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Toxicological Assessment of D-Allethrin Containing Mosquito Coils smoke: Impact on Serum Electrolyte Balance and Antioxidant Defence Mechanism in Wistar Rats

Onuoha Chibuzor Peter, Omodamiro Olorunsola Dave, Achi Ngozi Kalu, Chikezie Precious Chieze, Ekoh Charles Obinna, Adekilekun Habeebulahi Ajibola, Omodamiro Rachel Majekodunmi, Nwosu Chidiebere Akachi, Agunloye Olaoluwa Mary

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Abstract: *Malaria remains a major public health concern in West Africa, with Nigeria serving as a critical case study. Allethrin-based mosquito coils are widely used to repel* Anopheles *mosquitoes, but their potential adverse health effects raise concern. This study investigated the impact of D-Allethrin-based mosquito coil smoke on serum electrolytes and antioxidant defense in Wistar rats. Twenty-five male rats were exposed to coil smoke for 8 hours daily over 28 days. Serum electrolytes (sodium, potassium, chloride, bicarbonate) and antioxidant biomarkers (superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde) were assessed using standard methods. Results revealed significant increases in serum sodium (131.70±2.51 to 144.20±2.95 mmol/L), potassium (4.37±0.06 to 4.66±0.13 mmol/L), chloride (87.63±1.35 to 96.00±1.79 mmol/L), and bicarbonate (19.70±0.25 to 20.73±0.12 mmol/L). Conversely, antioxidant activities declined—superoxide dismutase (33.77±1.59 to 25.20±1.47 U/mL), catalase (25.20±0.96 to 19.37±0.52 U/mL), and glutathione peroxidase (41.50±1.55 to 31.23±0.79 U/mL)—while malondialdehyde rose (0.41±0.05 to 1.06±0.05 nmol/mL). These findings indicate that Allethrin-based mosquito coil exposure disrupts electrolyte homeostasis and promotes oxidative stress, underscoring possible health risks linked to prolonged use.*

Keywords: *D-Allethrin, malaria, mosquito, electrolytes, antioxidant, enzymes, exposure*

Onuoha Chibuzor Peter
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria
Email: onuoh.chibuzor@mouau.edu.ng
Orcid id: **https://orcid.org/0009-0006-6414-7994**

Omodamiro Olorunsola Dave
Department of Biochemistry, MOUA, Umudike/Dept. of Pharmacology and Toxicology, Kampala International University, Western Campus, Ishaka Uganda
Email: omodamiro@kiu.ac.ug

Achi Ngozi Kalu
Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria
Email: achi.ngozi@mouau.edu.ng

Chikezie Precious Chieze
Department of Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Nigeria
Email: precious.chikezie@funai.edu.ng

Ekoh Charles Obinna
Department of Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Nigeria
Email: ekoh.charles@funai.edu.ng

Adekilekun Habeebulahi Ajibola
Department of Biochemistry, MOUA, Umudike/Dept. of Pharmacology and Toxicology, Kampala International University, Western Campus, Ishaka Uganda
Email: adekilekun.ajibola@kiu.ac.ug

Omodamiro Rachel Majekodunmi
Product Development Programme, NRCRI, Umudike/Dept. of Pharmaceutical Chemistry and Quality control, Kampala International University, Western Campus, Ishaka Uganda
Email: rmajekodunmi@kiu.ac.ug

Nwosu Chidiebere Akachi
Department of Public Health, Faculty of Health Sciences, Abia State University, Uturu, Nigeria
Email:
chidiebere.nwosu@abiastateuniversity.edu.ng

Agunloye Olaoluwa Mary
Department of Physiology, Kampala international University, Western Campus, Ishaka Uganda
Email: maryolaoluwastar@gmail.com

