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(RESEARCH ARTICLE)

Histopathological effects of Amoxicillin-Clavulanic acid on the liver of Albino mice

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Abstract

The present study examined whether Amoxicillin-Clavulanic acid induces lesions in the liver tissues of albino mice. The experiment took place in the laboratories of the State Company for Drugs Industry and Medical Applications, Samarra, Iraq (SDI), and ran from March (December 2024) to April 2025. Thirty mice, 8–10 weeks old and weighing 23–27 g, were divided into 6 groups of five. The liver weight of animals treated with Amoxicillin-Clavulanic acid was significantly higher than that of the control group ($P < 0.01$). Microscopic examination of the livers from the groups treated with Amoxicillin-Clavulanic acid revealed histological lesions, including necrosis and degeneration. The hepatocyte columns were disorganized toward the central vein, whose walls were thickened, while the hepatic arteries were congested. Bile ducts were surrounded by fibers, and lymphocytes and neutrophils had infiltrated the tissue in comparison with the control group.

Keywords: Amoxicillin; Clavulanic acid; Liver; Histopathology; Albino mice; Hepatotoxicity

1. Introduction

Antibiotics are still among the drug classes prescribed most often worldwide. Within this group, beta-lactam/beta-lactamase inhibitor combinations are central to the treatment of bacterial infections because they act against a broad range of organisms. Marketed as Amoxicillin-Clavulanic acid, amoxicillin-clavulanic acid pairs amoxicillin, a semisynthetic aminopenicillin, with clavulanic acid, a beta-lactamase inhibitor that broadens its activity against resistant, beta-lactamase-producing organisms [1]. It is also one of the most widely used oral antibiotics globally, prescribed for respiratory, urinary, skin, and soft-tissue infections. Extensive clinical use around the world has generally established the combination as safe and well tolerated; in most patients, reported liver-enzyme elevations have been transient and infrequent [2,3].

Despite this favorable safety profile, amoxicillin-clavulanic acid is the drug most often implicated worldwide in drug-induced liver injury (DILI) [4]. Population-based estimates suggest that clinically apparent liver injury occurs in roughly one of every 2,300 to 2,400 prescriptions [5,6]. Recent pharmacovigilance analyses from 2020–2024 likewise show a disproportionate rise in hepatobiliary adverse-event reports with the amoxicillin-clavulanate combination compared with amoxicillin alone [2]. Symptoms generally appear days to weeks after treatment begins, though the injury may not become evident until the antibiotic course has ended. The clinical pattern can be purely cholestatic, mixed, or predominantly hepatocellular, especially in younger patients [5,3].

The mechanism behind this hepatotoxicity remains unclear. Current evidence, however, points more toward an immune-mediated process than a straightforward dose-dependent one. Eosinophilic tissue infiltration, signs of hypersensitivity, and links to particular human leukocyte antigen (HLA) class II haplotypes all support the view that immune injury drives the hepatic response. The clavulanate component appears to be more responsible than amoxicillin by itself, as recurrence after re-exposure to amoxicillin without clavulanate is rare [5]. Genetic studies add another piece to the explanation: additional risk variants, including a pathogenic ERAP2 mutation reported in a recent case with

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unusual autoimmune features, may help account for why clinically apparent injury develops in only a minority of exposed individuals [4].

Experimental animal studies have added a tissue-level view to the clinical picture, showing what the injury looks like structurally. In a controlled rat model, amoxicillin-clavulanate administration led to measurable increases in serum liver enzymes and oxidative stress markers, while antioxidant capacity fell. Histopathological examination also showed hepatic injury and a reduced liver injury score. Pretreatment with a protective antioxidant compound reversed these biochemical and histological changes and restored markers of hepatoprotective signaling pathways [7]. Alongside the biochemical disturbances reported in human case series [3,6], these findings indicate that amoxicillin-clavulanate causes reproducible changes in hepatic tissue architecture—changes that animal histopathology can capture and quantify.

Taken together, these clinical and experimental results identify amoxicillin-clavulanic acid as a hepatotoxic agent with both clinical significance and demonstrable mechanistic activity. Still, much of the existing evidence comes from human case reports or rat biochemical assays, while detailed histopathological descriptions of hepatic architectural changes in mice remain relatively scarce. Because this antibiotic is widely used, sometimes without supervision, a clearer account of how it affects liver structure continues to matter clinically and for public health. The present study therefore examined the histopathological effects of amoxicillin-clavulanic acid (Amoxicillin-Clavulanic acid) on the livers of albino mice, documenting the structural and cellular changes induced by the drug.

