

## Research Paper

# Protective Effects of Lycopene against Propoxur Induced Liver Injury Mediated by Up Regulation of Xanthine oxidase/ Uric Acid Signaling

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## ABSTRACT

### **Background:**

Propoxur, a carbamate pesticide, induces liver injury through oxidative stress and inflammation, primarily via the xanthine oxidase/uric acid signaling pathway, leading to increased reactive oxygen species (ROS) production and hepatic damage. Lycopene, a powerful antioxidant in tomatoes, counters oxidative stress and inflammation, but its protective role against propoxur-induced liver toxicity remains underexplored. This study evaluates lycopene's potential in mitigating pesticide-induced liver injury.

### **Methodology:**

Thirty male Wistar rats (average weight: 165 g) were divided into six groups (A–F) of five rats each. Group A served as the control, while Groups B and C were exposed to propoxur (3 ppm) for 30 days, with exposure durations of 5 minutes and 1 hour daily, respectively. Group D received lycopene (10 mg/kg) for 30 days, while Groups E and F were co-treated with propoxur and lycopene.

### **Results:**

Histological analysis revealed liver damage in propoxur-exposed rats, marked by significant weight loss and elevated liver enzymes (ALP, ALT, AST), indicating liver dysfunction. Oxidative stress markers, such as malondialdehyde (MDA) and ROS, were elevated, alongside an increase in interleukin-6 (IL-6), indicating oxidative and inflammatory damage.

**Conclusion:**

*Propoxur exposure resulted in liver injury, oxidative stress, inflammation, and weight loss in Wistar rats, highlighting its toxic effects. Lycopene's antioxidant and anti-inflammatory properties show promise in protecting against pesticide-induced liver toxicity, warranting further investigation into its therapeutic applications.*

**KEYWORDS:** Lycopene, Propoxur, Liver injury, Oxidative stress, Inflammation

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**INTRODUCTION**

Liver problem remains a significant global health issue, with various environmental toxins, pharmaceuticals, and industrial chemicals contributing to its onset. Among these, pesticides, particularly organophosphates and carbamates, are widely used in agriculture and domestic settings to control pests, but they pose substantial risks to human health and the environment. Propoxur, a carbamate pesticide, is commonly used to manage household and agricultural pests. Despite its effectiveness in pest control, exposure to propoxur has been shown to induce various toxic effects, including liver damage, neurotoxicity, and oxidative stress<sup>1</sup>. The liver, as a primary organ responsible for detoxification, is particularly vulnerable to the toxic effects of propoxur, which can lead to severe hepatic dysfunction. Given the increasing use of propoxur and other pesticides, understanding the mechanisms underlying its hepatotoxicity is crucial for developing effective protective strategies<sup>1</sup>.

Propoxur's toxicity is primarily attributed to its ability to inhibit cholinesterase, an enzyme critical for proper nerve function. This inhibition disrupts neurotransmission, leading to neurotoxic effects, but it also affects other organs, particularly the liver, where the detoxification of xenobiotics occurs. When exposed to propoxur, the liver experiences a series of biochemical and structural changes, including an increase in the levels of liver enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST). Elevated levels of these enzymes are indicative of hepatocellular damage and are commonly used as biomarkers for liver injury<sup>2</sup>. Furthermore, oxidative stress plays a pivotal role in the pathogenesis of liver injury caused by propoxur. Propoxur exposure leads to the generation of reactive oxygen species (ROS), which overwhelm the liver's antioxidant defense mechanisms, resulting in lipid peroxidation, DNA damage, and mitochondrial dysfunction<sup>3</sup>. The accumulation of ROS also triggers the activation of inflammatory pathways,

contributing to further hepatic injury. Inflammatory cytokines such as interleukin-6 (IL-6) are released in response to oxidative stress, amplifying the damage and impairing the liver's ability to regenerate<sup>4</sup>.

In light of these detrimental effects, there has been growing interest in identifying natural compounds with potential hepatoprotective properties. Lycopene, a carotenoid found in tomatoes, watermelon, and other red fruits, has emerged as a promising candidate due to its powerful antioxidant and anti-inflammatory effects. Lycopene is known to scavenge free radicals, reduce oxidative stress, and modulate inflammatory pathways, all of which make it a potential therapeutic agent for protecting against liver injury. Studies have shown that lycopene can alleviate oxidative damage in various organs, including the liver, by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase<sup>5, 6</sup>. Additionally, lycopene has been found to suppress the production of pro-inflammatory cytokines, such as IL-6, which are implicated in the progression of liver damage<sup>7</sup>.

