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Comprehensive Assessment of Lead Bioaccumulation in *Helix aspersa* (Müller, 1774), Snails: A Study of Histopathological and Biochemical Impacts

S. Nadji[1], F. Boulkenafet[1], S. Benzazia[1], L. Mellahi[1], F. Bououza[1], F.A. Al-Mekhlafi[2], M.S. Al-Khalifa[2], S. Lambiase[3]

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ABSTRACT

Background: The snail *Helixa spersa* is considered a relevant bioindicator of soil pollution by metallic elements due to its resistance and accumulation capabilities.

Methods: This study aims to determine the dose-response relationship between different concentrations of lead and its toxic effects on juvenile *H. aspersa* through a semi-static ecotoxicity test under controlled conditions. In addition, continuous monitoring of carbohydrates, lipids and proteins of the snails affected by this metal was evaluated along with a histopathological study of the hepatopancreas.

Result: The two-month lead exposure in *H. aspersa* resulted in significant alterations in biochemical and histological parameters. Lead concentrations in the hepatopancreas and foot exhibited a dose-dependent increase, with higher levels observed in the former. Elevated lead concentrations (1000 µg/g and 1500 µg/g of soil) led to substantial protein increase in both organs, while even lower doses displayed considerable protein elevation in the hepatopancreas. Carbohydrate levels were significantly lower in all contaminated groups compared to controls. Increasing lead concentrations caused a reduction in lipid levels. Histological analysis revealed distinct alterations, including excretory cell hypertrophy, tubule clustering and inflammatory changes in the hepatopancreas of treated snails. Severe histological damage, including inflammatory infiltrates, cellular debris and necrosis, were observed at higher lead concentrations (1000 and 1500 µg/g). These findings emphasize the impact of lead exposure on biochemical profiles and organ histology in *H. aspersa*, highlighting the potential ecological implications of heavy metal contamination.

Key words: Accumulation, Foot, *Helix aspersa*, Hepatopancreas, Lead, Soil.

INTRODUCTION

Soil contamination has been a prominent focus of research and efforts to address it for the past two decades (Byrne, 2021). Soils concentrate all emitted pollutants, including metallic elements (Facchinelli *et al.*, 2001). The presence of heavy metals in soils is particularly problematic due to their non-biodegradability compared to organic pollutants and their toxicity (Olaniran *et al.*, 2013). They have a toxicological impact on plants, daily consumer products and humans (Gove *et al.*, 2001). These pollutants are considered hazardous to the environment due to their persistence and ability to bioaccumulate in living organisms (Ali *et al.*, 2019)

In both aquatic and terrestrial habitats, invertebrates have long been used to evaluate the quality of ecosystems. as members of terrestrial ecosystems, snails can integrate many sources of pollution (soil, atmosphere, vegetation) via several routes (digestive, respiratory and/or cutaneous). Small grey snails (*H. aspersa*) are important ecological indicators of metal and organic pollution in the terrestrial environment, using biomarkers as a natural biological tool to signal pollution and harmful chemical bioavailability. They are complementary tools for assessing ecosystem quality (McCarthy and Shugart, 1990). As a result, they enable early detection of physiological dysfunctions in individuals, identifying the harmful effect before it becomes visible (Van der Oost *et al.*, 2003).

H. aspersa has been widely used as a biological model to evaluate the toxic effects of different soil pollutants (Gimbert *et al.*, 2006, De Vaufleury *et al.*, 2006, Druart *et al.*, 2011). Therefore, *H. aspersa* is used in the present study to

[1]Department of Natural and Life Sciences, University of 20th August 1955 Skikda, 21000 Skikda, Algeria.
[2]Department Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[3]Department of Public Health, Experimental and Forensic Medicine, University of Pavia, 27100 Pavia, Italy.

Corresponding Author: F. Boulkenafet, Department of Natural and Life Sciences, University of 20th August 1955 Skikda, 21000 Skikda, Algeria. Email: f.boulkenafet@univ-skikda.dz

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evaluate the lead accumulation in the foot and hepatopancreas of the juvenile snails under controlled laboratory conditions. Furthermore, the biochemical composition (proteins, carbohydrates and lipids) in the two considered organs are examined with histological study of the hepatopancreas.

