Medical Technology Journal of Applied Science (MTJAS)

Volume 2, Issue 2, 2026, Page No: 13-19
Website: https://mtjas.com.ly/index.php/mtjas/index

Paracetamol-Induced Splenic Toxicity and the Protective Role of Vitamin C in Albino Rats

Ahlaam M. Khalid[1], Eda M.A. Alshailabi [2]*, Ola A. Abdalally[3]

[1] Higher Institute of Medical Sciences and Technology, Laboratory Department, El-Beyda, Libya.
[2] Omar Al-Mukhtar University, Zoology Department, Faculty of Science, El-Beyda, Libya.
[3] Almahara institute for the medical and managerial sciences, El-Beyda, Libya.

*Corresponding author: eda.muftah@omu.edu.ly

Received: January,03, 2026	Accepted: February, 01, 2026	Published: February, 02, 2026

Abstract:
Paracetamol (PCM) is widely used as an analgesic and antipyretic agent; however, overdose can result in systemic toxicity, primarily mediated through oxidative stress. This study aimed to evaluate the histopathological effects of PCM on the spleen of albino rats and to assess the potential protective effect of vitamin C (VC) as an antioxidant. Twenty-eight albino rats were randomly assigned to five groups: control, VC (500 mg/kg), PCM (500 mg/kg), PCM + VC co-treatment, and a prophylactic group receiving VC before PCM administration. Histological examination of the spleen revealed alterations in the PCM-only group, including lymphoid depletion in the white pulp and congestion in the red pulp. Co-administration of VC attenuated these changes, while pre-treatment with VC provided the greatest preservation of splenic architecture. These findings suggest a potential protective benefit of VC against PCM-induced oxidative damage in the spleen.

Keywords: Paracetamol, Spleen, Vitamin C, Albino rats, Histopathology.

Introduction

Paracetamol (acetaminophen, PCM) is one of the most widely used analgesic and antipyretic agents worldwide due to its efficacy and general safety at therapeutic doses. However, overdose or prolonged high-dose administration can cause systemic toxicity, primarily affecting the liver, kidneys, and, increasingly recognized, the spleen—a vital organ of the immune system (Mandal *et al.,* 2016; Kennon-McGill & McGill, 2017; Alshailabi *et al.,* 2021). PCM-induced toxicity is mediated through the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes cellular glutathione (GSH) and triggers oxidative stress, mitochondrial dysfunction, and cellular apoptosis or necrosis (Jaeschke *et al.,* 2018; Alshailabi *et al.,* 2021). While hepatic toxicity due to PCM overdose has been extensively studied, the effects on immune organs, such as the spleen, remain relatively underexplored. Evidence indicates that the spleen can act as an extramedullary source of inflammatory cells in response to acetaminophen-induced liver injury, highlighting its crucial role in immune cell production and inflammatory responses (Mandal *et al.,* 2016). The spleen consists of two major compartments: white pulp, which is rich in lymphocytes and contributes to adaptive immune responses, and red pulp, responsible for blood filtration, iron recycling, and removal of damaged erythrocytes. Disruption of these structures due to oxidative stress or toxic insults can lead to architectural disorganization, lymphoid depletion, and impaired immune function (Elmore, 2006). Experimental studies have demonstrated that reactive oxygen species (ROS) play a central role in mediating these histological changes, suggesting that oxidative stress is a key mechanism underlying PCM-induced tissue injury (Du *et al.,* 2016; Elduob *et al.,* 2023). ROS are naturally generated during cellular metabolism and participate in essential cellular processes. However, excessive accumulation of ROS causes oxidative damage (OD) to membranes, proteins, and DNA. Antioxidants counteract these effects by neutralizing ROS and limiting oxidative injury, thereby protecting cellular structures and enhancing the body's defense mechanisms against oxidative stress (Alshailabi, 2016; Kozlov *et al.,* 2024). Among these, Vitamin C (VC, ascorbic acid) is a potent water-soluble antioxidant that scavenges free radicals, regenerates other antioxidants such as vitamin E, and contributes to maintaining intracellular GSH levels (Lee *et al.,* 2023; Alshailabi *et al.,* 2024; Ogunleye *et al.,* 2025). In various experimental models, VC has been shown to reduce oxidative stress, inhibit lipid peroxidation (LPO), and improve histological architecture in tissues exposed to toxic agents, including the liver, kidney, and heart (Alshailabi *et al.,* 2021; Ogunleye *et al.,* 2025).

