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Effects of Zeolite Administration on Kinetics of White Blood Cell in ICR Laboratory Albino Mice after Sub-Chronic Cadmium and Lead Administration

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Abstract: The accumulation of heavy metals in the body is associated with an increased immune response manifested by pathological changes in white blood cell (WBC) counts. This study aimed to examine the impact of cadmium (Cd) and lead (Pb) exposure on the WBC kinetics of laboratory ICR albino mice over a subchronic intoxication period and to assess the efficacy of zeolite administration in modulating detoxification processes. Five variants of laboratory experiments were performed over 45 days: 1) food + water (control); 2) food + (water+$Cd(NO_3)_2$); 3) food + (water+$Pb(NO_3)_2$); 4) (food + clinoptilolite) + (water+$Cd(NO_3)_2$) and 5) (food + clinoptilolite) + (water+$Pb(NO_3)_2$). Haematological analyses were conducted on days 0, 15, 30 and 45. Pb and Cd intoxication resulted in pathological alterations of WBC kinetics, with inconsistent effects in different WBC subtypes but more pronounced in granulocyte. We observed a tendency for a decrease in WBC counts at day 15 and an increase at day 30, followed by normalisation at day 45 in all experimental groups. Pb – more immunotoxic to mouse WBCs. The study on the effects of clinoptilolite (hydrated alkali aluminosilicate, one of the most abundant minerals in the zeolite family) administration suggests insufficient efficiency on leukopoiesis in ICR albino mice. Further research is required to elucidate the potential mechanisms of action of clinoptilolite-mediated detoxification in the context of immune responses to heavy metal poisoning.

Key words: cadmium, lead, naturally modified clinoptilolite, white blood cells kinetics

Introduction

Environmental contamination by toxic metals and other hazardous chemical elements is an ever-present hazard for humans and terrestrial biota. Soil, water and air contamination with heavy metals such as cadmium, lead, chromium, copper, zinc, mercury and arsenic is of particular concern (Dolanc et al., 2023). Lead (Pb) and cadmium (Cd) remain important pollutants globally and in the European Union (EU) due to their direct toxicity in animals and plants and their progressive accumulation in food chains at each level (EEA, 2019). Despite implementing robust measures, the challenge of eliminating toxic substances from soil and water sources persists. The gradual elimination of these substances

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and their high accumulation rate at higher levels of food chains is anticipated to present significant challenges in the coming decades.

Finding suitable sorbents with detoxifying properties will help address several environmental and public health risk issues. In recent years, natural and modified zeolites, especially clinoptilolites, have found increasing application in this field. The crystal structure of zeolites, combined with their porous, rough surface, makes them very suitable as sorbents for toxic cations such as Cs^{+}, NH_4^{+}, Cu^{2+}, Zn^{2+}, Sr^{2+}, Cd^{2+}, Hg^{2+}, Cr^{3+} and Pb^{2+} from aqueous solutions (Burns et al. 1990, Langella et al. 2000). There is also a growing interest in studying their sorption capabilities in solving environmental problems affecting living organisms not only in Europe but also globally (Pavelic 2002; Kavak & Ülkü 2015, Cerri et al. 2016,Tomečková et al. 2016). Bulgarian clinoptilolite sorbents demonstrated efficacy against intoxication with lead nitrate ($Pb(NO_3)_2$) in laboratory mice (Beltcheva et al. 2012, Topashka-Ancheva et al. 2012, Beltcheva et al. 2015). Previously we have established protective effects of modified natural clinoptilolite from the "Beli Plast" deposit in ICR laboratory mice subchronically exposed to $Cd(NO_3)_2$ (Beltcheva et. al. 2022).

Cadmium and lead are highly hazardous metals with long-term effects and can potentially cause organ damage even at low concentrations. Lead has been demonstrated to exert a deleterious effect on liver function and haematopoiesis, with the potential to cause hypochromic anaemia due to blocking the enzymes aminolevulinic acid dehydratase (ALAD) and ferrochelatase (Goyer & Rhyne 1973,Zelikoff & Thomas 1998). Meanwhile, Cd has been shown to induce gene toxicity and kidney failure (Wlostowski et al. 2010). It is known that acute or chronic exposure to cadmium and lead results in haematological changes in mammals and humans. A common consequence of chronic poisoning with these two heavy metals is anaemia, which is regarded as one of the earliest indications of Cd and Pb poisoning in animals. In these cases, the white blood cell (WBC) count is typically within the normal range, whereas the red blood cell (RBC) count is reduced (Powolny et al. 2023). Moreover, significantly elevated neutrophil and diminished lymphocyte counts have been documented in rabbits subjected to intoxication as compared to control subjects. However, no notable discrepancy in total WBC counts – observed between the two groups (Stowe et al. 1972). These findings indicate that Cd may influence the form elements in WBCs by disrupting their production and/or destruction, thereby affecting their transformation. This potential mechanism of action for the two heavy metals – corroborated in the early 1970s by demonstrating direct stimulation of lymphocytes by Cd and Pb in cultured human leukocytes (Utakoji 1972; Shenker et al. 1977). While there is experimental evidence on the induction of changes in WBC pools by Pb and Cd in test animals, it is contradictory (Zelikoff & Thomas 1998). For example, Ohsawa & Kawai (1981) reported a Cd-induced increase in lymphocyte production in ICR mice and Sprague-Dawley rats during subchronic exposure, while Koller et al. (1976) found a suppression of immune function and antibody production after Pb and Cd exposure. In any case, most experimental evidence on the effects of Pb and Cd on WBC counts is at least three decades old and there is no direct evidence of the impacts of zeolite administration on leukopoiesis during non-acute exposure of animals to Pb and Cd, which necessitates the current study. Our aim – to assess the effects of subchronic exposure to Pb and Cd on leukopoiesis in laboratory mice and the potential protective effects of clinoptilolite supplementation on WBC kinetics.

