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Bucillamine as a Novel Oral Dithiol Chelator for Lead and Nickel Detoxification in Rats

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Abstract
Chronic occupational exposure to toxic metals such as lead and nickel represents a major health concern due to their cumulative toxicity and long-term biological effects. Common chelating agents, including DMPS and EDTA, are limited by parenteral administration and potential adverse effects. Bucillamine, a cysteine-derived dithiol drug, may represent an orally active alternative with combined chelating and antioxidant properties. In this study, the efficacy of bucillamine was evaluated in rats co-exposed to lead acetate (15 mg/kg/day) and nickel chloride (10 mg/kg/day), and compared with DMPS. Blood metal concentrations were determined by ICP-MS, together with oxidative stress markers, liver and kidney function parameters, and histopathological evaluation. Bucillamine at 60 mg/kg significantly reduced blood lead and nickel levels by 58% and 54%, respectively, improved plasma thiol status, and reduced lipid peroxidation without inducing hepatic or renal toxicity. These findings support the potential of bucillamine as an effective orally active agent.

Keywords: Bucillamine, Lead, Nickel, Oxidative stress, DMPS, Rats

I. Introduction

Heavy metal exposure remains a major global toxicological concern due to its serious health effects and environmental persistence [1–3]. Recent reports highlight that chronic exposure to toxic metals through industrial activities, contaminated water, and urban pollution continues to pose significant public health challenges worldwide [4,5]. Chelation therapy remains the primary strategy for managing metal intoxication, and extensive research has addressed its mechanisms and limitations [6,7].

Among toxic metals, lead (Pb) is particularly hazardous because of its neurotoxic, hematological, renal, and cardiovascular effects, especially in vulnerable populations [8–10]. Similarly, nickel (Ni) poses significant health risks, with documented carcinogenic and genotoxic potential in both experimental and occupational settings [11,12].

Conventional chelating agents, including EDTA, DMSA, and DMPS, have been widely evaluated for heavy metal detoxification [13,6]. However, these agents are limited by parenteral administration, suboptimal selectivity, and the potential redistribution of toxic metals [7,14]. In addition, growing evidence suggests that traditional chelators may not adequately address oxidative damage associated with metal toxicity [15,16].

Environmental exposure to heavy metals leads to systemic accumulation and chronic toxicity, mediated in part through oxidative stress and disruption of cellular redox homeostasis [17,3]. Long-term exposure to carcinogenic metals such as nickel, arsenic, and chromium has been associated with molecular and epigenetic mechanisms of carcinogenesis [18,12]. Consequently, antioxidant-based strategies have gained increasing attention as complementary approaches to chelation therapy [19,16].

Recent advances in metal detoxification research have emphasized the need for multifunctional agents that combine metal-chelating capacity with intrinsic antioxidant properties to improve therapeutic outcomes [14,16].

Bucillamine (BUC) is a cysteine-derived dithiol drug approved in Japan for the treatment of rheumatoid arthritis and is characterized by two reactive sulfhydryl groups [20]. Thiol-containing compounds have been reported to exert dual actions by chelating metals and modulating oxidative pathways [15]. Oxidative stress plays a central role in heavy metal toxicity, as reflected by disturbances in thiol redox balance and enhanced lipid peroxidation, commonly assessed using plasma thiols and malondialdehyde (MDA) levels [21,22].

Despite its favorable chemical structure, the role of bucillamine in combined Pb and Ni toxicity has not been previously investigated. The present study therefore evaluates the hypothesis that bucillamine can provide effective detoxification through simultaneous metal chelation and

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attenuation of oxidative stress. Accordingly, the comparative efficacy and safety of bucillamine versus DMPS were assessed in a rat model of combined Pb–Ni exposure, with emphasis on metal clearance, redox biomarkers, biochemical parameters, and histopathological findings

II. Materials and Methods

2.1. Experimental animals and ethical approval

Seventy-two adult male Rattus norvegicus (Wistar strain; family Muridae) rats (250–280 g) were obtained from an accredited animal house facility and housed under standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 55 ± 10%, 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. Animals were acclimatized for one week prior to experimentation.

