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Protective role of Spirulina against lead-induced genotoxicity in mice evaluated by the comet assay

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Abstract

Objective

This investigation aimed to evaluate the genotoxic effects of lead (Pb) exposure in murine blood cells and to evaluate the protective potential of spirulina against lead-induced DNA damage applying the alkaline single-cell gel electrophoresis (comet) assay.

Materials and Methods

Eighty mice were randomly assigned to four experimental groups: control (standard drinking water), Pb-exposed (0.01 mg/L lead acetate in drinking water), spirulina-only (3.0 g/kg spirulina in drinking water), and combined Pb + spirulina group. The treatment period lasted three months. Blood samples were gathered post-treatment, and DNA strand breaks in leukocytes were analyzed applying the comet assay. The slides were prepared applying three layers of agarose, lysed, and subjected to electrophoresis under alkaline conditions. Nuclei were stained with ethidium bromide, and DNA damage was quantified applying fluorescence microscopy and Comet Assay Software Project (CASP).

Results

Lead exposure meaningfully improved the percentage of cells exhibiting medium and high DNA damage compared to controls, denoting pronounced genotoxic effects. The Pb group showed a sharp decrease in undamaged cells (63.20 ± 4.61%) and a marked improve in highly damaged cells (28.00 ± 2.99%). In contrast, the Pb + spirulina group demonstrated a notable reduction in

DNA damage, with 60.30 ± 5.65% of cells showing no damage and only 26.00 ± 2.98% exhibiting high damage. The protective effect of spirulina was statistically meaningful when compared to the Pb-only group, bringing DNA damage levels close to those observed in the control group. These results align with previous reports suggesting that spirulina's antioxidant properties can neutralize free radicals, limit oxidative stress, and stabilize DNA integrity.

Conclusions

Chronic exposure to low-dose lead acetate induces substantial DNA damage in murine blood cells, primarily through oxidative mechanisms and interference with DNA repair systems. However, spirulina supplementation at 3.0 g/kg effectively mitigates this damage, highlighting its role as a protective agent against lead-induced genotoxicity. These results underscore the potential of spirulina as a natural intervention for reducing the harmful effects of environmental lead exposure. Further investigations are recommended to investigate spirulina's effectiveness across varying dosages and exposure durations, and its possible application in public health strategies aimed at combating heavy metal toxicity.

Keywords: Comet assay, DNA damage, lead toxicity, oxidative stress, Spirulina

Paper Type: Research Paper.

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Introduction

Lead (Pb), a ubiquitous and hazardous heavy metal, stays a meaningful public health concern due to its toxicity, even at exposure levels previously deemed safe (Ali et al., 2024). In industrialized regions, individuals face increasing levels of ambient lead contamination. The World Health Organization (WHO) recommends a maximum blood lead concentration of 40

µg/dL for occupational exposure when lead accumulation exceeds critical thresholds (Salman & Dawood, 2021). Recent investigations have revealed novel insights into lead's genotoxicity, demonstrating its capability to disrupt DNA damage repair mechanisms, leading to comutagenicity at lower concentrations and direct mutagenicity or blastogenesis at higher levels (Golbamaki et al., 2015). Lead has been shown to yield positive results in assays evaluating enzyme prevention, DNA synthesis fidelity, mutagenesis, chromosomal aberrations, carcinogenesis, and teratogenicity (Kayaaltı et al., 2015). In developed regions, escalating ambient lead contamination poses meaningful risks, exclusively when concentrations reach critical thresholds (Pan et al., 2022; Saber et al., 2015). Lead generates reactive oxygen species (ROS) that damage DNA, creating base alterations, substitutions, alkali-labile sites, and single- or double-strand breaks (Kumar et al., 2023). The alkaline single-cell gel electrophoresis (SCGE) assay, commonly known as the comet assay, is a highly sensitive method for evaluating DNA damage in blood leukocytes. Its capability to detect genetic damage at the individual cell level across nearly all eukaryotic cell types has made it a valuable tool for short-term genotoxicity experimenting and human biomonitoring (Alyasiri et al., 2018; Cordelli et al., 2021). In vivo investigations of lead compounds have generated inconsistent results, with some demonstrating meaningful DNA damage in mice exposed to lead nitrate (Sharma & Sharma, 2024). Spirulina, a nutrient-rich cyanobacterium, is distinguished for its potent antioxidant properties, which protect proteins, DNA, and chromosomes from oxidative damage and neutralize free radicals (Hassan et al., 2024; Martemucci et al., 2022). Investigations suggest that spirulina may prevent lead absorption and accumulation, reduce lipid peroxidation, and prevent degradation of DNA, RNA, and proteins (Salem & Ismail, 2021; Al-Obaidi et al., 2021; Atiyah & Hamod, 2021). The investigation of DNA damage in animals is critical, as it impacts lifespan, health, and species survival (Mohammadabadi et al., 2024). Such research enhances our understanding of how animals respond to genetic damage, providing insights that can improve human health and support the conservation of endangered species (Nejad et al., 2024). DNA damage contributes to premature aging, numerous diseases, and reduced lifespan in animals. By studying these effects, we can identify protective mechanisms against aging and disease. Efficient DNA repair systems in some animals confer resistance to environmental stressors and extend longevity. Investigating DNA damage and repair mechanisms can inform genetic engineering strategies to enhance desirable traits, like disease resistance or extended lifespan (Barazandeh et al., 2016). For endangered species, understanding DNA damage and repair capabilities can guide conservation efforts to prevent extinction (Mohammadabadi et al., 2010). Furthermore, studying the DNA of extinct species may enable their revival applying advanced genetic technologies in the future. This investigation aims to investigate the impact of lead on DNA damage in murine blood cells