Materials and Methods

Experimental animals

A total of 120 male ICR laboratory mice, aged 6-8 weeks, were randomly and equally assigned to one of five experimental groups. Animals were placed in individually ventilated cages following the EU requirements (Directive 2010/63/EU): at a standard temperature between 20°C and 22°C, a humidity of 45–60% and a 12-hour day/night cycle. They were fed on ISO-2000-certified standard rodent food.

In accordance with our objectives, the five variant groups were as follows: 1) control ‑ food + water; 2) food + (water + $Cd(NO_3)_2$); 3) food + (water + $Pb(NO_3)_2$); 4) (food + 12.5 wt.% powdered modified natural clinoptilolite) + (water+$Cd(NO_3)_2$); 5) (food + 12.5 wt.% powdered modified natural clinoptilolite) + (water + $Pb(NO_3)_2$). The final concentrations of Cd and Pb nitrate forms were as follows: 0.00125 M for $Cd(NO_3)_2$) and 0.00250 M for $Pb(NO_3)_2$. The mice had access to food and water *ad libitum*. Each experiment lasted for 45 days after one week of acclimatisation. The blood samples were collected from each tested group at the beginning, 15^{th}, 30^{th} and 45^{th} experimental days.

All experiments were conducted in accordance with Ordinance No. 20/01.11.2013 on the protection and welfare of animals and the Animal Protection Act of 31 January 2008 of the Republic of Bulgaria.

Heavy metal bioaccumulation

The Cd and Pb bioaccumulation in the liver and kidneys of the tested animals – measured using a Perkin Elmer SCIEX DRCe ICP-MS system with a cross-flow nebuliser. Todorova et al. (2023) provide a detailed description of the methodology.

Clinoptilolite

The modified clinoptilolite used in this study – collected from the volcanogenic sedimentary clinoptilolite-rich tuff deposit "Beli Plast" in the Eastern Rhodopes, Bulgaria. The clinoptilolite – ground and sieved and the fraction below 0.15 mm – separated for treatment. Clinoptilolite – exchanged into its sodium form by stirring 100 g of the natural sample and 1000 ml of 1 M $NaNO_3$ solution at 500 rpm. The treatment – performed in a closed bottle in an oven heated at 80°C. The $NaNO_3$ solution – replaced daily by a fresh one. This procedure – repeated 12 times. The sample – – hed with deionised water to remove the occluded salt and dried at 60°C in an oven. The mineral composition of the zeolitised tuff – determined by powder X-ray diffraction (PXRD). The chemical composition of the major elements in wt.% – 71.08 SiO_2, 0.12 TiO_2, 10.88 Al_2O_3, 0.96 Fe_2O_3(t), 0.09 MnO, 1.03 MgO, 2.78 CaO, 0.31 Na_2O, 2.76 K_2O, 0.15 SO_3, while ignition losses i.e. adsorbed and chemically bonded water – about 11.61 wt.%. The values of the oxides of the exchangeable cations CaO, K_2O and MgO after Na-exchange decrease were 1.23, 2.32 and 0.72, respectively. The Na_2O content increased significantly and reached 2.57 wt.%.

Haematology

Blood samples were collected from the tail vein in MicroTube EDTA.K3 0.5 mL microtubes and processed automatically using a MindRay BC-30Vet automated veterinary haematologyanalyser. Among the parameters analysed, the most representative markers for white blood cells were selected and processed further: WBC (total white blood cells), LYM (lymphocytes), GRAN (granulocytes) and MON (monocytes).

Statistics

The statistical significance of mean differences between the tested parameters – calculated using Prism software version 9.0 (GraphPad Software, San Diego, CA, USA). Both univariate and multivariate statistical analyses were performed. The haematological data underwent a normality test ($p < 0.001$) and we utilised one-way ANOVA. In case ANOVA showed significant differences, post hoc analysis – performed using Tukey's test. $P < 0.05$ – considered to be statistically significant.

