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Original Research Article

Toxicological Assessment of Pesticide Exposure in Wistar Rats: An Acute and Sub-Acute Inhalation Study

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Abstract

Background: Pesticide ubiquity and extensive applications have attendant public health implications. This study examines the immediate and prolonged inhalation toxicity of various commonly used insecticides in male Wistar rats. ***Methods***: 192 male Wistar rats were utilized and kept in regular housing. The study was carried out in three stages: a preliminary test, an acute test, and a sub-acute. Wistar rats, housed in an improvised chamber, were exposed to varying doses of insecticides in each phase to assess their toxicities to insecticides. For comparativeness, we withdrew 57 of the rats from exposure to ascertain possible recovery. The study included monitoring behavioral changes, recording mortality rates, and conducting necropsies to evaluate organ pathology. Dose-response relationships were analyzed to determine lethal doses (LD_{50}) and potential sublethal effects. ***Results***: Overall, we found a dose-related increase in the severity of the response, which potentially indicates an alteration of a specific biochemical process. The range-finding test assessed the effects of DD Force, Sniper, Industrial Camphor, Edible Camphor, Kerosene, and combined pesticides. It revealed significant physiological impacts followed by mortalities at specific dosage levels. Subsequent acute toxicity testing determined LC_{50} values for each insecticide. Rats showed a dose-dependent health deterioration following sub-acute exposure, particularly in the combined pesticide group, indicating synergistic toxicity. ***Conclusion***: Exposure to the combined pesticide at high doses was associated with severe toxicities in the tested rats. This evidence suggests that commonly used pesticides could potentiate adverse health outcomes when results are extrapolated in humans.

Keywords: Pesticides, Acute Toxicity, Sub-Acute Toxicity, Lethality, Dose Response Relationship, Mortality Rate, Environmental Toxicity.

INTRODUCTION

Exposure to pesticides presents substantial health hazards to both humans and animals, resulting in immediate and long-term harmful consequences (Damalas and Eleftherohorinos, 2011). Pesticides are widely employed in agriculture, domestic products, and industrial applications, leading to considerable environmental pollution. Comprehending the toxicological consequences of these substances is essential for safeguarding public health, especially in areas with extensive pesticide utilization (Pathak *et al*., 2022). This study examines the toxicological impacts of typical pesticide components on male Wistar rats, offering valuable information about their possible dangers and promoting safer techniques for handling and applying pesticides (Ahmad *et al*., 2024).

Animal models, such as Wistar rats, are extremely important in toxicological research because they share similar physiological and genetic characteristics with humans (Domínguez-Oliva *et al*., 2023). In this study, we utilized a comprehensive methodology, which involved doing range-finding tests, assessing acute toxicity, and conducting sub-acute exposure experiments**.** These approaches facilitated a thorough assessment of the lethal concentrations, dose-response relationships, and sub-chronic effects of pesticides. This provided a strong framework for comprehending the possible hazards linked to pesticide exposure (Ajayi and Akhigbe, 2020). Insecticide toxicity in exposed rats manifests as tremors, lethargy, loss of coordination, hyperactivity, labored breathing, excessive salivation, reduced appetite, seizures, ataxia, diarrhea, pupil dilation, and, in severe cases, coma. The inhalation route was selected for this investigation to replicate real-life situations in which humans may breathe in pesticide aerosols or vapors. A single oral dose of the combination of cypermethrin and endosulfan was dissolved in dimethyl sulfoxide (DMSO) in a ratio of 1:1 and administered orally at the concentration of 165 mg/kg body weight (b.w), 330 mg/kg b.w, 660 mg/kg b.w, and 1320 mg/kg b.w to experimental animals. LD_{50} was calculated according to the method described by Miller and Tainter (1994) and was observed as 691.83 mg/kg b.w for this combination. A single dose of test article at 165 mg/kg b.w did not reveal any toxic signs or behavioral alterations, hence considered as no observed adverse effect level.

The widespread use and extensive application of pesticides have significant public health implications. Previous research has predominantly focused on oral and dermal exposure, leaving a paucity of data on the inhalation route—a common exposure pathway for humans. We hypothesize that Inhalation exposure to commonly used pesticides induces significant toxicological effects in male Wistar rats, manifested through physiological, biochemical, and behavioral changes. This study addresses a critical research gap by evaluating the toxicological effects of acute and sub-acute inhalation exposure to widely used pesticides in male Wistar rats.