and evaluate the protective effects of spirulina against lead toxicity applying the single-cell gel electrophoresis (comet) assay.

Materials and methods

Experimental design: This experimental investigation evaluated the protective effects of spirulina on mice exposed to lead acetate in heavy metal-contaminated drinking water. Eighty mice were housed in plastic containers calculating 20 × 50 × 75 cm in a designated area of the Department of Public Health, College of Veterinary Medicine, University of Baghdad. Following a one-week acclimatization period, mice were prepared tap water and commercial feed pellets, constituting the standard diet, without restriction. The living conditions were maintained in climate-controlled chambers at 20–25°C with a 10–14-hour light/dark cycle. A ventilator was consistently applied to exchange air in the rooms, and waste in the containers was replaced daily to ensure hygiene. The mice were divided into four groups: Group 1 served as the control, receiving standard drinking water; Group 2 received drinking water containing 0.01 mg/L lead acetate dissolved in distilled water; Group 3 received drinking water supplemented with 3.0 g/kg water-soluble spirulina; Group 4 received simultaneous administration of 0.01 mg/L lead acetate and 3.0 g/kg spirulina in their drinking water. The spirulina suspension was prepared to ensure uniform incorporation into the drinking water, maintaining the specified dosage throughout the investigation period.

Blood serum collection: The treatments were administered for three months. Each mouse was anesthetized applying chloroform, and blood samples were gathered via direct cardiac puncture. The blood was transferred to sterile, dry, and clean gel tubes and permited to clot at room temperature for a brief period. The samples were then centrifuged at 4000 rpm for 15 minutes to separate the clear sera. Applying a micropipette, the sera were transferred to Eppendorf tubes, which were stored in a deep freezer at -20°C until subsequent biochemical experiments were managed.

Detection of DNA strand breaks: DNA strand breaks in murine blood cells were quantified applying the single-cell gel electrophoresis (SCGE) assay, commonly referred to as the comet assay. Slides were initially coated with 100 µL of 0.5% normal agarose in phosphate-buffered saline (PBS) and stored at 4°C for 15 minutes to solidify. A mixture of 750 µL of 1% low-melting-point agarose at 37°C and 100 µL of blood was prepared, and 85 µL of this mixture was applied as a second layer on the slide. A third layer of 75 µL of 1% low-melting-point agarose was added applying a similar procedure. The slides, prepared at the Department of Public Health, College of Veterinary Medicine, University of Baghdad, were immersed in a freshly prepared lysis solution

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containing 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl (pH 10.0, adjusted with NaOH), 1% N-lauroyl sarcosine, 1% Triton X-100, and 10% DMSO for 2–3 hours. Subsequently, the slides were placed in an electrophoresis buffer (1 mM Na_2EDTA, 300 mM NaOH) for 20 minutes to permit DNA unwinding, followed by electrophoresis at 25 V and 40 mA for 20 minutes. The slides were neutralized, and the nuclei were stained with 50–100 µL of ethidium bromide (5 mg/L). A fluorescence microscope was applied to calculate the extent of DNA damage. Across at least two-thirds of the gel slide surface, the numbers of viable and compromised cells (DNA comets) were enumerated to identify the proportion of damaged cells. Two independent blood samples from each mouse were analyzed to ensure robust data collection.