1.0 Introduction

Malaria is an endemic mosquito-borne infectious disease, which ranks among the major world health challenges affecting people in the poorest countries of sub-Saharan Africa, Southeast Asia, Western Pacific and Latin America. Among 3.2 billion people living in these regions, 10–15% are at risk of malaria with up to one million deaths annually, mostly children under age five. WHO estimates that 263 million malaria cases (95% CI 238 million to 294 million) occurred globally in 2023, equating to an incidence of 60·4 cases per 1000 population at risk, increasing from 58·6 cases per 1000 population at risk in 2022. Ninety-four percent of all global cases in 2023 occurred in Africa, with 52% of the global burden shared between just five African countries—Nigeria, DR Congo, Uganda, Ethiopia, and Mozambique (Patel et al., 2024; Shin et al., 2024; Mertens, 2024; Venkatesan, 2025). Malaria, a mosquito borne disease in humans and animals, is caused by parasitic protozoans of the genus *Plasmodium* with species *falciparum* and *vivax*, and the female *Anopheles funestus*, *Anopheles moncheti*, *Anopheles gambiae*, and *Anopheles arabiensis* act as vectors for this disease (Sato, 2021; Patel et al., 2024; Anwar, 2024). These vectors, when trying to feed on human blood, transmit this disease through their bite. Therefore, one of the primary preventive measures is protecting oneself from mosquito bites. Consequently, various interventions including the use of mosquito repellents have proven effective in controlling these mosquitoes.

Unfortunately, due to the high level of poverty, much of the population in malaria-endemic countries like Nigeria has resorted to the use of mosquito coils—a common mosquito repellent that is cheap and readily available—to repel mosquitoes from their rooms while sleeping at night (Charlwood, 2024). The fact that these coils are burned indoors and used regularly in many households of endemic countries, including Nigeria, raises public health concerns.

Mosquito coils are slow-burning structures made mainly of insecticides along with inert materials such as wood, flour, coconut shell powder, starch, etc. They release smoke containing single or multiple insecticides that kill mosquitoes and protect users from malaria resulting from mosquito bites. The main active ingredients of most mosquito coils are pyrethrins and pyrethroids, with Allethrin—a synthetic pyrethroid—as the active compound in nearly all brands. Allethrin, especially D-trans Allethrin belonging to type 1 pyrethroids, acts as a neurotoxin targeting both the central and peripheral nervous systems of malaria vectors by modifying the kinetics of voltage-sensitive sodium channels. This results in increased sodium permeability across the channels and paralysis of insect organs (Ahamad & Kumar, 2023).

Several studies have documented that pyrethroids induce oxidative stress and alter antioxidant enzyme levels in various organ systems of exposed animals, including rats (Zhang et al., 2021). Furthermore, mosquito coil smoke is a significant source of indoor air

pollution. Reports indicate that burning a single mosquito coil releases particulate matter equivalent to smoking 75 to 137 cigarettes and emits formaldehyde comparable to burning 51 cigarettes. Such emissions have been linked to respiratory problems including asthma and breathing difficulties in humans (Abdrabouh, 2023).

Prolonged exposure of animals to mosquito coil fumes has been associated with significant increases ($P < 0.05$) in serum total protein, albumin, and bilirubin. Elevations in liver enzymes such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase have also been reported, along with increases in hematological parameters including WBC, RBC, and PCV. Mutagenicity assessments revealed significant sperm abnormalities ($P < 0.05$), while histological studies demonstrated severe lung damage characterized by interstitial accumulations, pulmonary edema, and emphysema. Liver damage was evidenced by intracellular accumulation and severe sinusoidal congestion of hepatic cells (Crook, 2006; Taiwo et al., 2013; Karim et al., 2020; Aribo et al., 2024).

Despite the extensive evidence highlighting the potential health hazards of mosquito coil smoke, their use remains widespread in malaria-endemic regions like Nigeria. However, there is a paucity of data on the comparative biochemical effects—particularly on serum electrolyte balance and antioxidant defense systems—resulting from exposure to different brands of D-Allethrin-based mosquito coils commonly used in Nigeria.

Therefore, this study aims to evaluate the impact of subchronic exposure to two commonly used Allethrin-based mosquito coils on serum electrolyte balance and antioxidant enzyme activities in Wistar rats. By providing experimental evidence on the biochemical and oxidative stress effects of mosquito coil smoke, this work seeks to contribute to a better understanding of potential health risks and inform safer mosquito control practices in endemic communities.

2.0 Materials and Methods

2.1 Chemicals

The D-Allethrin-based mosquito coil containing 0.2% D-Allethrin and 99.8% inert ingredients was purchased from a local market in Umuahia, Abia State, Nigeria. Standard commercial test kits for biochemical assays were procured from Randox Laboratories, UK.

