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Hepatoprotective, antioxidant, and anti-inflammatory properties of quercetin in paracetamol overdose-induced liver injury in rats

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Abstract

Acute liver injury is a severe clinical condition with potentially fatal consequences commonly caused by viral infections, medications, toxins, and drug overdoses. Among these, paracetamol (acetaminophen) overdose is a leading cause of hepatic failure due to its narrow therapeutic index, resulting in oxidative stress and hepatocyte apoptosis. Quercetin, a flavonoid found abundantly in vegetables and herbs, has demonstrated antioxidant, hepatoprotective, and anti-inflammatory properties. This study evaluates the hepatoprotective role of quercetin in mitigating liver damage induced by paracetamol overdose in an experimental rat model. A total of 28 male rats were randomly divided into four groups (n = 7 per group): (i) normal control (distilled water and saline), (ii) paracetamol-induced liver injury group (2 g/kg paracetamol intraperitoneally), (iii) paracetamol + quercetin group (50 mg/kg quercetin orally and 2 g/kg paracetamol intraperitoneally), and (iv) quercetin-only group (50 mg/kg quercetin intraperitoneally). Blood and liver samples were analyzed for liver enzymes (glutamate pyruvate transaminase [GPT], glutamate oxaloacetate transaminase [GOT]), inflammatory markers (nuclear factor kappa B [NF-κB], tumor necrosis factor-alpha [TNF-α]), apoptotic markers (cysteine-aspartic acid protease 3 [Caspase-3], B-cell lymphoma 2 [BCL2]), oxidative stress markers (malondialdehyde [MDA], glutathione [GSH]), and histological changes. Paracetamol administration significantly elevated GPT, GOT, NF-κB, TNF-α, caspase-3, and MDA levels whereas reducing BCL2 and GSH levels, indicating hepatic injury and oxidative stress. In contrast, results showed that quercetin treatment significantly mitigated these changes, demonstrating its potential hepatoprotective effects. Histological analysis further confirmed that quercetin reduced hepatic damage compared to the paracetamol-only group. These findings suggest that quercetin exerts a protective effect against paracetamol-induced liver injury by reducing oxidative stress, inflammation, and apoptosis.

Keywords: Liver injury; Oxidative stress; Paracetamol; Quercetin; Apoptosis; Inflammatory markers

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1. Introduction

A wide variety of viral infections, toxic substances, specific drugs, and herbal or novel pharmaceutical products can result in acute liver injury (ALI). ALI pathogenesis comprises a complex array of centrally interconnected cascading processes – hepatocellular degeneration, biliary stasis, and inflammation – that ultimately lead to clinical manifestations, including jaundice and hepatic function impairment.[1] Given the liver's central role in metabolism, protein synthesis, and detoxification, liver disease may lead to severe systemic consequences and should be considered a critical determinant of body homeostasis.[2]

Paracetamol overdose is considered one of the most important causes of ALI as it is strongly related to fatal hepatic failure.[3] Paracetamol is one of the most popular analgesic agents worldwide for its ability to relieve pain and reduce fever. It is a safe and efficient treatment when used within therapeutic doses. However, the drug has a relatively low therapeutic index and can induce toxicity with relatively minor deviations from the recommended dosage. A high propensity of overdose and subsequent liver damage is directly associated with a narrow therapeutic index, where the margin between an effective and a toxic dose is small. The hepatotoxic effects of paracetamol are a consequence of metabolic activation in the liver through the cytochrome P450 enzymatic system, leading specifically to the generation of N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive toxic metabolite.[4] The potentially toxic metabolite NAPQI is effectively scavenged by the readily available liver antioxidant glutathione (GSH) through conjugation with GSH to form an inactive conjugate, which is excretable and thus non-toxic. This activity restores cellular homeostasis from any aberration that may result in cell damage. However, in the event of an overdose, NAPQI is generated at a rate that exceeds the scavenging capacity of GSH, thus resulting in the depletion of GSH reserves. This condition compromises the normal detoxification processes, allowing more NAPQI to diffuse into the hepatocytes.

Once formed, NAPQI covalently binds to cellular proteins, interfering with their normal function and triggering a cascade of harmful downstream processes. The resulting oxidative stress damages essential cellular components – lipids, proteins, and nucleic acids – with the mitochondria exhibiting some of the earliest changes in acetaminophen-induced liver toxicity. Mitochondrial dysfunction further increases reactive oxygen species (ROS) production, a condition described as a vicious cycle of damage. In addition, fragmentation of nuclear DNA – an indication of cell injury – caused by NAPQI accumulation results in the activation of apoptotic pathways, leading to necrosis and apoptosis of hepatocytes. This eventually culminates in liver cell damage and loss of liver function, a condition that, if left untreated, will lead to acute liver failure. Oxidative stress is defined as the condition that results from the disequilibrium between the generation of ROS and the body's capacity to detoxify these reactive products readily.[5] Primary roles in the pathophysiology of various liver disorders, including ALI, can be attributed to oxidative stress. Flavonoids, including quercetin, can neutralize ROS to protect against cellular damage, mainly in hepatic tissues. In this regard, flavonoids, including quercetin, may protect the liver cells from oxidative stress, reduce inflammation, and enhance liver tissue regeneration through an antioxidative mechanism. In this context, the biotransformations and biological effects of quercetin make it a potential candidate with highly effective multipreventive effects against hepatic lesions. Hence, quercetin, a flavonoid that exists naturally in many fruits and vegetables, such as apples, grapes, onions, and broccoli, is an attractive prospect in treating liver diseases, providing preventive and therapeutic effects in hepatic lesions.[6] Flavonoids are one of the most studied classes of bioactive compounds and are effective against oxidative stress-induced cellular damage, particularly in hepatic tissues.[7] Quercetin is known to neutralize the formation of ROS, downregulate pro-inflammatory cytokines and lipid peroxidation, and enhance the activities of endogenous antioxidant enzymes, including superoxide dismutase and catalase.[8,9]

