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Alpha-lipoic acid as a therapeutic agent in ibuprofen-induced liver fibrosis in a rat model

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MAIN POINTS

- Ibuprofen-induced liver injury caused hepatocyte degeneration, sinusoidal dilatation, collagen accumulation, and increased the expression of TLR4, TGF-β, and α-SMA.
- ALA treatment significantly reduced TLR4 and α-SMA expression and alleviated sinusoidal dilatation and fibrosis, although its effect on TGF-β, was limited.
- The therapeutic potential of ALA lies in its ability to suppress inflammatory signaling and hepatic stellate cell activation, suggesting that ALA may serve as a protective strategy against NSAID-induced liver fibrosis.

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ABSTRACT

Aim: Ibuprofen (IBU), a commonly used nonsteroidal antiinflammatory drug, may cause hepatotoxicity and fibrosis with prolonged or high-dose use. Alpha-lipoic acid a natural antioxidant, has shown protective effects against oxidative stress and liver injury. The potential therapeutic role of ALA in IBU-induced liver fibrosis was evaluated.

Materials and Methods: Thirty male Wistar Albino rats were divided into five groups (n = 6): control, sham (corn oil), IBU (200 mg/kg by oral gavage for 21 days), IBU + ALA (IBU for 21 days followed by ALA 100 mg/kg by oral gavage for 7 days), and ALA (100 mg/kg for 7 days). Liver tissue was analyzed histopathologically with hematoxylin-eosin and Masson's trichrome staining and immunohistochemically for TLR4, TGF-β, and α-SMA expression. Data were analyzed using GraphPad Prism 10, with normality assessed by Shapiro-Wilk, group comparisons by analysis of variance or Kruskal-Wallis, and $p < 0.05$ considered significant.

Results: IBU caused significant hydropic degeneration, sinusoidal dilatation, and perivascular collagen accumulation compared with controls ($p<0.001$). ALA treatment significantly reduced sinusoidal dilatation ($p<0.01$) and perivascular fibrosis ($p<0.001$) significantly but had no significant effect on hydropic degeneration. Immunohistochemistry showed increased TLR4, TGF-β, and α-SMA expression in the IBU group ($p<0.001$). ALA significantly decreased TLR4 ($p<0.001$) and α-SMA ($p<0.001$), while reduction in TGF-β was not statistically significant.

Conclusion: Although ALA alleviates IBU-induced liver fibrosis by suppressing inflammatory signaling (TLR4) and hepatic stellate cell activation (α-SMA), its effect on TGF-β-mediated fibrogenesis is limited. These findings suggest that ALA may help prevent nonsteroidal anti-inflammatory drug-induced liver injury, but further studies with longer treatment durations are needed.

Keywords: Ibuprofen, Alpha-lipoic acid, Fibrosis, Rat

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INTRODUCTION

Ibuprofen (IBU) was first introduced in the United Kingdom in 1969 and became internationally available as a prescription medication in the 1970s [1]. IBU is a nonsteroidal antiinflammatory drug that non-selectively inhibits cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis. This mechanism exerts anti-inflammatory, analgesic, and antipyretic effects [2,3]. IBU is rapidly absorbed from the gastrointestinal tract at approximately 80%, reaching a maximum plasma concentration within 1-2 hours after oral administration and demonstrating 99% plasma protein binding. It is metabolized in the liver and excreted by the kidneys, with an elimination half-life ranging from 1.6 to 2.5 hours [4]. While IBU is commonly used for musculoskeletal disorders, osteoarthritis, and rheumatoid arthritis, high doses and long-term use of international bitterness unit have been associated with hepatotoxicity, including drug-induced fibrosis [2,3,5]. Therefore, liver fibrosis may develop secondary to chronic IBU-related hepatic injury, impairing liver function through excessive extracellular matrix accumulation [6].

Fibrosis is characterized by abnormal and excessive extracellular matrix deposition during chronic tissue injury and represents a maladaptive wound-healing response extension [7]. Although initially protective, uncontrolled fibrosis disrupts organ structure and function. Hepatocytes, hepatic stellate cells, sinusoidal endothelial cells, and immune cells play ma-

jor roles in the progression of fibrosis in the liver. Advanced fibrosis and cirrhosis are associated with severe vascular remodeling, which results in impaired hepatocyte perfusion and liver dysfunction [8].

