Immunomodulatory Effects of *Moringa oleifera* Seed Extract and Nano Zinc Oxide on Benzene-Induced Toxicity *In-Vivo*

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Abstract

Background: Benzene, an aromatic hydrocarbon found in crude oil and petrol, shows significant health risks due to its ubiquitous presence in the environment. Exposure to benzene leads to various health issues, including hematological disorders and increased cancer risk. In recent years, interest has grown in identifying natural compounds with antioxidant properties to mitigate benzene toxicity. This study aimed to investigate the effects of Moringa oleifera (MO) seed extract on benzene-induced changes in key hematological parameters in a rat model. Methods: Fresh Moringa seeds were extracted and processed, and adult Wistar rats were divided into six groups, including controls and those exposed to benzene alone or treated with Moringa seed extract, nano zinc oxide, or their combination. Blood samples were collected at different intervals to assess white blood cell counts, immunoglobulin levels, and other hematological markers. Results: Treatment with benzene led to significant decreases in total leukocyte counts and alterations in differential leukocyte percentages, indicating immune system suppression. However, treatment with Moringa seed extract, alone or in combination with nano zinc oxide, mitigated these effects, restoring white blood cell counts and improving immune parameters. Additionally, Moringa extract treatment was associated with enhanced levels of immunoglobulins IgG and IgM. Conclusion: Moringa seed extract shows promise in increasing immunity and white blood cell counts after benzene exposure. Further research is needed to explore its potential as a therapeutic agent against benzene toxicity.

Keywords: Benzene toxicity, Moringa oleifera, Nano zinc oxide, Immunomodulation, Wistar rats

Significance | This study addressed the critical need for antidotes against benzene toxicity, highlighting Moringa oleifera's potential immunomodulatory effects.

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Introduction

Benzene, a component of crude oil and petrol, is a pervasive environmental pollutant originating from both natural and anthropogenic sources (Schettgen et al., 2009). It is widely used in various industries, leading to significant exposure among chemical workers, oil pipeline operators, and automotive repair personnel (Weisel, 2010). Humans can be exposed to benzene through inhalation, skin contact, contaminated food and water, and both active and passive smoking (Goldstein & Shalat, 2000). The adverse health effects of benzene are well-documented and include skin irritation, respiratory issues, central nervous system depression, and hematological toxicity (Zhang et al., 2014). Chronic exposure, even at low levels, can lead to severe hematological disorders and an increased risk of various cancers, including acute myeloid leukemia, multiple myeloma, and non-Hodgkin's lymphoma (Hayes et al., 2001; Schnatter et al., 2005; Kirkeleit et al., 2008).

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Given the severity of benzene toxicity, particularly its impact on the blood and bone marrow, there is a growing interest in identifying natural compounds with antioxidant properties that can mitigate these harmful effects (Gupta & Flora, 2005; Rajkumar et al., 2010). In this context, the seeds of Moringa oleifera (MO), known for their nutritional and medicinal benefits, have shown promise in reducing oxidative stress and toxicity in various models (Ajibade et al., 2012, Rolla et al. 2023, Rolla et al. 2024). Commonly referred to as the horseradish tree or drumstick tree, Moringa oleifera is cultivated throughout India for its edible parts and therapeutic potential (Jahn, 1988; Anonymous, 2009).

This study aimed to investigate the effects of benzene exposure on the immune system and blood cells in adult Wistar rats and to evaluate the potential protective effects of Moringa oleifera seeds. Given the limited research on natural antidotes for benzene toxicity, this study is significant in exploring new avenues for mitigating the adverse health effects associated with benzene exposure.

Materials and Methods

Chemicals and Reagents

Commercially available benzene was purchased from S D Fine Chemicals, India.

Moringa Seeds Extraction

Fresh, uncrushed Moringa seeds were obtained from the local market. The seeds were cleaned, dried under direct sunlight, and crushed using a mechanical grinder. The seeds were then hand-cleaned to remove foreign matter such as other seeds, stones, and small stems. Subsequently, the seeds were dried at 50°C for 12 hours in an oven and ground into a fine powder. The powder was stored until use.

For extraction, Moringa seed powder was mixed with hexane (10:1 m/v) at 60-80°C using a Soxhlet apparatus. This extraction process was repeated for 6 hours. The hexane was then distilled using a distillation group and concentrated using a hot plate microscope. The extract was dried in air at a temperature of 40±2°C.

Animal Study

Seventy-two adult Wistar rats were used in this study. The animals were divided into six groups, each containing twelve rats, as follows:

Group 1 (G1) - Negative Control: Received only water and feed.

Group 2 (G2) - Positive Control: Received benzene 400 mg/kg body weight orally for 14 consecutive days.

Group 3 (G3): Received Moringa seed extract 150 mg/kg body weight for 28 days.

Group 4 (G4): Received benzene 400 mg/kg body weight orally for 14 consecutive days followed by Moringa seed extract 150 mg/kg body weight for 28 days.

