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TOXICITY OF LEAD-ACETATE ON EXPERIMENTAL RATS INFECTED WITH *ESCHERICHIA COLI*

Maja MANOJLOVIC[1], Radoslav DEKIC[1], Svjetlana LOLIC[1]*, Aleksandar IVANC[2], Ivica RADOVIC[3]

[1]Faculty of Science and Mathematics, University of Banja Luka, Bosnia and Herzegovina
[2]State University of Novi Pazar, Serbia
[3]Faculty of Security Studies,University of Belgrade, Serbia
*Corresponding author: svjetlanalolic@gmail.com

Abstract

Due to the increased concentration caused by emission in traffic, lead is an important issue in environmental protection. Its toxicity depends on its chemical form administrated to the animal, the route of administration and the frequency and duration administered to animals. Bacterial infection caused by *Escherichia coli* may also lead to serious pathological processes in the body, such as sepsis or meningitis and to a variety of systemic infections, such as infections of urinary or gastrointestinal tract. The aim of study was to determine individual and synergistic toxic effects oflead-acetate and effects of infection with *E.coli* on haematological and morphometric characteristics of Wistar rats.It has been found that intoxication with lead-acetate caused a significant decrease in number of erythrocytes and leukocytes. Animals that had also been infected with *E. coli* after intoxication with lead-acetate had statistically significant reduction of number of erythrocytes and slightly increasing of number of leukocytes Mass index of kidney, liver and spleen showed statistically significant differences between individuals of the control group and individuals intoxicated with lead-acetate, while values of the mass of the heart had not statistically significant differences.

Keywords: *Lead-acetate, toxicity, Escherichia coli, blood count.*

Introduction

Lead has been used since prehistoric times, so it is a very widely distributed in the environment. Knowledge of its general toxic effects is three millennia old, and yet lead exposure continues to be a major global problem, especially in developed urban areas and third world countries. It is one of thee arliest discovered poisons (Lidsky&Schneider, 2003). Lead is emitted into the atmosphere from natural and anthropogenic sources. Natural emission includes resuspension by wind, volcanic eruptions, forest fires, sea aerosol and biogenic sources. Lead emission into the atmosphere is not entirely natural, because it is partly caused by anthropogenic deposition of lead in history (Nriagu & Pacyna, 1989). Main emission sources of lead are burning of fossil fuels, production of non-ferrous metals, iron and steel, production of cement and disposal of industrial and urban waste. For a long time the largest source of anthropogenic lead was a leaded fuel, so organisms that live near highways represent a kind of determinants of lead exposure (Strömberg*etal,* 2003). The same situation is with organisms that live near specific factories and mines.

Lead affects negative on many organ systems. Numerous experiments with laboratory animals show that lead causes changes in the function of the placenta and fetal development. It also causes various neurological disorders and behavioral changes (Seddik *et al*., 2010), decrease in body weight and length, and increase in the mass of internal organs (Sood *et al*., 2008).

According to previous research, the effects of lead-acetate on blood picture of rats are reduced number of red blood cells (anemia), increased (leukocytosis) or decreased (leukopenia) number of white blood cells, monocytosis, eosinopenia, neutrophilia and thrombocytosis. Some scientists believe that anemia sideropenica can be associated with metabolic interaction of lead with iron and copper. Increased number of leukocytesis associated with the inflammatory effect of lead on lymphatic organs (Noori Mugahi *et al*., 2003). For kidneys and liver of rats treated with lead-acetate was recorded increase of the organ weight/body weight index (Abdel-Moneim*etal*., 2011).

Bacterial infection caused by *Escherichia coli* may also lead to serious pathological processes in body, such as sepsis or meningitis, and to a variety of systemic infections, such as infections of urinary or gastrointestinal tract (Brock *et al*, 1994). Sepsis and the cancellation of many physiological functions of body can have fatal effects.

The aim of study was to determine individual and synergistic toxic effects of lead-acetate and effects of infection with *E.coli* on hematological and morphometric characteristics of Wistar rats.

Materials and methods

The experiment was realized in a stable and laboratory of the Faculty of Science and Mathematics, University of Banja Luka (Republic of Srpska, Bosnia and Herzegovina). There were used wistar rats of the same age, approximate weight of the body and equal representation of the sexes. Rats were divided into eight groups of ten specimens. All groups were kept in plexiglas cages with 12-hour light mode, at air temperature 22°C (± 2), with food and water ad libitum.