All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and complied with national regulations for animal experimentation. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Zagazig University, Egypt (Approval No: ZU-IACUC/3/F/229/2025, approved on 26 November 2025), and the study was conducted at the Nuclear Materials Authority, Cairo, Egypt.

2.2. Chemicals and reagents

Lead(II) acetate trihydrate (≥99%), nickel(II) chloride hexahydrate (≥98%), bucillamine (≥98%), and sodium 2,3-dimercapto-1-propanesulfonate (DMPS, ≥98%) were purchased from Sigma-Aldrich/Merck (Germany). Ellman's reagent (DTNB) and TBARS assay kits were obtained from Sigma-Aldrich. Nitric acid (trace metal grade, 69%) and hydrogen peroxide (30%) were used for sample digestion prior to metal analysis.

All solutions were prepared using ultrapure deionized water, and all glassware and polypropylene tubes were acid-washed to prevent trace metal contamination.

III. Experimental design and treatment protocol

After acclimatization, rats were orally administered lead acetate (15 mg/kg/day) and nickel chloride (10 mg/kg/day) for seven consecutive days to induce combined heavy metal exposure. Following this exposure period, administration of both metals was discontinued. Animals were then randomly divided into six experimental groups (n = 12 per group) and treated with DMPS or bucillamine for an additional seven days.

Group I: Vehicle control
Group II: Pb–Ni exposure
Group III: Pb–Ni + DMPS (30 mg/kg/day, intraperitoneal)
Group IV: Pb–Ni + Bucillamine (10 mg/kg/day, oral)
Group V: Pb–Ni + Bucillamine (30 mg/kg/day, oral)
Group VI: Pb–Ni + Bucillamine (60 mg/kg/day, oral)

Treatments were continued for an additional seven days. Oral administration was performed by gavage, while intraperitoneal injections were conducted under aseptic conditions. Body weight, food intake, and general clinical behavior were monitored daily.

3.1. Sample collection and biochemical analysis

At the end of the treatment period, rats were fasted overnight and euthanized under deep anesthesia. Blood samples were collected via cardiac puncture using trace metal–free syringes. Plasma was separated by centrifugation and stored at −20 °C until analysis.

Blood lead and nickel concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS) following nitric acid digestion. Plasma total thiols were determined using Ellman's method, and lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the TBARS assay. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and blood urea nitrogen (BUN) were measured using standard commercial diagnostic kits.

3.2. Histopathological examination

Liver and kidney tissues were excised, rinsed in ice-cold saline, and fixed in 10% neutral buffered formalin. Tissue samples were processed using standard paraffin embedding techniques, sectioned at 4–5 µm thickness, and stained with hematoxylin and eosin (H&E) for microscopic examination.

3.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 26, IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

