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Research Article

Investigating the Hepatorenal Toxicity of Co-Exposure of Cadmium and Arsenic Via Food Chain in Wistar Rats: A Study on Oxidative Stress Markers and Kidney Function Parameters

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ABSTRACT
Heavy metal contamination through the food chain poses significant health risks; however, the combined effects of simultaneous cadmium and arsenic exposure on liver and kidney function remain poorly understood. This study investigated the hepatorenal toxicity of singular and combined exposure to cadmium and arsenic across the food chain in *Wistar* rats, emphasizing on oxidative stress markers and indications of kidney function. The *Wistar* rats were divided into four groups: control, arsenic-treated, cadmium-treated, and combined treatment groups. Body weight increase, organ-to-body weight ratios, enzymatic activities (monoamine oxidase, sulfite oxidase, aldehyde oxidase, and xanthine oxidase) and plasma biomarkers (creatinine and urea) were assessed. Exposure to individual metals markedly reduced body weight gain (arsenic: 39.27g; cadmium: 37.62g compared to control: 77.50g, $p<0.05$), although combined exposure showed no significant decrease (80.07g). The ratio of kidney weight to body weight significantly increased across all treatment groups. Cadmium treatment and combined exposure markedly elevated aldehyde oxidase activity in the liver while diminishing it in the kidneys. Cadmium alone markedly diminished liver xanthine oxidase activity, whereas combined exposure substantially increased it (0.83 vs. 0.41 min/g weight wet, $p<0.05$). Plasma creatinine levels significantly increased exclusively with cadmium therapy (2.57 mg/dl versus control: 2.20 mg/dl). Exposure to cadmium and arsenic exhibited intricate correlations. For instance, it produced contrasting effects on body weight while enhancing specific oxidative enzymes. The results demonstrate that co-exposure assessment is crucial for clarifying heavy metal toxicity mechanisms and developing appropriate risk assessment strategies for food chain contamination.

Keywords: Cadmium-arsenic interaction; Food chain contamination; Heavy metals; Hepatorenal toxicity; Oxidative stress: *Wistar* rats

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INTRODUCTION

Heavy metals may infiltrate the body by inhalation, ingestion, and dermal absorption following their release into the environment (Asagba, 2010). Cadmium and arsenic are acknowledged toxic elements that can damage the liver and kidneys with extended occupational or environmental exposure (Balali-Mood *et al*., 2021). The worldwide problem of toxic heavy metal pollution poses significant risks to the health of both humans and animals (Jomova *et al*., 2025).

Researchers have reported that the detrimental element Cd originates from soil, rock phosphate

fertilizer, and the tobacco plant (Diakparomre *et al*., 2024). Mining and industrialization have elevated cadmium concentrations in soils, sediments, and water (Umair *et al*., 2024; Diakparomre *et al*., 2023). Cadmium (Cd) is prevalent in the environment at hazardous levels due to heightened industrial, volcanic, and agricultural activities, coupled with its persistence, leading to human exposure by inhalation or ingestion (Hossain *et al*., 2019). Cadmium is not an essential element for biological systems; its absorption is enabled by detoxification via membrane stabilization and binding (Ezedom *et al*., 2016). The Agency for Toxic Substances and Disease Registry (ATSDR, 2022) compiled a list of the 275 most deleterious compounds. Arsenic and cadmium rank among the seven most hazardous wastes on the list. Cadmium is regarded as one of the most hazardous environmental elements due to its potential to harm several organs, its prolonged retention in the body (10 to 30 years), and its carcinogenic properties (Genchi *et al*., 2020; Charkiewicz *et al*., 2023; Diakparomre *et al*., 2023). Cadmium was identified as a pollutant at 776 of the 1,467 locations on the EPA National Priorities List. Even in minimal quantities, it has been shown to be significantly detrimental to living cells and tissues. Cadmium is a subterranean metal. It did not enter the air, water, or food in significant quantities until it was discovered in zinc deposits (Hossain *et al.*, 2019).