Twenty specimens were receiving lead-acetate (concentration of 1500 ppm) by water ad libitum for 14 days. After that period they got 0,2 ml of saline solution by intraperitoneal injection. Ten specimens were sacrificed 24 hours after injection (T1-24 test group), and another ten specimens were sacrificed 72hours after injection (T1-72 test group). Other twenty specimens received by intraperitoneal injection 0,2 ml of saline solution with $3x10^7$ CFU/ml of bacteria *Escherichia coli* ATCC 11755. The number of bacteria was determined by standard optical density method. Ten of them were sacrificed 24 hours after injection (T2-24 test group), and ten 72 hours after injection (T2-72 test group).The next twenty specimens were used to monitor the synergistic effects of intoxication by lead-acetate and infection with *E. coli*. They were treated with lead-acetate (concentration of 1500ppm), for a period of 14 days. After that bacterial infection was caused by intraperitoneal injection of 0.2 ml saline solution with *Escherichia coli* ($3x10^7$ CFU/ml). Ten specimens were sacrificed 24 hours later (T3-24test group), and ten 72 hours after injection (T3-72 test group). Control group, consisted of twenty animals, got intraperitoneal injection of 0.2 ml 0,9% saline solution. Ten of them were sacrificed after 24 hours (C-24control group) and ten after 72 hours (C-72 control group).

Before blood sampling animals were anesthetized by intramuscular injection of ketamine (concentration of 50 mg/kg). Blood was taken by cardiac puncture with a needle (diameter 1.2 mm) and a syringe (volume 2 ml) and delayed in vacuum blood collection tubes with anticoagulant K_3EDTA. The following hematological parameters were determined: the number of red blood cells per liter of blood, the number of leukocytes per liter of blood, hematocrit, hemoglobin concentration, MCV (the average volume of a red blood cell), MCH (the average mass of hemoglobin per red blood cell), MCHC (the average concentration of hemoglobin per liter of a red blood cell) and differential blood count.

Number of formed elements of blood was determined by counting in aThoma chamber using the appropriate solution (Hayem’s solution, Türk’s solution). Hematocrit was determined by micromethod with capillary hematocrit tubes and hematocrit centrifuge with reader (5 minutes

at 16000 rpm). Hemoglobin concentration was determined by method according to Drabkin with a colorimeter (Colormetar 254) and Drabkin's reagent. Hematological indices (MCV, MCH and MCHC) were determined by calculating the standard formulas. Differential blood count was determined by differentiating the blood smears that were stained by method according to Pappenheim (Ivanc & Dekić, 2006).
In accordance with the instructions of Institutional Animal Care and Use Committee, all animals were sacrificed by decapitation under deep anesthesia ("Guide for the Care and Use of Laboratory Animals", 1996). Internal organs (heart, liver, kidneys, spleenand testes) were removed, measured and fixed in the shortest possible time.
The results were statistically analyzed with IBM SPSS ver. 20.0 software package. Differences among groups were determined with ANOVA statistical test, and multiple comparisons were determined with LSD test. Confidence intervals were stated at the 95% confidence level.

Results and discussion

Comparative analysis of morphometric parameters within groups of specimens under the same treatment did not show any significant differences in regard to the time interval (24 hours and 72 hours after injection). Results for morphometric parameters are summarized into four groups according to the treatment (Table 1). Comparisons of body weight gain, kidney and liver weight index showed statistically significant differences between the animals of the control group and animals of the T1 and T3 groups which had been treated with lead-acetate. Body weight gain was significantly lower for the test specimens of the T1 and T3 test groups (p=0.000/0.000), and the kidney and liver weight indices were significantly increased (p=0.000/0.000 and p=0.000/0.001). The values of the lien weight index were significantly increased at the animals of the T1 test group (p =0.001). Heart weight index had statistically significant difference only between the control and T3 test group (p =0.008). There were no statistically significant differences between control and T2 test group treated just with *E.coli*.