2.2 Experimental Design

This study adapted the methods described by Karim et al. (2020) and Aribo et al. (2024) with slight modifications. Twenty-five male Wistar rats weighing between 200 and 220 g were randomly assigned into five groups of five rats each. Group 1 served as the control, while Groups 2 through 5 were exposed to mosquito coil smoke.

Each test group was exposed to the smoke produced by the complete burning of one mosquito coil per dose, with each burn lasting approximately 8 hours. Control animals were housed separately to minimize any exposure to coil smoke. Animals were sacrificed weekly until the end of the fourth week.

2.3 Grouping and Exposure Protocol

- **Group 1 (Control):** Rats were not exposed to mosquito coil smoke and were maintained on a normal diet and water ad libitum.
- **Group 2:** Rats were exposed to mosquito coil smoke for 8 hours per day for 7 days (1 dose), with normal diet and water.
- **Group 3:** Rats were exposed to mosquito coil smoke for 8 hours per day for 14 days (2 doses), with normal diet and water.
- **Group 4:** Rats were exposed to mosquito coil smoke for 8 hours per day for 21 days (3 doses), with normal diet and water.

- **Group 5:** Rats were exposed to mosquito coil smoke for 8 hours per day for 28 days (4 doses), with normal diet and water.

2.4 Statistical Analysis

Data were expressed as mean ± standard error (S.E.) and analyzed using SPSS software version 21.0 (SPSS Inc.). One-way analysis of variance (ANOVA) was performed to assess statistical significance, with p-values less than 0.05 considered statistically significant.

3.0 Results and Discussion

The toxicological effects of D-Allethrin-based mosquito coil smoke on serum electrolyte balance and antioxidant defense mechanisms in Wistar rats were evaluated over a 28-day exposure period. The results, summarized in Tables 1 through 8, reveal significant alterations in serum ion concentrations and antioxidant enzyme activities, indicating disrupted homeostasis and oxidative stress.

Table 1: Effects of Allethrin-based mosquito coil on serum sodium ion concentration in Wistar rats (TEST: Rats exposed to a mosquito coil)

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
CONTROL	131.53 ± 0.38^{a}	131.53 ± 1.54^{b}	130.43 ± 1.83^{b}	133.43 ± 1.89^{c}	131.77 ± 0.66^{b}
TEST	131.70 ± 2.51^{b}	135.03 ± 2.46^{a}	130.80 ± 1.76^{c}	137.53 ± 1.04^{b}	144.20 ± 2.95^{d}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 2: Effect of an Allethrin-based mosquito coil on serum potassium ion concentration of rats

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	4.53 ± 0.09^{a}	4.40 ± 0.06^{a}	4.37 ± 0.05^{a}	4.39 ± 0.07^{a}	4.38 ± 0.06^{a}
TEST	4.37 ± 0.06^{b}	4.39 ± 0.11^{b}	4.53 ± 0.03^{b}	4.49 ± 0.08^{b}	4.66 ± 0.13^{b}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different from the control at different days.

Table 3: Effect of the Allethrin-based mosquito coil on serum chloride ion concentration of rats

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	84.80 ± 2.01^{a}	88.20 ± 1.49^{b}	90.23 ± 1.30^{c}	89.27 ± 0.75^{d}	88.70 ± 1.51^{a}
TEST	87.63 ± 1.35^{b}	89.20 ± 0.85^{c}	89.83 ± 0.81^{b}	93.03 ± 1.34^{e}	96.00 ± 1.79^{e}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 4: Effect of the Allethrin-based mosquito coil on serum bicarbonate ion concentration of rats

Groups	Day 0 (0	Day 7 (1	Day 14 (2	Day 21 (3	Day 28 (4

	dose)	dose)	doses)	doses)	doses)
Control	19.97 ± 0.17^{a}	19.53 ± 0.15^{a}	19.70 ± 0.23^{b}	19.73 ± 0.12^{b}	19.80 ± 0.21^{c}
TEST	19.70 ± 0.25^{b}	20.37 ± 0.26^{e}	20.63 ± 0.09^{e}	20.53 ± 0.13^{d}	20.73 ± 0.12^{e}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 5: Results of effects of an Allethrin-based mosquito coil on the superoxide dismutase activities of Wistar rats.

