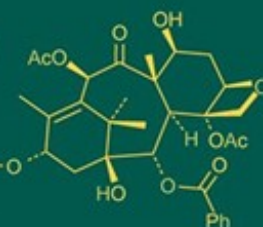
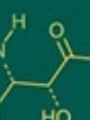
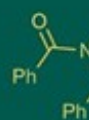


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Assessment of lead-cadmium induced pathophysiological alterations in albino Wistar rats

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ABSTRACT

Lead (Pb) and cadmium (Cd) are ubiquitous and non-biodegradable pollutants which possess a great concern to animal and human health. The present work was designed to study ameliorative and therapeutic effect of *Moringa oleifera* against combined lead and cadmium induced toxicity in male albino Wistar rats. The experiment was conducted on 30 male albino wistar rats of with 6-8 weeks age having body weight of 180-200 g. These rats were maintained as per the standard CCSEA guidelines and the study protocol was approved by IAEC, Co.V.Sc. & A.H., Jabalpur. Rats exposed to lead acetate @ 150 mg/kg b.wt. and cadmium chloride @ 5 mg/Kg b.wt. showed various adverse effects such as hematological changes, elevated liver enzymes. Administration of *Moringa oleifera* mitigated these effects, with better results observed at a dose of 250 mg/kg compared to 400 mg/kg. These findings suggest that *Moringa oleifera* has potential as a protective agent against lead and cadmium toxicity.

Keywords: Cadmium, Kidney, Lead, Liver, *Moringa Oleifera*

Introduction

Environmental pollution, driven largely by industrialization and mining, is a pressing global issue, with elemental contaminants like lead (Pb) and cadmium (Cd) posing substantial threats to human and animal health. These toxic metals are pervasive and persistent, with exposure primarily occurring through consumption and inhalation. Efforts to mitigate their impact are crucial for safeguarding well-being and environmental sustainability (Pandya *et al.*, 2010; Andjelkovic *et al.*, 2019 and Arya *et al.*, 2021) [2, 1]. Ingesting or inhaling lead (Pb) can result in severe toxicity, adversely affecting nearly all organs and bodily systems. Its primary impacts encompass damage to the hepatic, nervous, hematopoietic, renal, reproductive, and skeletal systems (Ayodele, 2012) [3]. Exposure to cadmium causes morphological and functional harm, particularly affecting the renal and testicular systems, with additional impacts on the hepatic, lung, and gastrointestinal tract. Both lead and cadmium exposure have been linked to metabolic disruptions and an increased risk of various cancers such as lung, kidney, pancreatic, and prostate cancer (Ebrahimi *et al.*, 2020) [4]. Their coexistence of lead and cadmium can lead to broader impacts on various organs beyond their individual effects. These metals induce a spectrum of toxic changes, from acute poisoning to chronic adverse effects on multiple organs, with oxidative stress being the primary mechanism of toxicity (Bas *et al.*, 2021) [5]. Even, it is one of major cause of cancers in present scenario (Ebrahimi *et al.*, 2020 and Michalczyk *et al.*, 2023) [4]. Various components of the plants have traditionally been utilized to address diverse health issues particularly in South Asia (Velaga *et al.*, 2014 and Nakata *et al.*, 2022) [6]. *Moringa oleifera*, commonly known as drumstick, is a medicinal plant grown in tropical and subtropical regions. Its various parts of plants rich in essential minerals, proteins, vitamins, β -carotene, amino acids, and various phenolics, exhibits pharmacologically active compounds such as zeatin, quercetin, β -sitosterol, caffeoylquinic acid, and kaempferol are utilized for medicinal purposes, has antioxidant, antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antidiabetic, hepatoprotective, antibacterial, and antifungal properties (Elblehi *et al.*, 2019; Elgawish *et al.*, 2019 and Guan *et al.*, 2021) [7, 8].

Materials and methods

Reagents

CdCl₂ analytical grade and (Pb(NO₃)₂ AR > 99.0%), were obtained from Supriti trader Madhya Pradesh.

