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Comparative Protective Effect of Alpha-Lipoic Acid and Melatonin Against Acetaminophen-Induced Liver Injury in Male Rats

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Abstract: The present study evaluated the efficacy of alpha-lipoic acid, melatonin, and their co-administration in preventing liver injury caused by an acetaminophen overdose in a rat model. **Methods:** Forty-two rats were allocated into six groups: normal control (0.75% carboxymethyl cellulose, CMC), negative control (acetaminophen 3 g/kg), positive control (N-acetylcysteine 100 mg/kg), alpha-lipoic acid (100 mg/kg), melatonin (10 mg/kg), and a combination of alpha-lipoic acid and melatonin (100 mg/kg + 10 mg/kg, respectively). Treatments were administered once daily for 14 days before a single oral hepatotoxic dose of acetaminophen (3 g/kg). Liver biochemical parameters (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and total protein), oxidative stress indicators (hepatic and serum reduced glutathione, GSH), inflammatory cytokines (interleukin-6 and interleukin-10), and liver histopathology were assessed 48 hours post-toxication. **Results:** Acetaminophen overdose caused elevated serum levels of ALT, AST, ALP, and total bilirubin; decreased total protein and GSH; increased IL-6 and IL-10; and marked histopathological alterations. Alpha-lipoic acid reversed these changes. Melatonin improved most parameters, particularly hepatic GSH levels, but had limited effects on ALP and total protein. The combination therapy produced the most complete histological preservation. Conclusions: Alpha-lipoic acid provides comprehensive hepatoprotective activity against acetaminophen-induced toxicity, both alone and in combination with melatonin. While melatonin contributed localized antioxidant benefits, particularly in hepatic tissue, the combination therapy offered the most pronounced structural protection, supporting the therapeutic potential of dual antioxidant strategies.

Keywords: Acetaminophen; Hepatotoxicity; Alpha-lipoic acid; Melatonin; N-acetyl cysteine; Hepatoprotective; Acute liver injury

Introduction

The liver is a key organ responsible for maintaining physiological homeostasis within the body. Functions such as metabolic control, protein biosynthesis, detoxification of exogenous compounds, and immune regulation are primarily carried out by hepatocytes [1] . Owing to its essential role in the biotransformation of xenobiotics, the liver is equipped with an extensive array of enzymes that participate in both phase I oxidation and phase II conjugation reactions [2]. However, these metabolic reactions can also yield reactive intermediates, which may provoke hepatic damage by inducing oxidative stress and impairing mitochondrial function [3].

Liver disorders represent a group of conditions marked by hepatocyte damage, immune cell infiltration, and activation of hepatic stellate cells. These pathological processes progressively disrupt normal liver architecture, ultimately leading to impaired hepatic function [4]. Common liver diseases include viral hepatitis, non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, and drug-induced liver injury (DILI), which has become a major contributor to both acute and chronic liver diseases [1]. Each year, liver diseases account for approximately two million deaths worldwide, with cirrhosis being the leading cause. The remaining deaths are mainly due to conditions such as viral hepatitis and liver cancer [5].

DILI develops when the liver adversely responds to toxins, drugs, natural supplements, or other chemicals. According to pathogenic mechanisms, DILI is traditionally categorized into intrinsic, idiosyncratic, and, more recently, indirect types [6]. Intrinsic DILI is dose-related and predictable (as in the case of acetaminophen toxicity), with excess drug metabolism generating reactive intermediates that covalently bind to cellular proteins to form toxic adducts, thereby impairing hepatocellular function and tissue integrity. Idiosyncratic DILI, in contrast, is unpredictable, often immune-mediated, and typically occurs only in genetically susceptible individuals. It involves activation of the adaptive immune system in recognition of drug-protein adducts [6, 7]. Oxidative stress is a primary mechanism of DILI, with many drugs or their metabolites generating reactive oxygen species (ROS) that exceed the cell's antioxidant capacity, leading to mitochondrial injury and ultimately cell death through apoptosis or necrosis [8].