MATERIALS AND METHODS

Following ISO (2018) criteria, a semi-static ecotoxicity test was conducted During the period from March to April 2022, in the Animal Biology Laboratory of the Department of Natural and Life Sciences at the University of Skikda. Juvenile snails were exposed to lead-contaminated soil at varying concentrations (100, 500, 1000 and 1500 μg/g of soil). The snails were placed in polystyrene boxes with 100 g of dry soil covering the bottom, which was then moistened to 50% of its water-holding capacity. The specimens were divided into two groups: control groups and groups treated with the tested concentrations, each group with 10 individuals. The soil, the sole source of contamination, was renewed once a week throughout the test duration. After the experimental period, the snails were euthanized and dissected. The hepatopancreas and foot tissues were collected for lead and metabolite assessment and sections of the hepatopancreas were prepared for histological examination.

Lead analysis

The hepatopancreas and foot tissues were individually placed in screw-cap tubes and then dried in the oven at 80°C for 24 hours. Subsequently, the dried fragments were weighed and 4 ml of 65% nitric acid were added to each tube. The tubes were carefully sealed and placed in the oven at 60°C for 72 hours to complete tissue digestion under pressure. Following digestion, each sample was diluted to a volume of 19 ml with distilled water and stored at 4°C until analysis (Cœurdassier, 2001). Metal concentrations in the various samples were determined using inductively coupled plasma atomic emission spectrophotometry (ICP-AES), In the analysis laboratory of the SONATRACH Skikda oil refining complex.

Metabolites extraction and measurement

The technique described by Shibko *et al.* (1966) was used to extract metabolites (carbohydrates, lipids and proteins) from the tissues investigated. Carbohydrates were measured using the method proposed by Goldsworthy *et al.*, (1972) and proteins that of Bradford (1976).

Histological study

For the histological study of hepatopancreatic fragments, the tissues of the control and treated groups, were fixed in 10% formalin for 24 hours, dehydrated in increasing alcohol baths and then embedded in a paraffin block. Sections of 5ìm thickness were made using a microtome (Leitz, Germany). These sections were stained with hematoxylin and eosin (HandE) following the criteria of Martoja and Martoja, (1967). Finally, they were observed and photographed using a light microscope.

Statistical analysis

All the results were expressed as mean ± SE and analyzed using Student's t test with the Minitab program (version-15) comparing each treated group with the control. The significance levels of $p \leq 0.05$ was considered.

RESULTS AND DISCUSSION

Gastropod mollusks are well-known for their ability to accumulate heavy metals, including Cd, Cu, Pb and Zn. By virtue of this property, snails have been used as bioindicators of heavy metal contamination (Beeby and Richmond, 2002; Viard *et al*., 2004; Notten *et al*., 2005). Most gastropods acquire critical or hazardous heavy metals through food consumption and absorption via the digestive epithelium or the skin (Marigómez *et al*., 1998). Once they pass through various biological barriers like skin and digestive epithelium, metallic elements circulate within the organism via the hemolymph and distribute among various organs where they are stored. Heavy metals are considered truly toxic agents, disrupting certain enzymatic systems and metabolic and physiological activities in both humans and animals (Nedjoud *et al*., 2016).

Lead levels in the hepatopancreas and foot

After two months of therapy, the data presented in Fig 1 illustrate the progression of lead concentration in the hepatopancreas and foot of the treated groups, revealing a significant difference ($P \leq 0.001$) between the concentrations of the control group and the treated groups with different doses. On the other hand, it is evident that lead levels in both the organs tend to increase in a dose-dependent manner. However, the highest concentration was recorded in the hepatopancreas compared to the foot. Following the exposure of a population of juvenile *H. aspersa* to soil contaminated with increasing doses of lead, a non-essential element for living organisms, the accumulation and toxic effects of this metal in the hepatopancreas and foot of the snails were demonstrated (Carbone and Faggio, 2019). Further, the data revealed that lead levels in hepatopancreas and foot increased in a dose-dependent manner in the treated snails under the laboratory conditions. The high concentrations of lead in the snails' organs indicate significant metal accumulation due to the ingestion of contaminated soil particles and/or the diffusion of the metal through the epidermal epithelium of the foot during the experiment. Additionally, the lead levels in the hepatopancreas of *H. aspersa* was found to be substantially higher than those in the foot.

