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# HEPATOPROTECTIVE EFFECTS OF *Alstonia boonei* AGAINST ASPIRIN-INDUCED LIVER INJURY IN ALBINO RATS

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## ABSTRACT

The liver is essential for metabolism and detoxification but is highly vulnerable to drug-induced injury. Aspirin has been reported to induce hepatic alterations, particularly at prolonged or high doses, primarily through oxidative stress mechanisms, while *Alstonia boonei* possesses documented hepatoprotective properties. This study evaluated the hepatoprotective effects of *A. boonei* against aspirin-induced hepatic toxicity in albino rats. Thirty rats were assigned into six groups (n = 5). Group I served as control; Group II received aspirin (10 mg/kg); Group III received *A. boonei* (1000 mg/kg); Groups IV-VI received aspirin (10 mg/kg) combined with *A. boonei* ethanolic extract (1000, 1500, and 2000 mg/kg), orally for 14 days respectively. Data were analyzed using one-way ANOVA ($p < 0.05$). Liver enzymes (AST, ALT, ALP), total and conjugated bilirubin were assessed, and hepatic tissues were examined histologically. Animals were weighed before and after experimentation including organ weight. Aspirin administration produced mild biochemical alterations characterized by reduced serum AST (12.67 ± 2.67 U/L), ALT (11.00 ± 0.58 U/L), and ALP (52.67 ± 15.71 U/L) compared with control values of 36.00 ± 24.00 U/L, 17.50 ± 5.50 U/L, and 56.00 ± 2.00 U/L, respectively. Total bilirubin showed a slight increase (0.37 ± 0.07 mg/dL), while conjugated bilirubin remained unchanged (0.10 ± 0.00 mg/dL). Histology showed mild hepatocellular stress after aspirin, with cytoplasmic granulation and sinusoidal prominence. *A. boonei* co-treatment improved hepatic structure dose-dependently, with near-normal architecture at the moderate dose and minimal stress at the highest dose, indicating hepatoprotective potential.

**Keywords:** *Alstonia Boonei*, Aspirin, Hepatotoxicity, Liver Injury, Oxidative Stress, Drug-Induced Liver Injury

## INTRODUCTION

The liver is the largest internal organ and a central metabolic hub responsible for detoxification, xenobiotic biotransformation, nutrient metabolism, bile production, and maintenance of systemic homeostasis (Wang *et al*., 2025a). Its unique dual blood supply, high metabolic rate, and extensive exposure to endogenous and exogenous compounds make it particularly vulnerable to toxic injury (Wang *et al*., 2025a; Hora and Wuestefeld, 2023). Hepatocytes play an essential role in carbohydrate, lipid, and protein metabolism, but these same metabolic functions increase their susceptibility to reactive intermediates, oxidative stress, and inflammation triggered by drugs and environmental agents (Berumen *et al.,* 2021; Villanueva-Paz *et al*., 2021). Drug-induced liver injury remains a major cause of morbidity worldwide and accounts for a significant proportion of acute liver failure cases and continues to be a challenge in both clinical practice and toxicological research (Kobayashi *et al.,* 2023; Hassan *et al.,* 2025).

Aspirin is one of the oldest and most widely used nonsteroidal anti-inflammatory drugs valued for its analgesic, antipyretic, anti-inflammatory, and cardioprotective properties. Despite its therapeutic importance, aspirin is well documented to induce hepatic toxicity, particularly at prolonged or high-dose exposures (Huang *et al.,* 2025; Jiang *et al.,* 2025). Aspirin's primary mechanism involves irreversible inhibition of cyclooxygenase, reducing prostaglandin synthesis. Its metabolic conversion in the liver generates salicylate and other intermediates capable of impairing mitochondrial function, increasing reactive oxygen species, disrupting ATP production, and triggering hepatocellular injury (Arif and Aggarwal, 2023; Visagie *et al.,* 2024; Borgne-Sanchez and Fromenty, 2025). Reports show that aspirin-induced hepatotoxicity can manifest as elevated serum transaminases, oxidative stress, lipid peroxidation, mitochondrial dysfunction, and inflammation even at doses commonly used in experimental models (Elshaer and Lizaola-Mayo, 2024; Kim *et al.,* 2023; Skat-Rørdam *et al*., 2025). These mechanisms highlight the need for safe and effective hepatoprotective interventions, particularly those derived from medicinal plants with strong antioxidant profiles (Villanueva-Paz *et al.,* 2021; Chen *et al.,* 2025).