Results

Heavy metals bioaccumulation

The heavy metal loadings in the animal's liver and kidneys exhibited notable differences (Table 1). The kidneys were identified as the most sensitive organs,

Table 1. Heavy metals loading in the liver and kidneys (mg/kg dry weight) of tested animals

Days	Cadmium ($\bar{x}$ ± SD) (Cd group)				Lead ($\bar{x}$ ± SD) (Pb group)			
	after Cd exposure		after clinoptilolite administration		after Pb exposure		after clinoptilolite administration	
	Liver	Kidneys	Liver	Kidneys	Liver	Kidneys	Liver	Kidneys
0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.59 ± 0.19	2.5 ± 0.59	0.59 ± 0.19	2.5 ± 0.59
15	56.2 ± 1.1^{a,d}	53.1 ± 1.4^{a}	44.2 ± 1.6^{a}	39.1 ± 0.9^{a}	41.89 ± 1.27^{a,d}	149.3 ± 4.52^{a,d}	12.56 ± 1.57^{a}	22.15 ± 11.45^{a}
30	96.0 ± 3.6^{a,b,d}	104.0 ± 2.6^{a,b}	78.0 ± 2.1^{a,b}	74.6 ± 1.8^{a,b}	58.37 ± 2.45^{a,b,d}	269.66 ± 4.79^{a,d}	9.45 ± 0.99^{a}	17.45 ± 3.49^{a}
45	173.0 ± 1.9^{a,b,c,d}	260.0 ± 3.1^{a,b,c,d}	90.0 ± 2.3^{a,b,c}	100.0 ± 2.4^{a,b,c}	67.78 ± 3.06^{a,b,d}	537.1 ± 3.89^{a,b,c,d}	4.92 ± 1.69^{a,b}	10.39 ± 1.52^{a,b}

Values with a different superscript letter indicate a significant difference between groups at each time point: (a) between day 0 and days 15, 30 and 45; (b) between day 15 and days 30 and 45; (c) between day 30 and day 45; (d) between groups before and after clinoptilolite administration. Values with a, b, c and d are significant at $p < 0.001$.

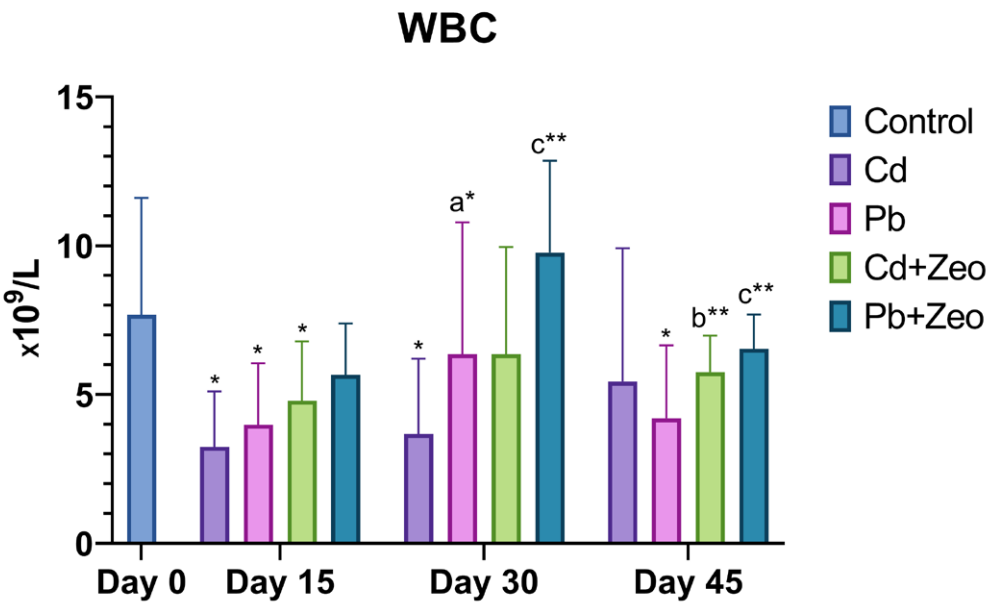

Fig.1. White blood cell kinetics during the experimental trial. The asterisks indicate the statistical differences between the control and the experimental groups on day 15 ($p < 0.05$). The superscript 'a' demonstrates the statistically significant difference ($p < 0.05$) between WBC values on days 15 and 30 following Pb exposure, while the superscript 'b' illustrates the significant difference ($p < 0.01$) between days 15 and 45 after Cd + Zeo exposure. The superscript 'c' depicts the significant differences ($p < 0.01$) between the WBC values at day 15 following Pb exposure and days 30 and 45 after Pb and zeolite exposure.

exhibiting the highest Cd and Pb accumulation (173.0 ± 1.9 mg/kg Cd and 537.1 ± 3.89 mg/kg Pb for groups 2 and 3 on the 45th day, Table 1). Furthermore, the differences in Cd and Pb concentrations between the experimental and control groups were statistically significant ($p < 0.0001$). The administration of clinoptilolite to subchronically metal-exposed animals resulted in statistically significant alterations in the concentrations of the examined metals. A significant reduction in the levels of Cd and Pb – observed in both the kidney and the liver ($p < 0.001$).

Haematology

Cadmium and Pb-induced alterations in the counts of WBC observed in control and experimental groups are shown in Fig.1. In comparison with the control group, the experimental trial revealed that the leukopenia induced by Cd – observed on days 15 and 30, whereas Pb-induced leukopenia – noted at the beginning and end of the trial. No statistically significant differences were observed between WBC counts on different experimental days in the animals exposed only to Cd. Their values remained low in comparison to the control throughout the experiment. The analysis demonstrated that only on day 15, the experimental animals administered $Cd(NO_3)_2$ in the drinking water and clinoptilolite in the food (group 4) exhibited statistically significant differences ($p < 0.05$) in comparison to the control group. A significant increase ($p < 0.05$) – observed in the experimental group solely exposed to Pb on day 30 compared to day 15. This – followed by a further decrease on day 45, which – not statistically significantly different from the control values. By the end of the experimental phase, a tendency towards compensation for the observed changes emerged. Nevertheless, this process – not entirely efficacious.