The protective effects of lycopene against liver injury have been investigated in several experimental models, particularly in the context of hepatotoxicity induced by chemicals and toxins. Lycopene's ability to modulate the xanthine oxidase/uric acid signaling pathway, a crucial pathway in oxidative stress and inflammation, further supports its potential therapeutic application<sup>8</sup>. Xanthine oxidase is an enzyme that generates ROS and contributes to the inflammatory response, and its inhibition by lycopene may help reduce oxidative damage and inflammation in the liver. Furthermore, lycopene has been shown to improve liver function by reducing the levels of liver enzymes such as ALP, ALT, and AST, which are commonly used as markers of liver injury<sup>8</sup>. Given its antioxidant, anti-inflammatory, and liver-protective properties, lycopene presents a promising adjunct in mitigating the toxic effects of propoxur.

This study aims to explore the protective effects of lycopene against propoxur-induced liver injury in Wistar rats. Specifically, the study will focus on evaluating the modulation of oxidative stress markers, liver enzyme levels, in response to lycopene administration. By investigating the mechanisms through which lycopene mitigates propoxur-induced liver damage, this research may provide valuable insights into the development of novel therapeutic strategies for preventing pesticide-induced hepatotoxicity. Moreover, this study could contribute to the broader understanding of how natural compounds can be used to combat environmental toxin-induced organ damage, with potential implications for human health.

## MATERIALS AND METHOD

### Procurement of Compounds and Animals

All compounds used (Propoxur and lycopene) were procured from Adebayo Ige and Sons, Osogbo and verified at Pharmacology Department, University of Ilorin, Ilorin. Experimental animals used for this research were procured from the Animal Holdings College of Health Science, Osun State University, Osogbo. The animals were allowed access to food and ad libitum. They were given two weeks to get used to the lab condition before the study started. The study followed the rules set by the Health Research Ethics Committee at the College of Health Sciences, University of Ilorin, Ilorin, Nigeria, and complied with the National Institute of Health guidelines for caring for and using lab animals.

### Experimental Design

A total number of thirty male Wistar rats, with an average weight of 165g, were used in the study. The rats were randomly divided into six groups (A, B, C, D, E, and F), each consisting of five rats. Group A served as the control. Group B was exposed to Propoxur for 5 minutes at 3 ppm for 30 days. Group C was exposed to Propoxur for 1 hour at 3 ppm for 30 days. Group D received Lycopene at 10 mg/kg for 30 days. Group E was exposed to Propoxur for 5 minutes at 3 ppm and received Lycopene at 10 mg/kg for 30 days. Group F was exposed to Propoxur for 1 hour at 3 ppm and received Lycopene at 10 mg/kg for 30 days. All treatments were administered orally.

### Sacrifice of Experimental Animals, Sample Collection and Hormonal Assay

Blood was withdrawn from the apex of the heart (left ventricle) of the thirty male adult wistar rats, which were first anesthetized with 80 mg/kg of ketamine hydrochloride, 12 hours after the last administration just according to Saha *et al.*, 2005<sup>9</sup>. The blood was then dispensed into red-topped tubes for hormonal analysis. The liver was excised following an abdominal incision, and they were fixed in Neutral buffer Formalin for histological analysis. It was then dehydrated progressively in stronger alcohols, cleared in Xylene and infiltrated in paraffin wax, before being embedded in molten paraffin wax. A rotary microtome was then used to slice the paraffin block containing the tissue into 4 µm thick sections. The sections were then transferred to a glass slide, floated in a water bath set at 40 degrees Celsius, and stained with hematoxylin and eosin dyes

### Hormonal Assay

Serum samples were assayed for MDA, Interleukin in batches with the control sera at both physiological and pathological levels by the standard Quantitative Enzyme-Linked Immunosorbent Assay (ELISA) technique with microwell kit which was manufactured by Syngened. The manufacturer instructions that accompanied the assay kits were strictly adhered to.

### Measurement of Body Weight

The weights of the animals were obtained upon arrival and on weekly basis using digital weighing balance scale in order to account for possible results in physical changes in rats upon administration (Propoxur and Lycopene) at regular intervals. The weights are checked for the comparison of possible changes from the initial weight and kept in record.

### STATISTICAL ANALYSIS

The mean and standard error of mean (S.E.M) of all data were calculated. Comparison of means was made by one way analysis of variance (ANOVA) using Graphpad Prism 8. Tukey's test was used to adjust for multiple comparisons. P value < 0.05 was considered to be statistically significant.

### PCSK-9 Assay

Plasma proprotein convertase subtilisin/ kexin type 9 (PCSK-9) levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (manufacturer details). The assay was performed according to the manufacturer's instructions, and absorbance was recorded at 450 nm using a microplate reader (Zhao *et al.*, 2021)<sup>10</sup>.

