PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 29 (2) 525-531 Feb. 2025

Moringa Oleifera Extract Mitigates Arsenic Trioxide-Induced Gastric Toxicity in Wistar Rats

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ABSTRACT: Toxic substances like arsenic trioxide pose a significant threat to gastric health, often leading to severe complications or even death. The search for natural remedies has brought attention to *Moringa oleifera*, renowned for its medicinal properties and bioactive compounds. Hence, the objective of this paper was to evaluate the protective effects of aqueous extract of *Moringa oleifera* on arsenic trioxide-induced gastric toxicity in adult Wistar rats using appropriate standard procedures after treating the Wistar rats with varying concentrations of arsenic trioxide as follows: Group A (control) received only feed and water, Group B was treated with 10 mg/kg arsenic trioxide, Groups C-E received arsenic trioxide plus 200 mg/kg (low dose), 400 mg/kg (intermediate dose), or 800 mg/kg (high dose) of *Moringa oleifera*, respectively, while Group F received only 800 mg/kg *Moringa oleifera*. Treatments were administered orally for 28 days. The stomach weight (g) across the different groups was as follows: [A = 1.30 ± 0.25, B = 1.87 ± 0.09, C = 1.33 ± 0.07, D = 1.77 ± 0.20, E = 1.50 ± 0.10, and F = 1.57 ± 0.17 P = 0.1486, P > 0.05]**,** organosomatic index [A = 0.0069 ± 0.0009, B = 0.0071 ± 0.0004, C = 0.0079 ± 0.0004, D = 0.0086 ± 0.0005, E = 0.0089 ± 0.0009, and F = 0.00797 ± 0.0007**,** P = 0.3180, P > 0.05], initial body weight (g) [A = 174.0 ± 12.64, B = 262.6 ± 9.628, C = 157.7 ± 6.087, D = 180.0 ± 11.87, E = 153.5 ± 8.047, and F = 185.3 ± 13.65. After treatment, the final body weight (g) for the respective groups was **[**172.4 ± 12.00, 265.2 ± 1.463, 160.6 ± 9.563, 192.2 ± 13.56, 157.6 ± 9.642, and 189.0 ± 10.29] respectively, indicating variations in weight changes across the groups. The statistical values obtained were converted into graphical representation in form of bar charts. Results showed no significant differences in body weight, organ weight, or organosomatic index across groups. However, histological examination revealed that *Moringa oleifera* extract provided notable ameliorative and protective effects against arsenic-induced gastric damage. These findings provide evidence of the therapeutic potential of *Moringa oleifera* in managing gastric toxicity caused by toxicants like arsenic trioxide, offering promise for natural, plant-based interventions.

DOI: https://dx.doi.org/10.4314/jasem.v29i2.23

Cite this Article as: CALMDAY-OMBO, D; ELUEHIKE, N (2025) Moringa Oleifera Extract Mitigates Arsenic Trioxide-Induced Gastric Toxicity in Wistar Rats. *J. Appl. Sci. Environ. Manage.* 29 (2) 525-531

Dates: Received: 23 December 2024; Revised: 27 January 2025; Accepted: 09 February 2025; Published: 28 February 2025

Keywords: Gastric toxicity; Arsenic trioxide; *Moringa oleifera*; histological analysis; Wistar rats.

Medicinal plants have been integral to human health for centuries, serving as sources of bioactive compounds for pharmacological purposes (Nasim *et al.*, 2022). Globally, the use of medicinal herbs dominates traditional healthcare systems, with plant-derived extracts forming the basis of numerous modern drugs (Salmerón-Manzano *et al.*, 2020). *Moringa oleifera*, a drought-resistant tree native to India's sub-Himalayan regions, stands out for its medicinal and nutritional properties (Nasim *et al.*, 2022). This versatile plant has been utilized for culinary, medicinal, and nutraceutical applications

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due to its bioactive components, including essential amino acids and carotenoids (Innih and Calmday-Ombo, 2024; Sundaram and Babu, 2024). Despite extensive research on its general therapeutic potential, limited studies focus on its impact on gastric health. Conversely, arsenic trioxide, a toxic inorganic compound, poses a severe threat to human health, causing tissue and organ damage upon exposure (Innih and Calmday-Ombo, 2024; Olukayode and Innih, 2024).

