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Original Research Article

Assessment of Dichlorvos, Cypermethrin, Synthetic Camphor, Pinenes, and Kerosene: Impact on Testosterone Levels, Glutathione Concentration, and Testicular Histology in Male Wistar Rats

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Abstract: Pesticides are integral to modern agriculture and public health but pose significant risks to human and environmental health. This study investigates the impact of selected pesticides on male reproductive health via inhalation in an improvised chamber. Male Wistar rats were assigned to groups and exposed to individual pesticides and a combined mixture to determine lethal doses. Tissue samples from 54 rats were analyzed histologically and biochemically for effects on testes and epididymides, with measured serum testosterone and GSH levels. Sniper consistently showed the highest significant decrease in GSH levels across all exposure levels ($p < 0.05$). A significant dose-dependent relationship was observed in the recovery groups from 25% to 75% exposure ($p < 0.05$). Pesticide exposure led to a substantial decrease in serum testosterone levels in male Wistar rats at 25% (all pesticides, $p < 0.05$), 50% (DD Force, Sniper, Kerosene, Combined, $p < 0.05$), and 75% (DD Force, Industrial Camphor, Edible Camphor, Kerosene, $p < 0.01$) concentrations. Escalating damage in testicular and epididymal structures, with a more pronounced severity at higher exposure levels, including epithelial degeneration, Sertoli cell vacuolation, seminiferous tubule derangement, and ductal atrophy. Increased exposure to high doses of pesticides induces oxidative stress in male Wistar rats which could potentiate decreased testosterone levels and adversely impact the histological architecture of the testes and epididymides. Pesticide detoxifications are somewhat evident following withdrawal from the environmental insults. Significant and persistent damage occurred at higher levels, suggestive of substantial reproductive toxicity following exposure.

Keywords: Pesticides, Testosterone, Glutathione, Testicular histology, Reproductive toxicity, Oxidative stress, Wistar rats.

INTRODUCTION

Pesticides are integral to modern agriculture for crop protection and yield enhancement, yet their ubiquitous use raises concerns about potential health and environmental impacts. Male Wistar rats are widely utilized in toxicological studies due to their physiological similarity to humans, making them ideal models for assessing the reproductive effects of pesticide exposure (Sultana *et al.,* 2020). This study focuses on Dichlorvos (DD Force), Cypermethrin (Snipper), Synthetic Camphor, Pinenes (Edible Camphor), and Kerosene, commonly formulated and extensively used in developing countries for their cost-effectiveness and efficiency (Jallow *et al.,* 2022). Dichlorvos, an organophosphate insecticide, acts as an acetylcholinesterase inhibitor, disrupting insect neurotransmission and potentially affecting non-target organisms (Sultana *et al.,* 2020). Cypermethrin, a synthetic pyrethroid, exerts neurotoxic effects by prolonging sodium channel activation, impacting neuronal function and insect mortality (Gupta *et al.,* 2021). Synthetic Camphor and Pinenes are aromatic compounds used in pesticide formulations for their repellent and preservative properties, while Kerosene serves as a solvent and carrier in pesticide mixtures (Jallow *et al.,* 2022). The formulations of pesticides used in developing countries often comprise a mixture of

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synthetic chemicals and organic compounds, tailored to local agricultural and economic conditions. Unlike standardized pesticide products that undergo rigorous testing and regulatory scrutiny, these locally formulated mixtures may vary widely in composition and concentration (Mostafalou and Abdollahi, 2017). This variability could potentially introduce unique health risks because the interactions between different chemical constituents can amplify toxicity or create unforeseen health effects. Moreover, the lack of standardized formulations and quality control in local production processes can lead to inconsistent pesticide potency and unintended environmental contamination (Jallow *et al.*, 2022).

Individual chemicals and combined exposure to these chemicals, often encountered in homes, agricultural, and industrial settings, pose a significant health risk to humans and wildlife. Inhalation, a common route of exposure in occupational and environmental contexts, allows these chemicals to bypass metabolic barriers and directly impact systemic physiology. In male reproductive health, pesticide exposure has been linked to disruptions in hormone regulation, oxidative stress, and histological alterations in testicular tissues (Mostafalou and Abdollahi, 2017). Pesticide exposure can disrupt male reproductive health through interconnected pathways involving testosterone regulation, oxidative stress, and histological changes in the testes. Testosterone, essential for spermatogenesis and reproductive function, can be affected by pesticides such as Dichlorvos and Cypermethrin (Gupta *et al.*, 2021). Simultaneously, these chemicals induce oxidative stress, leading to reduced glutathione levels, which are critical for protecting testicular tissues from damage (Agrawal and Sharma, 2019). Histological evaluations often reveal structural alterations in the seminiferous tubules and impaired spermatogenesis as indicators of reproductive toxicity linked to pesticide exposure (Sultana *et al.*, 2020; Agrawal and Sharma, 2019). Experimental studies have established a link between pesticide chemicals and adverse reproductive outcomes, including reduced sperm quality, testicular cancer, and other reproductive abnormalities (Diamanti-Kandarakis *et al.*, 2009; La Merrill *et al.*, 2020). These chemicals often act as endocrine disruptors by inhibiting the activity of androgens, which are critical for male reproductive development (Sikka & Wang, 2008). Research indicates a decline in human semen quality and fecundity over recent decades, potentially related to increased exposure to environmental toxins such as pesticides (Levine *et al.*, 2017). Pesticides vary widely in their chemical composition, mechanisms of action, and toxicity profiles. Typically, they consist of one or more active ingredients responsible for pesticidal activity and inert ingredients that facilitate handling but were previously thought to be non-toxic. However, emerging evidence suggests that these inert components can also contribute to toxicity (Mesnage *et al.*, 2014). Globally, over 700 active pesticide ingredients are in use, each with distinct chemical and toxicological properties (Casida & Durkin, 2013). Certain pesticides, such as chlorpyrifos and malathion, function as inhibitors of acetylcholinesterase, leading to increased acetylcholine levels, which subsequently suppresses the release of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). This suppression can inhibit gametogenesis and steroidogenesis, thereby affecting testosterone synthesis and potentially leading to erectile dysfunction (Pohanka, 2011; Terasawa & Fernandez, 2001). Organophosphates and other pesticides can induce oxidative stress by generating reactive oxygen species (ROS), causing damage to reproductive tissues (Bal *et al.*, 2012). For instance, atrazine and imidacloprid have been shown to reduce glutathione levels, contributing to oxidative stress (Sasidhar *et al.*, 2014). The reduction in testosterone levels may occur via inhibition of FSH and LH release or direct interference with steroidogenesis (Dehkhargani *et al.*, 2011; Slimani *et al.*, 2011).