Natural compounds continue to attract increasing interest in biomedical research, as approximately 80% of the global population relies on natural or traditional medicines for health care purposes [9]. Alpha-lipoic acid (ALA) was first identified in the 1930s and later isolated from liver tissue in the 1950s as a thiol-containing molecule with strong antioxidant characteristics [10-12]. ALA is considered a potent antioxidant and mitochondrial cofactor with notable antiinflammatory properties [13]. It is naturally present in various vegetables and is particularly abundant in mitochondria-rich animal tissues such as the liver, heart, and kidneys [14,15]. In addition, all anatomical parts of plants contain polyphenolic compounds, particularly flavonoids, which constitute approximately 60% of naturally occurring polyphenols and display diverse pharmacological activities [16]. Multiple studies have demonstrated the therapeutic benefits of ALA in conditions such as diabetes, atherosclerosis, neurodegenerative diseases, joint disorders, and AIDS [17]. ALA exerts hepatoprotective effects by decreasing oxidative stress through free radical scavenging and regenerating endogenous antioxidants, thereby preventing cellular injury and maintaining hepatic function [18].

Toll-like receptor 4 (TLR4) and transforming growth factor-beta (TGF-β) signaling pathways play key roles in liver fibrogenesis. TLR4 contributes to hepatic injury under certain pathological conditions, such as alcoholic and non-alcoholic fatty liver disease, but its inactivation does not reduce injury in bile duct ligation or CCl4-induced fibrosis models [14, 19-21]. TGF-β is a major fibrogenic cytokine that activates hepatic stellate cells (HSCs) through the TGF-β/Smad pathway, leading to myofibroblast transformation and excessive extracellular matrix production [22-25]. Additionally, TGF-β interacts with Notch, Wnt/β-catenin, and YAP/TAZ pathways, further amplifying inflammation, cellular injury, and fibrosis progression [26-29]. HSCs, located in the space of Disse, lose stored vitamin A during activation and begin expressing α-smooth muscle actin (α-SMA), producing large amounts of extracellular matrix [30-33].

In this study, liver fibrosis will be experimentally induced in rats by high-dose IBU administration, and the potential therapeutic effects of ALA on this fibrotic process will be investigated. Histopathological evaluations and immunohistochemical analyses will be performed to assess morphological and cellular responses. This study aimed to comprehensively evaluate the ability of alpha-lipoic acid to attenuate IBU-induced hepatic injury and fibrosis.

MATERIALS AND METHODS

Animals and the experimental protocol

This study was conducted at the Kirsehir Ahi Evran University Experimental Research Center. Procurement, care, and experimental modeling of the animals were conducted in the same center. The Kirsehir Ahi Evran University Local Animal Experiments Ethics Committee approved the experimental studies prior to their initiation (Approval number: 5/10, Date: 06/03/2025). A total of 30 male Wistar Albino rats, weighing 220 ± 20 g were used in this study. Before and during the experiment, the animals were housed under a 12-hour light/12-hour dark cycle at a constant temperature of 22-24 °C. Standard pellet chow and tap water were provided ad libitum without any restriction. Animal experiments were performed according to the Animal Research: Reporting In Vivo Experiments guidelines.

Experimental groups and treatments used

The animals were randomly assigned to 5 groups using simple randomization, each consisting of 6 rats [34].

Group 1 (control group): No procedure was applied to this group for 4 weeks.

Group 2 (sham group): Corn oil, which was used as the solvent for ALA, was administered to the rats by oral gavage for 4 weeks.

Group 3 (IBU group): The rats received 200 mg/kg IBU [35] by oral gavage for 21 days, followed by a 7-day drug-free period.

Group 4 (IBU + ALA group): The rats received 200 mg/kg IBU for 21 days, followed by oral administration of 100 mg/kg ALA for 7 days [36].

Group 5 (ALA group): No procedure was performed on the rats during the first 21 days. For the last 7 days, 100 mg/kg ALA was administered orally.

At the end of the study, the rats were anesthetized with xylazine (10 mg/kg, intraperitoneally) and ketamine (60 mg/kg, intraperitoneally), tissue samples were collected, and the animals were sacrificed by cervical dislocation. Liver tissue were dissected under deep anesthesia. A portion of the liver tissue was fixed in formaldehyde for histopathological and immunohistochemical evaluations.