Hence, further research is needed to study the potential hepatoprotective effects of quercetin.[10] Available literature has shown that flavonoids, such as quercetin, exhibit liver-protective effects, with good bioavailability profiles further supporting their possible candidacy as promising agents. Such treatment allows the maintenance of mitochondrial function and the prevention of hepatocyte apoptosis, two critical factors for an effective strategy to reduce acetaminophen-induced liver injury.[11,12] By reducing oxidative damage, preserving mitochondrial function, and preventing hepatocyte apoptosis, quercetin may serve as an effective strategy for reducing drug-induced liver injury. Therefore, this study aims to elucidate the hepatoprotective properties of quercetin in mitigating paracetamol-induced liver damage using an experimental rat model.[13,14]

2. Materials and methods

2.1. Materials

The details of the materials used in this study are tabulated in Table 1.

2.2. Methods

This study was approved by the Scientific Research Ethics Committee at Al-Kafeel University (Approval No. 2022 – 7).

Table 1. Materials used in this study

Chemicals	Manufacturer	Country of origin
Quercetin	Alps pure life sciences	India
Paracetamol	Kabri life sciences	India
Ketamine	Pfizer	United States
Xylazine	Santa Cruz Biotechnology	United States

Twenty-eight male Sprague-rats (obtained from the University of Kufa/College of Science) weighing 200 – 250 g were randomly divided into four groups ($n = 7$): (i) normal control group: Received distilled water and saline, (ii) paracetamol group: Received 2 g/kg of paracetamol.[15] (iii) paracetamol + quercetin group: Received 50 mg/kg of quercetin and 2 g/kg of paracetamol, and (iv) the quercetin-only group was administered with 50 mg/kg of quercetin intraperitoneally.

After 24 h of treatment, the animals were sacrificed with 90 mg/kg of ketamine and 10 mg/kg of xylazine 15 to obtain blood and liver samples for histological and biochemical analyses.[16] After 24 h of treatment, the animals were sacrificed to obtain blood and liver samples for histological and biochemical analyses.

2.3. Biochemical analysis

Liver function was comprehensively assessed by determining the serum levels of two principal hepatic enzymes: Glutamate pyruvate transaminase (GPT), known as alanine aminotransferase, and glutamate oxaloacetate transaminase (GOT), referred to as aspartate aminotransferase. These enzymes are important as elevated levels in the blood are usually a sign of hepatocellular damage and liver injury due to different pathological conditions, particularly on the hepatocytes.

To further analyze the inflammatory response, the expression levels of nuclear factor kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) were quantified using enzyme-linked immunosorbent assay. NF-κB is one of the most important transcription factors for the transcription of many pro-inflammatory cytokines, with TNF-α being one of them. TNF-α is a pro-inflammatory cytokine significantly related to liver inflammation mediated by other immune responses. Upregulation of these markers is often associated with processes involving inflammation, oxidative stress, and immune activation in liver disease.

Cysteine-aspartic acid protease 3 (caspase-3) and B-cell lymphoma 2 (BCL2) levels were determined to assess apoptotic activity. Caspase-3 is an executioner caspase in the apoptotic pathway. It is hypothesized to play a major role in apoptosis by proteolytically cleaving vital cellular proteins, leading to cell death. BCL2 is an anti-apoptotic protein that inhibits apoptosis by blocking the function of an activated apoptotic protease. The relative expression of these markers indicates the degree of hepatocyte apoptosis, a relatively common phenomenon in liver injury and disease development.

Oxidative stress was assessed by determining levels of malondialdehyde (MDA) and GSH. MDA, a product of lipid peroxidation, serves as a prominent marker of oxidative cellular membrane damage, reflecting the degree of injury induced by oxidative stress. GSH is a key endogenous antioxidant that maintains cellular redox balance by scavenging ROS and ROS-associated oxidative damage. A decrease in GSH level and an increase in MDA reflect an imbalance in the oxidative defense system that may lead to liver dysfunction and disease development. These biochemistry parameters, reflecting liver function, inflammation, apoptosis, and oxidative stress, were evaluated to better understand the pathophysiological mechanisms underlying liver injury and disease progression.

2.4. Histopathological examination

Liver tissues were fixed with 10% formalin to preserve the cellular architecture and prevent putrefaction. After fixation, tissues were embedded in paraffin for easy and precise sectioning. The thin liver tissue sections were prepared and mounted on glass slides, followed by hematoxylin and eosin (H&E) staining. H&E staining is a common histological technique, staining the nuclei of cells deep blue or purple, enhancing nuclear morphology, and staining the cytoplasm and extracellular matrix, providing a distinguishable observation of tissue components.

Histological assessment was performed to observe changes in the architecture of the liver, with a special focus on major pathological features. These assessments included evidence of hepatocyte necrosis, which included loss of cellular integrity and nuclear fragmentation. The presence of inflammatory cells, when observed, served as an indicator of an immune response to hepatic injury. Additional histological evaluations involved vascular atony and passive hyperemia, the passive accumulation of blood within the liver sinusoids, which may indicate circulatory disturbance or tissue damage. Collectively, these histological parameters provided a comprehensive understanding of the extent and nature of liver injury, offering valuable insights into the associated tissue pathology.