*Alstonia boonei* is an ethnomedicinal plant widely used across West and Central Africa and has gained increasing scientific interest due to its broad pharmacological properties. Its stem bark and leaves contain bioactive compounds such as flavonoids, alkaloids, triterpenoids, phenolic acids, and iridoids that exhibit antioxidant, anti-inflammatory, analgesic, antimalarial, and hepatoprotective effects (Sharma and Goyal, 2022; Mishra *et al.,* 2025; Olanlokun *et al.,* 2021). Recent studies report that extracts of *A. boonei* can modulate redox balance, suppress pro-inflammatory cytokines, stabilize cellular membranes, and improve biochemical markers of hepatic injury in experimentally induced hepatotoxicity models (Adesina *et al.,* 2025; Okpashi *et al.,* 2022; Taiwo et al., 2019; Uroko *et al.,* 2020). These properties position A. boonei as a promising natural agent for mitigating oxidative and inflammatory liver damage including that caused by NSAIDs such as aspirin (Gonfa *et al.,* 2024; Li *et al.,* 2025).

Given the rising global interest in plant-derived therapeutic agents and the urgent need for safer hepatoprotective alternatives, there is currently limited experimental evidence on the hepatoprotective effects of *A. boonei* against non-steroidal anti-inflammatory drug–induced hepatic injury, particularly aspirin-associated liver alterations (Gonfa *et al.,* 2024; Chen *et al.,* 2025). This study was therefore designed to evaluate the hepatoprotective potential of *A. boonei* against aspirin-induced hepatic changes in rats, thereby addressing

this knowledge gap and providing experimental evidence to support its traditional medicinal use.

## MATERIALS AND METHODS

### Study Location

The study was conducted in a controlled laboratory environment at the Department of Medical Laboratory Science, University of Benin, Nigeria, following internationally accepted guidelines for the care and use of laboratory animals. Conditions were maintained to provide adequate ventilation, controlled temperature, and minimal external stress. All procedures adhered to ethical principles for experimental research to ensure humane handling throughout the study (Borgne-Sanchez and Fromenty, 2025; Elshaer and Lizaola-Mayo, 2024; OECD, 2022). Ethical approval for animal experimentation was obtained from the Medical and Allied Faculty of Science Animal Ethics Committee (MAFSAEC), University of Benin, with approval number MAFSAEC-025-01/09/0003.

### Plant Collection and Extract Preparation

Fresh leaves of *A. boonei* were collected and authenticated by a qualified plant taxonomist, with a voucher specimen (UBH-A591) deposited in the institutional herbarium. The leaves were thoroughly washed, air-dried at room temperature, and pulverized into a fine powder using a mechanical grinder. Extraction was performed by cold maceration in ethanol for 72 hours, during which the solution was periodically stirred to enhance extraction efficiency. The mixture was filtered through muslin cloth, and the filtrate was concentrated under reduced pressure using a rotary evaporator with the water bath set at 60°C. Concentration was performed under vacuum to lower the effective boiling point of ethanol and minimize thermal exposure of heat-sensitive phytoconstituents. Any remaining moisture was removed by freeze-drying to further preserve thermolabile compounds. The extract was stored in airtight containers and refrigerated until administration. These methods follow established extraction protocols for *A. boonei* and related species (Mishra *et al*., 2025; Sharma and Goyal, 2022; Uroko *et al.,* 2020).

### Drug Preparation and Administration

Aspirin tablets were sourced from a certified pharmaceutical supplier. Fresh drug solutions were prepared daily at a dose of 10 mg/kg, a concentration previously shown to induce mild hepatic alterations appropriate for evaluating hepatoprotective effects (Huang *et al.,* 2025; Jiang *et al.,* 2025; Arif and Aggarwal, 2023). Extract doses were selected based on $LD_{50}$ findings and literature demonstrating their safety and hepatoprotective potential in rodent models (Adesina *et al*., 2025; Taiwo *et al.,* 2019; Uroko *et al.,* 2020). All substances were administered orally using calibrated gavage needles for 14 consecutive days.

### Acute Toxicity Study ($LD_{50}$ Determination)

The acute toxicity assessment followed OECD guidelines for acute oral toxicity testing using stepwise dose administration and observation for toxicity signs and mortality for up to 72 hours (OECD, 2022). The extract produced no mortality and showed no behavioral distress, confirming its wide safety margin and suitability for subsequent dosing in the main experiment (Chen *et al*., 2025; Gonfa *et al.,* 2024).