Histopathological evaluation of the liver

Tissue samples from each experimental group were preserved in 10% formaldehyde solution for histological examination. After 72 h in formaldehyde, the tissues were rinsed with running tap water, processed through a series of increasing alcohols, and cleared with xylene. Subsequently, they were embedded in paraffin, and paraffin blocks were formed. Polylysine-coated slides were used to mount 5-μm sections of the blocks. The prepared slides were then paraffin-free with xylene using a standard histological staining process,

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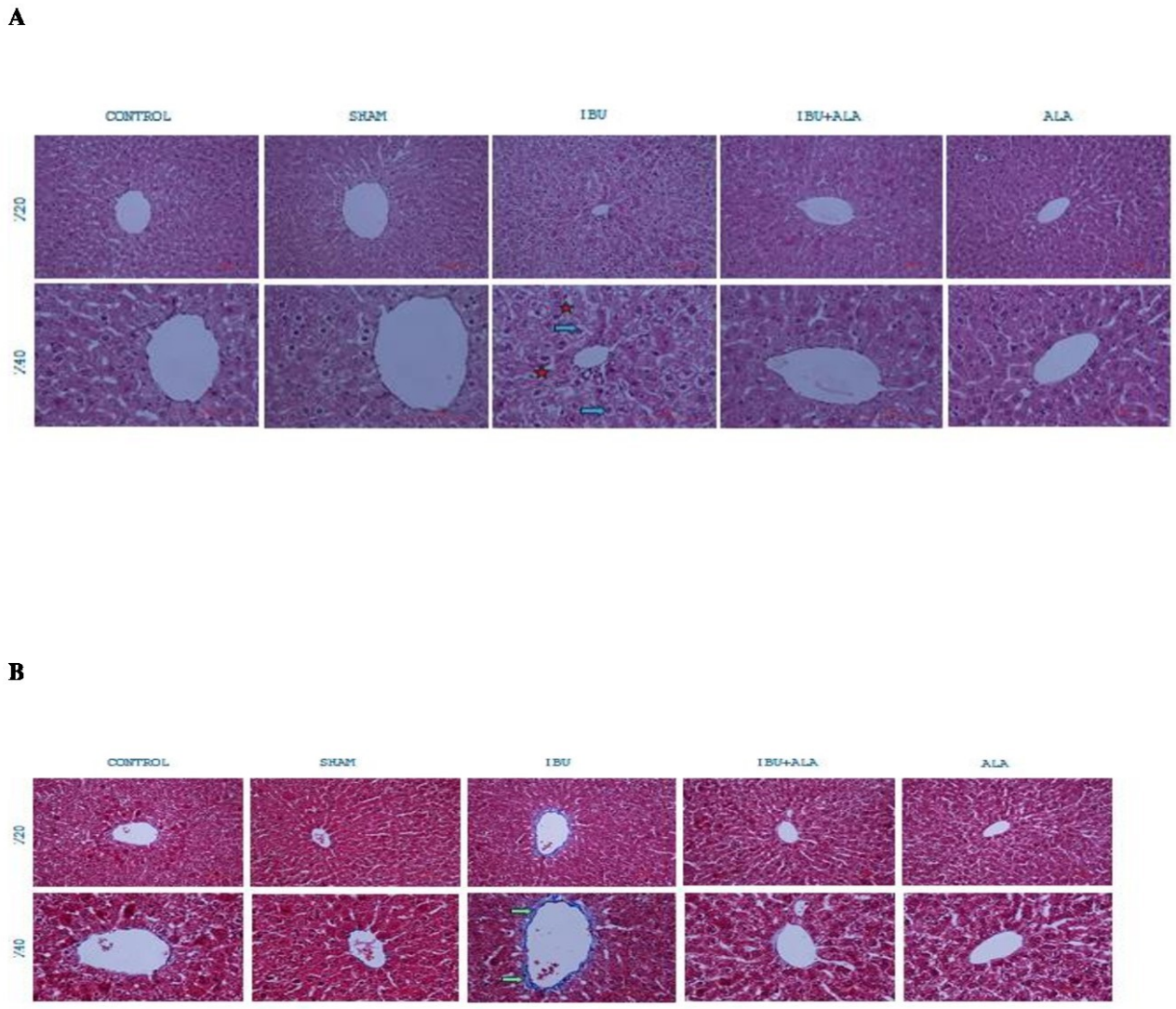

Figure 1. Light microscopic findings in rat liver tissue. (Nikon Eclipse Si, Tokyo, Japan,X200 and X400). The IBU group of HE stained histological section is shown as hydropicdegeneration (blue arrow), sinusoidal dilatation (red star). 1B. Light microscopic findings inrat liver tissue. (Nikon Eclipse Si, Tokyo, Japan, X200 and X400). The IBU group of MTstained histological section is shown as perivascular fibrosis (green arrow).

passed through a graded alcohol series (100%, 96%, 80%, 70%, and 50%), and rinsed in water. The sections were stained with hematoxylin and eosin (H&E) to detect the overall histopathological structure, and Masson's trichrome (MT) was used to assess connective tissue collagen development. They were run through a rising alcohol series, then xylene, and finally covered with a coverslip after dropping entella. They were examined under a light microscope (Nikon Eclipse Si, Tokyo, Japan). The liver tissue was examined for hydropic degeneration, sinusoidal dilatation, and perivascular fibrosis. Each criterion obtained a semi rating on a scale of 0 to 3 (0 =none, 1 = mild, 2 = moderate, and 3 = severe).