The stomach, the broadest portion of the alimentary canal, plays a central role in digestion with its anatomical, histological, and functional adaptations. Located mainly in the left hypochondrial region and extending into the epigastric and umbilical regions, it is divided into the fundus, body, and pyloric portion, with a typical adult capacity of 1.5 liters (Chaudhry *et al.*, 2019). It connects proximally to the esophagus via the cardiac orifice and distally to the duodenum through the pyloric orifice, facilitated by sphincters. Blood supply arises primarily from the celiac artery, forming vascular arcades along its curvatures, while venous drainage channels into the portal vein. Lymphatic drainage, meticulously classified for clinical relevance, converges at the celiac nodes via diverse routes. The stomach's wall includes specialized layers for mechanical and chemical digestion, with gastric glands secreting enzymes like pepsin and hormones like gastrin. It digests proteins, triglycerides, and selectively absorbs substances like alcohol, contributing to chyme formation and regulated gastric emptying (Hsu and Lui, 2023). The intrinsic factor, critical for vitamin B12 absorption, highlights its vital functions, enabling survival even post-gastrectomy with supplementation.

Given the growing interest in phytomedicine as an alternative to conventional drugs, this study aims to evaluate the protective effects of *Moringa oleifera* aqueous extract on arsenic trioxide-induced gastric toxicity in adult Wistar rats.

MATERIALS AND METHOD

Collection and Authentication of Moringa oleifera: The leaves of *Moringa oleifera* used in this research work were collected from the Nursing Hostel, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. The plant was identified and authenticated by a plant taxonomist in the Department of Plant Biology and Biotechnology, University of Benin. The herbarium specimen was assigned voucher number UBH-M218.

Preparation of Moringa oleifera Extract: The leaves were air-dried to prevent degradation of the phytocompounds. After drying, the leaves were ground into a fine powder using a pestle and mortar. The powdered leaves were weighed on an electric weighing balance, and the required amount was extracted using solvents. The extraction was performed by refluxing the plant material with ethanol and methanol for 4 hours. The filtrates were then concentrated using a rotary evaporator at 40°C, followed by drying in a water bath at 50°C to obtain the final extract. The dried extract was stored at 4°C until use.

Collection of Experimental Animals: Thirty (30) adult Wistar rats, weighing between 180–250g, were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria. They were exposed to controlled environmental temperature (28 ± 2°C), relative humidity (50 ± 5%) and 12-hour light-dark cycle. The rats were acclimatized for two weeks and were fed with Top Feeds growers' mash and clean water. Their weight was measured twice weekly throughout the study.

Method of Administration/Choice of Dosage: Ten grams (10 g) of arsenic trioxide crystals were dissolved in 100 ml of distilled water to prepare an arsenic trioxide solution. Similarly, 10 g of *Moringa oleifera* extract was dissolved in 100 ml of distilled water and administered orally to Wistar rats at doses of 200, 400 and 800 mg/kg body weight after determining the LD50 using the method described by Lorke (1983).

The dosage was administered via an orogastric tube to ensure accurate delivery of the extract. The animals were weighed before and after the administration, with constant access to water and feed during the experiment (Olukayode *et al.*, 2024).

Experimental Design: The thirty (30) rats were randomly assigned into six groups (A–F), each consisting of five rats. The groups were treated as follows:

- Group A: 1 mL of distilled water
- Group B: 10 mg/kg of Arsenic Trioxide (toxicant) only
- Group C: 200 mg/kg of *Moringa oleifera* extract and 10 mg/kg of Arsenic Trioxide
- Group D: 400 mg/kg of *Moringa oleifera* extract and 10 mg/kg of Arsenic Trioxide
- Group E: 800 mg/kg of *Moringa oleifera* extract and 10 mg/kg of Arsenic Trioxide
- Group F: 800 mg/kg of *Moringa oleifera* extract only

Method of Sample Collection: At the end of the 4-week treatment period, the rats were weighed and sacrificed under chloroform anesthesia. Their stomachs were harvested immediately and fixed in 10% formal saline for further histological analysis.

Histological ProcedureParaffin Tissue Processing: The harvested stomach tissues were first fixed in 10% formal saline, dehydrated through a graded series of ethanol (70%, 90%, and absolute ethanol), followed by clearing with xylene, infiltrated with molten paraffin wax at 65-70°C. After embedding, the tissues were allowed to cool and solidify into blocks. The tissue blocks were sectioned into 5-micron thick ribbons using a rotary microtome.