### Liver Enzyme Assays (ALT, AST, GGT)

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) were determined using colorimetric spectrophotometric methods with commercially available diagnostic kits (manufacturer details). Enzyme activity was measured at specific wavelengths using a spectrophotometer, following the procedure described by Reitman and Frankel (1957)<sup>11</sup> for ALT and AST and Szasz (1969)<sup>12</sup> for GGT.

### Lipid Peroxidation (MDA Assay) and Glutathione Peroxidase (GPx) Activity

The extent of lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid

reactive substances (TBARS) assay (Ohkawa *et al.*, 1979)<sup>13</sup>. Briefly, liver homogenates were reacted with thiobarbituric acid (TBA) at 95°C for 60 minutes, and the absorbance of the resulting pink chromogen was measured at 532 nm.

GPx activity was determined using a coupled enzyme assay based on the oxidation of reduced glutathione (GSH) in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP<sup>+</sup> was monitored spectrophotometrically, following the method of Paglia and Valentine (1967)<sup>14</sup>.

## RESULTS

### BIOCHEMICAL RESULTS

#### Nuclear Factor Kappa Light Chain Enhancer of Activated B Cells

The results in Figure 1 show that Propoxur exposure increases NF-kB levels in a time dependent manner, which indicates inflammatory response. Lycopene alone does not induce inflammation and when co-administered with Propoxur reduces NF-kB levels compared to Propoxur only groups. This showed that lycopene has protective, anti-inflammatory effects against Propoxur induced toxicity.

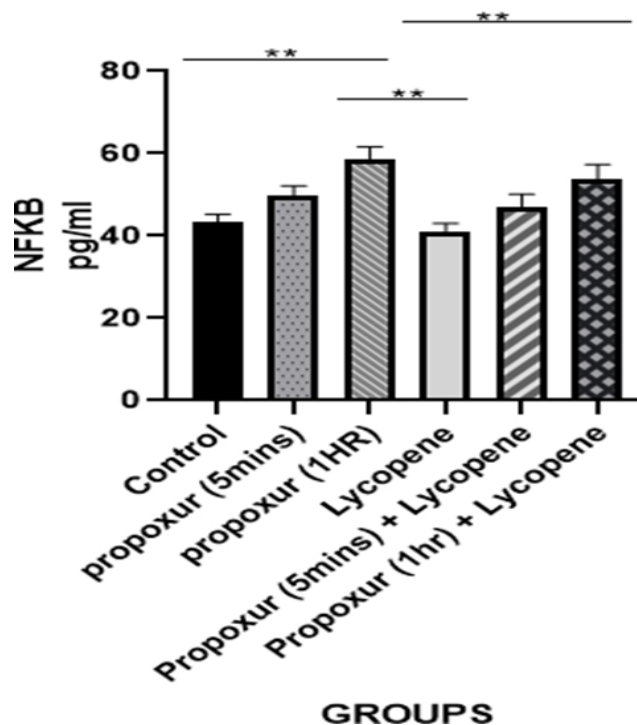
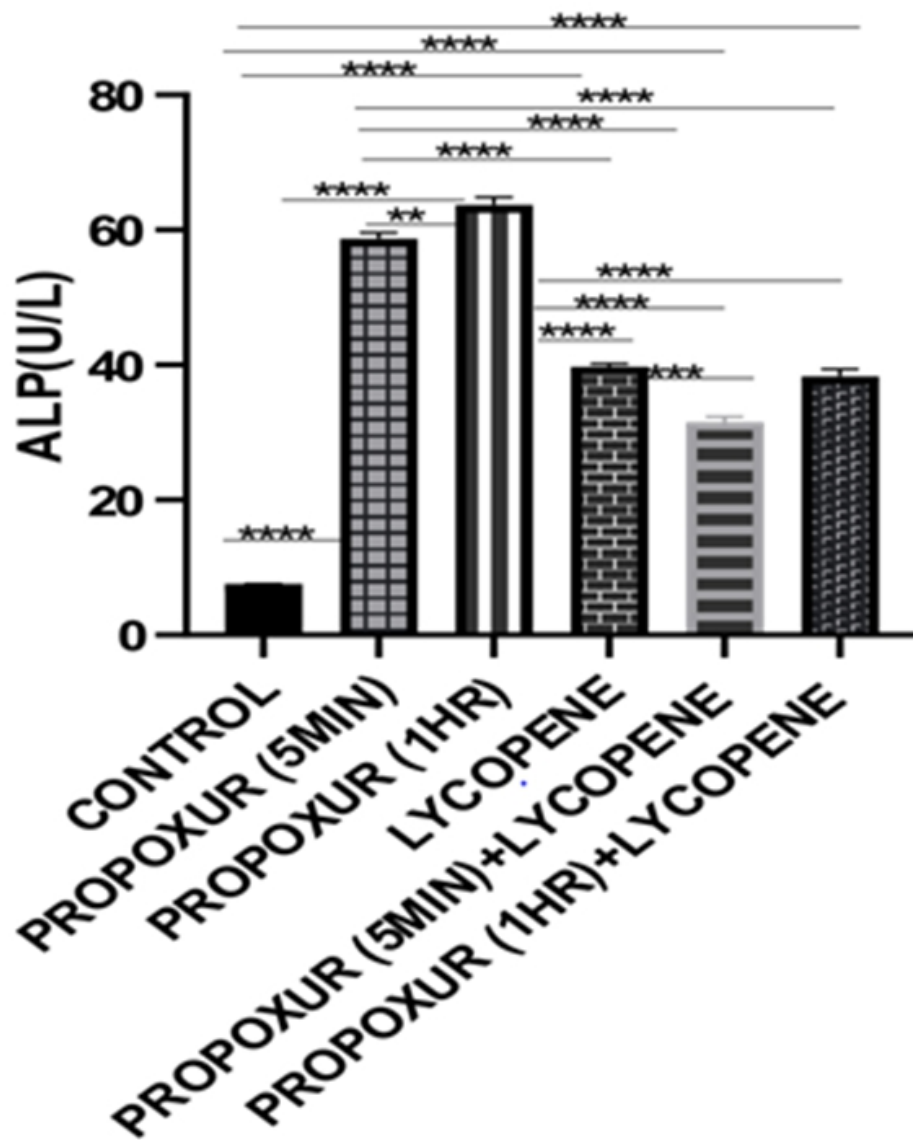


