 $$

Note: See Table 2

After analyzing the data of biochemical and hematologi- cal studies of the blood of rats, it can conclude that the ani- mals of the III study group, which in addition to the toxic agent lead acetate, received a feed additive based on leonardite, to a greater extent had closer values to the intact rats of group I than the similar group IV animals treated with lignite. These changes are credibly explained by the presence of a relatively more extensive amount of fulvic acids in the composition of the feed additive from leonardite; while using their donor-acceptor abilities, they can bind some part of lead ions at a particular stage of me- tabolism, coupled with the adaptogenic effect of other sub- stances of

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humic nature presented in composition.

4. Conclusions

1. It has been established that feed additives based on leonardite and lignite can affect changes in the morphologi- cal parameters of the blood of rats treated with lead acetate, such as hemoglobin, hematocrit, erythrocyte count, MCV, MCHC, platelet count. These parameters were close to the values of the intact rats' group, which indicates a possible anti-anemic effect of these humic feed additives.

2. The influence of feed additives from leonardite and lignite in laboratory rats of groups which 21 days of the experiment were also affected by a toxic agent caused nor- malization to the group of intact animals of indicators of the state of protein metabolism (serum protein, albumins, urea, creatinine), especially activity of enzymes AST and ALT. There was also a positive effect on triglycerides, total cho- lesterol, calcium, ALP, and inorganic phosphorus.

3. The dependence of the number of fulvic acids in feed additives on the adaptogenic functions of the rat's organism has been assessed. It has been revealed that the use of a feed additive derived from leonardite in a group of laboratoryrats, which contains a higher number of fulvic acids com- pared to lignite, resulted in relatively superior blood analysisresults in comparison to intact animals.

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