Results and discussion

Table 1 and Figure 1A show the impact of lead exposure on DNA damage in murine blood cells, showing a meaningful difference in the percentage of damaged cells between the lead-exposed group and the control group. These results indicate that the blood cells of mice were adversely affected by lead administered at a concentration of 0.01 mg/L in drinking water. The extent of lead-induced DNA damage observed in this investigation is consistent with the results announced by Ghazi and Al-Qaiym (2023) and AlSalman and Dawood (2023). Lead exposure is known to generate free radicals, which can induce DNA strand breaks and lead to alterations in genetic material.

Table 1. Percentages of DNA damage evaluated by the comet assay in murine blood cells

Groups	No Damage (%)	Low Damage (%)	Medium Damage (%)	High Damage (%)
Control	90.00 ± 2.55^{a}	3.50 ± 4.50^{b}	3.00 ± 1.70^{c}	3.50 ± 1.40^{b}
Pb	63.20 ± 4.61^{b}	2.80 ± 1.01^{b}	6.00 ± 1.20^{bc}	28.00 ± 2.99^{a}
Spirulina	91.00 ± 5.12^{a}	4.00 ± 0.90^{c}	3.00 ± 1.04^{c}	2.00 ± 0.80^{b}
Pb + Spirulina	60.30 ± 5.65^{b}	9.30 ± 0.98^{a}	4.70 ± 0.98^{c}	26.00 ± 2.98^{a}

*** Different superscript letters within the same column indicate statistically meaningful differences ($p < 0.05$).**

Statistical analysis of the data showed in Table 1 indicates that *Spirulina* meaningfully reduced lead-induced toxicity in mice co-administered with lead and *Spirulina*. The percentage of DNA damage, as evaluated by the comet assay, in the Pb + *Spirulina* group was comparable to that of the control group, consistent with the results of Chu (2011). Nakata et al. (2021) announced that *Spirulina* supplementation mitigates lead poisoning in mice, exclusively when

administered via drinking water following lead exposure—a result further corroborated by Sorelle et al. (2023). These investigations, together with our current results, affirm that *Spirulina* functions as an effective protective agent against lead toxicity. Gargouri et al. (2016) also support the role of *Spirulina* in neutralizing free radicals, thereby preventing lead-induced DNA damage. As shown in Table 1, the extent of DNA damage in the Pb + *Spirulina* group was meaningfully lower than that observed in the Pb-only group, further reinforcing the protective effects of *Spirulina* supplementation. These results are consistent with previous investigations demonstrating that *Spirulina* reduces DNA damage and offers protection against environmental lead toxicity (Gargouri et al., 2020).

