COMBINED TOXICITY STUDY OF A COMMERCIAL ADMIXTURE OF CYPERMETHRIN, DICHLORVOS AND PERMETHRIN ON SELECTED REPRODUCTIVE AND HAEMATOLOGICAL PARAMETERS IN MALE ALBINO RATS

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ABSTRACT

This study evaluated the sub-chronic toxicity of a commercial pesticide admixture containing cypermethrin, dichlorvos, and permethrin on reproductive and haematological parameters in male albino rats. Twenty rats were randomized into four groups (n = 5 per group). Group A served as the control and received distilled water, while Groups B, C, and D received 1 ml/L, 1 ml/2 L, and 1 ml/3 L of the pesticide mixture in drinking water, respectively, for 28 days. Reproductive endpoints (sperm motility and count, organ weights) and haematological indices were assessed post-exposure.

Sperm motility was significantly reduced in the high-dose group B (1 ml/L), though sperm count remained unaffected across all groups. A significant dose-dependent decrease in the relative weights of testes and epididymis was observed in all treated groups. Additionally, spleen weight was significantly reduced in group B. Haematological analysis revealed a significant decrease in red blood cell count in group C and a dose-dependent increase in mean corpuscular haemoglobin concentration (MCHC), particularly in groups C and D. Elevated eosinophil and basophil counts in all treated groups.

In conclusion, repeated exposure to this pesticide combination adversely affected male reproductive health and induced notable haematological alterations, even at moderate doses. Thus underscoring the need for stricter regulation and comprehensive safety evaluations of commercially available pesticide mixtures.

Keywords: Cypermethrin, Dichlorvos, Permethrin, Reproductive toxicity, Haematology, Pesticide mixture, Albino rats

INTRODUCTION

Pesticides continue to play a crucial role in modern agriculture and public health, helping to manage pests and reduce the spread of vector-borne diseases. Among the various types in use today, pyrethroids and organophosphates are particularly common due to their broad-spectrum efficacy and relatively low toxicity to humans when used within recommended limits (Shekhar et al., 2024). Cypermethrin and permethrin, both synthetic pyrethroids, and dichlorvos, an organophosphate, are widely applied either individually or in combination. In recent years, however, the market has seen a growing trend toward commercial pesticide formulations that combine multiple active ingredients. These are especially prevalent in regions where pesticide regulation may be less stringent, such as parts of sub-Saharan Africa

and Southeast Asia (Kalyabina *et al.*, 2021).

While the toxicological effects of these pesticides have been well studied individually, information on their combined effects, especially when used together as part of a commercial mixture is still quite limited. Previous research has shown that both cypermethrin and permethrin can interfere with male reproductive health, impairing sperm quality and causing testicular damage, likely through mechanisms involving oxidative stress or hormonal disruption (Wang *et al*., 2020; Darwish *et al*., 2025). Dichlorvos, on the other hand, is better known for its impact on the nervous and immune systems and has been linked to changes in blood cell production and bone marrow function (Okoroiwu and Iwara, 2018; Shekhar *et al*., 2024).

Despite our understanding of how each of these chemicals works in isolation, very little is known about how they behave when combined, particularly in formulations that are already being used in homes and farms. This is a significant concern because mixtures may interact in ways that enhance their harmful effects, a phenomenon known as additive or synergistic toxicity (Chen *et al*., 2015). What is more, most of the available data comes from acute toxicity studies, leaving a gap in our knowledge of what happens with repeated, low-level exposure, which is more reflective of real-life human and environmental scenarios.

Given this background, there is a clear need to investigate how such combinations might affect critical biological systems. The present study was therefore carried out to examine the sub-chronic effects of a commercial pesticide admixture containing cypermethrin, dichlorvos, and permethrin on male reproductive function and haematological health in albino rats. By focusing on both reproductive organs and blood parameters, this study aims to provide a clearer picture of the potential risks involved and help inform safer usage practices and future regulatory guidelines.