Figure 1: NFKB Analysis among the Groups

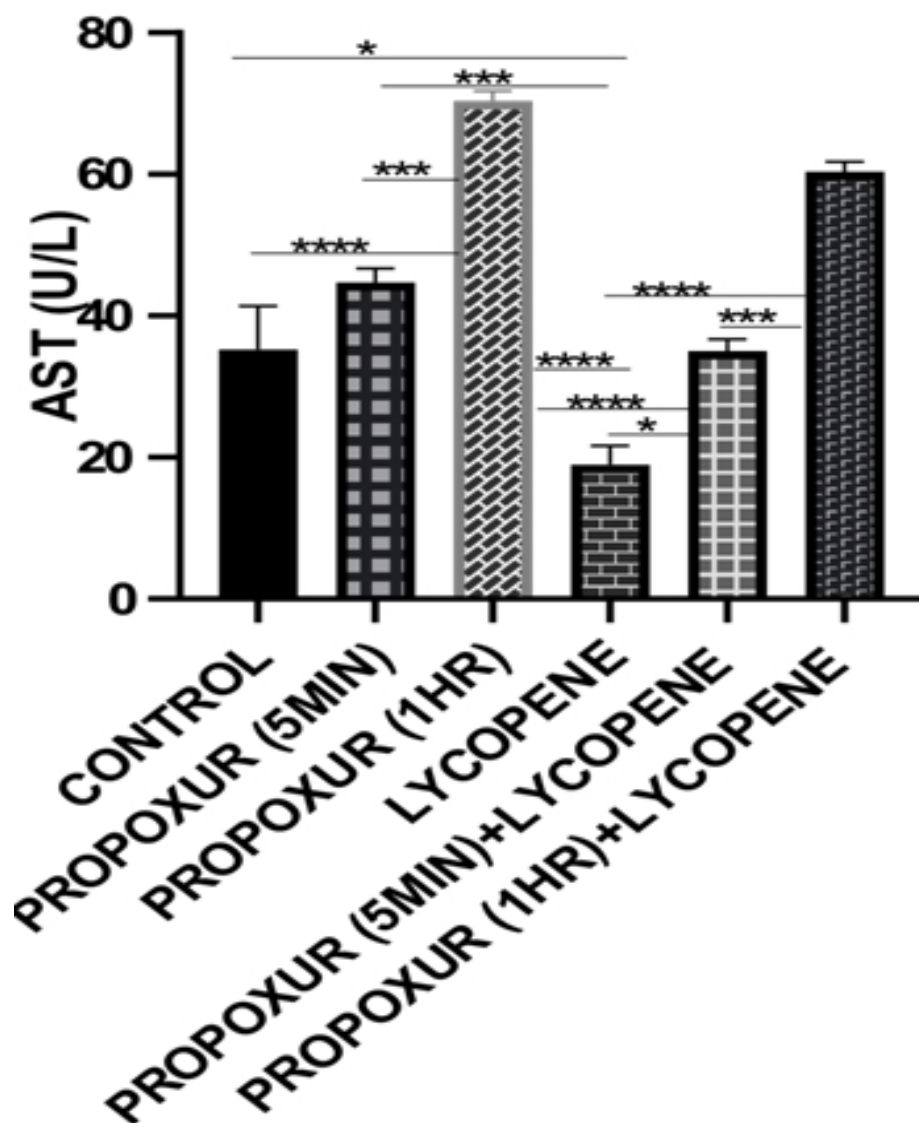
## Alkaline phosphatase (ALP)



**Figure 2:** Alkaline phosphatase Activity across the Experimental Groups

The results in Figure 2 show that Propoxur exposure significantly increases ALP activity in a time-dependent manner, indicating liver damage. Lycopene alone does not affect ALP levels and when combined with Propoxur, it reduces ALP activity compared to Propoxur only group.