Effect of lead on hepatopancreas and foot biochemical parameters

Protein levels

The lead-contaminated snails exhibited a dose-dependent increase in protein level compared to control animals.

However, even at lower dose, this rise becomes considerable in the hepatopancreas (Fig 2). Across all species investigated thus far, the hepatopancreas consistently contains the highest concentrations of Cd, Pb and Zn (Cooke *et al.*, 1979, Dallinger and Wieser, 1984). It appears that the digestive system is also implicated in the storage of metals, with the foot serving as a site of transient accumulation in connection with cutaneous absorption. (Dallinger and Wieser, 1984, Chabicovsky *et al.*, 2004). The metals are subsequently redirected to the hepatopancreas, either definitively for excretion over varying periods or temporarily. In the presence of lead, the evolution of total protein levels in the two organs of treated snails increase in a dose-dependent way. These findings are congruent with those of (Nedjoud *et al.,* 2016) following the findings of (Besnaci *et al.*, 2016), who demonstrated that total protein level has significantly increased after 28 days of treatment of snails with metal dust and those of exposing an adult population of *H. aspersa* to iron oxide nanoparticle toxicity. This phenomenon could be considered an early biomarker of exposure to chemical contaminants. This increase in proteins may be explained by the accumulation of Pb in the tissues. Proteins are primarily involved in cell structure and can also be bound to toxins, serving as transport proteins (Cui *et al.*, 2010). Metals can bind to proteins that require a metal ion as part of their structure (hemoglobin, hemocyanin,

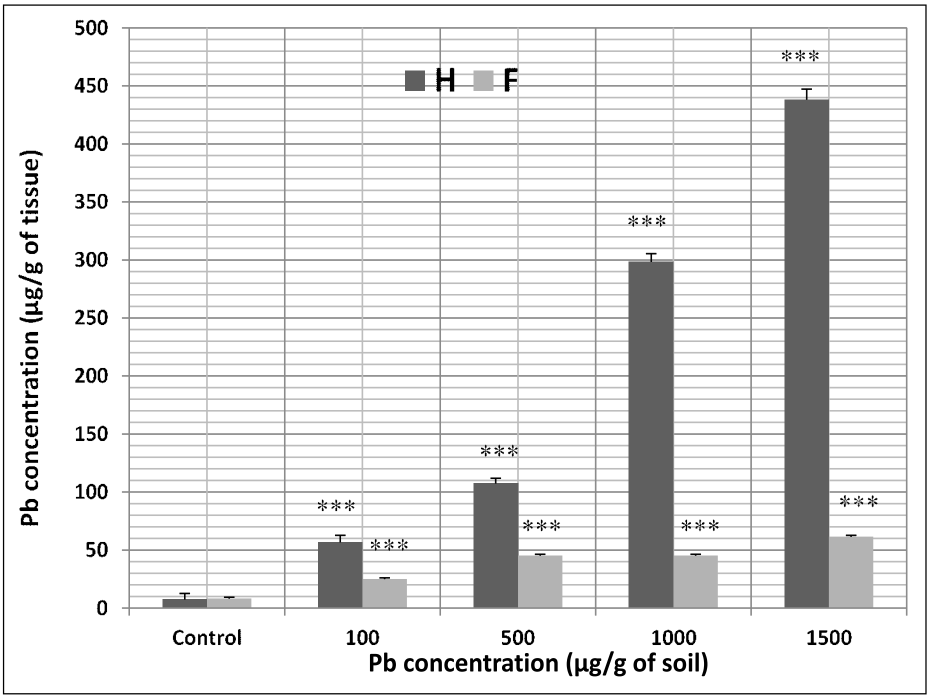


Fig 1: Evolution of Pb concentrations in hepatopancreas (H) and foot (F) of *H. aspersa*.

