

SYNERGISTIC EFFECTS OF PERSISTENT ORGANIC POLLUTANTS (POPs) AND HYDROGEN PEROXIDE ON SOME OXIDATIVE STRESS MARKERS IN WISTAR RATS

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Received: 16-05-2025

Accepted: 03-06-2025

<https://dx.doi.org/10.4314/sa.v24i3.12>

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Journal Homepage: <http://www.scientia-african.uniportjournal.info>

Publisher: [Faculty of Science, University of Port Harcourt.](#)

ABSTRACT

Persistent organic pollutants (POPs) and oxidative stress are key contributors to cellular dysfunction and disease progression. This study investigates the synergistic effects of POPs, specifically benzo(a)pyrene and naphthalene, in combination with hydrogen peroxide (H_2O_2) on oxidative stress markers in a comparative experimental model with two treatment durations: Batch 1 (4 weeks) and Batch 2 (8 weeks). Biochemical assessments included superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx). The results revealed significant oxidative stress in groups exposed to POPs alone, with MDA levels increasing from 2.70 ± 0.28 (control) to 6.40 ± 0.14 in Batch 1 and from 2.60 ± 0.42 to 9.45 ± 0.35 in Batch 2, indicating progressive lipid peroxidation. This was accompanied by a decline in GSH levels (3.35 ± 0.21 to 2.35 ± 0.21 in Batch 2), SOD (18.20 ± 0.28 to 10.40 ± 0.14 in Batch 2), and CAT (17.20 ± 0.28 to 12.65 ± 0.21 in Batch 2), confirming oxidative stress-induced depletion of antioxidant defenses. Exposure to H_2O_2 alone exacerbated oxidative damage, with MDA levels reaching 8.30 ± 0.42 in Batch 1 and 10.30 ± 0.28 in Batch 2, along with a significant reduction in GSH (2.35 ± 0.21 in Batch 2). Co-exposure to POPs and low-dose H_2O_2 resulted in moderate oxidative stress, as seen in MDA levels of 9.20 ± 0.28 (Batch 1) and 12.50 ± 0.28 (Batch 2). High-dose H_2O_2 combined with POPs induced severe oxidative damage, with the highest MDA levels (10.30 ± 0.28 in Batch 1 and 14.35 ± 0.21 in Batch 2) and a sharp depletion in GSH (1.10 ± 0.14 in Batch 2). Vitamin C supplementation provided partial protection, lowering MDA levels to 4.45 ± 0.35 (Batch 1) and 3.60 ± 0.28 (Batch 2), while restoring some antioxidant enzyme activities, particularly SOD and GPx. However, its effectiveness diminished in Batch 2, indicating that prolonged oxidative stress may reduce antioxidant efficacy. These findings highlight a dose- and duration-dependent synergistic effect between POPs and H_2O_2 , with protracted exposure exacerbating oxidative damage. The study emphasizes the potential risks associated with combined environmental toxicants and oxidative stressors in inducing cellular damage and the need for effective antioxidant interventions.

Keywords: Persistent Organic Pollutants, Oxidative Stress, Biochemical Indices, Genotoxicity, Wistar Rats, Environmental Toxicology

INTRODUCTION

Persistent organic pollutants (POPs) are a class of environmental contaminants that persist in ecosystems due to their chemical stability, resistance to degradation, and bioaccumulation in living organisms (Akhtar et al., 2021). These pollutants, including polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene and naphthalene, pose significant health hazards due to their capacity to induce oxidative stress, alter cellular homeostasis, and contribute to disease pathogenesis (Mallah et al., 2022). Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense mechanisms of cells, leading to biomolecular damage that affects lipids, proteins, and DNA (Juan et al., 2021). Among the key ROS, hydrogen peroxide (H_2O_2) plays a dual role as both a signaling molecule in normal physiological processes and a cytotoxic agent when present in excessive amounts (Andrés et al., 2022). The interaction between exogenous toxicants such as POPs and endogenous oxidants like H_2O_2 could potentially amplify oxidative stress, leading to progressive cellular dysfunction and disease initiation (Lind et al., 2021).