Hematoxylin and Eosin Staining Method: The tissue sections were deparaffinized with xylene and rehydrated through alcohol and water, stained with hematoxylin for 10 minutes, washed in running tap water, and counterstained with eosin for 5-10 minutes, dehydrated through graded alcohols and cleared in xylene. The sections were mounted with DPX under a cover slip (Drury and Wallington (1980).

Photomicrography: The stained sections were examined under a Leica DM750 research microscope attached to a Leica CC50 digital camera. Digital images were captured at magnifications of 40x and 400x.

Ethical Clearance: Ethical clearance for the experiment was obtained from the relevant institutional ethics committee, following national and international guidelines for the care and use of laboratory animals.

Statistical Analysis: The data were analyzed using GraphPad Prism version 8.1. One-way analysis of variance (ANOVA) was performed, with data expressed as mean ± standard error of the mean (SEM). Post-hoc analysis was conducted using the Least Significant Difference (LSD) test. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Body Weight: There were no significant differences (P>0.05) in stomach weight and organosomatic index observed across the treatment groups in comparison with control, as shown in figures 1–2, and there was no significant difference between initial and final weight across the groups(P>0.05) as shown in figure 3.

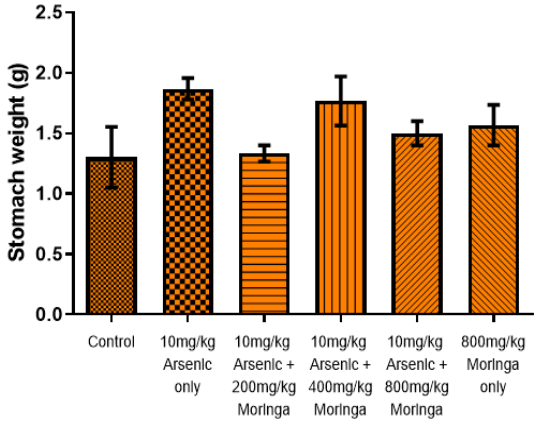

Fig 1: stomach weight in Arsenic trioxide intoxicated Wistar rat treated with *Moringa oliefera* extract. There were no significant differences across the groups (P>0.05)

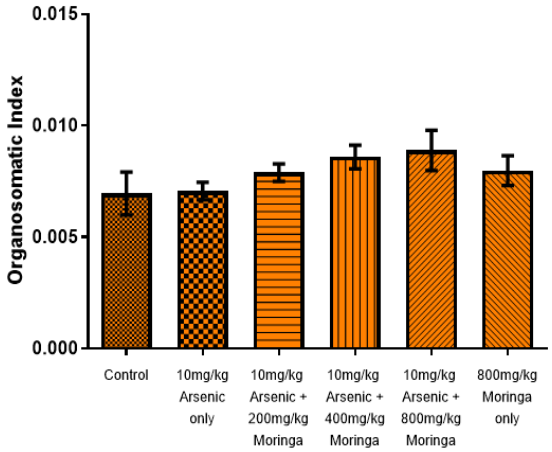

Fig 2: Organosomatic index in Arsenic trioxide intoxicated Wistar rat treated with *Moringa oliefera* extract. There was no significant difference across the groups (P>0.05)

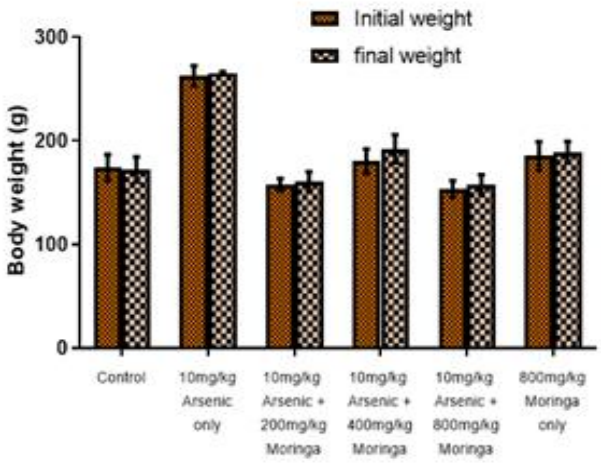

Fig 3: body weight in Arsenic trioxide intoxicated Wistar rat treated with *Moringa oliefera* extract.
There was no significant difference between initial and final weight across the groups(P>0.05).

