INTERNATIONAL JOURNAL OF
INTEGRATIVE AND
MODERN MEDICINE

# Evaluating the Dose-Dependent Hepatotoxic Effects of Acetaminophen in Wistar Rats

***Araa Mahdi Hussein Ai-Rubaye***
*Forensic Medicine Department - Forensic Toxicology Unit, M.SC Biology NeuroSience &Toxicology*

**Annotation:** Acetaminophen (APAP), a widely used analgesic and antipyretic, is known for its hepatotoxic potential when consumed in high doses. This study aims to evaluate the dose-dependent hepatotoxic effects of acetaminophen in Wistar rats by assessing changes in liver enzymes, histopathological findings, and biochemical markers of oxidative stress. Thirty adults male Wistar rats were divided into five groups (n=6): control, low-dose (500 mg/kg), medium-dose (1,000 mg/kg), high-dose (1,500 mg/kg), and toxic dose (2,000 mg/kg). The study demonstrated a significant increase in liver enzyme levels, oxidative stress markers, and histopathological damage at higher doses of APAP, indicating its dose-dependent hepatotoxicity.

**Keywords:** Acetaminophen, Liver, Rats.

## Introduction

Acetaminophen (APAP), also known as paracetamol, is one of the most commonly used over-the-counter analgesic and antipyretic medications globally. It is preferred for its effectiveness in reducing pain and fever, and its safety profile when used at recommended doses. Unlike nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen has minimal gastrointestinal side effects and no significant anti-inflammatory properties, making it a first-line treatment for mild-to-moderate pain and fever (1).

However, acetaminophen's therapeutic window is narrow, meaning the difference between a therapeutic and a toxic dose is relatively small. When consumed in excessive amounts, APAP undergoes bioactivation to a highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (2). In normal therapeutic doses, NAPQI is detoxified by conjugation with glutathione, a potent intracellular antioxidant. However, at toxic doses, the liver's glutathione reserves are depleted, resulting in the accumulation of NAPQI, which leads to oxidative stress, mitochondrial dysfunction, and ultimately, hepatocyte necrosis. This hepatotoxicity is a leading cause of acute liver failure, especially in developed countries where acetaminophen is readily accessible (3).

Rodent models, particularly Wistar rats, are widely used to study drug-induced liver injury (DILI) due to their physiological and metabolic similarities to humans (4). This study aims to assess the dose-dependent hepatotoxic effects of acetaminophen in Wistar rats by evaluating biochemical markers of liver function, oxidative stress parameters, and histopathological changes. Understanding the dose-dependent effects of acetaminophen-induced liver injury is crucial for improving therapeutic interventions and preventing toxic overdoses, particularly in patients at higher risk of hepatotoxicity.

## Materials and Methods

### Animals

Thirty adult male Wistar rats weighing between 200-250 grams were obtained for the study. The rats were housed in standard laboratory conditions with a 12-hour light-dark cycle and given free access to food and water. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee.

### Experimental Design

The rats were divided into five groups (n=6) and administered acetaminophen dissolved in saline orally, based on the following dose regimens:

- **Group 1 (Control):** Normal saline (vehicle)
- **Group 2 (Low dose):** 500 mg/kg acetaminophen
- **Group 3 (Medium dose):** 1,000 mg/kg acetaminophen
- **Group 4 (High dose):** 1,500 mg/kg acetaminophen
- **Group 5 (Toxic dose):** 2,000 mg/kg acetaminophen

The treatment was administered once daily for 7 days. At the end of the treatment period, rats were anesthetized, and blood samples were collected for biochemical analysis. Livers were harvested for histopathological examination and oxidative stress marker analysis.

### Biochemical Analysis

The following biochemical markers were measured in serum:

- **Alanine aminotransferase (ALT)**
- **Aspartate aminotransferase (AST)**
- **Alkaline phosphatase (ALP)**
- **Total bilirubin**

These were measured using kits purchased from Biolabo company. The measuring of these kits was done according to the company instructions.

### Oxidative Stress Markers

Liver tissues were homogenized, and the following oxidative stress markers were analyzed:

- **Malondialdehyde (MDA)** (a marker of lipid peroxidation)
- **Superoxide dismutase (SOD)**
- **Glutathione (GSH)** levels

**These were done according to** ( 5 ).

### Histopathological Examination

For histopathological evaluation, liver sections were stained with hematoxylin and eosin (H&E). The degree of liver damage was assessed based on cellular degeneration, necrosis, and infiltration of inflammatory cells.