Numerous studies have established that exposure to POPs disrupts redox homeostasis by increasing oxidative stress markers and impairing the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Lee et al., 2021). Lipid peroxidation, a critical consequence of oxidative stress, is commonly assessed by measuring malondialdehyde (MDA), a byproduct of membrane lipid degradation (Ying et al., 2025). Similarly, glutathione peroxidase (GPx) serves as a crucial antioxidant enzyme that mitigates oxidative damage by neutralizing peroxides (Demirci-Çekiç et al., 2022). The interplay between these biomarkers provides insights into the extent of oxidative stress and the efficiency of cellular antioxidant defenses in response to toxicant exposure. While individual exposure to POPs or H_2O_2 has been

well-documented, studies evaluating their combined effects remain limited (D'Souza et al., 2024). Given that both are capable of inducing oxidative stress through distinct but overlapping pathways, their co-exposure may exacerbate oxidative damage in a dose- and duration-dependent manner.

The understanding of the synergistic effects of POPs and H_2O_2 is crucial, particularly in the context of prolonged environmental and occupational exposure. Chronic exposure to POPs has been linked to a range of adverse health effects, including carcinogenesis, neurotoxicity, immunosuppression, and metabolic disorders, all of which have been associated with oxidative damage as an underlying mechanism (Ahmed et al., 2021). Similarly, excess H_2O_2 accumulation within cells can lead to oxidative modification of macromolecules, impair cellular function, and ultimately contribute to disease progression. The potential interaction between these two toxicants raises concerns about their cumulative impact on cellular health, necessitating a comparative study to elucidate their combined effects on oxidative stress biomarkers (Nagy et al., 2020).

Given the increasing global concern regarding environmental pollutants and their impact on human health, research into the mitigation of oxidative stress-induced toxicity has gained prominence. Antioxidants, both endogenous and exogenous, play a significant role in counteracting oxidative stress (Pisoschi et al., 2021). Vitamin C, a potent water-soluble antioxidant, has been shown to enhance cellular defense mechanisms by scavenging free radicals and regenerating other antioxidants. Its potential protective role against oxidative damage induced by POPs and H_2O_2 warrants investigation, particularly in determining whether it can effectively counteract the synergistic oxidative effects observed in co-exposure scenarios. However, previous studies have suggested that prolonged exposure to high levels of oxidative stress may impair the efficacy of antioxidants, indicating a possible threshold beyond which

antioxidant intervention becomes ineffective (Forman & Zhang, 2021).

This study aims to comparatively assess the synergistic effects of benzo(a)pyrene, naphthalene, and hydrogen peroxide on oxidative stress markers over two different treatment durations. By evaluating the biochemical alterations in SOD, CAT, GSH, MDA, and GPx levels, the study seeks to determine the extent of oxidative stress induced by individual and combined exposures. Additionally, the study explores the potential protective role of vitamin C in mitigating oxidative damage and assesses whether its effectiveness diminishes under prolonged exposure conditions. The findings of this study will contribute to a deeper understanding of the interplay between environmental toxicants and oxidative stress, providing valuable insights into the risks associated with combined exposure and highlighting the need for effective antioxidant-based therapeutic strategies.

METHODOLOGY

Experimental Animals and Housing

Adult male Wistar rats which weighed between 150–200 g were used in this study (Ghasemi et al., 2021). The rats were housed under standard laboratory conditions, with a 12-hour light/dark cycle and free access to standard rat feed and water.

Determination of LD₅₀ (Pilot Study)

A stock solution of hydrogen peroxide (H₂O₂) was prepared by dissolving 3 g in 100 mL of distilled water, yielding a concentration of 30 g/L. Based on preliminary calculations, the low-dose administration of H₂O₂ was determined to be 0.5 mL, corresponding to 505 mg/L, while the high-dose administration was set at 1.5 mL, equivalent to 1115 mg/L.

According to literature, the reported LD₅₀ for benzo(a)pyrene is between 200–500 mg/kg (Susheela et al., 2008). To establish a safe working concentration, a pilot study was conducted using four Wistar rats, each receiving 200 mg/kg of benzo(a)pyrene.

Observed mortality necessitated a reduction in dosage to 100 mg/kg for the main study. Similarly, the LD₅₀ for naphthalene is documented at 1100 mg/kg. A pilot study conducted at this concentration resulted in mortality, leading to a revised working concentration of 550 mg/kg for the study.