Acetaminophen (APAP) is a widely used therapeutic agent, primarily for its analgesic and antipyretic effects. While safe at recommended doses, overdose is associated with severe hepatic injury and is a major cause of acute liver failure globally [9]. Its hepatotoxicity is primarily driven by oxidative imbalance and mitochondrial dysfunction, which are central to its mechanism of toxicity [10]. However, the specific molecular

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pathways involved in APAP-induced hepatotoxicity remain incompletely elucidated. At therapeutic doses, 60–90% of APAP is metabolized via glucuronidation and sulfation, while 5–15% is processed by cytochrome P450 enzymes into N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive metabolite [11]. In overdose situations, excessive NAPQI formation leads to oxidation of protein thiols and elevated ROS levels [12].

Increased free radical production within hepatocytes promotes inflammation and exacerbates cellular damage during APAP-induced hepatotoxicity [13]. Despite its varied clinical presentation, N-acetylcysteine (NAC) remains the standard treatment, with liver transplantation considered in severe cases. Nevertheless, the limitations of current therapies—particularly the reduced efficacy of NAC when administered late after overdose—have driven interest in identifying additional compounds that may reduce susceptibility to both unintentional and intentional APAP-induced liver injury [14].

Natural compounds have demonstrated significant hepatoprotective activity against APAP-induced liver injury by modulating oxidative stress, inflammation, and reactive metabolite formation [12]. Phytochemicals such as silymarin, resveratrol, and curcumin offer promising therapeutic alternatives due to their multifaceted biological properties [15]. Among these, alpha-lipoic acid (ALA) and melatonin have been the most extensively studied, largely because of their potent antioxidant and anti-inflammatory actions.

ALA, also known as thioctic acid, is a naturally occurring dithiol compound with a strong safety profile. It is present in various foods, synthesized endogenously, and functions as a mitochondrial cofactor in carbohydrate and lipid metabolism [16]. ALA mitigates oxidative stress and inflammation by chelating metals and regenerating antioxidants such as glutathione (GSH), ascorbate, and tocopherol. Its potent redox activity and amphiphilic nature enhance cellular uptake, supporting its protective effects on liver tissue [17].

Melatonin, a pineal gland-derived neurohormone, is involved in the regulation of multiple physiological processes, including sleep, circadian rhythm synchronization, reproductive function, and immune modulation [18]. It exhibits diverse pharmacological effects, notably antioxidant, anti-inflammatory, anticancer, anti-apoptotic, and immunomodulatory properties [19]. Acting as a potent scavenger of free radicals, melatonin enhances the activity of glutathione peroxidase, which converts reduced glutathione to its oxidized form. This process facilitates the breakdown of hydrogen peroxide (H_2O_2) into water, thereby preventing the generation of highly reactive hydroxyl radicals by removing their precursors [20].

Given these insights, the present study aimed to explore the hepatoprotective capacity of alpha-lipoic acid, melatonin, and their combination, using an acetaminophen-induced liver injury model to evaluate their antioxidant and anti-inflammatory effects in rats.

Materials and Methods

Materials

Alpha-lipoic acid (250 mg) and melatonin (20 mg) capsules were obtained from NOW FOODS (USA); N-acetylcysteine (600 mg) capsules from Life Extension (USA); acetaminophen (Doliprane, 1 g tablets) from Sanofi (France); carboxymethyl cellulose (sodium salt) from TM Media (India); chloroform from Thomas Baker (India); and formaldehyde from Chem-Lab NV (Belgium).

Experimental Animals

The present study was performed at the College of Pharmacy, University of Basrah, involving 42 male albino rats weighing 180–220 g. The animals were maintained in conventional laboratory cages under controlled temperature (21 ± 3°C) and a 12-hour light/dark photoperiod, with food and water available ad libitum. They underwent a two-week adaptation period to minimize stress and maintain physiological stability.

Experimental Design

Forty-two male rats were randomly assigned to six experimental groups (seven rats per group). Treatments were administered once daily via oral gavage for 14 consecutive days as pretreatment prior to APAP administration. Group 1 (Normal Control) received 0.75% carboxymethyl cellulose (CMC) solution for the entire 16-day period to maintain consistency with the overall study timeline and sacrifice schedule. Group 2 (Negative Control) received 0.75% CMC for 14 days, followed by a single oral dose of APAP (3 g/kg) on day 15 to induce hepatotoxicity [21], selected as a sublethal dose previously reported in rats. The remaining groups received their respective treatments dissolved in 0.75% CMC [22] for 14 days, Group 3 (Positive Control) received N-acetylcysteine (100 mg/kg) [23], Group 4 was treated with alpha-lipoic acid (100 mg/kg) [24], Group 5 received melatonin (10 mg/kg) [25], and Group 6 received a combination of alpha-lipoic acid (100 mg/kg) and melatonin (10 mg/kg). On day 15, following a 12-hour fasting period, a single oral dose of APAP (3 g/kg) was administered to all groups, except the normal control.