Despite extensive research on dermal, and oral toxicity (Nantia *et al.*, 2018; Sardar *et al.*, 2023), studies examining the individual and combined effects of Dichlorvos, Cypermethrin, Synthetic Camphor, Pinenes, and Kerosene on reproductive health outcomes are limited, particularly via inhalation exposure involving withdrawal study in animal models. In this study present study, we aim to evaluate the impacts of individual and combined pesticide exposure on testosterone levels, glutathione concentration, and testicular histology in male Wistar rats via inhalation. The elucidation of these mechanisms through the findings will not only contribute to the reproductive toxicity of pesticide mixtures but also ascertain possible reversible effects or recovery after cessation of the treatment.

METHODS

Selection and Preparation of Pesticides

For this study, the pesticides used included DD Force (Dichlorvos), Sniper (Bifenthrin), industrial camphor (Terpenoid), edible camphor (Turpentine), and kerosene, all sourced from a market in Sabo, Ile-Ife, Osun State. These pesticides were chosen based on their common usage and potential impact on reproductive health, as reported in the literature. The rats were exposed to individual pesticide constituents and then combined in a ratio of 1:2:3 (25%, 50%, and 75% respectively).

Animal Experiment Design and Sampling

In this study, one hundred and ninety-two (192) male Wistar rats (*Rattus norvegicus*), aged 8-10 weeks and weighing between 250-300 grams, were purchased from Animal House, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The rats were weighed and kept in cages at 22^0 C and 50 ± 10% humidity housed under a natural light-dark cycle of 12 hours at room temperature and humidity. Throughout the study, the rats had *ad libitum* access to standard rat pellets and water. The

drinking water was refreshed every 3 d in this study. After a one-week acclimatization phase, twenty-one (21) out of the total rats were randomly split into seven groups, comprising three rats per group, and exposed to each chemical constituent and the combined pesticide for 24 hours in a range-finding test aimed at determining the lethal dose of the commercial formulation that would result in 100% mortality in rats (Akande *et al.*, 2024). Toxicity signs were observed and recorded daily. Body weight was recorded weekly throughout the study duration. The control group rats were fed with commercialized pellets. The pesticides were combined to promote synergistic interactions in line with what is sold and used by the local community. The range-finding test involved testing DD Force, Sniper, Industrial Camphor, Edible Camphor, Kerosene, and mixed pesticides at specific quantities: 250 ml, 50 ml, 0.63 g, 63 g, 750 ml, and 700 ml, respectively, based on their available quantities.

Following the range-finding test, we calculated the 96-hour median lethal concentration (LC_{50}) using the Spearman-Karber method. 54 rats were divided into six groups, three in each (A, B, C), exposed to different doses of the pesticides. Rats in the control group (n=3) were exposed to natural air, without pesticide exposure (Table 1). The dosages were varied for the animals in each group for each pesticide and the combined. Based on the outcome, we were able to deduce the ideal dose for the main study.

Experimental Groups and Exposure Protocol

Table 1: Acute Toxicity Wistar Rats Exposed to Pesticides

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

The acute toxicity data was used to determine the concentrations for the sub-acute investigation. in which a total of 114 male Wistar rats were divided into seven groups (A-G) based on their LC50 values. The pesticide mixture ratios were established using the results from the range-finding test. The sub-acute pesticide mixture ratio obtained from the range finding test was derived from Spearman-Kabal's arithmetic.

The equation is given by:

$$LD_{50} = \frac{LD_{100} - \sum (A \times B)}{N}$$

LD_{50} …… Highest Concentration
N…… No of Animals
A…… Dose Difference
B…… Mean Mortality
∑…… Summation

Pesticide mixture ratios were established following the range-finding test results (Akande *et al.*, 2024).

Rats were exposed to DD Force, Sniper, industrial camphor, edible camphor, kerosene, and a mixed pesticide mixture in an improvised poorly ventilated inhalation chamber for four hours daily, with three-day intervals, over four weeks. To prevent mortalities in the sub-acute test, the doses were kept constant i. e based on the ones used in the range finding test, whereas both the frequency and duration were altered. The mixture (combined) comprised 750 ml of kerosene, 250 ml of DD Force, 50 ml of Sniper, 63 grams of ground industrial camphor, and 63 grams of ground edible camphor. These components were thoroughly mixed in a plastic bowl for homogeneity and allowed to dissolve completely before use. Out of the 114 Wistar rats exposed to the pesticide mixture, 57 were withdrawn from exposure for two weeks to assess potential recovery. During this period, the rats were provided with rat pellets and water ad libitum. Subsequently, they were sacrificed to evaluate the effects of the pesticides on their reproductive profiles, comparing the recovered rats to those in the treated groups. At the end of the

experimental period, rats were euthanized via cervical dislocation, and blood samples were obtained through cardiac puncture. Blood from both control and treated animals was collected in plain tubes and allowed to clot at room temperature. The clotted blood samples were centrifuged at 3000 rpm for 10 minutes using a cold centrifuge to separate the serum. Serum, the fluid component of blood devoid of clotting factors, was obtained. It contains all proteins not involved in clotting, along with electrolytes, antibodies, antigens, hormones, and any exogenous substances. This serum was utilized for hormonal assays and Glutathione level determination using the Enzyme-Linked Immunosorbent Assay (ELISA) method, strictly following the manufacturer's instructions (Mansour and Mossa, 2011). To ensure sample integrity, serum samples were stored at -80°C until analysis.

Tissue Collection and Processing

Histological Analysis: The testes and epididymides were carefully dissected and fixed in Bouin's fluid for 24 hours. Fixed tissues were then dehydrated, embedded in paraffin wax, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin (H&E) for histopathological examination under a light microscope.

Biochemical Analysis

Testosterone Levels: Serum testosterone levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Glutathione (GSH) Concentration: GSH levels in serum were quantified using a colorimetric assay based on the reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and subsequent spectrophotometric analysis.