2. Material and methods

2.1. Animals and housing

Thirty male albino mice (*Mus musculus*), weighing 23–27 g and aged 8–10 weeks, were obtained from the National Center for Drug Control and Research, Baghdad. Animals were housed five per cage (30 × 15 × 8 cm) at 25 ± 2 °C, fed a standard laboratory pellet diet with water *ad libitum*, and allowed one week for acclimatization before the start of the experiment.

2.2. Drug and dose calculation

Amoxicillin-Clavulanic acid (Julmentin from Julphar), 625 mg/tablet, formulated for adult human bacterial infections, was used. The therapeutic and overdose concentrations for mice were calculated from the human clinical dose using the body-weight-based interspecies conversion equation of Paget and Barnes [8]:

$$X_1 \times Y_2 = X_2 \times Y_1$$

where X_1 = average adult human body weight, X_2 = average mouse body weight (25 g), Y_1 = human drug concentration, and Y_2 = the corresponding required concentration for the animals. One tablet was dissolved in 90 ml distilled water, and each mouse received 0.2 ml/g of solution orally, corresponding to 0.446 mg/B.W./day (therapeutic dose) or 0.669 mg/B.W./day (overdose).

2.3. Experimental design

Animals were divided into six groups of five mice each. Groups 1 and 2 (therapeutic and overdose, respectively) and their control (Control 1) were treated for 14 days; Groups 3 and 4 (therapeutic and overdose, respectively) and their control (Control 2) were treated for 21 days. Control groups received water and standard diet only, for the corresponding duration.

Tissue slide processing: Animals were euthanized by cervical dislocation, after which a longitudinal abdominal incision was made for dissection. The livers were removed and weighed, fixed in 10% formalin for 24 hours, then preserved in 70% ethanol before routine histological processing and eosin staining, according to standard histopathological technique [9].

Statistical analysis: The data were analyzed in Minitab (under SPSS) and Microsoft Excel XP. Results were reported as mean ± SD, together with minimum and maximum values. Differences among groups were tested using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to compare the means [10]. Results were considered non-significant at $P > 0.05$, significant at $P < 0.05$, and highly significant at $P < 0.01$.

3. Results

3.1. Effect of Amoxicillin-Clavulanic Acid on Liver Weight

The present study showed a highly significant increase ($P < 0.01$) in the liver weight of mice treated with Amoxicillin-Clavulanic acid compared with the control group, at both the therapeutic and overdose concentrations, and at both durations of exposure (14 and 21 days). Table (1) and Table (2) present the means and standard deviations of liver weight for each experimental group.

Table 1 Means ± standard deviations of liver weight in different groups of adult mice (for 14 days).

Groups	Liver weight Mean ±SD
Control	1.51 ± 0.07
Amoxicillin-Clavulanic acid (0.446 mg)	1.91 **± 0.08
Amoxicillin-Clavulanic acid (0.669mg)	1.83 **± 0.11

** Highly significant difference compared with control ($P < 0.01$).

Table 2 Means ± standard deviations of liver weight in different groups of adult mice (for 21 days).

Groups	Liver weight Mean ±SD
Control	1.57 ± 0.07
Amoxicillin-Clavulanic acid (0.446 mg)	1.87 **± 0.08
Amoxicillin-Clavulanic acid (0.669mg)	1.89 **± 0.12

** Highly significant difference compared with control ($P < 0.01$).

The increase in liver weight was dose- and duration-dependent, with the overdose groups showing a slightly greater increase than the therapeutic-dose groups at both time points, and the 21-day exposure groups showing marginally higher values than their 14-day counterparts.

3.2. Histological Effects of Amoxicillin-Clavulanic Acid on the Liver

Microscopic examination of liver sections from the control group revealed a normal hepatic architecture: the central vein and hepatocytes were arranged radially around it in the typical cord-like pattern, hepatocytes were normal in shape and size, and the hepatic sinusoids and Kupffer cells appeared normal (Fig. 1 and Fig. 2).