## Results

### Biochemical Parameters

The effects of acetaminophen on liver function were evaluated by measuring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin. The results show a clear dose-dependent increase in these biochemical markers, which are indicators of liver injury.

**ALT Levels**: In the control group, ALT levels were normal at 30 ± 5 U/L. The low-dose group (500 mg/kg) showed a modest increase in ALT to 45 ± 6 U/L. A significant rise in ALT was observed in the medium-dose group (1,000 mg/kg) with levels reaching 80 ± 10 U/L. The high-dose group (1,500 mg/kg) exhibited even higher ALT levels of 150 ± 15 U/L, while the toxic-dose group (2,000 mg/kg) had a dramatic elevation to 250 ± 20 U/L, indicating substantial liver damage at higher doses.

**AST Levels**: Similar to ALT, AST levels increased progressively with higher doses of acetaminophen. The control group had normal AST levels of 28 ± 4 U/L. The low-dose group showed a mild increase to 50 ± 7 U/L, while the medium-dose group reached 90 ± 12 U/L. The high-dose group exhibited AST levels of 160 ± 18 U/L, and the toxic-dose group showed a marked increase to 300 ± 25 U/L, reflecting significant hepatocellular damage.

**ALP Levels**: ALP levels were also elevated in a dose-dependent manner. The control group had an ALP level of 100 ± 12 U/L. In the low-dose group, ALP levels increased slightly to 120 ± 15 U/L, and in the medium-dose group, they rose to 180 ± 20 U/L. A sharp increase in ALP was observed in the high-dose group (250 ± 30 U/L), with the toxic-dose group showing a considerable elevation to 400 ± 35 U/L, indicating cholestasis and possible bile duct involvement in liver damage.

**Total Bilirubin**: Bilirubin, a marker of liver function and bile excretion, also increased with acetaminophen dose. The control group had a total bilirubin level of 0.5 ± 0.1 mg/dL. The low-dose group had a minor increase to 0.8 ± 0.1 mg/dL, while the medium-dose group reached 1.2 ± 0.15 mg/dL. In the high-dose group, bilirubin levels rose to 2.0 ± 0.25 mg/dL, and in the toxic-dose group, there was a substantial increase to 3.5 ± 0.3 mg/dL, suggesting severe impairment in bile metabolism and excretion due to extensive liver damage.

Overall, the dose-dependent increases in ALT, AST, ALP, and bilirubin levels indicate progressive liver injury, with the most severe damage observed at the highest doses of acetaminophen. These biochemical findings are consistent with acetaminophen-induced hepatotoxicity, with higher doses leading to more pronounced liver damage (Table 1).

**Table 1:** Changes in liver enzyme levels in different groups.

| Parameter | Control | Low Dose (500 mg/kg) | Medium Dose (1,000 mg/kg) | High Dose (1,500 mg/kg) | Toxic Dose (2,000 mg/kg) |
|---|---|---|---|---|---|
| **ALT (U/L)** | 30 ± 5 | 45 ± 6 | 80 ± 10 | 150 ± 15 | 250 ± 20 |
| **AST (U/L)** | 28 ± 4 | 50 ± 7 | 90 ± 12 | 160 ± 18 | 300 ± 25 |
| **ALP (U/L)** | 100 ± 12 | 120 ± 15 | 180 ± 20 | 250 ± 30 | 400 ± 35 |
| **Total Bilirubin (mg/dL)** | 0.5 ± 0.1 | 0.8 ± 0.1 | 1.2 ± 0.15 | 2.0 ± 0.25 | 3.5 ± 0.3 |

**Oxidative Stress Markers**

The effects of acetaminophen on oxidative stress markers in liver tissues were assessed by measuring levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH). The results demonstrate a dose-dependent increase in oxidative stress, reflected by elevated MDA levels, and a corresponding decline in antioxidant defenses, as indicated by decreased SOD and GSH levels.

**MDA Levels**: Malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress, showed a progressive increase with higher doses of acetaminophen. The control group exhibited normal MDA levels of 5 ± 0.5 nmol/mg. In the low-dose group (500 mg/kg), MDA levels rose slightly to 7 ± 0.6 nmol/mg. The medium-dose group (1,000 mg/kg) showed a further increase to 10 ± 0.8 nmol/mg, while the high-dose group (1,500 mg/kg) demonstrated a substantial rise to 15 ± 1.2 nmol/mg. The toxic-dose group (2,000 mg/kg) had the highest MDA levels at 25 ± 2.0 nmol/mg, indicating severe oxidative stress and lipid peroxidation in the liver.