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IV. Results

4.1. Blood metal concentrations

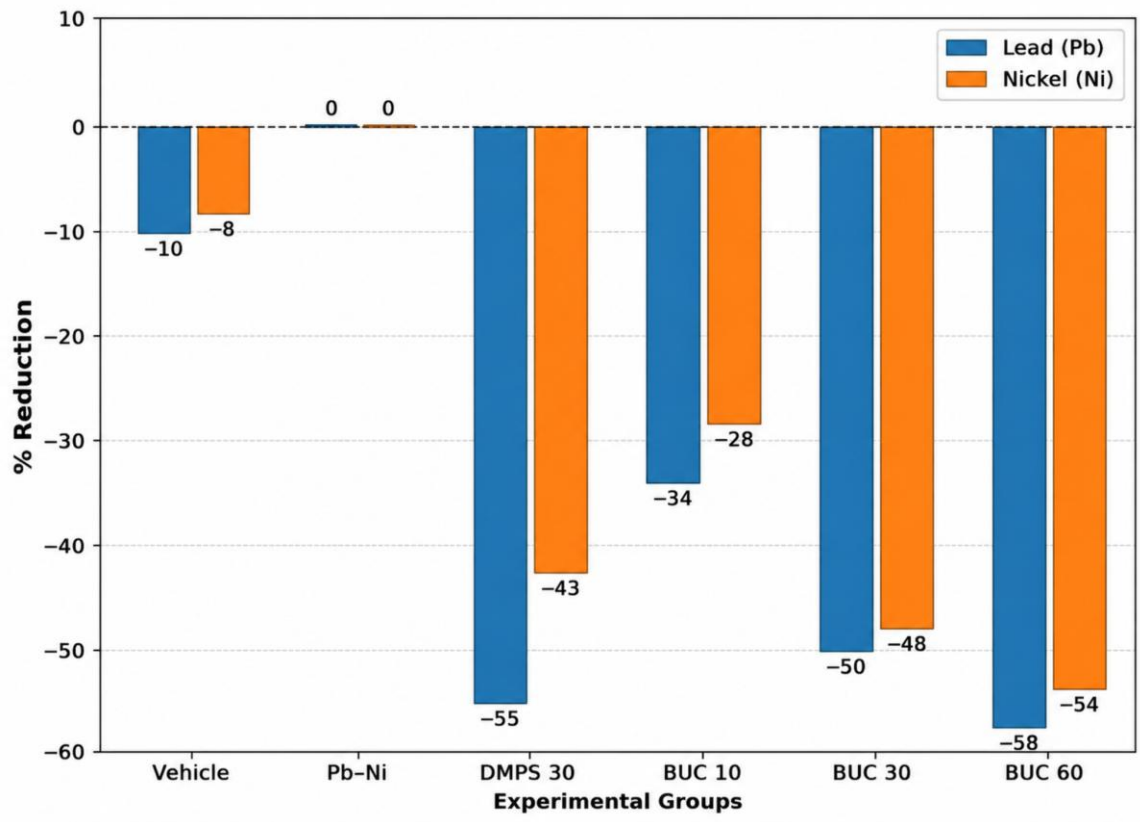
Oral administration of lead acetate and nickel chloride resulted in a pronounced elevation of circulating Pb and Ni levels in vehicle-treated animals, confirming successful induction of combined metal exposure (Tab. 1). Treatment with DMPS and bucillamine significantly reduced blood metal concentrations compared with the vehicle group ($p < 0.05$ vs. vehicle). Bucillamine exhibited a clear dose-dependent effect, with the highest dose (60 mg/kg) producing the greatest reduction in both Pb and Ni levels. Notably, bucillamine reduced blood Pb to levels comparable with DMPS, while achieving superior nickel removal. The percentage reduction relative to post-exposure baseline values is illustrated in Fig. 1.

Tab. 1. Blood lead (Pb) and nickel (Ni) concentrations (µg/dL, mean ± SD) in experimental groups

Group	Pb (µg/dL)	Ni (µg/dL)
Vehicle	62.3 ± 8.1	47.5 ± 6.9
Pb–Ni exposure	120 ± 10	95 ± 9
DMPS 30	28.0 ± 5.3	27.1 ± 4.5
BUC 10	41.2 ± 6.8	34.3 ± 5.1
BUC 30	31.0 ± 5.9	24.7 ± 4.0
BUC 60	26.2 ± 4.7	21.9 ± 3.8

Compared with Vehicle (Pb 62.3 ± 8.1; Ni 47.5 ± 6.9), DMPS lowered Pb and Ni to 28.0 ± 5.3 and 27.1 ± 4.5, respectively. BUC exhibited dose-dependent reductions, with BUC 60 achieving 26.2 ± 4.7 (Pb) and 21.9 ± 3.8 (Ni).

Fig. 1. Blood Pb and Ni reduction (Day 7). Values represent mean % change from baseline; BUC shows dose-dependent decreases, with BUC 60 outperforming DMPS for Ni.



Data are presented as mean ± SD (n = 3), and error bars represent standard deviation (SD).