Experimental Design and Animal Groupings

A total of 56 Wistar rats were weighed and distributed into seven groups of eight Wistar rats per group:

- **Group 1 (Control):** Corn oil and water (vehicle control).
- **Group 2:** Benzo(a)pyrene (100 mg/L) + naphthalene (550 mg/L) (PAHs) only.
- **Group 3:** H₂O₂ (1.0 mL) (oxidative stress inducer) only.
- **Group 4:** Benzo(a)pyrene (100 mg/L) + naphthalene (550 mg/L) (PAHs) + low-dose H₂O₂ (0.5 mL).
- **Group 5:** Benzo(a)pyrene (100 mg/L) + naphthalene (550 mg/L) (PAHs) + low-dose H₂O₂ (1.5 mL).
- **Group 6:** H₂O₂ (1 mL) + Vitamin C (100 mg/L).
- **Group 7:** Benzo(a)pyrene (100 mg/L) + naphthalene (550 mg/L) (PAHs) + low-dose H₂O₂ (1.0 mL) + Vitamin C (100 mg/L).

Treatment Administration

Administration of toxicants [(Benzo(a)pyrene (100 mg/L), naphthalene (550 mg/L) (PAHs), H₂O₂)] and treatment of the intoxicated groups with Vitamin C was carried out in two batches (batch one and batch two). In batch one, animals were administered [(Benzo(a)pyrene (100 mg/L), naphthalene (550 mg/L) (PAHs), H₂O₂)] and treatment of the intoxicated groups with Vitamin C for four weeks whereas for batch two, administration of [(Benzo(a)pyrene (100 mg/L), naphthalene (550 mg/L) (PAHs), H₂O₂)] and treatment of the intoxicated groups with Vitamin C was for eight weeks.

- **Benzo(a)pyrene and Naphthalene (POPs):** Administration was done via oral gavage for 4–8 weeks.
- **Hydrogen Peroxide (H₂O₂):** Administered via oral at varying doses corresponding to the experimental groups.
- **Vitamin C:** Administered orally at a dose of 100 mg/kg as an antioxidant agent.

Sample Collection

For oxidative stress analysis, the kidney tissues of the batch one and batch two treated Wistar rats were carefully excised and rinsed in ice-cold phosphate-buffered saline (PBS) which ensured the removal of excess blood. The kidney samples, specifically collected from the renal cortex, were used to assess the following oxidative stress markers namely superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA) and glutathione peroxidase (GPx).

Oxidative Stress Marker Analysis

Oxidative stress markers were analyzed by measuring the activity levels of key antioxidant enzymes and lipid peroxidation indicators (Demirci-Çekiç et al., 2022). Superoxide dismutase (SOD) activity was assessed based on its ability to catalyze the dismutation of superoxide radicals into hydrogen peroxide and oxygen. Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide into water and oxygen. Glutathione (GSH) levels were quantified to evaluate the cellular antioxidant defense system, as GSH plays a critical role in neutralizing reactive oxygen species. Malondialdehyde (MDA) concentration, an indicator of lipid peroxidation, was measured using a thiobarbituric acid reactive substances (TBARS) assay. Glutathione peroxidase (GPx) activity was analyzed to determine its role in reducing peroxides and protecting cells from oxidative damage. These biochemical assessments provided insights into the extent

of oxidative stress and the impact of toxicant exposure on antioxidant defense mechanisms.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way ANOVA followed by post-hoc Tukey's test. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 represents the synergistic effects of persistent organic pollutants and hydrogen peroxide on oxidative stress markers for batch 1 and batch 2 administration of POPs and hydrogen peroxide for four and eight weeks respectively. The oxidative stress markers revealed distinct variations across the different experimental groups over the two treatment durations. In Batch 1, the control group maintained relatively stable levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx), while in Batch 2, these markers exhibited a slight increase, reflecting a normal physiological response over time. The group administered Benzo(a)pyrene and naphthalene (PAHs) alone in Batch 1 showed increased oxidative stress, characterized by elevated MDA and GPx levels, with corresponding reductions in antioxidant enzyme activities compared to the control. This trend was exacerbated in Batch 2, where SOD, CAT, and GSH levels were further reduced, while MDA and GPx increased significantly, indicating progressive oxidative damage with prolonged exposure.