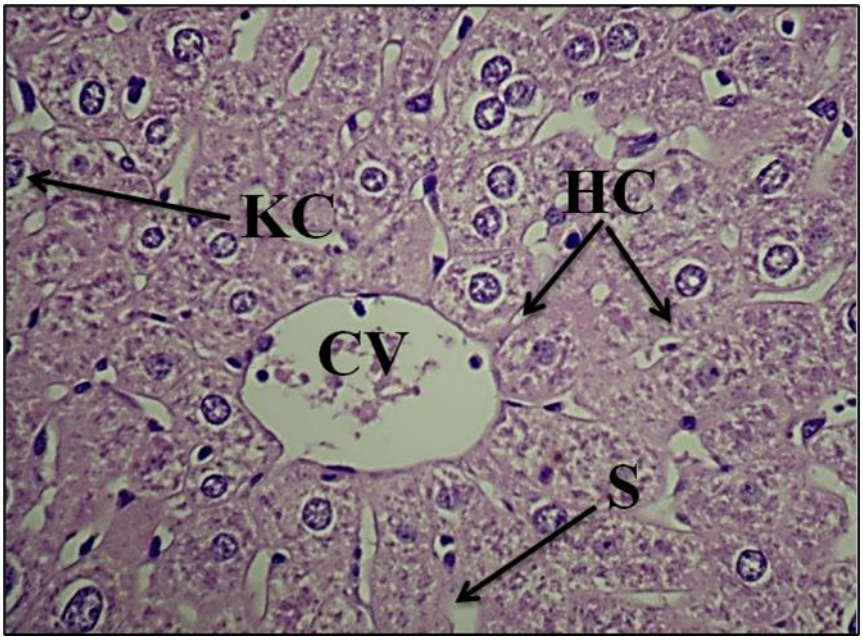

Figure 1 The liver of the control group displayed the central vein (CV), hepatocytes (HC), Kupffer cells (KC), and sinusoids (S) (H&E 400X).

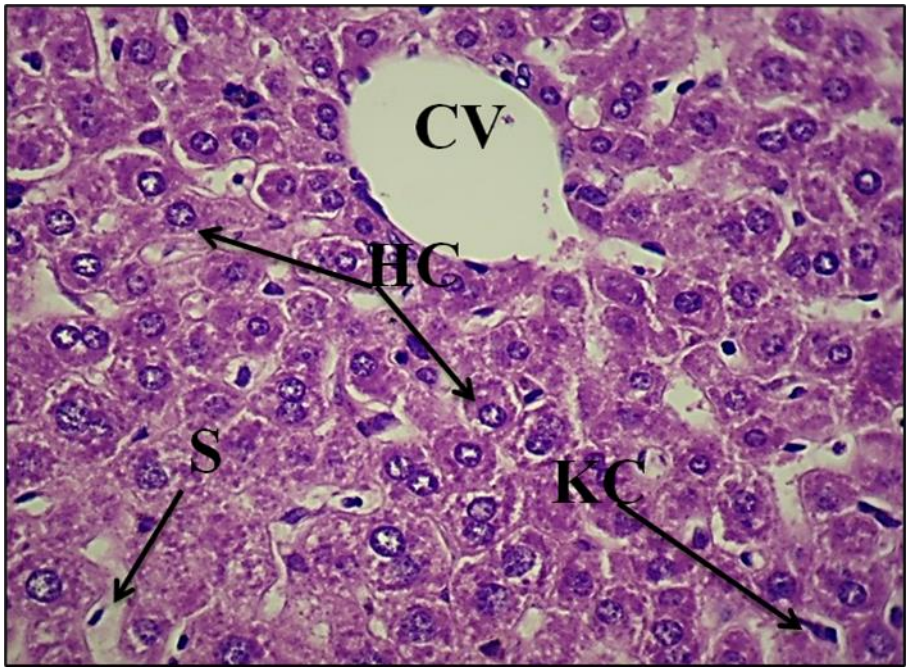

Figure 2 The liver tissue from the control group displayed a central vein (CV), hepatocytes (HC), Kupffer cells (KC), and sinusoids (S) (H&E 400X).