METHODOLOGY

Animal Care and Use

A total of 192 male Wistar rats, aged 8-12 weeks and weighing 150-200g, were acquired from the Animal Holding Unit at the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria. The study followed the standards of the Declaration of Helsinki for animal research and received approval from the Institute of Public Health, OAU, along with an ethical clearance certificate. The rats were kept in a controlled environment with a 12-hour light/dark cycle, at room temperature and humidity. They had unrestricted access to conventional rat pellets and water. They underwent a two-week acclimatization period before the commencement of the trial. An inhalation chamber specifically developed for the purpose allowed for controlled exposure to the various pesticide constituents (Anderson *et al*., 2020; Cao *et al*., 2015).

Toxicity Tests

Experimental Design

The research was carried out in three stages: a preliminary test to determine the range, a test to assess immediate effects, and a test to evaluate prolonged effects, with the main emphasis on the latter. Male Wistar rats were subjected to different pesticide components in a handmade wooden breathing chamber that had inadequate ventilation. The chamber, made of wood with a clear glass lid, facilitated the application of insecticides during the exposure process. The container consisted of seven partitions, with each partition accommodating rats that were subjected to distinct substances: Sniper, DD Force, industrial camphor, edible camphor, kerosene, and mixed pesticides. The seventh compartment functioned as the control group, consisting of rats that were not subjected to pesticide exposure.

1. Range-Finding Test

This stage involved determining the precise dosage of each pesticide for the conclusive experiment. The objective was to determine the lethal dose of the commercial formulation that would result in 100% mortality in rats that were exposed to it. A total of twenty-one male Wistar rats were separated into seven groups, with each group consisting of three rats. These rats were then subjected to individual and combination pesticides for 24 hours. The pesticides were combined to promote synergistic interactions.

Table 1: Pesticide Constituents Formulation for Range Finding Test

Pesticides	Quality available for formulation	Exact quantity tested (1/4th)
DD force	1000ml	250ml
Sniper	200ml	50ml
Industrial Camphor	250g	0.63g
Edible Camphor	250g	63g
Kerosene	3000ml	750ml
Combined	4000ml	700ml

The range-finding test involved testing DD Force, Sniper, Industrial Camphor, Edible Camphor, Kerosene, and mixed pesticides at certain quantities: 250ml, 50ml, 0.63g, 63g, 750ml, and 700ml, respectively, based on their accessible amounts.

2. Acute Toxicity Test

After conducting the range-finding test, we computed the 96-hour median lethal concentration (LC_{50}) using the Spearman-Karber method. This test yielded the requisite concentrations for the following tests.

Groups and Doses: Rats were divided into groups (A, B, C), each exposed to different doses of the pesticides. Group A served as the control with no pesticide exposure. The doses varied for each pesticide, tested at three different concentrations.

Table 2: Acute Toxicity Wistar Rats Exposed to Pesticides

Pesticide Constituents	Groups	No of Animals	Dose of stock concentration
Natural air	A	3	Nil
Sniper	A B C	3 3 3	50 100 200
DD force	A B C	3 3 3	62.5 ml 125 ml 250ml
Industrial camphor	A B C	3 3 3	63 g 126 g 252 g
Edible camphor	A B C	3 3 3	63 g 126 g 252 g
Kerosene	A B C	3 3 3	87.5 ml 175ml 350 ml
Combined pesticide **[**sniper (50mls), DD force (250mls), Industrial camphor (63g), Edible camphor (63g) and Kerosene (750mls)**]**	A B C	3 3 3	87.5 ml 175 ml 350 ml

3. Sub-Acute Test

The acute toxicity data was utilized in this phase to establish the concentrations for the sub-acute investigation. A total of 114 male Wistar rats were categorized into seven groups (A-G) according to their LC_{50} values. The pesticide mixture ratios were determined based on the results of the range-finding test. Rats were subjected to pesticide exposure in an inadequately ventilated inhalation chamber for four hours per day, with three-day intervals, over four weeks.

Exposure Groups: Group A (control) was exposed to ambient air, whereas Groups B-G were subjected to specific substances: DD Force, Sniper, industrial camphor, edible camphor, kerosene, and a mixed pesticide mixture, respectively.