Group 5 (G5): Received benzene 400 mg/kg body weight orally for 14 consecutive days followed by nano zinc oxide for 28 days.

Group 6 (G6): Received benzene 400 mg/kg body weight orally for 14 consecutive days followed by Moringa seed extract 150 mg/kg body weight plus nano zinc oxide for 28 days.

Blood Collection

Blood samples were collected to estimate white blood cells (WBCs), immunoglobulin G (IgG), and immunoglobulin M (IgM) at three time points: day 0 (baseline), after 14 days, and at the end of the experiment. A total of 2 ml of blood was drawn from each rat using EDTA tubes for WBC estimation, which was conducted using an automated blood analyzer. Another 2 ml of blood was used for serum separation for IgG and IgM estimation.

Immunoglobulin Estimation

Serum IgG and IgM levels were measured using ELISA kits (Mybiosource Company). The procedure was performed according to the manufacturer's instructions.

Statistical Analysis

The data were analyzed using SPSS version 23. Descriptive statistics and inferential tests were applied to assess the differences between groups.

Results and Discussion

Hematological Parameters

The current study demonstrated significant hematological alterations due to benzene exposure and subsequent treatment with Moringa seed extract and nano zinc oxide. Total leukocyte count (TLC) significantly decreased in the benzene-exposed group (G2) compared to all other groups (Table 1). However, a significant increase in TLC was observed in groups treated with Moringa seed extract, nano zinc oxide, or their combination (G4, G5, G6) compared to G2, indicating a therapeutic effect of these treatments against benzene-induced toxicity.

These findings align with the known hematotoxic effects of benzene, which include leucopenia due to the direct cytotoxicity of its metabolites on bone marrow (Smith et al., 2000; Tsai et al., 2004). White blood cells play a critical role in immune defense, and their reduction reflects a compromised immune system's ability to combat infections and other pathogens (Qu et al., 2002; Lan et al., 2004).

The differential leukocyte count revealed a significant decrease in neutrophils, monocytes, eosinophils, and basophils in the benzene-exposed group (G2) compared to the control group (G1). At the end of the experiment, groups treated with Moringa seed extract, nano zinc oxide, or their combination (G4, G5, G6) showed a significant increase in these cells compared to G2 (Tables 2, 4, 5, 6). Conversely, lymphocyte percentages increased in the benzene-exposed group and decreased following treatment, which indicates a shift towards a more balanced immune response post-treatment (Table 3). These results suggest the potential of Moringa oleifera

Table 1. Total leukocyte count ($\times 10^3$ /ul) in studied groups

	G1	G2	G3	G4	G5	G6
0 day	8.49±0.84Aa	8.51±0.2Aa	8.5±1.3Aa	8.42±0.6Aa	8.46±1.01Aa	8.49±0.4Aa
14 days of experiment	8.47±0.93Aa	5.01±1.6Bb	8.48±0.5Aa	5.22±1.3Cb	5.09±1.8Cb	5.3±1.26Cb
At end of exp.	8.50±1.054Aa	4.65±0.93Cc	8.44±1.284Aa	6.84±1.42Bb	7.48±1.36Bb	7.87±0.70Bb

Table 2. Neutrophil % in studied groups

	G1	G2	G3	G4	G5	G6
0 day	60.19±1.23Aa	60.13±1.46Aa	60.12±1.63Aa	59.94±0.8Aa	60.03±0.65Aa	59.98±0.94Aa
14 days of experiment	60.04±1.01Aa	45.88±3.74Bb	59.98±4.78Aa	43.79±8.92Cb	44.82±4.75Cb	42.95±6.1Cb
At end of exp.	60.26±1.94 Aa	40.11±2.86 Cc	58.76± 5.02Aa	53.33±3.02Bb	56.34±3.18 Bb	57.77±3.54Bb

Table 3. Lymphocyte % in studied groups

	G1	G2	G3	G4	G5	G6
0 day	30.88±1.63Aa	30.38±0.48Ca	30.52±1.06Aa	30.61±1.85Ca	30.59±0.93Ca	30.72±1.46Ba
14 days of experiment	30.79±1.01Ab	35.34±1.37Ba	30.29±1.82Ab	36.47±3.63Aa	36.81±5.74Aa	36.99±4.69Aa
At end of exp.	30.96±4.53Ac	37.21± 2.68Aa	30.49± 4.15Ac	34.33±2.82Bb	34.00±1.48Bb	31.04±2.94Bc

Table 4. Monocyte % in studied groups

	G1	G2	G3	G4	G5	G6
0 day	8.29±0.93Aa	8.33±0.89Ca	8.28±0.53Ca	8.39±0.18Aa	8.31±1.05Ca	8.23±0.84Ca
14 days of experiment	8.30±1.13Ab	9.21±1.52Ba	8.78±0.93Bb	6.37±1.32Bc	9.84±2.38Ba	9.93±1.79Ba
At end of exp.	8.357±1.00Ac	11.12±0.56Aa	9.08±1.36Ab	5.74±1.97Cc	11.22 ±2.37Aa	11.22±2.37Aa