Experimental design

The study was conducted for duration of 6 months from August 2023 to January 2024 on 30 male albino wistar rats of 6-8 weeks age having body weight of 180-200 g which were maintained as per the standard CCSEA guidelines. The study protocol was approved by IAEC, Co.V.Sc. & A.H., Jabalpur and consisted 5

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experimental groups, with six male rats each. Group I rats served as control animals and received standard feed and water only. Group II rats were administered with lead acetate and cadmium chloride separately, dissolved in distilled water at a dose rate of @150 mg/kg b.wt. and @5 mg/kg b.wt. respectively orally with intubation canula for 30 consecutive days. Group III rats (Ameliorative group) were administered with *Moringa oleifera* @ 250 mg/kg b.wt orally, 2 weeks prior to lead cadmium administration till end of the experimental period. Group IV rats (Therapeutic group) were administered with *Moringa oleifera* @400 mg/kg from day 10th of intoxication till experimental period. Group V rats were given with *Moringa oleifera* @400 mg/kg b.wt. for 30 consecutive days.

A. Preparation of aqueous extract of *Moringa oleifera*

Fresh leaves of *Moringa oleifera* were procured in bulk from JNKVV Jabalpur, M.P. Leaves were cleaned, shade-dried at room temperature and grounded into fine powder form. One gram of leaf powder was boiled in 25 ml sterile distilled water for 10 minutes. The extract was then cooled and filtered through Whatman filter paper no. 1 and volume of the filtrate was adjusted to 10 ml by adding sterile distilled water. Prepared extract at concentration of 100 mg/ml used as stock solution. To dose group III rats the stock extract was diluted in 1:4 dilution (1 part of stock extract + 3 part of d/w) to make concentration of 25 mg/ml. To dose group IV rats stock extract was diluted to 1:2.5 (1 part of extract + 1.5 part of d/w) to make the concentration of 40 mg/ml. These rats were dosed @ 1 ml/100 gm. Aqueous extracts were prepared fresh immediately before use.

B. Clinical observation

All the rats belonging to various groups were closely observed on a daily basis for the development of any clinical signs and symptoms

C. Collection of samples

- Blood – blood samples were collected in vial with anticoagulant on 10th and 30th day of lead cadmium intoxication for haemato-biochemical examination and estimating lead and cadmium level.

Processing of blood for estimation of lead and cadmium level

1 ml of blood sample was well mixed with 7 ml of concentrated nitric acid and 1 ml of hydrogen peroxide in a 50 ml conical flask before being transferred to the digesting assembly (Kelpus, KES-06-INLE) for 2-3 hours. The digested samples were rinsed with triple glass distilled water to get the volume to 25 ml (Welna *et al.*, 2011) [9]. Furthermore, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) (Agilent technologies; ICP-MS/MS QQQ-8900) was utilized to estimate lead in digested blood samples, with an argon flame as a fuel. Blood samples were processed and thawed to room temperature. Prior to analyzing samples, the equipment was calibrated using six known concentration standards (5, 10, 25, 50, 75, and 100 ppb).

Statistical analysis

Data gathered from study was analyzed using one-way Analysis of Variance (ANOVA). The mean values were compared by Fisher pair wise comparison for 5 per cent level of significance (Snedecor and Coc

Results

The result of experimental investigation, effect of *Moringa oleifera* on lead acetate and cadmium chloride toxicity in adult male albino wistar rats are presented below. Parameters includes such as haemato-biochemical changes, estimation of blood lead and cadmium level

Clinical observations

Group II showed anorexia, dullness, abdominal respiration, hunched back posture rough hair coat from the second week until the end of the experiment. Two rats of group II showed rashes on skin near to neck area. Sporadic rat showed intermittently loose faeces in group II, III, IV and V initial day of experiment. Two rats out of six in group II showed intermittent incoordination in last week of experiment. Group I rat's did not shows any clinical symptoms throughout the study period.