The potential role of VC in protecting the spleen from PCM-induced oxidative damage has not been fully elucidated, providing a strong rationale for the present study. Beyond directly neutralizing ROS, antioxidants such as VC may modulate signaling pathways involved in cellular survival and apoptosis. For example, by reducing mitochondrial oxidative stress, VC can prevent the release of pro-apoptotic factors such as cytochrome c, thereby preserving tissue integrity and immune function (Rössig *et al.,* 2001; Alshailabi *et al.,* 2024). Given the spleen's central role in immunity and hematopoiesis, interventions that mitigate oxidative stress may have profound implications for maintaining systemic immune competence during drug-induced stress (Manful *et al.,* 2025). This study aims to investigate the histopathological effects of PCM on the spleen of albino rats and to evaluate the protective potential of VC, both when administered concurrently with PCM and as a prophylactic pre-treatment. Understanding these interactions may provide insights into the therapeutic use of antioxidants in mitigating drug-induced organ toxicity and preserving immune function.

Material and Methods

Experimental Animals and Design

Twenty-eight adult albino rats of comparable body weights (200–250 g) were obtained from the Central Animal House, College of Veterinary Medicine, University of Omar Al-Mukhtar, El-Beyda, Libya. Animals were maintained under standard laboratory conditions (22–25 °C, 12 h light/12 h dark cycle) with free access to food and water. All experimental procedures were performed in compliance with institutional and international guidelines for the ethical use of laboratory animals.

After a one-week acclimatization period, rats were randomly assigned to five groups (n = 7 per group):

1. Control Group: received distilled water orally throughout the experimental period.
2. VC Group: received VC at a dose of 500 mg/kg/day orally for two weeks (Başeğmez *et al.,* 2025).
3. PCM Group: received PCM at 500 mg/kg/day orally for two weeks (Venkatesan *et al.,* 2014).
4. VC + PCM (Co-treatment) Group: received both VC and PCM simultaneously at the above doses for two weeks.
5. Protective VC (Pre-treatment) Group: received VC at 500 mg/kg/day orally for one week before PCM administration (500 mg/kg/day) for one additional week.

At the end of the experimental period, animals were humanely euthanized, and spleen samples were collected for histopathological analysis.

Histopathological Examination of Spleen

Spleen tissue samples from all groups were immediately fixed in 10% neutral buffered formalin. Following routine tissue processing, samples were embedded in paraffin, and 5 µm sections were cut and stained with hematoxylin and eosin (H&E) for light microscopic examination (Bancroft & Gamble, 2008).

Histopathological evaluation focused on the white pulp and red pulp architecture, including lymphocyte density, pulp organization, and evidence of congestion or cellular deterioration. A semi-quantitative scoring system was employed to grade the severity of splenic lesions as absent (–), mild (+), moderate (++), or severe (+++) according to the criteria adapted from (Landmann *et al.,* 2021; Alshailabi *et al.,* 2024).

All observations were conducted blindly to prevent observer bias, and representative micrographs were captured for documentation and comparative analysis.

Semi-quantitative Histopathological Assessment

Semi-quantitative histopathological scoring data were expressed as descriptive observations and mean scores for each experimental group, as described previously, where the differences in lesion severity among groups were assessed based on the semi-quantitative scoring system. Results are presented descriptively in tables and illustrated with color-coded heatmaps (Landmann *et al.,* 2021). No inferential statistical analysis was performed, as the study was designed as a descriptive histopathological evaluation.