Table 5. Eosinophil % in studied groups

	G1	G2	G3	G4	G5	G6
0 day	0.33±0.04Aa	0.32±0.001Ca	0.33±0.02Ca	0.32±0.009Ca	0.32±0.02Aa	0.32±0.02Ca
14 days of experiment	0.32±0.01Ae	0.39±0.02Bc	0.36±0.003Bd	0.45±0.22Ba	0.33±0.08Ae	0.42±0.13Bb
At end of exp.	0.32±0.04Ad	0.45±0.09 Ac	0.42 ±0.11Ac	0.54± 0.07Aa	0.34±0.07Ad	0.48±0.06Ab

Table 6. Basophil % in studied groups

	G1	G2	G3	G4	G5	G6
0 day	0.029±0.001Aa	0.028±0.0001Aa	0.027±0.0004Aa	0.028±0.0008Ca	0.027±0.0001Ca	0.028±0.002Ca
14 days of experiment	0.028±0.003Ac	0.001±0.005Be	0.021±0.014Bd	0.039±0.01Bb	0.052±0.016Ba	0.035±0.03Bb
At end of exp.	0.028±0.04 Ac	0.00±0 Ce	0.014±0.03Cd	0.057±0.053Ab	0.071±0.04Aa	0.057±0.053Ab

Table 7. IgG in studied groups

	G1	G2	G3	G4	G5	G6
0 day	9.12±0.83Aa	9.13±0.73Aa	9.01±0.48Aa	8.93±0.31Aa	8.98±0.85Aa	9.05±0.92Aa
14 days of experiment	9.08±0.91Aa	6.47±2.78Bb	9.21±0.89Aa	6.28±1.07Bb	6.37±1.08Bb	6.26±1.65Bb
At end of exp.	9.01±0.37Aa	4.47±1.99Cb	9.30±1.19Aa	8.10± 0.65Aa	9.77±1.39Aa	9.52±1.87Aa

Table 8. IgM in studied groups

	G1	G2	G3	G4	G5	G6
0 day	0.81±0.08Aa	0.82±0.07Aa	0.79±0.02Aa	0.82±0.02Aa	0.80±0.09Aa	0.81±0.06Aa
14 days of experiment	0.81±0.01Aa	0.51±0.68Bb	0.84±0.09Aa	0.50±0.32Cb	0.53±0.28Bb	0.52±0.37Bb
At end of exp.	0.80±0.03Aa	0.30±0.16Cc	0.82±0.19Aa	0.62±0.37Bb	0.83±0.19Aa	0.82± 0.20Aa

and nano zinc oxide in ameliorating benzene-induced hematotoxicity (Hayes et al., 2001; Schnatter et al., 2005).

Immunoglobulin Levels

Both IgG and IgM levels significantly decreased at day 14 in the benzene-exposed group (G2) compared to the control group (G1). By the end of the experiment, there was a significant enhancement in IgG and IgM levels in the treated groups (G4, G5, G6) compared to G2 (Tables 7, 8). This improvement is consistent with previous studies indicating that benzene exposure leads to reduced serum immunoglobulin levels (Kirkeleit et al., 2006; Uzma et al., 2010).

Our findings on the therapeutic effects of Moringa oleifera align with those of Ojeka et al. (2018), who reported increased production of IgM, IgA, and IgG levels following treatment with Moringa extracts. The immunomodulatory properties of Moringa oleifera may enhance B cell activation and antibody production, thus bolstering the immune system's defense mechanisms (Abd-Elhakim et al., 2018; Frandson, 1981).

Implications for ZnO Nanoparticles

The study also highlighted the potential benefits of ZnO nanoparticles in combination with Moringa oleifera extract. ZnO nanoparticles have shown positive effects on the development and physiological performance in animals, improving parameters such as body weight, feed intake, and various blood metabolites (Reda et al., 2021; Feng et al., 2010). However, further research is necessary to determine the optimal dosages and long-term safety of ZnO nanoparticles in animal feed (Tang et al., 2016).

Conclusion

This study demonstrated that Moringa oleifera seed extract and ZnO nanoparticles individually and in combination can mitigate the hematological and immunological toxicity induced by benzene exposure. These findings provide a foundation for future research into the therapeutic applications of Moringa oleifera and ZnO nanoparticles in managing chemical-induced toxicity. Given the potential for ZnO nanoparticle toxicity at higher doses, careful consideration of dosage and long-term effects is warranted (Asomugha et al., 2015; Otitoju et al., 2014).

Author contributions

S.J.A., A.J.A.A., S.M.A.A. conducted the practical work and edited the manuscript, performed the statistical analysis, wrote the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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