2.5. Statistical analysis

Statistical calculations were performed using the Statistical Package for Social Sciences 24.0 software for the Windows operating system. Data are expressed as mean ± standard

error of the mean, where the number of samples per group (n) equals seven. Multiple comparisons were calculated using analysis of variance, and $p<0.05$ denotes statistical significance.

3. Results

3.1. Liver enzyme levels

Administration of paracetamol resulted in a highly significant increase in serum levels of GPT and GOT compared to the control group ($p<0.05$). This proves to be hepatotoxic because an increase in these serum transaminases is usually a result of liver damage. On the other hand, pretreatment with quercetin showed a highly considerable reduction in serum concentrations of both GPT and GOT, indicating the possible hepatoprotective effects of quercetin. These findings are statistically significant ($p<0.05$), suggesting that quercetin protects against paracetamol-induced hepatic injuries, as presented in Figures 1 and 2.

3.2. Oxidative stress enzymes

Paracetamol administration caused a significant increase in oxidative stress, as indicated by increased MDA and reduced GSH levels. These changes led to an increase in the lipid peroxidation process provoked by toxicity due to paracetamol. On the other hand, quercetin administration resulted in a protective effect against oxidative damage. Treatment with quercetin significantly decreased MDA levels and increased GSH levels ($p<0.05$), indicating its potent antioxidant effect (Figures 3 and 4).

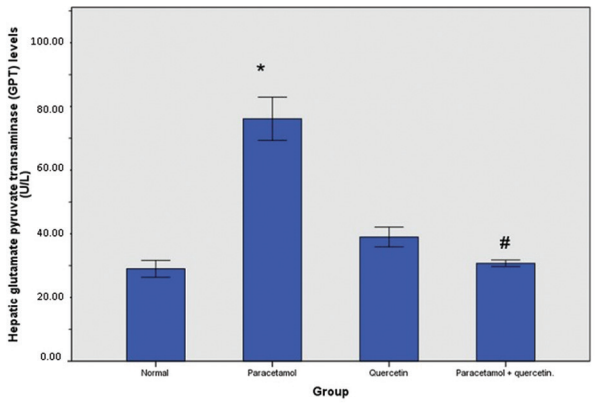


Figure 1. Hepatic glutamate pyruvate transaminase (GPT) levels (U/L). The average hepatic GPT levels (U/L) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

3.3. Inflammatory markers

The group treated with paracetamol showed a significant increase in hepatic NF-κB and TNF-α levels compared to the control group ($p<0.05$). These findings suggest that paracetamol administration induces a marked inflammatory response in the liver. However, treatment with quercetin notably reduced the elevated levels of these inflammatory markers, demonstrating its potential as an anti-inflammatory agent ($p<0.05$). This attenuation of the inflammatory response highlights the therapeutic effects

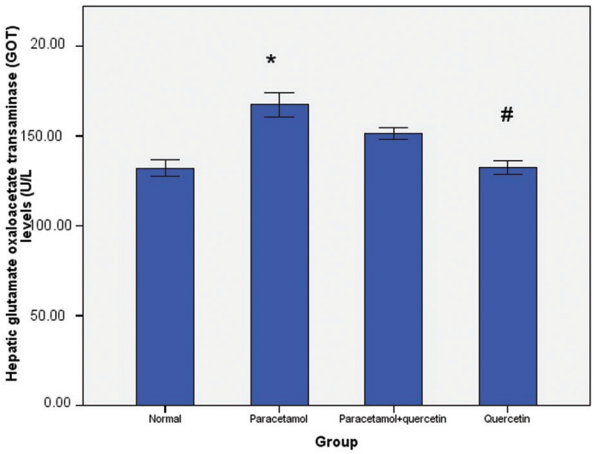


Figure 2. Hepatic glutamate oxaloacetate transaminase (GOT) levels (U/L). The average hepatic GOT levels (U/L) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

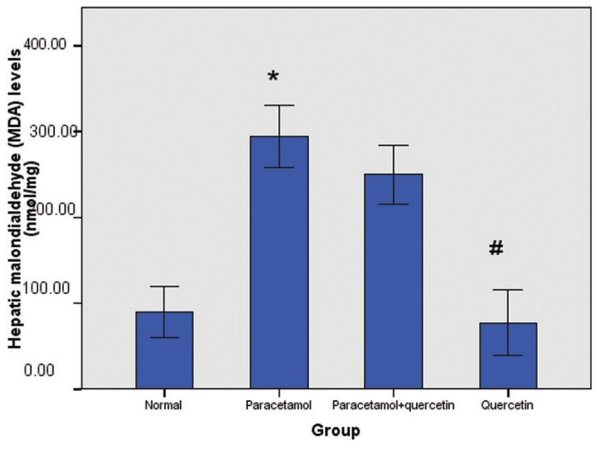


Figure 3. Hepatic malondialdehyde (MDA) levels (nmol/mg). The average hepatic MDA levels (nmol/mg) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