Statistical Analysis

Analyses were performed using the Statistical Package for Social Sciences (SPSS) version 25.0 or Prism (version 5.03; Graph Pad Software Inc.,). For comparisons between groups, using One-way ANOVA followed by the Newman- Keuls test was performed, and adjusted p-value levels of <0.05 were considered significant. The significance levels were $*p \leq 0.05$, $**p \leq 0.01$ and $p***\leq 0.001$. A $p < 0.05$ was accepted as the level of statistical significance. All analyses were performed on the individual rat or in independent experiments, as indicated in the text.

Ethical Considerations

This study was conducted in strict compliance with ethical guidelines for animal research and received approval from the Institute of Public Health (IPH), Obafemi Awolowo University. We tried all we could to minimize animal suffering and ensure the welfare and ethical treatment of the animals throughout the experiment.

RESULTS

Evaluation of Oxidative Stress in Exposed and Recovered Rats following Pesticide Exposure

The effects of selected pesticides on serum Glutathione (GSH) levels in exposed rats were evaluated at different concentrations. At 25% exposure, Sniper showed a significant decrease in GSH levels (-0.10 ± 0.01, $p < 0.005$) compared to the control (1.03 ± 0.14). DD Force (-0.10 ± 0.01, $p < 0.005$), Sniper (-0.09 ± 0.01, $p < 0.005$), and Kerosene (0.33 ± 0.18, $p < 0.005$) exhibited significant declines in GSH levels at 50% exposure, while other pesticides showed non-significant increases compared to the control. At 75% exposure, Industrial Camphor caused a significant decrease in GSH levels ($p < 0.005$). Sniper consistently resulted in the highest significant decrease across all concentration levels ($p < 0.005$), while Edible Camphor and Kerosene showed non-significant increases compared to the control (Figure 1). Following withdrawal from pesticide exposure, recovery rats exhibited significant increases in GSH levels at 25% exposure: Edible Camphor (2.13 ± 0.13, $p < 0.005$), Industrial Camphor (1.97 ± 0.15, $p < 0.005$), and Kerosene (1.87 ± 0.21, $p < 0.005$) compared to control (1.04 ± 0.27), indicating decreased oxidative stress. At 50% exposure, only Industrial Camphor significantly increased GSH levels ($p < 0.005$). At 75% exposure, no significant increases were observed for any pesticide ($p > 0.005$). The recovery groups showed a dose-dependent relationship, with GSH levels increasing from 25% to 75% exposure, suggesting partial detoxification compared to rats that were not withdrawn (Figure 2).

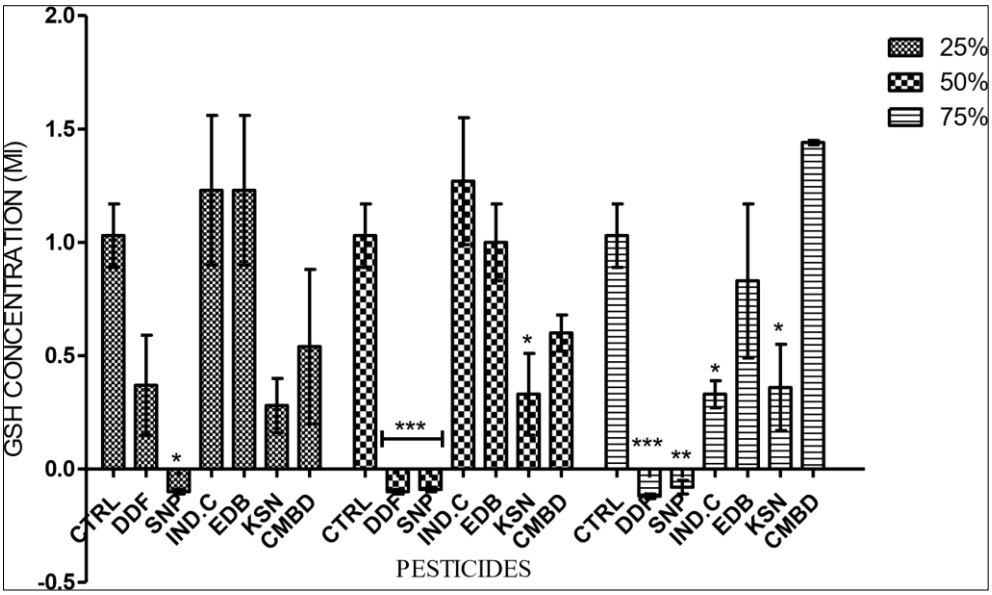

Figure 1: Effect of Selected Pesticides on Serum Level of Glutathione (GSH) in the Exposed Rats

Data are expressed as mean ± S.E.M. CTRL – CONTROL; DDF- DD FORCE, SNP – SNIPER; IND.C-INDUSTRIAL CAMPHOR; EDB- EDIBLE CAMPHOR; KSN- KEROSENE; CMBD – COMBINED. * ($p<0.01$), ** ($p < 0.001$) *** ($p<0.0001$). Values without (*) indicate no significant difference compared with control. (One way ANOVA followed by post-tests)

