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Morphological Alterations In Blood Crystals Under The Influence Of Heavy Metals: A Comparative Study In Three Experimental Groups

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Abstract: In recent years, the impact of heavy metals in the environment on animal and human health has received considerable attention. Heavy metals can adversely affect various physiological systems, including the hematological system. This study investigated structural changes in blood plasma in the form of crystal morphology resulting from exposure to heavy metals. The toxic effects of lead, mercury, cadmium, and cobalt on animal organisms were assessed. During the study, the effects of heavy metals on blood composition, red blood cells, their morphology, and functions were analyzed.

In this study, juvenile male Wistar rats, weighing 60–70 g, were administered a diet containing heavy metals for a duration of 21 days. At the end of this period, blood samples were collected from the animals and analyzed under laboratory conditions. The shape of erythrocytes, their aggregation, and signs of hemolysis were examined. Additionally, changes in the color and density of blood plasma were recorded. Morphological alterations in blood crystals were analyzed as indicators reflecting the physiological impact of heavy metals.

In the experimental groups, alterations in the morphology of blood crystals were observed. In blood samples from lead-exposed rats, large and irregular protein aggregates appeared in the central zone, symmetry was disrupted in the radial area, and uneven fissures were observed in the periphery. In rats exposed to mercury, deposits were observed in the central zone, interrupted lines in the radial area, and large crystals formed in the periphery. The number of crystals in the blood of exposed animals increased significantly. In the control group, blood crystals were normal, flat, and disk-shaped, with no deformation or aggregation observed. Blood crystals in healthy rats were ordered and smooth, indicating preserved protein–electrolyte balance. In the lead- and mercury-exposed groups, central aggregates, radial disruptions, and irregular peripheral crystals reflected disturbed protein–lipid balance and impaired cellular metabolism. These results indicate that blood crystal morphology can serve as a sensitive and diagnostic tool for evaluating toxic stress and the effects of heavy metals.

Keywords: Heavy metal, blood cell, protein, denaturation, synergetics, microscope, crystallization.

Introduction: Environmental contamination with heavy metal ions has led to serious changes in recent years. In biological systems, heavy metal ions entering through the food chain cause significant alterations in

the organisms of humans and animals. Among heavy metals, elements such as lead, mercury, copper, zinc, and cadmium accumulate in living organisms and adversely affect physiological functions. These elements primarily damage the blood, liver, kidneys, heart, and nervous system [26]. Blood is the most vital fluid of the organism, and changes occurring in its composition provide an opportunity to evaluate the impact of toxic substances. Heavy metal ions decrease the activity of cell membranes in blood cells, cause denaturation of hemoglobin molecules, and lead to the formation of hemoglobin crystals [15]. In recent years, the field of biophysics, biology, ecology, and medicine has introduced the concept of synergetics—the study of how complex systems can self-organize into structured formations and functions without external influence or control. This concept, first introduced to science by Hermann Haken in the 1970s, is now applied in studying cellular, tissue, and fluid (e.g., blood plasma) structures in biological systems.

Heavy metals affect animal organisms to varying degrees. Their effects occur acutely and cause several changes in the blood plasma. In livestock, metals such as copper, lead, zinc, and cadmium alter the albumin-to-globulin ratio in blood plasma. This has been proven to disrupt liver function and decrease the immune system's efficiency [20].

METHODS

For the experiment, 18 healthy male laboratory rats (Rattus norvegicus) weighing 60–70 g were selected. The animals were randomly divided into three experimental groups:

1.Healthy (control)

