

# The Protective Role of Alpha-Lipoic Acid against Paracetamol-Induced Hematotoxicity in Male Rabbits

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## Abstract:

**Background:** Paracetamol (PAR) is a widely used analgesic that can cause hematological toxicity at high doses through oxidative stress. Alpha-lipoic acid (ALA) is a potent antioxidant known for its cytoprotective properties. **Objective:** This study aimed to evaluate the protective effects of ALA against PAR-induced hematological alterations in male rabbits. **Methods:** Male rabbits were divided into four groups: Control, PAR-treated, ALA-treated, and a combination group (PAR+ALA). Hematological parameters, including Red Blood Cells (RBCs), White Blood Cells (WBCs), Hemoglobin (Hb), Hematocrit (HCT), and Platelets (PLAT), were analyzed. **Results:** The administration of PAR led to a significant decrease in RBC count ( $5.36 \pm 0.111 \times 10^6 \mu\text{L}$ ) and WBC count ( $6.9 \pm 0.37 \times 10^6 \mu\text{L}$ ), suggesting myelosuppression or oxidative damage. In contrast, ALA supplementation markedly improved the erythropoietic profile, significantly increasing RBCs ( $6.79 \pm 0.258 \times 10^6 \mu\text{L}$ ) and HCT ( $45.04 \pm 0.783\%$ ). Interestingly, the PAR+ALA combination group showed a significant recovery in RBC and WBC levels compared to the PAR-only group, indicating a clear protective role for ALA. While Hb and Platelet levels showed no statistically significant differences across groups ( $p > 0.05$ ), a declining trend in platelets was observed in the PAR group, which was stabilized by ALA co-treatment. **Conclusion:** The findings demonstrate that alpha-lipoic acid effectively mitigates the hematotoxic effects of paracetamol in male rabbits. This protection is likely due to ALA's antioxidant capacity, which preserves red cell membrane integrity and supports bone marrow function.

**Keywords:** Paracetamol, Alpha-Lipoic Acid, Antioxidants, Male Rabbits, Oxidative Stress.

## Introduction

The blood-forming system is a highly sensitive target for toxic compounds, and hematological parameters are critical indicators of the health status in both humans and experimental animals. Among commonly used drugs, Paracetamol (Acetaminophen, PAR) is widely prescribed as an analgesic and antipyretic [1]. Although considered safe at therapeutic doses, overdosage or chronic administration can lead to severe side effects, including hepatotoxicity and hematological disturbances such as anemia and leucopenia [2]. These effects are primarily attributed to the induction of oxidative stress and the depletion of cellular antioxidants, leading to damage in blood cell membranes and bone marrow suppression [3-5]. To counteract these toxic effects, there has been a growing interest in using potent antioxidants. Alpha-Lipoic Acid (ALA), also known as thioctic acid, is a naturally occurring disulfide compound that acts as a powerful biological antioxidant. It is unique because of its solubility in both water and fat, allowing it to provide protection in various cellular compartments [6]. ALA plays a vital role in recycling other antioxidants, such as vitamins C and E and glutathione, and has been shown to protect red blood cells (RBCs) from oxidative hemolysis and maintain the integrity of hematopoietic tissues [7]. Given the potential of ALA to mitigate oxidative damage, this

study was designed to investigate the possible protective influence of ALA on the hematological profile of male rabbits exposed to paracetamol. The study specifically evaluates changes in red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), and hematocrit (HCT) to determine the efficacy of ALA in stabilizing the blood system against PAR-induced toxicity.

## Materials and Methods

A total of twenty adult male rabbits were used in this study. The animals were housed in a controlled environment with a 12-hour light/dark cycle and provided with a standard pellet diet and water *ad libitum*. All procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals. The rabbits were randomly assigned to four equal groups ( $n = 5$  per group): Group 1 (CON): Served as the control group and received a normal saline solution. Group 2 (PAR): Rabbits were treated with Paracetamol to induce hematotoxicity. Group 3 (ALA): Rabbits received Alpha-Lipoic Acid supplementation. Group 4 (PAR + ALA): Rabbits were co-treated with Paracetamol and Alpha-Lipoic Acid to evaluate the protective effect. At the end of the experimental period, blood samples were collected from the marginal ear vein of each rabbit. The samples were gathered in EDTA-containing tubes to prevent clotting and were immediately