**SOD Levels**: Superoxide dismutase (SOD), an important antioxidant enzyme, showed a dose-dependent reduction in activity. The control group had normal SOD levels of 120 ± 15 U/mg. In the low-dose group, SOD levels decreased to 110 ± 12 U/mg, and in the medium-dose group, they dropped further to 90 ± 10 U/mg. The high-dose group exhibited a more pronounced decline to 70 ± 8 U/mg, while the toxic-dose group had the lowest SOD levels at 50 ± 5 U/mg, indicating a significant impairment of the liver's antioxidant defenses at higher acetaminophen doses.

**GSH Levels**: Glutathione (GSH), a critical antioxidant responsible for detoxifying reactive metabolites, also decreased with increasing acetaminophen doses. The control group had normal GSH levels of 20 ± 2 nmol/mg. The low-dose group showed a slight decrease to 18 ± 2 nmol/mg, and the medium-dose group displayed a more pronounced reduction to 15 ± 1.5 nmol/mg. GSH levels were further reduced in the high-dose group (10 ± 1.2 nmol/mg), and the toxic-dose group had the lowest levels at 5 ± 0.5 nmol/mg, indicating severe depletion of GSH and a reduced capacity to detoxify the reactive metabolite NAPQI.

In summary, the dose-dependent increase in MDA levels and the corresponding decrease in SOD and GSH levels highlight the oxidative stress induced by acetaminophen in liver tissues. The marked reduction in antioxidant defenses at higher doses suggests that acetaminophen-induced hepatotoxicity is closely linked to oxidative damage, particularly at doses exceeding 1,000 mg/kg (Table 2).

**Table 2: Oxidative stress marker levels in liver tissues.**

| Parameter | Control | Low Dose (500 mg/kg) | Medium Dose (1,000 mg/kg) | High Dose (1,500 mg/kg) | Toxic Dose (2,000 mg/kg) |
|---|---|---|---|---|---|
| **MDA (nmol/mg)** | 5 ± 0.5 | 7 ± 0.6 | 10 ± 0.8 | 15 ± 1.2 | 25 ± 2.0 |
| **SOD (U/mg)** | 120 ± 15 | 110 ± 12 | 90 ± 10 | 70 ± 8 | 50 ± 5 |
| **GSH (nmol/mg)** | 20 ± 2 | 18 ± 2 | 15 ± 1.5 | 10 ± 1.2 | 5 ± 0.5 |

**Histopathological Findings**

Histopathological analysis revealed standard hepatic architecture in the control group (Fig. 1). In the low-dose group, minimal cellular changes were observed (Fig. 2). However, the medium-dose group exhibited mild cellular degeneration (Figure 3). In contrast, the high-dose group showed pronounced hepatic necrosis and inflammatory infiltration (Figure 4). The toxic-dose group displayed severe hepatic necrosis, widespread cellular damage, and massive inflammatory cell infiltration (Figure 5).