Histological Results: Arsenic Trioxide Effects: Histological analysis revealed that arsenic trioxide exposure led to notable gastric tissue damage, including cellular degeneration, inflammation, and loss of normal tissue architecture as observed in plate 3 and 4. These observations align with the known toxic effects of arsenic on gastrointestinal tissues, likely due to its ability to induce oxidative stress and inflammatory responses.

Protective Role of *Moringa oleifera*: Co-administration of *Moringa oleifera* extract showed dose-dependent amelioration of gastric damage. At 200 mg/kg, mild improvements were observed, with reduced cellular degeneration (plates 3 and 4) while at 400 mg/kg, significant preservation of tissue structure was evident, with fewer signs of inflammation and damage (plates 5 and 6). At 800mg/kg, nearly complete restoration of normal histological architecture was noted, with minimal evidence of degeneration or infiltration (plates 7 and 8).

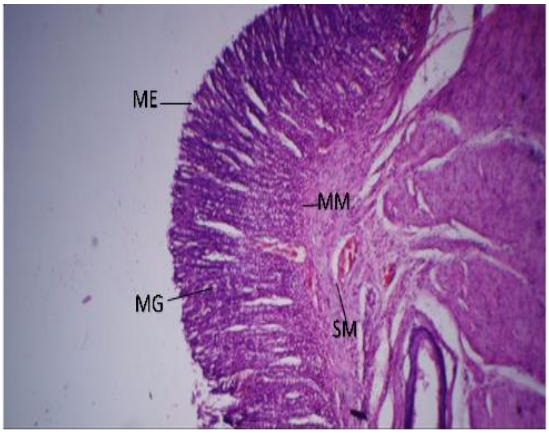

Plate 1: Rat stomach. Control. Showing pitted gastric mucosal lining (ME), glands (MG), muscularis mucosa, (MM), submucosa (SM), all normal: H and E x40.

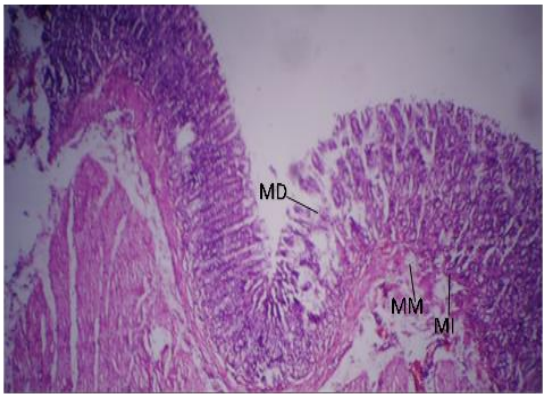

Plate 2: Rat stomach given Arsenal only showing: mucosal devitalization (MD), muscularis mucosal degeneration (MM) and inflammation (MI): H and E x40.

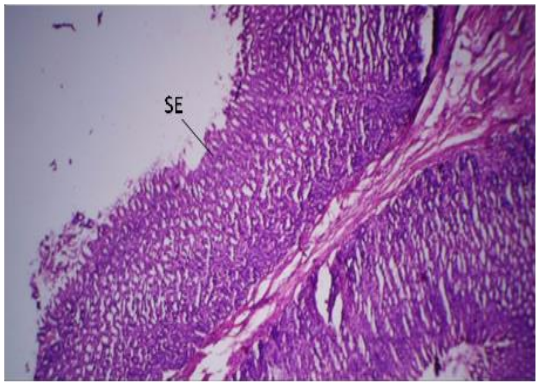

Plate 3: Rat stomach given 200mg extract + Arsenic showing: superficial mucosal erosion (SE): H and E x40

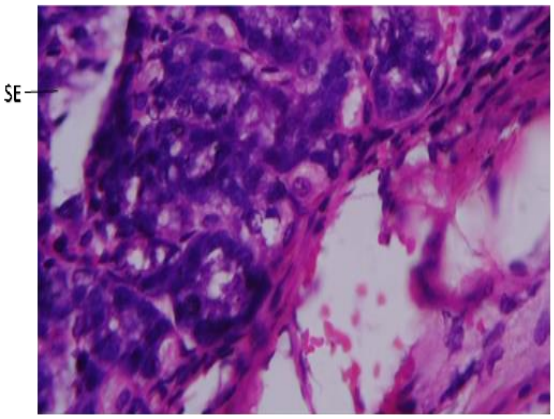

Plate 4: Rat stomach given 200mg extract + Arsenic showing: superficial mucosal erosion (SE): H and E x400

