UMSHA Press

AJMB
Avicenna Journal of Medical Biochemistry

Avicenna J Med Biochem. 2025;13(1):27-35. doi:10.34172/ajmb.2630

http://ajmb.umsha.ac.ir

Original Article

Protective Effects of *Vernonia amygdalina* Extract Against 1-Nitropyrene-Induced Hepatopulmonary Oxidative Injury in Rats

Omorede Ikponmwosa-Eweka[1*], Ikenna C. Maduako[2]

[1]Department of Medical Biochemistry, University of Benin, Benin City, Nigeria
[2]Chemoprevention and Toxicology Laboratory, Department of Medical Biochemistry, College of Medical Sciences, Benson Idahosa University, Benin City, Nigeria

Article history:
Received: August 2, 2025
Revised: September 16, 2025
Accepted: September 21, 2025
ePublished: October 31, 2025

***Corresponding author:**
Omorede Ikponmwosa-Eweka,
Email: omorede.aguebor@uniben.edu

Abstract

Background: 1-Nitropyrene (1-NP), an environmental pollutant derived from nitro-polycyclic aromatic hydrocarbons, poses serious environmental hazards. *Vernonia amygdalina* (VA), commonly known as bitter leaf, is widely used in folk medicine due to its pharmacological properties.

Objectives: This study investigated the pharmacological role of VA in 1-NP-induced hepatopulmonary oxido-inflammatory stress responses in a murine model.

Methods: Experimental animals were randomly divided into five groups of seven rats each and orally treated with 1-NP (250 mg/kg) alone or in combination with VA (50 mg/kg and 100 mg/kg) for 7 consecutive days. Then, glutathione, reactive oxygen and nitrogen species, nitric oxide (NO), and lipid peroxidation levels were biochemically assessed alongside the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase-4 (GPX4), glutathione-S-transferase (GST), and myeloperoxidase (MPO). Eventually, the enzyme-linked immunosorbent assay was utilized to evaluate regulated upon activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β).

Results: VA administration markedly ameliorated 1-NP-induced oxido-inflammatory hepatopulmonary damage by upregulating enzymatic and non-enzymatic antioxidants while diminishing proinflammatory proteins as observed by the reduced levels of RANTES, TNF-α, IL-1β, NO, and MPO activities.

Conclusion: Overall, VA prevented 1-NP-induced hepatopulmonary damage by enhancing antioxidant enzymes and inhibiting proinflammatory markers, thereby protecting the organs.

Keywords: 1-Nitropyrene, *Vernonia amygdalina*, Hepatopulmonary, RANTES, GPX4

Please cite this article as follows: Ikponmwosa-Eweka O, Maduako IC. Protective effects of *Vernonia amygdalina* extract against 1-nitropyrene-induced hepatopulmonary oxidative injury in rats. Avicenna J Med Biochem. 2025;13(1):27-35. doi:10.34172/ajmb.2630

Background

1-Nitropyrene (1-NP) is a widely studied nitro-polycyclic aromatic hydrocarbon and ubiquitous by-product of the incomplete combustion of diesel fuels. Therefore, it is found in diesel exhaust from vehicles, which is associated with health-related diseases (1). Additionally, it can be found in the air in the indoor kitchens during cooking periods (2). Human exposure primarily occurs through the inhalation of 1-NP-containing particulate matter or the ingestion of contaminated food and water (3), resulting in various negative health consequences.

The biotransformation of 1-NP produces reactive metabolites, forming deoxyribonucleic acid (DNA) and protein adducts that can cause cellular damage and toxicity (4). These adducts contribute to neoplastic transformation, amplify pro-inflammatory signaling, induce oxidative stress (OS), and trigger cellular apoptosis. The production of reactive oxygen and nitrogen species (RONS) is a major cause of 1-NP toxicity; these species overwhelm the antioxidant defenses, causing progressive damage to macromolecules, such as lipids, proteins, and DNA (5). This chronic OS disrupts cellular functions and has been linked to genotoxicity, manifesting as mutations and apoptosis in hepatic cells (6). In the liver, the hepatic metabolism of 1-NP via nitro-reduction exacerbates toxicity, compromising membrane integrity and releasing intracellular enzymes into circulation, indicative of hepatotoxicity (7). Similarly, in the lungs, 1-NP

increases intracellular reactive oxygen species (ROS) and upregulates pro-inflammatory biomarkers in epithelial cells, thereby contributing to pulmonary inflammation and injury (8). This further highlights the role of 1-NP in hepatopulmonary-related diseases.

Most synthetic drugs are often associated with adverse side effects (9), thereby increasing the call for complementary and alternative medicine for tolerable therapeutic alternatives (10). Naturally occurring phenolic compounds have antioxidative, anti-inflammatory, and chemopreventive properties. They are also involved in free radical scavenging and metal chelating activities, thereby inhibiting oxido-inflammatory stress. Phytochemicals, particularly polyphenols, flavonoids, and phenolic compounds, have gained attention for their antioxidant, anti-inflammatory, and chemopreventive properties (11).

Vernonia amygdalina (VA), commonly known as bitter leaf, is a perennial shrub of the Asteraceae family native to sub-Saharan Africa and is extensively used in traditional African medicine (12). Rich in secondary metabolites (e.g., saponins, tannins, alkaloids, and glycosides) and bioactive compounds (e.g., vernodalin and vernonioside), VA exhibits potent antioxidant and anti-inflammatory effects (13). Studies have demonstrated that VA has the ability to scavenge hazardous free radicals and inhibit pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF-α). It can also reduce prostaglandin production (PGE1 and PGE2), conferring protection against various health conditions (14). VA extracts have shown efficacy in preventing aflatoxin B1-induced hepatotoxicity and tetrachloromethane-induced liver damage in rats, restoring hepatic function and mitigating oxidative damage (15). Furthermore, a growing body of scientific data has confirmed the anti-nausea, anti-diabetic, and gastrointestinal ameliorative properties of VA, thus highlighting the therapeutic efficacy of VA (16). In spite of these many therapeutic capabilities associated with VA, its mechanism of action in preventing environmental pollutants, such as 1-NP-induced tissue injury, has not been fully unraveled yet.