The group exposed to hydrogen peroxide (H₂O₂) alone in Batch 1 exhibited a further rise in MDA levels, suggesting heightened lipid peroxidation, while antioxidant enzyme activities showed a moderate decline. By Batch 2, oxidative stress intensified, with a more pronounced reduction in SOD, CAT, and GSH, alongside elevated MDA and GPx levels. The administration of PAHs with low-dose H₂O₂ in Batch 1 resulted in a moderate increase in MDA and GPx, with SOD and

CAT showing reduced activities, indicative of an additive oxidative stress effect. This pattern became more pronounced in Batch 2, with a significant suppression of antioxidant enzymes and heightened MDA levels, reflecting cumulative oxidative damage.

In the group treated with PAHs and high-dose H₂O₂, Batch 1 results showed severe oxidative stress, as evidenced by significantly elevated MDA levels and a marked decrease in GSH. This effect was even more severe in Batch 2, where GSH depletion was more pronounced, and MDA reached its highest levels, indicating progressive oxidative injury over the extended exposure period. The administration of vitamin C alongside H₂O₂ in Batch 1 demonstrated a partial protective effect, as seen in reduced MDA levels and a modest improvement in antioxidant enzyme activities.

In Batch 2, this protective effect persisted but was less pronounced, suggesting that prolonged oxidative stress exposure might limit the efficacy of antioxidant intervention.

The group receiving a combination of PAHs, H₂O₂, and vitamin C in Batch 1 exhibited partial amelioration of oxidative stress markers, with moderate reductions in MDA and a slight improvement in SOD, CAT, and GPx activities. In Batch 2, however, the protective effects of vitamin C appeared more constrained, with persistent oxidative stress indicators despite some recovery in antioxidant enzyme activities. These findings indicate that while vitamin C provides some level of mitigation against oxidative damage, its effectiveness is influenced by the duration and intensity of oxidative stress exposure.

Table 1: Synergistic Effects of Persistent Organic Pollutants and Hydrogen Peroxide on Oxidative Stress Markers

Entry No	Grouping	Batch 1					Batch 2				
		SOD	CAT	GSH	MDA	GPx	SOD	CAT	GSH	MDA	GPx
1	Positive/ Vehicle control group	14.70 ± 0.14 ^a	16.35 ± 0.21 ^a	3.35 ± 0.21 ^a	2.70 ± 0.28 ^a	8.65 ± 0.35 ^a	18.20 ± 0.28 ^a	17.20 ± 0.28 ^a	5.95 ± 0.21 ^a	2.60 ± 0.42 ^a	16.20 ± 0.28 ^a
2	Benzo(a)pyrene + naphthalene (PAHs) only	15.10 ± 0.14 ^a	18.20 ± 0.14 ^b	4.90 ± 0.14 ^b	6.40 ± 0.14 ^b	17.20 ± 0.14 ^b	10.40 ± 0.14 ^b	12.65 ± 0.21 ^b	3.35 ± 0.21 ^b	9.45 ± 0.35 ^b	11.35 ± 0.21 ^b
3	Oxidative stress inducer (H ₂ O ₂) only	16.20 ± 0.14 ^b	17.40 ± 0.14 ^c	3.65 ± 0.21 ^a	8.30 ± 0.42 ^c	13.40 ± 0.56 ^c	15.40 ± 0.14 ^c	10.70 ± 0.28 ^c	2.35 ± 0.21 ^c	10.30 ± 0.28 ^c	9.35 ± 0.21 ^c
4	Benzo(a)pyrene + naphthalene (PAHs) + low dose H ₂ O ₂	15.40 ± 0.14 ^a	16.98 ± 0.28 ^d	3.85 ± 0.21 ^a	9.20 ± 0.28 ^d	11.50 ± 0.42 ^d	7.10 ± 0.14 ^d	9.45 ± 0.07 ^d	1.60 ± 0.14 ^d	12.50 ± 0.28 ^d	7.60 ± 0.28 ^d
5	Benzo(a)pyrene + naphthalene (PAHs) + high dose H ₂ O ₂	16.40 ± 0.14 ^b	18.55 ± 0.49 ^c	4.08 ± 0.14 ^c	10.30 ± 0.28 ^e	13.30 ± 0.42 ^c	14.15 ± 0.21 ^c	10.30 ± 0.28 ^c	1.10 ± 0.14 ^c	14.35 ± 0.21 ^e	5.55 ± 0.35 ^e
6	Oxidative stress inducer (H ₂ O ₂) + Antioxidant treatment [(Vitamin C) (ascorbic acid)]	15.30 ± 0.14 ^a	16.95 ± 0.21 ^d	3.58 ± 0.21 ^a	4.45 ± 0.35 ^f	12.55 ± 0.35 ^e	13.20 ± 0.28 ^f	9.20 ± 0.28 ^d	5.35 ± 0.21 ^a	3.60 ± 0.28 ^f	8.70 ± 0.28 ^f
7	Benzo(a)pyrene + naphthalene (PAHs) + Oxidative stress inducer (H ₂ O ₂) + Antioxidant treatment [(Vitamin C) (ascorbic acid)]	14.70 ± 0.14 ^a	14.75 ± 0.07 ^f	3.92 ± 0.14 ^a	5.75 ± 0.07 ^g	16.50 ± 0.42 ^b	13.85 ± 0.21 ^{ef}	9.40 ± 0.28 ^d	3.35 ± 0.21 ^b	3.45 ± 0.49 ^d	7.20 ± 0.28 ^d