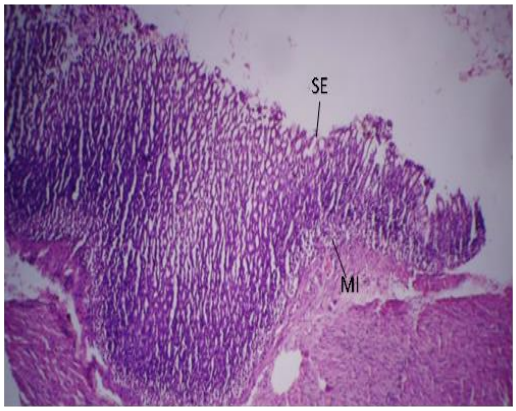

Plate 5: Rat stomach given 400mg extract + Arsenic showing: superficial mucosal erosion (SE), mural infiltrates of inflammatory cells (MI): H and E x40

Arsenic trioxide is a highly toxic compound that poses significant risks to human and animal health (Innih and Calmday-Ombo, 2024). Its toxic effects are primarily mediated through the induction of oxidative stress, which disrupts cellular redox balance, leading to the generation of reactive oxygen species (ROS) and subsequent damage to proteins,

lipids, and DNA. It binds to sulfhydryl groups in proteins, impairing enzyme functions and interfering with mitochondrial activity, which compromises energy production and triggers cell death pathways such as apoptosis and necrosis (Hu *et al.*, 2020).

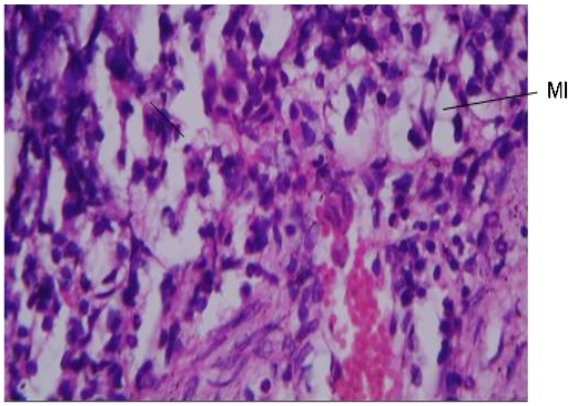

Plate 6: Rat stomach given 400mg extract + Arsenic showing: superficial mucosal erosion (SE), mural infiltrates of inflammatory cells (MI): H and E x400

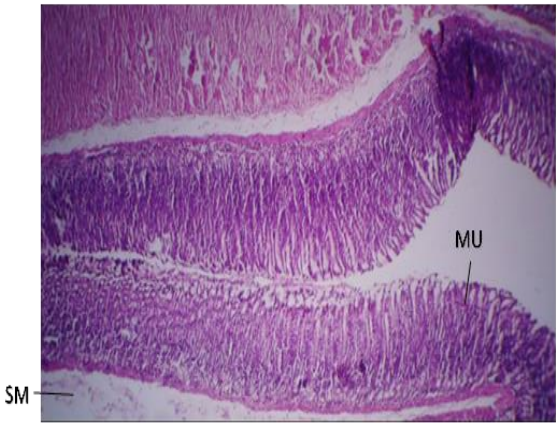

Plate 7: Rat stomach given 800mg extract showing: mucosal (MU), sub mucosa (SM), all normal: H and E x40

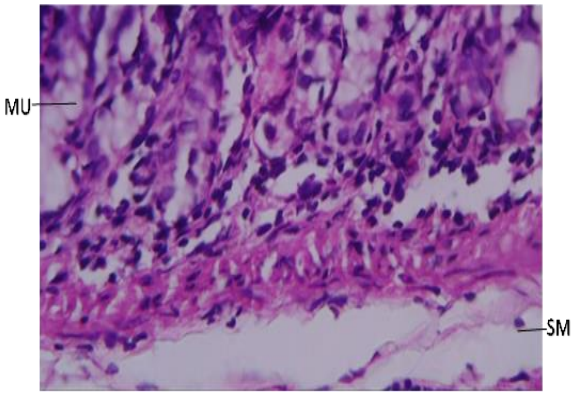

Plate 8: Rat stomach given 800mg extract showing: mucosal (MU), sub mucosa (SM), all normal: H and E x400