Results

Histopathological Findings in the Spleen

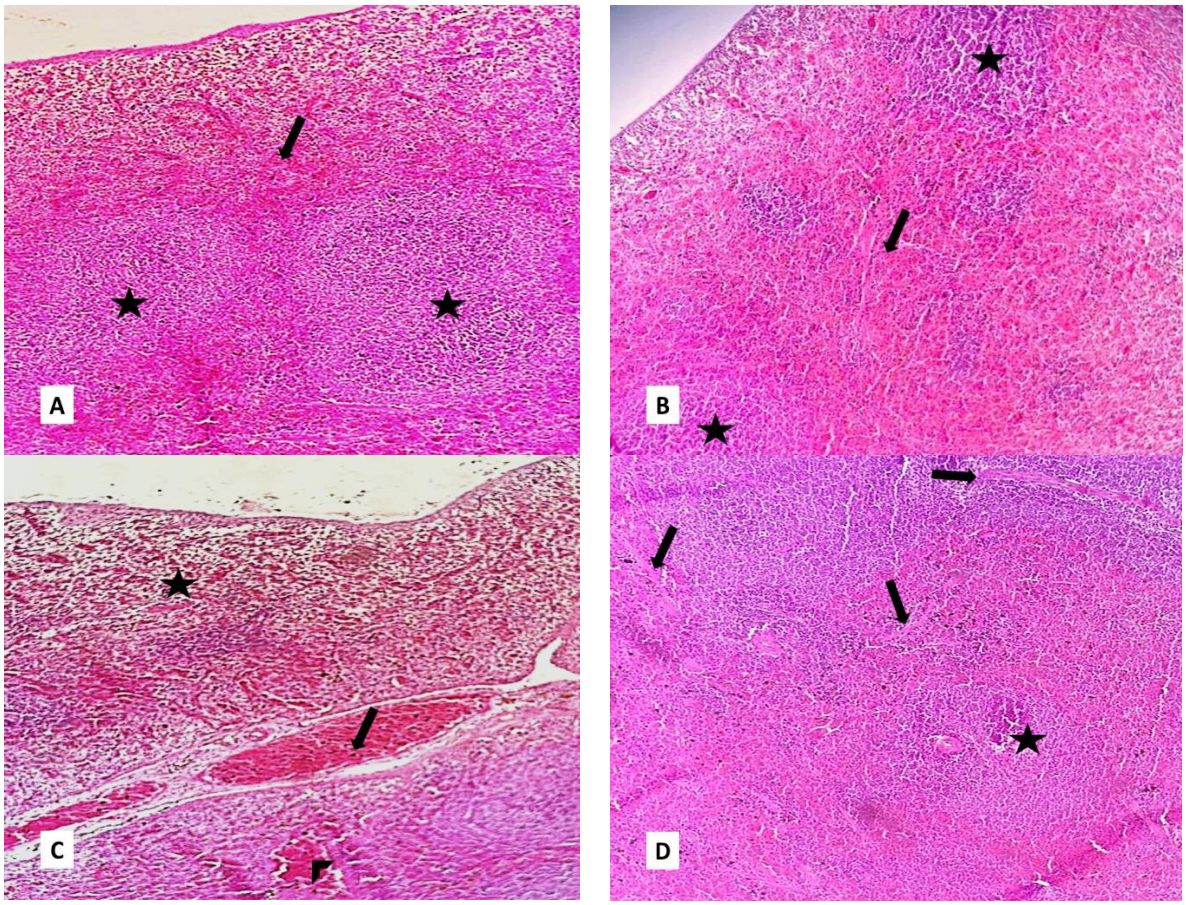
Histological examination of spleen sections from the control group revealed a normal splenic architecture, characterized by a clear distinction between the white pulp and red pulp compartments (Figure 1A). The white pulp contained well-organized lymphoid follicles with normal lymphocyte density surrounding central arterioles, while the red pulp exhibited intact sinusoids and typical cellular composition. Similarly, sections from the VC-treated group displayed preserved architecture, with no detectable histopathological alterations (Figure 1B).

In contrast, the PCM-treated group showed pronounced splenic alterations. Disorganization of the white pulp was accompanied by a marked reduction in lymphocyte density, while the red pulp exhibited significant congestion, sinusoidal dilation, and degenerative changes in splenic parenchymal cells. Focal areas of hemorrhage and partial disruption of the splenic framework were also observed. These observations corresponded to moderate to severe (++/+++) semi-quantitative scores for white pulp integrity, red pulp congestion, and cellular deterioration (Figure 1C–D, Table 1).

Spleen sections from the co-treatment group (PCM + VC) demonstrated moderate histopathological changes, including mild vacuolar deterioration of the red pulp, moderate sinusoidal congestion, and small focal necrotic areas. Semi-quantitative scoring indicated mild to moderate (+/++) alterations across all parameters, suggesting a partial protective effect of VC (Figure 1E–F, Table 1).