Therefore, this study aims to better understand the ameliorative effects of the ethanolic extract of VA on 1-NP-induced hepatopulmonary injury in a murine model.

Materials and Methods

Plant Source and Extraction

Freshly harvested VA leaves were purchased from Bodija Market, Ibadan. Taxonomic verification was performed at the Department of Botany, University of Ibadan, where a voucher specimen (UI-02567) was deposited. The leaves were air-dried under ambient conditions, pulverized, and defatted with 2.5 L of n-hexane. Subsequently, the defatted material was extracted with 2.5 L of 75% ethanol using Soxhlet extraction. The ethanolic extract was concentrated to dryness at 50°C using a rotary evaporator, yielding 7.8% extract (17). For administration, the extract was reconstituted in corn oil (2 g/50 mL) and orally given to rats via gavage.

Chemicals and Reagents

1-NP (>96% purity) was purchased from AK Scientific, Inc. (California, USA). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, glutathione peroxidase 4 (GPX4), and regulated on activation, normal T cell expressed and secreted (RANTES) were obtained from Elabscience, China. Serum levels of liver enzymes aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were determined using commercial kits sourced from RANDOX. All reagents were of the highest commercially available purity (British Drug Houses, Poole, Dorset, UK), unless otherwise specified.

Experimental Animals

In total, 35 female Wistar albino rats weighing 180–200 g were sourced from the animal house of the Department of Anatomy, University of Benin. The animals were kept in standard laboratory cages in a controlled environment with a 12-12-hour light/dark cycle. They were provided with rat chow and water ad libitum. A 7-day acclimation period was observed. Experimental protocols followed the National Institute of Health (1985) guidelines and received ethical clearance (approval No. LS21046) from the Life Science Ethics Committee.

Animal Grouping and Treatment

Experimental animals were randomized into 5 groups of equal size (n = 7), each receiving a distinct treatment protocol:

- Group A (Control): corn oil (2 mL/kg body weight)
- Group B: 1-NP (250 mg/kg body weight)
- Group C: VA (100 mg/kg body weight)
- Group D: 1-NP (250 mg/kg) + VA (50 mg/kg body weight)
- Group E: 1-NP (250 mg/kg) + VA (100 mg/kg body weight)

1-NP, dissolved in corn oil, was orally given through gavage for seven consecutive days, with the dose selected based on a preliminary dose-response study in which different doses of 1-NP (100, 150, 200, 250, and 300 mg/kg) were examined. Finally, 250 mg/kg was selected as a dose that caused consistent biochemical and histological damage without mortality (data are not shown). In addition, previously published in vivo studies of 1-NP and related nitro-polycyclic aromatic hydrocarbon reports were considered (18), and VA was co-administered at the specified doses to assess its protective effects (19).

Tissue Collection and Sample Preparation

Rats were humanely euthanized via cervical dislocation 24 hours after the final treatment. Then, blood samples were collected, allowed to clot, and centrifuged at 4000 g for 10 minutes to obtain serum. The organs of interest were

excised, thoroughly rinsed in ice-cold 1.15% potassium chloride solution, blotted to remove excess fluid, weighed, and homogenized in phosphate buffer kept on ice. To obtain post-mitochondrial supernatants, homogenates were spun at 10000 g for 15 minutes at 4°C, with the produced fraction preserved at −20°C for biochemical assays.

Assessment of Serum Liver Enzyme

RANDOX commercial kits were used to assess the serum levels of AST, ALT, and ALP to evaluate hepatocyte damage, following the manufacturer's instructions (20).

Assessment of Hepatic and Pulmonary Oxidative Stress

Liver and lung homogenates were assessed for OS-related biomarkers. The enzymatic activity of superoxide dismutase (SOD) was determined by its capacity to inhibit adrenaline autoxidation, recorded at 480 nm, using 30–40 µg protein/mL in carbonate buffer (pH: 10.2) with absorbance recorded every 30 seconds for 150 seconds (21). In addition, catalase (CAT) activity was quantified by monitoring hydrogen peroxide (H_2O_2) breakdown at 270 nm, using 1.2 mL of buffer, 5 µL of H_2O_2, and 25 µL of sample, with absorbance measured every 30 seconds for 3 minutes (22). Moreover, reduced glutathione (GSH) levels were determined using 5,5'-dithiobis (2-nitrobenzoic acid) (Ellman's reagent), forming a yellow chromophore determined at 412 nm (23). Glutathione peroxidase (GPX) activity was assessed by measuring GSH consumption in the presence of H_2O_2, with residual GSH quantified using Ellman's reagent (24). Additionally, glutathione-S-transferase (GST) activity was evaluated by monitoring the conjugation of 1-chloro-2,4-dinitrobenzene with GSH at 340 nm (25). Furthermore, lipid peroxidation (LPO) was quantified as thiobarbituric acid reactive substances via the malondialdehyde–thiobarbituric acid reaction, measured at 532 nm (26).

Quantification of Reactive Oxygen and Nitrogen Species

RONS levels in liver and lung tissues were estimated using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation to fluorescent 2',7'-dichlorofluorescein (DCF) (27). A reaction mixture containing 10 µL homogenate, 5 µL DCFH-DA (5 µM final concentration), 150 µL 0.1 M potassium phosphate buffer (pH: 7.4), and 35 µL distilled water was monitored for fluorescence (excitation: 488 nm; emission: 525 nm) every 30 seconds for 10 minutes using a SpectraMax plate reader. Finally, the DCF production rate was expressed as a percentage of the control.

Quantification of Pro-Inflammatory Markers

Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration, was measured following an established protocol (28). In addition, nitric oxide (NO) levels, indicated by nitrite concentration, were quantified using the Griess reagent (29). Using commercial ELISA kits (Elabscience, China), tissue levels of GPX4 and cytokines (TNF-α, IL-1β, and RANTES) were determined following the provided instructions.