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4.2. Oxidative stress biomarkers

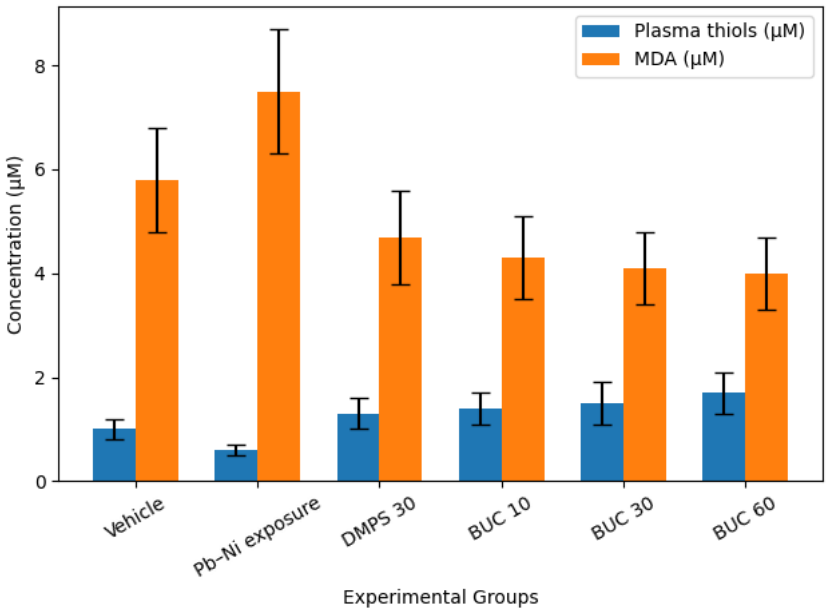
Combined Pb–Ni exposure induced marked oxidative imbalance, characterized by depletion of plasma thiols and elevation of lipid peroxidation products (Tab. 2). Bucillamine treatment resulted in dose-dependent restoration of plasma thiol levels, reaching the highest values at 60 mg/kg ($p < 0.05$ vs. vehicle). Concurrently, MDA levels were significantly reduced ($p < 0.05$), indicating attenuation of lipid peroxidation. These effects were more pronounced with bucillamine than with DMPS (Fig. 2).

Tab. 2. Oxidative stress markers: plasma thiols and MDA levels (µM, mean ± SD)

Group	Plasma Thiols	MDA
Vehicle	1.0 ± 0.2	5.8 ± 1.0
Pb–Ni exposure	0.6 ± 0.1	7.5 ± 1.2
DMPS 30	1.3 ± 0.3	4.7 ± 0.9
BUC 10	1.4 ± 0.3	4.3 ± 0.8
BUC 30	1.5 ± 0.4	4.1 ± 0.7
BUC 60	1.7 ± 0.4	4.0 ± 0.7

BUC 60 increased plasma thiols to 1.7 ± 0.4 versus Vehicle 1.0 ± 0.2 and reduced MDA to 4.0 ± 0.7 versus Vehicle 5.8 ± 1.0.

Fig. 2. Oxidative stress markers. BUC increased plasma thiols and reduced MDA compared with Vehicle and DMPS.



Data are presented as mean ± SD (n = 3), and error bars represent standard deviation (SD).

4.3. Liver and kidney function parameters

Biochemical indices of hepatic and renal function are summarized in (Tab. 3). Vehicle-treated animals showed mild elevations in ALT, AST, creatinine, and BUN, consistent with metal-induced systemic stress. Bucillamine administration produced dose-related improvements in all measured parameters, with values remaining within normal physiological ranges and comparable to those observed with DMPS treatment ($p < 0.05$ vs. vehicle). Trends in liver enzyme activities across experimental groups are illustrated in (Fig. 3).

Tab. 3. Liver and kidney function parameters (mean ± SD)