A study on acute toxicity was conducted to measure the concentration of various components of the pesticides given to Wistar rats for a sub-acute toxicity study. A total of 114 adult male rats were categorized into seven groups (A-G) according to the concentration derived from the 96-hour median lethal concentration (LC_{50}). The sub-acute pesticide mixture ratio was calculated using Spearman-Kabal's arithmetic based on the results of the range-finding test.

The equation is given by:

$$LD_{50} = \frac{LD_{100} - \Sigma(A \, X \, B)}{N}$$

LD50…………………………Highest Concentration
N………………………………. No of Animals
A……………………………… Dose Difference
B……………………………… Mean Mortality
Σ……………………………… Summation

Table 3: Sub-acute Pesticides Formulation Ratio obtained from Range Finding Test (Spearman Kabal's Arithmetic Method)

Group	Dose	Dose difference (A)	Dead Animals	Mean Mortality (B)	Product ∑ A X B	No of Animals
Control	0	0	0	0	0	0
Sniper	50	0	0	0	0	3
	100	50	1	0.5	25	
	200	100	3	2	200	
DD Force	62.5	0	0	0	0	3
	125	62.5	0	0	0	
	250	125	3	1.5	187.5	
Industrial Camphor	63	0	0	0	0	3
	126	63	0	0	0	
	252	126	2	1	126	
Edible Camphor	63	0	0	0	0	3
	126	63	0	0	0	
	252	126	2	1	126	
Combined Pesticides	87.5	0	0	0	0	3
	175	87.5	3	1.5	131.25	
	350	175	3	3	525	

Combined Pesticide Preparation

Pesticides were purchased from a market in Ile-Ife, Osun State. Seven hundred and fifty milliliters of kerosene were mixed with two hundred and fifty milliliters of DD Force, fifty milliliters of Sniper, sixty-three grams of ground industrial camphor, and sixty-three grams of ground edible camphor. The mixture was thoroughly homogenized and allowed to dissolve completely before use.

Recovery Groups

Of the 114 Wistar rats exposed to the pesticides, 57 were withdrawn for two weeks for recovery. These rats were fed with rat pellets and water ad libitum. They were later sacrificed, and the effects of the pesticides on their reproductive profiles were assessed in comparison with the treated groups.

DISCUSSION

Two fundamental principles underlie all descriptive animal toxicity studies. First is that the effects observed in laboratory animals exposed to a substance can be relevant to humans when appropriately interpreted, and secondly, exposing experimental animals to high doses of toxic substances is crucial for identifying potential hazards in humans, as the likelihood of observing effects increases with higher doses or exposures (Hayes., 2016; Moudgal *et al*., 2020; Slob *et al*., 2020). The three-phase methodology, consisting of range-finding, acute, and sub-acute toxicity studies, facilitated a thorough assessment of the fatal concentrations, dose-response relationships, and enduring impacts of these compounds.

The range-finding test determined the fatal dosages of each pesticide by identifying the levels that resulted in 100% mortality in rats that were exposed to them. The findings revealed that Sniper, DD Force, industrial camphor, consumable camphor, and combination pesticides displayed significant toxicity at certain concentrations as similarly reported in previous research (Islam *et al*., 2021). Regardless of the dosage, the range-finding test revealed that kerosene had no notable impact. These data highlight the variation in toxicity levels among different pesticides, emphasizing the significance of determining precise dose limits for each chemical.

We utilized Spearman-Karber's approach to determine the 96-hour median lethal concentration (LC_{50}) values in the acute toxicity test. The research indicated that pesticides such as Sniper, DD Force, industrial camphor, and consumable camphor had unambiguous dose-dependent effects, whereby larger doses resulted in elevated fatality rates. As an example, the lethal concentration (LC_{50}) for Sniper was determined to be 200 ml, resulting in the death of all three rats in the group. These findings validate the immediate harmful effects of these pesticides and emphasize the urgent requirement for strict regulations to prevent potentially fatal high-dose exposures (Islam *et al*., 2021).

The sub-acute toxicity test, which exposed rats to pesticides over four weeks, provided insights into the long-term effects of inhalation exposure. The findings revealed substantial and proportional negative impacts on the well-being of the rats, specifically in the cohort exposed to a combination of pesticides. The mortality rates were higher and the health concerns were more evident in this group, indicating a synergistic effect when multiple pesticides are combined. This discovery is of utmost importance as it accurately reflects real-life situations when people may come into contact with combinations of many pesticides, which could result in more severe health consequences compared to exposure to just one pesticide (Anderson *et al*., 2020).