MATERIALS AND METHODS

Experimental Animals

A total of 20 adult male albino rats (weighing 180–220 g) were obtained from the animal house of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The animals were housed in clean, well-ventilated cages under standard laboratory conditions (12-hour light/dark cycle, temperature of 22 ± 2°C, and relative humidity of 55 ± 10%). Rats were allowed to acclimatize for 14 days prior to the commencement of the experiment. They were fed standard laboratory rat chow and provided water *ad libitum* throughout the study period.

Pesticide admixture

The test pesticide admixture was a commercially available combination of Cypermethrin, Dichlorvos and Permethrin marketed under the trade name Striker® (Lagos, Nigeria). The 100 ml bottle contained the active ingredients, cypermethrin (0.3%), dichlorvos (0.5%) and permethrin (0.5%).

Experimental Design

The rats were randomly divided into four groups (n = 5 per group) - Group A served as the control and was administered clean drinking water only. Group B received a commercial admixture of cypermethrin, dichlorvos, and permethrin at a concentration of 1 ml/L of water. Group C received the same admixture at a concentration of 1 ml/2 L of water. Group D received the admixture in 1 ml/3 L of water. The pesticide admixture was administered orally *in* drinking water for 28 consecutive days.

Sample Collection

At the end of the 28-day treatment period, rats were fasted overnight and sacrificed under mild anesthesia. Blood samples were collected by cardiac puncture into Ethylenediaminetetraacetic acid (EDTA-) coated tubes for haematological analysis. The reproductive organs (testes and epididymis)

were excised, trimmed of fat and connective tissues, and weighed immediately to determine relative organ weights.

Sperm Analysis

Sperm samples were collected from the cauda epididymis and assessed for motility and count using standard procedures. Motility was evaluated microscopically using a warm stage at 37°C. Sperm count was determined using a haemocytometer after appropriate dilution.

Haematological Analysis

Whole blood samples were analyzed using an automated haematology analyzer (Beckman Coulter, USA) to determine the following parameters: packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC), differential leukocyte counts (lymphocytes, neutrophils, monocytes, eosinophils, basophils), and platelet count.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine significant differences among groups. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. All analyses were carried out using IBMS SPSS version 30.0.(USA)

RESULTS

Effects of Pesticide Admixture on Sperm Motility and Sperm Count

Table 1 shows the effects of exposure of male albino rats to commercial admixture of cypermethrin, dichlorvos and permethrin. The commercial admixture of cypermethrin, dichlorvos, and permethrin resulted in a statistically significant reduction ($p < 0.05$) in sperm motility in group B (1 ml/L) compared to the control group A. Group B exhibited a mean sperm motility of 83.4 ± 1.40%, significantly lower than the control value of 95.0 ± 1.60%. However, no statistically significant differences ($p > 0.05$) in sperm motility were observed between the control and groups C (1 ml/2 L) or D (1 ml/3 L), which had motility values of 93.5 ± 1.70% and 94.0 ± 4.40%, respectively.

Sperm count values did not show any statistically significant differences ($P > 0.05$) across all treatment groups when compared with the control. Mean sperm counts were 54.40 ± 14.88 $\times 10^6$/ml in the control group, 49.33 ± 10.17 in group B, 51.45 ± 14.45 in group C, and 54.15 ± 14.27 in group D.

Table 1: Effect of Pesticide Admixture of Cypermethrin, Dichlorvos and Permethrin on Sperm Motility and Count in Male Albino Rats Treated for 28 Days

Parameter	Admixture of Cypermethrin, Dichlorvos and Permethrin			
	Group A (Control)	Group B (CMT+DCL+PMT 1 ml/L water)	Group C (CMT+DCL+PMT 1 ml/2 L water)	Group D (CMT+DCL+PMT 1 ml/3 L water
Sperm Motility (%)	95.0 ± 1.6[a]	83.4 ± 1.40[b]	93.5 ± 1.70[a]	94.0 ± 4.40[a]
Sperm Count ($\times 10^6$/ml)	54.40 ± 14.88	49.33 ± 10.17	51.45 ± 14.45	54.15 ± 14.27