Group	ALT	AST	Creatinine	BUN
Vehicle	72 ±10	110 ±15	0.82 ±0.1	21 ±3

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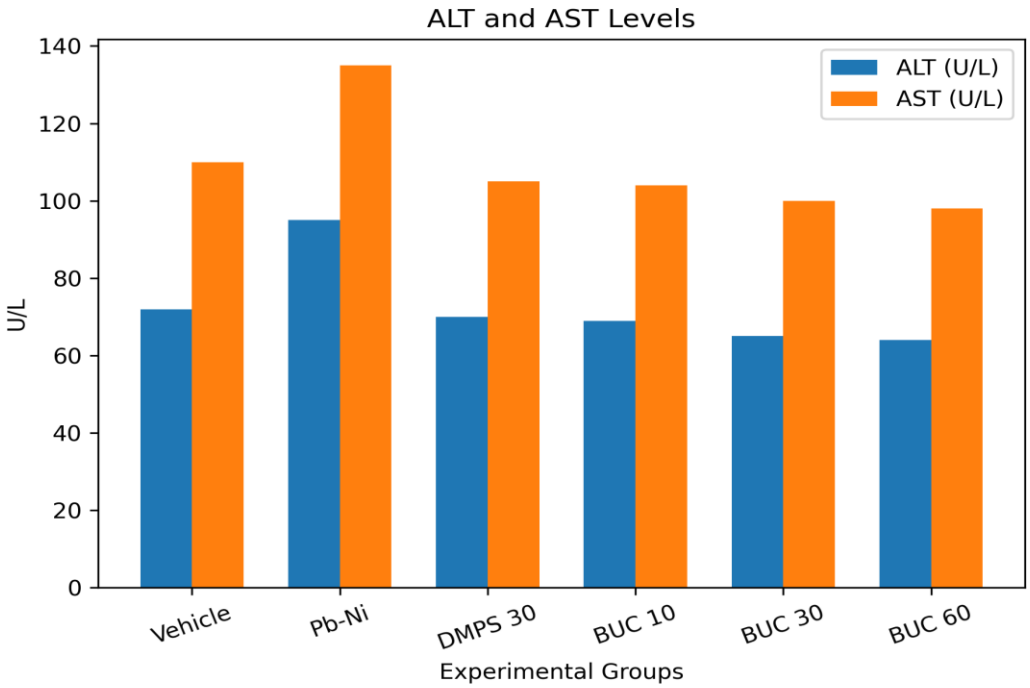
Pb–Ni exposure	95 ±12	135 ±18	1.2 ±0.2	32 ±4
DMPS	70 ±12	105 ±14	0.80 ±0.1	20 ±3
BUC 10	69 ±11	104 ±13	0.80 ±0.1	20 ±3
BUC 30	65 ±11	100 ±13	0.79 ±0.1	19 ±3
BUC 60	64 ±10	98 ±12	0.77 ±0.09	18 ±2

No hepatotoxicity or nephrotoxicity was observed with BUC; ALT/AST, creatinine, and BUN remained within normal ranges comparable to DMPS.

Fig. 3. Liver function enzymes (ALT, AST). Enzyme activities remained stable across groups with a trend toward improvement with BUC



Data are presented as mean ± SD (n = 3), and error bars represent standard deviation (SD).

4.4. Histopathological findings

Histopathological examination of kidney sections from vehicle-treated animals revealed marked tubular degeneration, cytoplasmic vacuolation, and loss of normal renal architecture. DMPS-treated animals showed partial structural recovery, whereas bucillamine at 60 mg/kg preserved near-normal renal morphology with minimal pathological alterations. Representative photomicrographs are presented in Figs. 4 and 5.

Fig. 4Representative photomicrographs of kidney histology in experimental groups (H&E staining, ×400).