### Experimental Animals and Grouping

Thirty healthy albino rats weighing between 130 and 180 g were used for the study. The animals were housed in clean cages under a 12-hour light/dark cycle and provided standard pellet feed and water *ad libitum*. A two-week acclimatization period was allowed prior to experimentation to ensure physiological stability (McGill and Jaeschke, 2018; Hassan *et al.,* 2025; Skat-Rørdam *et al.,* 2025). The rats were randomly assigned into six groups of five animals each (n=5) as follows. Group I served as the control and received no treatment. Group II received aspirin at a dose of 10 mg/kg body weight. Group III received *A. boonei* extract at a dose of 1000 mg/kg body weight. Group IV received aspirin at 10 mg/kg combined with *A. boonei* extract at 1000 mg/kg. Group V received aspirin at 10 mg/kg combined with *A. boonei* extract at 1500 mg/kg. Group VI received aspirin at 10 mg/kg combined with *A. boonei* extract at 2000 mg/kg. All treatments were administered orally by gavage. Randomization minimized experimental bias and ensured comparability across groups. The sample size (n = 5 per group) was considered adequate for an exploratory in vivo study based on prior comparable hepatotoxicity and hepatoprotective experiments, providing biological replication for one way ANOVA comparisons of serum liver enzymes and histopathological outcomes, while adhering to the ethical principle of reduction under the 3Rs (Replacement, Reduction and Refinement) framework.

### Biochemical Analysis

At the end of the treatment period, the animals fasted overnight and were sacrificed humanely. Blood samples were collected via cardiac puncture, allowed to clot, and centrifuged to obtain serum. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and conjugated bilirubin were determined using standardized enzymatic colorimetric assay kits obtained from Randox Laboratories Ltd., Crumlin, United Kingdom, according to the manufacturer's instructions. These biomarkers serve as sensitive indicators of hepatocellular integrity and cholestatic function and are widely used in assessing drug-induced hepatic injury (Arif and Aggarwal, 2023; Li *et al.,* 2025; Villanueva-Paz *et al*., 2021).

### Histopathological Processing

Liver tissues were excised immediately after sacrifice, rinsed in saline to remove excess blood, weighed with a measuring scale, and fixed in 10% neutral buffered formalin. Tissue processing followed standard paraffin embedding protocols, after which sections approximately 5 µm thick were prepared using a rotary microtome. Hematoxylin and eosin staining was employed to evaluate hepatocyte morphology, sinusoidal structure, cellular degeneration, and inflammatory changes. Haematoxylin and eosin staining remains the gold standard for structural assessment in hepatic toxicology (Borgne-Sanchez and Fromenty, 2025; Skat-Rørdam *et al*., 2025; Berumen *et al*., 2021). Histology slides of the liver were examined using a Leica DM750 binocular microscope. Expert pathologists, who were blinded to the sample identities, independently interpreted the slides to ensure unbiased assessment.

### Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical analyses were conducted using GraphPad Prism (version 9.0, GraphPad Software Inc., San Diego, CA, USA). A p-value of less than 0.05 was considered statistically significant. This analytical approach is widely applied in

toxicological and pharmacological research to detect treatment effects while accounting for biological variability (Hassan *et al.,* 2025; McGill and Jaeschke, 2018).

## RESULTS AND DISCUSSION

Evaluation of body weight changes showed that body weight varied across experimental groups, with some groups showing weight gain while others exhibited slight weight reduction (Table 1). Rats in the aspirin-only group showed a decrease in body weight from 154.67 ± 8.28 g to 146.67 ± 4.68 g over the experimental period. In contrast, groups treated with *A. boonei* demonstrated comparatively better weight maintenance and gain, particularly the extract-only group, which increased from 137.33 ± 2.44 g to 154.00 ± 4.93 g, and the aspirin plus 1500 mg/kg extract group, which increased from 135.00 ± 8.00 g to 144.00 ± 8.08 g (Table 1). These trends suggest improved physiological tolerance and metabolic stability in extract-treated animals. The magnitude of body weight change is an important indicator in toxicological studies, as reduced weight gain or weight loss often reflects systemic toxicity, metabolic disruption, or poor feed utilization (Hassan *et al.,* 2025; McGill and Jaeschke, 2018). The absence of adverse weight trends in *A. boonei*-treated groups supports the conclusion that the extract did not interfere with normal physiological processes and may have mitigated physiological stress associated with aspirin exposure. Liver weights showed no statistically significant differences across all groups, with values remaining within a narrow range (5.13 ± 0.22 g to 5.70 ± 0.60 g), as shown in Table 1. This finding indicates that the treatments did not induce gross hepatic enlargement or atrophy. The stability of liver weights further supports the interpretation that the observed effects of aspirin and *A. boonei* were primarily biochemical and histological rather than grossly structural, consistent with reports that early or mild hepatic injury may not be accompanied by significant organ weight changes (Gonfa *et al*., 2024).