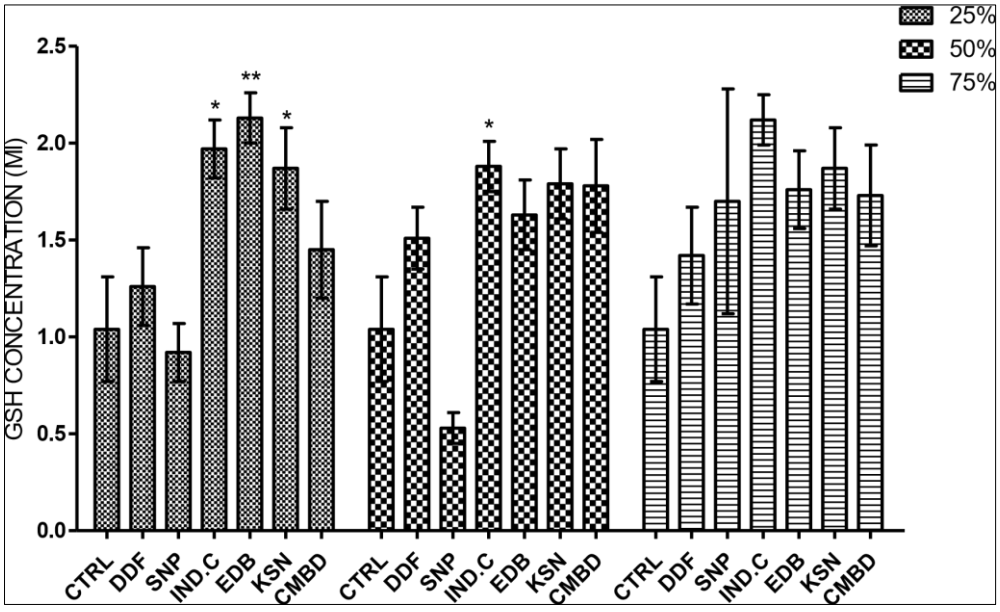

Figure 2: Effect of Selected Pesticides on Serum Level of Glutathione (GSH) in the Recovery Rats

Data are expressed as mean ± S.E.M. CTRL – CONTROL; DDF- DD FORCE, SNP – SNIPER; IND.C-INDUSTRIAL CAMPHOR; EDB- EDIBLE CAMPHOR; KSN- KEROSENE; CMBD – COMBINED. * ($p<0.01$), ** ($p < 0.001$) *** ($p<0.0001$). Values without (*) indicate no significant difference compared with the control. (One-way ANOVA followed by post-tests)

The Effects of Pesticide Exposure on Serum Testosterone Levels in Exposed and Recovered Rats

Evaluation of Oxidative Stress in Exposed and Recovered Rats following Pesticide Exposure

We next investigated the effects of varying concentrations of pesticide exposure to assess potential hormonal disruptions. There was a significant change observed across the groups and at all levels of concentrations in the exposed rats (Figure 3). At 25% concentration, DD Force, Industrial, and Edible Camphor showed no significant difference when compared with the control (0.44 ± 0.02). At 50% there was no significant difference in Industrial and Edible Camphor when compared to the control. However, DD Force shows a significant difference. Sniper, Kerosene, and Combined Pesticides. DD Force, Sniper, Industrial, and Edible Camphor showed no significant difference at

75% level of concentration whereas, Kerosene and combined showed a significant change.

Figure 4 shows the effects of pesticides on the hormonal level of the testosterone in the exposed and recovery rats. At 25% exposure there was a significant decrease in all the pesticides compared to control (0.43 ± 0.04). A significant difference was observed in 50% exposure groups when compared to control. However, at 75% concentration, a significant decrease was observed in DD Force, Industrial Camphor, Edible Camphor, and Kerosene with $p < 0.01$ while Sniper and combined pesticides showed no significant difference. There was a dose- dependent change observed only in Kerosene and Sniper.

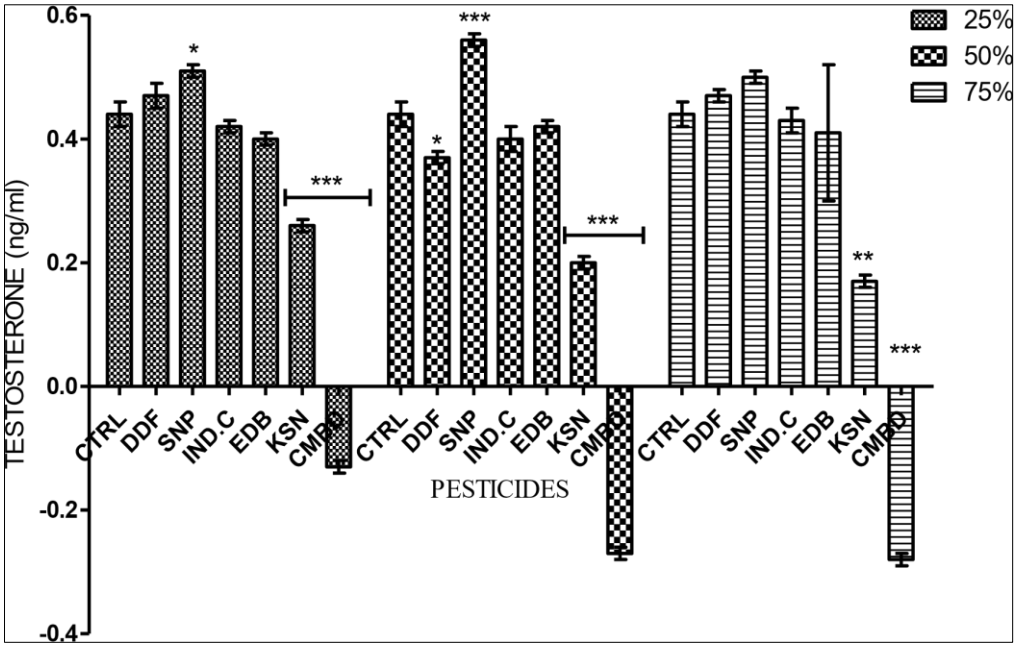

Figure 3: Effects of Pesticides on the Serum Testosterone Level in the Exposed Rats

Data are expressed as mean ± S.E.M. CTRL – CONTROL; DDF- DD FORCE, SNP – SNIPER; IND.C-INDUSTRIAL CAMPHOR; EDB- EDIBLE CAMPHOR; KSN- KEROSENE; CMBD – COMBINED. * ($p<0.01$), ** ($p < 0.001$) *** ($p<0.0001$). Values without (*) indicate no significant difference compared with control. (One way ANOVA followed by post-tests)

