

**SJMLS - 10(1) - 021****Propoxur-Induced Nephrotoxicity and Haematotoxicity: Ameliorative Role of Ascorbic Acid in Adult Male Wistar Rats**

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**Abstract**

Propoxur, a widely used carbamate insecticide, has raised concerns due to its potential toxicity to non-target organisms. Oxidative stress has been implicated as a key mechanism in pesticide-induced toxicity. Ascorbic acid (Vitamin C), a potent antioxidant, may offer protection against such toxicity. This study aimed to investigate the nephrotoxic and haematotoxic effects of propoxur exposure in adult male Wistar rats and evaluate the potential protective role of vitamin C supplementation. The objectives included assessing changes in body weight, biochemical parameters (SOD, ROS, TNF- $\alpha$ ), haematological indices, kidney function markers, and histological alterations in response to propoxur exposure and vitamin C intervention. Propoxur exposure resulted in significant weight loss, increased ( $p > 0.05$ ) ROS levels, decreased ( $p < 0.05$ ) SOD activity and elevated uric acid levels. Histological examination revealed renal haemorrhage, tubular necrosis and glomerular congestion in propoxur-treated rats. Vitamin C supplementation ameliorated some of these effects, normalizing body weight, reducing ROS levels, enhancing SOD activity, and improving renal histology. Haematological parameters showed subtle changes, with significant alterations observed in Mean Corpuscular Volume (MCV) across all treatment groups. In conclusion, this study demonstrates that propoxur induces nephrotoxicity and subtle haematological alterations in adult male Wistar rats, primarily through oxidative stress mechanisms. Ascorbic acid (Vitamin C) supplementation exhibits significant protective effects against propoxur-induced toxicity, highlighting its potential as a preventive

intervention. These findings contribute to our understanding of pesticide-induced health risks and the role of antioxidant strategies in mitigating these effects, with important implications for public health and environmental toxicology.

**Keywords:** Propoxur, Carbamate insecticide, Oxidative stress, Vitamin C, Nephrotoxicity, Haematotoxicity, Antioxidant, Pesticide toxicity

**Introduction**

Propoxur is a carbamate insecticide used to control ants, roaches and hornets in and around residences and commercial food handling establishments. The formulations include aerosols, baits, emulsifiable concentrates, wettable powders formulated into aerosol cans, dust and powders, pest strips, shelf paper, ready-to-use solutions, granular baits, containerized baits, and pet flea collars. Studies using laboratory animals show propoxur generally to be of acute toxicity. It has been placed in Toxicity Category II (the second highest of the four categories) for effects via the oral route of exposure, and Toxicity Category III for the dermal and inhalation routes. Propoxur has been classified as a Group B, probable human 2 carcinogen (United States Environmental Protection Agency, EPA-738-F-97-009, August 1997). Increasing worries have been raised about its possible negative impacts on non-target organisms, including humans and animals (Sonal *et al.*, 2018). Among the organs susceptible to propoxur toxicity are the kidney and haematopoietic system. The kidney, crucial for filtration, reabsorption, and excretion, is vulnerable to xenobiotic-induced damage (Blessy *et al.*, 2017). Similarly, the haematopoietic system, responsible

for blood cell production, can be adversely affected by toxicant exposure (Hirabayashi and Inoue, 2010). The etiology of many diseases, including those brought on by xenobiotics, is linked to oxidative stress, which is defined as an imbalance between the generation of reactive oxygen species and the antioxidant defense system (Sultana *et al.*, 2016). Strong antioxidant vitamin C has demonstrated potential in reducing tissue damage caused by oxidative stress (Halliwell and Gutteridge, 2007). In this work, male Wistar rats are used to test the nephrotoxic and hemotoxic effects of propoxur, as well as the protective impact of vitamin C against these toxicities.