## Aspartate Aminotransferase (AST)



**Figure 3:** Level of Aspartate amino transferase (AST) Activity across Different Experimental Groups

The results in Figure 3 show that Propoxur exposure significantly increases AST activity in a time dependent manner, indicating liver damage. Lycopene alone does not affect AST levels and when combined with Propoxur, it reduces AST activity compared to Propoxur only groups.

## OXIDATIVE STRESS PARAMETERS

## Malondialdehyde(MDA)

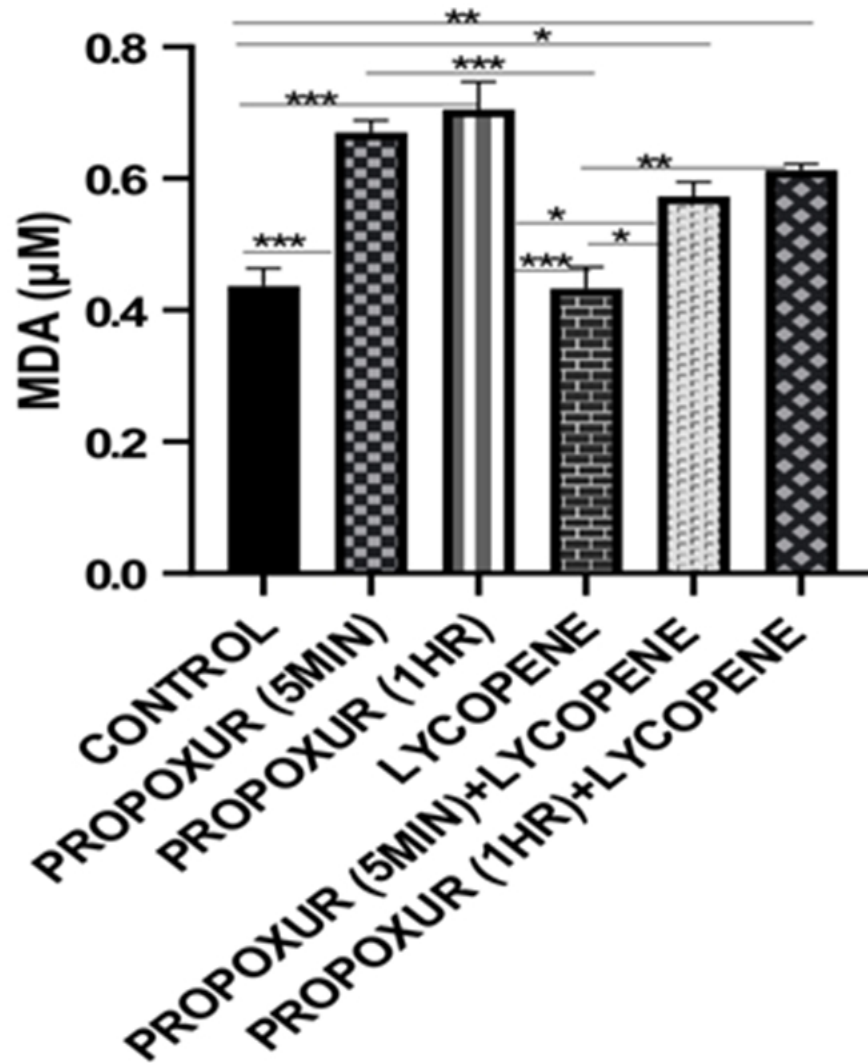


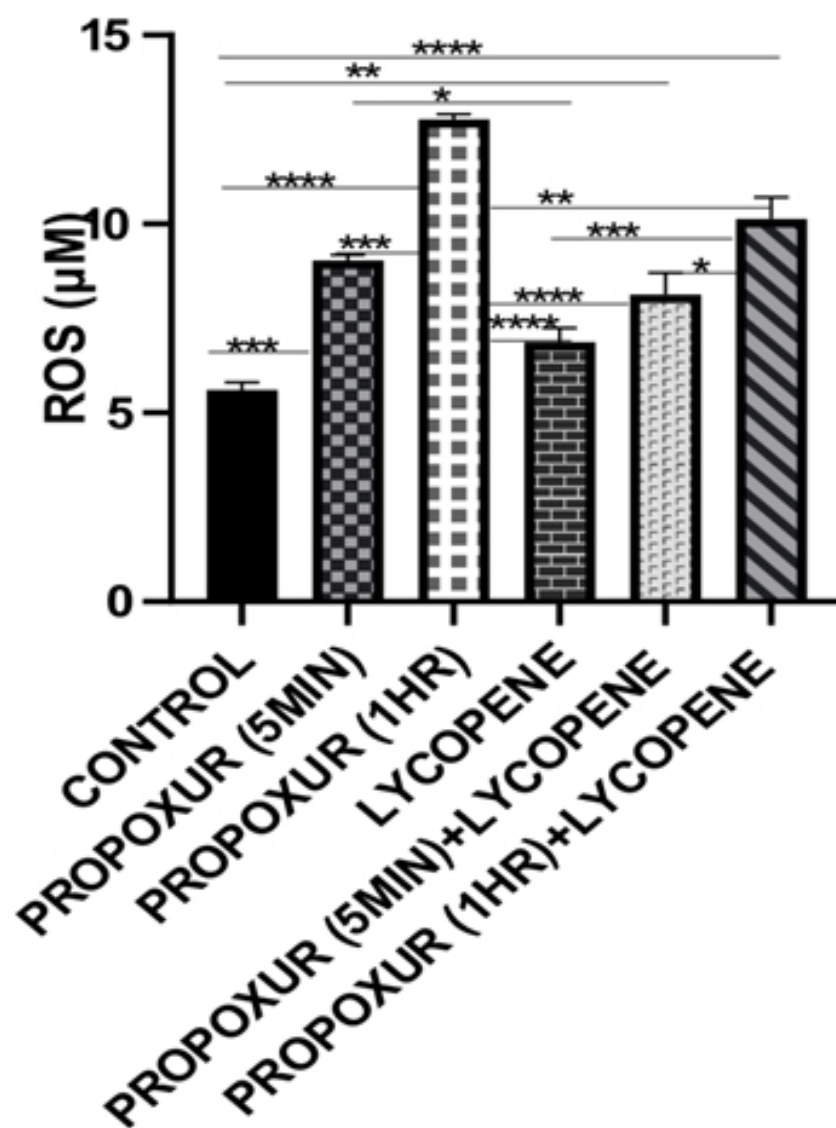
Figure 4: Level of MDA across the Experimental Groups

The results in Figure 4 show that Propoxur exposure significantly increases oxidative stress, as indicated by elevated MDA levels. However, Lycopene reduces these elevated MDA levels.

All data are represented as Mean  $\pm$  SEM

P < 0.05. (\* = p < 0.01, \*\* = p < 0.005, \*\*\* = p < 0.001).

## Reactive Oxygen Species (ROS)



**Figure 5:** Level of ROS across the Experimental Groups

The results in Figure 5 show that Propoxur exposure significantly increases ROS levels, with higher levels observed after prolonged exposure (1 hour). However, Lycopene reduces these ROS levels.

All data are represented as Mean  $\pm$  SEM

$p < 0.05$ . (\*= $p < 0.01$ , \*\*= $p < 0.005$ , \*\*\*= $p < 0.001$ , \*\*\*\*= $p < 0.0001$ ).

Interleukin-6 (IL-6)