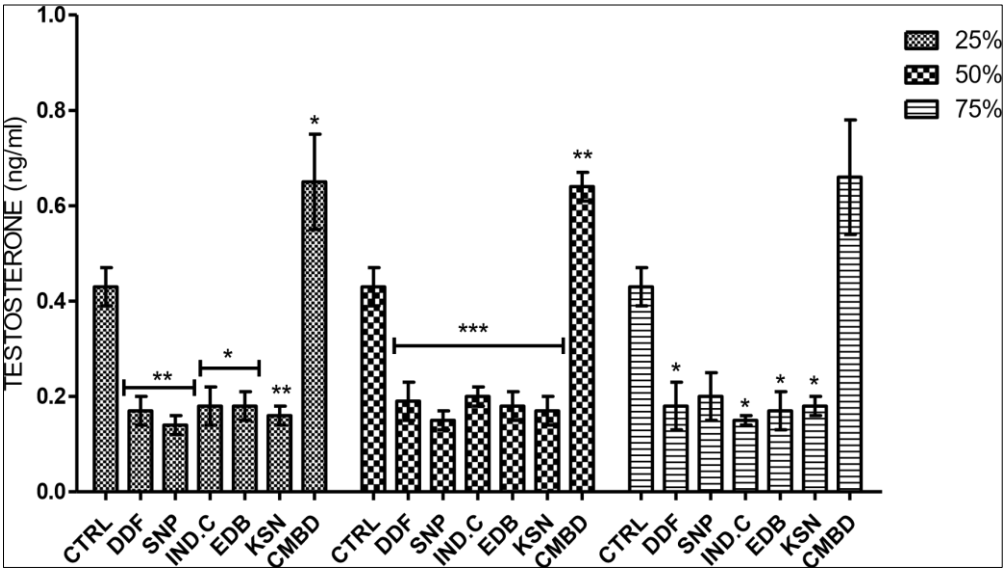

Figure 4: Effects of Pesticides on Serum Level of Testosterone in the Recovery Rats.

Data are expressed as mean ± S.E.M. CTRL – CONTROL; DDF- DD FORCE, SNP – SNIPER; IND.C-INDUSTRIAL CAMPHOR; EDB- EDIBLE CAMPHOR; KSN- KEROSENE; CMBD – COMBINED. * ($p<0.01$), ** ($p < 0.001$) *** ($p<0.0001$). Values without (*) indicate no significant difference compared with control. (One way ANOVA followed by post-tests)

Effects of the Pesticides on the Histology of Testes and Epididymis

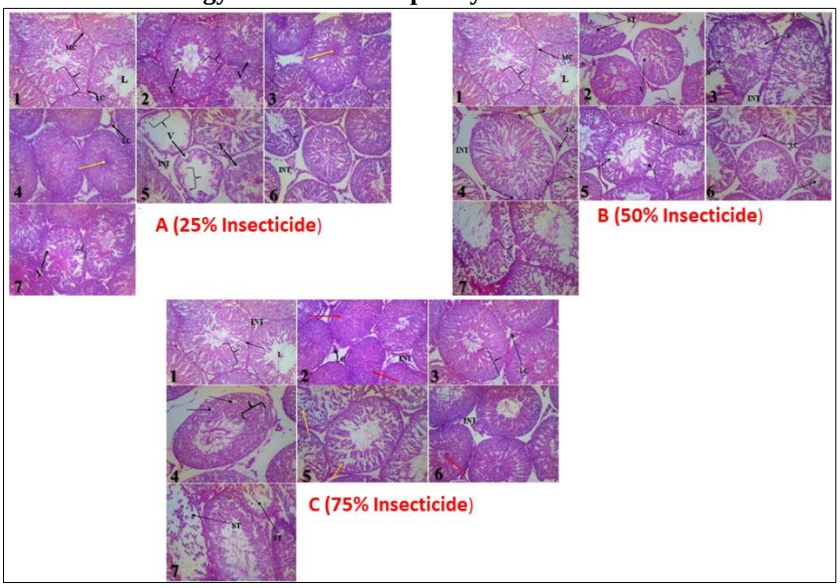

Plate 1: Representative photomicrographs of cross sections of rats' testes subjected to 25% dose of pesticides. H &E (x400)

Observations: In 1, intact seminiferous tubules enclosed by thick basal lamina and peritubular myoid cells (MC). *Seminiferous (germinal) epithelium is intact, spermatogenic cells and Sertoli cells are well arranged with the l*uminar surface being filled with elongating spermatids whose heads lie in the basal Sertoli cells cytoplasm (brace). The interstitium is normal with intact intertubular endocrine Leydig cells (LC). In 2 and 6 mild degenerative changes in tubular epithelium, with evident distortion in spermatogenic cells arrangement (brace) and vacuolation (V). The interstitum of 6 appear wide and almost empty (INT). In 3 and 4 germ cells are eroded into the lumen clogging it (yellow arrow) vacuolation degeneration in 3, sloughed germ cells in tubular lumen, in 4 (yellow arrow) and wide interstitium with Leydig cells (LC). In 5, degeneration and depletion of germinal epithelium characterized by vacuolation of basal Sertoli cells (V), spermatogenic and Sertoli cell loss (brace), a wide intestitium with Leydig cells atrophy (INT). In 7, degenerative changes in germinal epithelium characterized loss of epithelial tissue, disorganized arrangement of germ cells (Brace) and vacuolation. Stain –

1 – 7 represent control, DD force, Sniper, Industrial camphor, Edible camphor, kerosene, and combination respectively

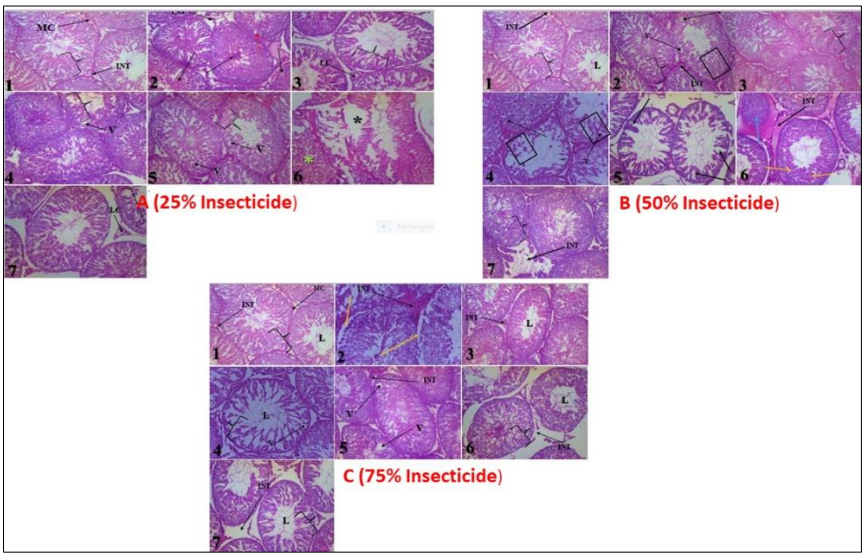

Plate 2: Representative light micrographs of cross section of rats' testes exposed to 25% concentration of pesticides and left to recover for 2 weeks post-treatment. H &E (x400)

Observation: (1) the normal arrangement of germ cells in discrete layers as they move up through the epithelium (brace). The interstitia are normal with visible Leydig cell nucleus (INT). Myoid cells are also distinct (MC).

