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Effect of Subclinical Lead Toxicity on Hematological and Serum Biochemical Profiles of Damani Sheep

Muhammad Umer Farooq[1], Muhammad Sajid[2], Aziz Ur Rehman[2], Amar Nasir[3], Habib Ullah[1], Muhammad Mubeen[1], Muneeb Ur Rahman[4] and Assar Ali Shah[1]*

[1]Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan 29111, Khyber Pakhtunkhwa, Pakistan
[2]Deprtment of Pathobiology, University of Veterinary and Animal Sciences, Lahore, (Jhang Comps)., Pakistan
[3]Deprtment of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan
[4]Civil Veterinary Hospital Gul Imam, 29420, District Tank, Khyber Pakhtunkhwa, Pakistan

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All authors contributed to the study conception, methodology, and analysis by MUF, MS, and AR. Material preparation, data collection, and analysis were performed by AN, HU, and MM. The first draft of the manuscript was written by MUF. AAS commented and edited on previous versions of the manuscript.

Key words
Damani sheep, Kidney, Liver, Oxidative stress, Haemato-biochemical alterations, Heavy metal

ABSTRACT

Heavy metals are known to have harmful effects on both human and animal health. Among these toxic metals, lead is particularly damaging, affecting the kidneys, brain, liver, and genetic integrity of both humans and animals. This study aimed to assess the levels of Pb in the serum of Damani sheep in the Dera Ismail Khan district. The investigation was carried out across three distinct areas within the district. Pb levels were measured using atomic absorption spectrophotometry, revealing concentrations above the permissible limit of 0.25 ppm in serum samples from sheep grazing in contaminated areas. This elevated Pb exposure correlated with significant alterations in hematological, serum biochemical, and antioxidant parameters. Notably, sheep from contaminated areas exhibited substantial reductions in red blood cells, total leukocyte count, hemoglobin, packed cell volume, and mean corpuscular volume compared to those grazing in uncontaminated areas. Furthermore, significant differences were observed in serum biochemical markers, including kidney function indicators (urea and creatinine), liver enzymes (AST and ALT), and oxidative stress biomarkers (SOD and GPx). Sheep from contaminated areas showed elevated levels of kidney and liver enzymes, coupled with a reduction in antioxidant levels. This study highlights the presence of Pb above permissible limits in the serum of Damani sheep, suggesting that consuming products from these animals could pose serious risks to public health.

INTRODUCTION

Heavy metals, characterized by an atomic density exceeding 6 g/cm³, are naturally occurring elements in the Earth's crust. While they are integral to various industrial and agricultural processes, their entry into the food chain poses significant health risks to humans and animals. Once inside the body, heavy metals can accumulate and cause a wide range of health issues, including organ damage, oxidative stress, and neurological disorders (Akan *et al*., 2010). The term heavy metal often refers to a group of metals and semimetals (metalloids) that are associated with environmental contamination and potential toxicity. Some of these metals, such as lead, are not required by the animal body and can lead to severe toxicity.

Heavy metals enter the soil ecosystem through natural processes like weathering and erosion of bedrock and ore deposits. However, human activities such as mining, smelting, and the use of agriculture-related chemicals significantly increase their presence in the environment (Zhong *et al*., 2018). Additionally, these metals can be introduced into the ecosystem through rainfall, which transports pollutants from the atmosphere to the soil and water bodies (Yaqub *et al*., 2019; Iroegbulem *et al*., 2023). The use of manure and various mineral fertilizers further encourages the deposition of heavy metals in the soil (Hu *et al*., 2019), where they are absorbed by plants,

* Corresponding author: assaralishah@yahoo.com
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subsequently entering the food chain (Ugulu *et al*., 2019). Contaminated food and water are primary sources of heavy metal accumulation in animals, which can then be transferred to humans through the consumption of animal products. This accumulation disrupts numerous metabolic processes in the human body, leading to severe health issues (Sharaf *et al*., 2020). Among the routes of exposure, inhalation and ingestion are particularly lethal, as they facilitate direct entry of these toxic metals into the bloodstream (Noksi *et al*., 2021).