**Table 1: Effect of aspirin and *A. boonei* on body weight and liver weight of rats after administration across all groups**

| Parameters | Group A | Group B | Group C | Group D | Group E | Group F | F-value | p-value |
|---|---|---|---|---|---|---|---|---|
| Initial body weight (g) | 101.67 ± 1.20 | 154.67 ± 8.28 | 137.33 ± 2.44 | 119.33 ± 5.78 | 135.00 ± 8.00 | 106.33 ± 4.41 | 4.05 | 0.022 |
| Final body weight (g) | 126.00 ± 0.58 | 146.67 ± 4.68 | 154.00 ± 4.93 | 126.33 ± 5.70 | 144.00 ± 8.08 | 114.33 ± 5.67 | 3.76 | 0.028 |
| Liver weight (g) | 5.70 ± 0.06 | 5.47 ± 0.49 | 5.70 ± 0.60 | 5.13 ± 0.22 | 5.70 ± 0.60 | 5.60 ± 0.15 | 0.28 | 0.910 |

Values are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA. A p-value < 0.05 was considered statistically significant.

The graphical trends shown in Figures 1-5 corroborate the biochemical values presented in Table 2, demonstrating numerical variations in liver function markers following aspirin exposure and co-treatment with *A. boonei*. As shown in Figure 1 and Table 2, AST values varied across experimental groups, with lower mean values in aspirin-treated rats relative to controls and numerical changes in co-treated groups. Figure 2 and Table 2 show similar numerical variations in ALT across groups. The effects on ALP depicted in Figure 3 reflected the numerical trends in Table 2, with modest reductions following aspirin exposure and numerical changes with co-treatment. These patterns are consistent with early or mild hepatic stress, which may occur without enzyme elevation, and suggest that *A. boonei* exerted a stabilizing influence on hepatocellular integrity, likely related to its antioxidant and membrane-stabilizing phytoconstituents (Adesina *et al.,* 2025; Sharma and Goyal, 2022). Total bilirubin trends shown in Figure 4 corresponded with Table 2, showing minor fluctuations without marked elevation, while conjugated bilirubin values in Figure 5 and Table 2 remained unchanged across all groups. The preservation of bilirubin conjugation and biliary excretory function indicates the absence of significant cholestasis or biliary obstruction despite mild biochemical modulation of transaminases and ALP. Similar patterns of mild biochemical alteration without bilirubin impairment have been reported in early or low-grade NSAID-induced hepatic stress (Arif and Aggarwal, 2023; Borgne-Sanchez and Fromenty, 2025; Skat-Rørdam *et al*., 2025; Villanueva-Paz *et al*., 2021). Collectively, the concordance between Table 2 and Figures 1-5 supports a numerical, dose-related modulation of aspirin-associated hepatic changes by *A. boonei* without inducing additional biochemical disturbance, consistent with its hepatoprotective potential (Chen *et al*., 2025; Hu *et al.,* 2025; Liao *et al.,* 2024).