(2) Vacuolar degeneration of basal Sertoli cells (red arrow), epithelial apoptosis (black arrow), disrupted epithelium and sloughed germ cells (black arrow) wide interstitium with visible Leydig cell nucleus (INT). (3, 7) Degenerative changes in germinal epithelium (brace), loss of germ cells and wide interstitium with few Leydig cells (LC). (4, 5) disorganized germinal epithelium with degenerated spermatogenic cells in some tubules (brace). Vacuolar degeneration of basal and adluminar Sertoli cells is visible (V). (Recovery is evident when compared). (6) Seminiferous epithelium sloughing (asterisk), germ cells erosion into the lumen (left ST – green asterisk), interstitium and Leydig cells are indistinct.

NB: ***[1 – 7] stand for control, DD force, Sniper, Industrial camphor, Edible camphor, kerosene, and combination respectively***

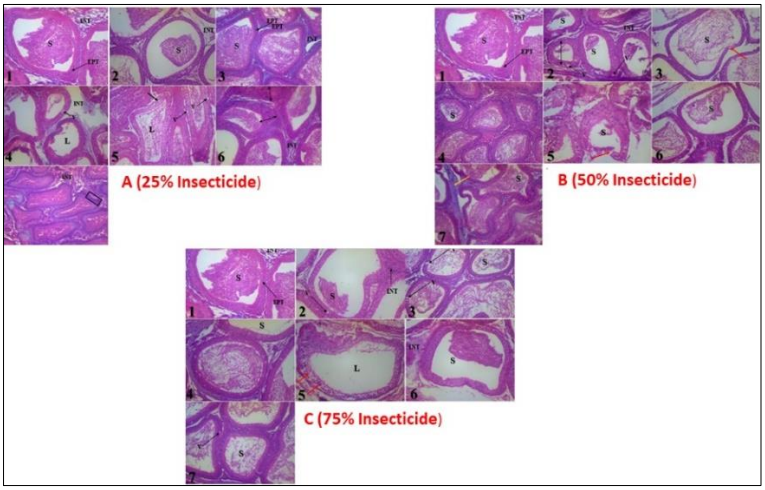

Plate 3: Representative light micrographs of cross section of rats' epididymis exposed to 25% concentration of pesticides. H &E(x400)

Observation: (1) intact epididymal epithelium with distinct cell types, normal sperm content and intestitium. (2) reduced intraluminal spermatozoa (S), scanty interstitial connective tissue (INT). (3) Appears very close to control (4, 5). Empty epididymal lumen in 4, presence of immature testicular germ cells or cell debris in the luminal contents of 5 (L), degenerative vacuolar changes in epididymal duct cells (V). Sparse / loss of interstitial connective tissue in _4'. Also note indication of epithelial apoptosis in 5 (black arrow). (6) Abnormally thickened epithelium with indications of hypercellularity (double arrow). The connective tissues in the interstitium are distinct (INT). (7) Epithelium is reduced in thickness, with increased cellularity (rectangle), sperm content of the epididymis is relatively normal but the connective tissue of the interstitum appears clumpsy (INT).

NB: ***[1 – 7] stand for control, DD force, Sniper, Industrial camphor, Edible camphor, kerosene, and combination respectively***

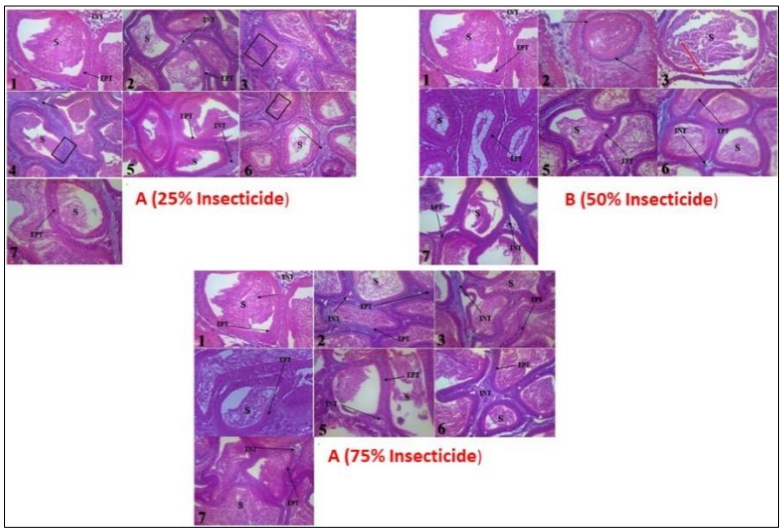

Plate 4: Representative light micrographs of cross section of rats' epididymis exposed to 25% concentration of pesticides and left to recover for 2 weeks posttreatment. H&E (x400)

Observation: (1) intact epididymal epithelium with normal sperm content and intestitial connective tissue (2, 4, 5, 6) Ductal atrophy of the epididymis with reduced sperm content compare to control. Sign of interstitial fibrosis in _4, 5, 6'. Also note increased cellularity and sloughed cell nucleus in 3, 4, 6. (rectangle) (7) epithelial cellularity appears normal, although the content of the lumen is fewer compare to control. (Some cases were recovered while some new features came up)

NB: ***[1 – 7] stand for control, DD force, Sniper, Industrial camphor, Edible camphor, kerosene, and combination respectively.***

DISCUSSION

Past research findings have shown that environmental toxins cause oxidative stress by overproduction of reactive oxygen species (ROS). While ROS plays a crucial role in the defense mechanism against pathological conditions, excessive production can harm tissues (Puppel *et al.,* 2015). In an actual sense, a depleted antioxidant system provokes oxidative stress, triggering inflammation and influencing epigenetic functions and apoptotic events. Consequently, these chemicals affect steroidogenesis, deteriorate sperm quality, and damage male reproductive organs (Hussain *et al.,* 2024).