Pathological Analysis

Liver and lung tissues were fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. Further, sections (5 µm) were stained with hematoxylin and eosin for histoarchitectural assessment. Eventually, a blinded pathology expert evaluated the slides to ensure unbiased analysis of tissue integrity and pathological changes.

Statistical Analysis

The obtained data were analyzed using the analysis of variance for group comparisons, followed by a Bonferroni post-hoc test to identify statistically significant differences, performed with GraphPad Prism 8 software. Significance was accepted at $P < 0.05$.

Results

Vernonia amygdalina Mitigates 1-Nitropyrene-Induced Hepatic Injury

Exposure to 1-NP induced significant liver injury, as indicated by the elevated serum levels of hepatic transaminases. Based on the results (Table 1), rats treated with 1-NP alone exhibited significantly ($P < 0.05$) elevated levels of ALT, AST, and ALP in comparison with the control group, reflecting early signs of hepatic injury. These elevations were remarkably ($P < 0.05$) attenuated in a dose-dependent manner following the administration of VA at 50 mg/kg and 100 mg/kg.

Restoration of Antioxidant Defenses Disrupted by 1-Nitropyrene in Hepatopulmonary Tissues

Exposure to 1-NP significantly impaired antioxidant defenses in hepatic and pulmonary tissues. As shown in Figure 1, the administration of 1-NP alone to rats considerably ($P < 0.05$) decreased the activities of antioxidant enzymes SOD, CAT, GPX, and GST compared to the control group. These reductions were significantly ($P < 0.05$) reversed in a dose-dependent manner by co-treatment with VA at 50 mg/kg and 100 mg/kg.

Suppression of 1-Nitropyrene-Induced Hepatopulmonary Redox Status

Treatment with 1-NP alone noticeably ($P < 0.05$) reduced GSH levels while markedly increasing LPO and GPX-4 levels in the hepatopulmonary tissues of treated rats compared to controls (Figure 2). Conversely, the administration of VA significantly ($P < 0.05$) elevated GSH levels while decreasing LPO and GPX-4 levels in these tissues compared to the rats treated with 1-NP alone.

Mitigation of 1-Nitropyrene-induced Hepatopulmonary Inflammatory Response and Reactive Oxygen and Nitrogen Species Generation

Figures 3 and 4 illustrate the impact of 1-NP and VA on pro-inflammatory markers. Rats exposed to 1-NP alone

Table 1. Effect of *Vernonia Amygdalina* on the Liver Function Biomarkers in Treated Rats

Biomarkers	Control	1-NP Alone	VA Alone	1-NP+VA-50	1-NP+VA-100
AST (U/L)	41.36±0.16	79.62±0.29*	42.08±0.12	57.08±0.27[a]	48.11±0.14[b]
ALT (U/L)	20.22±0.10	63.52±0.24*	21.16±0.19	52.01±0.20[a]	32.86±0.31[b]
ALP (U/L)	30.25±0.25	60.97±0.20*	31.01±0.22	50.15±0.27[a.]	41.15±0.18[b]

Note. VA: Vernonia amygdalina; AST: Aspartate transaminase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; SD: Standard deviation; 1-NP: 1-Nitropyrene. Values are expressed as means±SD (n=7 rats). *$P<0.05$ vs. control, [a, b]$P<0.05$ vs. 1-NP alone.

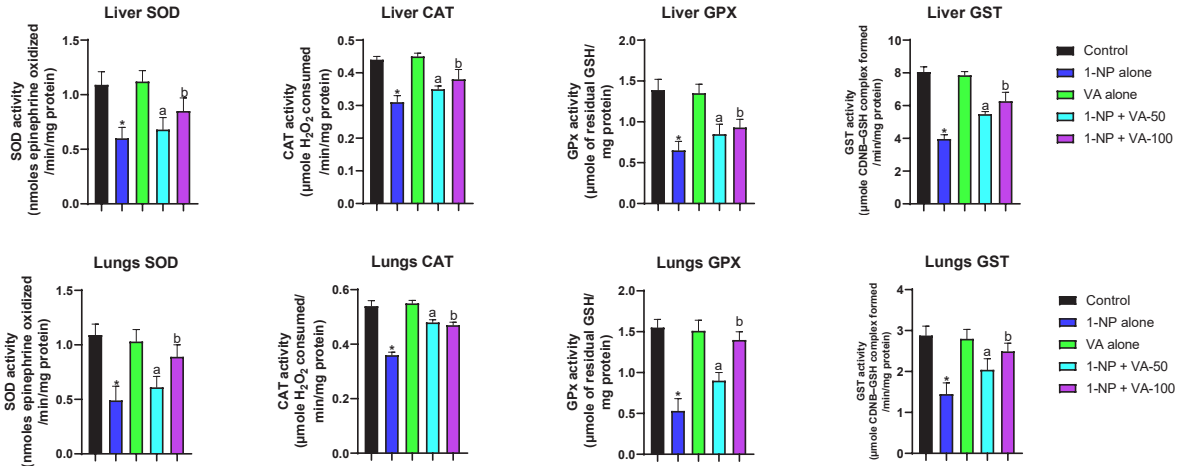

Figure 1. Effect of VA on the Activities of SOD, CAT, GPX, and GST in the Liver and Lungs of 1-NP-Treated Rats After 7 Days. *Note*. VA: *Vernonia amygdalina*; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; GST: Glutathione-S-transferase; 1-NP: 1-Nitropyrene; SD: Standard deviation. Each bar represents means±SD (n=7 rats). *$P<0.05$ vs. control, [a, b]$P<0.05$ vs. 1-NP alone