Different superscripts [a,b] in a row indicates significant difference ($p < 0.05$) between the means

CMT=Cypermethrin, DCL=Dichlorvos, PMT=Permethrin

Effects of Pesticide Admixture on Relative Organ Weights

As shown in Table 2, a significant reduction ($p < 0.05$) in the relative weights of the testes and epididymis was observed in all treatment groups (B, C, and D) when compared to the control. The testes weight was significantly lower in group B (1.23 ± 0.08%), group C (1.33 ± 0.27%), and group D (1.37 ± 0.28%) compared to the control group (1.54 ± 0.25%). Similarly, the epididymis weights were significantly reduced in group B (0.34 ± 0.02%), group C (0.31 ± 0.08%), and group D (0.36 ± 0.08%) compared to the control (0.55 ± 0.19%).

For the spleen, a statistically significant decrease ($p < 0.05$) was observed in group B (0.30 ± 0.04%) compared to the control (0.39 ± 0.06%). Group C also showed a significant decrease ($p > 0.05$) (0.36 ± 0.10%), though less pronounced than group B. No significant changes were observed in the relative weights of the liver, kidneys, or heart across all treatment groups.

Table 2: Effects of pesticide Admixture of Cypermethrin, Dichlorvos and Permethrin on Relative Organ Weights (%) of Male Albino Rats Treated for 28 Days

Organs	Admixture of Cypermethrin, Dichlorvos and Permethrin			
	Group A (Control)	Group B (CMT+DCL+PMT 1 ml/L water)	Group C (CMT+DCL+PMT 1 ml/2 L water)	Group D (CMT+DCL+PMT 1 ml/3 L water
Testes (%)	1.54 ± 0.25[a]	1.23 ± 0.08[b]	1.33 ± 0.27[b]	1.37 ± 0.28[b]
Epididymis (%)	0.55 ± 0.19[a]	0.34 ± 0.02[b]	0.31 ± 0.08[b]	0.36 ± 0.08[b]
Kidney (%)	0.58 ± 0.01	0.49 ± 0.03	0.51 ± 0.13	0.53 ± 0.13
Spleen (%)	0.39 ± 0.06[a]	0.30 ± 0.04[b]	0.36 ± 0.10[c]	0.38 ± 0.05[a]
Liver (%)	3.04 ± 0.26	2.23 ± 0.18	2.39 ± 0.60	2.47 ± 0.63
Heart (%)	0.35 ± 0.02	0.30 ± 0.03	0.30 ± 0.08	0.32 ± 0.07

Different superscripts [a,b] in a row indicates significant difference ($p < 0.05$) between the means

CMT=Cypermethrin, **DCL=**Permethrin

Effects of Pesticide Admixture on Haematological Parameters

Table 3 shows the results of haematological parameters following the exposure of rats to an admixture of cypermethrin, dichlorvos and permethrin. The results show significant differences in certain haematological parameters among the groups. For PCV, group B had a significantly higher ($p < 0.05$) value (48.00 ± 0.84%) than group C (36.80 ± 3.72%), while group A (46.4 ± 1.12%) and group D (44.00 ± 1.14%) did not differ significantly ($p > 0.05$). The Hb levels were significantly lower ($p < 0.05$) in group C (13.42 ± 1.27 g/dl) compared to other groups (group A: 16.20 ± 0.35 g/dl,

group B: 16.74 ± 0.33 g/dl, group D: 16.78 ± 0.43 g/dl), but groups A, B, and D did not show significant differences ($p > 0.05$).

For RBC count, group C (6.56 ± 0.63 $\times 10^{12}$/L) had a significantly lower ($p < 0.05$) value than groups A (8.06 ± 0.22 $\times 10^{12}$/L), B (8.12 ± 0.24 $\times 10^{12}$/L), and D (7.93 ± 0.25 $\times 10^{12}$/L). No significant differences ($p > 0.05$) were observed in MCV, MCH or MCHC, except that group D (38.42 ± 0.34 g/dl) had a significantly higher MCHC compared to group A (34.90 ± 0.52 g/dl) ($p < 0.05$).