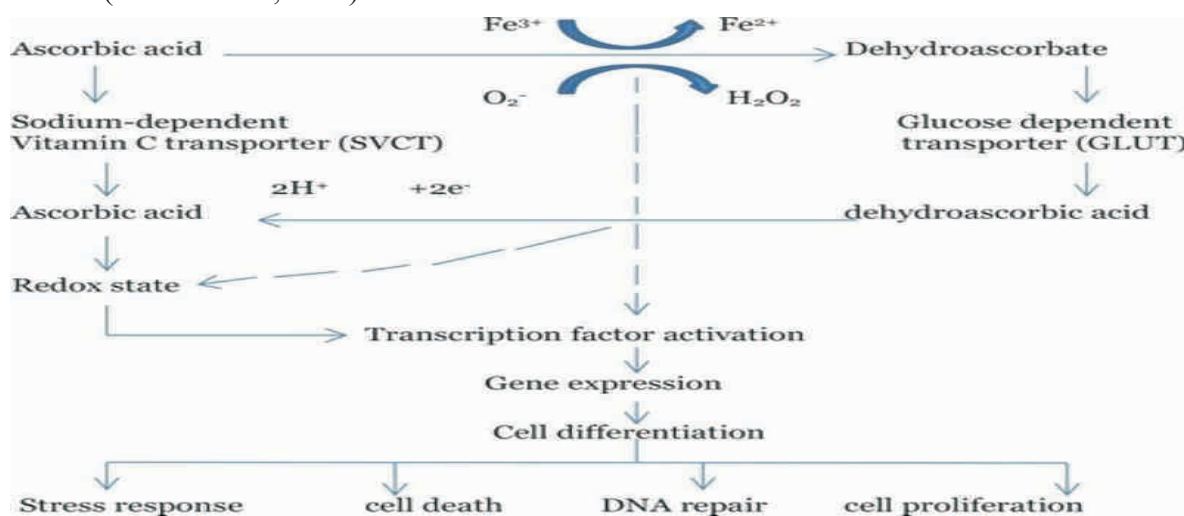
The kidney, a critical organ responsible for maintaining fluid and electrolyte balance, as well as waste elimination, is susceptible to damage from xenobiotics (Sharma *et al.*, 2018). Exposure to toxic substances can induce renal dysfunction characterized by alterations in renal function tests, histological changes, and oxidative stress (Kadhim *et al.*, 2024). Similarly, the haematopoietic system, responsible for blood cell production, is vulnerable to the toxic effects of chemicals. Disruptions in this system can manifest as anemia, leukocytosis, or thrombocytopenia (Khan *et al.*, 2019).

Oxidative stress, a condition arising from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, has been implicated in the pathogenesis of various diseases, including those induced by xenobiotics (Sultana *et al.*, 2008). Excessive ROS

can damage cellular components, leading to lipid peroxidation, protein oxidation, and DNA damage (Halliwell and Gutteridge, 2007). Antioxidants, such as vitamin C, play a crucial role in neutralizing ROS and protecting cells from oxidative damage (Pizzino *et al.*, 2017).

Given the potential toxicity of propoxur and the importance of the kidney and haematopoietic system, this study aims to investigate the effects of propoxur on renal and haematological parameters in male Wistar rats. Furthermore, the study will evaluate the protective efficacy of vitamin C against propoxur-induced toxicity. By elucidating the underlying mechanisms of propoxur-induced renal and haematological dysfunction, this research contributes to a better understanding of the health risks associated with pesticide exposure and informs strategies for mitigating their adverse effects.

The transfer of a hydrogen atom to lipid radicals, the elimination of molecular oxygen, and the quenching of singlet oxygen are the main mechanisms via which ascorbic acid functions as an antioxidant (Du *et al.*, 2012). Ascorbic acid's antioxidant mechanism also includes aqueous radical scavenging and the regeneration of  $\alpha$ -tocopherol from teco-pheroxyl radical species (Padayatty and Levine 2016). Ascorbic acid's power to increase iron absorption from the diet is attributed to its ability to bind and decrease transition metal ions, particularly  $Fe^{3+}$  and  $Cu^{2+}$ , according to Padayatty and Levine (2016).



**Figure 1: Possible mode of action of vitamin C in human cells (Du *et al.*, 2012).**

### **Role in Detoxification**

Ascorbic acid, another name for vitamin C, is an essential component of various bodily detoxification processes. By scavenging free radicals and reactive oxygen species (ROS), vitamin C functions as a strong antioxidant, shielding cells from oxidative damage (Carr and Frei, 1999; Padayatty and Levine, 2016). Vitamin C functions as an electron donor to renew other antioxidants like vitamin E, glutathione (GSH), and coenzyme Q10, enhancing the overall antioxidant capacity of the body (Carr and Frei, 1999; Padayatty and Levine, 2016).

Vitamin C contributes to the regeneration of GSH, a crucial intracellular antioxidant involved in detoxification processes, by reducing oxidized glutathione (GSSG) back to its reduced form (GSH) (Padayatty and Levine, 2016). The availability of vitamin C ensures the continuous recycling of GSH, which is essential for neutralizing toxins and reactive metabolites (Padayatty and Levine, 2016).