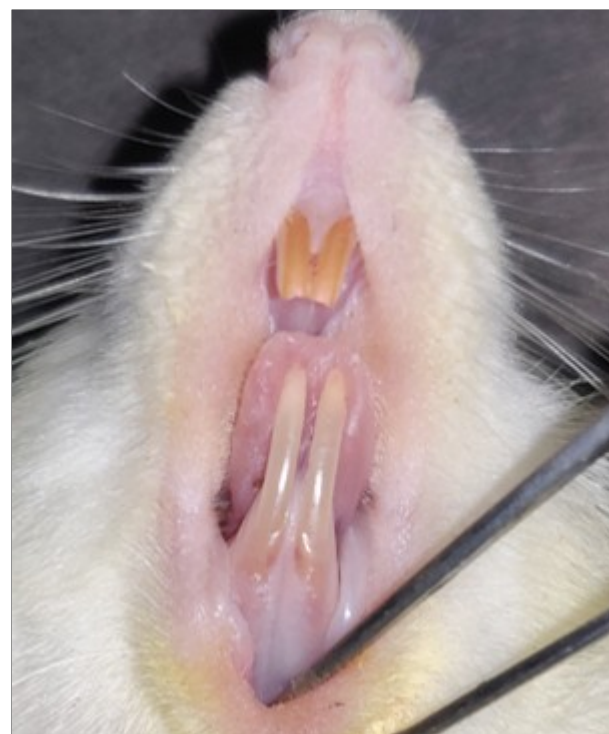


Figure 1. Pale buccal mucous membrane of group II dam administered with lead acetate @ 1%

A) Haematology

Results of haematological examination on day 10 and 30 of lead cadmium intoxication are presented in table 01 and 02.

Table 1. Haematology of rats of different groups on day 10

Parameter	Group I	Group II	Group III	Group IV	Group V
Hb (g/dl)	14.01 ^a ±0.16	12.87 ^b ±0.14	13.80 ^a ±0.211	12.06 ^b ±0.25	14.01 ^a ±0.21
TEC (x10 ⁶ /μl)	07.27 ^a ±0.16	05.26 ^b ±0.16	06.96 ^b ±0.08	05.46 ^b ±0.09	07.60 ^a ±0.130
PCV (%)	42.09 ^a ±2.22	37.84 ^a ±2.55	41.54 ^a ±0.91	37.34 ^a ±0.98	41.67 ^a ±1.60
TLC (x10 ³ /μl)	07.32 ^a ±0.49	07.62 ^a ±0.18	07.52 ^a ±0.22	07.57 ^a ±0.43	08.82 ^a ±1.36
Lymphocytes (%)	76.78 ^a ±0.99	66.32 ^b ±0.95	77.65 ^a ±0.27	66.10 ^b ±0.90	76.15 ^a ±1.89

(continued on next page)

Neutrophils (%)	20.66 ^b ±0.84	30.33 ^a ±0.71	20.53 ^b ±0.63	31.67 ^a ±1.54	20.41 ^b ±0.48
Monocytes (%)	01.14 ^a ±0.16	01.52 ^a ±0.11	01.47 ^a ±0.07	01.10 ^a ±0.10	01.54 ^a ±0.16
Eosinophils (%)	01.42 ^a ±0.26	01.36 ^a ±0.19	01.35 ^a ±0.17	01.15 ^a ±0.15	01.86 ^a ±0.20

Mean value with different superscript differ significantly ($P < 0.05$) in row

On day 10th significant decrease in haemoglobin and TEC in group II (12.87 ± 0.14 g/dl and $05.26 \pm 0.16 \times 10^6/\mu\text{l}$) and group IV (12.06 ± 0.25 g/dl and $05.46 \pm 0.09 \times 10^6/\mu\text{l}$) as compared to other groups (group I, III and V). Significant increased neutrophil and decrease

lymphocytes noted in group II and IV as compared to others. Group III showed improvement as compared to group II.