In the prophylactic (pre-treatment) group, spleen architecture was relatively well-preserved. White pulp regions were clearly defined around central arterioles, while red pulp was diffusely distributed. Nonetheless, a slight reduction in lymphoid follicle density and moderate red pulp congestion were observed, along with a prominent focal necrotic area. Semi-quantitative scoring reflected mild to moderate (+/++) lesions, indicating that pre-treatment with VC conferred better protection than concurrent administration (Figure 1G–H, Table 1).

Collectively, these findings indicate that PCM induces significant histopathological alterations in the spleen, affecting both the white and red pulp compartments. In contrast, VC, particularly when administered prophylactically, provides substantial protective effects against PCM-induced splenic injury (Figures 1 & 2, Table 1).



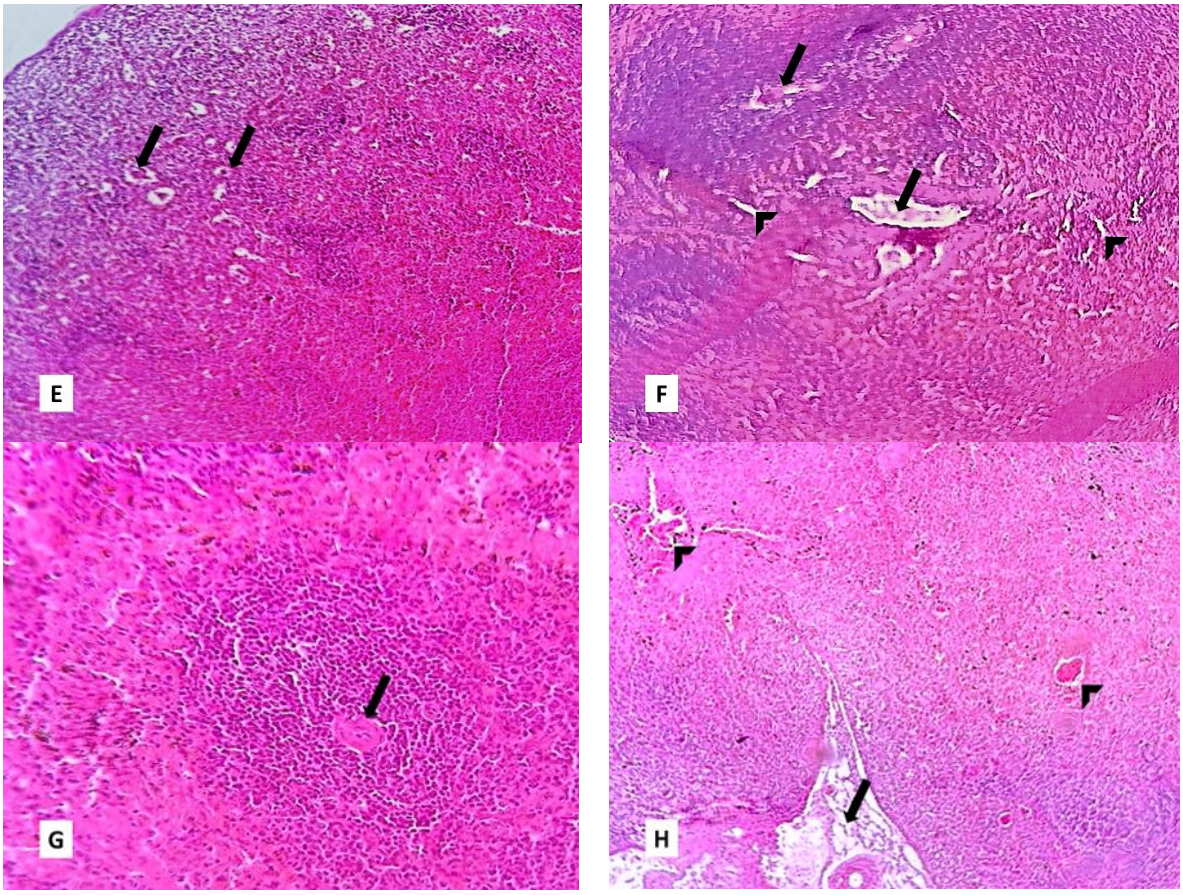


Figure 1: Histopathological changes in splenic tissue of experimental groups.
Sections of the spleen from the control group and the VC group (A & B) revealed a typical splenic architecture, characterized by a clear distinction between the white pulp (stars) and red pulp (arrows) regions. Lymphoid cells were regularly distributed within the white pulp (H&E, x100).