On day 17 (i.e., 48 hours after the APAP toxic dose), rats were anesthetized with chloroform before sacrifice. Chloroform was used exclusively for brief anesthesia during sacrifice, and at this minimal exposure, it does not induce hepatotoxicity. Blood was collected from the posterior vena cava, and the samples were then transferred into gel tubes and left at ambient temperature for 1 hour to coagulate. Subsequently, the samples were spun using a centrifuge at 5000 rpm for 10 minutes. The separated serum was placed into Eppendorf tubes for biochemical analysis. Following dissection, the liver was excised and split into two segments: One segment was fixed in 10% formaldehyde for histopathological processing, and the other was preserved in phosphate-buffered saline (PBS) for GSH assessment.

Biochemical Analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using colorimetric kits (Elabscience, Cat. Nos. E-BC-K236-M and E-BC-K235-S, USA). Alkaline phosphatase (ALP) and total bilirubin were assessed with Mindray kits (China) via the IFCC enzymatic and VOX vanadate oxidation methods, respectively. Serum and hepatic glutathione (GSH) levels were quantified by ELISA kits (ELK Biotechnology, Cat. No. ELK8577; Elabscience, Cat. No. E-EL-0026), based on absorbance inversely related to GSH levels, with hepatic values normalized to total protein. Serum interleukin-6 and interleukin-10 were measured using specific sandwich ELISA kits (ELK Biotechnology, Cat. Nos. ELK1158 and ELK1144), following the manufacturer's instructions.

Histopathological Examination

Liver specimens were gently rinsed with cold normal saline and fixed in 10% neutral buffered formalin for 48 hours. The samples were then processed using standard histological protocols as described by Suvarna et al. [26], including dehydration through a graded ethanol series, clearing in xylene, and embedding in paraffin wax. Sections approximately 4–5 µm thick were cut using a rotary microtome. These sections were stained with haematoxylin and eosin (H&E) and examined under a light microscope (Genex, USA) at 20× magnification to assess hepatic architecture and identify structural damage or

pathological changes. Histopathological evaluation was conducted descriptively on representative sections from two rats per group, providing qualitative insights into the extent of liver injury.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). A one-way ANOVA was used to assess differences among group means, followed by Tukey's multiple comparison test for pairwise analysis. A p-value < 0.05 was considered statistically significant. Data analysis was performed using GraphPad Prism software, version 8 (USA).

Results

Hepatic Enzyme Markers

Figures 1A–C demonstrate a substantial elevation in serum ALT, AST, and ALP levels in the negative control group (Group 2) compared with the normal control group (Group 1) ($p < 0.0001$). All intervention groups significantly reduced ALT and AST relative to Group 2. Notably, the most pronounced ALT reductions were observed in the alpha-lipoic acid (Group 4) and combination treatment (Group 6) ($p < 0.0001$; Figure 1A), both outperforming the positive control (Group 3). Regarding AST, only Group 6 exhibited a significant decline versus Group 3 ($p < 0.001$; Figure 1B). For ALP, all interventions except melatonin monotherapy (Group 5) significantly reduced levels relative to Group 2 ($p < 0.05$; Figure 1C), with no statistically meaningful differences among the treated groups.