This oxidative stress triggers inflammation, apoptosis, and necrosis, causing extensive tissue and organ damage, particularly in the gastrointestinal system, liver, kidneys, and cardiovascular system. Prolonged exposure to arsenic trioxide is associated with chronic health conditions, including cancer, cardiovascular diseases, and neurotoxicity, while acute exposure can result in severe gastric

The study assessed the protective effects of *Moringa oleifera* aqueous extract at three dosages-200 mg/kg (low dose), 400 mg/kg (intermediate dose), and 800 mg/kg (high dose)-against arsenic trioxide-induced gastric toxicity. Results showed no significant differences in body weight (Figure 3), stomach weight (Figure 1), or organosomatic index (Figure 2) across the treatment groups. Group B (arsenic trioxide only) and Groups C-E (arsenic trioxide with varying *Moringa oleifera* doses) exhibited comparable weight parameters to the control group (Group A), as well as Group F (*Moringa oleifera* only).

Histological analysis revealed the protective effect of *Moringa oleifera* in mitigating arsenic-induced gastric damage, with clear dose-dependent improvements. Plate 3 and Plate 4 (arsenic trioxide-only group) displayed significant cellular degeneration, inflammation, and disrupted gastric architecture. Co-administration of *Moringa oleifera* at 200 mg/kg (low dose) exhibited mild improvements with reduced cellular degeneration (Plates 5 and 6). A more pronounced protective effect was observed at 400 mg/kg (intermediate dose) with better-preserved gastric structure (Plates 7 and 8), while the 800 mg/kg (high dose) group showed near-complete restoration of normal gastric architecture, with minimal evidence of inflammation or cellular damage.

These findings align with previous research, where arsenic trioxide toxicity was reported to impair gastric tissues primarily through oxidative stress and inflammatory pathways (Zhao *et al.*, 2022). Interestingly, in studies involving chronic arsenic exposure, significant weight loss has been observed due to reduced nutrient absorption and anorexia (Handali and Rezaei, 2021; Fatoki and Badmus, 2022)). However, in this study, the absence of significant weight changes suggest that the 28-day exposure may not have been sufficient to induce systemic metabolic effects, or that dietary intake was adequately maintained.

The efficacy of *Moringa oleifera* in reversing arsenic-induced damage is consistent with its documented antioxidant and anti-inflammatory

properties (Khalid *et al.*, 2024; Mahaveerchand and Ajees, 2024). The dose-dependent amelioration explains the potential of higher doses (800 mg/kg) to provide significant gastric protection. Similar findings in the literature support *Moringa oleifera*'s ability to mitigate oxidative stress-related damage in various tissues, suggesting that its bioactive compounds play a central role in this protective mechanism (Alia *et al.*, 2022). These results emphasize the therapeutic promise of *Moringa oleifera* as a natural remedy for mitigating arsenic-induced gastric toxicity and emphasize its potential application in addressing toxicant-induced gastric pathologies.

Conclusion: Moringa oleifera aqueous extract demonstrated significant dose-dependent protective effects against arsenic trioxide-induced gastric toxicity in Wistar rats. While no significant changes were observed in body and organ weights, histological analysis confirmed notable gastric tissue preservation, particularly at higher doses. These findings prove *Moringa oleifera'*s therapeutic potential as a natural intervention against arsenic-induced gastric damage.

Future studies should investigate *Moringa oleifera*'s molecular mechanisms, validate its efficacy in humans, and standardize dosage to enhance its therapeutic potential for managing arsenic-induced gastric toxicity.

Declaration of Conflict of Interest: The authors declare no conflict of interest.
Data Availability Statement: Data are available upon request from the corresponding author.