Spleen sections from the PCM-treated group demonstrated marked histopathological alterations, including disorganization of the white pulp, accompanied by a noticeable reduction in lymphocyte density with congestion and dilation of the vascular sinusoids within the red pulp (arrows), and degenerative changes in immune cells, along with mild disruption of the fundamental splenic architecture (stars) (C & D). Also, massive hemorrhage (head arrow) (C) (H&E, x100).
Spleen sections from the co-treatment group revealed marked histopathological alterations, including mild vacuolar deterioration in the red pulp (arrows) (E), congested, dilated splenic sinuses (arrows) with necrotic areas (head arrows) (F) (H&E, x100).
Spleen sections from the pre-treatment group revealed marked histopathological alterations, including preserved architecture, ill-defined spleen tissue with diffuse red pulp, which surrounds clearly defined white pulp with a central arteriole (arrow) (G) (H&E, x400), a reduction in lymphoid follicle density with congestion and dilation of splenic sinuses (head arrows) with a large focal area of necrosis (H) (arrow) (H&E, x100).

Table 1: Semi-quantitative Histopathological Scoring.

Group	White pulp integrity	Red pulp congestion	Cellular deterioration
Control	–	–	–
VC	–	–	–
PCM	++/+++	++/+++	++/+++
Co-treatment (PCM + VC)	+/++	+/++	+/++
Pre-treatment (VC → PCM)	+/++	+/++	+/++

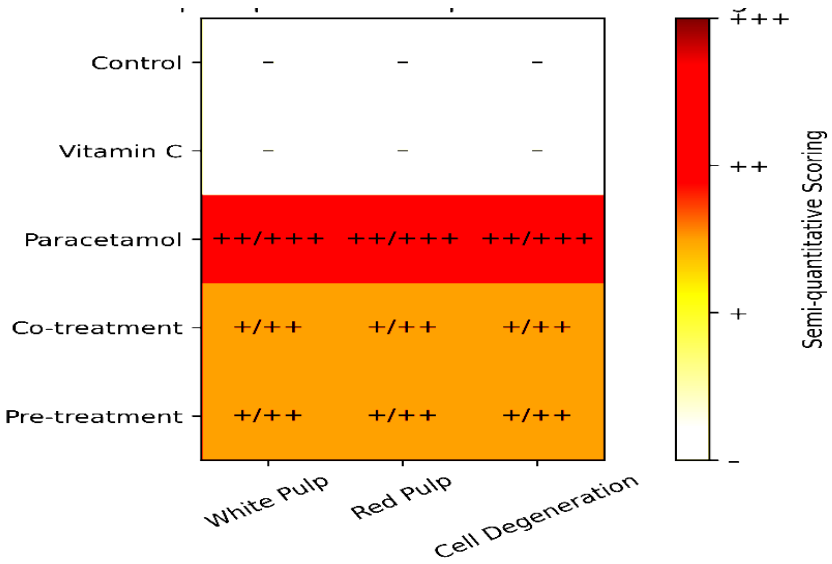


Figure 2: Descriptive representation of semi-quantitative histopathological scoring of splenic lesions across experimental groups.