Worthy of note is the mortalities observed in the range-finding test indicate respiratory failure linked to inhibition of brainstem respiratory centers, bronchoconstriction, increased bronchial secretion, and flaccid paralysis of respiratory muscles. Researchers commonly explore these effects by conducting thorough observations, histopathological examinations, and occasionally neurophysiological assessments. These methods are crucial for unraveling the mechanism of action and assessing the potential implications for human health (Klaassen et al 2019). The observed signs of recovery in the rats suggest detoxification of pesticides after a 30-day withdrawal period. They also indicate reduced toxicity effects on the rats' reproductive profiles. However, exposure to Sniper, kerosene, and combined pesticides at certain levels (25%, 50%, and 75%) showed significant decreases, highlighting potential harm to male reproductive hormones. Pesticides with no significant effects pose less threat to reproductive profiles during recovery.

The investigation revealed many health concerns associated with the acute and sub-acute toxic effects. Even when present in lesser amounts, the act of breathing in pesticides can result in notable negative impacts on health, such as respiratory problems, harm to the nervous system, and higher rates of death (Sapbamrer *et al*., 2024). The observed synergistic toxicity resulting from combined pesticide exposure underscores the potential hazards of cumulative exposure to several pesticides (García-García *et al*., 2016; Hassaan and El Nemr, 2020). This is especially alarming for agricultural laborers, persons living close to fields that have been treated with pesticides, and those who use household pesticides without sufficient ventilation or safety precautions (Damalas and Eleftherohorinos, 2011).

The available data on reproductive health, while not extensively covered in this discussion, indicate that prolonged exposure to these pesticides may have harmful impacts on reproductive function (Fucic *et al*., 2021; Pathak *et al*., 2022). This necessitates the need for additional research. The results necessitate enhanced safety protocols, such as the implementation of personal protective equipment (PPE), adequate ventilation during pesticide handling, and strict adherence to recommended application dosages to mitigate health hazards.

In addition to human health, the environmental consequences of pesticide toxicity are substantial. The study's findings reveal significant mortality rates, indicating the potential for substantial ecological consequences, especially for species not intended as targets. Excessive use of pesticides such as Sniper and DD Force can result in the contamination of air, water, and soil, which in turn can cause more extensive ecological disturbances. This can affect biodiversity, disrupt food chains, and result in long-term environmental degradation (Ahmad *et al*., 2024; Bernardes *et al*., 2015; Kim *et al*., 2017; Mahmood *et al*., 2016).

The study's findings support the implementation of more stringent environmental restrictions and the use of sustainable pest management strategies. Integrated Pest Management (IPM) strategies, which emphasize the use of biological controls and reduced chemical applications, could mitigate the environmental impact while effectively managing pest populations.

CONCLUSION

In conclusion, this study emphasizes the pronounced inhalation toxicity of commonly used insecticides in male Wistar rats. The findings indicate dose-dependent health effects and potential synergistic toxicity, particularly with combined pesticide exposure. The knowledge and evidence from this study will inform policy and enhance practices that aid the understanding of adverse health outcomes and foster proper use of insecticides especially in homes.

RECOMMENDATIONS

The recommendations stemming from this study include conducting further research on the effects of inhalation exposure to commonly used pesticides in Nigeria on pregnancy and its outcomes. Public education on pesticide usage and handling should emphasize their adverse effects on reproductive health. Encouraging minimal pesticide exposure and promoting the use of safety measures to mitigate inhalation risks is crucial. Public awareness campaigns on the reproductive health implications of pesticides and their environmental impact should emphasize information and education as cost-effective tools. Governments should establish rigorous enforcement and compliance mechanisms for pesticide regulations. Supporting monitoring programs to gather data for policymaking and consumer protection laws is essential. Coordinated efforts among academic, private, non-governmental, and international organizations are needed to address environmental concerns collaboratively. Promotion of adequate ventilation during pesticide application in homes, offices, and public spaces to minimize inhalation risks is recommended. Further studies are necessary to elucidate the effects of current environmental, household, or occupational pesticide exposures on male reproductive health and their underlying physiological mechanisms.

Credit: AOA conceptualized the study, carried out the experiment and wrote the first draft of manuscript. TOA and OSA designed and supervised the research. OPA carried out the experiment, and contributed to manuscript writing.

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