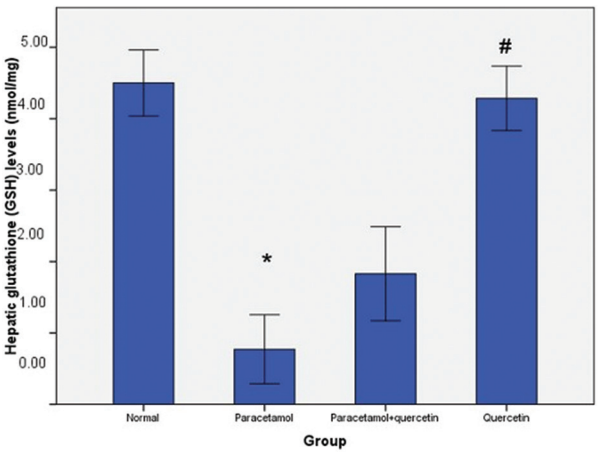


Figure 4. Hepatic glutathione (GSH) levels (nmol/mg). The average hepatic GSH levels (nmol/mg) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

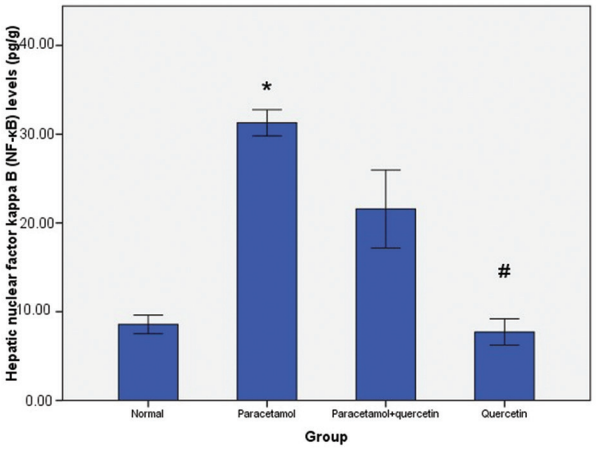


Figure 5. Hepatic nuclear factor kappa B (NF-κB) levels (pg/g). The average hepatic NF-κB levels (pg/g) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

of quercetin in mitigating the pro-inflammatory cytokine and transcription factor signaling pathways involved in liver inflammation (Figures 5 and 6).

3.4. Apoptotic markers

Paracetamol administration led to a marked increase in caspase-3 levels and a significant reduction in the expression of BCL2, key markers associated with the induction of apoptosis. These findings suggest that paracetamol may promote cell death through apoptotic pathways. In contrast, treatment with quercetin resulted in a notable decrease in caspase-3 levels, alongside restoration of BCL2 expression to near-normal levels ($p<0.05$). This observed effect strongly supports the anti-apoptotic potential of quercetin, highlighting its capacity to modulate apoptotic signaling pathways (Figures 7 and 8).

3.5. Histopathological findings

Histological analysis of liver tissue sections demonstrated distinct differences among the experimental groups. In the control group (Figure 9A), the hepatic architecture appeared normal, with well-preserved hepatocytes, clearly defined lobular structures, and intact central veins and sinusoids. No signs of cellular damage, inflammation, or vascular abnormalities were observed.

In contrast, the paracetamol-treated group (Figure 9B) exhibited extensive hepatocellular necrosis, characterized by widespread loss of cellular integrity, nuclear pyknosis, and karyorrhexis. Inflammatory cell infiltration was prominent, particularly around the central veins and periportal regions, indicating a severe inflammatory

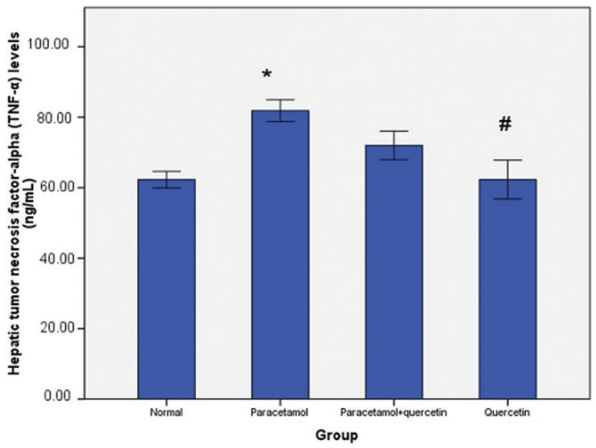


Figure 6. Hepatic tumor necrosis factor-alpha (TNF-α) levels (ng/mL). The average hepatic TNF-α levels (ng/mL) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

response. In addition, significant congestion of hepatic blood vessels was evident, suggesting vascular impairment and circulatory disturbances due to paracetamol-induced toxicity.

The group receiving paracetamol and quercetin (Figure 9C) displayed moderate histological alterations, with notably reduced hepatocyte necrosis and inflammation compared to the paracetamol-only group. The hepatic lobular structure was partially preserved, and signs of tissue regeneration were apparent, suggesting a protective

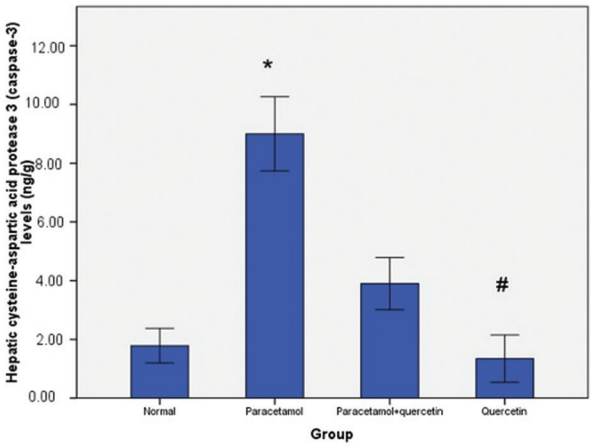


Figure 7. Hepatic cysteine-aspartic acid protease 3 (caspase-3) levels (ng/g). The average hepatic caspase-3 levels (ng/g) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$. Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