Discussion

The present study investigated the histopathological effects of PCM on the spleen of albino rats and evaluated the potential protective role of VC as an antioxidant. The results clearly demonstrate that PCM induces significant alterations in splenic architecture, including disorganization of the white pulp, reduction in lymphocyte density, congestion and dilation of red pulp sinusoids, and degenerative changes in parenchymal cells. In some sections, focal hemorrhage was also observed. These findings indicate that the spleen, a central organ in immune surveillance and erythrocyte clearance, is highly susceptible to PCM-induced oxidative stress, extending the well-established knowledge of PCM hepatotoxicity to immune tissues (Mandal *et al.,* 2016; Kennon-McGill & McGill, 2017; Alshailabi *et al.,* 2021).The underlying mechanism of PCM-induced splenic injury is likely mediated by the reactive metabolite NAPQI, which depletes intracellular GSH, disrupts mitochondrial function, and triggers oxidative stress, apoptosis, and necrosis of splenic cells (Du *et al.,* 2016; Kennon-McGill & McGill, 2017; Jaeschke *et al.,* 2018). The semi-quantitative scoring results (++/+++) reflected severe damage in white pulp integrity, red pulp congestion, and cellular deterioration, corroborating the histological observations (Elmore, 2006). Lymphoid depletion in the white pulp may compromise adaptive immune responses, whereas red pulp congestion could impair blood filtration and the removal of damaged erythrocytes, potentially leading to systemic immunological consequences (Mandal *et al.,* 2016). On the other hand, VC demonstrated a notable protective effect against PCM-induced splenic injury. In the co-treatment group (PCM + VC), histological alterations were attenuated, as evidenced by mild vacuolar degeneration, moderate sinusoidal congestion, and limited necrotic foci (+/++). The prophylactic (pre-treatment) group exhibited the most preserved splenic architecture, with largely intact white pulp and reduced red pulp congestion. This superior protective effect observed in the pre-treatment group is likely attributable to priming of the antioxidant defense system, replenishment of intracellular glutathione (GSH), and stabilization of mitochondrial function before PCM exposure (Lee *et al.,* 2023; Kozlov *et al.,* 2024; Alshailabi *et al.,* 2024). These findings are consistent with previous reports demonstrating that VC protects hepatic, renal, and cardiac tissues from chemically induced oxidative damage by scavenging free radicals, inhibiting lipid peroxidation, and enhancing cellular antioxidant capacity (Alshailabi *et al.,* 2021; Ogunleye *et al.,* 2025). Comparing our results with the limited literature on PCM's effects on lymphoid organs, the current study supports the notion that PCM toxicity is not restricted to the liver but also extends to immune tissues, potentially impairing systemic immunity. Moreover, the results emphasize the dose- and timing-dependent efficacy of antioxidants, where preemptive administration is more effective than concurrent treatment. These observations underline the importance of prophylactic antioxidant strategies in mitigating organ-specific toxicity. Moreover, the clinical implications of these findings are significant. Since PCM overdose is a common cause of drug-induced organ injury, adjunctive antioxidant therapy may provide a practical approach to preserve immune organ function, particularly in patients at risk of systemic oxidative stress. Additionally, the data reinforce the role of VC as a safe, widely available, and potent antioxidant that could complement standard interventions. Limitations of the present study include reliance on semi-quantitative scoring rather than quantitative morphometric or molecular assessments such as measurement of ROS, GSH content, LPO, or pro-inflammatory cytokines. Functional assays assessing immune competence, such as lymphocyte proliferation or cytokine production, were not performed and would provide deeper insight into the functional consequences of histological alterations. Future

studies should explore dose-dependent effects of PCM and VC, investigate other antioxidants, and incorporate molecular and functional analyses to optimize protective strategies and validate translational relevance.

Conclusion

PCM induces significant histopathological alterations in the spleen of albino rats, including white pulp disorganization, lymphoid depletion, red pulp congestion, and cellular degeneration, reflecting oxidative stress-mediated tissue injury. VC demonstrated a protective effect, with pre-treatment providing greater preservation of splenic architecture compared to concurrent administration. These findings suggest a potential protective benefit of VC against drug-induced OD in immune organs. Further studies incorporating molecular and functional assessments are warranted to optimize antioxidant-based interventions and assess their translational relevance.

References

Alshailabi, E. M. A. (2016). Effects of omeprazole on healing of non-steroidal anti-inflammatory drug (NSAID)-induced peptic ulceration in rats and protective role of indole-3-carbinol. *Journal of Sciences and Humanities Studies, 12*, 174–188.

Alshailabi, E. M. A., Abdalally, O. A., & Majeed, S. F. (2021). Histopathological study on the protective effect of vitamin C against paracetamol-induced acute hepatic damage in rat. *Global Libyan Journal, 53*, 1–15.

Alshailabi, E. M., Abdalally, O. A., & Mohammed, F. A. (2024). The protective role of ascorbic acid on the testis tissue damage induced by paracetamol in albino rats. *Al-Kitab Journal for Pure Sciences, 8*(1), 19–28. https://doi.org/10.32441/kjps.08.01.p3

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques* (6th ed.). Churchill Livingstone, Elsevier.