Following 15 and 30 days of Cd exposure, GRAN (Fig. 2) exhibited a statistically significant decline in numbers compared to the control values ($p < 0.001$). Conversely, a notable increase in the number of cells – observed on day 45, compared to day 15 ($p < 0.05$). The alterations in the mean GRAN counts observed in the Pb-exposed animals were analogous to those observed in the total WBC data. In test groups 2 and 3, there – a statistically significant reduction in the mean values on the 15th day, followed by a significant increase on the 30th. After the experimental period, a tendency towards compensation for the changes – evident on the 45th day. A similar pattern of GRAN change – observed in the groups following the administration of the clinoptilolite. The number of GRANs decreased on day 15, exhibited a significant increase on day 30 and on day 45, there – a rise that did not reach control values.

The kinetics of LYM (Fig. 3) demonstrated a statistically significant difference in comparison to

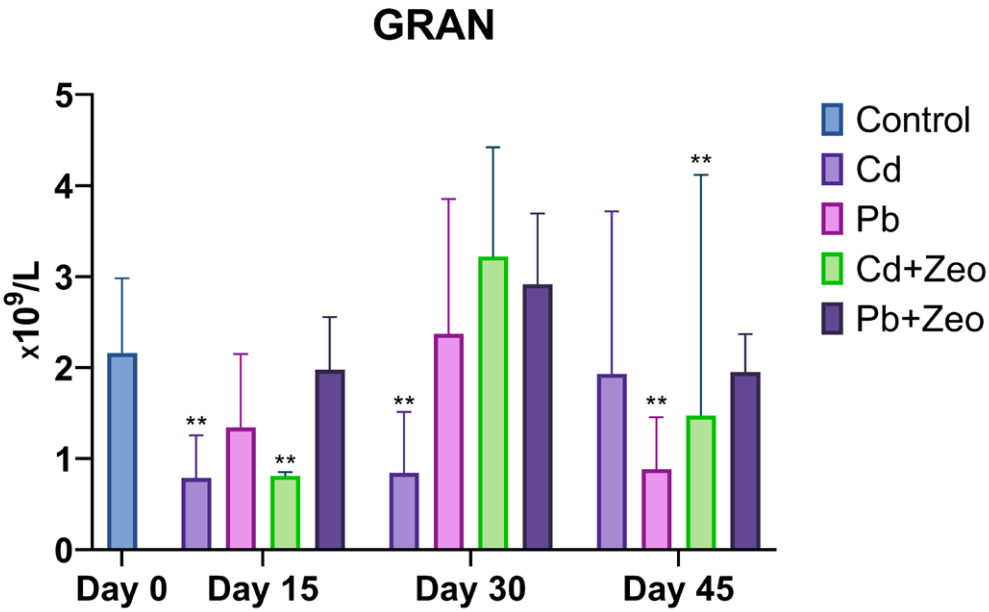

Fig.2. Granulocyte kinetics during the experimental trial. The asterisks indicate the statistically significant differences ($p < 0.01$) between the control granulocyte counts and those on the other experimental time points after Cd and Pb exposure.

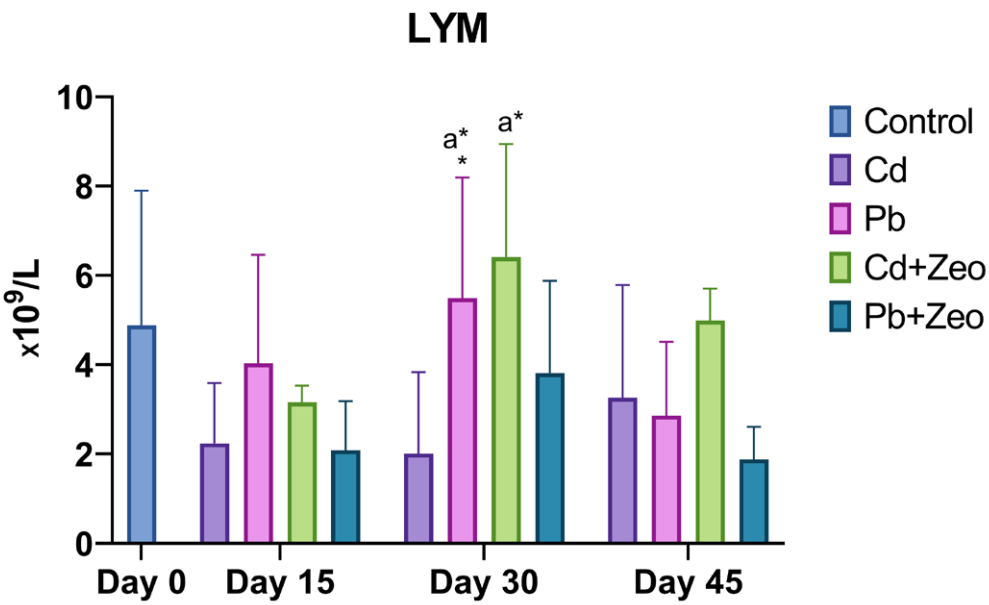

Fig.3. Lymphocyte kinetics during the experimental trial. The asterisks indicate the statistical differences between the control and the experimental groups on day 30 ($p < 0.05$). The superscript 'a' demonstrates the statistically significant difference ($p < 0.05$) between lymphocyte values on days 15 and 30 following following Cd + Zeo and Pb exposure.