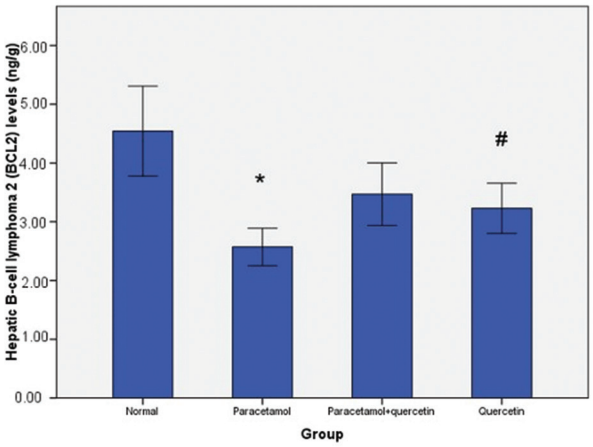


Figure 8. Hepatic B-cell lymphoma 2 (BCL2) levels (ng/g). The average hepatic BCL2 levels (ng/g) among the four groups, expressed as mean ± standard error of the mean, are significant at $p<0.05$.
Notes: *refers to statistical significance between paracetamol versus the control group at $p<0.05$. #refers to statistical significance between quercetin versus the paracetamol group at $p<0.05$.

effect of quercetin in mitigating paracetamol-induced liver damage. While some inflammatory cell infiltration and vascular congestion remained, the overall histological appearance indicated a substantial improvement in hepatic architecture.

The quercetin-only group (Figure 9D) exhibited liver histology nearly indistinguishable from the control group, with well-organized hepatocytes, intact sinusoidal spaces, and minimal or no evidence of necrosis, inflammation, or congestion. This finding suggests that quercetin alone does not exert any deleterious effects on liver morphology and may contribute to maintaining normal hepatic function. These histopathological changes are comprehensively illustrated in Figure 9.

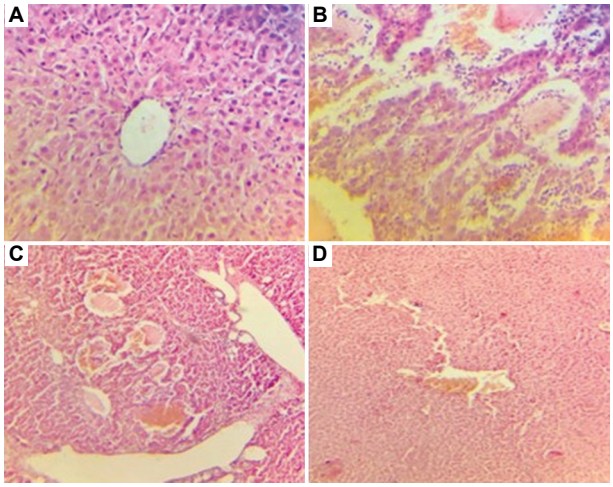


Figure 9. Histopathological findings. (A) Normal group. (B) Paracetamol group. (C) Paracetamol + quercetin group. (D) Quercetin group. A: ×400 magnification, 40 µm scale bar; B: ×400 magnification 40 µm scale bar; C: ×200 magnification 20 µm scale bar; D: ×100 magnification 10 µm scale bar.

4. Discussion

Due to its widespread availability and perceived safety, excessive or prolonged use of paracetamol poses a significant health risk, primarily through hepatotoxicity.[17] Paracetamol overdose is a leading cause of acute liver failure, a condition that has become a major global public health concern.[18]

Quercetin is a naturally occurring bioflavonoid in most fruits and vegetables[19] with extensive pharmacological properties. Multiple studies have reported quercetin as an effective agent in preventing and slowing the process of chronic disease development, such as chronic neurodegenerative diseases, cardiovascular conditions, and cancer.[20,21]

One of the key mechanisms underlying quercetin's protective efficacy lies in its potent antioxidant activity. It effectively neutralizes free radicals, thereby reducing oxidative stress and inhibiting lipid peroxidation – crucial mechanisms in cellular damage and disease development. In addition, quercetin enhances the activity of antioxidant enzymes such as superoxide dismutase, catalase, and GSH peroxidase. By increasing these enzymatic activities, quercetin helps protect hepatocytes from oxidative damage and maintains cellular homeostasis.[22]

In addition to its antioxidant properties, quercetin exhibits significant anti-inflammatory effects. It functions

as an inhibitor of NF-κB, a transcription factor that regulates the expression of genes involved in inflammatory responses. Through this mechanism, quercetin suppresses the production of pro-inflammatory cytokines. Furthermore, quercetin downregulates the expression of the two inflammatory mediators, cyclooxygenase-2 and inducible nitric oxide synthase. These actions contribute to the attenuation of hepatic inflammation and confer a protective effect against liver injury.[23,24]

Treatment with paracetamol significantly increased the levels of GOT, GPT, TNF-α, NF-κB, and caspase-3 in the paracetamol-only group, indicating evident liver injury and necrosis. A significant rise in MDA levels was observed, suggesting that increased lipid peroxidation can cause tissue damage. Reduced levels of GSH and BCL2 were also observed in this study. These findings aligned with previous studies,[13] which reported elevated serum GOT and GPT levels as urine markers and MDA levels in tissue homogenates.[25]

Quercetin may also alleviate hepatic complications instigated by paracetamol. The manifestations of hepatic complications are mostly revealed by enhanced serum GOT and GPT activities and a rise in MDA level, a direct marker for oxidative stress and liver damage.[26] The normalization of biochemical parameters following quercetin administration strongly suggests its restorative potential in mitigating liver toxicity. These findings align with earlier reports that demonstrated a marked decrease in the elevated plasma levels of GOT and GPT activities and MDA levels following quercetin treatment before hepatic ischemia-reperfusion injury.[27] The results provide evidence for quercetin's therapeutic potential in hepatoprotection, including its efficacy against paracetamol-induced liver injury.