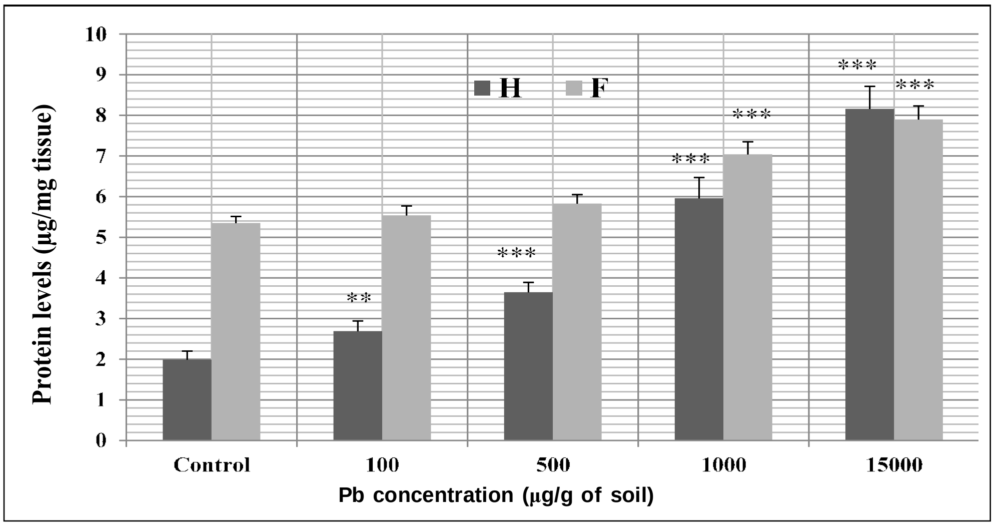


Fig 2: Protein levels in hepatopancreas (H) and foot (F) of *H. aspersa*.

etc.) and to transport/store proteins that are crucial for the control of metal homeostasis or detoxification., binding to certain metals, especially heavy metals, more or less precisely (lead-binding proteins in some species) (Cœurdassier, 2001).

Carbohydrate levels

In comparison to the carbohydrate levels in the control groups, the carbohydrate levels in all four contaminated groups are considerably lower at all tested concentrations and also in both the organs examined in the present study (Fig 3). These results are consistent with those of El Wakil and Radwan, (1991). Decrease in carbohydrate levels under the influence of metal stress implies a disturbance of carbohydrate metabolism (Nzengue, 2008). Eissa *et al.*, (2002) reported that the harmful effect of chemical compounds could be attributed to increased energy use and/or altered cell organelles (of treated snails) and may interfere with protein synthesis. This drop in carbohydrates might be attributed to the oxidation of the proteins of metal ions, leading to the release of aldehydes and hydrogen peroxide (El Wakil and Radwan, 1991).

Lipid levels

Increasing the concentration of lead has led to a reduction in lipid levels in both examined tissues compared to the control group. The lowest concentration reached was 1.9 and 2.2 µg/mg at 1500 µg/g in the foot and hepatopancreas, respectively, while in the control group, the concentration was 5.45 and 7.8 µg/mg in the foot and hepatopancreas, respectively. The statistical analysis showed significant differences between the concentrations (1500, 1000 and 500 µg/g) and the control group, while there were no significant differences between the concentration of 100 and the control group (Fig 4). These results are supported by the findings of Padmaja and Rao (1994) and they are of the view that after carbohydrates, lipids are the primary energy source provided to tissues when needed. According to