Each value in the Table represents the mean value of duplicates ± SD

Values with different superscripts indicate statistically significant differences ($p < 0.05$) within a column

The same superscripts within a column indicate no significant difference ($p > 0.05$)

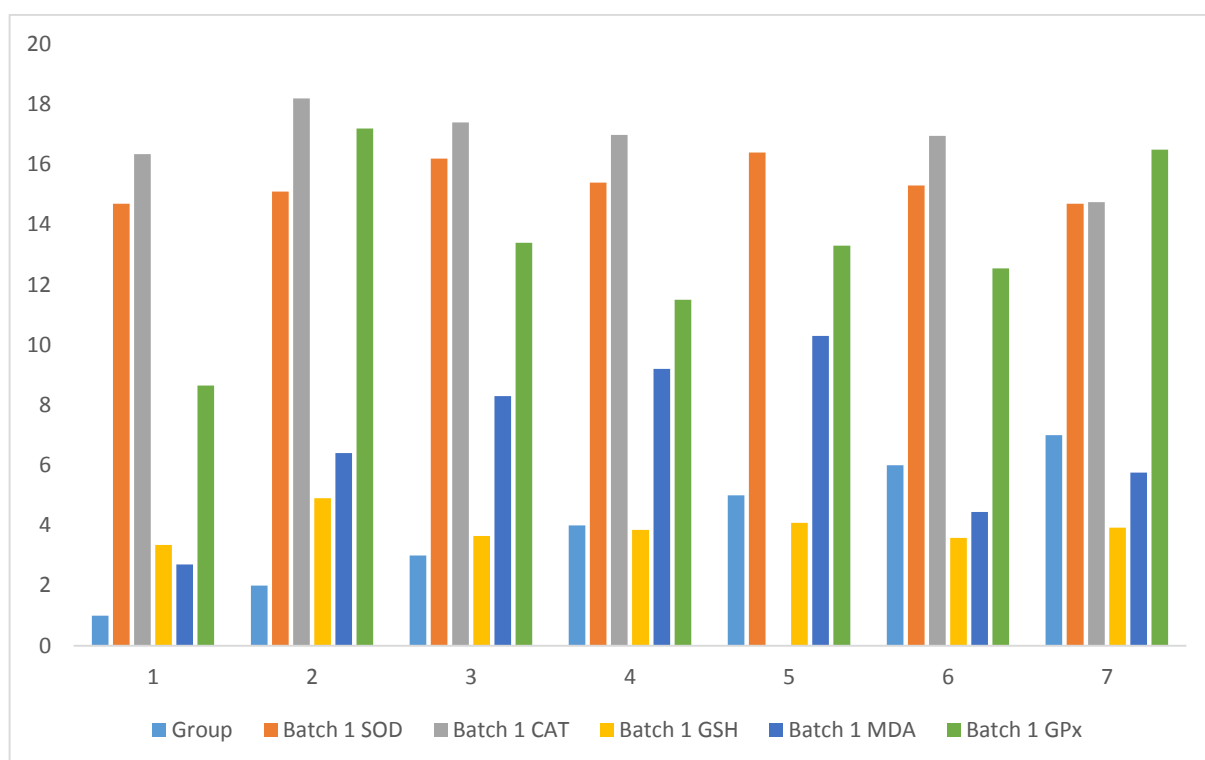


Figure 1: Bar chart representation of Batch 1 oxidative stress (SOD, CAT, GSH, MDA and GPx) across Experimental Groups in Wistar Rats Exposed to Persistent Organic Pollutants (POPs) and Oxidative Stress Inducers with Antioxidant Treatment

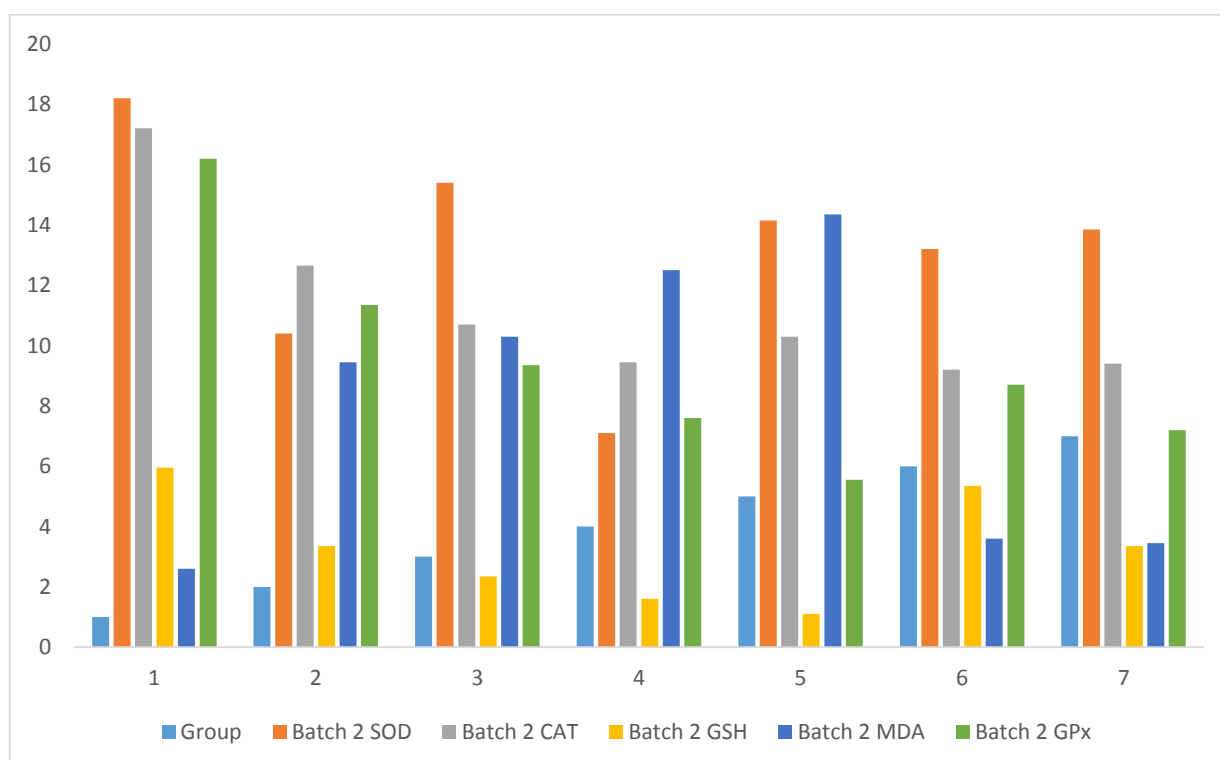


Figure 2: Bar chart representation of Batch 2 oxidative stress (SOD, CAT, GSH, MDA and GPx) across Experimental Groups in Wistar Rats Exposed to Persistent Organic Pollutants (POPs) and Oxidative Stress Inducers with Antioxidant Treatment

DISCUSSION

Oxidative stress is a well-recognized mechanism underlying cellular damage, particularly in response to environmental toxicants such as polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs). The current study demonstrated significant oxidative alterations following PAH and hydrogen peroxide (H_2O_2) exposure, with variations across experimental groups and exposure durations. A comparison with previous studies reveals both consistencies and divergences in findings, particularly in terms of oxidative damage progression, antioxidant defense mechanisms, and the efficacy of intervention strategies.