Arsenic exhibits complex chemistry and forms several compounds. Arsenic possesses several valence or oxidation states, resulting in diverse behaviours of its compounds in biological systems. Arsenic compounds are utilized in dyes and pigments, as preservatives for animal hides, in glass production, as pesticides for agriculture, and in certain pharmaceuticals (ATSDR, 2006). Arsenic (As) belongs to group V of the periodic table, which comprises nitrogen, phosphorus, antimony, and bismuth. It occurs naturally in flora, soil, and subterranean water sources. Arsenic exists in both inorganic and organic compounds, each exhibiting distinct physical and chemical characteristics. The toxicity of inorganic arsenic compounds significantly differs from that of organic arsenic compounds. Organic arsenic compounds exhibit low toxicity. The potential harmful effects of arsenic on animal and human health depend on the content of inorganic arsenic in food. Inorganic arsenic comprises arsenite (As (III)) and arsenate (As (V)). A methyl group can bond to them to form monomethylarsonic acid (MMA (V)), or it can be removed, as observed in arsenic acid, which has two methyl groups. The metabolism of inorganic arsenic entails a two-electron reduction from valency 5 to 3, requiring glutathione, followed by the formation of organic arsenic at valency 5 through oxidative methyl group addition (Hughes, 2002). Organic arsenic is less toxic than inorganic arsenic (Medina-Pizzali *et al*., 2018). Arsenic is detrimental to numerous organs, with the kidneys potentially being the most impacted. The severity of arsenic poisoning depends on various factors, including dosage, individual susceptibility, and age (Jomova *et al*., 2025).

The liver and kidneys, tasked with metabolizing nearly all xenobiotics in the body, are typically compromised by the toxic effects of cadmium (Cd), which induce oxidative stress, disrupt the functionality of essential metals, and result in damage (Peana *et al*., 2022). Metals ingested through contaminated food are absorbed and sent through the circulation to organs such as the liver, the body's detoxification centre, and the kidneys, the primary excretory organs. Metals can persist in these organs, disrupt metabolic functions, and trigger harmful pathophysiological processes (Mukherje *et al*., 2022). Individuals can acquire cadmium pollutants from the atmosphere and the food chain. Upon ingestion, cadmium (Cd) is absorbed and transported to the liver by the body's intestinal protein metallothionein. Subsequently, it rapidly disseminates to other organs, with the kidneys being the primary organ adversely affected by cadmium (Asagba, 2009). The kidneys play a crucial role in regulating electrolyte and fluid levels within cells, as well as eliminating metabolic waste from the body. Thus, the functional integrity of the kidneys is crucial for sustaining overall homeostasis. Any medication that damages the kidneys may negatively impact overall body metabolism (Michael *et al*., 2014).

Aldehyde oxidase (AO; EC 1.2.3.1) is an enzyme found in the cells of various animals, including humans. It belongs to the xanthine oxidase family and contains unique metal and vitamin components (molybdenum and flavin) that facilitate chemical interactions. The primary function of AO is to transform aldehydes and other nitrogenous cyclic molecules into carboxylic acids. It uses oxygen from the ambient air and produces hydrogen peroxide (H_2O_2) as a natural byproduct (Dalvie and Di, 2019). The liver contains

the highest concentration of this enzyme, which also facilitates the breakdown of endogenous molecules and exogenous compounds, such as pharmaceuticals or toxins, in other organs. Notably, not all animals have functional AO; for example, dogs entirely lack this enzyme, which explains their distinct responses to certain drugs in comparison to humans (Wu *et al*., 2022).