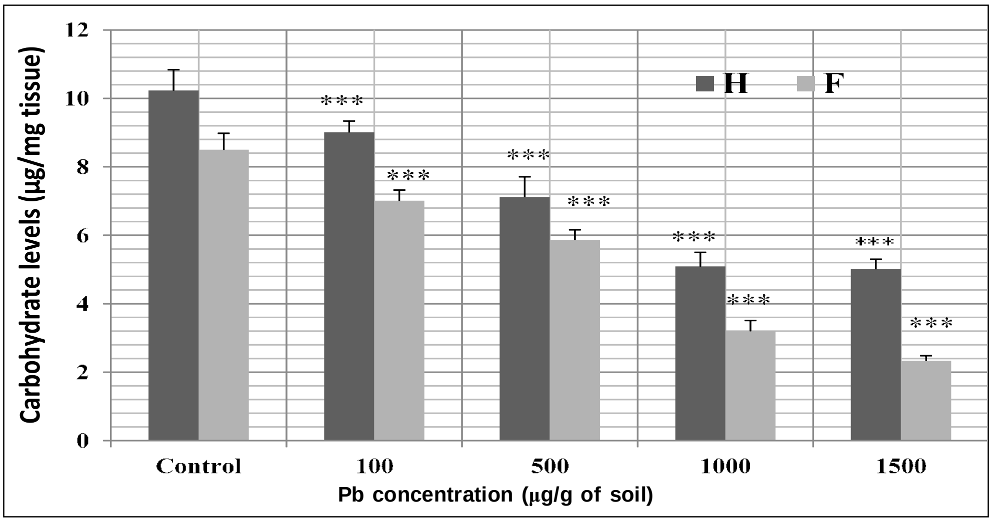


Fig 3: Carbohydrate levels in hepatopancreas (H) and foot (F) of *H. aspersa.*

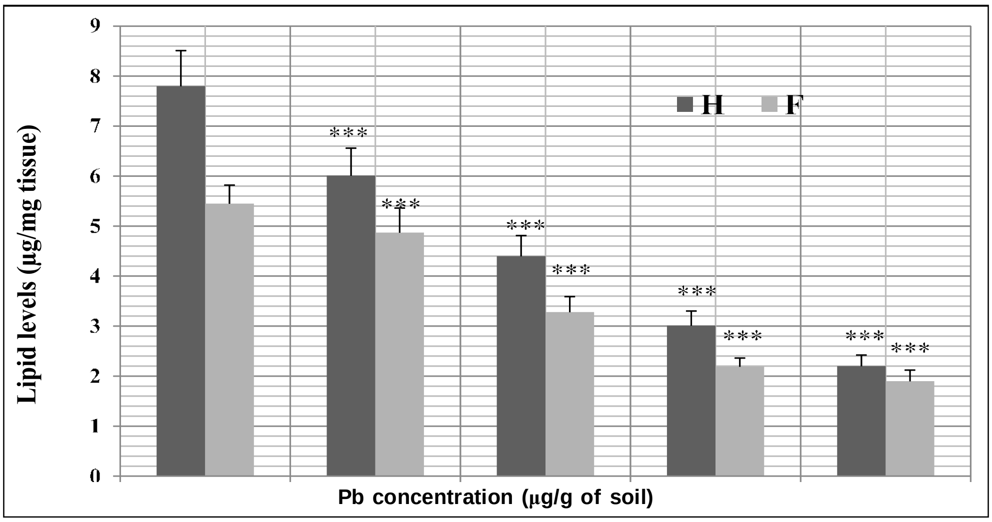
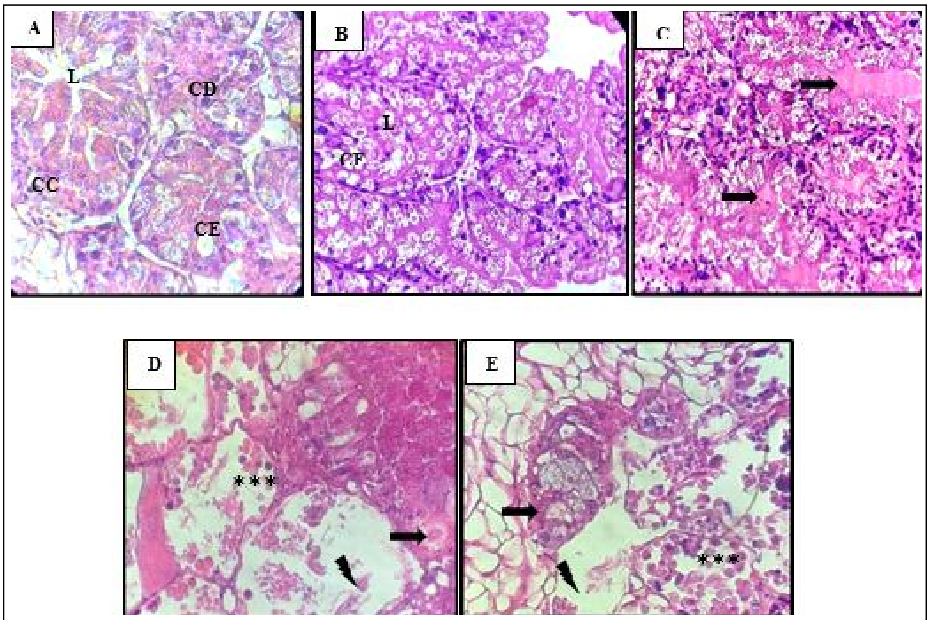


Fig 4: Lipid levels in hepatopancreas (H) and foot (F) of *H. aspersa.*



CC: Calcium cell, CD: Digestive cell, CE: Excretory cell, L: Lumen of the acini, Inflammatory infiltrates, : Necrosis, *** : Cellular debris.