the control in group 3 (following Pb exposure) and group 4 (with $Pb(NO_3)_2$ and clinoptilolite supplementation) at day 30. Lead and Cd were observed to exert indirect immunotoxic effects on LYM, while Cd exposure resulted in a notable mild lymphopenia compared to Pb exposure. Administration of zeolite – associated with an increased lymphocyte count on day 30 in group 4. The count of MON remained relatively stable throughout the experimental period for all tested groups compared to the control (Fig. 4).

The results of the haematological parameter analyses suggested that the subchronic Pb and Cd exposure can cause varying degrees of inflammatory injury in ICR albino mice. The established patterns of WBC kinetics demonstrated a tendency towards a reduction in WBC score values at day 15

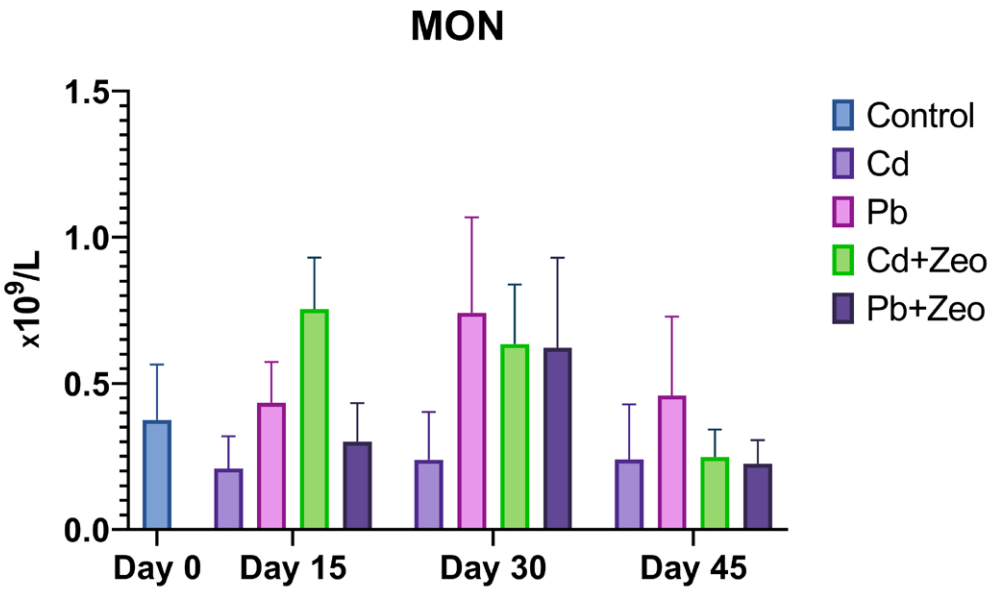

Fig. 4. Monocyte kinetics during the experimental trial.

and an increase at day 30, followed by normalisation at day 45 for all experimental groups. The white blood cells and their main subtypes of mice from groups 4 and 5 with clinoptilolite supplementation exhibited alterations analogous to those observed in the mice from the Cd and Pb-exposed groups. This kinetic pattern indicated that the action of clinoptilolite – not sufficiently efficient.

Discussion

The haematopoietic system is one of the most sensitive systems for assessing the toxicity of environmental toxins in humans and animals (Yuan et al. 2014). Haematological indices can indicate environmental or toxic stressors, causing significant alterations in the animal's haematopoiesis. WBCs are among the most sensitive indicators of animal health, such as the immune system status (Powolny et al. 2023). LYM, the effectors of acquired immunity, proliferate in response to antigenic stimuli and have a long life span in blood. At the same time, their numbers decrease (lymphopenia) during immunosuppression by glucocorticoids or immunosuppressive infections (Stockham & Scott 2002).

Cadmium and Pb are known to cause haematological changes, mainly in the erythropoiesis in mammals and humans exposed to acute or chronic intoxication (Beltcheva et al. 2022). The higher number of white blood cells following Cd toxicity may be due to increased free radicals, decreased antioxidant activity or suppression of nonspecific and specific immune responses, as well as systemic inflammation (El-Boshy et al. 2015).The *in vitro* investigation of the associations of Cd exposure with peripheral blood cell subtype counts and indices in Cd-poisoned mice demonstrated that Cd increased the number of white blood cells and the number of neutrophils in mice, while reducing the number of monocytes and the number of lymphocytes (Dong et al. 2024). Other research demonstrated that Cd administration in albino mice suppressed humoral immunity and total WBC count (Rehman et al. 2017). Conversely, Cd exposure induces a small and statistically insignificant reduction in circulating WBCs (Huang et al. 2022).