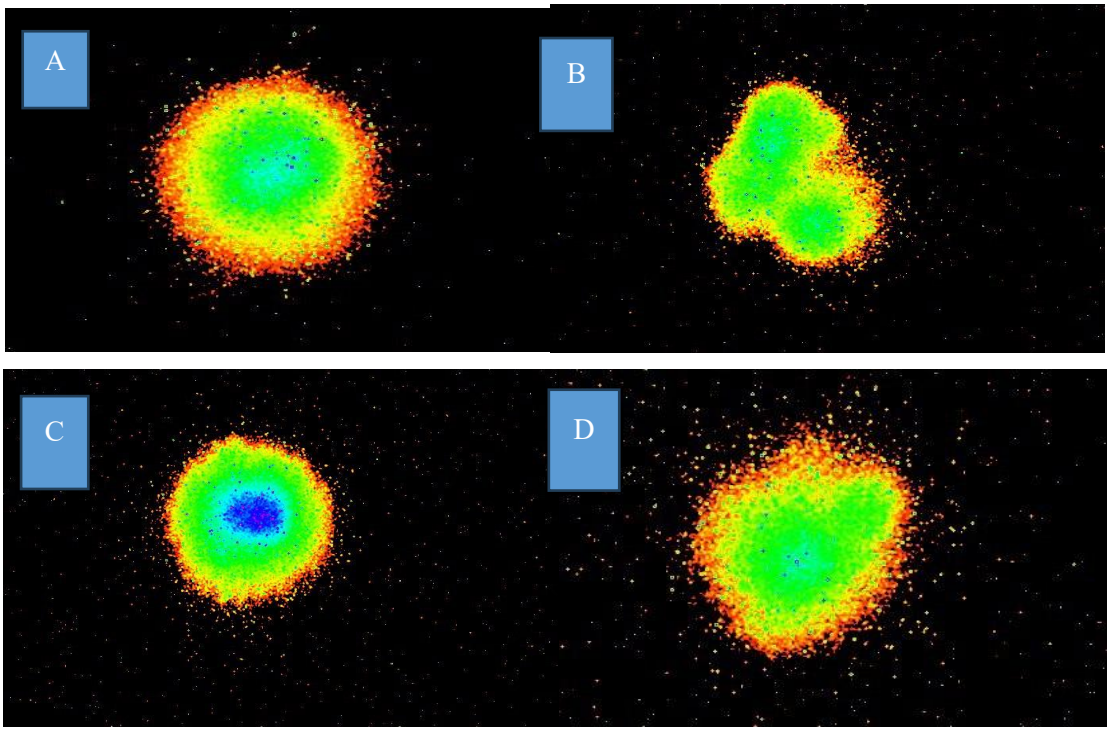

Figure 1. Alkaline comet assay images showing varying levels of DNA damage in white blood cells of mice after three months of treatment. (A) Negative control, (B) 0.01 mg/L lead (Pb), (C) 3.0 g/kg spirulina, (D) combination of 0.01 mg/L Pb and 3.0 g/kg spirulina in drinking water. Images were acquired at 200× magnification and analyzed applying the Comet Assay Software Project (CASP) (Końca et al., 2003).

Lead (Pb) is known to form covalent bonds with triphosphate groups in DNA and proteins, potentially preventing the synthesis of nucleic acids and proteins. Previous investigations have demonstrated that heavy metals can alter the molecular structure of DNA. Specifically, Pb exhibits a high affinity for phosphate groups in the DNA backbone, which may induce structural

distortions and genomic instability, potentially resulting in mutations. These interactions are believed to underlie the mechanism by which $Pb(NO_3)_2$ induces cleavage of single-stranded DNA. Additionally, Pb may prevent DNA repair processes by interfering with essential repair enzymes (Breaker & Joyce, 1994). Lead has also been shown to disrupt enzymatic repair mechanisms, thereby impairing DNA replication and repair pathways. Its strong affinity for sulfhydryl groups can improve lipofuscin synthesis, promote lipid peroxidation, and deplete intracellular glutathione levels, all of which contribute to compromised DNA integrity (Xiong et al., 2021). Data from our comet assay affirm that $Pb(NO_3)_2$ acts as a genotoxic agent, inducing meaningful DNA damage. Given the widespread industrial and environmental apply of lead and its compounds, proper handling and regulation are critical to minimizing its genotoxic effects.

Conclusions: This investigation prepares compelling evidence that chronic exposure to lead (Pb) at a concentration of 0.01 mg/L in drinking water induces meaningful DNA damage in murine blood cells. The comet assay results revealed a marked improve in medium and high levels of DNA damage in the Pb-exposed group compared to the control, underscoring lead's genotoxic potential even at low environmental concentrations. The mechanism of this genotoxicity is likely multifactorial, involving Pb's high affinity for phosphate and sulfhydryl groups, which can disrupt DNA structure, interfere with enzymatic repair pathways, and intensify oxidative stress at the cellular level. Importantly, our results demonstrate that co-administration of *Spirulina platensis* at a dose of 3.0 g/kg effectively ameliorated lead-induced DNA damage. The Pb + *Spirulina* group exhibited DNA damage levels statistically comparable to those of the negative control, supporting the hypothesis that *Spirulina* acts as a potent protective agent. Its rich antioxidant profile, containing phycocyanin, vitamins, and essential trace elements, likely contributes to its capability to scavenge free radicals, maintain redox balance, and enhance DNA repair processes. These results align with and expand upon previous investigations that have highlighted the protective effects of *Spirulina* against heavy metal toxicity. Furthermore, our investigation adds valuable evidence supporting the apply of dietary supplements like *Spirulina* as potential countermeasures against environmental genotoxins. Given the increasing global concern over lead pollution in water, soil, and food sources, the implications of these results are far-reaching. There is a critical need for cost-effective, natural, and accessible interventions to mitigate the health risks posed by lead exposure. *Spirulina*, being a broadly available and nutritionally valuable microalga, shows a hopeful candidate for such interventions, especially in vulnerable populations with high environmental exposure. Future research should aim to elucidate the precise molecular pathways involved in *Spirulina*'s protective effects, evaluate long-term outcomes in different tissues and organ systems, and evaluate its effectiveness in combination with other natural antioxidants or

chelators. Additionally, human epidemiological and clinical investigations are needed to validate the translational potential of these results in real-world settings. In conclusion, this investigation reinforces the urgent need for proactive strategies to combat the genotoxic effects of environmental lead exposure and positions *Spirulina platensis* as a viable, natural bio-protective agent worthy of further investigation.

Author contributions

A.A.Z.S. conducted the laboratory experiments and documented the results. M.F.H. and A.A.Z.S. contributed to data analysis, manuscript writing, and conceptual development. All authors reviewed and approved the final version of the manuscript.