The present study found that the control group maintained stable levels of oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx). The control group in Batch 1 had SOD level of 14.70 ± 0.14 and CAT of 16.35 ± 0.21 , while in Batch 2, SOD and CAT were 18.20 ± 0.28 and 17.20 ± 0.28 , respectively. These values are consistent with prior research suggesting that endogenous antioxidant defenses efficiently regulate reactive oxygen species (ROS) under normal conditions (Halliwell & Gutteridge, 2015). The slight increase in oxidative markers over time in Batch 2 aligns with studies indicating that aging or prolonged exposure to even mild oxidative conditions can subtly increase oxidative stress levels due to the gradual accumulation of ROS (Sies, 2017).

PAH exposure alone (Benzo(a)pyrene + naphthalene) resulted in significantly altered oxidative stress markers, as evidenced by an increase in MDA (6.40 ± 0.14 in Batch 1 and 9.45 ± 0.35 in Batch 2) and GPx (17.20 ± 0.14 in Batch 1 and 11.35 ± 0.21 in Batch 2). This aligns with Xue et al. (2021), who demonstrated that PAHs generate ROS through redox cycling, leading to oxidative damage. The observed depletion of SOD (15.10 ± 0.14 in Batch 1 and 10.40 ± 0.14 in

Batch 2) and CAT (18.20 ± 0.14 in Batch 1 and 12.65 ± 0.21 in Batch 2) reflects the exhaustion of primary antioxidant defenses, a pattern also seen in xenobiotic-induced oxidative stress (Brigelius-Flohé & Maiorino, 2013).

Hydrogen peroxide exposure alone led to increased oxidative damage, as indicated by the elevated MDA (8.30 ± 0.42 in Batch 1 and 10.30 ± 0.28 in Batch 2) and reduced antioxidant enzyme activities, particularly SOD (16.20 ± 0.14 in Batch 1 and 15.40 ± 0.14 in Batch 2). These findings support those of Winterbourn (2018), who emphasized that excessive H_2O_2 exposure overwhelms cellular antioxidant defenses, leading to oxidative damage. The observed decrease in GSH (3.65 ± 0.21 in Batch 1 and 2.35 ± 0.21 in Batch 2) is particularly significant, as GSH is crucial for counteracting oxidative stress, and its depletion indicates an overwhelming ROS burden (Ighodaro & Akinloye, 2018).

The combined exposure to PAHs and low-dose hydrogen peroxide produced an additive oxidative stress effect, as observed through further reductions in SOD (15.40 ± 0.14 in Batch 1 and 7.10 ± 0.14 in Batch 2) and CAT (16.98 ± 0.28 in Batch 1 and 9.45 ± 0.07 in Batch 2). The concurrent rise in MDA (9.20 ± 0.28 in Batch 1 and 12.50 ± 0.28 in Batch 2) highlights the heightened oxidative burden. These results are consistent with Wang et al. (2021), who demonstrated that environmental pollutants act synergistically to exacerbate oxidative stress.

In the PAH and high-dose hydrogen peroxide group, oxidative stress was even more severe, with a marked rise in MDA (10.30 ± 0.28 in Batch 1 and 14.35 ± 0.21 in Batch 2) and sharp declines in GSH (4.08 ± 0.14 in Batch 1 and 1.10 ± 0.14 in Batch 2). This aligns with Rinna and Forman (2008), who emphasized the role of GSH in counteracting oxidative damage and its depletion as an indicator of severe oxidative stress. The progressive decline in antioxidant defenses in Batch 2 further corroborates studies suggesting that prolonged oxidative stress leads to irreversible

cellular damage, apoptosis, or necrosis (Chaudhary et al., 2023).