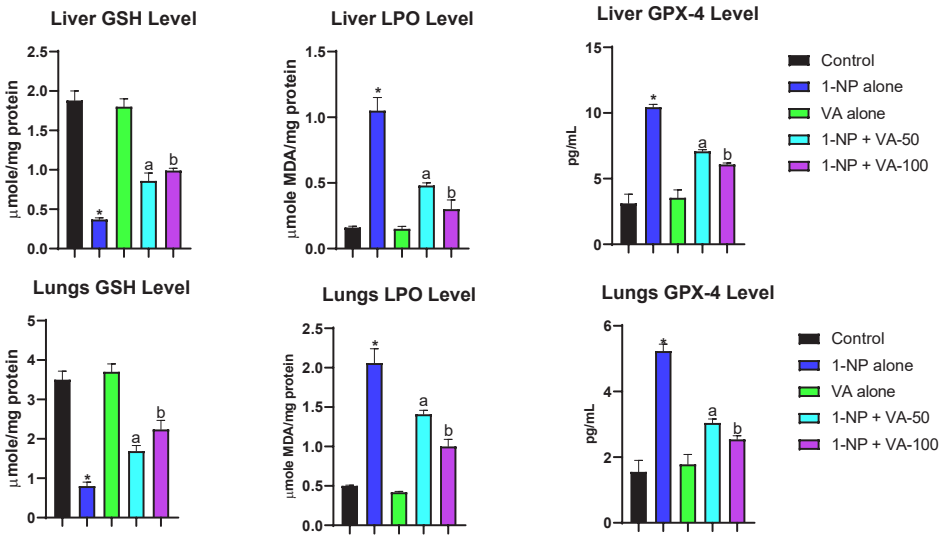

Figure 2. Effect of VA on the Levels of GSH, LPO, and GPX-4 in the Liver and Lungs of 1-NP-Treated Rats After 7 Days. *Note*. VA: *Vernonia amygdalina*; GSH: Glutathione; LPO: Lipid peroxidation; GPX-4: Glutathione peroxidase; 1-NP: 1-Nitropyrene; SD: Standard deviation. Each bar illustrates means±SD (n=7 rats). *$P<0.05$ vs. control, [a, b]$P<0.05$ vs. 1-NP alone

showed significantly ($P<0.05$) elevated MPO activity, NO, and RONS levels compared to controls, reflecting heightened inflammation and immune cell activation. Furthermore, the tested dose of 1-NP alone markedly increased the levels of TNF-α, IL-1β, and RANTES when compared to the control group. However, co-treatment with VA (50 mg/kg and 100 mg/kg) remarkably ($P<0.05$) reversed these elevations, reducing MPO activity and NO, RONS, TNF-α, IL-1β, and RANTES levels in a dose-dependent manner.

Restoration of Hepatopulmonary Histoarchitectural Integrity in 1-Nitropyrene-Challenged Rats

Liver tissue from rats treated with 1-NP alone displayed severe pathological changes, including obstructed central veins, distorted brush borders, and distended

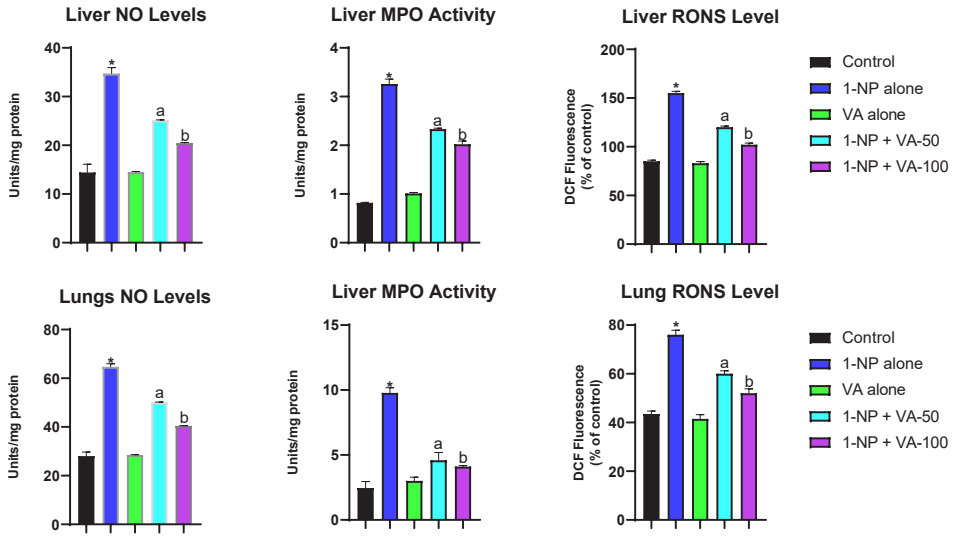

Figure 3. Effect of VA on the Levels of NO, RONS, and MPO Activity in Liver and Lungs of 1-NP-Treated Rats After 7 Days. *Note*. VA: *Vernonia amygdalina*; NO: Nitric oxide; RONS: Reactive oxygen and nitrogen species; MPO: Myeloperoxidase; 1-NP: 1-Nitropyrene; SD: Standard deviation. Each bar depicts means ± SD (n = 7 rats). $^{*}P < 0.05$ vs. control, $^{a, b}P < 0.05$ vs. 1-NP alone