Fig 5: Histological sections of the hepatopancreas: (A) Control snails (B) Snails treated with a dose of 100 µg/g of Pb, (C) Snails treated with a dose of 500 µg/g of Pb, (D) Snails treated with a dose of 1000 µg/g of Pb and (E) Snails treated with a dose of 1500 µg/g of Pb. (Magnification 40×).

Nzengue (2008), metals such as iron and copper have been widely used as initiators of lipid oxidation.

Histological examination of the hepatopancreas

Fig (5A) shows the histology of the hepatopancreas of control snails, which displays a digestive epithelium composed of lobules that create a collection of acini bound together by connective tissue. The epithelium is made up of three types of cells: digestive cells, excretory cells and calcium cells. Lead particles cause histological alterations (Fig 5B) in the group treated with 100 µg/g, demonstrating excretory cell hypertrophy and tubule clustering as a result of acini lumen constriction. Similar changes were also observed in the group treated with 500 µg/g of lead (Fig 5C), along with enlargement of hemolymphatic gaps between tubules, degeneration of the acini's basement membrane and an inflammatory appearence of the tissue. These alterations are more severe in the treated groups with 1000 and 1500 ìg/g (Fig 5D and E), with inflammatory infiltrates and cellular debris throughout the tissue and necrosis affecting the connective tissue and digestive tubule membranes.

Earlier observations by Zaldibar *et al*. (2007) also revealed the structural changes in the hepatopancreas (Zaldibar *et al.*, 2007), who showed a high relative number of calcium cells and hypertrophy of intercellular spaces in the digestive gland of terrestrial snails under chemical stress (metallic or otherwise). Studies by Besnaci *et al.*, (2016) demonstrated undeniable tissue alterations in the digestive gland and kidney of *H. aspersa* in response to the toxicity of iron oxide nanoparticles at the investigated doses (1, 2 and 3 mg of flour) administered through digestion, leading to structural alterations like enlargement of hemolymphatic spaces between tubules, inflammatory infiltrates and cellular necrosis. The degree and frequency of reported lesions varies depending on the organ and the species under consideration and they are more obvious at greater doses. (Adams *et al*., 2018; Adams *et al*. 1990; Świergosz-Kowalewska *et al.*, 2007).

These changes might be caused by lipid disturbances, in which free radicals generated cause structural and functional abnormalities in the cell, as well as membrane permeability associated to the creation of lipid peroxides (Lawton and Donaldson, 1991). All components can be damaged, including lipids, proteins and the membrane (Halliwell and Chirico, 1993), affecting DNA and causing pathologies (Curtin *et al.*, 2002).

CONCLUSION

In conclusion, this study demonstrates that juvenile *H. aspersa* snails are sensitive to the presence of lead particles in the soil. We have shown unambiguously that the snail is capable of accumulating the lead in hepatopancreas and foot, with a higher accumulation in the hepatopancreas. The biochemical composition is affected by lead exposure, with a significant increase in proteins and a decrease in carbohydrate and lipid levels. The histological examination of the hepatopancreas confirms the metal accumulation with important qualitative alterations even at the lowest tested concentration.

These findings emphasize the possible impact of lead on snails while also raising concerns about the consequences on other creatures in the environment. The findings also highlight the need of monitoring heavy metal pollution in the ecosystem, as well as possible consequences for biodiversity and ecological health. On the other hand, further research is needed to understand the long-term effects of lead exposure on snails and its potential consequences in the ecosystem. This research adds to the expanding body of knowledge on heavy metal contamination and its effects on the ecosystem, offering important data for risk assessments and environmental conservation initiatives.

Authors' contribution

SN, FB and BF designed the study, FA and BS conducted data analyses and wrote the manuscript. LM performed light microscopy experiments. MS and SL helped in writing the manuscript and conducted data analyses.

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Ethical approval

The conducted research is not related to either human or animal use.

Data availability statement

All the data is available within the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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