Table 2. Haematology of rats of different groups at day 30

Parameter	Group I	Group II	Group III	Group IV	Group V
Hb (g/dL)	14.42 ^a ±0.70	10.90 ^c ±0.19	14.14 ^a ±0.31	13.26 ^b ±0.16	14.41 ^a ±0.31
TEC ($\times 10^6/\mu\text{l}$)	07.54 ^a ±0.12	03.76 ^c ±0.14	06.39 ^b ±0.09	06.70 ^b ±0.10	07.65 ^a ±0.12
PCV (%)	44.77 ^a ±1.46	30.82 ^b ±0.39	43.35 ^a ±0.95	40.72 ^a ±1.69	44.94 ^a ±2.35
TLC ($\times 10^3/\mu\text{l}$)	07.46 ^a ±0.45	07.96 ^a ±0.17	07.76 ^a ±0.15	10.12 ^a ±0.94	09.74 ^a ±1.39
Lymphocytes (%)	74.99 ^a ±1.11	55.81 ^c ±0.61	74.87 ^a ±1.72	63.83 ^b ±0.74	76.21 ^a ±0.7
Neutrophils (%)	21.83 ^c ±0.91	40.50 ^a ±0.84	22.58 ^c ±0.77	33.05 ^b ±0.56	21.07 ^c ±0.490
Monocytes (%)	01.52 ^a ±0.1	01.69 ^a ±0.05	01.31 ^a ±0.08	01.62 ^a ±0.06	01.45 ^a ±0.11
Eosinophils (%)	01.66 ^a ±0.18	01.63 ^a ±0.18	01.30 ^a ±0.18	01.50 ^a ±0.18	01.38 ^a ±0.26

Mean value with different superscript differ significantly ($P < 0.05$) in row

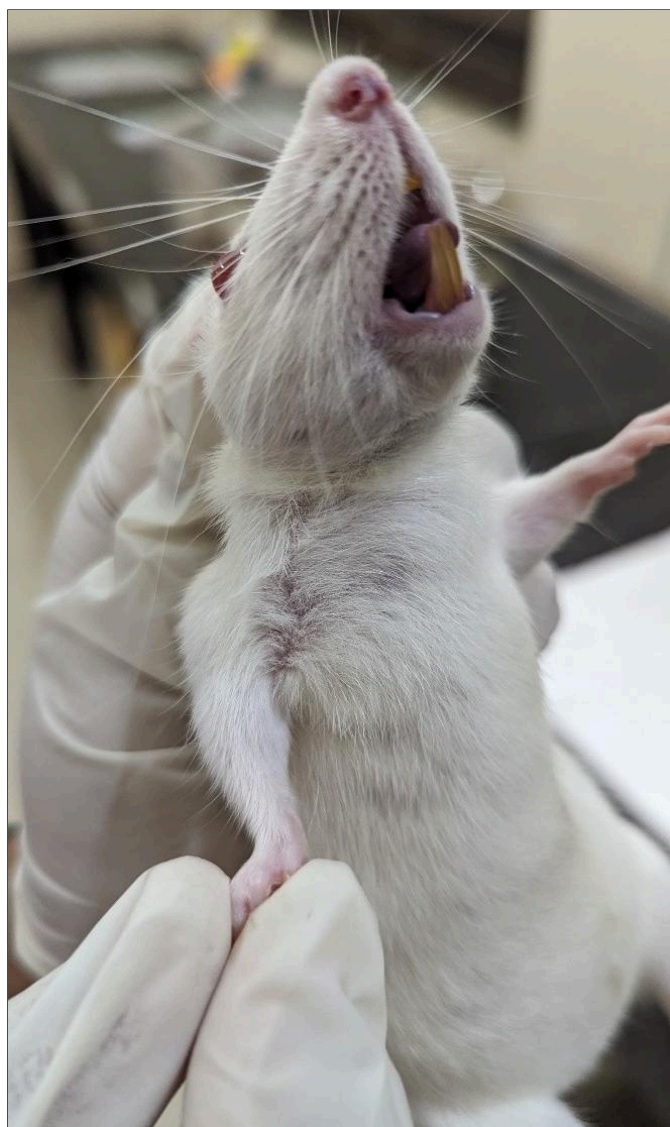


Figure 2. Sporadic rashes on neck of a rat administered with lead and cadmium (G II)

Day 30th of lead and cadmium intoxication showed significant reduction in haemoglobin concentration, TEC and PCV, lymphocytes along with increase neutrophils values of group II rats (10.90 ± 0.19 g/dl, $3.76 \pm 0.14 \times 10^6/\mu\text{l}$, $30.82 \pm 0.39\%$, $55.81 \pm 0.61\%$ and $40.50 \pm 0.84\%$ respectively) as compared to group I and rats who received *Moringa olifera* extract in group III, IV and V. Group III and IV rats showed significant improvement as compared to group II.