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processed for hematological analysis. The complete blood count (CBC) was performed using an automated hematology analyzer. The parameters measured included: Red Blood Cell count (RBC), White Blood Cell count (WBC), Hemoglobin concentration (Hb), Hematocrit (HCT) or Packed Cell Volume (PCV) and Platelet count (PLAT): Statistical Analysis Data were expressed as Mean  $\pm$  Standard Error (SE). Statistical significance was determined using one-way Analysis of Variance (ANOVA), followed by a suitable post-hoc test (e.g., Tukey or Duncan) to compare differences between groups. A p-value of less than 0.05 ( $p < 0.05$ ) was considered statistically significant.

## Results

The effects of paracetamol (PAR), alpha-lipoic acid (ALA), and their combination on male rabbits' red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (HCT), and platelet count (PLAT) are shown by the hematological analysis in Table 1 and Figures 1–5. The mean Hb values in the ALA group ( $14.48 \pm 0.13$  g/dL) were marginally higher than those in the control group ( $14.03 \pm 0.15$  g/dL) and PAR group ( $13.93 \pm 0.22$  g/dL), indicating an erythropoietic or protective effect of ALA on hemoglobin synthesis, even though there was no statistically significant difference in hemoglobin levels among the experimental groups ( $p > 0.05$ ). There were notable differences in the RBC count between the groups. When paracetamol was administered, the RBC count significantly decreased ( $5.36 \pm 0.111 \times 10^6/\mu\text{L}$ ), suggesting that PAR may have a hemolytic or myelosuppressive impact that is linked to either bone marrow suppression or oxidative stress.

On the other hand, ALA markedly increased the RBC count ( $6.79 \pm 0.258 \times 10^6/\mu\text{L}$ ), which could be due to its antioxidant capacity to promote erythropoiesis and repair red cell membranes. It's interesting to note that the PAR+ALA combo group had better RBC levels ( $6.51 \pm 0.352 \times 10^6/\mu\text{L}$ ), which may indicate that ALA lessens the hematotoxic effects of PAR. The trend for white blood cell numbers was comparable. WBCs were considerably lower in the PAR group ( $6.9 \pm 0.37 \times 10^3/\mu\text{L}$ ) than in the control group ( $8.36 \pm 0.11 \times 10^3/\mu\text{L}$ ), which may have been brought on by bone marrow toxicity or immunosuppression brought on by PAR. Nonetheless, WBC levels in the ALA ( $8.4 \pm 0.12 \times 10^3/\mu\text{L}$ ) and PAR+ALA ( $8.26 \pm 0.18 \times 10^3/\mu\text{L}$ ) groups stayed close to normal, confirming ALA's immunomodulatory or protective function. Although there were no statistically significant changes in platelet counts (PLAT) across any of the groups, the PAR group had a declining trend ( $244.27 \pm 11.72 \times 10^3/\mu\text{L}$ ), which may indicate moderate thrombocytopenia. The combination group maintained a platelet count of  $256.42 \pm 9.42 \times 10^3/\mu\text{L}$ , while ALA supplementation slightly increased platelet counts ( $262.60 \pm 12.79 \times 10^3/\mu\text{L}$ ), confirming the idea of hematological stability with ALA co-treatment. Lastly, the therapies had a considerable impact on the hematocrit (HCT) levels. Administration of ALA markedly increased HCT ( $45.04 \pm 0.783\%$ ), suggesting improved control of plasma volume or red cell mass. The lowest HCT ( $40.26 \pm 0.712\%$ ) was obtained with PAR therapy, suggesting its suppressive effect on erythropoiesis. The HCT levels of the PAR+ALA group were intermediate ( $42.84 \pm 0.626\%$ ), confirming ALA's partial protective impact.

**Table 1.** Shifts Male rabbits treated with PRA, ALA, and their combination had complete blood counts that included red blood cells "RBCs", white blood cells "WBCs", hemoglobin "Hb", packed cell volume "PCV", platelets, and hemoglobin "Hb".