Monoamine oxidase (MAO) is an enzyme often situated in the outer membrane of mitochondria, the cellular powerhouses. It regulates the amounts of crucial brain chemicals known as monoamines. These encompass neurotransmitters such as dopamine, serotonin, norepinephrine, epinephrine, and histamine. MAO functions as a regulatory mechanism by eliminating surplus monoamines through oxidative deamination. It removes an amine group from the molecule and substitutes it with a carbonyl group. It uses oxygen to accomplish this, resulting in hydrogen peroxide (H_2O_2) and ammonia (NH_3) as byproducts. This process prevents the excessive accumulation of neurotransmitters, which could otherwise disrupt normal brain and bodily functions (Bortolato *et al*., 2008).

Xanthine oxidase (XO) is a crucial enzyme in purine metabolism, facilitating the degradation of substances such as ATP and DNA for the reutilization of their components. It is a member of the xanthine oxidoreductase family and is present in several tissues, with the liver and intestines exhibiting the highest concentrations. The primary function of XO is to convert hypoxanthine into xanthine and then transform xanthine into uric acid. This process relies on oxidation reactions that utilise oxygen as the terminal electron acceptor. These reactions produce hydrogen peroxide (H_2O_2) and superoxide anion ($O_2\bullet^-$) as byproducts. Uric acid is a common metabolic byproduct; nevertheless, excessive accumulation can lead to complications such as gout or nephrolithiasis. Excessive reactive oxygen species from an overactive XO can induce oxidative stress, inflammation, and tissue damage. Consequently, individuals frequently employ XO inhibitors such as allopurinol and febuxostat to manage gout, hyperuricemia, and certain cardiac conditions (Aziz & Jamil, 2023)

Sulfite oxidase is located in the intermembrane space of mitochondria in several organs. It functions most effectively in the liver and kidneys. The role is crucial, as sulfite accumulation can damage proteins, DNA, and cell membranes. Sulfite oxidase insufficiency is an uncommon yet severe disorder that occurs when this enzyme is either lacking or functioning inadequately. This may occur due to genetic mutations or issues with the synthesis of its molybdenum cofactor. Sulfite and a related compound, S-sulfocysteine, may accumulate in the body, resulting in this condition, which can cause developmental delays, seizures, and neurological damage (Feng *et al*., 2007).

The concentration of urea in a fluid is referred to as urea concentration. Blood (Blood Urea Nitrogen, BUN) and urine are the primary fluids utilised for this measurement. This illustrates the quantity of urea produced by the liver through the urea cycle and the amount of urea excreted by the kidneys. Assessing urea concentration is an essential diagnostic tool in medical and veterinary contexts for evaluating renal function, hepatic function, hydration levels, and protein metabolism (Weiner *et al*., 2014).

Creatinine is a byproduct produced by the metabolism of creatinine phosphate in muscle tissue. Creatinine phosphate is crucial for energy storage during muscle contraction; hence, healthy individuals produce creatinine at a relatively constant rate primarily influenced by their muscle mass. Creatinine is produced in muscle tissue and then enters the bloodstream. The kidneys excrete it, with minimal reabsorption occurring. This is why individuals frequently assess creatinine levels in blood and urine to evaluate renal function (Wyss and Kaddurah-Daouk, 2000).

Individuals rarely encounter a singular metal. Cadmium and arsenic frequently co-occur in contaminated food chains, particularly in rice, seafood, and vegetables from polluted regions. Most toxicological research concentrates on the effects of individual metals, leading to a restricted comprehension of the interactions among many metals, despite their presence in actual co-exposure scenarios. This study investigated the hepatorenal toxicity from both individual and combined exposure to cadmium and arsenic down the food chain in *Wistar* rats, emphasizing oxidative stress markers and kidney function indicators.