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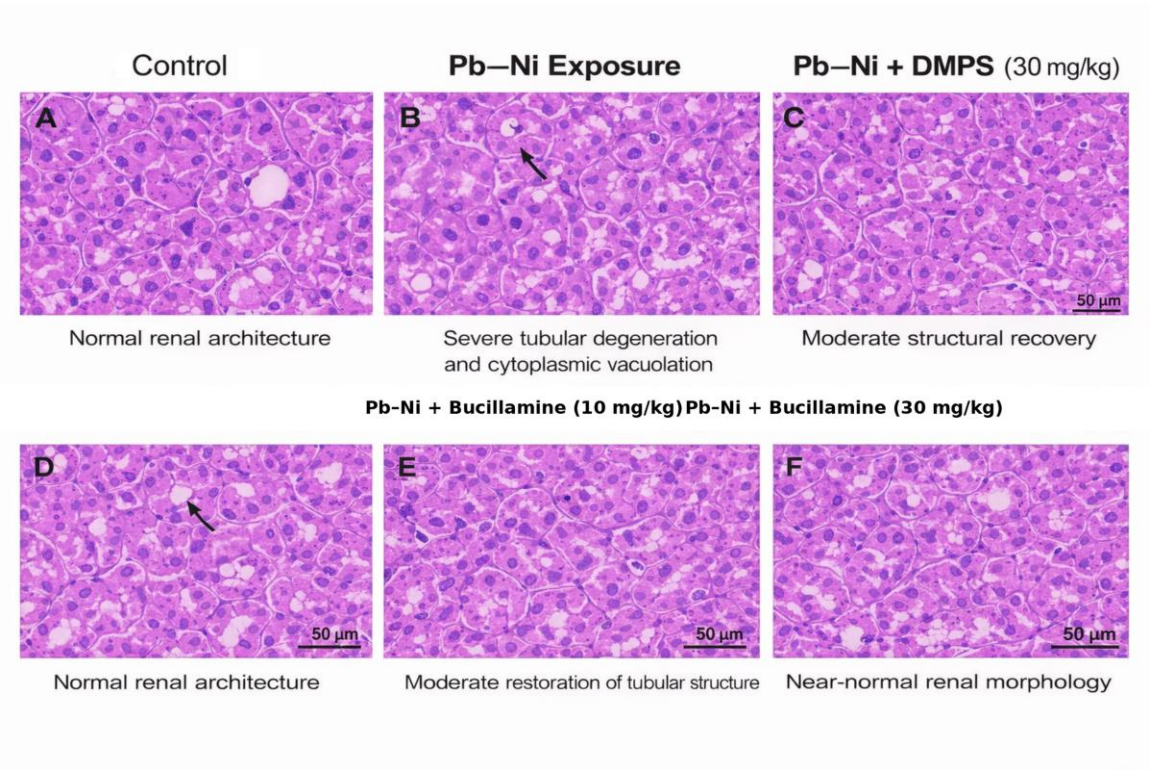

(A) Control group showing normal renal architecture.
(B) Pb–Ni exposed group showing severe tubular degeneration and cytoplasmic vacuolation.
(C) Pb–Ni + DMPS (30 mg/kg) showing moderate structural recovery.
(D) Pb–Ni + Bucillamine (10 mg/kg) showing mild improvement in tubular morphology.
(E) Pb–Ni + Bucillamine (30 mg/kg) showing moderate restoration of tubular structure.
(F) Pb–Ni + Bucillamine (60 mg/kg) showing near-normal renal morphology.
Fig. 5. **Fig. 4. Representative photomicrographs of kidney histology in experimental groups (H&E staining, ×400).**

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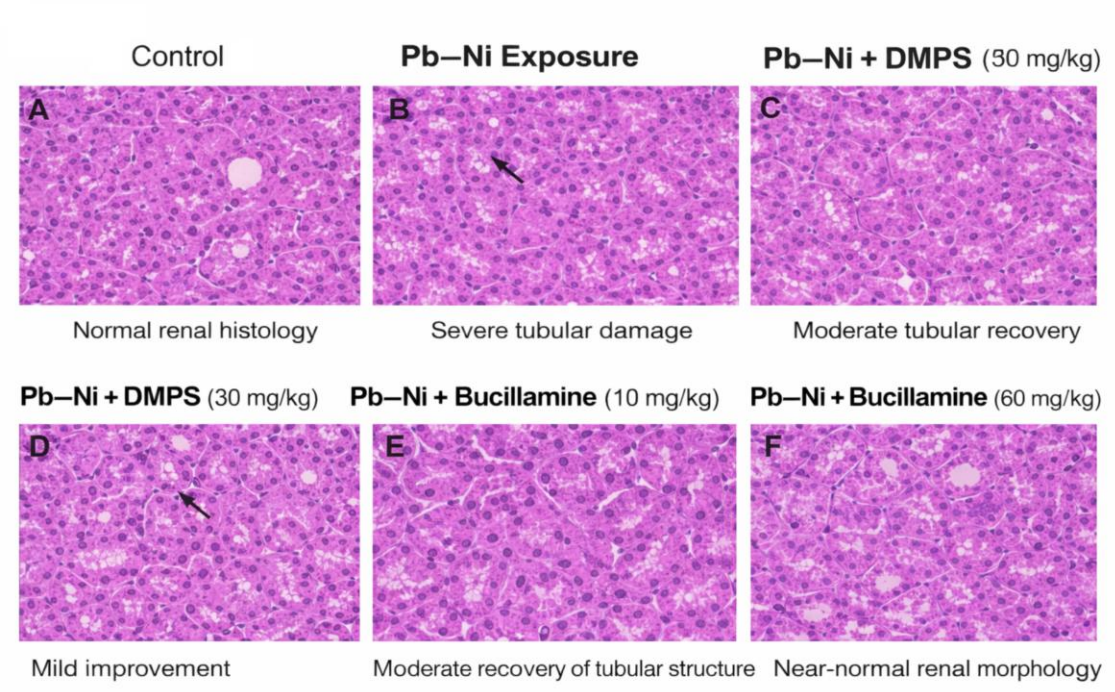