It has been demonstrated that vitamin C increases the expression and activity of phase II detoxification enzymes, such as quinone reductase (NQO1) and glutathione S-transferases (GSTs), which aid in the conjugation and removal of toxins and xenobiotics (Huang *et al.*, 2006; Sharma *et al.*, 2010). Vitamin C aids in the detoxification of numerous environmental contaminants, heavy metals, and carcinogens by increasing the activity of these enzymes (Huang *et al.*, 2006; Sharma *et al.*, 2010).

According to Swaran and Vidhu, vitamin C has the ability to chelate metals, binding to harmful metals including lead, mercury, and cadmium and promoting their elimination from the body. Vitamin C inhibits the bioavailability of hazardous metals by chelation, reducing their build-up and harmful effects on tissues and organs (Swaran and Vidhu, 2010).

Vitamin C plays a crucial role in supporting the immune system, enhancing the function of immune cells involved in detoxification processes, such as macrophages and neutrophils (Carr and Maggini, 2017). By bolstering immune

function, vitamin C aids in the identification and elimination of pathogens, toxins, and foreign substances, contributing to overall detoxification processes (Carr and Maggini, 2017).

### **Materials and Methodology**

Approved by the University's Departmental Research and Ethical Review Committee, this study was carried out in 2023 on twenty (20) adult male Wistar rats at the Animal Holding Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin.

### **Animal Care**

The animals were housed in comfortable wire gauzed plastic cages bedded with sawdust and were fed with standard laboratory diet (pellet feed). They were fed daily, and their cages were constantly cleaned in order to keep them healthy and prevent diseases and infections that may erupt from poor hygiene. All the rats were handled in accordance with the standard guide for the care of the laboratory animals. During the experiment, the rules and guidelines of the Institutional Animal Care and Use Committee (IACUC) were strictly followed throughout the handling of animals.

Propoxur and vitamin C were administered to male Wistar rats in an experimental setting as part of the study. The method of administration for Propoxur was inhalation, whereas Vitamin C was given orally. Four groups of rats were created: one for control, one for Propoxur, one for Vitamin C, and one for both Propoxur and Vitamin C. The duration of the treatment was 56 days. The rats were put to sleep at the end of the experiment, and their kidneys were removed for biochemical and histological examination. The University of Ilorin Teaching Hospital's Histopathology Laboratory handled and examined the kidney samples. The kidneys underwent fixation, dehydration, clearing, impregnation, embedding, trimming, and sectioning, mounting, dewaxing, and staining using Haematoxylin and Eosin techniques.

### **Superoxide Dismutase Activity**

The superoxide dismutase activity was analyzed using SOD Assay Kit-WST (SOD-19160 water-soluble tetrazolium salt, Sigma-Aldrich, USA) as previously described.

**Reactive Oxygen Species Activity**

The Reactive Oxygen Species (ROS) levels were measured using a ROS Assay Kit-DCFH-DA (DCFH-Diacetate) (Sigma-Aldrich, USA) following the manufacturer's instructions.

**Tumour Necrosis Factor alpha Activity**

Using a TNF-alpha ELISA Kit (Sigma-Aldrich, USA), the levels of the tumor necrosis factor alpha (TNF-alpha) were measured in accordance with the given methodology.

**Evaluation of Gross and Histological Changes**

The kidney of each rats were examined for any gross lesion. Tissue samples were then prepared using the modified method for histological examination.

**Statistical Analysis**

The data obtained were expressed as mean ± SEM and then subjected to one-way analysis of variance (ANOVA) followed by Turkey's post-hoc multiple comparison test. GraphPad prism version 8.03 for windows (GraphPad Software, San Diego, California, USA) was used to analyze all the data. Values of p < 0.05 were considered significant.

**General Observation and Results**

During the course of this work, morphological and histological effects of Propoxur and Vitamin C were observed. All necessary data and observations were appropriately documented.