MATERIALS AND METHODS

Animal Model

A total of 16 male Wistar rats, each weighing between 135 and 140 g, were procured from the Animal House

at the Faculty of Pharmacy, Delta State University, Abraka, for the study. The selection of male rats aims to reduce hormonal fluctuations that may influence the outcomes. The rats were acclimatized to the laboratory environment for a duration of 10 days prior to the initiation of the experiment. The rats were separately kept in conventional stainless-steel cages within a well-ventilated environment that maintains a 12-hour light/dark cycle in line with the National Institute of Health (NIH) handbook (NIH 1985) for animal care and handling with the ethical approval number ETH12/13/PG214572. The rats had unrestricted access to standard rat food and clean drinking water during the experiment, with the exception of the treatment groups specified below. Four experimental diets were formulated to examine metal exposure across the food chain. The three experimental diets included milled fish contaminated with cadmium (Cd), arsenic (As), or a mixture of both metals, functioning as the dietary metal source. The control group was fed a meal consisting of milled fish that had not been previously exposed to cadmium or arsenic. Supplementary nutritional constituents were maize starch (Livestock Feed Depot, Warri), a multivitamin/mineral formulation (Vetindia Pharmaceuticals Limited, India), vegetable oil (locally procured, Abraka, Nigeria), analytical grade cellulose, and refined granulated sugar (Abraka market).

Experimental Design

The study employed a four-group experimental design, with 16 *Wistar* rats randomly allocated to each group. Each rat was individually housed in a metabolic cage for the full three-month length of the trial. The designated diets included a control diet, a cadmium (Cd) test diet, an arsenic (As) test diet, or a combined Cd+As test diet, with all animals granted unrestricted access to water. The diet formulation contain 20% protein (fish), 55% carbohydrate source (corn), 10% fat (groundnut oil), 10% fiber (cellulose), 5% multivitamin/mineral mix. For the individual metals 10 mg/ml of cadmium and 10 mg/ml of arsenic was incorporated into the fish water while for the combined dosage, 5 mg/ml and 5 mg/ml of cadmium and arsenic were incorporated into the fish water.

Tissue and Blood Sample Collection

Upon completion of the three-month exposure period, all animals were subjected to a three-hour fast prior to weighing. They were subsequently euthanized humanely using chloroform anaesthesia. During anaesthesia, blood samples were obtained by heart puncture using a hypodermic syringe and needle. The obtained blood was promptly transferred to heparinized tubes and gently agitated. Plasma was subsequently extracted by centrifuging the blood at 4000 rpm for 10 minutes. Subsequent to blood collection, the liver and kidneys were swiftly removed, placed on ice, and weighed individually. Segments of liver, heart, and kidney tissues were subsequently homogenized to create 10% homogenates. The homogenates were centrifuged at 4000 rpm for 10 minutes to get clean supernatants, which were subsequently used for biochemical tests.

Determination of Various Biochemical Parameters

The supernatants derived from liver, kidney, testes, heart, and brain tissues were examined for the activities of sulfite oxidase (SO), aldehyde oxidase (AO), monoamine oxidase (MO), and xanthine oxidase (XO). The SO, AO, MO and XO activity were evaluated according to the methodology outlined by Ezedom and Asagba (2016). Urea concentration and serum creatinine were carried out according to the method of Orororo and Asagba (2022).

Data Analysis

The results were presented as mean ± SEM. Statistical analysis was conducted using one-way analysis of variance (ANOVA) with the software package SPSS version 21 (SPSS Inc., Cary, NC, USA). The least significant difference (LSD) test was employed to assess the mean difference between the experimental and control groups. Statistical significance was defined as $P<0.05$.

RESULTS

Effect of Food chain Mediated Metal Exposure on Weight Gain

The separate and combined effect of arsenic and cadmium on body weight gain and organ/body weight ratio of rats is presented in Table 1. There was no significant difference in body weight gain of rats administered with the combined dose (80.07 g) when compared with the control (77.50 g) but a significant decrease in body weight gain was observed in rats administered with arsenic (39.27 g) and cadmium (37.62 g) at $p < 0.05$. Thus, this study shows that arsenic and cadmium has no influence on body weight gain when combined. There was no significant difference in the liver/body weight ratio of rats in all treatment group (combined and separate treatment

with arsenic and cadmium) when compared to the control ($p < 0.05$). On the other hand, the organ/body weight ratio for kidney was significantly increased for separate and combined treatment with arsenic and cadmium ($p < 0.05$).