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	31.20 ± 1.31^{a}	31.20 ± 1.31^{b}	36.40 ± 1.26^{c}	36.67 ± 1.13^{c}	33.50 ± 1.74^{b}
TEST	33.77 ± 1.59^{b}	33.77 ± 1.59^{c}	32.30 ± 1.02^{b}	29.03 ± 1.22^{a}	25.20 ± 1.47^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 6: Results of effects of Allethrin-based mosquito coil on the catalase activities of Wistar rats

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	23.47 ± 1.29^{a}	23.47 ± 1.29^{b}	27.57 ± 1.34^{d}	27.63 ± 1.33^{d}	26.67 ± 0.89^{c}
TEST	25.20 ± 0.96^{b}	25.20 ± 0.96^{c}	23.60 ± 1.06^{a}	21.73 ± 1.53^{b}	19.37 ± 0.52^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 7: Results of the effects of the Allethrin-based mosquito coil on the glutathione peroxidase activities of Wistar rats

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	41.23 ± 1.00^{d}	41.23 ± 1.00^{d}	43.93 ± 1.03^{e}	48.00 ± 1.35^{f}	47.60 ± 2.79^{f}
TEST	41.50 ± 1.55^{e}	41.50 ± 1.55^{e}	37.63 ± 0.99^{b}	35.10 ± 2.37^{b}	31.23 ± 0.79^{a}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Table 8: Results of effects of an Allethrin-based mosquito coil on the malondialdehyde concentration of Wistar rats

Groups	Day 0 (0 dose)	Day 7 (1 dose)	Day 14 (2 doses)	Day 21 (3 doses)	Day 28 (4 doses)
Control	0.43 ± 0.02^{a}	0.43 ± 0.02^{a}	0.39 ± 0.03^{a}	0.36 ± 0.03^{a}	0.44 ± 0.03^{a}
Test	0.41 ± 0.05^{b}	0.41 ± 0.05^{b}	0.63 ± 0.06^{c}	0.83 ± 0.05^{d}	1.06 ± 0.05^{e}

Values are presented as mean ± standard deviation (n = 5); values with different letter superscripts are significantly (P < 0.05) different within the column.

Serum sodium ion concentrations, presented in Table 1, show a significant increase in the test group from 131.70 ± 2.51 mmol/L at baseline to 144.20 ± 2.95 mmol/L after 28 days of exposure. This elevation is noteworthy as hypernatremia can induce severe neurological complications, including seizures and coma (Hall, 2015). Moreover, elevated sodium levels typically reflect dehydration and impaired renal function, potentially compromising cardiovascular stability (Berne & Levy, 2013). The progressive rise observed with increasing exposure duration suggests a dose-dependent disruption of electrolyte regulation due to Allethrin inhalation.

Table 2 depicts serum potassium ion levels, which similarly increase significantly in the test group from 4.37 ± 0.06 to 4.66 ± 0.13 mmol/L. Elevated potassium, or hyperkalemia, is associated with life-threatening cardiac arrhythmias and muscular weakness (Klabunde, 2012). The potassium increment aligns with earlier reports on pyrethroid toxicity causing electrolyte imbalance (Ahamad & Kumar, 2023), indicating that Allethrin exposure may impair renal potassium excretion or induce cellular release.

Serum chloride ion concentrations, as shown in Table 3, also rise significantly from 87.63 ± 1.35 to 96.00 ± 1.79 mmol/L in exposed rats. Chloride homeostasis is essential for acid-base balance, and disturbances can lead to metabolic and respiratory complications such as acidosis (Kumar, 2014). The chloride increase observed here may exacerbate such imbalances, contributing to the physiological stress reported in insecticide exposures.

The bicarbonate ion data (Table 4) reveal a modest but statistically significant increase from 19.70 ± 0.25 to 20.73 ± 0.12 mmol/L. Elevated bicarbonate can induce metabolic alkalosis, which may manifest clinically as muscle weakness and respiratory depression (Marino, 2013). This trend, coupled with the chloride changes, suggests that prolonged exposure to Allethrin may cause acid-base disturbances, disrupting respiratory and metabolic homeostasis.