Liver immunohistochemical evaluation

The immunohistochemical staining method is performed by linking a marker with specialized antigenic identification characteristics to the cell surface. In this study, we examined the immunohistochemical expression of TLR4, TGF-β and α-SMA in liver tissue from rats with IBU-induced liver fibrosis model. TLR4 (Proteintech; Cat No. 19811-1 AP, 1:400), TGF-β (Proteintech, Cat No. 21898-1 AP, 1/250) and α-SMA (Proteintech, Cat No. 80008-1RR) primary antibodies were used at specific dilutions for the identified markers. The streptavidin-biotin kit (Ultravision Polyvalent (Rabbit-Mouse), Horseradish Peroxidase (HRP) Kit, 125 ml from Thermo Fisher Scientific (Waltham, Massachusetts, USA) was used to apply the avidin-biotin peroxidase method for the immunohistochemical staining method. The sections on polylysine slides were deparaffinized in xylene, dehydrated by maintaining them in each of the decreasing alcohol series, and then kept in distilled water for immunohistochemical staining. Subsequently, the sections were boiled in a microwave oven at 600 W with citrate buffer for antigen retrieval, washed again with phosphate-buffered saline (PBS), and treated with 3% hydrogen peroxide (H_2O_2) to inhibit endogenous per-

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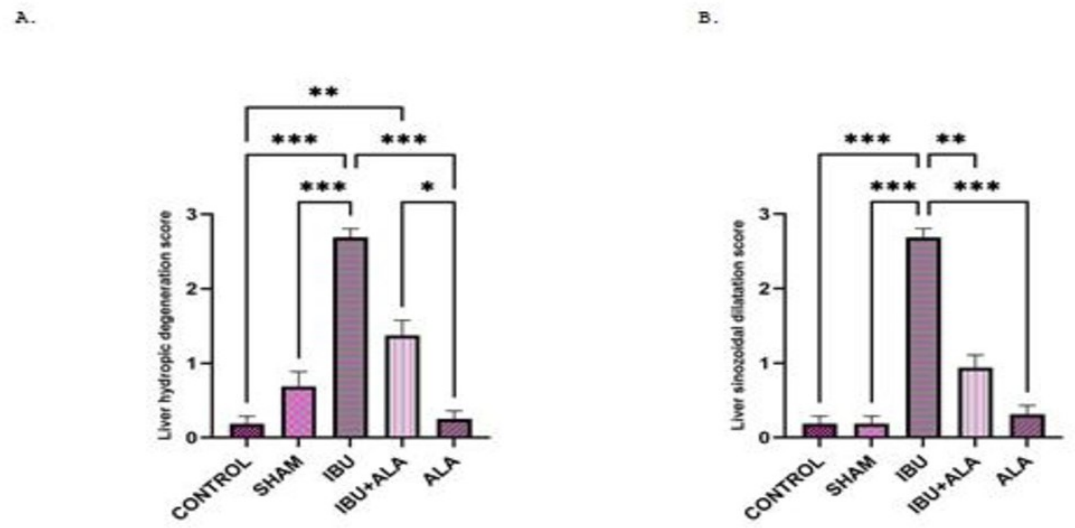

Figure 2. Graphical representation of hydropic degeneration in rat liver tissue groups. *;p<0.05, **; p<0.01, ***; p<0.001. Values are represented as mean ± SD using ANOVA andKruskal-Wallis followed as a post-hoc test Bonferroni and Dunn's test, respectively. 2B.Graphical representation of sinusoidal dilatation in rat liver tissue groups. *; p<0.05, **;p<0.01, ***; p<0.001. Values are represented as mean ± SD using ANOVA and Kruskal-Wallis followed as a post-hoc test Bonferroni and Dunn's test, respectively.