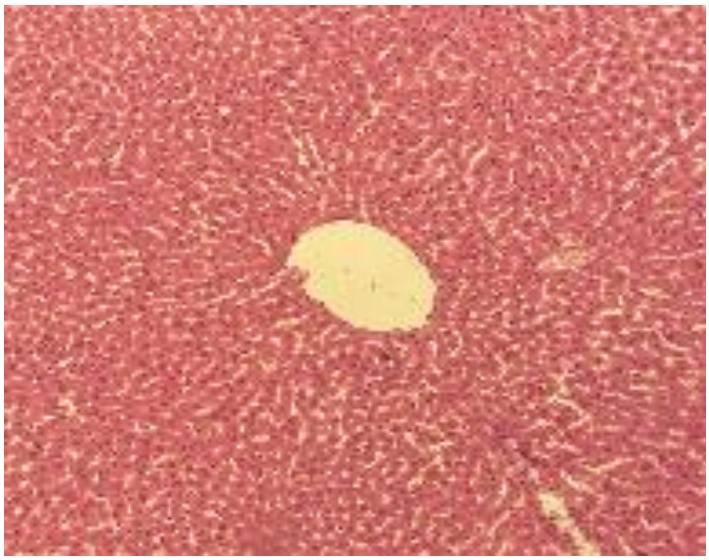

**Figure 1. Histopathological analysis revealed standard hepatic architecture in the control group, H & E stain**

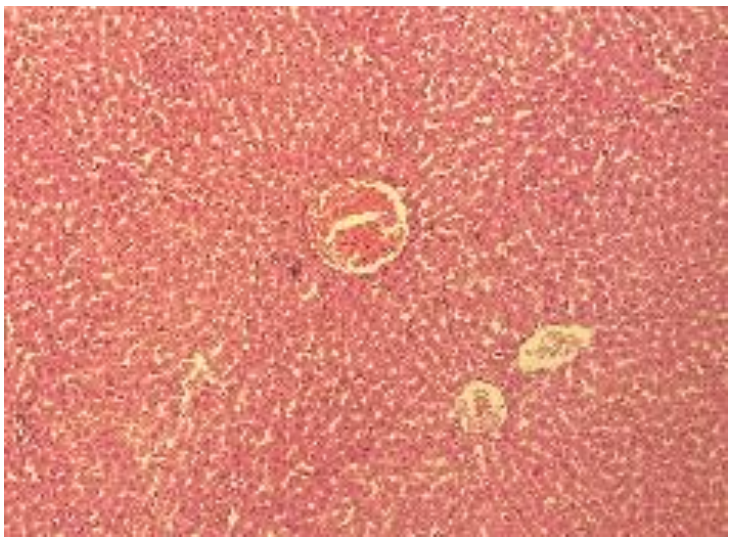

**Figure 2. In the low-dose group, minimal cellular changes such as blood vessel congestion, H & E stain**

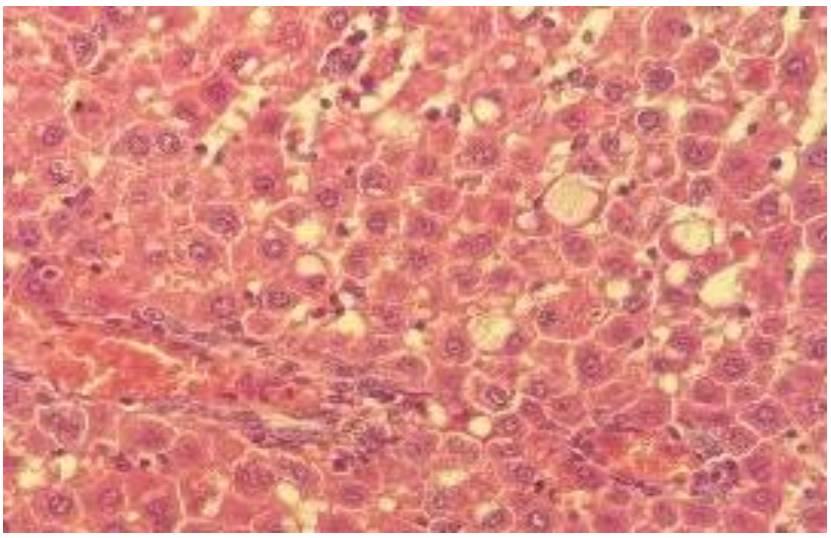

**Figure 3. medium-dose group exhibited mild cellular degeneration, H & E stain**

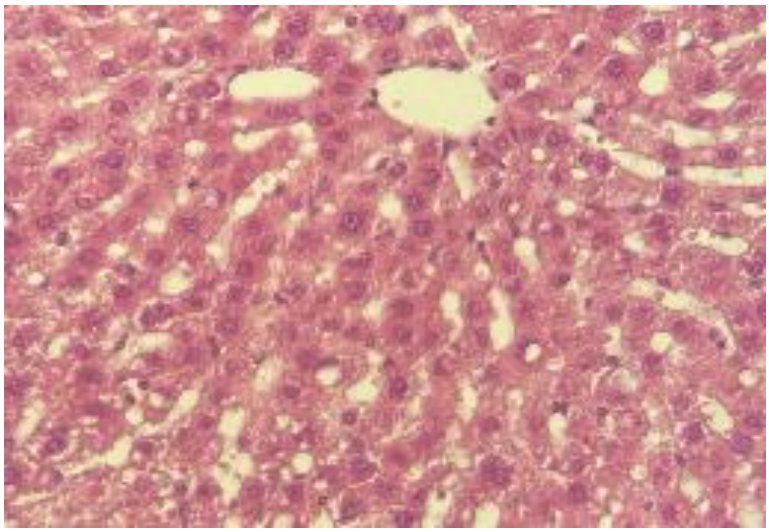

**Figure 4. high-dose group showed pronounced hepatic necrosis and inflammatory infiltration**

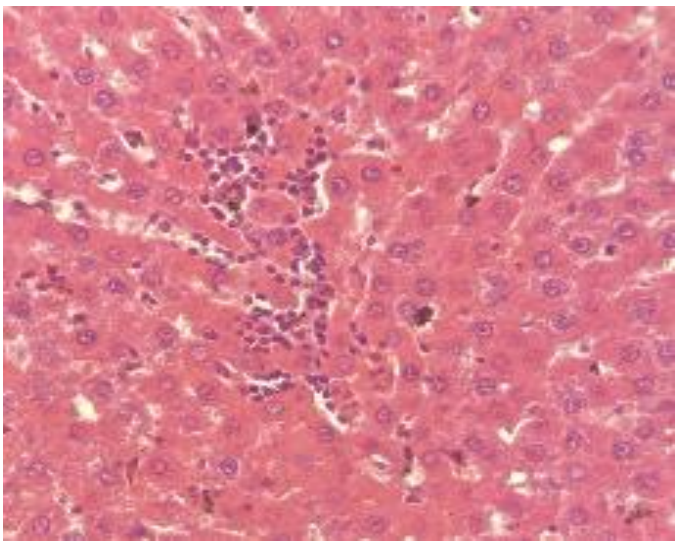

**Figure 5. toxic-dose group displayed severe hepatic necrosis, widespread cellular damage, and massive inflammatory cell infiltration**

## Discussion

The present study aimed to evaluate the dose-dependent hepatotoxic effects of acetaminophen in Wistar rats. Our findings showed that acetaminophen administration led to significant biochemical, oxidative, and histopathological changes, particularly at higher doses, thus confirming its dose-dependent hepatotoxic potential.

### Biochemical Markers of Liver Injury

A dose-dependent increase in liver enzymes, including ALT, AST, and ALP, was observed in this study. These enzymes are commonly used biomarkers for assessing liver function and hepatocellular integrity. ALT and AST are primarily released into the bloodstream when hepatocytes are damaged, making them reliable indicators of liver injury (6). The significant elevation in these enzymes in the medium, high, and toxic dose groups suggests that acetaminophen induces hepatic injury in a dose-dependent manner. Additionally, the rise in total bilirubin in higher doses is indicative of impaired liver function, as bilirubin metabolism and excretion are compromised during liver injury (7).