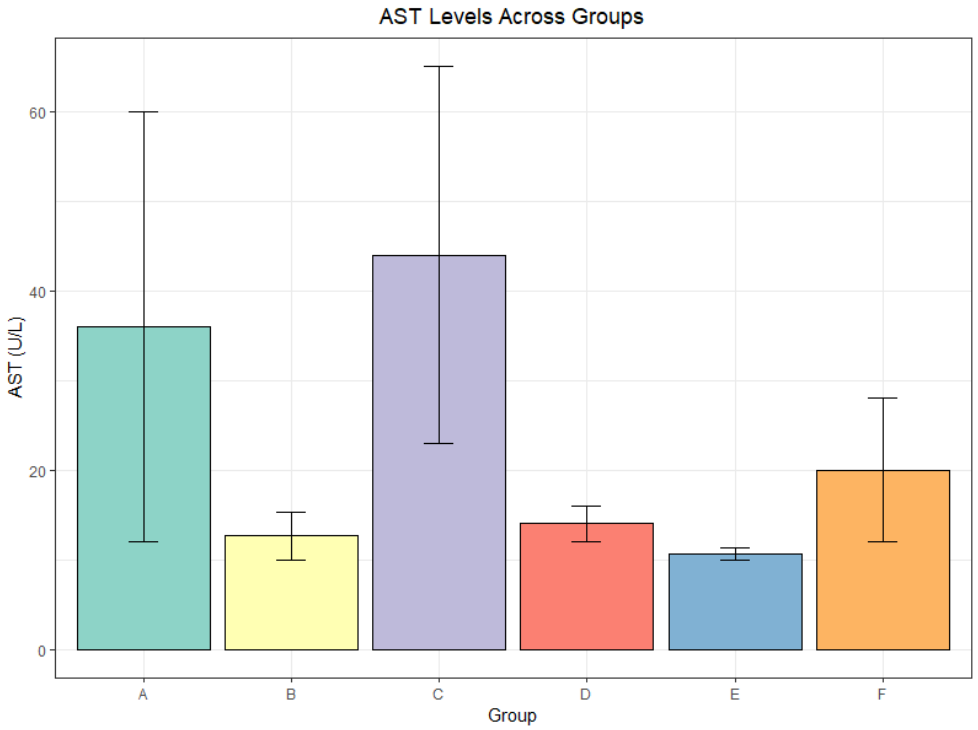


Figure 1: Aspartate aminotransferase (AST) levels across experimental groups. AST levels were reduced in aspirin-treated and co-treated groups relative to control and extract-only groups.

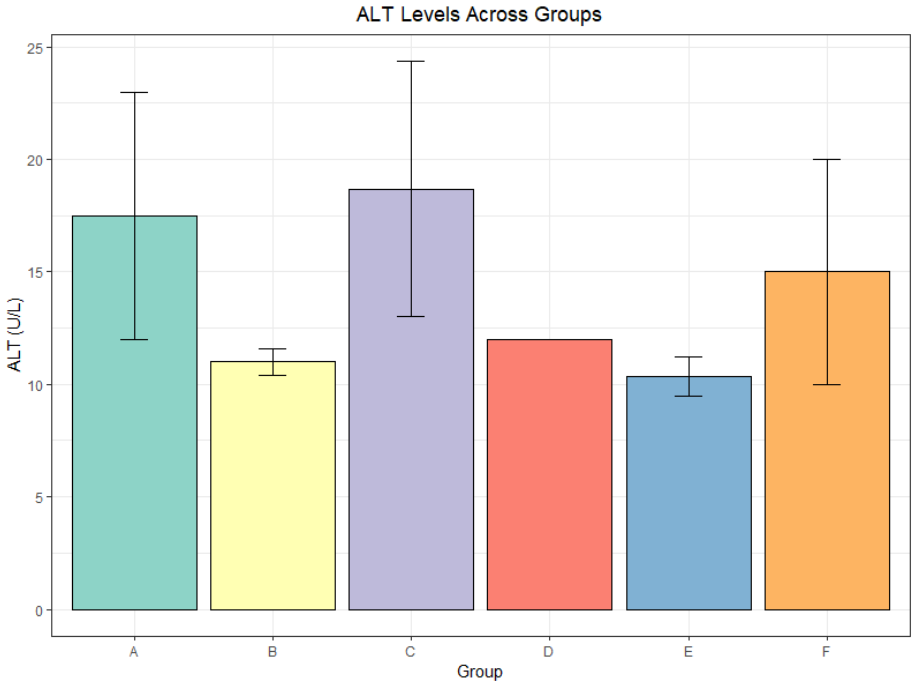


Figure 2: Alanine aminotransferase (ALT) levels across experimental groups. ALT levels were lower in aspirin-treated and co-treated groups compared with control and extract-only groups, with partial normalization at higher extract doses.