Lead in particular, is notorious for causing unintentional toxicity in domestic animals, especially in industrial regions of the world (Hassan *et al*., 2023). Elevated levels of Pb can have devastating effects on vital organs such as the kidneys and brain, potentially leading to death (ATSDR, 2007). Pb and other heavy metals induce oxidative stress by generating reactive oxygen and nitrogen species, which cause significant damage to the liver, nervous system, kidneys, and genetic material in both animals and humans. This oxidative stress is closely associated with disruptions in antioxidant processes, including the inhibition of key antioxidant molecules like glutathione (GSH) and enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase. The oxidative stress induced by Pb can also result in DNA damage (Jubril *et al*., 2017). Furthermore, Pb exposure has been linked to the development of hypertension and alterations in the hypothalamic-pituitary-adrenal (HPA) axis in humans, affecting stress response and overall health (Alvarez-Velazquez *et al*., 2024).

The detrimental effects of Pb are not limited to mammals; they have also been observed in birds, which exhibit symptoms such as drooping wings, paralyzed legs, loss of vision, bent necks, and the presence of blood in fecal matter (Platt, 2006). Animals, in general, serve as effective bioindicators of environmental pollution, reflecting the health of the ecosystem they inhabit (Omoniwa *et al*., 2017). The present study was conducted to detect the levels of Pb in Damani sheep and to assess its impact on various blood and serum biochemical parameters. Understanding the extent of Pb contamination in these animals is crucial, as it provides insights into the broader environmental and public health implications associated with heavy metal exposure.

MATERIALS AND METHODS

Study area

This study was conducted in the Dera Ismail Khan district, located in the Khyber Pakhtunkhwa province of Pakistan, with geographical coordinates of 31.831482° N latitude and 70.911598° E longitude. Three distinct grazing areas were selected for the study: Area 1 was cultivated land irrigated with the River Indus receiving city's sewage water discharged into it. Area 2 was irrigated with industrial wastewater from the Chashma Sugar Mill. Area 3 was thought to be free from heavy metal contamination, irrigated with underground water extracted via tube wells in the Village Malana.

Sheep and blood sampling

A total of 120 Damani sheep were selected from these three distinct study areas, with each area contributing 40 sheep (20 males and 20 females) chosen randomly. The selected sheep were tagged and subjected to appropriate vaccination and deworming schedules.

Blood samples (3ml) were collected from the jugular vein of each sheep using disposable syringes. The blood was then divided into two samples: One was transferred to an EDTA-coated vacutainer for hematological analysis, and the other was placed in a gel-coated tube for serum collection. These samples were then transported to the laboratory for further analysis.

Estimation of Pb concentration

One milliliter of serum from each sample was digested according to method described by Matusiewicz and Mroczkowska (2003). The digested samples were diluted with distilled water up to the 20 ml mark of a graduated cylinder and stored at -18°C for subsequent heavy metal (Pb) analysis.

Calibrated standards were prepared from commercially available stock solutions (Applichem®) in the form of an aqueous solution (1000 ppm). Highly purified deionized water was used to prepare the working standards. All glassware used in the analytical process was immersed in 8N HNO_3 overnight and thoroughly rinsed with deionized water before use.

Pb concentrations were measured using atomic absorption spectrophotometry (AAS) at the Hitech Laboratory, University of Agriculture, Faisalabad, following the conditions outlined in AOAC (1990). The specific instrumental operating conditions for Pb analysis were as follows: wavelength (nm), 283.3; slit width (nm), 1.3; lamp current (mA), 7.5; burner head, standard type; flame, Air-C_2H_2; burner height (mm), 7.5; oxidant gas pressure (flow rate) (kpa), 160; fuel gas pressure (flow rate) (kpa), 7.