Başeğmez, M., Eryavuz, A., & Demirel, H. H. (2025). Effects of vitamin C supplementation on total antioxidant status, inflammation, and histopathological changes in aged rats. *Journal of Biochemical and Molecular Toxicology, 39*(6), e70324. https://doi.org/10.1002/jbt.70324

Du, K., Ramachandran, A., & Jaeschke, H. (2016). Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. *Redox Biology, 10*, 148–156. https://doi.org/10.1016/j.redox.2016.10.001

Elduob, R. E. A., Alshailabi, E. M. A., & Efkeren, S. M. (2023). The protective effects of rutin and stem cells against the kidney function changes induced by paracetamol in rats. *Sirte University Scientific Journal, 13*, 53–58. https://doi.org/10.37375/susj.v13i2.2501

Elmore, S. A. (2006). Enhanced histopathology of the spleen. *Toxicologic Pathology, 34*(5). https://doi.org/10.1080/0192623060086552

Jaeschke, H., Duan, L., Akakpo, J. Y., Farhood, A., & Ramachandran, A. (2018). The role of apoptosis in acetaminophen hepatotoxicity. *Food and Chemical Toxicology, 118*, 709–718. https://doi.org/10.1016/j.fct.2018.06.025

Kennon-McGill, S., & McGill, M. R. (2017). Extrahepatic toxicity of acetaminophen: Critical evaluation of the evidence and proposed mechanisms. *Journal of Clinical and Translational Research, 3*(3), 297–310. https://doi.org/10.18053/jctres.03.201703.005

Kozlov, A. V., Javadov, S., & Sommer, N. (2024). Cellular ROS and antioxidants: Physiological and pathological role. *Antioxidants, 13*(5), 602. https://doi.org/10.3390/antiox13050602

Landmann, M., Scheibner, D., Graaf, A., Gischke, M., Koethe, S., Fatola, O. I., Raddatz, B., Mettenleiter, T. C., Beer, M., Grund, C., Harder, T., Abdelwhab, E. M., & Ulrich, R. (2021). A semiquantitative scoring system for

histopathological and immunohistochemical assessment of lesions and tissue tropism in avian influenza. *Viruses, 13*(5), 868. https://doi.org/10.3390/v13050868

Lee, E., Park, H.-Y., Kim, S.-W., Kim, J., & Lim, K. (2023). Vitamin C and glutathione supplementation: A review of their additive effects on exercise performance. *Physical Activity and Nutrition, 27*(3), 36–43. https://doi.org/10.20463/pan.2023.0027

Mandal, M., Gardner, C. R., Sun, R., Choi, H., Lad, S., Mishin, V., Laskin, J. D., & Laskin, D. L. (2016). The spleen as an extramedullary source of inflammatory cells responding to acetaminophen-induced liver injury. *Toxicology and Applied Pharmacology, 304*, 110–120. https://doi.org/10.1016/j.taap.2016.04.019

Manful, C. F., Fordjour, E., Ikumoinein, E., Abbey, L., & Thomas, R. (2025). Therapeutic strategies targeting oxidative stress and inflammation: A narrative review. *BioChem, 5*(4), 35. https://doi.org/10.3390/biochem5040035

Ogunleye, O. D., Afolabi, O. A., Saka, W. A., Olusola, B. O., Ajike, R. A., Oladokun, O. O., Hammed, S. O., Hezekiah, O. S., & Adedeji, O. J. (2025). Possible role of vitamins C and E co-administration in the prevention of testicular ischemia–reperfusion injury following surgical repair of torsion of the testis. *Frontiers in Nutrition, 12*. https://doi.org/10.3389/fnut.2025.1660240

Rössig, L., Hoffmann, J., Hügel, B., Mallat, Z., Haase, A., Freyssinet, J. M., Tedgui, A., Aicher, A., Zeiher, A. M., & Dimmeler, S. (2001). Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. *Circulation, 104*(18), 2182–2187. https://doi.org/10.1161/hc4301.098284

Venkatesan, P. S., Deecaraman, M., Vijayalakshmi, M., & Sakthivelan, S. M. (2014). Sub-acute toxicity studies of acetaminophen in Sprague Dawley rats. *Biological & Pharmaceutical Bulletin, 37*(7), 1184–1190. https://doi.org/10.1248/bpb.b14-00066