This observation aligns with previous studies, which reported similar biochemical changes following acetaminophen overdose in animal models. The liver injury mechanism is primarily driven by excessive NAPQI production, which depletes glutathione and allows reactive oxygen species (ROS) to accumulate, causing oxidative damage to cellular components such as lipids, proteins, and DNA (8).

### Oxidative Stress and Its Role in Hepatotoxicity

One of the key findings of this study is the significant alteration in oxidative stress markers, particularly the increase in malondialdehyde (MDA) and the decrease in antioxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH) (9). MDA is a well-established marker of lipid peroxidation, which occurs when ROS attack the lipid membranes of cells, leading to cellular dysfunction and necrosis. The observed increase in MDA levels in higher acetaminophen doses supports the role of oxidative stress in APAP-induced hepatotoxicity (10,11).

Conversely, the reduction in SOD and GSH levels suggests a depletion of the liver's antioxidant defenses in response to acetaminophen overdose. SOD is an essential enzyme that neutralizes superoxide radicals, while GSH directly detoxifies NAPQI. The reduction in these antioxidant defenses at higher doses exacerbates oxidative stress, further contributing to hepatocellular injury (12,13).

These results are consistent with previous studies demonstrating that oxidative stress plays a critical role in acetaminophen-induced liver injury (14,15). NAPQI production increases as acetaminophen

doses rise, leading to rapid depletion of glutathione and an inability of the liver to counterbalance the oxidative stress. Therapeutic interventions, such as N-acetylcysteine (NAC), work by replenishing glutathione levels and are commonly used in clinical practice to treat acetaminophen poisoning (16).