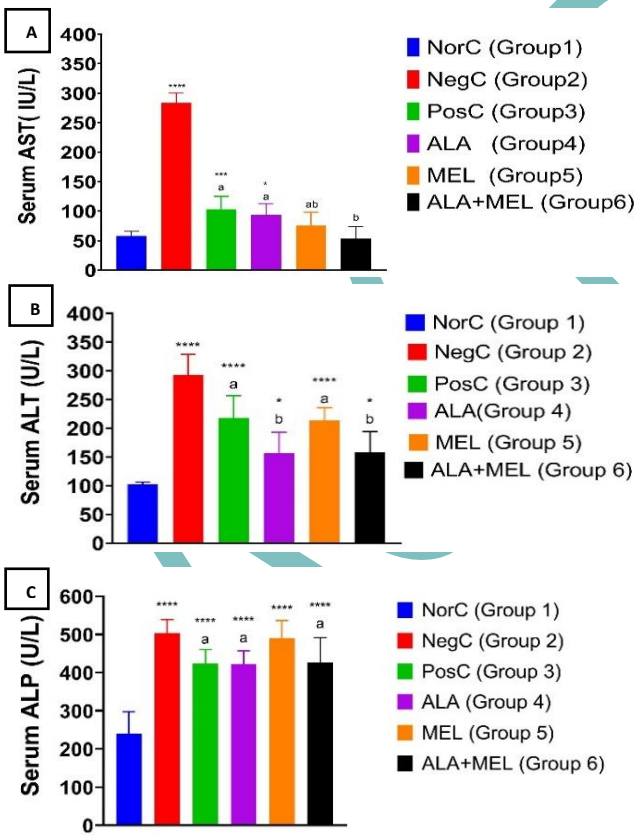

Figure (1): Serum levels of A) ALT, B) AST, and C) ALP following experimental treatments in rats with acetaminophen-induced liver injury. (*) denotes statistical significance in comparison with Group 1. Different letters indicate a statistical significance relative to Group 2

Biliary Function Marker

Assessment of serum total bilirubin levels (Figure 2) revealed a marked increase in Group 2 compared to Group 1 ($p < 0.0001$). All treatment groups significantly lowered bilirubin, with Group 4 (ALA) and Group 6 (ALA + melatonin) showing levels comparable to Group 3 (NAC) ($p > 0.05$), whereas Group 5 (melatonin alone) remained significantly elevated ($p < 0.05$).

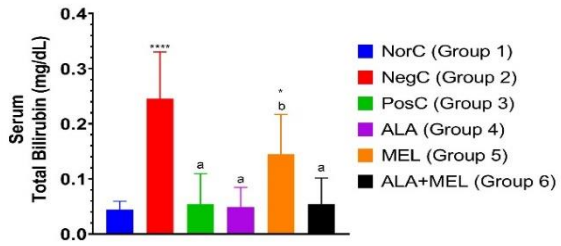

Figure (2): Serum levels of A) ALP and B) total bilirubin following experimental treatments in rats with acetaminophen-induced (*) denotes statistical significance in comparison with Group 1. Different letters indicate a statistical significance relative to Group 2

Protein Synthesis

As shown in Figure 3, hepatic total protein was significantly diminished in Group 2 in contrast to Group 1 ($p < 0.05$). Treatment with all agents, excluding melatonin (Group 5), resulted in significant restoration of hepatic protein levels relative to Group 2, with no statistical differences among the treated groups.

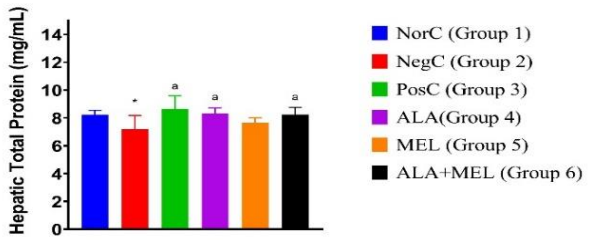

Figure (3): Hepatic total protein levels following administration of experimental therapies in rats with acetaminophen-induced liver injury. (*) denotes statistical significance in comparison with Group 1. (a) indicates statistical significance relative to Group 2.

Oxidative Stress Markers

An overview of GSH concentrations in serum and liver tissues across all groups is provided in Figures 4A and 4B. Group 2 showed a significant decrease in GSH levels in both compartments compared to Group 1 ($p < 0.0001$). All treatment groups significantly restored both serum and hepatic GSH levels to varying extents relative to Group 2. Administration of ALA (Group 4) resulted in a markedly higher serum GSH concentration than that recorded in Group 3 ($p < 0.05$, Figure 4A). Furthermore, in liver tissue, all treatment groups surpassed Group 3 in enhancing GSH levels ($p < 0.0001$, Figure 4B), with the most substantial increase detected in the melatonin group (Group 5).