**Body Weights**

**Table 1: Initial, final and relative body weights of experimental animals**

Group	IBW(g)	FBW (g)	RW (%)
CONTROL	120 ± 0.49	178 ± 5.43	48.3 ± 4.08
PROPOXUR	116 ± 0.40	133 ± 7.80	14.7 ± 6.98
VITAMIN C	100 ± 0.49	162 ± 10.90	62.0 ± 10.42
VITAMIN C + PROPOXUR	112 ± 0.45	156 ± 8.34	39.3 ± 7.19

Key:

Significant decrease at p > 0.05 when compared to control group

IBW: Initial body weight; FBW: Final body weight; RW: Relative body weight

Values are mean ± S.E.M. n = 5 in each group

Control 2ml/kg body weight

Propoxur (3 ppm/body weight) 56 days

Vitamin C (10mg/kg body weight) 56 days

Vitamin C (10mg/kg body weight) + Propoxur (3ppm/body weight) 56 days

Biochemical Assay

**Table 2: Concentration of SOD, ROS and TNF-alpha of experimental animals**

Parameters /Groups	Control	Propoxur	Vitamin C	Vitamin C + Propoxur
SOD (U/mL)	1.74 ± 0.04	1.55 ± 0.03**	1.49 ± 0.02*	1.87 ± 0.09*
ROS (uM)	5.35 ± 0.27	11.81 ± 0.64*	6.52 ± 0.38*	0.35 ± 0.01
TNF-alpha (pg/ml)	13.16 ± 0.27	6.84 ± 0.46**	7.97 ± 0.31*	9.29 ± 0.39*

Key:

\*Represents significant decrease at p < 0.05 when compared to control group

\*\*Represents significant decrease at p < 0.01 when compared to control group

SOD: Superoxide dismutase; ROS: Reactive oxygen species; TNF-alpha: Tumor necrosis factor alpha

Values are mean ± S.E.M. n = 5 in each group

Control (2ml/kg body weight) 56 days

Propoxur (3 ppm/body weight) 56 days

Vitamin C (10mg/kg body weight) 56 days

Vitamin C (10mg/kg body weight) + Propoxur (3ppm/body weight) 56 days

**Superoxide Dismutase (SOD)**

The graph in figure 3 shows that the differences in the mean of the SOD levels across all the experimental animals were statistically significant when compared to the control with P-value (0.0029). A Tukey post hoc test revealed that this significance in the differences is between the control group and propoxur group (p-value 0.0394), Vitamin C group (p-value 0.0223). Also, there is significance between the differences in the mean of Propoxur and Vitamin C plus Propoxur group (p-value 0.0063) and Vitamin C and Vitamin C plus Propoxur (Vit C + Propoxur) group (p-value 0.0046). Analysis of variance (ANOVA) with mean ±SEM was used.

**Reactive Oxygen species ROS (uM)**

The graph in figure 4 indicates that the differences in the mean of the SOD levels across all experimental animals was statistically significant when compared to the control with P-value (<0.0001).

**Tumor Necrosis Factor TNF-alpha (pg/ml)**

The graph in figure 5 indicates that the differences in the mean of the SOD levels across all experimental animals were statistically significant when compared to the control with P-value (<0.0001). A Tukey post hoc test revealed that this significance in the differences is between the control group and propoxur group (p-value <0.0001), Vitamin C (p-value 0.0003), Vitamin C plus Propoxur (Vit C + Propoxur) group (p-value 0.0017). Also, there is significance between the differences in mean of Propoxur and Vitamin C plus Propoxur group (p-value 0.0175). Analysis of variance (ANOVA) with mean ±SEM was used.

**Haematological Parameters**

The graphs below represent the levels of Hematological parameters in all experimental animals

Parameters	Control	Propoxur	Vitamin C	Vitamin C + Propoxur
WBC (x10 <sup>3</sup> /μL)	4.63 ±0.44	4.30 ±0.29	4.38 ±0.78	4.28 ±0.15
RBC (x10 <sup>6</sup> /μL)	7.15 ±0.07	7.73 ±0.56	7.19 ±0.29	6.03 ±1.29
HGB (g/dL)	10.23 ±0.51	10.50 ±1.50	10.30 ±0.29	9.35 ±0.73
HCT (%)	36.80 ±0.65	46.40 ±2.05	41.83 ±2.30	35.85 ±7.32
MCV (fL)	50.68 ±0.99	59.20 ±1.75*	59.28 ±1.12*	61.58 ±1.86*
MCH (pg)	14.23 ±0.81	12.60 ±1.39	14.48 ±0.19	19.63 ±5.20
MCHC (g/dL)	28.10 ±1.60	21.18 ±2.32	24.63 ± 0.71	31.18± 7.10
PLT (x10 <sup>3</sup> /μL)	443.00 ±68.17	495.80 ±98.27	505.80 ±107.90	1008 ±336.20

**Key:**

\*Represents significant increase p>0.05 in MCV when compared to control group

WBC: White blood cell; RBC: Red blood cell; HGB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin MCHC: Mean corpuscular haemoglobin concentration; PLT: Platelet

Values are mean ± S.E.M. n = 5 in each group.