In Group IV significant decrease haemoglobin concentration, TEC and PCV, lymphocytes along with increase neutrophils values (13.26 ± 0.16 g/dl, $6.70 \pm 0.10 \times 10^6/\mu\text{l}$, $63.83 \pm 0.74\%$ and $33.05 \pm 0.56\%$ respectively) compared to group I, III and V.

Haematological values of group I, V and III are statistically comparable to each other except decrease TEC noted in group III.

B) Serum biochemistry

Biochemical examination including, ALT, AST, BUN, creatinine and total protein, on day 10th and day 30th are presented in table 03 and 04.

On day 10th of lead and cadmium intoxication, serum samples showed examined biochemical parameters were comparable between the group I and group V rats, who received *Moringa olifera* extract only.

Statistical significant increase value of ALT, AST and BUN were recorded in group II, III and IV compared to group I. These parameters did not show any significant difference between the group II and IV at this time point, Group III had significant lower values of ALT, AST and BUN compared to group II and IV.

Significant decreased value of total protein noted in group II and III and IV compared to group I, but it is significantly lower in group II compared to group III and IV. Creatinine showed increase in group II, though this value is within species range.

Table 3. Biochemical parameters of rats of different group on day 10

Parameter	(Mean±SE)				
	Group I	Group II	Group III	Group IV	Group V
ALT(U/L)	25.28 ^c ±0.31	47.38 ^b ±4.29	34.69 ^b ±1.47	52.72 ^a ±1.72	25.81 ^c ±0.39
AST(U/L)	73.74 ^c ±0.32	102.50 ^a ±5.84	86.84 ^b ±2.10	107.22 ^a ±5.09	71.913 ^c ±0.91
BUN(mg/dL)	13.77 ^d ±0.92	37.17 ^a ±1.49	27.40 ^c ±1.55	32.33 ^b ±0.09	14.35 ^{cd} ±1.14
TP(g/dL)	07.06 ^a ±0.07	05.44 ^c ±0.26	06.38 ^b ±0.13	06.21 ^b ±0.18	07.39 ^a ±0.14
Creatinine(mg/dL)	00.32 ^b ±0.01	00.46 ^a ±0.02	00.32 ^b ±0.07	00.34 ^b ±0.006	00.36 ^b ±0.01

Mean value with different superscript differ significantly ($P < 0.05$) in row

Table 4. Biochemical parameters of rats of different group on day 30

Parameter	(Mean±SE)				
	Group I	Group II	Group III	Group IV	Group V
ALT(U/L)	32.81 ^d ±2.91	69.89 ^a ±0.46	42.78 ^c ±1.50	57.03 ^b ±1.96	26.66 ^d ±0.40
AST(U/L)	74.9 ^c ±0.79	118.92 ^a ±3.03	79.83 ^c ±2.49	88.67 ^b ±3.64	74.03 ^c ±1.04
BUN(mg/dL)	15.86 ^c ±0.75	47.45 ^a ±3.14	17.46 ^c ±0.09	37.37 ^b ±1.12	15.88 ^c ±1.14
TP(g/dL)	07.11 ^{ab} ±0.08	04.82 ^c ±0.16	07.26 ^a ±0.14	07.45 ^a ±0.15	07.44 ^a ±0.06
Creatinine(mg/dL)	00.33 ^b ±0.07	00.48 ^a ±0.015	00.33 ^b ±0.01	00.34 ^b ±0.00	00.35 ^b ±0.01

Mean value with different superscript differ significantly ($P < 0.05$) in row

Significant decreased value of total protein noted in group II and III and IV compared to group I, but it is significantly lower in group II compared to group III and IV. Creatinine showed increase in group II, though this value is within species range. On day 30 of lead and cadmium intoxication, serum samples showed examined biochemical parameters were comparable between the group I and group V rats. Statistical significant increase value of ALT, AST and BUN were recorded in group II, III and IV compared to group I. Group III had significant improvement in values of ALT, AST and BUN compared

to group II and IV on day 30 of examination. Whereas group IV had significant lower value compared to group II and higher compared to group III.