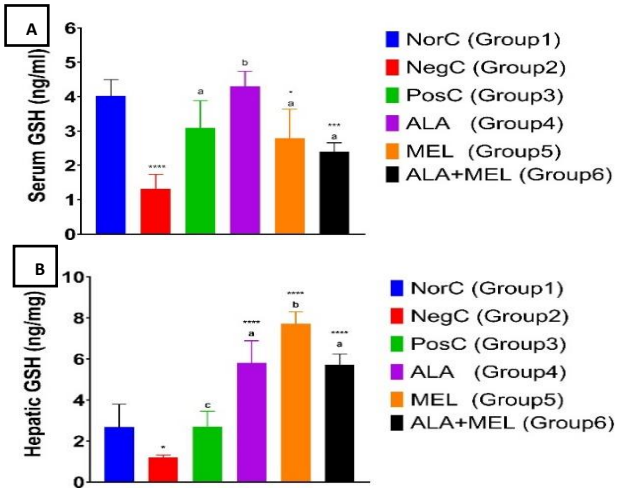

Figure (4): Serum (A) and hepatic (B) levels of reduced glutathione (GSH) following experimental therapies in rats with acetaminophen-induced liver injury. (*) denotes statistical significance in comparison with Group 1. Different letters indicate a statistical significance relative to Group 2.

Inflammatory Cytokines

Figures 5A and 5B illustrate a significant rise in serum levels of IL-6 and IL-10 in Group 2 compared to Group 1 ($p < 0.0001$). All therapeutic interventions significantly downregulated these cytokines, albeit to different extents relative to Group 2. Comparisons among the treated groups showed no significant differences.

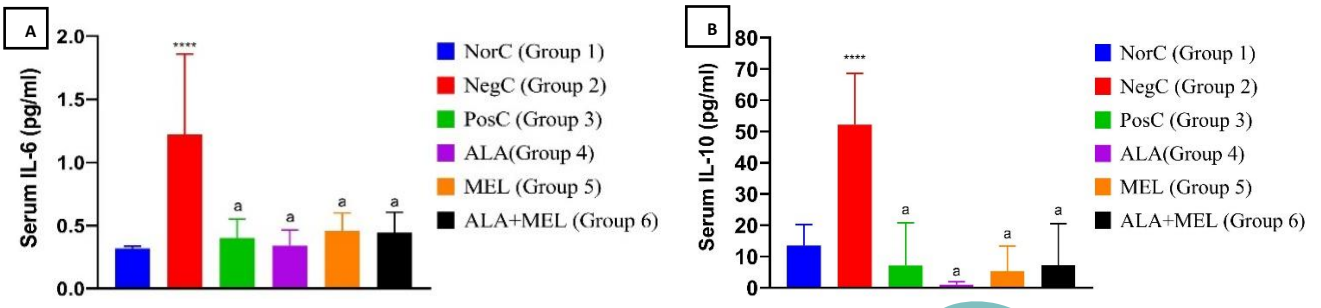

Figure (5): Serum levels of A) IL-6 and B) IL-10 following experimental treatments in rats with acetaminophen-induced liver injury. (*) denotes statistical significance in comparison with Group 1. Different letters indicate statistical significance relative to Group 2.

Histopathological Findings

The histopathological analysis of liver tissues is summarized in Figure 6. Group 2 exhibited pronounced hepatic injury (Figure 6B), characterized by disrupted liver architecture, in comparison with Group 1 (Figure 6A), which retained normal hepatic structure. Among the treatment groups, the positive control (Group 3, Figure 6C) and the combination therapy (Group 6, Figure 6F) preserved hepatic architecture most effectively. Moderate amelioration was observed in the ALA (Group 4, Figure 6D) and melatonin (Group 5, Figure 6E) groups, as evidenced by reduced vascular abnormalities and decreased hepatocellular vacuolation