**Histopathological Evidence of Hepatotoxicity**

The histopathological examination of liver tissues provided further evidence of dose-dependent hepatotoxicity. While the control and low-dose groups showed minimal or no liver damage, the medium, high, and toxic dose groups exhibited increasing degrees of hepatocyte necrosis, cellular degeneration, and inflammatory infiltration. The high-dose group showed extensive hepatic necrosis and evidence of steatosis (fatty liver), while the toxic dose group presented with massive necrosis and widespread infiltration of inflammatory cells (17,18).

These histopathological changes mirror the clinical features of acetaminophen-induced acute liver injury in humans (19). Hepatocyte necrosis is the hallmark of acetaminophen toxicity and results from oxidative damage, mitochondrial dysfunction, and disruption of cellular homeostasis (20). Inflammatory cells, particularly neutrophils and macrophages, are recruited to the site of injury as part of the liver's response to tissue damage, further exacerbating the injury through the release of pro-inflammatory cytokines and ROS (21).

**Clinical Implications and Future Directions**

The results of this study have important implications for understanding the mechanisms of acetaminophen-induced liver injury and its clinical management (22, 23,24). The dose-dependent nature of hepatotoxicity emphasizes the need for caution when using acetaminophen, especially in individuals with pre-existing liver conditions, chronic alcohol use, or other risk factors for liver disease. Additionally, these findings underscore the importance of timely intervention with antioxidant therapies such as NAC, which can replenish glutathione levels and mitigate oxidative stress (25,26).

Further research should focus on identifying biomarkers that predict susceptibility to acetaminophen-induced hepatotoxicity, particularly in populations at higher risk. Additionally, exploring alternative therapeutic strategies to enhance liver regeneration and prevent liver failure following acetaminophen overdose remains an area of interest.

**Conclusion**

This study highlights the dose-dependent hepatotoxic effects of acetaminophen in Wistar rats, as demonstrated by significant elevations in liver enzymes, oxidative stress markers, and histopathological damage at higher doses. The findings provide insight into the mechanisms of acetaminophen-induced liver injury and emphasize the importance of early intervention in cases of overdose to prevent severe hepatotoxicity. Future studies should aim to elucidate further the pathways involved in acetaminophen toxicity and explore potential therapeutic interventions to mitigate its harmful effects.

**References:**

1. Albano, E. (2006). Alcohol, oxidative stress, and free radical damage. Proceedings of the Nutrition Society, 65(3), 278-290. https://doi.org/10.1079/PNS2006495
2. Andrade, R. J., Lucena, M. I., Fernández, M. C., Vega, J. L., & Camargo, R. (2005). Drug-induced liver injury: An analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology, 129(2), 512-521. https://doi.org/10.1053/j.gastro.2005.05.006
3. Beier, J. I., & McClain, C. J. (2010). Mechanisms and cell signaling in alcoholic liver disease. Biological Chemistry, 391(11), 1249-1264. https://doi.org/10.1515/BC.2010.127
4. Bhushan, B., & Apte, U. (2019). Liver regeneration after acetaminophen hepatotoxicity: Mechanisms and therapeutic opportunities. The American Journal of Pathology, 189(3), 719-729. https://doi.org/10.1016/j.ajpath.2018.11.012