Control (2ml/kg body weight) 56 days

Propoxur (3 ppm/body weight) 56 days

Vitamin C (10mg/kg body weight) 56 days

Vitamin C (10mg/kg body weight) + Propoxur (3ppm/body weight) 56 days

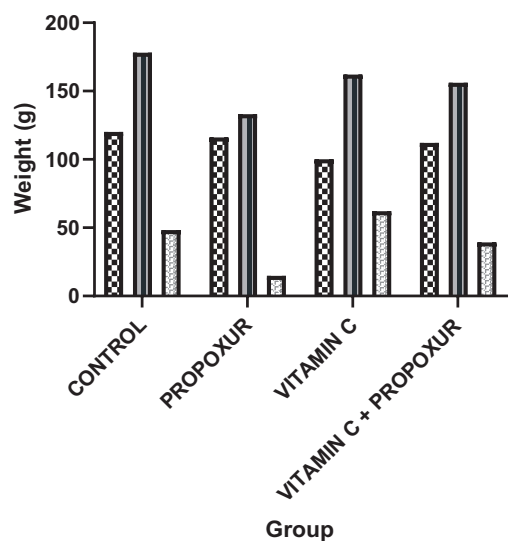


Figure 2: BODY WEIGHTS

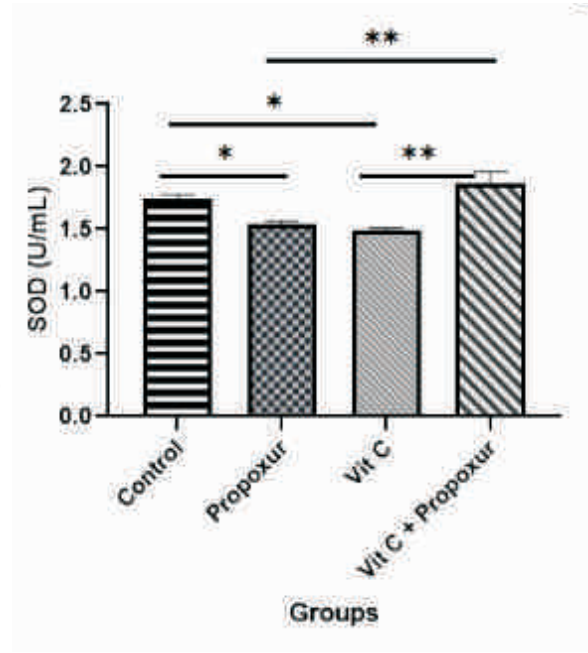


Figure 3: SOD

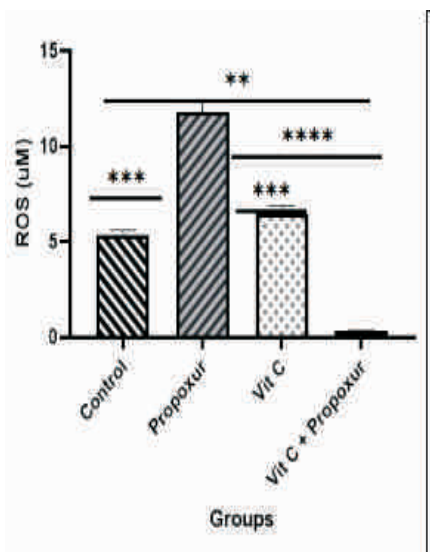


Figure 4: ROS

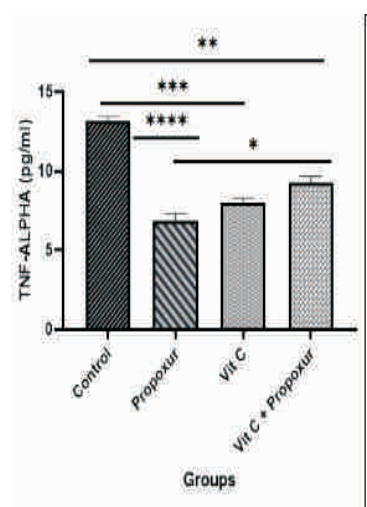
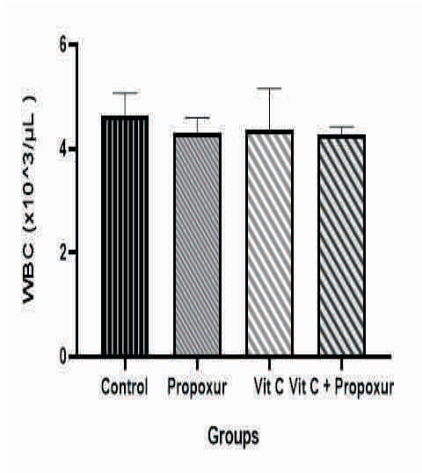
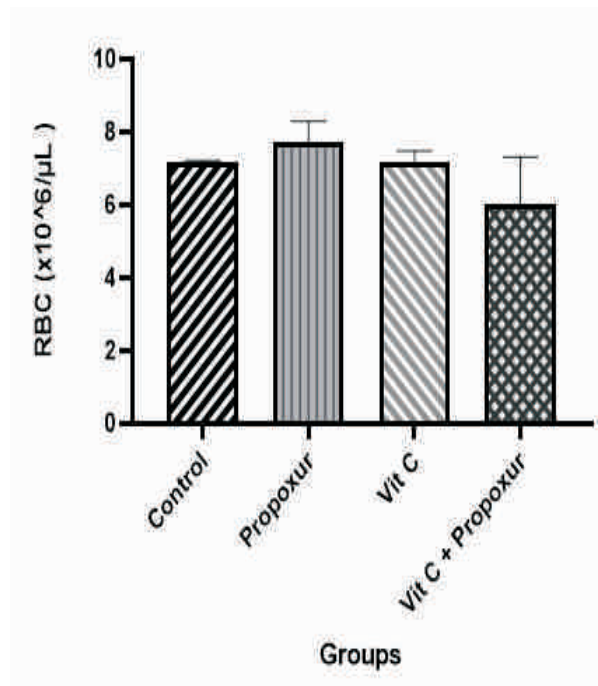


Figure 5: NF-ALPHA