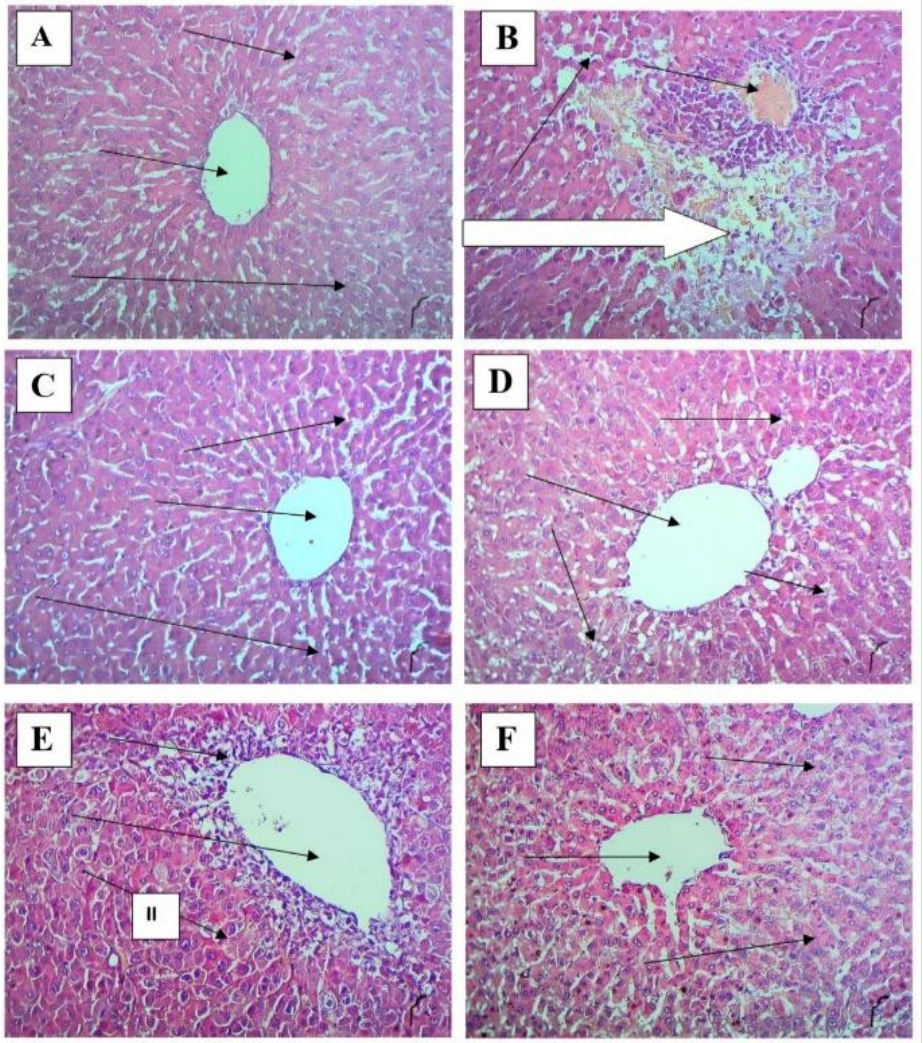

Figure (6): Microscopic examination of liver sections from rats (H&E, ×20). A) Group 1: normal hepatic cords radiating from the central vein as single plates of hepatocytes, within normal liver zones. B) Group 2: acute hepatitis with central vein congestion, intense granulomatous infiltrates, loss of radial cord arrangement, and prominent coagulative necrosis. Hydropic degeneration with vacuolization is also noted. Sinusoids show severe damage with congestion. C) Group 3: normal hepatocytes with intact central vein, hepatic cords, and sinusoids. D) Group 4: marked dilation of the central vein with vacuolated hepatocytes. Small lipid droplets and large cytoplasmic vacuoles are present. Sinusoids appear mostly normal. E) Group 5: varicosity and dilation of the central vein with endothelial lining damage. Hepatic cords in zone II appear normal, and sinusoids are generally preserved. F) Group 6: preserved hepatic cord architecture with pink-staining hepatocyte plates and normal-appearing sinusoids.