Parameter	Experimental groups			
	CON	PAR	ALA	PAR+ALA
Hb (g/dl)	$14.03 \pm 0.15^a$	$13.93 \pm 0.22^a$	$14.48 \pm 0.13^a$	$14.08 \pm 0.19^a$
RBC $\times 10^6$ ( $\mu\text{L}$ )	$6.06 \pm 0.108^{bc}$	$5.36 \pm 0.111^c$	$6.79 \pm 0.258^a$	$6.51 \pm 0.352^b$
WBC $\times 10^3$ ( $\mu\text{L}$ )	$8.36 \pm 0.11^a$	$6.9 \pm 0.37^b$	$8.4 \pm 0.12^a$	$8.26 \pm 0.18^a$
PLAT $\times 10^3$ ( $\mu\text{L}$ )	$284.49 \pm 9.879^a$	$244.27 \pm 11.721^a$	$262.60 \pm 12.793^a$	$256.42 \pm 9.421^a$
HCT $\times 10^3$ ( $\mu\text{L}$ )	$40.31 \pm 0.354^c$	$40.26 \pm 0.712^{bc}$	$45.04 \pm 0.783^a$	$42.84 \pm 0.626^{ab}$

Values are expressed as means  $\pm$  SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c) were significantly different,  $p < 0.05$ .

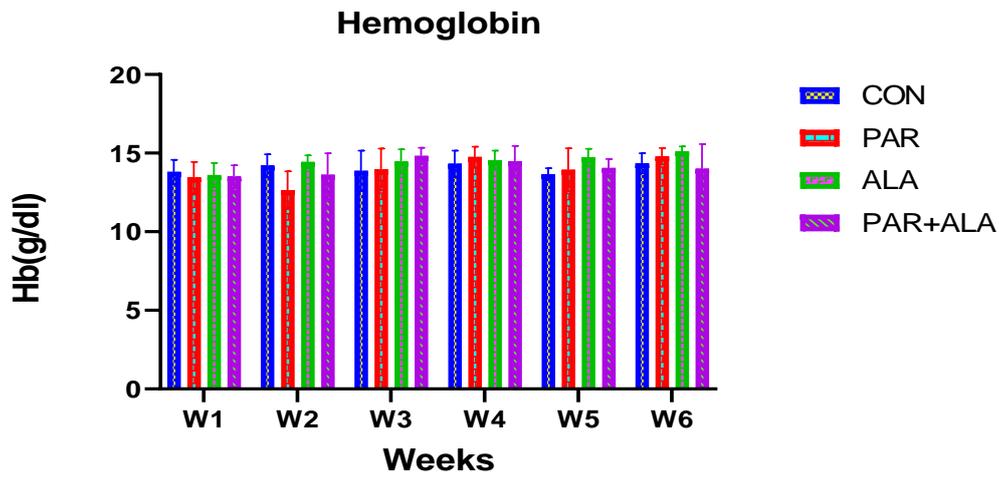


Figure 1. Changes in hemoglobin Hb during treatment of male rabbits with PRA, ALA, and their combination.