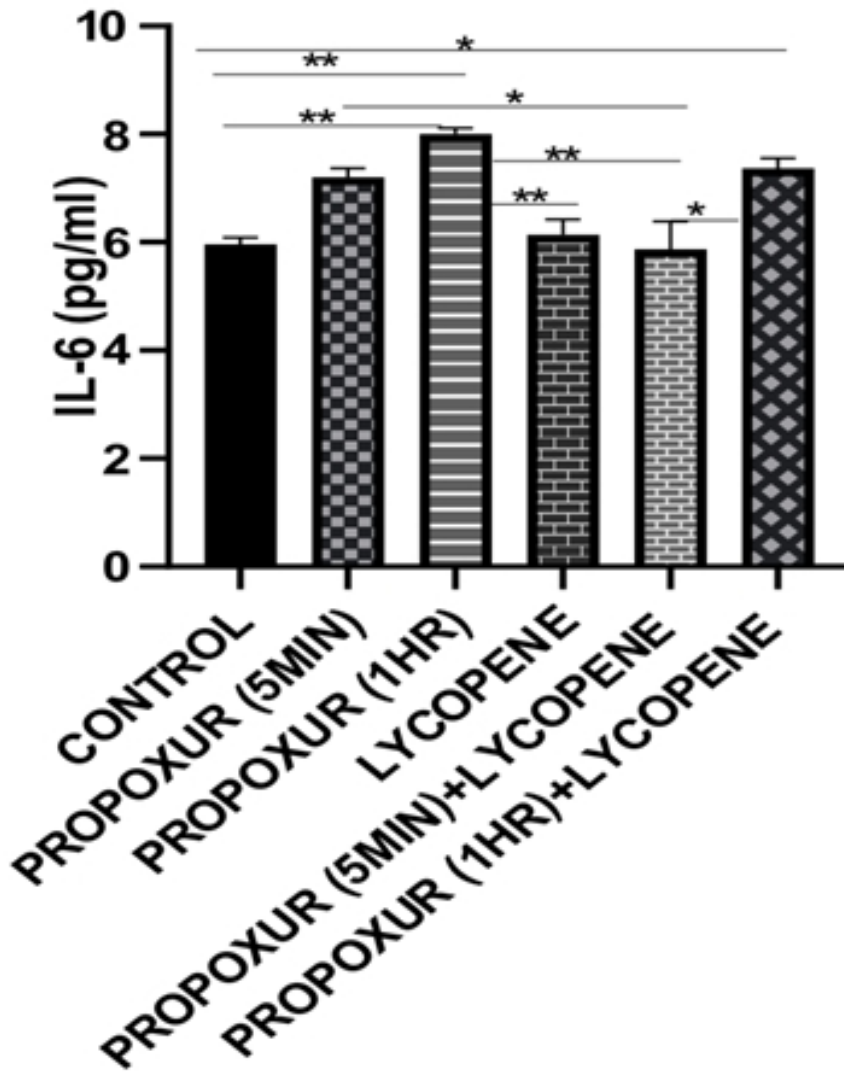


Figure 6: Level of IL-6 across all Experimental Levels

The results in Figure 6 show that Preprosur exposure increases IL-6 levels indicating inflammation, with higher levels observed after prolonged exposure (1 hour). However, Lycopene reduces IL-6 levels.

All data are represented as Mean ± SEM

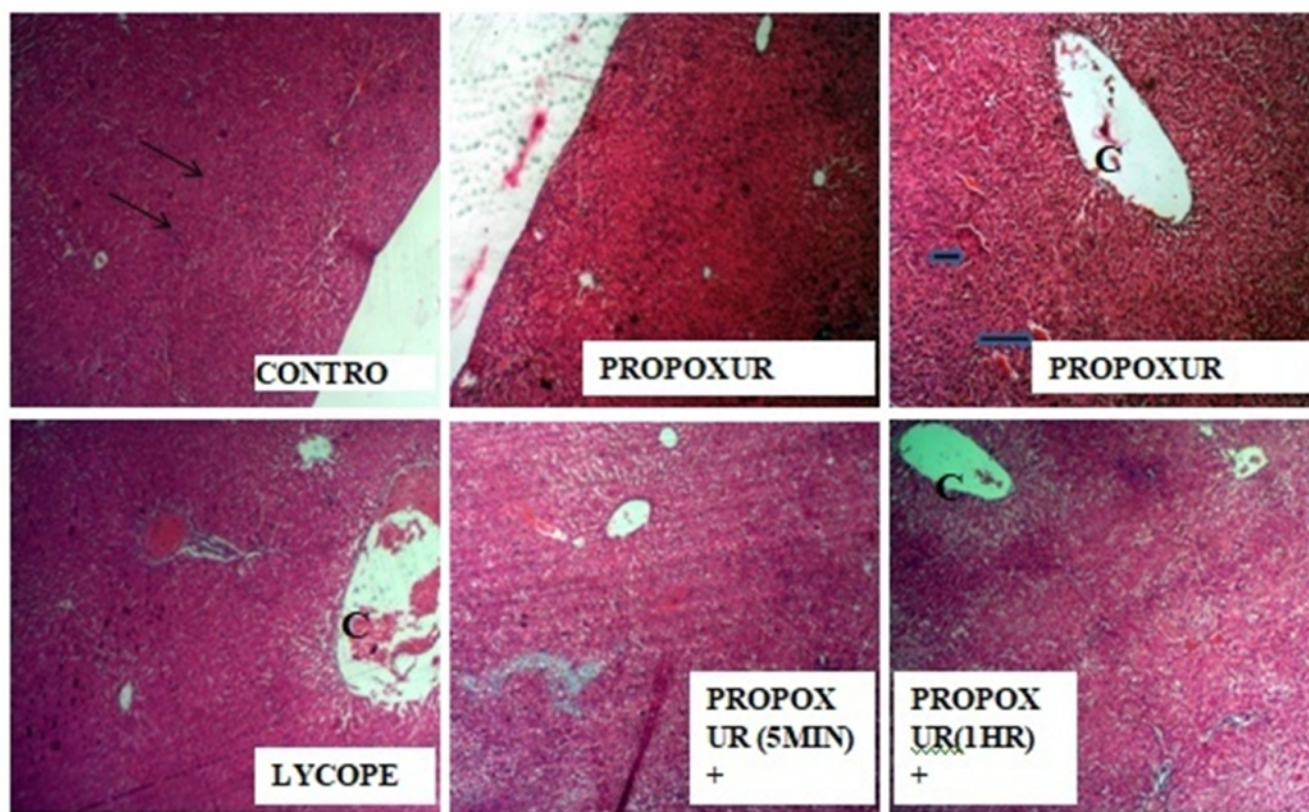
p<0.05. (\*=p<0.01, \*\*=p<0.005).