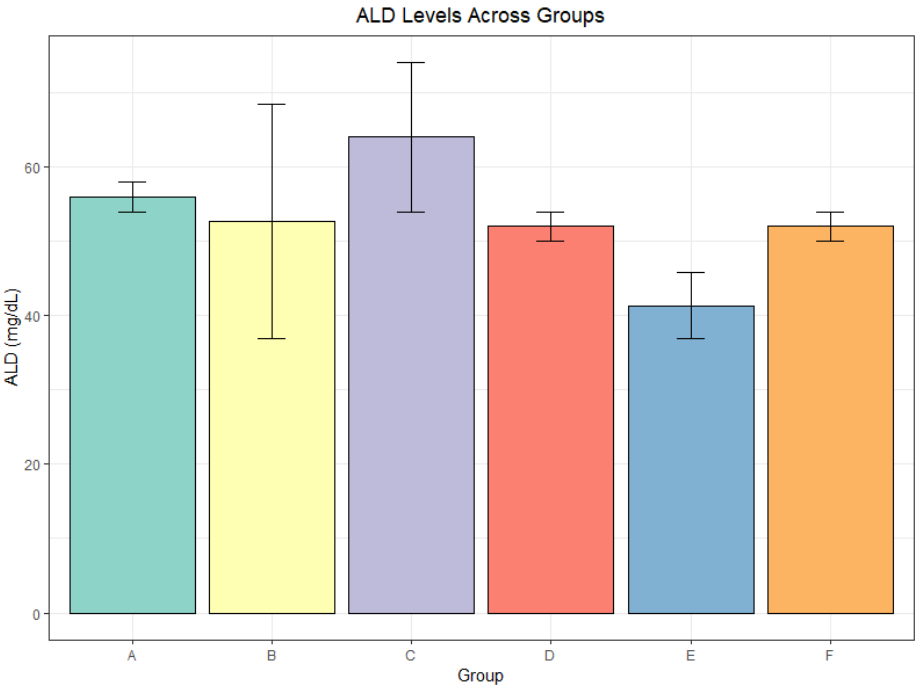


Figure 3: Alkaline phosphatase (ALP) levels across experimental groups. ALP activity showed modest reductions in aspirin-treated and co-treated groups relative to control and extract-only groups.

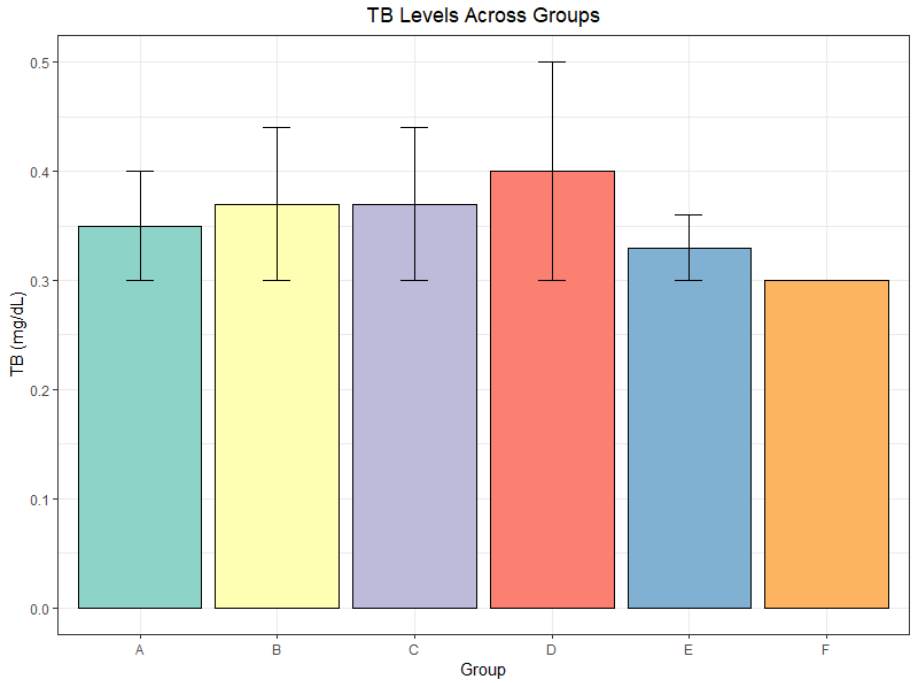


Figure 4: Total bilirubin levels across experimental groups. Total bilirubin levels showed no statistically significant differences across groups.