The NF-κB pathway plays a key role in the development of hepatocellular damage, mainly due to its sensitivity to various external factors, such as ROS, cytokines, or shear stress in the vasculature.[28] The pathway is the main promoter of inflammation and stress response within the hepatocyte, and its activation exacerbates hepatocellular damage, as depicted in Figure 10.

In the present study, levels of NF-κB were significantly elevated in the active control group compared to the normal group, indicating that the upregulation of this pathway strongly relates to the progression of liver injury. However, quercetin treatment effectively mitigated the activation of NF-κB in the active control group, preventing further hepatic damage. This finding is consistent with another study, which found that treatment with quercetin following the induction of hepatic injury caused a notable reduction in NF-κB activation. The previous study found that treatment with quercetin resulted in the

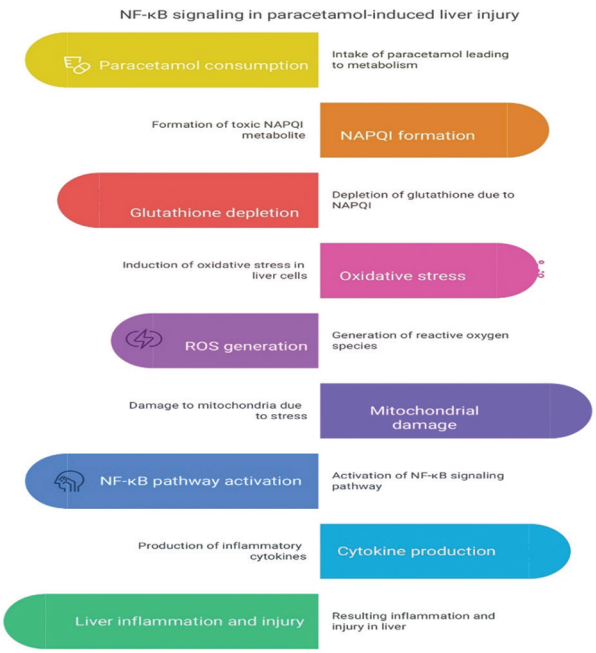


Figure 10. The role of nuclear factor kappa B in liver injury
Abbreviations: NAPQI: N-acetyl-p-benzoquinone imine; NF-κB: Nuclear factor kappa B; ROS: Reactive oxygen species.

downregulation of NF-κB-dependent mRNA expression, further supporting quercetin's protective role against hepatocellular inflammation and damage.[29]

TNF-α is one of the key cytokines in the immune response, particularly in liver injury, where its local effects are significant. Upregulated shortly after cellular injury, TNF-α initiates the inflammatory cascade, modulating tissue responses to injury. This cytokine actively recruits and activates immune cells such as neutrophils, macrophages, and T lymphocytes, which in turn produce a wide array of pro-inflammatory mediators, including cytokines, chemokines, and enzymes. This cascade of events amplifies tissue damage, further compromising liver function by eliciting strong host defenses. TNF-α has been shown to play a dual role, both in protective immune responses and immune-mediated pathological tissue injury, as indicated by its complex interactions between various cell types.[30]

The present study showed a significant increase in the levels of TNF-α in the paracetamol-treated groups compared to the untreated control. The elevation of TNF-α is a key marker for inflammation and hepatic injury in paracetamol-induced liver toxicity. In contrast, rats treated with quercetin revealed a significant reduction in TNF-α expression, highlighting its therapeutic potential in reducing inflammation associated with liver damage. These findings align with similar studies,[31] which indicated that quercetin protects against liver injury.

The liver-protective effects of quercetin are primarily attributed to its potent antioxidant properties. It is assumed that quercetin scavenges free radicals, thus reducing lipid peroxidation and protecting the liver from damage. By blocking the induction of oxidative stress, quercetin may help prevent liver damage. In addition, quercetin's ability to reduce inflammatory cytokines and apoptosis in hepatocytes through NF-κB-dependent pathways further emphasizes its protective role. Since NF-κB is a key regulator of inflammation and cell survival, its inhibition by quercetin could help reduce inflammation and tissue damage. Therefore, these findings, based on a rat model of liver injury, suggest that quercetin could be a promising therapeutic agent for severe liver injuries in the future.[31]

In our study, a significant rise in the levels of caspase-3 in the paracetamol-treated group was observed compared to the untreated normal group. This suggests that paracetamol induces caspase-3 activation, which is often associated with apoptosis. The group that received paracetamol also showed a significant decrease in the levels of BCL2 compared to the control group. Thus, paracetamol may enhance pro-apoptotic signaling whereas suppressing anti-apoptotic actions.

On the contrary, the quercetin-treated group showed significantly lower levels of caspase-3 activation, suggesting a protective effect against apoptosis. These results align with the findings of previous studies, whereby pretreatment with quercetin[32,33] preserved histological architecture and regulated immune expressions of caspase-3 and BCL2, returning them to baseline levels in the liver and kidney. These findings further support that quercetin may regulate apoptosis-related pathways, making it useful in preventing paracetamol-induced organ damage.[33]

Histological examination of liver sections from the normal group revealed well-preserved hepatic lobular architecture, consistent with normal liver function and structural integrity. In contrast, the paracetamol-treated control group exhibited features of liver injury, including grade 2 histological changes such as prominent congestion in the centrilobular and midzonal areas, hemorrhage with focal areas of hepatocyte necrosis, and inflammation. These changes are significantly related to liver dysfunction and tissue damage. Similarly, the control group showed signs of grade 1 acetaminophen-induced liver toxicity. Histological features of this group, such as centrilobular and midzonal congestion and hemorrhage, indicate the early stages of liver damage.