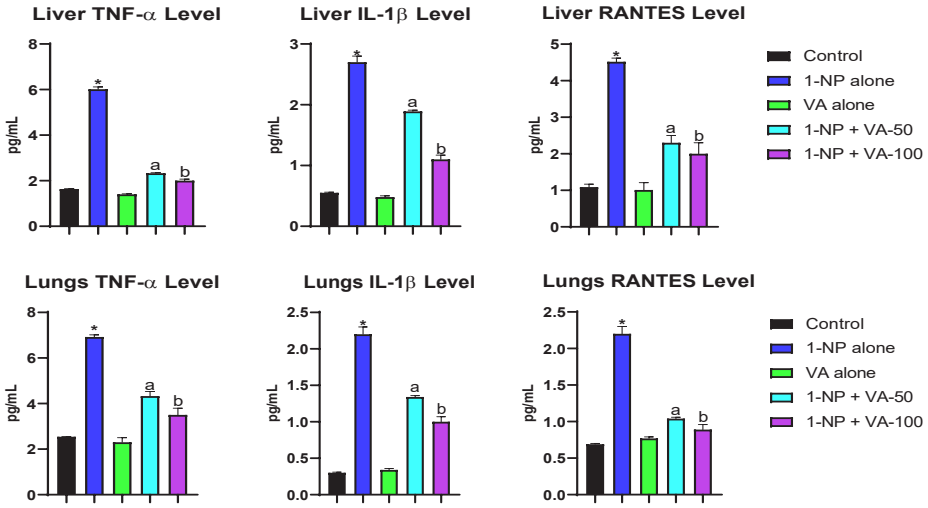

Figure 4. Effect of VA on the Levels of TNF-α, IL-1β, and RANTES in the Liver and Lungs of 1-NP-Treated Rats After 7 Days. *Note*. VA: *Vernonia amygdalina*; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1beta; RANTES: Regulated upon activation, normal T cells expressed and secreted; 1-NP: 1-Nitropyrene; SD: Standard deviation. Each bar displays means ± SD (n = 7 rats). $^{*}P < 0.05$ vs. control, $^{a, b}P < 0.05$ vs. 1-NP alone

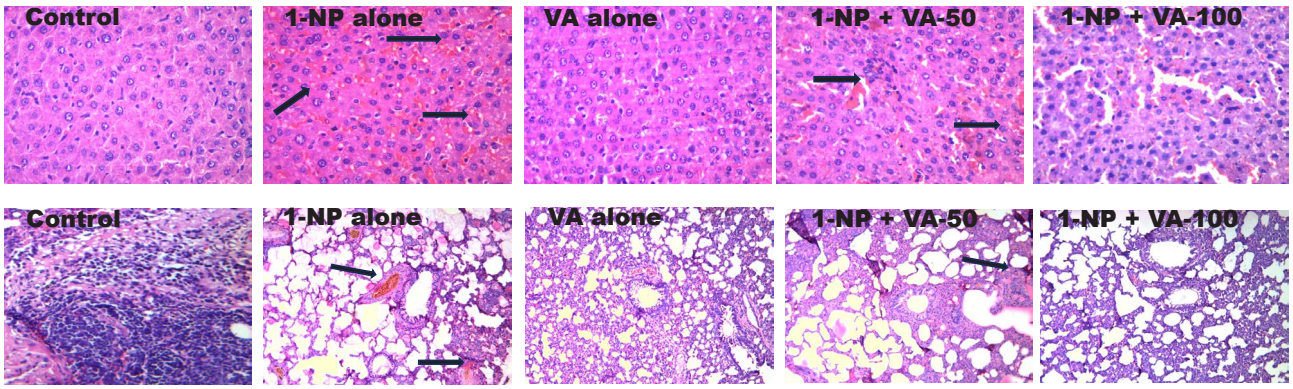

Figure 5. Histopathological Sections of Liver and Lung Tissues. *Note*. VA: *Vernonia amygdalina*; 1-NP: 1-Nitropyrene. In the liver, 1-NP alone caused pronounced degeneration, focal necrotic lesions, and infiltration of inflammatory cells. VA alone had no noticeable effect, appearing similar to the control. However, co-exposed rats showed only a slight presence of inflammatory cells. In the lungs, rats exposed to 1-NP alone exhibited significant inflammatory changes, including swollen arterioles and perivascular infiltration of lymphocytes and neutrophils. Rats treated with VA alone demonstrated lung morphology comparable to controls. Co-exposure to 1-NP and VA resulted in mild arteriole swelling and limited perivascular accumulation of lymphocytes and neutrophils (hematoxylin and eosin staining, × 400).

sinusoidal spaces, indicative of significant ($P<0.05$) hepatic damage (Figure 5). Similarly, lung tissue exhibited prominent inflammatory changes (e.g., swollen arterioles and perivascular accumulation of lymphocytes and neutrophils) compared to the control group. These findings confirm the hepatopulmonary damage caused by the toxic effects of 1-NP. In contrast, co-treatment with VA (50 mg/kg and 100 mg/kg) significantly ($P<0.05$) restored the histological architecture of both organs to near-normal. In the liver, the administration of VA ameliorated sinusoidal distension, normalized the brush border, and cleared central vein obstruction. Ultimately, treatment with VA reduced inflammation, restored normal arteriole structure, and promoted thin-walled vasculature in the lungs, closely resembling the histology of the control group.

Discussion

1-NP, a well-recognized marker of combustion-derived particulate matter exposure, has garnered increasing attention for its significant contribution to particulate matter-related toxicity (30). Exposure to 1-NP is known to provoke OS, inflammation, and endothelial dysfunction, which generally result in tissue injury and impaired immune responses. This toxicity is further intensified by its metabolic conversion into reactive intermediates (e.g., 6-hydroxy-1-NP, 1-aminopyrene, and N-acetyl-1-aminopyrene) that generate ROS and reactive nitrogen species, exacerbating oxidative damage and chronic inflammation (31).

The present study assessed the effects of VA, a natural compound with anti-inflammatory and antioxidant properties, on hepatopulmonary OS and inflammation induced by exposure to 1-NP in rats. The administration of 1-NP alone elevated serum hepatic transaminases, which are key indicators for evaluating liver toxicity and necrotic injury (32). However, the concurrent administration of VA reduced serum transaminase levels (ALT, ALP, and AST) in a dose-dependent manner, highlighting the liver-protective effect of VA. Our results are consistent with those reported in a related study (33).

Free radicals generated by OS can overpower the enzymatic and non-enzymatic antioxidant defenses of cells, leading to macromolecular damage, heightened lipid peroxidation, and amplified production of RONS (34). Elevated RONS levels further drive the onset and advancement of tissue injuries and various disorders affecting the liver and lungs. Typically, increased RONS and LPO levels coincide with diminished activities of enzymatic antioxidants (e.g., SOD, CAT, GPX, and GST) and GSH levels (35). Our findings indicated that 1-NP alone markedly reduced antioxidant enzyme activities and GSH levels in the liver and lungs while elevating LPO and RONS levels. This 1-NP-induced OS may contribute to injuries associated with hepatopulmonary injury. In contrast, rats co-treated with VA exhibited significant enhancements in enzymatic antioxidant activities (SOD, CAT, GPX, GST) and GSH levels. On the other hand, co-exposure to VA and 1-NP led to a notable decrease in LPO and RONS levels in the treated rats. The protective effects of VA may be due to its intrinsic antioxidant properties (36).