Effect of Food Chain Mediated Exposure on Monoamine Oxidase

Table 2 presents the effect of arsenic, cadmium and combination of both metals on tissue monoamine oxidase activity of experimental rats. The study shows that the MAO activity was not influenced by metals in these organs. There was no significant difference in the liver MAO activity of arsenic (24.64 units/g tissue), cadmium (23.86 units/g tissue) and combined dosage of cadmium and arsenic (23.55 units/g tissue) treated group when compared to the control (22.33 units/g tissue). Also, there was no significant difference in the kidney MAO activity of the arsenic (28.41 units/g tissue), cadmium (29.62 unit/g tissue), Arsenic + Cadmium (33.73 unit/g tissue) when compared to the control (32.97 units/g tissue).

Table 1: Body Weight Gain and Organ/Body Weight Ratio of Rats Exposed to Cadmium and Arsenic via the Food Chain

Parameter (g)	Control	Arsenic	Cadmium	Arsenic + cadmium
Body weight	77.50 ± 7.59^{a}	39.27 ± 11.65^{b}	37.62 ± 5.85^{c}	80.07 ± 5.39^{a}
Liver/bodyweight ratio	0.034 ± 0.20^{a}	0.0350 ± 0.05^{a}	0.0305 ± 0.28^{a}	0.0339 ± 0.33^{a}
Kidney/bodyweight ratio	0.006 ± 0.03^{a}	0.008 ± 0.04^{b}	0.007 ± 0.02^{c}	0.007 ± 0.01^{c}

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$

Table 2: Effect of Food Chain Mediated Exposure on Monoamine Oxidase

Parameter (units/g tissue)	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	22.33 ± 1.58^{a}	24.64 ± 0.82^{a}	23.86 ± 2.51^{a}	23.55 ± 1.48^{a}
Kidney	32.97 ± 2.08^{a}	28.41 ± 1.37^{a}	29.62 ± 2.15^{a}	33.73 ± 2.56^{a}

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$.

Effect of Food Chain Mediated Exposure on Sulphite Oxidase

Table 3 represents the effect of arsenic, cadmium and combination of both metals on tissue sulphite oxidase activity of experimental rats. There was no significant difference in the sulphite oxidase activity in liver when compared to control (17.69units/g tissue) in rats treated with arsenic (19.38 units/g tissue), cadmium (18.82 unit/g tissue) and cadmium + arsenic (19.33 units/g tissue). Similarly, kidney sulphite oxidase was not significantly different from control (17.30 units/g tissue) in rats treated with arsenic (16.29 unit/g tissue), cadmium (18.82unit/g tissue) or arsenic plus cadmium (16.96units/g tissue).

Effect of Food Chain Mediated Exposure on Aldehyde Oxidase

Table 4 represents the effect of arsenic, cadmium and combination of both metals on tissue aldehyde oxidase activity of experimental rats. The liver aldehyde oxidase activities were not significantly different from control (23.99 µmol benzoate/g) in rats treated with arsenic (23.05 µmol benzoate/g), but was significantly increased in rats treated with cadmium (26.80 µmol benzoate/g) or combination of cadmium and arsenic (30.37 µmol benzoate/g). On the other hand kidney AO activity was not significantly different from control (28.93 µmol benzoate/g) in rats treated with arsenic (27.21 µmol benzoate/g), but was significantly decreased in rat administered cadmium (25.40±0.90µmol benzoate/g) and cadmium plus arsenic (25.60±1.57µmol benzoate/g).

Table 3: Sulphite Oxidase Activity in Rats Exposed to Cadium and Arsenic

Parameter (units/g tissue)	Control	Arsenic	Cadmium	Arsenic+ Cadmium
Liver	17.69 ± 0.22^{a}	19.38 ± 0.81^{a}	18.82 ± 0.38^{a}	19.33 ± 0.63^{a}
Kidney	17.30 ± 0.16^{a}	16.29 ± 0.39^{a}	18.82 ± 0.58^{a}	16.96 ± 0.39^{a}

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at ($P<0.05$), values not sharing a common superscript in same row differs at $P<0.05$.