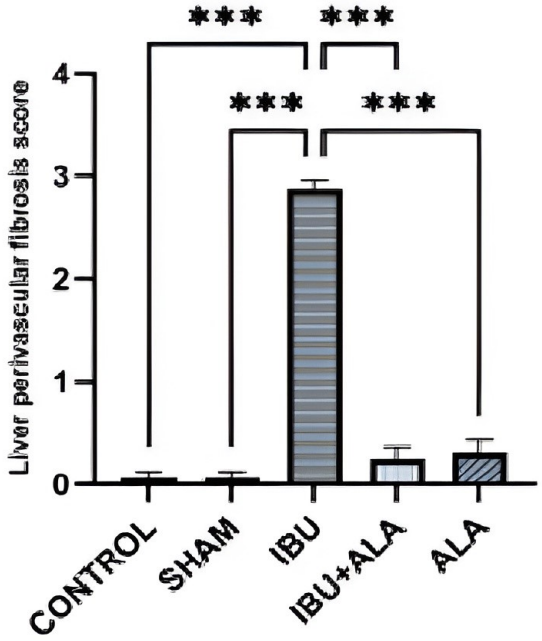

Figure 3. Graphical representation of perivascular fibrosis in rat liver tissue groups. *;p<0.05, **; p<0.01, ***; p<0.001. Values are represented as mean ± SD using ANOVA andKruskal-Wallis followed as a post-hoc test Bonferroni and Dunn's test, respectively.

oxidase activity. Block serum was then applied to the non-antigenic areas for 10 min at room temperature. The tissues in the preparations were covered with the prepared primary antibody, which was then incubated at +4°C overnight. After washing, the sections were treated with a secondary antibody, including biotin. To make immunoreactivity apparent, the sections were next coated with diaminobenzidine (DAB) substrate (Diaminobenzidine chromogen and substrate system, 125 ml from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Liver sections stained with immunohistochemistry were examined under a light microscope, and microscopic images of a number of randomly chosen areas were obtained. The immunoreactivity intensity of the markers that appeared in the images was measured using ImageJ software. The total immunoreactivity was evaluated using the color thresholding feature of ImageJ.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 10. The Shapiro–Wilk test was used to assess the normality of the data distribution. For comparisons among multiple groups, one-way analysis of variance or the Kruskal-Wallis test was performed depending on the distribution characteristics. Post hoc analyses were performed using the Bonferroni test following analysis of variance and the Dunn test following Kruskal–Wallis analysis. Data are presented as mean ± SD and median (Q1-Q3). A p-value < 0.05 was considered statistically significant.

RESULTS

Light microscopy findings

Figure 1 illustrates 2 different scale levels (X20 and X40), and H&E staining was used to evaluate liver histology in different groups. In the control group, hepatocytes in the liver tissue were coordinated with the central vein, maintaining the

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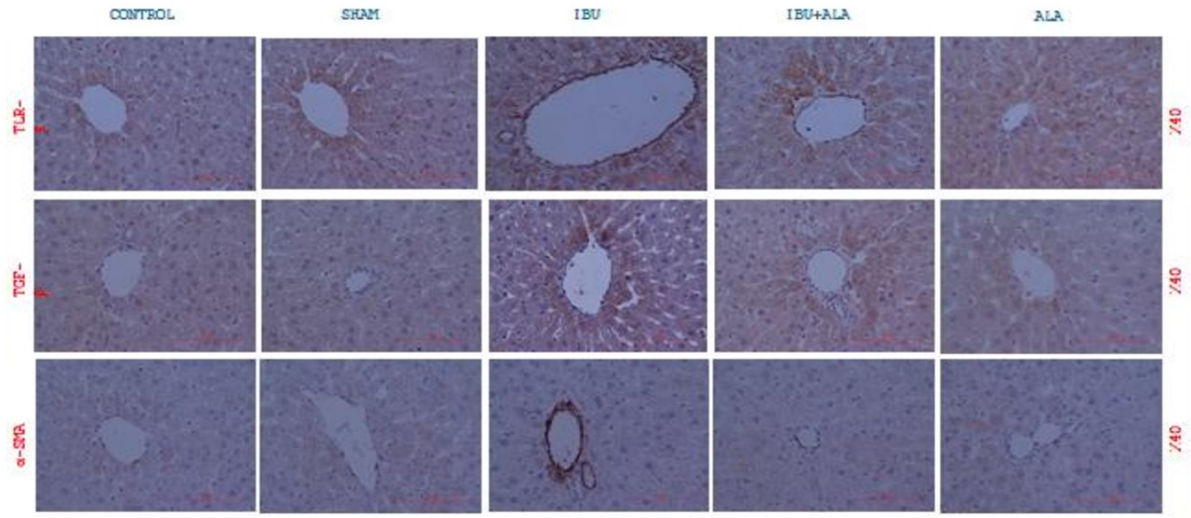

Figure 4. Immunohistochemical light microscopic findings of TLR-4, TGF-β and α-SMAmarkers in rat liver tissue of all groups. (Nikon Eclipse Si, Tokyo, Japan, X400). Browncolored areas with DAB indicate positivity.