Significant decreased value of total protein and increase creatinine value noted in group II compared to group I and other groups.

C) Blood lead and cadmium level

At the end of the experiment, blood samples from all groups were analysed for concentration of lead and cadmium, summarized in table 05.

Table 5. Blood lead and cadmium level (ppm) in rats of different groups

Group	Number of animals	Blood lead level (ppm) (Mean±SE)	Blood cadmium level (ppm) (Mean±SE)
I	06	0.006 ^d ±0.002	0.003 ^d ±0.0076
II	06	2.940 ^a ±0.120	0.140 ^a ±0.0065
III	06	0.377 ^c ±0.001	0.013 ^c ±0.0001
IV	06	0.806 ^b ±0.040	0.022 ^b ±0.0001
V	06	0.082 ^d ±0.001	0.007 ^{cd} ±0.0001

Mean value with different superscript differ significantly ($P < 0.05$) in column

The findings from the current investigation indicate that rats exposed to lead acetate and cadmium chloride only exhibited noticeable presence of lead and cadmium in their blood samples. The mean±SE values for lead concentration (ppm) in blood samples of experimental rats of group I, II, III, IV, and V were 0.0060 ± 0.0002 , 2.940 ± 0.120 , 0.377 ± 0.001 , 0.806 ± 0.040 , and 0.082 ± 0.001 ppm respectively and cadmium concentration 0.003 ± 0.0076 , 0.140 ± 0.0065 , 0.013 ± 0.0001 , 0.022 ± 0.001 and 0.007 ± 0.00 ppm respectively. Group I and V had negligible but comparable concentration of lead and cadmium.

Group II rats showed significant higher blood lead and cadmium concentration as compared to group I, III, IV and V. Group III and IV rats showed significant lower blood lead and cadmium level as compared to group II.

Discussion

Niranjan *et al.* (2017) [10] and Alam *et al.* (2021) both reported the presence of quercetin in *Moringa oleifera* extract. *Moringa oleifera* leaves contain protein, vitamins, β -carotene, amino acids, and pheno-

lic components such as zeatin, quercetin, β -sitosterol, caffeoylquinic acid, and kaempferol, minerals and antioxidant that improve its nutritional and therapeutic properties in a variety of disease states, as well as metal toxicity (Niranjan *et al.*, 2017; Kumar *et al.*, 2021) [10, 11].

Lead inhibits calcium and zinc-dependent enzymatic metabolic processes, causing liver and kidney damage, as well as oxidative stress-induced catabolic states that produce muscle waste and cachexia (Marchlewicz *et al.*, 2004; Yuan *et al.*, 2014) [12]. Cadmium also affects glycogenesis enzymes, leading to glycogen depletion in the liver and muscles, as well as alterations in energy metabolism (Markiewicz-Gorka *et al.*, 2015) [13]. Our findings on the altered hematological values induced by combined lead and cadmium administration to rats are consistent with those of Mahaffey *et al.* (1981) [14], Yuan *et al.* (2014) [12], Andjelkovic *et al.* (2019) [1], and Choudhuri *et al.* (2021) [15].

Anaemia caused by lead and cadmium oral administration. After ingestion, these metals are dispersed in the blood and bind to RBC and proteins. Anaemia can be caused by decreased hemoglobin synthesis or hemolysis of adult and immature erythrocytes, both of which have a shorter lifespan. Lead played a key part in this process by blocking

the enzymes amino-levulinic acid dehydrase and ferrochelatase, which are essential for heme production. Lead has a long-term effect on heme production via inhibiting cytoplasmic and mitochondrial enzymes. Lead poisoning is also thought to interfere with hematopoietic progenitor growth, copper metabolism, and erythropoietin production (Andjelkovic *et al.*, 2019; Daku *et al.*, 2019) [1, 16].