Discussion

Acetaminophen overdose is a well-established experimental model for drug-induced liver injury. Its hepatotoxicity mainly arises from N-acetyl-p-benzoquinone imine (NAPQI) accumulation, a reactive metabolite that triggers oxidative stress, mitochondrial dysfunction, hepatocyte necrosis, and immune activation [27]. In this study, male rats received a single sublethal dose of APAP (3 g/kg), consistent with previous reports indicating an LD50 of approximately 3.7 g/kg in rats [28]. This dose produced sustained hepatic damage, enabling assessment of the hepatoprotective efficacy of various treatments.

A key indicator of hepatic injury is the leakage of intracellular enzymes into the bloodstream due to compromised membrane integrity. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are sensitive markers of hepatocellular damage [29]. Group 2 exhibited significant elevations in ALT and AST, confirming acute liver injury as previously reported [30, 31]. All treatment groups demonstrated substantial enzyme reductions compared to Group 2. In Group 3, N-acetylcysteine scavenged NAPQI and replenished intracellular glutathione (GSH) [32]. Compared to Group 3, Groups 4 and 6 showed greater decreases in both enzymes, likely attributable to ALA's scavenging of hydroxyl radicals, transition metal chelation, and stabilization of the mitochondrial respiratory chain [24, 33]. Group 5 produced a comparable ALT reduction to Group 3 but a more pronounced AST decrease, reflecting melatonin's antioxidant properties and regulation of calcium homeostasis [34]. AST levels were lowest in Group 6, indicating superior hepatocellular and mitochondrial protection with combined therapy [35].

Cholestatic markers, alkaline phosphatase (ALP) and total bilirubin, were also assessed. Group 2 exhibited significant elevations, indicative of biliary tract injury [36]. ALP elevation reflects cholestasis, while increased bilirubin results from disrupted synthesis, conjugation, or excretion [37]. Group 3 significantly reduced ALP and bilirubin, confirming NAC's hepatoprotective activity [38]. Similarly, Groups 4 and 6 showed significant reductions in both markers, highlighting ALA's antioxidant and anti-inflammatory actions [17, 39]. No significant differences were observed among Groups 3, 4, and 6, suggesting comparable efficacy. By contrast, Group 5 significantly decreased bilirubin, albeit less than other treatments, but failed to reduce ALP relative to Group 2, a finding that contrasts with earlier reports [40]. This discrepancy may be explained by melatonin's primary role in modulating oxidative stress rather than improving bile production or ductal repair.

Total protein, a marker of hepatic synthetic function, was significantly reduced in Group 2, indicating impaired protein synthesis and liver dysfunction, consistent with prior hepatotoxicity reports [10, 41]. Groups 3, 4, and 6 exhibited significant improvement, demonstrating effective restoration of hepatic biosynthesis. The absence of differences among these groups suggests that ALA alone exerts hepatoprotective effects comparable to NAC and its combination with melatonin. These results support the therapeutic roles of NAC and ALA in liver function recovery post-APAP injury [32, 36]. Conversely, Group 5 showed no significant benefit, underscoring melatonin's limited capacity to reverse synthetic dysfunction, contrary to Bhatti et al. [25].

Oxidative stress was further evaluated by measuring GSH levels in serum and liver tissue. Group 2 showed marked GSH depletion, confirming APAP-induced oxidative damage [42]. Group 3 demonstrated moderate hepatic and marked serum GSH restoration via cysteine donation and NAPQI inhibition, consistent with NAC's role as a GSH precursor [32]. Group 4 significantly increased GSH in both compartments, especially serum, attributed to ALA's stimulation of cysteine uptake, activation of γ-glutamylcysteine synthetase, and regeneration of GSH through dihydrolipoic acid [17]. This systemic increase in serum GSH emphasizes ALA's extracellular antioxidant effects. Group 5 achieved the highest hepatic GSH elevation, likely due to melatonin's mitochondrial stabilization and modulation of the nitric oxide pathway, conferring strong liver-specific protection [43]. Group 6 showed no additional enhancement over individual treatments, indicating a lack of synergistic effect on GSH replenishment.