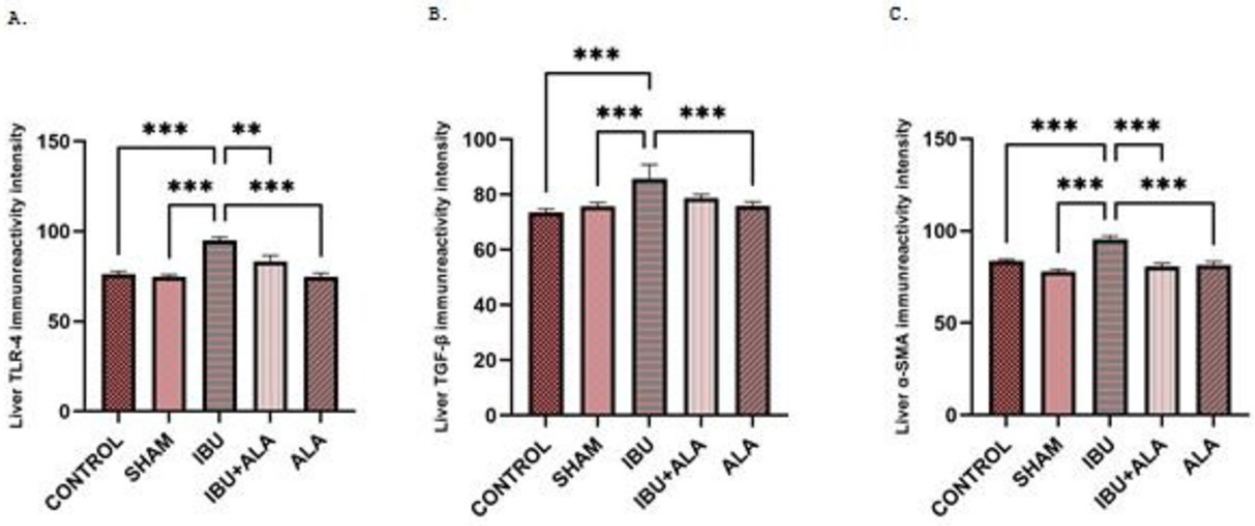

Figure 5. Graphical representation of TLR-4 (A), TGF-β (B) and α-SMA (C) markersreactivity of rat liver tissue groups. *; p<0.05, **; p<0.01, ***; p<0.001. Values arerepresented as mean ± SD using ANOVA and Kruskal-Wallis followed as a post-hoc testBonferroni and Dunn's test, respectively.

Table 1. Liver histopathological scores in experimental groups.

Liver Histoscore	Control (n=6)	SHAM (n=6)	IBU (n=6)	ALA (n=6)	IBU+ALA (n=6)	p value
Hydropic Degeneration	0.0 (0--1)	0.5 (0--2)	3.0 (2--3) ***	1.0 (0--1)	1.0 (0--3)	<0.001
Sinusoidal Dilatation	0.0 (0--1)	0.0 (0--1)	3.0 (2--3) ***	0.0 (0--1)	1.0 (0--2) **	<0.001
Perivascular Fibrosis	0.0 (0--1)	0.0 (0--1)	3.0 (2--3) ***	0.0 (0--1)	0.0 (0--1) ***	<0.001

Values are expressed as median (min–max). Statistical analyses were performed using ANOVA and Kruskal–Wallis tests, followed by Bonferroni and Dunn's post-hoc analyses. *** p<0.001 vs Control and SHAM. ** p<0.01 vs IBU.

cord structure. Sinusoids were clearly visible, and the liver tissue had a normal histological composition. The sham group had an identical structure to the control group, but there was slight vacuolization. The general structure of the ALA group was similar to that of the control group. In the IBU group, hepatocytes exhibited rich cytoplasmic transparency

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Table 2. Immunoreactivity intensity of fibrosis markers in experimental groups.