2.Almond-contaminated

3.Corn-contaminated

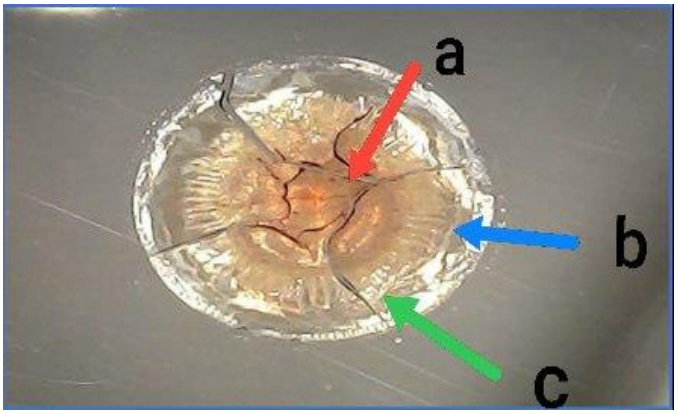
Each animal was fasted for 12 hours before the experiment but provided with drinking water. Blood samples were collected from the jugular vein using sterile syringes. During the sampling process, veterinary procedures were followed to minimize stress and pain [11]. No anticoagulants were used in the blood samples. The entire procedure was conducted in accordance with international Helsinki ethical standards [29]. From each sample, 20–30 µL of blood was placed at the center of a sterile glass slide using a micropipette. The drops were dried under natural conditions for 18–24 hours at a temperature of 22–25°C and relative humidity of 40–50% in a dust-free environment [5]. The dried blood drops were examined using a USB digital microscope at 40× magnification. Each drop's central (nuclear), radial, and peripheral zones were analyzed separately. Images were captured with a digital camera and analyzed using ImageJ software [3].

Morphological parameters—such as crack length, number of radial lines, and crystal density (%)—were determined and compared among the healthy, corn-contaminated, and almond-contaminated groups. Numerical data were processed in Excel, and differences between groups were evaluated using Student's t-test ($p < 0.05$ considered statistically significant).

The methodology was based on the blood drop drying and crystal morphology evaluation method developed by N.V. Shatenshtein (1952) and the pathocrystalloscopy principles of N.V. Shabalin and S.N. Shatokhina (2001) [33].

RESULTS AND DISCUSSION

In recent years, the study of crystallization processes in biological materials and the analysis of their morphology and structural properties have become an important direction in scientific research. The shape, size, and structure of crystals are directly related to the physicochemical state of the substance and external environmental factors [31]. The dried blood drops formed crystalline structures consisting of central, radial, and peripheral zones. Morphological variations in crystals are of significant diagnostic importance, as crystal shapes reflect the physiological or toxic state of the organism. By comparing the morphology of crystals obtained from different experimental groups, internal structural differences could be identified [4]. In this study, the distinctions between three crystal samples were analyzed, and their morphological variations were examined comparatively.



This approach provides new methods for detecting structural changes occurring in biological systems and assessing the effects of toxic factors [9].

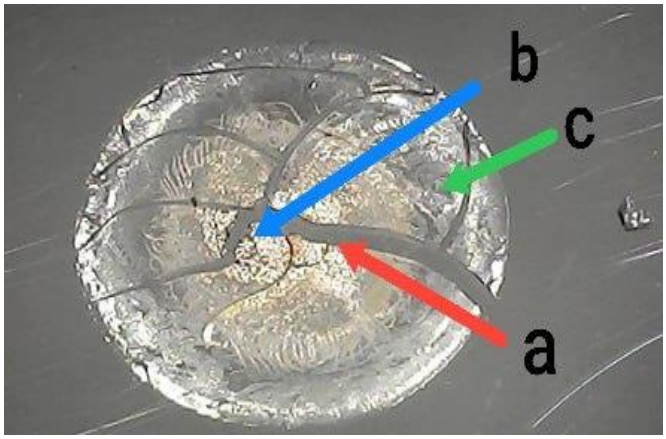
Figure1. Control group

a radial zone b-cenral zone c-periferic zone

In the blood samples of healthy rats, the results of

crystallographic analysis showed clearly contoured protein aggregates in the central zone, regular and symmetrical crystal growth in the radial zone, and uniformly thin cracks in the peripheral region [14]. This morphological structure indicates the biochemical stability of the blood, particularly the preservation of protein–electrolyte balance [18]. The presence of smooth crystal edges and orderly arrangement reflects the optimal state of plasma components and represents the outcome of homeostatic processes under physiological norm [6]. This condition confirms that, in healthy individuals, denaturation processes in the molecular and colloidal systems of the blood occur at a minimal level.