In this study, we evaluated the effects of various pesticides on serum Glutathione (GSH) levels in male Wistar rats. Sniper and DD Force significantly reduced GSH levels, indicating strong oxidative stress, likely due to direct GSH depletion and inhibition of antioxidant enzymes. At the same time, other pesticides caused varied, non-significant changes in GSH levels, suggesting differing oxidative stress impacts, with a dose-dependent recovery in GSH levels noted with Edible Camphor and the Combined pesticide group, demonstrated partial restoration of antioxidant defenses post-exposure, highlighting the complex interactions between pesticides and the antioxidant system. Several animal studies have demonstrated the adverse impact of pesticides on glutathione. According to Puppel *et al.,* (2015), environmental toxins can lead to oxidative stress by causing excessive production of reactive oxygen species (ROS). While ROS plays a crucial role in defending against pathological conditions, an overwhelming production can be harmful to tissues. Kefer *et al.,* (2009) further noted that ROS is implicated in male infertility, as high levels can impair sperm production and damage the sperm plasma membrane, resulting in sperm dysfunction. Exposure to various insecticides, such as Imidacloprid, Chlorpyrifos, Cypermethrin, and Propetamphos, induced significant biochemical and histopathological deformation. Environmental toxicants like pesticides enhance cellular and molecular mechanisms in ways that can vary with age, chronicity, and exposure dose (Hussain *et al.,* 2024). Mitochondria play a central role in regulating various functions of metabolic and cellular signaling, working together to maintain cell survival and homeostasis. Disruption of these biological systems by environmental toxicants can significantly impact these processes, leading to adverse reproductive effects (Duarte-Hospital *et al.,* 2022). Imidacloprid exposure during postnatal development downregulated steroidogenesis-related genes and antioxidants in testicular tissues, leading to reduced androgen production (Martín-Sánchez *et al.,* 2022). Chlorpyrifos exposure demonstrated dose-dependent testicular toxicity, evidenced by reduced levels of key reproductive hormones and increased oxidative stress markers. Lower doses led to significant damage to the seminiferous tubules and germ cells, while higher doses showed a revival of antioxidant defenses, suggesting a complex dose-response relationship. These results are consistent with previous research highlighting the oxidative damage and endocrine disruption caused by chlorpyrifos (Jaiswal *et al.,* 2016). The evaluation of Deltamethrin (DEL) and its combinations with other insect repellents revealed increased antioxidant enzyme activities and neurotransmitter levels, despite associated lung toxicities. In both acute and chronic toxicity trials of cypermethrin and propetamphos, significant oxidative stress and disruptions in drug-metabolizing enzymes were observed. Chronic exposure particularly highlighted increased levels of certain cytochrome P450 enzymes and decreased glutathione S-transferase activity, indicating prolonged toxicological impact. The combined administration exacerbated these effects, emphasizing the need for careful consideration of combined pesticide exposures in risk assessments (Bhardwaj *et al.,* 2021). These findings align with the observed effects of pesticides like Sniper and DD Force, which demonstrated significant decreases in GSH levels, indicating potent oxidative stress induction. This may occur through mechanisms involving direct GSH depletion, inhibition of antioxidant enzymes, and activation of inflammatory pathways. Other pesticides showed varied effects, with non-significant increases or fluctuations in GSH levels compared to controls, highlighting differential oxidative stress profiles among pesticide categories. The dose-dependent relationship observed in Edible Camphor and the combined pesticide group during recovery phases suggests partial restoration of antioxidant defenses post-exposure (Hamed *et al.,* 2018; Shah and Iqbal, 2019).

This study also examined the impact of various pesticides on serum testosterone levels in male Wistar rats, revealing significant alterations influenced by both pesticide type and concentration levels. Testosterone, the primary male hormone, is crucial for sexual vigor and secondary sexual characteristics. Disruption of Leydig cell viability can impair testicular steroidogenesis, leading to disturbances in spermatogenesis and fertility issues. In studies, exposure of mouse Leydig cells to Aroclor 1242 reduced cell viability and inhibited testosterone synthesis by suppressing 3 beta-hydroxysteroid dehydrogenase (3β-HSD) and 17 beta-hydroxysteroid dehydrogenase and (17β-HSD) enzymes (Aydin and Erkan, 2017). Similarly, organochlorines

have been shown to alter androgen-binding proteins and stimulate these enzymes, increasing H_2O_2 levels in adult male rats (Saradha *et al.,* 2008). Such exposures can negatively impact Leydig cell function, reducing testosterone production and sperm count, potentially leading to infertility, testicular cancer, and other reproductive disorders. At 25% concentration, DD Force, Industrial Camphor, and Edible Camphor did not significantly differ from control levels, indicating a relatively moderate impact on testosterone production at lower exposures. However, at 50% concentration, DD Force exhibited a significant decrease in testosterone levels, suggesting a threshold effect where higher pesticide concentrations begin to affect hormone regulation more noticeably. Sniper, Kerosene, and the Combined pesticide group consistently showed significant decreases at all concentrations, indicating a robust suppression of testosterone production across exposure levels. This corroborates the findings of Ahmad *et al.,* (2017) on the effects of chlorpyrifos (CPF) and cypermethrin (CYP) on testosterone levels in adult male albino rats. Both individual and combined exposures to these pesticides significantly reduced serum testosterone levels compared to the control group. This decline was associated with histopathological changes in the testes, including necrosis, degeneration, and decreased spermatogenic cell numbers. The combined treatment of CPF and CYP resulted in more severe reductions in testosterone and more pronounced testicular damage than either pesticide alone, indicating a synergistic toxic effect on the reproductive system. Mechanistically, pesticides such as Sniper and Kerosene may disrupt testosterone synthesis through interference with enzymes involved in steroidogenesis or by inducing oxidative stress and inflammation in the testicular tissue. The observed dose-dependent response in Kerosene and Sniper underscores their potency in disrupting hormonal balance. According to Delorenzi and Adan, 2024, both malathion and diazinon could act as reproductive toxicants, influencing sperm quality, hormonal concentrations, oxidative stress levels, and causing histopathological damage in reproductive organs. Exposure to these pesticides is associated with reduced testosterone levels, attributed to increased acetylcholine stimulation via muscarinic receptors in the testes, which diminishes steroidogenic activity in Leydig cells (Elayan *et al.,* 2013; Terasawa and Fernandez, 2001). This decrease in testosterone levels in rodents correlates with reduced fertility, suggesting potential reproductive toxicity in humans.