Vitamin C exhibited partial protective effects against oxidative stress, as evidenced by reduced MDA levels (4.45 ± 0.35 in Batch 1 and 3.60 ± 0.28 in Batch 2) and improved antioxidant enzyme activities, particularly GPx (12.55 ± 0.35 in Batch 1 and 8.70 ± 0.28 in Batch 2). These findings are consistent with Frei (2019), who highlighted vitamin C's role as a potent scavenger of free radicals and its ability to regenerate other antioxidants such as vitamin E. However, the reduced protective effect of vitamin C in Batch 2, where SOD and CAT levels were 13.20 ± 0.28 and 9.20 ± 0.28 , respectively, suggests that prolonged oxidative stress might limit its efficacy, aligning with studies by Jacob et al. (2017), which suggested that vitamin C's antioxidant potential is dose-dependent.

The combination of PAHs, hydrogen peroxide, and vitamin C resulted in moderate reductions in MDA (5.75 ± 0.07 in Batch 1 and 3.45 ± 0.49 in Batch 2) and slight improvements in SOD (14.70 ± 0.14 in Batch 1 and 13.85 ± 0.21 in Batch 2), CAT (14.75 ± 0.07 in Batch 1 and 9.40 ± 0.28 in Batch 2), and GPx (16.50 ± 0.42 in Batch 1 and 7.20 ± 0.28 in Batch 2). However, oxidative stress markers remained elevated in Batch 2, suggesting that vitamin C alone may not be sufficient for long-term protection. Muscolo et al. (2024) proposed that combining multiple antioxidants with distinct mechanisms of action may be required for optimal protection against prolonged oxidative stress.

The findings of this study largely align with previous research on PAH- and hydrogen peroxide-induced oxidative stress, reinforcing the notion that prolonged exposure exacerbates oxidative damage and depletes antioxidant reserves. However, some variations exist in terms of the extent of oxidative stress and the efficacy of antioxidant interventions. The observed progressive depletion of antioxidant enzymes and increased lipid peroxidation with prolonged exposure underscore the need for further

research into multi-antioxidant therapies. Future studies should explore the molecular pathways underlying oxidative stress responses to better inform intervention strategies against environmental toxicant-induced oxidative damage.

CONCLUSION

This study provides compelling evidence that oxidative stress plays a central role in the toxicity of PAHs and hydrogen peroxide, with prolonged exposure leading to progressive oxidative damage. The findings reveal that PAHs induce oxidative stress by generating reactive oxygen species (ROS), depleting antioxidant reserves, and increasing lipid peroxidation, while hydrogen peroxide exacerbates oxidative damage through direct ROS-mediated cellular injury. The cumulative effects of these toxicants were more pronounced in long-term exposure (Batch 2), emphasizing the importance of duration in oxidative stress progression.

Although vitamin C exhibited protective effects against oxidative damage, its efficacy diminished over extended exposure, highlighting the limitations of single antioxidant interventions in combating chronic oxidative stress. The study underscores the need for a more comprehensive antioxidant strategy, potentially involving synergistic combinations of antioxidants, to effectively counteract prolonged oxidative damage.

Conclusively, these findings reinforce the critical role of oxidative stress in environmental toxicant-induced cellular dysfunction and suggest that prolonged exposure to PAHs and oxidative stressors can lead to irreversible cellular damage. Future research should explore targeted antioxidant therapies and molecular mechanisms to develop effective strategies for mitigating oxidative damage in environmentally exposed populations.

Ethical Consideration

All animal trial techniques were directed as per local and worldwide controls and a similar law

regulated by the Bureau of Animal Experiment Licensing, University of Port Harcourt, Rivers State, Nigeria. The ethical approval identification code of this study is UPH/CEREMAD/REC/MM105/094.

Conflict of Interest

The authors have no conflict of interest to declare in connection with this article.

CRediT authorship contribution statement

Amadi, C. S^{1*} : Data acquisition, bench work, data analysis and drafting of manuscript; **Anacletus, F. C¹**: Conceptualization, Supervision, Writing - review & editing, Validation; **Ohiri, R. C¹**: Supervision of student, Writing - review & editing

Funding: None

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