Table 1. Morphometrical parameters: organ weight indices and body weight gain

	Weight index in % (organ weight/body weight x100)				
Group	Kidneys	Liver	Lien	Heart	Body weight gain in %
Test 1	0.42±0.09	3.46±0.56	0.26±0.07	0.34±0.04	8.57±3.36
Test 2	0.30±0.02	3.06±0.40	0.23±0.03	0.34±0.03	16.02±5.84
Test 3	0.38±0.05	3.43±0.36	0.22±0.03	0.35±0.03	7.71±2.58
Control	0.29±0.02	3.00±0.22	0.20±0.03	0.33±0.03	18.72±6.19

The obtained values for the control group were in accordance with the references (Sood *et al*., 2008), while the observed changes in the values of morphometrical parameters for test specimens were less than expected. For larger changes of these parameters is necessary longer exposure to lead-acetate intoxication (Sood *et al*., 2008; Abdel-Moneim *et al*., 2011).
The average number of erythrocytes per liter of blood (Table 2) in control groups of rats was 7.77×10^{12} (C-24) and 7.66×10^{12} (C-72). The observed values are in accordance with reference value for the Wistar rats which amounts 5-10 x 10^{12}/l (Sharp and LaRegina, in 1998, Pritchett and Corning, 2004). Significant reduction in number of red blood cells with regard to control group is observed in all the test groups. Maximal deviations were reached in T3 24 and T3 72 groups treated with lead-acetate and infected with *Escherichia coli*. ANOVA analysis showed statistically significant differences in number of red blood cells among the groups according to the treatment (p=0.000), and multiple comparisons showed significant

decrease in number of red blood cells in all test groups (C>T1>T2>T3). Animals treated just with a lead-acetate (T1-24 and T1-72) had lower values of RBC in regard to the control group (p=0.000), which corresponds to literature data (Noori Mugahi *et al*., 2013). Also, the number of red blood cells has been decreasing during the infection with *Escherichia coli* (T2-24 and T2-72) in regard to the control group (p =0.000). Time of exposure to the treatment in all groups There were no statistical significant differences in RBC values between T-24 and T-72 groups under the same treatment.

Table 2. Hematological values (Mean±SD) of Wistar rats exposed to lead-acetate and *E.coli*

Group	h	RBC count $1x10^{12}$	Hemoglobin (g/l)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/l)
T1	24	5.71±2.15	98.89±9.88	0.300±0.04	62.957±31.23	20.223±8.76	334.491±63.73
	72	6.21±8.97	95.19±9.41	0.277±0.06	45.441±11.72	15.686±3.02	360.389±101.29
T 2	24	5.85±6.73	102.85±9.76	0.235±0.03	40.798±7.44	17.648±2.84	436.399±50.13
	72	5.55±6.96	90.37±12.00	0.242±0.06	43.594±9.62	16.557±3.38	393.830±102.72
T 3	24	4.67±5.80	83.70±24.02	0.282±0.05	61.375±13.42	18.225±5.85	301.568±92.62
	72	4.27±6.23	94.81±15.64	0.298±0.04	70.977±14.79	22.818±6.07	325.774±72.78
Cont.	24	7.77±5.60	130.74±14.81	0.314±0.05	40.451±6.28	16.869±1.91	420.205±30.39
	72	7.66±3.24	132.22±11.59	0.342±0.03	44.672±4.21	17.268±1.49	391.293±65.63

Hematological parameters hematocrit, MCV and MCH of control group were a little lower than reference values (Sharp & La Regina, 1998), but within normal limits for untreated Wistar rats (Brkić *et al*., 2011). Values of MCHC were slightly increased. Rats of the test groups had decreased values of hemoglobin (p=0.000 for all test groups in comparison to the control) and hematocrit (p<0.05 for all test groups), while values of MCV, MCH and MCHC had different changes depending to the treatment.