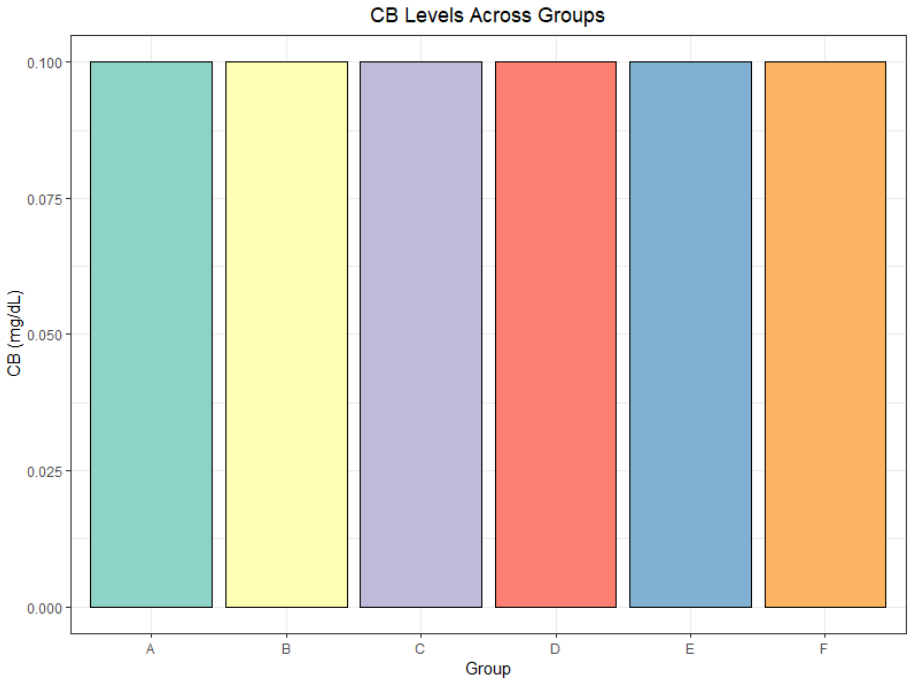


Figure 5: Conjugated bilirubin levels across experimental groups. Data are presented as mean ± SEM. One-way ANOVA; P < 0.05 considered statistically significant.

**Table 2: Effect of aspirin and *A. boonei* on liver function markers after administration across all groups**

| Parameters | Group A | Group B | Group C | Group D | Group E | Group F | p-value |
|---|---|---|---|---|---|---|---|
| AST (U/L) | 36.00 ± 24.00 | 12.67 ±2.67 | 44.00 ±21.00 | 14.00 ± 2.00 | 10.67 ± 0.67 | 20.00 ± 8.00 | 0.388 |
| ALT (U/L) | 17.50 ± 5.50 | 11.00 ±0.58 | 18.67 ± 5.67 | 12.00 ± 0.00 | 10.33 ± 0.88 | 15.00 ± 5.00 | 0.484 |
| ALP (U/L) | 56.00 ± 2.00 | 52.67 ±15.71 | 64.00 ±10.00 | 52.00 ± 2.00 | 41.33 ± 4.48 | 52.00 ± 2.00 | 0.675 |
| Total Bilirubin (mg/dL) | 0.35 ± 0.05 | 0.37 ± 0.07 | 0.37 ± 0.07 | 0.40 ± 0.10 | 0.33 ± 0.03 | 0.30 ± 0.00 | 0.923 |
| Conjugated Bilirubin (mg/dL) | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | — |

Values are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA. A p-value < 0.05 was considered statistically significant. Conjugated bilirubin showed no variation across groups; therefore, statistical comparison was not applicable.

Histological examination of liver sections across experimental groups (Plate 1 A-F) revealed largely preserved hepatic architecture. The control and *A. boonei* only groups exhibited normal hepatocyte morphology with intact hepatic cords and well-defined sinusoids, confirming the hepatic safety of the extract at the administered dose (Sharma and Goyal, 2022; Mishra *et al*., 2025). In contrast, rats treated with aspirin alone showed mild cytoplasmic granulation, subtle sinusoidal congestion, and early degenerative changes, indicative of low-grade hepatocellular stress. These alterations are consistent with aspirin-induced mitochondrial dysfunction and oxidative stress consistent with the mild biochemical alterations observed in this study (Berumen *et al.,* 2021; Borgne-Sanchez and Fromenty, 2025).