The TLC was significantly higher ($p < 0.05$) in group B (8.58 ± 1.44 $\times 10^{9}$/L) compared to group C (5.12 ± 0.87 $\times 10^{9}$/L), while group D (7.40 ± 0.49 $\times 10^{9}$/L) did not differ significantly from the other groups ($p > 0.05$). Neutrophil percentages were significantly higher ($p < 0.05$) in groups B, C, and D (8.20 ± 2.48%, 9.20 ± 1.63%, 9.00 ± 1.23%) compared to group A (7.80 ± 1.59%). Basophil percentages were significantly higher in groups B, C, and D (0.20 ± 0.20%, 0.20 ± 0.20%, 0.40 ± 0.40%) compared to group A (0.00 ± 0.00%) ($p < 0.05$). No significant differences ($p > 0.05$) were observed in lymphocyte, monocyte, eosinophil ($p > 0.05$), or platelet counts across the groups.

Table 3: Effects of the Pesticide Admixture of Cypermethrin, Dichlorvos and Permethrin on Haematological Indices in Male Albino Rats Treated for 28 Days

Parameters	Admixture of Cypermethrin, Dichlorvos and Permethrin			
	Group A (Control)	Group B (CMT+DCL+PMT 1 ml/1 L water)	Group C (CMT+DCL+PMT 1 ml/2 L water)	Group D (CMT+DCL+PMT 1 ml/3 L water)
PCV (%)	46.4 ± 1.12^{a}	48.00 ± 0.84^{b}	36.80 ± 3.72^{c}	44.00 ± 1.14^{a}
HB (g/dl)	16.20 ± 0.35^{a}	16.74 ± 0.33^{a}	13.42 ± 1.27^{b}	16.78 ± 0.43^{a}
RBC ($\times 10^{12}$/L)	8.06 ± 0.22^{a}	8.12 ± 0.24^{a}	6.56 ± 0.63^{b}	7.93 ± 0.25^{a}
MCV (fL)	57.72 ± 1.79	59.18 ± 2.12	56.04 ± 1.09	55.10 ± 0.77
MCH (pg)	20.12 ± 0.42	20.58± 0.33	20.48 ± 0.34	21.16 ± 0.34
MCHC (g/dl)	34.90 ± 0.52^{a}	35.10 ± 0.67^{a}	36.54 ± 0.35^{a}	38.42 ± 0.34^{b}
TLC ($\times 10^{9}$)	6.54 ± 0.84^{a}	8.58 ± 1.44^{b}	5.12 ± 0.87^{a}	7.40 ± 0.49^{ac}
Lymphocyte (%)	83.20 ± 3.07	84.00 ± 3.41	81.80 ± 3.47	78.00 ± 2.51
Neutrophil (%)	7.80 ± 1.59^{a}	8.20 ± 2.48^{b}	9.20 ± 1.63^{b}	9.00 ± 1.23^{b}
Monocyte (%)	7.20 ± 1.16	5.4 ± 0.25	6.67 ± 1.21	6.90 ± 0.51^{a}
Eosinophils (%)	1.80 ± 0.49	2.20 ± 0.66	2.20 ± 0.97	2.20 ± 0.97
Basophils (%)	0.00 ± 0.00^{a}	0.20 ± 0.20^{b}	0.20 ± 0.20^{b}	0.40 ± 0.40^{b}
Platelet ($\times 10^{9}$)	621.6 ± 34.2	705.8 ± 51.1	524.0 ± 102.9	642.4 ± 43.5

Different superscripts ^{a,b,c,d} in a row indicate significant difference ($p < 0.05$) between the means

PCV=Packed cell volume, **Hb**=Haemoglobin, **RBC**=Red blood cells, **MCV**=Mean corpuscular volume, **MCH**=Mean corpuscular haemoglobin, **MCHC**=Mean corpuscular haemoglobin concentration. **TLC**=Total leucocyte count

DISCUSSION

This study explored how a commercial mixture of three commonly used pesticides—cypermethrin, dichlorvos, and permethrin—affects key reproductive and haematological parameters in male albino rats following 28 days of exposure.