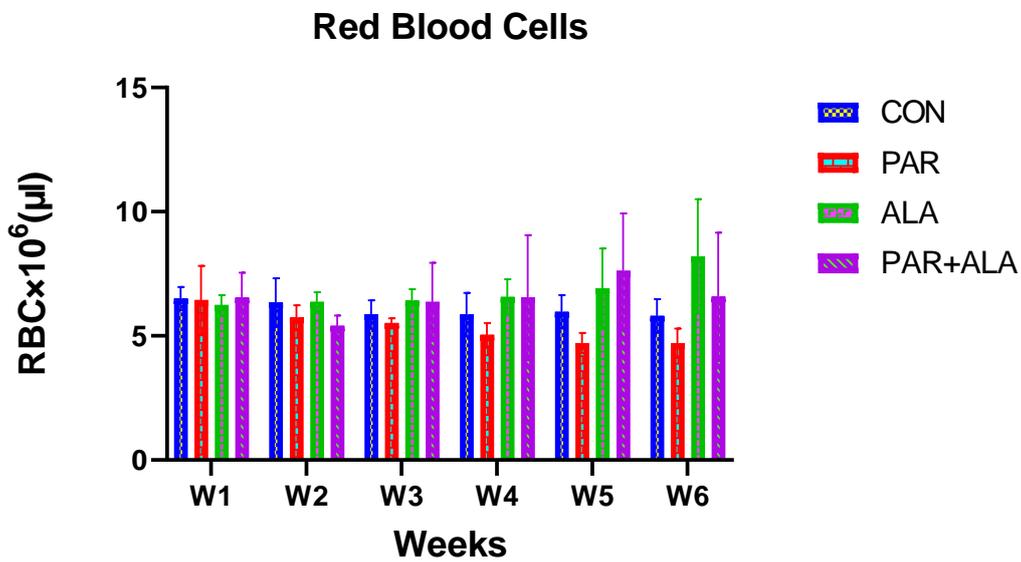


Figure 1. Changes in Red blood cell RBCs during treatment of male rabbits with PRA, ALA, and their combination.

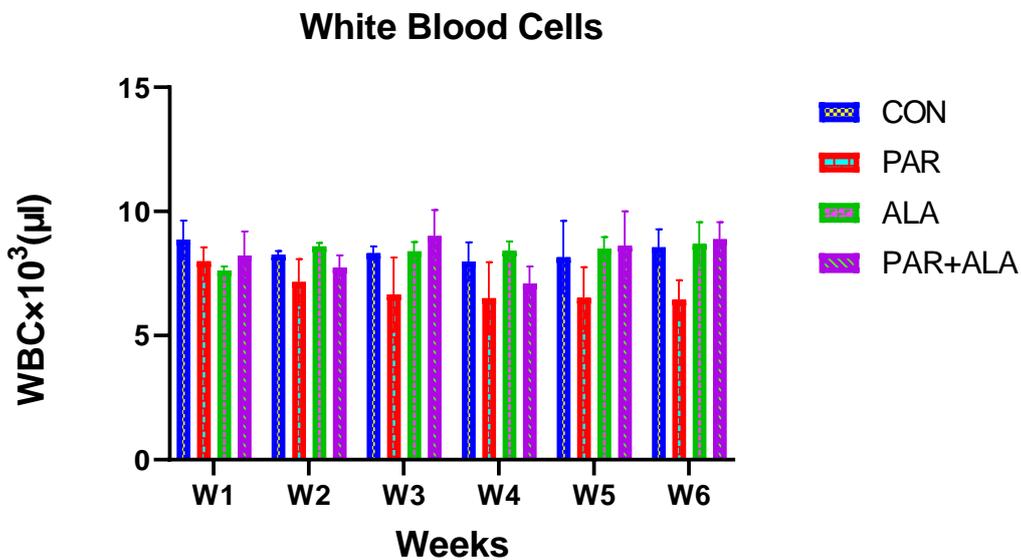


Figure 3. Changes in White blood cell WBCs during treatment of male rabbits with PRA, ALA, and their combination.

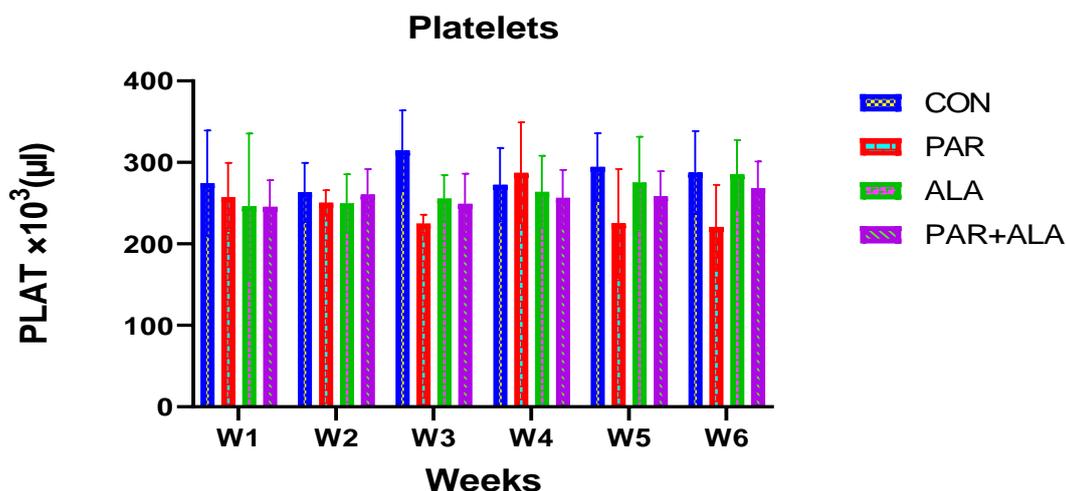


Figure 4. Alterations in platelets "plat" when male rabbits are treated with PRA, ALA, and their combination.