In mammals, significant Pb exposure in blood concentrations exceeding 1 mg/L induces statistically substantial and dose-dependent association with a high WBC count, indicating an initiated inflammatory response. Moreover, increases in WBC counts have also been documented in Pb-exposed mice, indicating that Pb – more immunotoxic to WBCs of mice. Classic experimental evidence has demonstrated that Pb exposure is immunotoxic, particularly reducing the ability of mice to resist bacterial and virus infections (Hemphill et al. 1971; Gainer 1974). This immunotoxicity is due to decreased humoral immunity, even though Pb induces pro-inflammatory cytokine signalling (Metryka et al. 2018). Various studies showed that Pb intoxication leads to neutrophilia and monocytosis, probably due to possible inflammations caused by this heavy metal (Hogan & Adams 1979).

In the current study, the leukogram revealed that exposure to Cd and Pb resulted in absolute

grano-philia with lymphopenia. At the same time, there – no change in the monocytes. The observed mild leukopenia – probably due to a drop in the neutrophils count, a significant part of granulocytes and the most common leukocytes. This is in accordance with several studies that established that Cd induces neutrophilia and lymphopenia in rats intoxicated with Cd (Djokic et al. 2014, Kataranovski et al 2009). The observed decrease in lymphocyte counts could be explained by heavy metals' indirect in vitro toxicity to lymphocytes. It may involve a reduction in proliferative ability (Dethloff & Bailey 1998), an impairment of antigen binding, a decrease in antibody production and an impairment of the cellular response (Niu et al. 2007). The observed neutrophilia and lymphopenia may be attributed to elevated serum cytokines-induced bone marrow neutrophil release and mobilisation and redistribution from tissues (marginal pool) into circulating neutrophils under basal and inflammatory conditions (El-Boshy et al. 2015). The observed increase in the absolute lymphocyte count may also be attributed to enhanced lymphocyte proliferation under the stimulatory effect of interferons (Niu et al. 2007).

In general, the WBC count is often the subject of debate and controversy regarding its interpretation. A high value has been identified as an indicator of optimal health status (Gustafsson et al. 1994). For example, Snoeijs et al. (2004) observed a heightened immunological response, including an elevated WBC count, in birds residing in relatively unpolluted sites along a metal contamination gradient. Tête et al. (2015) likewise documented a reduction in leukocytes with increasing Cd concentrations in the organs of wood mice. Furthermore, elevated WBC levels may indicate increased circulating granulocytes under stressful conditions, as observed in avian and mammalian species (Apanius 1998, Beldomenico et al. 2008). A reduction in WBC count indicates a decline in circulating granulocytes and a decrease in lymphocytes. This reduction could indicate a lower incidence of infection (Norris & Evans 2000).

In summary, the studied heavy metals caused damage to white blood cells, albeit with inconsistent effects across different WBC subtypes. One potential explanation for this outcome is that Cd and Pb impair the production of blood cells, prompting the body to mount defensive mechanisms.

The use of zeolites is becoming increasingly regarded as an effective method in the fight against the toxicological impact of heavy metal contamination (Dolanc et al. 2023). Clinoptilolites possess distinctive physicochemical characteristics, notably ion exchange and adsorption properties, which afford remarkable decontamination capabilities and reduce heavy metal burdens in industrial applications, animals and humans (Beltcheva et al. 2012).

The current results indicate that clinoptilolite materials benefit the concentration profile of metals in laboratory mice supplemented with the modified natural clinoptilolite. The observed decrease in the measured toxicants in the kidney and liver after 45 days of oral intake suggests a detoxification process, which may be attributable to the increase in particle size and active surface area of the zeolite material. This phenomenon has also been observed after long-term clinoptilolite administration in rats exposed to various heavy metals (Dolanc et al. 2023). The studies on haematopoiesis after clinoptilolite administration in mammals are rare. Beltcheva et al. (2022) and Topashka-Ancheva et al. (2012) demonstrated the positive effect of zeolite on the red blood cell kinetics, where the erythrocytes increased significantly. Studies on the clinoptilolite impact on small mammals' leukopoiesis are lacking. Martin-Kleiner et al. (2001) suggested the increased WBC count due to zeolite supplementation due to intestinal irritation and inflammation processes, which aligns with current research conclusions.

Conclusion

The results confirm the indirect Pb and Cd immunotoxicity. We found that Pb and Cd intoxication resulted in pathological alterations of WBC kinetics in mice, with inconsistent effects across different WBC subtypes but more pronounced in GRAN. Lead – more immunotoxic to mouse WBCs. The study on the effects of clinoptilolite administration suggested insufficient efficiency on leukopoiesis in ICR albino mice. Further investigations are needed to clarify the potential mechanisms of the clinoptilolite detoxification on the immune response to heavy metal poisoning.

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References

Apanius V. 1998.Stress and immune defense. Advances in the Study of Behavior 27: 133-153.

Beldomenico P. M., Telfer S., Gebert S., Lukomski L., Bennett M. & Begon M. 2008. The dynamics of health in wild field vole populations: a haematological perspective. Journal of Animal Ecology 77(5): 984-997.