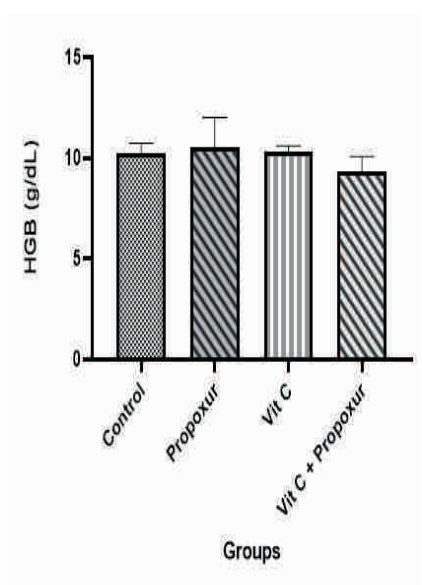
Control (2ml/kg body weight) 56 days  
 Propoxur (3ppm/body weight) 56 days  
 Vitamin C (100mg/kg body weight) 56 days  
 Propoxur (3ppm/body weight) + Vitamin C (100mg/kg body weight) 56 days



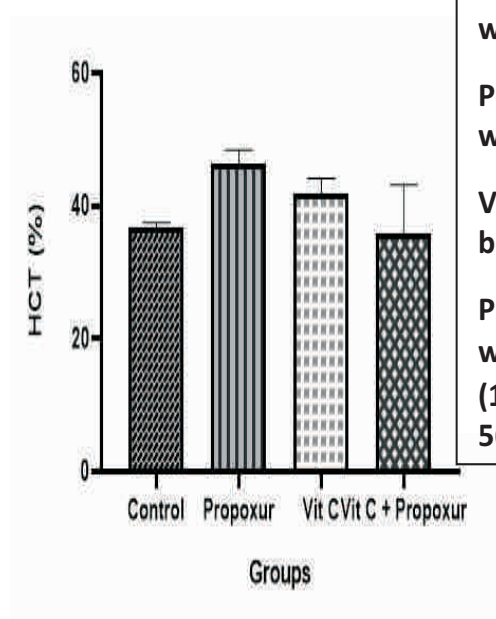
**Figure 6: WBC**



**Figure 7: RBC**



**Figure 8: HGB**



**Figure 9: HCT**

Control (2ml/kg body weight) 56 days  
 Propoxur (3ppm/body weight) 56 days  
 Vitamin C (100mg/kg body weight) 56 days  
 Propoxur (3ppm/body weight) + Vitamin C (100mg/kg body weight) 56 days