## HISTOLOGICAL RESULTS

Representative photomicrographs of rat liver by light microscope with H&E staining at X200 magnification. Control liver showing normal hepatocytes arranged in cords, obvious sinusoids. Propoxur (5 mins) and Propoxur (1 hour) section showing degenerated hepatocytes, congested sinusoids, distorted hepatic tissue structure and collection of

inflammatory cells. Lycopene treated rats showing similar architecture like the control. Propoxur (5minutes) + Lycopene, Propoxur (1hour) + Lycopene showed almost the normal appearance of hepatocytes around the central vein (CV) with few inflammatory cells.

Slim Arrow = Hepatocyte, CV = Central vein, Black Arrows = Inflammatory Cells



**Figure 7:** Photomicrograph of Histological Section of the Liver Stained with Hematoxylin and Eosin x200, done and arranged together using PowerPoint Software

## DISCUSSION

The findings of this study provide critical insights into the protective effects of lycopene against propoxur-induced liver injury. Propoxur, a widely used carbamate pesticide, has been shown to cause significant hepatotoxicity through mechanisms involving oxidative stress, inflammation, and disruption of hepatic function. In this study, the administration of propoxur led to marked histological damage to the liver, evidenced by cellular degeneration and necrosis. These structural changes align with previous studies that have demonstrated the hepatotoxic potential of propoxur due to its ability to generate reactive oxygen species (ROS) and disrupt liver enzyme activity<sup>2,3</sup>.

The observed increase in oxidative stress markers such as malondialdehyde (MDA) and ROS further supports the role of oxidative damage in propoxur-induced hepatotoxicity. The elevation of these markers indicates lipid peroxidation and a failure of the liver's antioxidant defense system, which is consistent with prior research (Rezaei *et al.*, 2019). Additionally, the elevated levels of liver enzymes, including alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), confirm liver dysfunction, as these enzymes are commonly released into the bloodstream during hepatic injury<sup>4</sup>. The inflammatory response, marked by an increase in interleukin-6 (IL-6), highlights the role of inflammation in exacerbating liver damage caused by propoxur exposure.

However, the co-administration of lycopene significantly mitigated the adverse effects of propoxur. Lycopene, a potent antioxidant, reduced oxidative stress by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes. This is consistent with findings from earlier studies that reported the ability of lycopene to attenuate oxidative damage in various models of chemical-induced organ injury<sup>5,7</sup>. The reduction in MDA and ROS levels observed in this study suggests that lycopene effectively counteracts lipid peroxidation and oxidative stress, thereby protecting the liver from damage.

Furthermore, lycopene's anti-inflammatory properties were evident in the significant reduction of IL-6 levels in the treated groups. This aligns with previous research showing that lycopene can suppress the production of pro-inflammatory cytokines, thereby mitigating inflammation-associated liver injury<sup>7</sup>. The improvement in liver enzyme levels, along with the preservation of liver histology in lycopene-treated groups, underscores its hepatoprotective effects. These findings

support the hypothesis that lycopene can modulate key pathways involved in oxidative stress and inflammation, such as the xanthine oxidase/uric acid signaling pathway, to protect against liver injury<sup>7,3</sup>. The weight loss observed in rats exposed to propoxur alone is indicative of systemic toxicity, which could be attributed to the metabolic burden and stress induced by the pesticide. Lycopene administration not only reduced liver damage but also helped maintain body weight, suggesting its role in improving overall metabolic health and reducing systemic toxicity.

## CONCLUSION

In conclusion, this study highlights the protective role of lycopene against propoxur-induced liver injury, primarily through the modulation of xanthine oxidase/uric acid signaling. Our findings underscore the potential of lycopene as a natural therapeutic agent in mitigating pesticide-induced oxidative stress and liver damage. This research contributes valuable insights into toxicology and antioxidant therapy, offering a basis for future studies and potential clinical applications.

**CONFLICT OF INTEREST:** None

**FINANCIAL SUPPORT:** None

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