Table 4: Aldehyde Oxidase Activity (µmol benzoate/g) in Rats Exposed to Cadmium and Arsenic

Parameter (µmol benzoate/g)	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	23.99 ± 1.97[a]	23.05 ± 2.37[a]	26.80 ± 1.37[b]	30.37 ± 1.76[b]
Kidney	28.93 ± 3.30[a]	27.21 ± 0.99[a]	25.40 ± 0.90[b]	25.60 ± 1.57[b]

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at (P<0.05), values not sharing a common superscript in same row differs at P<0.05

Xanthine Oxidase Activity (min/g wet weight) in Rats Exposed to Cadmium and Arsenic

The effect of arsenic, cadmium and combination of both metals on tissue xanthine oxidase activity of experimental rats is presented in Table 5. The liver xanthine oxidase (XO) activity was not significantly different from control (0.41 min/g wet weight) in rats treated with arsenic (0.41 min/g wet weight), but was significant decreased in rats treated with cadmium (0.29±0.024min/g wet weight). Conversely administration of both metals (0.83±0.16min/g wet weight) significantly increased liver XO activity when compared to control (p <0.05). The kidney, XO activity was not significantly different from control (0.37 min/g wet weight) in rats treated with arsenic 0.58 min/g wet weight), cadmium (0.45 min/g wet weight) and a combination of cadmium and arsenic (0.41 min/g wet weight).

Effect of Food Chain Mediated Exposure on Plasma Creatinine and Urea Concentrations

Table 6 presents the effect of arsenic, cadmium and combination of both metals on plasma creatinine and urea concentration of experimental rats. The creatinine concentration in the plasma of rats was not significantly different from the control (2.20 mg/dl) in rats treated with arsenic (2.34 mg/dl) and a combination of cadmium and arsenic (2.30 mg/dl) but was significantly increased in rats treated with cadmium (2.57 mg/dl). The urea concentration in the plasma of rats was not significantly different from the control (17.46 mg/dl) in rats treated with arsenic (18.84 mg/dl), cadmium (19.52 mg/dl) or the combination of both metals (19.79 mg/dl).

Table 5: Effect of Food Chain Mediated Exposure on Xanthine Oxidase (min/g wet weight)

Parameter	Control	Arsenic	Cadmium	Arsenic + Cadmium
Liver	0.41±0.04[a]	0.41±0.04[a]	0.29±0.024[b]	0.83±0.16[c]
Kidney	0.37±0.04[a]	0.58±0.14[a]	0.45±0.04[a]	0.41±0.04[a]

Values are expressed in Mean ± Standard error of Mean (SEM) N=4, significance at (P<0.05), values not sharing a common superscript in same row differs at P<0.05

Table 6: Creatinine and Urea in Plasma of Metal Treated Rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control	2.20 ± 0.025[a]	17.46 ± 1.69[a]
Arsenic	2.34 ± 0.11[a]	18.84±0.85[a]
Cadmium	2.57 ± 0.08[b]	19.52 ± 0.29[a] (11.76%)
Cadmium + Arsenic	2.30 ± 0.06[a]	19.79 ± 0.46[a]

Values are expressed in mean ± standard error of mean (SEM) N=4, significance at (P<0.05), values not sharing a common superscript in same column differs at P<0.05

DISCUSSION

The present study revealed differing impacts of arsenic and cadmium on body weight gain when administered separately and in combination. Exposure to arsenic and cadmium individually significantly reduced body weight gain compared to controls, although combined exposure showed no significant difference from controls. This evidence indicates potential antagonistic interactions among these metals regarding growth characteristics, along with previous research that suggests metal combinations may exhibit non-additive effects due to competing binding sites and altered toxicokinetics (Tchounwou *et al*., 2012). The reduced weight gain linked to individual metal exposure corresponds with studies demonstrating that arsenic and cadmium can disrupt metabolic functions, including glucose metabolism and lipid synthesis (Afridi *et al*., 2010, Satarug *et al*., 2010).