Beltcheva M., Metcheva R., Popov N., Teodorova S., Heredia-Rojas J., Rodríguez-De La Fuente A., Rodríguez-Flores L. & Topashka-Ancheva M. 2012. Modified natural clinoptilolite detoxifies small mammal organisms loaded with lead I. Lead disposition and kinetic model for lead bioaccumulation. Biological Trace Element Research 147 (1-3): 180-188.

Beltcheva M., Metcheva R., Topashka-Ancheva M., Popov N., Teodorova S., Heredia-Rojas J. A., Rodríguez-de la Fuente A. O. & Rodríguez-Flores L. E. 2015. Zeolites versus lead toxicity. Journal of Bioequivalence and Bioavailability 7 (1): 12-29.

Beltcheva M., Ostoich P., Alexieva I. & Metcheva R. 2022. Natural zeolites as detoxifiers and modifiers of the biological effects of lead and cadmium in small rodents: a review. BioRisk 17: 147-155.

Burns R. G., Wood V. M. & Morgenstein M. E. 1990, September. Sorption of cesium and strontium by zeolite single crystals. In: Mountain Site Characterization Project Radionuclide Adsorption Workshop at Los Alamos National Laboratory. 77 p.

Cerri G., Farina M., Brundu A., Daković A., Giunchedi P., Gavini E. & Rassu G. 2016. Natural zeolites for pharmaceutical formulations: Preparation and evaluation of a clinoptilolite-based material. Microporous and Mesoporous Materials 223: 58-67.

Dethloff G.M. & Bailey H.C. 1998.Effects of copper on immune system parameters of rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 17: 1807-1814.

Directive 2010/63/EU – European Union. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union L276/33.

Djokic J., Ninkov M., Mirkov I., Aleksandrov A.P., Zolotarevski L., Kataranovski D. & Kataranovski M. 2014. Differential effects of cadmium administration on peripheral blood granulocytes in rats. Environmental toxicology and pharmacology 37 (1): 210-219.

Dolanc I., FerhatovićHamzić L., Orct T., Micek V., Šunić I., Jonjić A., Jurasović J., Missoni S., Čoklo M. & Pavelić S. K. 2023. The Impact of Long-Term Clinoptilolite Administration on the Concentration Profile of Metals in Rodent Organisms. Biology 12 (2): 193.

Dong A., He H., Jing X., Zhang T., Ma Y., Wang X., Dong H., Liu W., Fan K., Huo J. 2024.Associations of Cadmium Exposure with Peripheral Blood Cell Subtype Counts and Indices in Cadmium-Poisoned Mice.Biological Trace Element Research.Available online: https://doi.org/10.1007/s12011-024-04271-9

EEA, European Environmental Agency. 2019. Available online: https://www.eea.europa.eu/publications/emep-eea-guidebook-2019

El-Boshy M.E., Risha E.F., Abdelhamid F.M., Mubarak M.S. & Hadda T.B. 2015. Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. Journal of Trace Elements in Medicine and Biology 29: 104-110.

Gainer J. H. 1974. Lead aggravates viral disease and represses the antiviral activity of interferon inducers. Environmental health perspectives 7: 113-119.

Goyer R.A. & Rhyne B.C. 1973. Pathological effects of lead. International Review of Experimental Pathology 12: 1-77.

Gustafsson E., Mattsson A., Holmdahl R. & Mattsson R. 1994. Pregnancy in B-cell-deficient mice: postpartum transfer of immunoglobulins prevents neonatal runting and death. Biology of reproduction 51 (6): 1173-1180.

Hemphill F. E., Kaeberle M. L. & Buck W. B. 1971. Lead suppression of mouse resistance to *Salmonella typhimurium.* Science 172 (3987): 1031-1032.

Hogan G. R. & Adams D. P. 1979.Lead-induced leukocytosis in female mice. Archives of toxicology 41: 295-300.

Huang H. Y., Lin Y. C. D., Cui S., Huang Y., Tang Y., Xu J.,Bao J., Li Y., Wen J., Zuo H., Wang W., Li J., Ni J., Ruan Y., Li L., Chen Y., Xie Y., Zhu Z., Cai X., Chen X., Yao L., Chen Y., Luo Y., LuXu S., Luo M., Chiu C.M., Ma K., Zhu L., Cheng G. J., Bai C., Chiang Y. C., Wang L., Wei F., Lee T. Y. & Huang H. D. 2022. miRTarBase update: an informative resource for experimentally validated miRNA–target interactions. Nucleic acids research 50 (D1): D222-D230.

Yuan G., Dai S., Yin Z., Lu H., Jia R., Xu J., Song X., Li L., Shu Y. & Zhao X. 2014. Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. Food and chemical toxicology 65: 260-268.

Kavak D. D. & Ülkü S. 2015.Kinetic and equilibrium studies of adsorption of β-glucuronidase by clinoptilolite-rich minerals. Process Biochemistry 50 (2): 221-229.

Kataranovski M., Janković S., Kataranovski D., Stošić J. & Bogojević D. 2009. Gender differences in acute cadmium-induced system inflammation in rats. Biomedical and Environmental Sciences 22 (1): 1-7.