Hematological and biochemical analysis

Blood samples collected from the sheep were analyzed using an automatic hematological analyzer from Balio Diagnostics. Serum tests, including urea, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), were conducted using commercially available kits from Randox Laboratories Ltd., UK.

Statistical analysis

The data were analyzed using one-way ANOVA, followed by the Least Significant Difference (LSD) test, with the Statistical Package for Social Sciences (SPSS, version 26).

RESULTS

The Pb levels in the serum samples of sheep grazing in contaminated areas (A1 and A2) were found to be above the permissible limit of 0.25 ppm, in contrast to the clean area (A3) (Fig. 1A). The mean hematological parameters of Damani sheep are presented in Table I. The total red blood cell (RBC) count, total leukocytic count (TLC), haemoglobin (Hb) concentration, packed cell volume (PCV), and mean corpuscular volume (MCV) in sheep grazing near contaminated areas river Indus and sugar mills were significantly lower than the clean area Malana ($p<0.05$). The mean values of serum biochemical parameters for Damani sheep grazing in the contaminated areas (A1 and A2) and the clean area (A3) are presented in Figure 1. Serum urea, creatinine, AST, and ALT levels in sheep grazing in the contaminated areas were significantly higher ($p<0.05$) than those in sheep grazing in the clean area. Oxidative stress, a key factor in cell damage, was evident in the reduced levels of oxidative stress parameters, specifically SOD and GPx, in sheep grazing in contaminated areas compared to those in the clean area ($p<0.05$).

Table I. Hematological parameters (Mean±SEM) of Damani sheep in area of Indus River and Malana.

Parameters	River Indus	Malana	Sugar mills	P values
RBC (10^6/µl)	8.29±0.17	9.87±0.33	8.01±0.35	0.001
TLC (10^3/µl)	6.36±0.34	11.39±1.72	6.28±0.47	0.002
Hb (gm/dl)	8.28±0.23	10.24±0.45	8.46±0.32	0.001
PCV (%)	24.49±1.06	29.03±1.13	23.45±0.89	0.002
MCV (fl)	25.18±0.64	29.00±0.78	24.65±1.13	0.001

DISCUSSION

The mean Pb values in both contaminated areas were significantly higher ($P<0.05$) than the permissible limit, as described by Radostits *et al*. (2006), indicating substantial Pb contamination in the regions surrounding the