Values of MCV were significantly increased at animals from groups T1-24 (p=0.001 and 0.007), T3-24 (p=0.002 and 0.013) and T3-72 (p=0.000 and 0.000) in comparison to the control groups (C-24 andC-72). MCH was significantly increased at animals from group T3-72 (p=0.011) in comparison to the control groups. MCHC values were significantly decreased at animals from group T3-24 in comparison to the both control groups (p=0.001 and 0.007), and from T3-72 group in comparison to C-72 group (p=0.010). Overall, values of hematological parameters in all treated groups showed different changes depending on the treatment and the time of exposure. According to Suradkar and associates (2009) reduced values of RBC, Hb, MCV, MCH and MCHC occur as a result of intoxication with lead-acetate, which overall results in hypochromic microcytic anemia. Changes are more evident at higher doses and longer exposure to this heavy metal. Lead inhibits synthesis of heme and shortens the life time of red blood cells. Results are increasing of red cells destruction and decreasing of hematological parameters values (Klassen, 2001).Values of white blood cells are given in Table 3. For control groups they are in accordance with reference values for the Wistar rats (Sharp & La Regina, 1998). Number of leukocytes was significantly increased at animals from groups T2-24 (p=0.000 and 0.000) and T2-72 (p=0.000 and 0.000) in comparison to the control groups (C-24 and C-72). Animals treated with lead-acetate had decreased number of white blood cells in comparison to all other groups (p=0.000-0.017). LSD test showed differences among number of WBC at animals from T2 groups in comparison to the control and all other test groups (p=0.000-0.003). Number of leukocytes was significantly higher 72 hours after infection with *E.coli* in comparison to T2-24 group (p=0.003). Different types of leukocytes showed significant differences among different experimental groups. The largest proportion of neutrophils was observed at animals from T3-

72 group (0.261) and lowest from T1-24 group (0.120). Comparing a multiple significance occurs between the specimens treated with lead acetate (24 T1) and *Escherichia* infected animals (p = 0.038 for 24 hours and 0.023 for 72 hours), and the dual treatment of the animals sacrificed after 72 hours (p = 0.000). The largest proportions of eosinophils and monocytes were observed at animals from control group. The largest proportion of lymphocytes was observed at animals treated with lead-acetate, indicating neutropenia and lymphocytosis. Basophils showed the lowest proportion (0.003) at animals infected with *E. coli*.

Table 3. Number of leucocytes and proportion of their specific types (Mean±SD)

Group		WBC count $1x10^9$	proportion of specific types of leukocytes				
			Neutrophil	Eosinophil	Basophil	Limphocyte	Monocyte
	h						
Test 1	24	5.42±4.69	0.120±0.06	0.007±0.01	0.023±0.02	0.846±0.06	0.004±0.01
	72	5.43±5.31	0.169±0.05	0.008±0.01	0.018±0.01	0.804±0.06	0.001±0.00
Test 2	24	6.74±4.93	0.182±0.04	0.001±0.00	0.003±0.01	0.812±0.04	0.002±0.00
	72	7.25±3.34	0.188±0.04	0.005±0.01	0.002±0.01	0.803±0.04	0.002±0.00
Test 3	24	6.02±1.25	0.169±0.09	0.003±0.01	0.026±0.01	0.801±0.09	0.001±0.00
	72	6.04±2.14	0.261±0.06	0.004±0.01	0.018±0.02	0.716±0.06	0.001±0.00
Control	24	5.84±2.69	0.193±0.09	0.029±0.03	0.032±0.02	0.734±0.10	0.012±0.01
	72	5.71±3.63	0.122±0.07	0.035±0.01	0.011±0.01	0.821±0.07	0.011±0.01

Noori Mugahi et al (2003) and Okediran et al (2010) noted that the total number of leukocytes in animals treated with lead-acetate significantly increased (leukocytosis), with expressed neutrophilia and monocytosis. On the other hand, Suradkar *et al* (2009), Ibrahim *et al* (2012) noted leukopenia and lymphopenia at higher doses of lead-acetate. Many scientists believe that the immune response of rats to intoxication with lead-acetate depends on received dose and rat's age and sex.

Conclusion

Results of morphometric analysis showed significant changes at rats treated with lead-acetate. Body weight gain was significantly lower and kidneys and liver weight indices were significantly higher. Lead also showed hematotoxic effect on animals. There has been a significant decrease of the total number of red blood cells, hemoglobin and hematocrit in comparison to the control group. Parameters of white blood cells also showed decreased values: number of leukocytes and proportions of neutrophilles, eosinophilles, basophilles and monocytes were lower in comparison to control group. Only proportion of limphocytes was significantly higer in comparison to control and T3 group. Animals infected with *E. coli* had lower values of RBC, hemoglobin and hematocrit and the highest values of WBC in comparison to control group, especially 72 hours after infection. Proportion of neutrophilles increased, but proportion of other WBC elements decreased. Animals treated with lead-acetate and infected with *E. coli* showed the largest changes of hematological parameters. Number of erythrocytes, concentration of hemoglobin and MCHC were the lowest and MCV was the highest in all groups of animals. Number of leukocytes was higher in comparison to control and T1 group, but lower in comparison to group infected with *E. coli*.

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