5. Blazka, M. E., Wilmer, J. L., Holladay, S. D., Wilson, R. E., & Luster, M. I. (1996). Role of proinflammatory cytokines in acetaminophen hepatotoxicity. Toxicology and Applied Pharmacology, 137(2), 268-280. https://doi.org/10.1006/taap.1996.0072
6. Chen, C., Krausz, K. W., Shah, Y. M., Idle, J. R., & Gonzalez, F. J. (2009). Serum metabolomics reveals irreversible effects of acetaminophen on liver function in mice. Journal of Proteome Research, 8(7), 3596-3604. https://doi.org/10.1021/pr900249m
7. Dahlin, D. C., Miwa, G. T., Lu, A. Y., & Nelson, S. D. (1984). N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. Proceedings of the National Academy of Sciences, 81(5), 1327-1331. https://doi.org/10.1073/pnas.81.5.1327
8. Davern, T. J., & Schiodt, F. V. (2006). Liver toxicity in clinical settings. Journal of Clinical Investigation, 116(4), 1010-1014. https://doi.org/10.1172/JCI28151
9. Ghosh, A., Sil, P. C. (2007). Anti-oxidative effect of a protein from Cajanus indicus L. against acetaminophen-induced hepatic disorder. Journal of Biochemical and Molecular Toxicology, 21(5), 258-264. https://doi.org/10.1002/jbt.20183
10. Hinson, J. A., Roberts, D. W., & James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. Handbook of Experimental Pharmacology, (196), 369-405. https://doi.org/10.1007/978-3-642-00663-0_12
11. Jaeschke, H., McGill, M. R., & Ramachandran, A. (2012). Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. Drug Metabolism Reviews, 44(1), 88-106. https://doi.org/10.3109/03602532.2011.602688
12. James, L. P., Mayeux, P. R., & Hinson, J. A. (2003). Acetaminophen-induced hepatotoxicity. Drug Metabolism and Disposition, 31(12), 1499-1506. https://doi.org/10.1124/dmd.31.12.1499
13. Larson, A. M. (2007). Acetaminophen hepatotoxicity. Clinics in Liver Disease, 11(3), 525-548. https://doi.org/10.1016/j.cld.2007.06.006
14. Lee, W. M. (2004). Acetaminophen and the U.S. acute liver failure study group: Lowering the risks of hepatic failure. Hepatology, 40(1), 6-9. https://doi.org/10.1002/hep.20386
15. Lemasters, J. J., Nieminen, A. L., Qian, T., Trost, L. C., Elmore, S. P., Nishimura, Y., & Herman, B. (1998). The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis, and autophagy. Biochimica et Biophysica Acta, 1366(1-2), 177-196. https://doi.org/10.1016/S0005-2728(98)00112-0
16. McGill, M. R., & Jaeschke, H. (2013). Metabolism and disposition of acetaminophen: Recent advances in relation to hepatotoxicity and diagnosis. Pharmaceutical Research, 30(9), 2174-2187. https://doi.org/10.1007/s11095-013-1007-9
17. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., & Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. Journal of Pharmacology and Experimental Therapeutics, 187(1), 185-194.
18. Myers, R. P., Shaheen, A. A., Faris, P., & Aspinall, A. I. (2008). Utilization rates, complications, and costs of paracentesis in the management of ascites: A population-based study. Hepatology, 48(1), 232-241. https://doi.org/10.1002/hep.22204
19. Nelson, S. D. (1990). Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Seminars in Liver Disease, 10(4), 267-278. https://doi.org/10.1055/s-2008-1040476
20. Ott, M., Gogvadze, V., Orrenius, S., & Zhivotovsky, B. (2007). Mitochondria, oxidative stress, and cell death. Apoptosis, 12(5), 913-922. https://doi.org/10.1007/s10495-007-0756-2

21. Patten, A. R., & Pourmotabbed, T. (1998). Inhibition of metalloproteinase activity with TIMP-1 prevents cell death following focal cerebral ischemia. Journal of Neurochemistry, 70(3), 668-675. https://doi.org/10.1046/j.1471-4159.1998.70030668.x

22. Ramachandran, A., & Jaeschke, H. (2019). Acetaminophen toxicity: Novel insights into mechanisms and future perspectives. Gene Expression, 19(3), 125-138. https://doi.org/10.3727/105221617X15099223168855

23. Reddy, J. K., & Rao, M. S. (2006). Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. American Journal of Physiology-Gastrointestinal and Liver Physiology, 290(5), G852-G858. https://doi.org/10.1152/ajpgi.00474.2005

24. Rouabhia, S., Milic, N., Abenavoli, L., & Morabito, G. (2014). Hepatotoxicity of paracetamol and related mortality rates. Journal of Gastrointestinal and Liver Diseases, 23(3), 233-240. https://doi.org/10.15403/jgld.2014.1121.233.ser

25. Thummel, K. E., Lee, C. A., Kunze, K. L., Nelson, S. D., & Slattery, J. T. (1993). Oxidation of acetaminophen to N-acetyl-p-benzoquinone imine by human cytochromes P450 2E1 and 1A2. Chemical Research in Toxicology, 6(4), 511-518. https://doi.org/10.1021/tx00035a006

26. Watkins, P. B., Kaplowitz, N., Slattery, J. T., Colonese, C. R., Colucci, S. V., Stewart, P. W., & Harris, S. C. (2006). Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: A randomized controlled trial. JAMA, 296(1), 87-93. https://doi.org/10.1001/jama.296.1.87