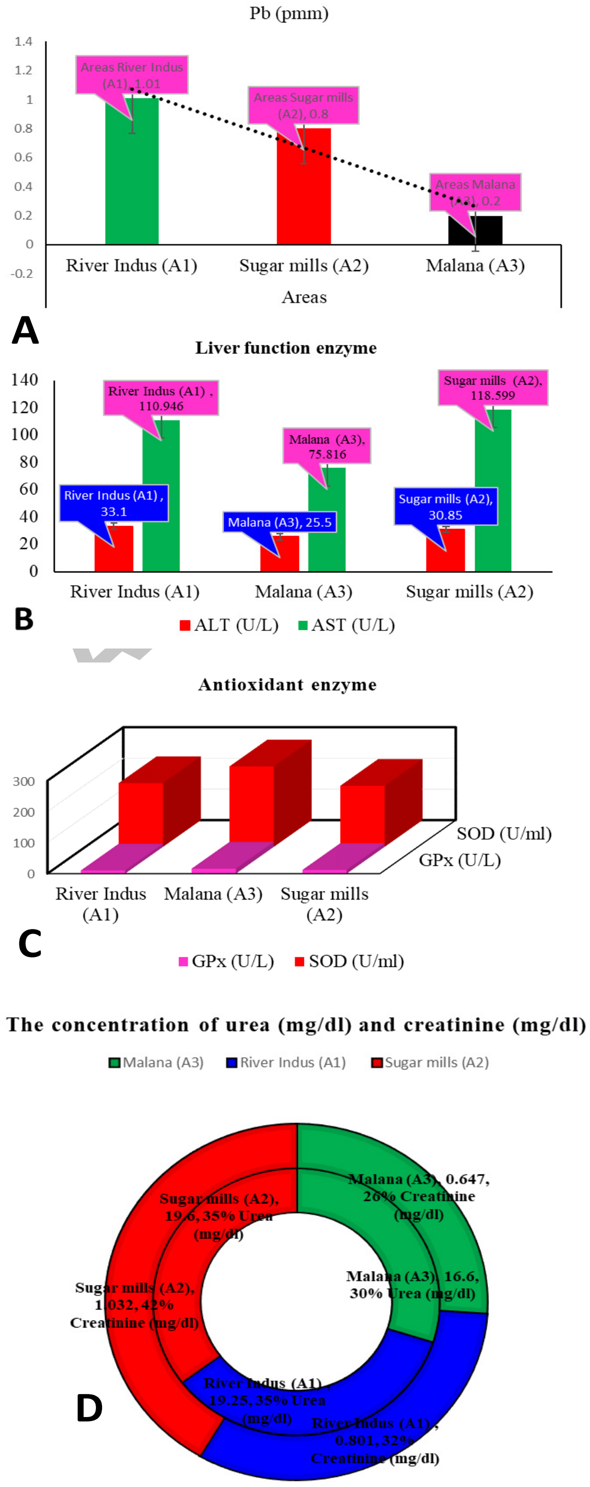

Fig. 1. The level of Pb (A), liver function enzyme (B); antioxidant enzyme (C), and renal function parameters (D) in blood serum of Damani sheep grazing in the three area (A1, A2 and A3).

River Indus and the sugar mills. These findings are consistent with a previous study by Sajid *et al.* (2018), which reported elevated Pb levels in the serum of Lohi sheep in the Jhang district. Sheep grazing in the contaminated regions near the River Indus and sugar mills exhibited a markedly lower RBC count ($p<0.05$) compared to those in the clean area of Malana. These findings align with Oraby *et al.* (2021), who observed similar results in Egyptian Ossimi sheep grazing in Pb-polluted areas. A reduction in RBC count was also reported by Sajid *et al.* (2017) in Lohi sheep in Jhang that were brought to the slaughterhouse. However, Shen *et al.* (2020) found no change in RBCs in Wumeng semi-fine wool sheep in southwest China, which may be due to breed differences or potential resistance to erythrocytic alterations. The TLC of sheep grazing in the contaminated areas near the River Indus and sugar mills was significantly lower than that of sheep in the clean area of Malana. This observation is consistent with Oraby *et al.* (2021), who reported a lower TLC in Pb-exposed Ossimi sheep in Egypt. In contrast, Shen *et al.* (2020) observed no change in TLC values in exposed and control groups of sheep, possibly due to breed variation or resistance to Pb-induced alterations.