(A) Control group showing normal renal histology.
(B) Pb–Ni–exposed group showing severe tubular damage and cytoplasmic vacuolation.
(C) Pb–Ni + DMPS (30 mg/kg) showing moderate tubular recovery.
(D) Pb–Ni + Bucillamine (10 mg/kg) showing mild improvement in tubular structure.
(E) Pb–Ni + Bucillamine (30 mg/kg) showing moderate restoration of renal architecture.
(F) Pb–Ni + Bucillamine (60 mg/kg) showing near-normal renal morphology.

V. Discussion

The present study demonstrates that bucillamine exerts significant protective effects against combined lead and nickel toxicity in rats. Oral administration of bucillamine resulted in dose-dependent reductions in circulating Pb and Ni levels, indicating effective systemic metal mobilization. The comparable efficacy to DMPS for lead removal and superior performance for nickel clearance highlight the strong affinity of dithiol-containing compounds toward divalent transition metals.

In addition to metal chelation, bucillamine markedly improved oxidative stress biomarkers. Restoration of plasma thiol levels and reduction of lipid peroxidation reflect effective modulation of redox homeostasis, a key mechanism underlying heavy metal toxicity. These antioxidant effects were more pronounced than those observed with DMPS, consistent with the presence of two reactive sulfhydryl groups in the bucillamine structure.

Biochemical assessments of liver and kidney function demonstrated that bucillamine did not induce hepatic or renal toxicity, even at the highest tested dose. Histopathological analysis further confirmed preservation of renal architecture and attenuation of metal-induced tissue injury. Collectively, these findings suggest that the combined chelating and antioxidant properties of bucillamine contribute to its protective efficacy.

In a translational context, it is important to consider the relationship between the experimental doses used in this study and clinically relevant dosing of bucillamine. Bucillamine is an approved oral drug for rheumatoid arthritis in Japan, typically administered at doses of 100–300 mg/day in humans [20,23]. When adjusted for body surface area, the doses used in the present study fall within a pharmacologically relevant range, supporting the potential translational significance of the findings.

Clinically, bucillamine is generally well tolerated, although reported adverse effects include proteinuria, dermatological reactions, and, less frequently, hepatic dysfunction [23,24]. The kidney has been identified as a potential target organ in some cases of long-term administration [24]. In the present study, no evidence of renal or hepatic toxicity was observed within the experimental

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timeframe, as indicated by biochemical and histopathological assessments.

However, it should be noted that the safety evaluation in this study is limited to selected biomarkers and short-term exposure. Therefore, further long-term and comprehensive toxicological investigations are required to fully establish the safety profile of bucillamine in the context of heavy metal detoxification.

The oral activity and favorable safety profile within the limits of the present experimental conditions represent important advantages over conventional chelators that require parenteral administration and lack intrinsic antioxidant capacity. These properties support its potential application as a multifunctional agent for mitigating biochemical and tissue damage associated with heavy metal exposure

VI. Conclusion

Bucillamine exhibited robust, dose-dependent chelating activity against lead and nickel in a rat model of combined exposure. At a dose of 60 mg/kg, its efficacy was comparable to DMPS for lead removal and superior for nickel clearance, while simultaneously restoring redox balance and reducing oxidative damage. The absence of hepatic or renal toxicity and the preservation of renal morphology further support its safety profile. These findings provide novel in vivo evidence supporting bucillamine as a promising orally active chelating agent for environmental and occupational heavy metal exposure.

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