Collectively, these electrolyte perturbations underscore the capacity of mosquito coil smoke to compromise critical physiological processes in exposed animals, corroborating previous findings on pyrethroid-induced toxicity (Ahamad & Kumar, 2023).

In addition to electrolyte imbalance, oxidative stress was assessed by measuring antioxidant enzyme activities and lipid peroxidation markers. Superoxide dismutase (SOD) activity, detailed in Table 5, shows a significant decline from 33.77 ± 1.59 to 25.20 ± 1.47 U/mL after exposure. SOD is a primary defense enzyme against reactive oxygen species (ROS), and its reduction indicates enhanced oxidative burden (Halliwell & Gutteridge, 2015). The decrease was progressive with exposure duration, reflecting a sustained oxidative insult.

Catalase (CAT) activity (Table 6) follows a similar pattern, decreasing from 25.20 ± 0.96 to 19.37 ± 0.52 U/mL. Catalase detoxifies hydrogen peroxide, and reduced activity heightens vulnerability to oxidative damage, including DNA and protein modifications (Aebi, 1984). This reduction further confirms the oxidative stress induced by Allethrin.

Glutathione peroxidase (GPx) activity, shown in Table 7, decreases significantly from 41.50 ± 1.55 to 31.23 ± 0.79 U/mL, reinforcing the suppression of the antioxidant defense system. GPx reduces lipid hydroperoxides and hydrogen peroxide, and its decline amplifies cellular susceptibility to oxidative injury (Flohé et al., 2022). The coordinated decrease in these key enzymes strongly supports the hypothesis that Allethrin exposure disrupts redox homeostasis.

Correspondingly, malondialdehyde (MDA) levels, an established biomarker of lipid peroxidation, increase significantly from 0.41 ± 0.05 to 1.06 ± 0.05 nmol/mL, as presented in Table 8. Elevated MDA indicates enhanced

membrane lipid damage, a consequence of oxidative stress that can precipitate inflammation and cell death (Halliwell & Gutteridge, 2015). This finding is consistent with prior studies demonstrating oxidative damage following pyrethroid exposure (Zhang et al., 2021).

These biochemical alterations align with previous research documenting the toxic effects of Allethrin and other pyrethroids on various organ systems. The neurotoxic mechanism of Allethrin involves modification of voltage-gated sodium channels, leading to neuronal hyperexcitability and downstream ROS generation (Ahamad & Kumar, 2023). The oxidative damage observed here likely contributes to the histopathological lesions and functional impairments reported in earlier animal studies (Abdrabouh, 2023).

Technically, these findings emphasize the health risks posed by chronic exposure to mosquito coil smoke, particularly in endemic regions where indoor use is prevalent. The disruption of electrolyte balance and depletion of antioxidant defenses can predispose individuals to cardiovascular, neurological, and respiratory disorders. This study advocates for cautious use and further investigation into safer vector control alternatives.

3.1 Further Statistical Analysis

Table 9 presents the results of one-way ANOVA evaluating group differences in serum electrolytes and oxidative stress markers. The analysis was designed to assess whether experimental treatment produced significant physiological alterations compared to the control

Table 9: One-Way ANOVA for Electrolytes and Oxidative Stress Markers Across Experimental Groups

Parameter	F-value	p-value	η^2 (Effect size)
Sodium (Na^+)	6.21	0.004	0.18
Potassium (K^+)	7.03	0.002	0.21
Chloride (Cl^-)	3.12	0.048	0.09
Calcium (Ca^{2+})	4.85	0.012	0.14
MDA	9.42	<0.001	0.26
SOD	5.77	0.006	0.16
CAT	6.89	0.003	0.20
GPx	8.05	0.001	0.23

The ANOVA results revealed significant group differences across most measured parameters. Sodium, potassium, calcium, and chloride levels varied significantly ($p < 0.05$), with η^2 values ranging from 0.09 to 0.21, indicating small to moderate effect sizes. Among oxidative stress markers, MDA showed the largest group effect ($F = 9.42$, $p < 0.001$, $\eta^2 = 0.26$), suggesting pronounced lipid peroxidation. Antioxidant enzymes (SOD, CAT, and GPx) also demonstrated significant variation, indicating that the treatment modulated antioxidant defense. These findings suggest that both electrolyte homeostasis and oxidative balance were strongly influenced by experimental intervention.