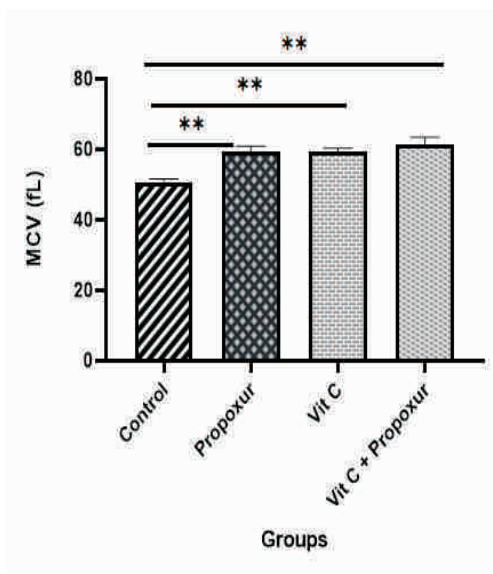


Figure 10: MCV

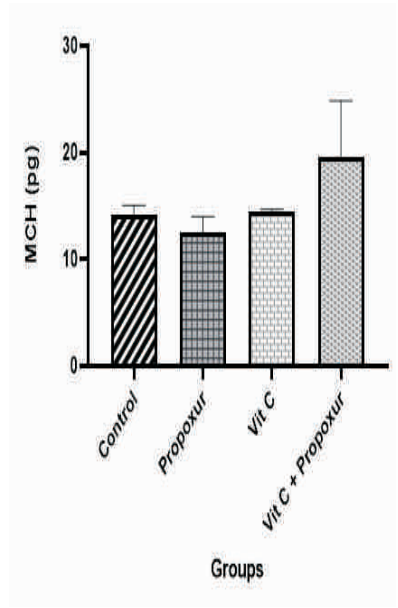
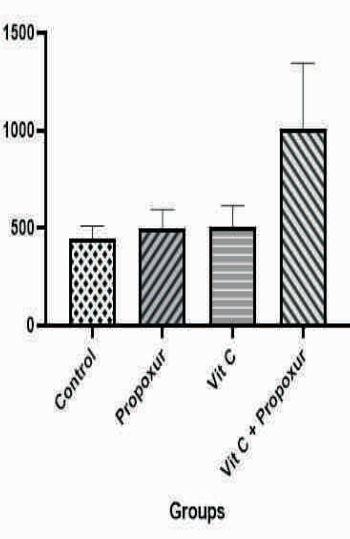
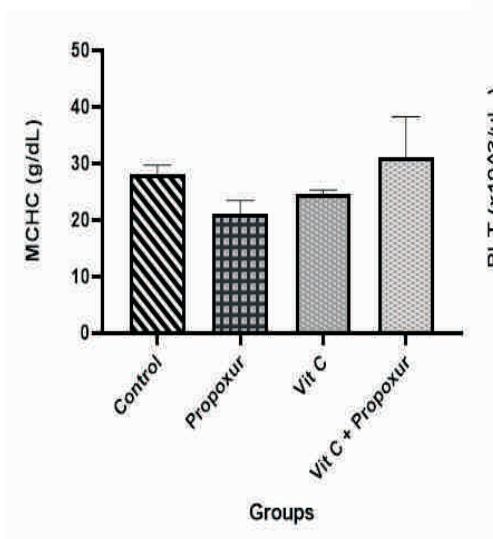