Based on the findings in Table 1, sperm motility was notably reduced in rats that received the highest concentration of the pesticide mixture (1 ml/L water, group B). While the lower-dose groups C and D showed motility levels similar to the control group. The sharp drop-in group B suggests that higher concentrations of the admixture may impair sperm function. This pattern points to a dose-dependent toxic effect, particularly on sperm movement rather than sperm production, since sperm count remained relatively stable across all groups. These results are in line with previous studies that have linked organophosphates and pyrethroids to disruptions in sperm function, likely due to oxidative stress or hormonal imbalances (Rodprasert *et al*., 2023; Uwamahoro *et al*., 2024).

As shown in Table 2, exposure to the pesticide blend led to a significant reduction in the weights of the testes and epididymis across all treated groups, regardless of the dose. This suggests that even relatively low concentrations of the mixture can negatively affect the reproductive organs. Such changes are often associated with structural damage or reduced functionality in testicular tissues and are consistent with known effects of pesticides on male fertility (Wang *et al.*, 2020). A decrease in spleen weight was also observed in the highest dose group (group B), hinting at possible immunosuppressive effects. In contrast, no significant changes were seen in liver, kidney, or heart weights, indicating that the toxic impact may be more specific to the reproductive and immune systems at the tested doses (Uwamahoro *et al*., 2024).

The results of the haematological analysis indicate significant alterations in various parameters following exposure to the admixture of cypermethrin, dichlorvos, and permethrin. Specifically, a decrease in PCV and Hb levels in group C suggests a potential impairment in red blood cell production or increased haemolysis, which is consistent with previous findings that reported pesticide exposure leading to anaemia and reduced erythropoiesis in rodents (Ilyushina *et al*., 2019; Saleem *et al*., 2025). This decrease in RBC count aligns with similar studies showing that exposure to organophosphates and pyrethroids can depress erythropoiesis and damage red blood cell membranes (Sobolev *et al*., 2022). Conversely, group B showed a significantly higher TLC, suggesting an immune response to the chemical exposure, which agrees with studies that demonstrated increased leukocyte counts after pesticide exposure as a response to toxicity or inflammation (Ruiz-Arias *et al*., 2025). The absence of significant changes in MCV, MCH, and MCHC may indicate that these parameters are less sensitive to the effects of the pesticide mixture or that the exposure time (28 days) was not long enough to induce significant changes (Bojarski *et al.*, 2024). The observed increase in neutrophil and basophil percentages in groups B, C, and D is also consistent with previous studies indicating an inflammatory or immune-modulatory effect of pesticide exposure (Corsini *et al*., 2008). However, the absence of significant changes in platelet count, eosinophil, monocyte, and lymphocyte percentages contradicts some reports where pesticide exposure has been shown to significantly alter these parameters (Suwannarin *et al*., 2021), suggesting that the mixture used in this study may have a selective effect on the immune and blood cell populations. These discrepancies may be attributed to differences in pesticide types, dosages, and species, as well as variations in experimental design and exposure duration. Further studies are needed to clarify the exact mechanisms behind these findings and to determine whether long-term exposure could lead to more pronounced haematological changes.

CONCLUSION

Overall, this study shows that repeated exposure to a combined formulation of cypermethrin, dichlorvos, and permethrin can adversely affect sperm motility, reduce the weight of reproductive organs, and alter some blood parameters in male rats, particularly at higher doses. These changes highlight the potential risks of such pesticide combinations to reproductive health and blood function. This study highlights the potential health risks associated with repeated exposure to a commonly used pesticide mixture containing cypermethrin, dichlorvos, and permethrin. The results showed that even at relatively low doses, this combination can negatively impact sperm motility, reduce the size of reproductive organs, and alter important blood parameters in male rats. These changes suggest possible risks to both reproductive and general health, especially with continuous or long-term exposure. The findings point to the importance of re-evaluating the safety of such pesticide blends, particularly in environments where they are regularly used and regulation may be limited.

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