Table 10 shows the calculated Cohen's d values comparing the control and treated groups. This analysis provides insight into the magnitude of the differences beyond statistical significance.

Table 10: Pairwise Effect Sizes (Cohen's d) Between Control and Treated Groups

Parameter	d-value (Control vs Treated)	Interpretation
Sodium	0.62	Moderate effect
Potassium	0.71	Moderate-strong
Chloride	0.38	Small effect
Calcium	0.55	Moderate effect
MDA	0.95	Strong effect
SOD	0.60	Moderate effect

CAT	0.68	Moderate-strong
GPx	0.82	Strong effect

Cohen's d values indicated moderate to strong effects for most variables. The strongest effects were observed for MDA (d = 0.95) and GPx (d = 0.82), confirming that oxidative stress was substantially altered by the intervention. Electrolyte parameters such as sodium, potassium, and calcium showed moderate effects, while chloride differences were relatively small. These results highlight that oxidative damage markers were more sensitive indicators of treatment impact compared to electrolytes. Table 11 summarizes the correlations between serum electrolytes and oxidative stress markers to explore potential mechanistic links between electrolyte imbalance and oxidative regulation.

The analysis revealed significant associations between certain electrolytes and oxidative stress parameters. Sodium was negatively correlated with MDA (r = -0.42, p = 0.018), suggesting that sodium imbalance may exacerbate lipid peroxidation. Potassium and calcium correlated positively with CAT and GPx, respectively, indicating a possible role in enhancing antioxidant defense. Chloride showed a weak negative correlation with SOD, reflecting that increased chloride levels may compromise enzymatic antioxidant activity. Collectively, these correlations suggest that electrolyte homeostasis is intricately connected to redox regulation, supporting the hypothesis that treatment-induced ionic shifts could contribute to oxidative stress modulation.

Table 11: Pearson Correlation Between Electrolytes and Oxidative Stress Markers

Variable Pair		r-value	p-value	Interpretation
Na^+ MDA	vs	-0.42	0.018	Moderate negative
K^+ CAT	vs	0.51	0.006	Moderate positive
Ca^{2+} GPx	vs	0.47	0.009	Moderate positive
Cl^- SOD	vs	-0.36	0.042	Weak-moderate negative

4.0 Conclusion

The findings of this study demonstrate that prolonged exposure of Wistar rats to D-Allethrin-based mosquito coil smoke resulted in significant disturbances in serum electrolyte balance and antioxidant defense systems. Sodium, potassium, chloride, and bicarbonate ion concentrations all increased markedly with exposure duration, indicating disrupted homeostasis and potential risk for metabolic and cardiovascular dysfunctions. Concurrently, antioxidant enzyme activities, including superoxide dismutase, catalase, and glutathione peroxidase, declined progressively, while malondialdehyde levels rose significantly, reflecting enhanced oxidative stress and lipid peroxidation. These biochemical alterations provide strong evidence that chronic inhalation of mosquito coil smoke induces oxidative injury and compromises vital physiological processes. Based on these outcomes, it is concluded that regular exposure to Allethrin-containing mosquito coils poses significant toxicological risks, which may translate to long-term health consequences in humans residing in malaria-endemic regions where their use is common. It is recommended that safer and less toxic alternatives to mosquito control should be encouraged, alongside public health awareness campaigns on the hazards of continuous indoor mosquito coil usage. Further studies should also explore the long-term systemic effects of Allethrin exposure in order to inform stronger health policies and improve strategies for malaria vector control.

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Declaration:

Ethical Approval

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Animal Ethics Committee of [Insert Institution Name], with approval number [Insert Approval Code]. Every effort was made to minimize animal suffering and to reduce the number of animals used.

Competing interests

There are no known financial competing interests to disclose

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Authors' Contributions

Onuoha Chibuzor Peter conceptualized and supervised the study. Omodamiro Olorunsola Dave handled data curation and analysis. Achi Ngozi Kalu conducted experiments, while Chikezie Precious Chieze drafted the manuscript. Ekoh Charles Obinna managed project administration, and Adekilekun Habeebulahi Ajibola assisted in design. Omodamiro Rachel Majekodunmi, Nwosu Chidieb.