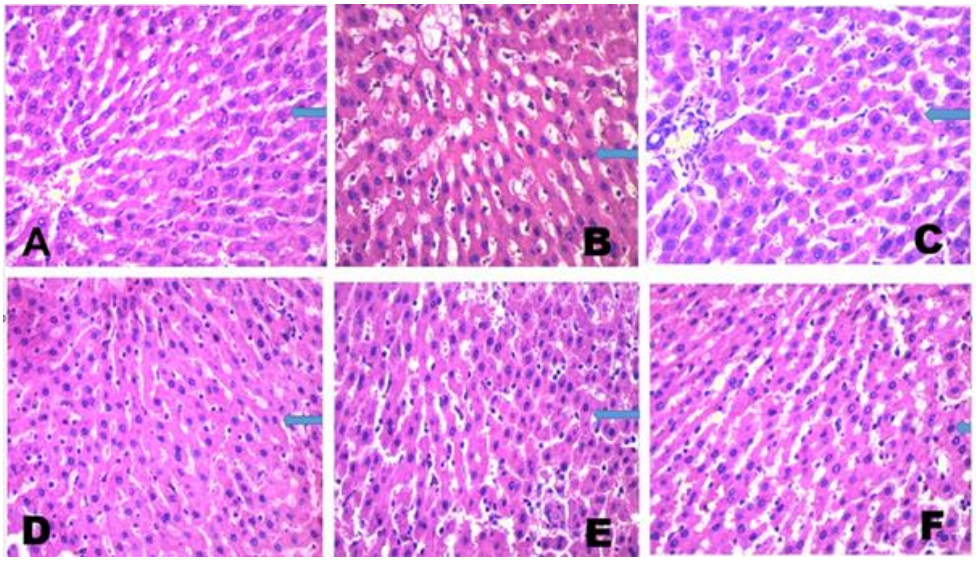


Plate 1: Representative histological sections of rat liver across experimental groups (H&E, ×400). (A) Normal hepatic architecture with well-organized hepatic cords and intact sinusoids. (B) Generally preserved architecture with mild cytoplasmic granulation and subtle sinusoidal prominence. (C) Normal hepatic histology comparable to control. (D–E) Well-preserved hepatic cords with minimal cytoplasmic alteration and near-normal architecture. (F) Largely preserved architecture with mild sinusoidal dilation and subtle cytoplasmic changes.

Co-administration of *A. boonei* produced dose-dependent histological improvement. Moderate doses (1000 and 1500 mg/kg) were associated with preservation and restoration of hepatic architecture, characterized by uniform hepatocytes, organized sinusoids, and minimal inflammatory infiltration. The 1500 mg/kg dose demonstrated the most notable recovery, closely corresponding with optimal biochemical improvement. These findings reflect the antioxidant, anti-inflammatory, and membrane-stabilizing properties of *A. boonei*, which have been shown to enhance endogenous antioxidant defenses and reduce lipid peroxidation in chemically induced hepatic injury (Adesina *et al*., 2025; Okpashi *et al.,* 2022; Taiwo *et al*., 2019; Uroko *et al.,* 2020). At the highest extract dose (2000 mg/kg), hepatic architecture remained largely preserved; however, mild sinusoidal dilation and subtle cytoplasmic changes were observed, suggesting minimal hepatic stress. This observation mirrors the slight attenuation in biochemical improvement at higher doses and supports a biphasic dose-response pattern commonly reported for phytomedicinal agents (Mishra *et al.,* 2025).

Overall, the integration of histological and biochemical findings confirms that *A. boonei* exerts significant hepatoprotective and reparative effects against aspirin-induced hepatic injury. The extract mitigated oxidative stress, preserved hepatocyte morphology, and supported functional recovery, reinforcing the therapeutic potential of medicinal plants in managing drug-induced liver damage associated with widespread NSAID use (Gonfa *et al.,* 2024; Kobayashi *et al.,* 2023; Villanueva-Paz *et al.,* 2021; Wang *et al.,* 2025a). Further studies are recommended to elucidate molecular mechanisms, long-term safety, and clinical translational potential (Chen *et al*., 2025; Gonfa *et al.,* 2024; Wang *et al.,* 2025b).

## CONCLUSION

*Alstonia boonei* demonstrated hepatoprotective potential against aspirin-associated hepatic alterations in rats, with co-treatment associated with numerical improvements in liver biomarkers and preservation of hepatic architecture. These findings support its potential as a natural hepatoprotective agent. Further studies are needed to elucidate molecular mechanisms, long-term safety, and translational relevance.

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