Data availability statement

The datasets generated and analyzed through the current investigation are available from the corresponding author upon reasonable request.

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Ethical Considerations

All animal procedures were conducted in accordance with ethical guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Baghdad (UM.VET.2024.141).

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Conflict of Interest

The authors declare no conflicts of interest related to this investigation.

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نقش محافظتی اسپیرولینا در برابر ژنوتوکسیسیتی ناشی از سرب در موش‌ها با استفاده از آزمون کومِت

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چکیده

هدف: این مطالعه با هدف بررسی اثرات ژنوتوکسیک مواجهه با سرب (Pb) در سلول‌های خونی موش‌ها و ارزیابی توان محافظتی اسپیرولینا در برابر آسیب DNA ناشی از سرب با استفاده از آزمون الکتروفورز ژل سلول منفرد قلیایی (آزمون کومِت) انجام شد.

مواد و روش‌ها: هشتاد موش به‌صورت تصادفی به چهار گروه آزمایشی تقسیم شدند: گروه کنترل (آب آشامیدنی استاندارد)، گروه مواجهه با سرب (۰/۰۱ میلی‌گرم/لیتر استات سرب در آب آشامیدنی)، گروه اسپیرولینا (۳ گرم/کیلوگرم اسپیرولینا در آب آشامیدنی) و گروه ترکیبی سرب + اسپیرولینا. دوره درمان سه ماه به طول انجامید. پس از پایان درمان، نمونه‌های خون جمع‌آوری و شکست‌های رشته‌ای DNA در لکوسیت‌ها با استفاده از آزمون کومت تحلیل شدند. اسلایدها با سه لایه آگارز تهیه، لیز و تحت الکتروفورز قلیایی قرار گرفتند. هسته‌ها با اتیدیوم بروماید رنگ‌آمیزی شدند و میزان آسیب DNA با استفاده از میکروسکوپ فلورسانس و نرم‌افزار CASPاندازه‌گیری شد.

نتایج: مواجهه با سرب به‌طور معنی‌داری درصد سلول‌های دارای آسیب متوسط و شدید DNA را نسبت به گروه کنترل افزایش داد که بیانگر اثرات ژنوتوکسیک شدید بود. در گروه سرب، کاهش چشمگیری در سلول‌های بدون آسیب (۶۳/۲۰±۴/۶۱) و افزایش قابل‌توجهی در سلول‌های با آسیب شدید (۲۸/۰۰±۲/۹۹) مشاهده شد. در مقابل، گروه سرب + اسپیرولینا کاهش قابل‌توجهی در آسیب DNA نشان داد، به‌طوری‌که ۶۰/۳۰±۵/۶۵ درصد از سلول‌ها بدون آسیب و تنها ۲۶/۰۰±۲/۹۸ دارای آسیب شدید بودند. اثر محافظتی اسپیرولینا از نظر آماری در مقایسه با گروه تنها سرب معنی‌دار بود و میزان آسیب DNA را به سطحی نزدیک به گروه

کنترل رساند. این نتایج با گزارش‌های قبلی هم‌راستا هستند که نشان می‌دهند خواص آنتی‌اکسیدانی اسپیرولینا می‌تواند رادیکال‌های آزاد را خنثی کرده، استرس اکسیداتیو را محدود نموده و یکپارچگی DNA را حفظ کند.

نتیجه‌گیری: مواجهه مزمن با دوز پایین استات سرب باعث آسیب قابل توجه به DNA سلول‌های خونی موش‌ها می‌شود، که عمدتاً از طریق مکانیزم‌های اکسیداتیو و تداخل در سیستم‌های ترمیم DNA اتفاق می‌افتد. با این حال، مکمل‌یاری با اسپیرولینا به میزان ۳.۰ گرم/کیلوگرم به‌طور مؤثری این آسیب را کاهش می‌دهد و نقش آن را به‌عنوان یک عامل محافظ در برابر ژنوتوکسیسیتی ناشی از سرب برجسته می‌سازد. این نتایج بر پتانسیل اسپیرولینا به‌عنوان یک مداخله طبیعی برای کاهش اثرات مضر مواجهه محیطی با سرب تأکید دارند. مطالعات بیشتر برای بررسی اثربخشی اسپیرولینا در دوزها و دوره‌های مختلف مواجهه، و کاربرد احتمالی آن در راهبردهای بهداشت عمومی برای مقابله با سمیت فلزات سنگین توصیه می‌شود.

کلمات کلیدی: آزمون کومت، آسیب DNA، سمیت سرب، استرس اکسیداتیو، اسپیرولینا

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