In contrast, liver sections from the treated groups showed marked histopathological alterations, including hepatocellular necrosis, degeneration, and disorganization of the hepatocyte cords radiating toward the central vein, with loss of the normal hexagonal (polygonal) lobular pattern of individual hepatocytes. Thickening of the walls of the central veins and hepatic arteries was observed, together with vascular congestion. Sclerosing cholangitis was noted, with bile ducts surrounded by fibrous tissue. Hyperemia of blood vessels was prominently associated with lymphocytic infiltration and leukocytic infiltration around, and even within, the blood vessel walls (Fig. 3 and Fig. 4).

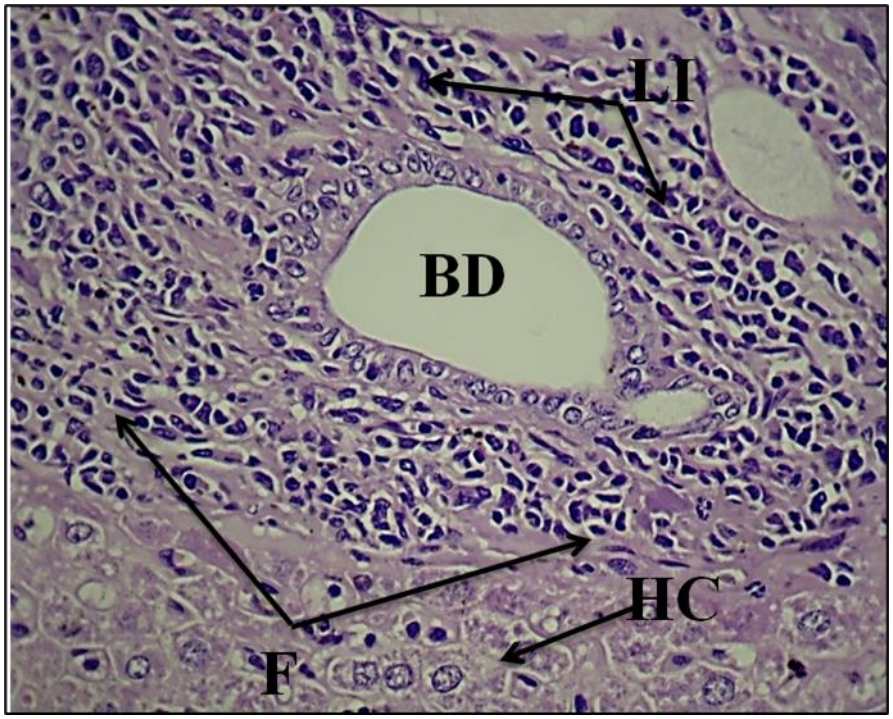

Figure 3 Liver with sclerosing cholangitis of mice administrated with (Amoxicillin-Clavulanic acid (0.446 mg/B.W**)** for 14 day, displayed bile duct (BD)surrounded by fibers (F) and with severing lymphocytes infiltration (LI) with hepatocytes (HC) (H&E 400 X).