Treatment with quercetin improved the liver condition, where the hepatic injury was reduced to grade 1. Liver tissues showed minimal congestion and inflammation; the few present were mostly in the centrilobular area. This suggests that quercetin may protect the liver from damage. These findings align with previous studies,[34,35] which concluded that quercetin could be a beneficial treatment solution for protecting hepatic cells against oxidative stress and cell injury. Quercetin's ability to regulate inflammatory pathways and decrease cellular damage further supports its protective effects on the liver. These histological improvements observed in the present study reinforce the potential therapeutic benefits of quercetin in liver injury.

5. Conclusion

The results of this study show that quercetin significantly improves liver histology, reduces the severity of hepatic injury, and exerts a protective effect against paracetamol-induced liver injury by reducing oxidative stress, inflammation, and apoptosis.

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None.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Yasmeen Ali Hussien
Formal analysis: Salim Fayze
Investigation: Yahiya Ibrahim Yahiya
Methodology: Salim Fayze
Writing – original draft: Yahiya Ibrahim Yahiya
Writing – review & editing: Yasmeen Ali Hussien

Ethics approval and consent to participate

The approval of the Scientific Research Ethics Committee at the Medical Sciences Center at Al-Kafeel University was obtained for the study protocol (approval date: November 15, 2022, decision number: 2022–7).

Consent for publication

Not applicable.

Availability of data

The details and supporting information can be obtained from the corresponding author upon reasonable request.

References

1. Chalasani NP, Maddur H, Russo MW, Wong RJ, Reddy KR, Practice Parameters Committee of the American College of

Gastroenterology. ACG clinical guideline: Diagnosis and management of idiosyncratic drug-induced liver injury. *Am J Gastroenterol*. 2021;116(5):878-898.

doi: 10.14309/ajg.0000000000001121

2. Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: An overview. *Front Physiol*. 2016;6:363.

doi: 10.3389/fphys.2015.00363

3. Zakaria ZA, Kamisan FH, Kek TL, Salleh MZ. Hepatoprotective and antioxidant activities of *Dicranopteris linearis* leaf extract against paracetamol-induced liver intoxication in rats. *Pharm Biol*. 2020;58(1):478-489.

doi: 10.1080/13880209.2020.1764058

4. Wang X, Wu Q, Liu A, *et al*. Paracetamol: Overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds *in vivo* and *in vitro*. *Drug Metab Rev*. 2017;49(4):395-437.

doi: 10.1080/03602532.2017.1354014

5. Ahmed HM, Shehata HH, Mohamed GS, Abo-Gabal HH, El-Daly SM. Paracetamol overdose induces acute liver injury accompanied by oxidative stress and inflammation. *Egypt J Chem*. 2023;66(3):399-408.

doi: 10.21608/ejchem.2022.134110.5474

6. Caparrotta TM, Antoine DJ, Dear JW. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. *Eur J Clin Pharmacol*. 2018;74(2):147-160.

doi: 10.1007/s00228-017-2356-6

7. Rotundo L, Pyrsopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol*. 2020;12(4):125-136.

doi: 10.4254/wjh.v12.i4.125

8. Li J, Chiew AL, Isbister GK, Duffull SB. Sulfate conjugation may be the key to hepatotoxicity in paracetamol overdose. *Br J Clin Pharmacol*. 2021;87(5):2392-2396.

doi: 10.1111/bcp.14642

9. Fujino C, Sanoh S, Katsura T. Variation in expression of cytochrome P450 3A isoforms and toxicological effects: Endo- and exogenous substances as regulatory factors and substrates. *Biol Pharm Bull*. 2021;44(11):1617-1634.

doi: 10.1248/bpb.b21-00332

10. Lewis PS. The Relevance of MicroRNAs and Circadian Rhythms in Drug Safety. University of Liverpool. [Doctoral Dissertation]; 2012.

11. Madrigal-Santillán E, Madrigal-Bujaidar E, Álvarez-González I, *et al*. Review of natural products with hepatoprotective effects. *World J Gastroenterol*. 2014;20(40):14787-14804.

doi: 10.3748/wjg.v20.i40.14787

12. Ullah A, Munir S, Badshah SL, *et al*. Important flavonoids and their role as a therapeutic agent. *Molecules*. 2020;25(22):5243.

doi: 10.3390/molecules25225243

13. Da Silva Pereira Saccol R, Da Silveira KL, Manzoni AG, *et al*. Antioxidant, hepatoprotective, genoprotective, and cytoprotective effects of quercetin in a murine model of arthritis. *Drug Chem Toxicol*. 2020;43(5):504-511.

doi: 10.1080/01480545.2019.1619095

14. Hawas UW, El-Ansari MA, Osman AF, Galal AF, Abou El-Kassem LT. Flavonoid constituents and protective efficacy of *Citrus reticulate* (Blanco) leaves ethanolic extract on thioacetamide-induced liver injury rats. *Biomarkers*. 2023;28(2):160-167.