This study found quite several clear-cut histopathological defects in both the treated animals and the rats withdrawn for possible recovery. The import of dose-response relationship was evident following the perturbations and the cellular deformations observed in testes and epididymides as the dose increases. The control group across all levels for the testes shows intact seminiferous tubules with well-organized spermatogenic and Sertoli cells, and normal Leydig cells. In contrast, DD Force (2) and kerosene (6) exhibit mild tubular degeneration and vacuolation, with a widened interstitial in kerosene at the lowest concentration. Sniper (3) and Industrial Camphor (4) display significant germ cell erosion and vacuolation, with sloughed cells in the lumen, indicating severe degeneration. Edible Camphor (5) presents marked germinal epithelium depletion and Leydig cell atrophy within an expanded interstitial. The combination group (7) shows the most pronounced damage, including epithelial loss and disorganized germ cells, hereby depicting severe impact of combined pesticide exposure on testicular structure. These architectural alterations worsened with increased dose. This aligns with the findings of Hariti *et al.,* (2024), who reported similar histopathological changes in testicular architecture following exposure to glyphosate-based herbicides in adult Wistar rats. In the Recovery Group (Plate 2), observations reveal partial recovery: restored normal germ cell arrangement in some tubules, reduced vacuolar degeneration and apoptosis compared to the exposed group, and partial restoration in Leydig cell population and interstitial structure. However, persistent histological impairments, such as disorganized epithelium and vacuolar changes in other tubules, indicate mixed results with partial recovery in some parameters but not complete restoration to control levels. The comparison suggests that while there are indications of recovery in certain histological parameters following a 2-week post-exposure period, the recovery is incomplete and variable across different pesticide exposures. The observed improvements in germ cell arrangement and reduction in some degenerative changes in the recovery group indicate a partial recovery trend. However, persistent vacuolation, disorganization of germinal epithelium, and incomplete restoration of Leydig cell populations suggest ongoing subclinical damage or slower recovery processes. This explains the lingering effects of pesticide exposure on testicular histology and the potential for long-term reproductive implications despite short-term recovery periods.

Similar trends of reproductive toxicity were observed in the histological architecture of the epididymides of the tested rats. The cross-sections of rats' epididymis at the lowest dose (25%) distinct histological changes. In control, the epididymal epithelium appears intact with normal sperm content and interstitium. Exposure to DD force shows reduced intraluminal spermatozoa and scant interstitial connective tissue. Similar observations near control levels are noted in Sniper and Industrial camphor, albeit with occasional empty lumens and the presence of immature germ cells or debris. Industrial camphor exhibits epithelial apoptosis, indicating cellular damage (black arrow). Abnormal thickening and hypercellularity are evident in Edible camphor, with distinct interstitial connective tissues. Combination exposure results in reduced epithelial thickness but increased cellularity, alongside clumpy interstitial connective tissue. On the contrary, a study from Sanabria *et al.,* (2015) on rat

sperm quality after subacute exposure to low doses of fungicide had no effect., where the authors investigated the effects of prochloraz (PCZ), a fungicide and androgen-receptor antagonist, on male reproductive parameters in adult male Wistar rats. They administered PCZ orally at doses of 10, 15, or 30 mg/kg bw/d for 4 consecutive days and evaluated various parameters including hormone concentrations, sperm evaluations, fertility, and histopathology of the testis and epididymis. The study concluded that even at these low doses and short exposure duration, PCZ did not induce reproductive toxicity nor compromise sperm quality compared to the control group. The light micrographs of rats' epididymis exposed and allowed to recover for 2 weeks post-treatment suggest some improvements alongside persistent or new histological features indicating ongoing tissue effects post-exposure. Observations reveal varied outcomes across different exposures: the control shows intact epithelium with normal sperm content and interstitial tissue. In nearly all pesticide mixtures administered, there's ductal atrophy with reduced sperm, interstitial fibrosis in some instances, and increased cellularity with sloughed cell nuclei. Notably, the combined displays relatively normal epithelial cellularity but reduced luminal content compared to the control. Partial recovery in certain histological parameters following the 2-week recovery period seems obvious. However, significant residual effects such as interstitial fibrosis and altered cellularity suggest that complete restoration to control levels was not achieved in all exposed groups. This highlights the complex and lingering impacts of pesticide exposure on epididymal morphology. Based on the study by Renata *et al.,* (2020), the effects of short-term and long-term exposure to methamidophos (MET) on male Swiss mice demonstrate significant impacts on spermatogenesis and reproductive parameters. Short-term exposure led to decreased epididymal weight and altered frequencies in spermatogenesis stages, while long-term exposure increased the frequencies of earlier spermatogenesis stages and reduced Sertoli cell counts. These findings enumerated the reproductive toxicity especially on short- and long-term effects on male fertility and spermatogenic processes. There is a paucity of information on withdrawal study of pesticides in reproductive toxicology. Nevertheless, reports are available about reversal of xenobiotics after about 7 to 30 days in various histology, enzymes, and blood. This study to some extent has demonstrated the recovery of tissue damage with the hormone level returning to near-normal. The recovery was faster in GSH, while the testosterone levels recovered to about 40% of the control values. However, recovery in the structure of the testes and epididymides was not very prominent, except that it appears that there are some reorganizations in some germ cells. This suffices to say that histological restorations take longer time than the GSH and testosterone levels.

CONCLUSION

Increasing exposure to high doses of pesticides leads to oxidative stress in male Wistar rats, contributing to reduced testosterone levels and adverse effects on testicular and epididymal histological architecture. Upon cessation of environmental exposure, biological and physiological detoxification processes in rats show a significant reduction in the effects of pesticides. While these findings provide valuable insights into the reproductive, biochemical, and hormonal impacts of commonly used pesticides, it is essential to recognize the limitations inherent in animal studies when extrapolating to human health. Nonetheless, the study demonstrates the critical need to consider the effects of pesticides on reproductive tissues when designing therapeutic strategies, particularly for organophosphate pesticide toxicity.

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