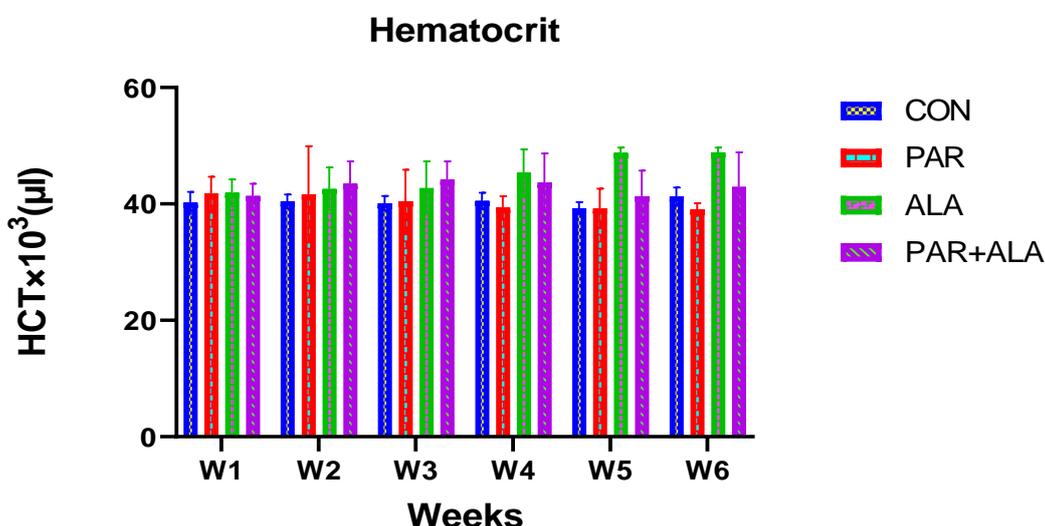


Figure 5. Alterations in hematocrit HCT when male rabbits are treated with PRA, ALA, and their combination.

## Discussion

The results of the present study demonstrate that paracetamol (PAR) administration induces significant hematological alterations, while alpha-lipoic acid (ALA) exerts a potent protective effect, maintaining blood parameters near physiological levels. The significant reduction in RBC count and HCT levels observed in the PAR group suggests a state of anemia, likely due to the oxidative stress induced by PAR metabolites. It is well-established that paracetamol overdose leads to the formation of N-acetyl-p-benzoquinone imine (NAPQI), which depletes glutathione (GSH) and causes oxidative damage to red blood cell membranes [8-20]. Furthermore, the decline in WBC count indicates a myelosuppressive effect, where PAR may interfere with leukopoiesis in the bone marrow or cause increased destruction of circulating leucocytes [21-30]. Interestingly, the ALA-treated group showed the highest RBC and HCT values. This enhancement can be attributed to ALA's role as a "universal antioxidant." ALA and its reduced form, dihydrolipoic acid (DHLA), help regenerate other antioxidants like Vitamin E and C, which are crucial for protecting

erythrocyte membranes from lipid peroxidation [31-42]. In the PAR+ALA group, the restoration of and WBC levels to near-control values highlights the antitoxic potential of ALA. By neutralizing NAPQI and replenishing intracellular glutathione stores, ALA effectively shielded the hematopoietic system from PAR-induced toxicity. This is consistent with findings by [43-44], who noted that ALA administration prevents oxidative stress-related damage in various tissues. Although Hb and PLAT levels did not show statistically significant changes ( $p > 0.05$ ), the declining trend in the PAR group points toward sub-clinical thrombocytopenia. The stabilization of these values in the combination group further reinforces the idea that ALA provides a "hematological buffer," ensuring that even under chemical stress, blood stability is maintained.

**Conclusion:** ALA effectively mitigates paracetamol-induced hematotoxicity by restoring blood cell counts and protecting against oxidative damage. These findings suggest that ALA is a potent protective agent for maintaining hematological stability during chemical stress.

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