REFERENCES

Alia, F; Putri, M; Anggraeni, N; Syamsunarno, M (2022). The Potency of Moringa oleifera Lam. as Protective Agent in Cardiac Damage and Vascular Dysfunction. *Fron. Pharm.*, *12*, 724439. DOI: https://doi.org/10.3389/fphar.2021.724439

Chaudhry, SR; Nataly, M; Peterson, DC (2019, August 10). *Anatomy, Abdomen and Pelvis, Stomach*. Nat. Lib. Med.; StatPearls Publishing. DOI: https://www.ncbi.nlm.nih.gov/books/NBK482334/

Drury, RAB; Wallington, EA (1980). *Carleton's histological technique.*Fatoki, JO; Badmus, JA (2022). Arsenic as an environmental and human health antagonist: A review of its toxicity and disease initiation. *J. Hazard. Mat. Adv. 5*, 100052. DOI: https://doi.org/10.1016/j.hazadv.2022.100052

Handali, S; Rezaei, M (2021). Arsenic and weight loss: At a crossroad between lipogenesis and lipolysis. *J. Trace Elem. Med. Bio.*, *68*, 126836. DOI https://doi.org/10.1016/j.jtemb.2021.126836

Hsu, M; Lui, F (2023, July 17). *Physiology, Stomach*. Nih.gov; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK535425/

Hu, Y; Li, J; Lou, B; Wu, R; Wang, G; Lu, C; Wang, H; Pi, J; Xu, Y (2020). The Role of Reactive Oxygen Species in Arsenic Toxicity. *Biomolecules*, *10*(2), 240. DOI: https://doi.org/10.3390/biom10020240

Innih, SO; Calmday-Ombo, D (2024). Effects of Aqueous Extract of *Moringa olifera* Leaves on Arsenic Trioxide Induced HepatoToxicity in Adult WistarRats. *Sok. J. Med. Lab. Sci. 9*(1), 104–113. DOI: https://doi.org/10.4314/sokjmls.v9i1.11

Khalid, F; Azmat, H; Khan, N (2024). Ameliorative effects of Moringa oleifera leaf extract against arsenic induced histo-biochemical alterations in Labeo rohita. *Ecotox. Environ. Saf.*, *287*, 117258. DOI: https://doi.org/10.1016/j.ecoenv.2024.117258

Lorke, D (1983). A new approach to practical acute toxicity testing. *Arch. Toxic.*, *54*(4), 275–287. https://doi.org/10.1007/bf01234480

Mahaveerchand, H; Ajees, A (2024). Environmental, industrial, and health benefits of Moringa oleifera. *Phyto. Rev.*, *23*, 1497–1556. DOI: https://doi.org/10.1007/s11101-024-09927-x

Nasim, N; Sandeep, IS; and Mohanty, S (2022). Plant-derived natural products for drug discovery: current approaches and prospects. *The Nucleus*, *65*(3), 399–411. DOI: https://doi.org/10.1007/s13237-022-00405-3

Olukayode, SB; Innih, SO (2024). Effects of Ethanolic Leaves Extract of Irvingia gabonensis on Arsenic Trioxide-Induced Liver damage in Wistar rats. *J. Appl. Sci. Environ. Manage. 28*(7), 1945–1950. DOI: https://doi.org/10.4314/jasem.v28i7.3

Olukayode, SB; Innih, SO; Calmday-Ombo, D (2024). Histopathological Assessment of Cardiac Tissue in Arsenic Trioxide-Induced Wistar rats: Effects of Ethanol Leaf Extract of *Irvingia gabonensis*. *J. Exp. Clin. Anat.*, *21*(2), 168–171. DOI: https://doi.org/10.4314/jeca.v21i2.4

Salmerón-Manzano, E; Garrido-Cardenas, JA; Manzano-Agugliaro, F (2020). Worldwide Research Trends on Medicinal Plants. *Intern. J. Environ Res. Pub. Heal*, *17*(10), 3376. DOI: https://doi.org/10.3390/ijerph17103376

Sundaram, D; Babu, NK (2024). Moringa oleifera: a review on nutritive significance and its medicinal application for children. *Intern. J. Comm. Med. Pub. Heal.*, *12*(1), 610–615. DOI: https://doi.org/10.18203/2394-6040.ijcmph20244081

Zhao, D; Yi, H; Sang, N (2022). Arsenic intake-induced gastric toxicity is blocked by grape skin extract by modulating inflammation and oxidative stress in a mouse model. *Ecotox. Environ. Saf.*, *233*, 113305–113305. DOI: https://doi.org/10.1016/j.ecoenv.2022.113305