Immunreactivity Intensity	Control (n=6)	SHAM (n=6)	IBU (n=6)	ALA (n=6)	IBU+ALA (n=6)	p value
TLR-4	76.17 ± 4.79	74.67 ± 3.86	95.02 ± 5.33	83.22 ± 10.07	74.69 ± 6.38	<0.001
TGF-β	72.69 (64.33–82.22)	75.79 (64.90–88.65)	89.90 (86.20–104.7)	75.65 (64.99–84.45)	79.73 (67.82–86.71)	<0.001
α-SMA	83.67 ± 2.70	78.07 ± 3.35	95.57 ± 4.91	81.54 ± 5.49	80.79 ± 5.08	<0.001

Values are expressed as mean ± SD or median (min–max). Statistical analyses were performed using ANOVA and Kruskal–Wallis followed by Bonferroni and Dunn's post-hoc tests. TLR-4: IBU group showed significantly higher values compared with Control and SHAM (***p<0.001). IBU+ALA group showed a significant reduction compared with IBU (**p<0.01). TGF-β: IBU group was significantly higher than Control and SHAM (***p<0.001). No significant difference between IBU and IBU+ALA groups (p>0.05). α-SMA: IBU group showed significantly higher values compared with Control and SHAM (***p<0.001). IBU+ALA group showed a significant reduction compared with IBU (***p<0.001).

and hydropic degeneration, represented as ballooned cells. In this group, hydropic degeneration showed a statistically significant increase compared to the Control (p<0.001), Sham (p<0.001) and ALA (p<0.001) groups. ALA treatment reduced this score compared with the IBU group, but this decrease was not significant (p>0.05) (Figures 1 and 2A) (Table 1).

The IBU group exhibited considerable sinusoidal dilatation. The outcome was significantly higher in the IBU group than in the Control (p<0.001), Sham (p<0.001), and ALA (p<0.001) groups. ALA treatment reduced this score compared with the IBU group, and the decrease was statistically significant (p<0.01) (Figures 1 and 2B) (Table 1).

MT staining was used to demonstrate the accumulation of collagen in the tissue. As shown in Figure 1B, an increase in connective tissue was observed in the perivascular section in the IBU group. This boost was especially noteworthy compared to the Control (p<0.001), Sham (p<0.001), and ALA (p<0.001) groups. The perivascular fibrosis score dropped significantly in the IBU + ALA group compared with the IBU group (p<0.001) (Figure 3; Table 1).

Immunohistochemical findings

When TLR4 reactivity in liver tissue was examined between groups, the Control, Sham, and ALA groups showed minimal reactivity, whereas TLR4 reactivity was noticeable in the portal areas and vascular walls in the IBU group (Figure 4). TLR4 reactivity was statistically significantly stronger in the IBU group than in the Control (p<0.001), Sham (p<0.001), and ALA (p<0.001) groups. In other words, with ALA treatment, the TLR4 intensity was significantly diminished compared to the IBU group (Figure 5A) (Table 2).

The control, sham, and ALA groups displayed low TGF-β reactivity when compared to the IBU group, which had particularly high levels in hepatocytes surrounding the sinusoidal and portal areas (Figure 4). The IBU group had significantly higher TGF-β reactivity than the Control (p<0.001), Sham (p<0.001), and ALA (p<0.001) groups at the same time. Compared with the IBU group, ALA treatment lessened TGF-β intensity, although this difference was not statistically significant (p>0.05) (Figure 5B) (Table 2).

The analysis of liver tissue α-SMA levels revealed weak expression in the control, sham, and ALA groups, but α-SMA positivity was identified in the IBU group, especially in the vessels (Figure 5). The IBU group demonstrated significantly higher α-SMA reactivity compared to the Control (p<0.001), Sham, (p<0.001) and ALA (p<0.001) groups. In the IBU+ ALA group α-SMA reactivity was significantly reduced compared with that in the IBU group (p<0.001) (Figure 5C) (Table 2).

DISCUSSION

IBU administration caused marked hepatocellular injury, including hydropic degeneration, sinusoidal dilation, and increased perivascular collagen accumulation. These histopathological changes were accompanied by significant increases in TLR4, TGF-β, and α-SMA immunoreactivity, indicating the activation of inflammatory and fibrotic pathways. Overall, these findings confirm that prolonged exposure to IBU damages hepatocytes and triggers fibrosis-associated signaling.

Alpha-lipoic acid treatment notably improved IBU-induced liver injury. Reductions in sinusoidal dilatation, perivascular collagen deposition, and TLR4 and α-SMA expression suggest that ALA protects the liver through multiple mechanisms. Although the decrease in TGF-β reactivity was not statistically significant, the downward trend indicates partial modulation of profibrotic activity. These results suggest that ALA attenuates IBU-mediated liver toxicity by targeting oxidative stress, inflammatory signaling, and fibrogenesis. ALA acts as a potent antioxidant, modulates NF-κB-dependent inflammatory cascades, and inhibits hepatic stellate cell activation [35]. Our findings support this multilevel hepatoprotective effect.