Increased NO production, indicative of nitrosative stress, is largely driven by inducible nitric oxide synthase, whose expression is enhanced by cytokines like IL-1β and TNF-α. These cytokines, produced by activated macrophages and other immune cells during 1-NP-induced inflammation, stimulate the expression of inducible nitric oxide synthase, leading to excessive production of NO (37). Moreover, increased MPO activity reflects the infiltration of neutrophils and other mononuclear cells, which are key players in the inflammatory cascade. IL-1β and TNF-α promote the recruitment and activation of these immune cells, thereby enhancing the release of MPO (38). Based on our results, rats treated with 1-NP alone exhibited elevated levels of OS and immune activation, contributing to the observed increases in MPO activity and the production of NO, TNF-α, and IL-1β. These cytokines amplify inflammation by activating immune cells and promoting the production of ROS and reactive nitrogen species, which aligns with our findings indicating increased nitrosative stress and mononuclear cell infiltration. However, the significant reduction in NO and MPO activity with VA co-treatment suggests that VA mitigates the inflammatory and nitrosative stress induced by 1-NP. This is likely due to the ability of VA to suppress the production or activity of pro-inflammatory cytokines (e.g., IL-1β and TNF-α). These cytokines are the upstream mediators of inflammation, driving NO production and immune cell recruitment, which can, in turn, lead to MPO activity. Its antioxidant and anti-inflammatory properties help alleviate the inflammatory and oxidative/nitrosative stress cascade. The ability of VA to reduce NO and MPO demonstrates that it interrupts this inflammatory loop, likely by suppressing IL-1β and TNF-α production or signaling. This is in line with existing literature documenting the protective and anti-inflammatory effects of VA (38,39).

GPX4 protects cells from oxidative damage by converting lipid hydroperoxides into non-toxic substances. It uses GSH as a cofactor to neutralize ROS and lipid peroxides. During inflammation, GPX4 typically mitigates OS; in other words, its expression or activity can increase in response to inflammatory stimuli as a compensatory mechanism to counteract heightened lipid peroxidation. However, persistent inflammation can deplete GSH, impairing the efficacy of GPX4 and exacerbating tissue injury (40-42). Regulated on activation, RANTES, also known as CCL5, is a chemokine that recruits immune cells, including T cells, monocytes, and eosinophils, to the sites of inflammation. It is produced by various cells, including macrophages and epithelial cells, in response to pro-inflammatory cytokines, such as TNF-α and IL-1β. In inflammation, RANTES amplifies inflammatory

responses by promoting the infiltration and activation of immune cells. Elevated RANTES levels are associated with chronic inflammatory conditions, including lung and liver disorders, where they contribute to tissue damage by sustaining immune cell recruitment and cytokine production (43,44).

Data from our study revealed that exposure to 1-NP alone in rats significantly elevates the levels of RANTES and GPX4 in the liver and lungs relative to the normal group. The significant increase in GPX4 and RANTES levels in rats exposed to 1-NP alone aligns with the observed elevations in TNF-α, IL-1β, MPO, and NO, indicating a coordinated inflammatory and OS response. However, the co-administration of VA could significantly reduce the levels of RANTES and GPX4 in the hepatopulmonary organs when compared to the control group. These findings underscore the therapeutic potential of VA in mitigating 1-NP-induced hepatopulmonary injury, which likely extends to reducing GPX4 and RANTES in rats treated with 1-NP. Our findings conform to those of earlier reports of VA inhibiting inflammatory cytokines (44,45).

Pathological evaluation provides insights into the integrity of hepatopulmonary tissue and disease progression, supporting and validating findings from biochemical analyses and tissue enzyme levels. Our histopathological data in 1-NP-treated rats alone confirmed severe liver and lung damage, characterized by congestion, inflammatory cell infiltration, swollen arterioles, and perivascular accumulation of lymphocytes and neutrophils compared to the normal group. These changes indicate a robust inflammatory and OS response elicited by 1-NP, which may potentially lead to hepatopulmonary injury. These histopathological changes are in line with our earlier biochemical results, where 1-NP alone increased serum transaminases (ALT, AST, and ALP), NO, MPO, TNF-α, IL-1β, and RANTES while decreasing antioxidant defenses (SOD, CAT, GPX, and GST) and the GSH level. Conversely, treatment with VA noticeably reduced the hepatopulmonary damage caused by 1-NP, restoring the tissue structure of the liver and lungs to a state closely resembling that of the control group.

Conclusion

Our findings demonstrated that 1-NP exposure alone induced hepatopulmonary oxidative injury in rats by triggering the production of reactive species and releasing inflammatory mediators, which disrupted the cellular antioxidant defense system. Remarkably, VA exerted a protective effect against 1-NP-induced hepatopulmonary damage by suppressing excessive production of inflammatory mediators, bolstering enzymatic and non-enzymatic antioxidant defenses, and mitigating the resulting OS and tissue injury. These results highlight the relevance of VA in mitigating environmental toxin-induced damage and warrant further investigation into its clinical applications.

Acknowledgements

The authors would like to thank the Institute for Advanced Medical Research and Training, particularly the Breast Cancer Laboratory at the University College Hospital, Ibadan.