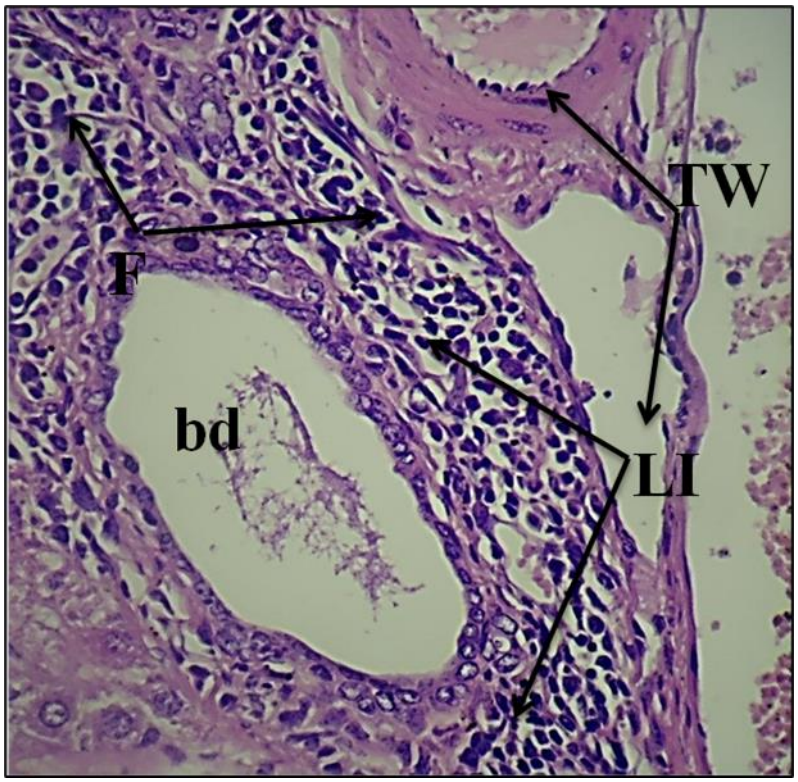

Figure 4 Liver with sclerosing cholangitis of mice administrated with (Amoxicillin-Clavulanic acid (0.669mg/B.W) for 14 day, displayed bile duct (bd) surrounded by fibers (F) and lymphocytes infiltration (LI) with thickening wall (TW) of central vein (H&E 400 X).

4. Discussion

4.1. Impact of Amoxicillin-Clavulanic Acid on Liver Weight

The research revealed a significantly significant increase in liver weight ($P < 0.01$) in mice treated with amoxicillin-clavulanic acid (AC) compared with the control group. The increase in hepatic mass (hepatomegaly) might be a hypertrophic and inflammatory reaction to the buildup of drug metabolites, followed by fluid retention (edema) in the hepatic parenchyma. In recent animal models of drug-induced liver damage (DILI) similar results were observed where AC exposure resulted in significant changes in organ weight related to progressive degenerative changes and vascular congestion [11, 12]. The morphometric alterations seen in this research are also consistent with the previous work of Ghimire [13] who reported that the treatment of amoxicillin causes significant weight variations in key organs such as liver and kidneys of murine models.

4.2. Histopathological and Structural Alterations

As shown in figures 1 and 2, the control group exhibited the normal hepatic architecture, but the groups treated with AC showed severe structural impairment. On microscopic inspection, there was extensive hepatocellular necrosis and hydropic degeneration, and hepatic cords were severely disordered. These histopathological features are consistent with the general clinical pattern of AC-induced hepatotoxicity, which remains the most frequently reported causative agent of DILI worldwide [14, 15].

There is a significant degree of microvascular involvement with significant vascular congestion, thickening of the central veins and hepatic arteries. The picture is further complicated by sclerosing cholangitis and periductal fibrosis. The dense lymphocytic and leukocytic infiltration around and inside the vasculature supports the concept that the damage is due to a severe inflammatory, immune-mediated response [16, 17]. Recent genetic studies have also identified specific human risk variants such as ERAP2 mutations that may predispose certain individuals to this amplified immune response following AC exposure [17].

4.3. Mechanisms of Hepatotoxicity

The resulting oxidative stress is believed to be the driving factor behind the cellular damage reported in our work, in particular necrosis and membrane breakdown. It has been reported that AC exposure decreases the internal antioxidant defense, including glutathione (GSH), and increases markers of lipid peroxidation such as malondialdehyde (MDA) at the same time [18, 19, 20]. This metabolic equilibrium is altered, disturbing cellular signaling pathways such SIRT1/Nrf2/NF-κB and eventually causing apoptosis [18, 20].

The inflammatory pattern seen in our animals including the lymphocyte recruitment is in accordance with findings that AC damages the liver architecture and induces the production of pro-inflammatory cytokines such as TNF-α [21]. ALT and AST are intracellular enzymes which may leak into the systemic circulation with cell deterioration, as widely reported in recent experimental and clinical literature [22, 23]. We found that the biliary changes and cholangitis are likewise similar to the cholestatic or mixed injury pattern typically reported in human case reports: individuals acquire quickly appearing jaundice and blockage of the biliary system after antibiotic treatment [24, 25].

5. Conclusion

The current investigation demonstrated considerable hepatotoxicity after administration of amoxicillin-clavulanic acid in albino mice. Liver weight was significantly elevated due to inflammation-induced hepatomegaly and vascular congestion. Histopathological investigation revealed significant structural damage with extensive hepatocellular necrosis, hydropic degeneration and loss of the normal hepatic architecture. Sclerosing cholangitis and extensive infiltration of inflammatory cells further suggest that cholestatic and immune-mediated processes are involved in the injury. The severity of hepatic damage was dose- and duration-dependent. This points to the need for clinical vigilance and monitoring during long-term antibiotic treatment.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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