Figure 11: MCH

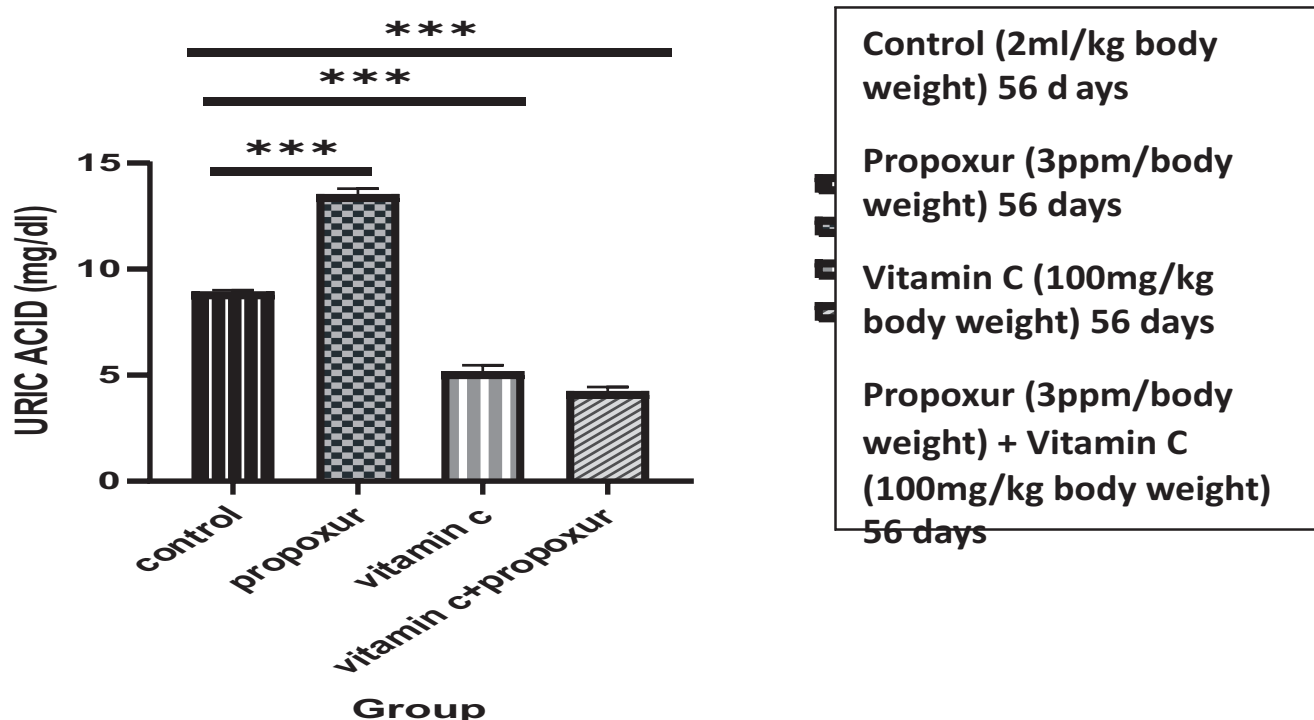


Control (2ml/kg body weight) 56 days  
 Propoxur (3ppm/body weight) 56 days  
 Vitamin C (100mg/kg body weight) 56 days  
 Propoxur (3ppm/body weight) + Vitamin C (100mg/kg body weight) 56 days

**Kidney Function Test**

**Uric Acid**

The Propoxur group shows a noticeable increase in the levels of Uric Acid compared to the Control group. Comparing the Propoxur group to the Vitamin group, the Propoxur group exhibits higher levels of Uric Acid. Vitamin C + Propoxur appears to have Uric Acid levels similar to or slightly lower than the Propoxur group, indicating that vitamin C may have a mitigating effect on the increase in Uric Acid caused by propoxur.



**Histological Presentation Analysis**

Treatment Groups	Observation
<b>Control Group</b>	Normal histological appearance with well-defined glomeruli and tubules.
<b>Propoxur-treated Group</b>	Tubular necrosis observed. Glomerular congestion present. Interstitial inflammation noted, indicative of significant renal damage. Findings consistent with biochemical evidence of oxidative stress and altered kidney function.
<b>Vitamin C plus Propoxur Group</b>	Amelioration of histological changes. Reduction in tubular necrosis and glomerular congestion. Decreased interstitial inflammation. Strong evidence for the protective effects of vitamin C against propoxur-induced nephrotoxicity.
<b>Vitamin C-only Group</b>	Normal kidney histology, similar to the control group. No adverse effects on renal structure observed. Supports the safety of vitamin C supplementation as a preventive measure against oxidative stress-induced kidney damage.

## Conclusion

The study investigated the nephrotoxic and haematotoxic effects of propoxur exposure in adult male Wistar rats and the protective role of vitamin C supplementation. The findings suggest that propoxur exposure led to weight loss, compromised antioxidant defense, increased ROS levels, and altered TNF- $\alpha$  levels. Vitamin C supplementation showed protective effects against propoxur-induced toxicity, enhancing antioxidant defenses and normalizing inflammatory responses. Haematological parameters remained stable, except for Mean Corpuscular Volume (MCV) changes. Uric acid levels indicated potential kidney function impact. Histological observations revealed significant renal damage in propoxur-treated rats, mitigated by vitamin C.

The study's limitations include a single dose and duration, male-only subjects limiting generalizability, and the need for further exploration of molecular pathways. The results emphasize the importance of antioxidant strategies in mitigating pesticide-induced health risks. Continued research is crucial to understand dose-dependent effects, molecular mechanisms, and application to human populations exposed to pesticides.

These findings provide valuable insights into pesticide toxicity and antioxidant interventions, highlighting the need for ongoing monitoring and regulation of pesticide use for public health and environmental protection.

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