Authors' Contribution

Conceptualization: Ikenna C. Maduako.
Data curation: Omorede Ikponmwosa-Eweka and Ikenna C. Maduako.
Formal analysis: Ikenna C. Maduako.
Funding acquisition: Omorede Ikponmwosa-Eweka.
Investigation: Omorede Ikponmwosa-Eweka and Ikenna C. Maduako.
Methodology: Ikenna C. Maduako.
Project administration: Omorede Ikponmwosa-Eweka.
Resources: Omorede Ikponmwosa-Eweka.
Software: Ikenna C. Maduako.
Supervision: Omorede Ikponmwosa-Eweka.
Validation: Omorede Ikponmwosa-Eweka.
Visualization: Ikenna C. Maduako.
Writing–original draft: Ikenna C. Maduako.
Writing–review & editing: Omorede Ikponmwosa-Eweka.

Competing Interests

The authors declare that there are no conflicting interests between the authors regarding this publication.

Data Availability Statement

Data will be made available upon reasonable request from the corresponding author.

Ethical Approval

All procedures adhered to the National Institute of Health guidelines for the care and use of laboratory animals (1985) and were approved by the Life Sciences Ethics Committee for Animal Research at the University of Benin, Nigeria (approval No. LS21046).

Funding

This is a self-funded study. The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this work.

References

1. Bandowe BA, Meusel H. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment–a review. Sci Total Environ. 2017;581-582:237-57. doi: 10.1016/j.scitotenv.2016.12.115.
2. Feng S, Shen X, Hao X, Cao X, Li X, Yao X, et al. Polycyclic and nitro-polycyclic aromatic hydrocarbon pollution characteristics and carcinogenic risk assessment of indoor kitchen air during cooking periods in rural households in North China. Environ Sci Pollut Res Int. 2021;28(9):11498-508. doi: 10.1007/s11356-020-11316-8.
3. Ochirpurev B, Eom SY, Toriba A, Kim YD, Kim H. Urinary 1-aminopyrene level in Koreans as a biomarker for the amount of exposure to atmospheric 1-nitropyrene. Toxicol Res. 2022;38(1):45-51. doi: 10.1007/s43188-021-00096-z.
4. Igwe CN, Ngwoke UN, Okugbo OT, Ikpowonsa-Eweka O, Ego CV, Omoyajowo EA, et al. Air quality and public health in metropolitan cities in Nigeria: implications. A review. Int J Res Sci Innov. 2025;12(2):50-60. doi: 10.51244/ijrsi.2025.12020006.
5. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002;30(6):620-50. doi: 10.1080/01926230290166724.