Recent studies have shown that the crystal structure of blood is highly sensitive to the ratio of proteins, lipids,



and trace elements, and even minor alterations are reflected in the morphology of the crystals. Therefore, the crystallographic method is increasingly recognized as an effective diagnostic tool for assessing the general physiological state of biological fluids [16, 22].

Figure2. Corn fed groups

a-radial zone b-central zone c-periferic zone

In the blood samples of rats poisoned with corn extract, the formation of large and irregular protein aggregates was observed in the central zone. These aggregates may be associated with erythrocyte degradation and the denaturation of protein–lipid complexes in the plasma [19]. In the radial zone, a loss of symmetry was detected. This observation, along with the appearance of dendritic and fractal-type growth structures, indicates that the colloidal properties of the blood have been disrupted [17, 32]. In the peripheral region, irregular cracks and dense network-like structures were formed. These findings suggest an imbalance in the protein–lipid structures of the blood plasma. The emergence of these characteristics indicates that the viscosity of blood plasma has been disturbed under the influence of heavy metal ions [13, 24, 23].

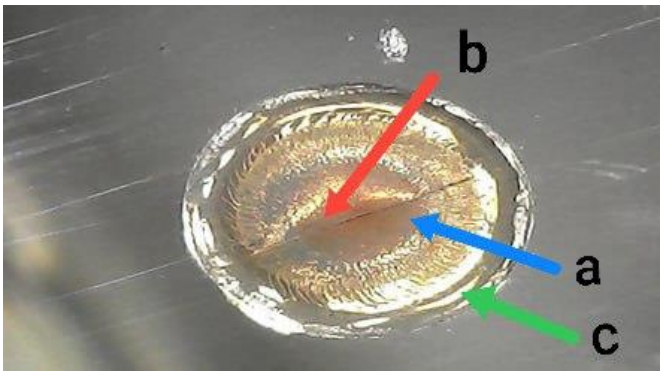


Figure3. Almond fed group

a-radial zone b-central zone c-periferic zone

Heavy metals (Pb, Hg, Cd) bind to the sulfhydryl (-SH) groups of proteins and enzymes within cells. Almonds, especially bitter almonds, contain a compound called amygdalin, which is converted into cyanide in the organism. Cyanide inhibits cellular respiration and blocks the utilization of oxygen in the body [1, 2]. As a result, cellular damage progresses gradually.

In the group of animals fed with almond-based feed containing heavy metals, precipitates were observed in the central zone, disrupted linear structures appeared in the radial zone, and large, distinctly visible crystal formations were formed in the outer peripheral region [3]. The irregular orientation of cracks indicates an alteration in the diffusion gradient during the drying process. This phenomenon is manifested due to physicochemical transformations of protein–ion complexes [30, 12].

CONCLUSION

The results of the conducted experimental study showed that during the drying process of blood samples from animals poisoned with heavy metal ions (Pb^{2+}, Cd^{2+}, Hg^{2+}) and plant toxins, the resulting crystal structures exhibited irregular, asymmetrical, and fractal morphologies. Such alterations are manifested in the physicochemical properties of the blood, including changes in pH levels and protein denaturation [7, 28].

In contrast, the blood drops of healthy animals demonstrated highly symmetrical, radially oriented, and clearly contoured crystal structures. This morphological organization indicates the stability of colloidal systems in the blood plasma and reflects a balanced state of proteins and ions [30, 21].

The method of blood drop drying and crystal morphology analysis can serve as a biophysical

diagnostic test that enables the early detection of toxic processes in the organism. It is cost-effective, rapid, and highly sensitive, making it a promising tool for physiological and toxicological assessments [27, 25, 8].

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