Koller L. D., Exon J. H. & Roan J. G. 1976.Humoral antibody response in mice after single-dose exposure to lead or cadmium. Proceedings of the Society for Experimental Biology and Medicine 151 (2): 339-342.

Langella A., Pansini M., Cappelletti P., De Gennaro B., De'Gennaro M. & Colella C. 2000. NH+ 4, Cu2+, Zn2+, Cd2+ and Pb2+ exchange for Na+ in a sedimentary clinoptilolite, North Sardinia, Italy. Microporous and Mesoporous Materials 37 (3): 337-343.

Martin-Kleiner I., Flegar-Meštrić Z., Zadro R., Breljak D., Janda S. S., Stojković R. ... & Boranić M. 2001. The effect of the zeolite clinoptilolite on serum chemistry and hematopoiesis in mice. Food and chemical toxicology, 39 (7): 717-727.

Metryka E., Chibowska K., Gutowska I., Falkowska A., Kupnicka P., Barczak K., Chlubek D. & Baranowska-Bosiacka I. 2018. Lead (Pb) exposure enhances expression of factors associated with inflammation. International journal of molecular sciences 19 (6): 1813.

Niu L., Strahotin S., Hewes B., Zhang B., Zhang Y., Archer D., Spencer T., Dillehay D., Kwon B., Chen L. & Vella A.T. 2007. Cytokine-mediated disruption of lymphocyte trafficking, hemopoiesis, and induction of lymphopenia, anaemia, and thrombocytopenia in anti-CD137-treated mice. The Journal of Immunology 178 (7): 4194-4213.

Norris K. & Evans M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behavioral Ecology 11 (1): 19-26.

Ohsawa M. & Kawai K. 1981.Cytological shift in lymphocytes induced by cadmium in mice and rats. Environmental Research, 24 (1): 192-200.

Pavelic K., Katic M., Sverko V., Marotti T., Bosnjak B., Balog T., Stojkovic R., Radacic, M., Colic M. & Poljak-Blazi M.

2002. Immunostimulatory effect of natural clinoptilolite as a possible mechanism of its antimetastatic ability. Journal of cancer research and clinical oncology 128: 37-44.

Powolny T., Scheifler R., Raoul F., Coeurdassier M., Fritsch C. 2023. Effects of chronic exposure to toxic metals on haematological parameters in free-ranging small mammals, Environmental Pollution 317: 120675.

Rehman H., Aziz A.T., Saggu S., VanWer, A.L., Zidan N. & Saggu S. 2017. Additive toxic effect of deltamethrin and cadmium on hepatic, hematological, and immunological parameters in mice. Toxicology and Industrial Health 33 (6): 495-502.

Shenker B.J., Matarazzo W.J., Hirsch R.L. & Gray I. 1977. Trace metal modification of immunocompetence: I. Effect of trace metals in the cultures on in vitro transformation of B lymphocytes. Cellular immunology 34 (1): 19-24.

Snoeijs T., Dauwe T., Pinxten R., Vandesande F. & Eens M. 2004. Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (*Parus major*). Archives of Environmental Contamination and Toxicology 46: 399-404.

Stockham S.L. & Scott M.A. 2002.Fundamentals of veterinary clinical pathology. Ames. Iowa State Press, 438, pp.446-450.

Stowe H.D., Wilson M. & Goyer R.A. 1972.Clinical and morphological effects of oral cadmium toxicity in rabbits. Arch. Path 94: 389.

Tête N., Afonso E., Bouguerra G. & Scheifler R. 2015. Blood parameters as biomarkers of cadmium and lead exposure and effects in wildwood mice (Apodemussylvaticus) living along a pollution gradient. Chemosphere 138: 940-946.

Todorova T., Alexieva I., Ostoich P., Dimitrova M., Boyadzhiev K., Lyubomirova V., Beltcheva M. 2023. Different Lead- and Cadmium-Induced Oxidative Stress Profiles in the Liver and Kidneys of Subchronically-Exposed Mice. Acta Zoologica Bulgarica 75 (3): 343-349.

Tomečková V., Reháková M., Mojžišová G., Wadsten T., Zelenáková K. & Komanický V. 2016.Spectral study of modified natural clinoptilolite with pharmacologically active escin and horse chestnut extract. Spectroscopy Letters 49 (2): 63-72.

Topashka-Ancheva M., Beltcheva M., Metcheva R., Rojas J., Rodriguez-De La Fuente A., GerasimovaTs., Rodríguez-Flores L. & Teodorova S. 2012. Modified natural clinoptilolite detoxifies small mammal organisms loaded with lead II: Genetic, cell, and physiological effects. Biological Trace Element Research 147 (1-3).

Utakoji T. 1972. Differential staining patterns of human chromosomes treated with potassium permanganate. Nature 239 (5368): 168-170.

Włostowski T., Dmowski K. & Bonda-Ostaszewska E. 2010. Cadmium accumulation, metallothionein and glutathione levels, and histopathological changes in the kidneys and liver of magpie (Pica pica) from a zinc smelter area. Ecotoxicology 19: 1066-1073.

Zelicoff J. T. & Thomas P. 1998. Immunotoxicology of environmental and occupational metals.CRC Press.

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