Neutrophilia may occur as a consequence of inflammation and the release of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) (Ebrahimi *et al.*, 2020) [4]. On day 10 of combined toxicity exposure, the biochemical parameters observed in rats from Group I were comparable to those of Group V, which received only *Moringa oleifera* extract.

Our findings on biochemical alterations are congruent with those of several researchers. In their research on combined lead and cadmium intoxication of rats, Mahaffey *et al.* (1981) [14], Dabak *et al.* (2009) [17], Yuan *et al.* (2014) [12], Klinova *et al.* (2020) [18], Bas *et al.* (2021) [5], Choudhuri *et al.* (2021) [15], and Huang *et al.* (2021) [19] all found identical changes in the biochemical parameters under investigation. Unlike us, Oladipo *et al.* (2016) [20] found a decrease in BUN and creatinine, while Andjelkovic *et al.* (2019) [1]

Liver enzymes AST, ALT, and ALP are cellular enzymes that are present in low amounts under normal conditions but become raised during hepatic damage, which can lead to reduced protein synthesis due to degeneration and necrosis. ALT is the earliest indicator of liver injury. BUN is produced in the liver and eliminated through the kidneys. Increased BUN levels indicate dysfunction or injury to either the liver, kidneys, or both. Creatinine is a sensitive measure of kidney damage; nevertheless, in the current investigation, it increased significantly but remained within the usual range of species reported after lead and cadmium administration. Lead and cadmium have been shown to cause free radical damage, which results in the degeneration of hepatocytes and renal tubular cells. (Yuan *et al.*, 2014; Bas *et al.*, 2021) [12, 5].

Our findings are in consistent with the reports of Wang *et al.* (2009) [21] and Klinova *et al.* (2020) [18], who similarly detected measurable concentrations of lead and cadmium in the blood of rats exposed to these heavy metals.

These metals are absorbed through the intestine and transferred to the bloodstream, where they attach to RBC and serum proteins. Lead remains in the body for 30 to 40 days, whereas cadmium can last up to 90 days, spread to various organs, accumulating mostly in the liver, kidney, and skeletal system. Higher concentrations of lead and cadmium in blood imply persistent exposure to these elements. The detected concentrations of these metals in the current investigation are beyond the permitted limit and exhibit significant harmful effects (Wang *et al.*, 2009; Klinova *et al.*, 2020) [21, 18].

The findings of the present study are consistent with those reported by Wang *et al.* (2009) [21], Markiewicz-Gorka *et al.* (2015) [13], Matović *et al.* (2015), Oladipo *et al.* (2016) [20], Andelković *et al.* (2019) [1], Bas *et al.* (2021) [5], Choudhuri *et al.* (2021) [15], and Tubaşkul *et al.* (2021).

On administration of *Moringa oleifera* leaf extract at a dose of 250 mg/kg for 15 days prior to lead and cadmium exposure, as an ameliorative treatment, and at 400 mg/kg for 10 days following intoxication, as a therapeutic treatment, resulted in notable improvements in haematological, biochemical, and histopathological alterations. The ameliorative administration of *Moringa oleifera* was more effective in attenuating the toxic effects than its therapeutic administration. Furthermore, treatment with *Moringa oleifera* at 400 mg/kg did not produce any adverse effects on the parameters evaluated in the rats.

Conclusion

Combined administration of lead acetate @ 150 mg/kg b.wt. and cadmium chloride @ 5 mg/kg b.wt showed adverse alterations in haematobiochemical and histopathological parameters with increased oxidative stress.

Moringa oleifera @ 250 mg/kg b.wt. and 400 mg/kg b.wt proven efficacy on adverse effects of lead acetate and cadmium chloride along

with reduced blood lead and cadmium level. Ameliorative use of *Moringa oleifera* proven to be better effective than therapeutic use of *Moringa oleifera* in present study.

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