In this study, the mean Hb levels in sheep from the contaminated areas near the River Indus and sugar mills were lower than those in Malana, where sheep were fed clean fodder. Similar findings were reported by Abeed *et al.* (2019) in a grazing flock of Awassi sheep in Iraq and in sheep grazing in urban areas compared to those in desert regions (control). Shen *et al.* (2020) also noted similar results in Wumeng semi-fine wool sheep, indicating hypochromic microcytic anemia. The PCV (%) was significantly lower in sheep from contaminated areas, corroborating the findings of Oraby *et al.* (2021), who reported a significant decrease in PCV in Pb-exposed sheep. This suggests that Pb exposure significantly reduces the percentage of PCV in affected animals. Similarly, the MCV of blood samples from sheep in contaminated areas (River Indus and sugar mills) was lower than in sheep from the clean area. This result aligns with the findings of Abeed *et al.* (2019) and Oraby *et al.* (2021) in different sheep breeds. Abubakar *et al.* (2020) also observed similar results in Pb-treated rats compared to control groups. In contrast, Pramesti *et al.* (2020) found an increase in MCV in Bali cattle in Indonesia, and Mohajeri *et al.* (2014) observed a similar increase in lactating cows reared near a Pb-Zn smelter in Zanjan Province, Iran. The increase in MCV in these cases may be attributed to species differences or the adaptation of animals living in those specific regions. The mean urea levels in Damani sheep from the River Indus and sugar mill areas were elevated compared to those from the clean area of Malana. These findings are consistent with those of Ganiyat *et al.* (2020), who reported increased urea levels in Pb-treated goats compared to a control group. Similarly, Kovacik *et al.* (2017) observed elevated urea levels in sheep in Slovakia, highlighting the impact of heavy metals on various hematological and serum biochemical parameters during the winter and spring seasons. Creatinine levels were also higher in sheep from the contaminated study areas compared to those from the clean area of Malana. This aligns with the findings of Oraby *et al.* (2021) in sheep and Abubakar *et al.* (2020) in Pb-induced rats. In contrast, Toyomaki *et al.* (2020) reported an almost negative correlation between creatinine levels and increasing Pb concentrations in dogs reared around the Pb-mining area in Kabwe, Zambia. This discrepancy may be due to species variation or differences in environmental pollution levels.

The AST levels were significantly higher in sheep from the contaminated areas than those from the clean area. Elevated AST levels have also been reported by Ganiyat *et al.* (2020) in Pb-treated goats. The ALT activity in Damani sheep grazing near the River Indus and sugar mills was markedly higher than in those from the clean area of Malana. Similar increases in ALT levels were observed by Oraby *et al.* (2021) in Pb-exposed sheep in Egypt, Abubakar *et al.* (2020) in Pb-induced rats, and Abeed *et al.* (2019). Elevated AST and ALT levels indicate liver damage caused by Pb exposure. The antioxidant SOD levels were significantly lower in sheep grazing in contaminated areas than in those grazing in the clean area, consistent with the findings of Oraby *et al.* (2021), who reported reduced enzyme activity in Pb-exposed sheep. GPx levels were also lower in sheep from both contaminated areas compared to those from the clean area of Malana. Similar results were noted by Soussi *et al.* (2018), who observed reduced GPx activity in Pb-treated rats. As key antioxidants, the reduction in SOD and GPx levels indicates that Pb exposure triggers the production of reactive oxygen species, leading to oxidative stress in animals.

CONCLUSION

Our study reveals that the Pb levels in the serum samples of Damani sheep from contaminated areas exceed permissible limits, leading to significant alterations in serum biochemical and hematological parameters compared to animals grazing in clean areas. While Damani sheep may exhibit some resistance to the toxic effects of Pb, the presence of this heavy metal in animal by-products such as milk and meat poses a potential risk to consumer health. Numerous studies have linked Pb contamination in drinking water, soil, and industrial waste to the development of cancers in various organs. It is imperative that the Pakistan Environmental Protection Agency (Pak-

EPA) takes serious measures to control hazardous element levels in affected regions and across the country to protect public health. Additionally, interventions should be implemented to mitigate Pb-induced hematological and serum biochemical disturbances in animals. Investigating the presence of toxic metals in the local human population is also crucial, given their exposure through the consumption of contaminated animal products, locally grown vegetables, and environmental pollutants.

DECLARATIONS

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IRB approval

The study was approved by College of Veterinary and Animal Sciences (CVAS), University of Veterinary and Animal Sciences (UVAS) Lahore, Advanced Studies and Research Board in its meeting held on 12-03-2021 (Reference number DAS/457).

Ethical approval

The present study received ethical approval from the Ethical Review Committee of College of Veterinary and Animal Sciences (CVAS) University of Veterinary and Animal Sciences (UVAS) Lahore, under reference number CVAS/ERC/118.

Statement of conflict of interest

The authors have declared no conflict of interest.

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