6. Asare N, Landvik NE, Lagadic-Gossmann D, Rissel M, Tekpli X, Ask K, et al. 1-Nitropyrene (1-NP) induces apoptosis and apparently a non-apoptotic programmed cell death (paraptosis) in Hepa1c1c7 cells. Toxicol Appl Pharmacol. 2008;230(2):175-86. doi: 10.1016/j.taap.2008.02.015.
7. Park EJ, Park K. Induction of pro-inflammatory signals by 1-nitropyrene in cultured BEAS-2B cells. Toxicol Lett. 2009;184(2):126-33. doi: 10.1016/j.toxlet.2008.10.028.
8. Belisario MA, Pecce R, Garofalo A, Sannolo N, Malorni A. Erythrocyte enzymes catalyze 1-nitropyrene and 3-nitrofluoranthene nitroreduction. Toxicology. 1996;108(1-2):101-8. doi: 10.1016/0300-483x(95)03293-o.
9. Farombi EO, Owoeye O. Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia* biflavonoid. Int J Environ Res Public Health. 2011;8(6):2533-55. doi: 10.3390/ijerph8062533.
10. Aruoma OI, Sun B, Fujii H, Neergheen VS, Bahorun T, Kang KS, et al. Low molecular proanthocyanidin dietary biofactor Oligonol: its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. Biofactors. 2006;27(1-4):245-65. doi: 10.1002/biof.5520270121.
11. Seeff LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH. Complementary and alternative medicine in chronic liver disease. Hepatology. 2001;34(3):595-603. doi: 10.1053/jhep.2001.27445.
12. Achuba FI. Role of bitter leaf (*Vernonia amygdalina*) extract in prevention of renal toxicity induced by crude petroleum contaminated diets in rats. Int J Vet Sci Med. 2018;6(2):172-7. doi: 10.1016/j.ijvsm.2018.07.002.
13. Danladi S, Hassan MA, Masa'ud IA, Ibrahim UI. *Vernonia amygdalina* Del: a mini review. Res J Pharm Technol. 2018;11(9):4187-90. doi: 10.5958/0974-360x.2018.00768.0.
14. Edo GI, Ugbune U, Ezekiel GO, Nwosu LC, Onoharigho FO, Agbo JJ. Medicinal plants used for the treatment of sexual dysfunction; ethnobotanical study and phytochemical analysis. Ecol Front. 2024;44(2):247-56. doi: 10.1016/j.chnaes.2023.05.008.
15. Iwo MI, Sjahlim SL, Rahmawati SF. Effect of *Vernonia amygdalina* Del. leaf ethanolic extract on intoxicated male Wistar rats liver. Sci Pharm. 2017;85(2):16. doi: 10.3390/scipharm85020016 .
16. Adeyanju AA, Oyenihi OR, Oguntibeju OO. Antioxidant-rich vegetables: impact on human health. In: Yildirim E, Ekinci M, eds. Vegetable Crops: Health Benefits and Cultivation. IntechOpen; 2022. doi: 10.5772/intechopen.101126.
17. Oriakhi K, Oikeh EI, Ezeugwu N, Anoliefo O, Aguebor O, Omoregie ES. In vitro antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves. J Pharm Bioresour. 2014;11(2):58-65. doi: 10.4314/jpb.v11i2.5.
18. Denda A, Tsutsumi M, Tsujiuchi T, Eimoto H, Konishi Y, Sato S. Induction of rat liver gamma-glutamyltranspeptidase-positive foci by oral administration of 1-nitropyrene. Cancer Lett. 1989;45(1):21-6. doi: 10.1016/0304-3835(89)90031-1.
19. Adaramoye OA, Akintayo O, Achem J, Fafunso MA. Lipid-lowering effects of methanolic extract of Vernonia amygdalina leaves in rats fed on high cholesterol diet. Vasc Health Risk Manag. 2008;4(1):235-41. doi: 10.2147/vhrm.2008.04.01.235.
20. Kaplan MM. Laboratory tests. In: Schiff L, Schiff ER, eds. Diseases of the Liver. 7th ed. Philadelphia: JB Lippincott; 1993. p. 108-44.
21. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-5.
22. Claiborne AL. Catalase activity. In: Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press; 1985. p. 383.
23. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.
24. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-90. doi: 10.1126/science.179.4073.588.
25. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-9. doi: 10.1016/s0021-9258(19)42083-8.
26. Varshney R, Kale RK. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol. 1990;58(5):733-43. doi: 10.1080/09553009014552121.
27. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol. 1982;78(3):206-9. doi: 10.1111/1523-1747.ep12506462.
28. Granell S, Gironella M, Bulbena O, Panés J, Mauri M, Sabater L, et al. Heparin mobilizes xanthine oxidase and induces lung inflammation in acute pancreatitis. Crit Care Med. 2003;31(2):525-30. doi: 10.1097/01.Ccm.0000049948.64660.06.
29. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8. doi: 10.1016/0003-2697(82)90118-x.
30. Li X, Yang H, Sun H, Lu R, Zhang C, Gao N, et al. Taurine ameliorates particulate matter-induced emphysema by switching on mitochondrial NADH dehydrogenase genes. Proc Natl Acad Sci U S A. 2017;114(45):E9655-64. doi: 10.1073/pnas.1712465114.
31. Schulte JK, Fox JR, Oron AP, Larson TV, Simpson CD, Paulsen M, et al. Neighborhood-scale spatial models of diesel exhaust concentration profile using 1-nitropyrene and other nitroarenes. Environ Sci Technol. 2015;49(22):13422-30. doi: 10.1021/acs.est.5b03639.
32. Liu CS, Tsai CS, Kuo CL, Chen HW, Lii CK, Ma YS, et al. Oxidative stress-related alteration of the copy number of mitochondrial DNA in human leukocytes. Free Radic Res. 2003;37(12):1307-17. doi: 10.1080/10715760310001621342.
33. Zachariah SK, Paul V, Mathews KS, Gopinath J, Celine TM, Rajeeve S. Hepatic transaminases as predictors of liver injury in abdominal trauma. Int Surg J. 2017;5(1):181-6. doi: 10.18203/2349-2902.isj20175891 .
34. Atangwho IJ, Edet EE, Uti DE, Obi AU, Asmawi MZ, Ahmad M. Biochemical and histological impact of *Vernonia amygdalina* supplemented diet in obese rats. Saudi J Biol Sci. 2012;19(3):385-92. doi: 10.1016/j.sjbs.2012.05.003.
35. Mao W, Liu X, Fan S, Zhang R, Liu M, Xiao S. Modulating oxidative stress: a reliable strategy for coping with community-acquired pneumonia in older adults. Front Med (Lausanne). 2025;12:1549658. doi: 10.3389/fmed.2025.1549658.
36. Edo GI, Onoharigho FO. Analysis of phytochemical constituents and antioxidant potential of bitter kola leaf extract towards bioactive food, nutrition and health resources. Org Med Chem Int J. 2022;11(5):555823. doi: 10.19080/omcij.2022.11.555823.
37. Wang WT, Liao SF, Wu ZL, Chang CW, Wu JY. Simultaneous study of antioxidant activity, DNA protection and anti-inflammatory effect of *Vernonia amygdalina* leaves extracts. PLoS One. 2020;15(7):e0235717. doi: 10.1371/journal.pone.0235717.
38. Kim ME, Lee JS. Advances in the regulation of inflammatory mediators in nitric oxide synthase: implications for disease modulation and therapeutic approaches. Int J Mol Sci. 2025;26(3):1204. doi: 10.3390/ijms26031204.

39. Asante DB, Henneh IT, Acheampong DO, Kyei F, Adokoh CK, Ofori EG, et al. Anti-inflammatory, anti-nociceptive and antipyretic activity of young and old leaves of Vernonia amygdalina. Biomed Pharmacother. 2019;111:1187-203. doi: 10.1016/j.biopha.2018.12.147.
40. Li XL, Liu YL, Liu JY, Zhu YY, Zhu XX, Zhang WW, et al. 1-Nitropyrene disrupts testicular steroidogenesis via oxidative stress-evoked PERK-eIF2α pathway. Ecotoxicol Environ Saf. 2023;259:115027. doi: 10.1016/j.ecoenv.2023.115027.
41. Forcina GC, Dixon SJ. GPX4 at the crossroads of lipid homeostasis and ferroptosis. Proteomics. 2019;19(18):e1800311. doi: 10.1002/pmic.201800311.
42. Yang W, Wang Y, Zhang C, Huang Y, Yu J, Shi L, et al. Maresin1 protect against ferroptosis-induced liver injury through ROS inhibition and Nrf2/HO-1/GPX4 activation. Front Pharmacol. 2022;13:865689. doi: 10.3389/fphar.2022.865689.
43. Peters VB, Matheis F, Erdmann I, Nemade HN, Muders D, Toubartz M, et al. Myeloperoxidase induces monocyte migration and activation after acute myocardial infarction. Front Immunol. 2024;15:1360700. doi: 10.3389/fimmu.2024.1360700.
44. Fu L, Zhao H, Xiang Y, Xiang HX, Hu B, Tan ZX, et al. Reactive oxygen species-evoked endoplasmic reticulum stress mediates 1-nitropyrene-induced epithelial-mesenchymal transition and pulmonary fibrosis. Environ Pollut. 2021;283:117134. doi: 10.1016/j.envpol.2021.117134.
45. D'Ambrosio D, Mariani M, Panina-Bordignon P, Sinigaglia F. Chemokines and their receptors guiding T lymphocyte recruitment in lung inflammation. Am J Respir Crit Care Med. 2001;164(7):1266-75. doi: 10.1164/ajrccm.164.7.2103011.