The histopathological changes align with those reported in previous studies. Panchal et al. (2022) observed portal inflammation, hepatocyte degeneration, central vein congestion, and neutrophil infiltration after exposure to IBU. Protective effects were observed with T. zeylanicus extract or silymarin [37]. Similarly, Gola et al. (2013) reported mild sinusoidal degeneration after 6 h of acute hypobaric hypoxia, which progressed to moderate dilation and vascular congestion after 24 h [38]. Kravchuk et al. (2004) found that ALA reduced fatty degeneration and inflammatory changes in alco-

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holic hepatitis, while fibrotic changes stabilized or decreased [39]. These studies support our findings and highlight the protective potential of ALA against drug-induced liver injury.

Perivascular collagen accumulation, assessed with Masson's trichrome staining, increased significantly in the IBU group, whereas ALA markedly reduced it. The TGF-β pathway, a key fibrosis driver, promotes hepatic stellate cell transdifferentiation into myofibroblasts via Smad-dependent and -independent pathways, enhances extracellular matrix synthesis, and sustains fibrogenesis through positive feedback [23, 24, 36]. The rise in TGF-β in the IBU group aligns with this mechanism. ALA reduced TGF-β levels, although not significantly, suggesting a limited effect on TGF-β-mediated fibrosis.

TLR4 links inflammation and fibrosis from an immune perspective. TLR4 is expressed in Kupffer cells, hepatocytes, and HSCs and recognizes PAMPs and DAMPs, triggering proinflammatory cytokine production [21]. TLR4 activation also sensitizes HSCs to TGF-β-induced fibrosis [40]. Elevated TLR4 expression in the IBU group supports the role of microbial and endogenous triggers in fibrosis. The significant reduction in TLR4 with ALA suggests suppression of inflammatory signaling and slowed fibrosis progression.

α-Smooth muscle actin (α-SMA) is a key marker of HSC activation. α-SMA rises during HSC transdifferentiation into myofibroblast-like cells, reflecting collagen-producing activity [30, 31]. IBU significantly increased α-SMA positivity, confirming HSC activation. ALA significantly reduced α-SMA, indicating suppression of HSC activation and limited fibrotic matrix deposition.

Overall, ALA provides multilevel hepatoprotection against IBU-induced liver injury through its combined antioxidant, antiinflammatory (via TLR4/NF-κB), and antifibrotic effects. This underscores its potential as a therapeutic adjuvant to mitigate NSAID-related hepatotoxicity. Future studies using oxidative stress markers, gene expression analysis, and dose-response evaluations will clarify the translational potential of this study in clinical settings.

Limitations

The current study provides important evidence for the hepatoprotective effects of alpha-lipoic acid against IBU-induced liver injury, although several limitations should be noted. The acute animal model may not fully replicate the complex progression of chronic liver fibrosis seen in clinical cases, potentially limiting the translational relevance of the findings. While ALA significantly suppressed key fibrotic markers, including TLR4 and α-SMA, its more modest effect on TGF-β signaling suggests that it may not completely block all fibrotic pathways. The absence of functional liver tests represents another limitation, as incorporating biochemical parameters, such as ALT and AST levels, could have strengthened the correlation between histological findings and actual liver function. Additionally, the study's single-dose design leaves questions unanswered regarding potential dose-dependent effects and the optimal therapeutic regimen. The exclusive use of an animal model also introduces uncertainties about human applicability due to known species differences in drug metabolism and fibrotic responses. These limitations highlight important areas for future investigation to better establish ALA's potential clinical utility in preventing drug-induced liver fibrosis while not diminishing the value of the current findings.

CONCLUSION

Our findings demonstrate that ALA exerts a notable therapeutic effect on IBU-induced liver fibrosis, particularly in inflammatory (TLR4) and HSC activation (α-SMA) markers, while its impact on TGF-β-mediated fibrogenesis appears more limited. Given the pathophysiological interplay between TGF-β, TLR4, and α-SMA in the literature, the differential effects of ALA on these pathways highlight the importance of targeted combination therapies in treatment strategies.

Ethics Committee Approval: Ethical approval for this study was obtained from the Ethics Committee of Kirsehir Ahi Evran University Local Animal Experiments (date: 06/03/2025, approval number: 5/10).

Informed Consent: Not necessary for this manuscript.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors have no conflicts of interest to declare.

Author Contributions: Concept: GÜ.; Design: GÜ.; Supervision: GÜ.; Materials: KTK.; Data Collection and/or Processing: KTK.; Analysis and/or Interpretation: GÜ., KTK.; Literature Review: GÜ.; Writing: GÜ., KTK. Critical Review: GÜ.

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