doi: 10.1080/1354750X.2022.2151645

15. Pingili RB, Vemulapalli S, Narra UB, Potluri SV, Kilaru NB. Diosmin attenuates paracetamol-induced hepato- and nephrotoxicity via inhibition of CYP2E1-mediated metabolism in rats. *J Appl Pharm Sci*. 2023;13(12):96-104.

doi: 10.7324/JAPS.2023.131212

16. Irwin MR, Curay CM, Choi S, Kiyatkin EA. Basic physiological effects of ketamine-xylazine mixture as a general anesthetic preparation for rodent surgeries. *Brain Res*. 2023;1804:148251.

doi: 10.1016/j.brainres.2023.148251

17. Ayoub SS. Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. *Temperature (Austin)*. 2021;8(4):351-371.

doi: 10.1080/23328940.2021.1886392

18. Offor SJ, Amadi CN, Chijioke-Nwauche I, Manautou JE, Orisakwe OE. Potential deleterious effects of paracetamol dose regime used in Nigeria versus that of the United States of America. *Toxicol Rep.* 2022;9:1035-1044.

doi: 10.1016/j.toxrep.2022.04.025

19. David AVA, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacogn Rev*. 2016;10(20):84-89.

doi: 10.4103/0973-7847.194044

20. Dajas F. Life or death: Neuroprotective and anticancer effects of quercetin. *J Ethnopharmacol*. 2012;143(2):383-396.

doi: 10.1016/j.jep.2012.07.005

21. Singh P, Arif Y, Bajguz A, Hayat S. The role of quercetin in plants. *Plant Physiol Biochem*. 2021;166:10-19.

doi: 10.1016/j.plaphy.2021.05.023

22. Elumalai P, Lakshmi S. Role of quercetin benefits in neurodegeneration. Adv Neurobiol. 2016;12:229-245.

doi: 10.1007/978-3-319-28383-8_12

23. Ezzati M, Yousefi B, Velaei K, Safa A. A review on anti-cancer properties of quercetin in breast cancer. *Life Sci*. 2020;248:117463.

doi: 10.1016/j.lfs.2020.117463

24. Bouhali IE, Tayaa H, Tahraoui A. Quercetin, a natural flavonoid, mitigates fenthion induced locomotor impairments and brain acetylcholinesterase inhibition in male Wistar rat. *Middle East J Sci Res*. 2015;23(1):55-58.

doi: 10.5829/idosi.mejsr.2015.23.01.9220

25. El Faras AA, Elsawaf AL. Hepatoprotective activity of quercetin against paracetamol-induced liver toxicity in rats. *Tanta Med J*. 2017;45(2):92-98.

doi: 10.4103/tmj.tmj_43_16

26. Anusha CS, Sini H, Prakashkumar B, Nevin KG. Mechanism of protection of rat hepatocytes from acetaminophen-induced cellular damage by ethanol extract of *Aerva lanata*. *Interdiscip Toxicol*. 2019;12(4):169-179.

doi: 10.2478/intox-2019-0021

27. Su JF, Guo CJ, Wei JY, Yang JJ, Jiang YG, Li AYF. Protection against hepatic ischemia-reperfusion injury in rats by oral pretreatment with quercetin. *Biomed Environ Sci*. 2003;16(1):1-8.

28. Rius-Pérez S, Pérez S, Martí-Andrés P, Monsalve M, Sastre J. Nuclear factor kappa B signaling complexes in acute inflammation. *Antioxid Redox Signal*. 2020;33(3):145-165.

doi: 10.1089/ars.2019.7975

29. Tieppo J, Cuevas MJ, Vercelino R, Tuñón MJ, Marroni NP, González-Gallego J. Quercetin administration ameliorates pulmonary complications of cirrhosis in rats. *J Nutr*. 2009;139(7):1339-1346.

doi: 10.3945/jn.109.105353

30. Williams JA, Manley S, Ding WX. New advances in molecular mechanisms and emerging therapeutic targets in alcoholic liver diseases. *World J Gastroenterol*. 2014;20(36):12908-12933.

doi: 10.3748/wjg.v20.i36.12908

31. Peng Z, Gong X, Yang Y, *et al*. Hepatoprotective effect of quercetin against LPS/d-GalN induced acute liver injury in mice by inhibiting the IKK/NF-κB and MAPK signal pathways. *Int Immunopharmacol*. 2017;52:281-289.

doi: 10.1016/j.intimp.2017.09.022

32. El-Bakry HA, El-Sherif G, Rostom RM. Therapeutic dose of green tea extract provokes liver damage and exacerbates paracetamol-induced hepatotoxicity in rats through oxidative stress and caspase 3-dependent apoptosis. *Biomed Pharmacother*. 2017;96:798-811.

doi: 10.1016/j.biopha.2017.10.055

33. Soliman MM, Gaber A, Alsanie WF, *et al*. Gibberellic acid-induced hepatorenal dysfunction and oxidative stress: Mitigation by quercetin through modulation of antioxidant, anti-inflammatory, and antiapoptotic activities. *J Food Biochem*. 2022;46(2):e14069.

doi: 10.1111/jfbc.14069

34. Liu X, Zhang Y, Liu L, *et al*. Protective and therapeutic effects of nanoliposomal quercetin on acute liver injury in rats. *BMC Pharmacol Toxicol*. 2020;21(1):11.

doi: 10.1186/s40360-020-0388-5

35. Ashkani-Esfahani S, Bagheri F, Azarpira N, *et al*. Protective effects of quercetin on thioacetamide-induced acute liver damage and its related biochemical and pathological alterations. *Egypt J Intern Med*. 2016;28:123-127.

doi: 10.4103/1110-7782.200965