The significant increase in the kidney-to-body weight ratio in all treatment groups indicates renal involvement, expected according to the nephrotoxic characteristics of both metals. Cadmium accumulates in the kidneys due to its great binding affinity for metallothionein proteins, which alters the structure and function of the kidneys (Järup & Åkesson, 2009). The preservation of liver-to-body weight ratios suggests that the liver may have enhanced adaptive mechanisms or that the duration of exposure was insufficient to provoke significant hepatic changes.

The minimal changes in MAO activity in liver and kidney tissues suggest that this enzyme system may demonstrate significant resistance to arsenic and cadmium damage at the given concentrations. This finding contradicts certain studies that have revealed altered MAO activity following heavy metal exposure (Abdelrazek *et al*., 2016, Mahmoudi *et al*., 2018). MAO activity, however, might vary depending on the duration of exposure, the dosage received, and the specific isoforms being evaluated. Maintaining constant MAO levels is essential, as this enzyme is crucial for neurotransmitter processing and cellular energy production.

Sulphite oxidase activity was constant across all treatment groups. This indicates that exposure to arsenic and cadmium may not directly impair this molybdenum-containing enzyme. Sulfite oxidase is essential for the metabolism of sulphur amino acids, and its preservation signifies the maintenance of basic metabolic pathways involving sulphur compounds (Garrett *et al*., 1998; Mendel and Schwarz, 2023).

The most notable finding was the tissue-specific response of aldehyde oxidase (AO) activity. Cadmium alone and the combo therapy markedly increased AO activity in liver tissue, whereas arsenic alone had no impact. Conversely, in renal tissue, both cadmium alone and the combination therapy significantly diminished AO activity. This differential response may suggest tissue-specific regulatory mechanisms and the distinct roles of AO in detoxification processes (Garattini & Terao, 2012). The increased hepatic AO activity may indicate an adaptive response to enhance detoxification, whereas the reduced renal activity may suggest enzyme inhibition or cellular damage.

The xanthine oxidase (XO) findings indicated that the metals interacted in a complex manner. Cadmium alone diminished liver XO activity, whereas the combination treatment markedly augmented it, suggesting synergistic effects. This enzyme participates in purine metabolism and can induce oxidative stress by producing superoxide and hydrogen peroxide (Battelli *et al*., 2016, Kotozaki *et al*., 2023). Increased XO activity due to concurrent metal exposure may exacerbate oxidative damage, a primary factor in metal poisoning.

The elevation of plasma creatinine levels in the cadmium-treated group indicates initial renal dysfunction, consistent with cadmium's established nephrotoxic properties (Nordberg *et al*., 2007). The lack of this increase in combination therapy suggests that arsenic may partially alleviate cadmium-induced renal injury, either through competitive mechanisms or altered distribution patterns.

Plasma urea levels remained consistent throughout the groups, indicating that the exposure time was insufficient to cause significant alterations in urea synthesis or excretion. Conversely, compensatory mechanisms may have maintained urea homeostasis despite metal exposure (Abdel-Moneim and Said, 2007, Andjelkovic *et al*., 2019, Genchi *et al*., 2020).

CONCLUSION

This work demonstrates that arsenic and cadmium exhibit intricate interactions when co-administered, resulting in effects that diverge from those seen with individual metal exposure. The maintenance of body weight growth and the varied enzyme responses suggest both antagonistic and synergistic interactions depending on the biological outcome assessed. These findings have significant implications for the risk assessment of metal combinations in environmental and occupational health situations, highlighting the necessity of considering interaction effects instead of only additive models.

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