Inflammation was assessed via serum IL-6 and IL-10 levels. Group 2 exhibited significant elevations of both cytokines, reflecting systemic inflammation. IL-6, produced by activated Kupffer cells, promotes acute-phase protein synthesis and neutrophil recruitment via STAT3 signaling [44, 45]. Notably, IL-10 levels were also elevated, contrasting earlier-phase studies reporting IL-10 suppression [46]. This does not contradict IL-10's well-established anti-inflammatory activity but rather reflects the 48-hour post-toxication sampling point, which likely captured a compensatory anti-inflammatory phase. Supporting this interpretation, Roth et al. (2023) demonstrated that elevated IL-10 in APAP-induced acute liver failure is associated with impaired immune responses and delayed tissue repair [47]. Group 3 significantly reduced both IL-6 and IL-10, demonstrating NAC's anti-inflammatory effects [48]. Group 4 elicited even greater suppression, likely through ALA's downregulation of pro-inflammatory genes and modulation of anti-inflammatory pathways [49]. Groups 5 and 6 exhibited cytokine reductions comparable to Group 3, with melatonin likely exerting its effects through inhibition of TLR4 and MAPK signaling pathways [50]. However, the combination (Group 6) did not confer additional benefits beyond single-agent treatments.

Histopathological analysis aligned with biochemical findings. Group 1 showed normal liver architecture with intact hepatic cords and sinusoids. Group 2 displayed extensive damage characterized by central vein congestion, granulomatous infiltration, hydropic degeneration, and coagulative necrosis, typical of acute hepatotoxicity [41]. Group 3 exhibited marked histological improvement, preserving hepatocyte structure and sinusoidal organization [38]. Group 4 demonstrated significant recovery, with mild vacuolization and central vein dilation persisting, consistent with previous ALA studies [33]. Group 5 presented better-preserved hepatic architecture in midzonal regions despite residual vascular injury, reflecting melatonin mitochondrial and anti-inflammatory effects [34]. Group 6 showed the most complete histological protection, with intact hepatocyte plates and well-defined sinusoids. Although biochemical data indicated similar or superior effects of ALA and melatonin alone, histology favored the combination, suggesting potential synergism [35].

Limitations

This study has several limitations. Histopathological evaluation was descriptive, non-quantitative, and unblinded, and standardized scoring was not applied. Oxidative stress assessment was limited to glutathione, without inclusion of other markers such as malondialdehyde (MDA). Molecular analyses, including CYP2E1 and apoptotic markers, were beyond the scope of the present work. No systematic evaluation of potential side effects was conducted, although no adverse effects were observed at the tested doses. In addition, only a single dose level of ALA (100 mg/kg) and melatonin (10 mg/kg) was examined, without a full dose–response assessment. Finally, the study included only male rats; therefore, sex-related differences were

not addressed. Future studies incorporating post-treatment models, broader biomarker panels, dose–response analyses, inclusion of both sexes, and clinical validation are warranted.

Conclusion

The findings of this study confirm that alpha-lipoic acid (ALA) provides substantial protection against acetaminophen-induced liver injury, as evidenced by improvements in hepatic enzymes, antioxidant status, inflammatory markers, and histopathological architecture. Melatonin conferred partial protection, primarily through the enhancement of hepatic GSH, but showed limited effects on synthetic and cholestatic functions. The combination of ALA and melatonin resulted in greater preservation of liver tissue, suggesting additive antioxidant activity at the histological level. While these findings support the therapeutic value of ALA as a primary hepatoprotective agent, the study was limited to a single dose level in a preclinical model. Future research should explore different dosing regimens, detailed safety profiling, and clinical evaluation to better define the translational potential of ALA and its combination with melatonin.

Disclosure Statements

- **Ethics approval and consent to participate:** Animal handling and experimental procedures adhered to the ethical standards outlined by the National Institutes of Health (NIH) for the use of laboratory animals. The research protocol was reviewed and approved by the Animal Ethics Committee at the College of Pharmacy, University of Basrah, under approval code EC64, issued on November 1, 2024.
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The corresponding author can be contacted to request access to the data, which will be made available upon reasonable request
- **Author's contribution:** Ausama Ayob Jaccob contributed to the study design and performed the data analysis. Huda Ayad Al-Mosawi and Muhsin S.G. Al-Mozie'l performed material preparation, experimentation, and data collection. Muhsin S.G. Al-Mozie'l also interpreted the histopathological findings. Huda Ayad Al-Mosawi wrote the initial draft of the manuscript, and all authors contributed to and commented on the final version. All authors read and approved the final manuscript.
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