



HISTOLOGICAL AND BIOCHEMICAL EFFECTS OF ETHYL-ACETATE BARK FRACTION OF *Alstonia boonei* ON CCL₄-INDUCED HEPATOTOXICITY IN ADULT MALE WISTAR RATS

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DOI: <https://doi.org/10.5281/zenodo.22687460>

How to cite this Article: Arayombo Babatunde Elijah, Adewole Olarinde Stephen, Ojo Surajudeen Kayode, Ale Temidayo Timothy, Ige Mokolade Samson. (2026). Histological and Biochemical Effects of Ethyl-Acetate Bark Fraction of *Alstonia boonei* on Ccl₄-Induced Hepatotoxicity In Adult Male Wistar Rats. European Journal of Biomedical and Pharmaceutical Sciences, 13(9), 286–297.

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Article Received on 15/08/2026

Article Revised on 05/09/2026

Article Published on 10/09/2026

ABSTRACT

Background: Carbon tetrachloride (CCl₄) induced hepatotoxicity is associated with oxidative stress, impaired liver function, and structural damage. *Alstonia boonei* is a medicinal plant rich in bioactive phytochemicals with reported antioxidant and hepatoprotective properties. This study investigated the Effects of the ethyl-acetate fraction of *A. boonei* (EAFAB) on CCl₄ -induced hepatotoxicity in adult male Wistar rats. **Methodology:** Forty two adult male Wistar rats were exposed to CCl₄ and treated orally with EAFAB (100, 200, or 400 mg/kg) or silymarin (100 mg/kg) for 14 days. Body weight was assessed, while serum total protein, aspartate aminotransferase (ALT), alanine aminotransferase (AST), and alkaline phosphatase (ALP) and hepatic superoxide dismutase (SOD) and malondialdehyde (MDA) were determined. Liver tissues were examined using hematoxylin and eosin and Reticulin stain. **Results:** CCl₄ administration significantly reduced body weight and serum total protein, increased ALT, AST, ALP, and MDA, and reduced SOD activity compared with the control group. EAFAB treatment improved body weight and total protein, reduced ALT and AST activities, and markedly decreased MDA concentration. ALP activity was also reduced following EAFAB treatment, although the difference was not statistically significant. EAFAB and silymarin improved SOD activity, but these changes were not statistically significant. Histologically, CCl₄ caused sinusoidal dilation, disorganization of hepatic cords, and fragmentation of the reticulin network, whereas EAFAB promoted restoration of hepatic architecture, with the 400 mg/kg dose showing the most pronounced improvement. **Conclusion:** EAFAB demonstrated promising hepatoprotective activity against CCl₄ -induced hepatic injury, particularly through attenuation of lipid peroxidation and improvement of biochemical and histological alterations.

KEYWORDS: *Alstonia boonei*, Ethyl-acetate, Carbon tetrachloride, Wistar rat, Liver.

INTRODUCTION

The liver is the largest internal gland in the human body, weighing approximately 1.5–1.8 kg in adults (Berthoud *et al.*, 2024). It is a vital organ involved in metabolism, protein synthesis, immune regulation, and detoxification of endogenous and exogenous compounds, with hepatocytes serving as its major functional cells (Santos *et al.*, 2024). Hepatic homeostasis is also maintained by non-parenchymal cells, including liver sinusoidal endothelial cells, hepatic stellate cells, cholangiocytes, and Kupffer cells, which contribute to hepatic function, bile regulation, tissue repair, and immune defense (Schulze *et al.*, 2019). These cellular components

maintain normal liver structure and function and influence the liver's response to toxic substances. However, because of its continuous exposure to metabolites, drugs, chemicals, and other xenobiotics, the liver is particularly susceptible to toxic injury (Ezhilarasan *et al.*, 2025). Hepatotoxicity may manifest as hepatocellular injury, inflammation, oxidative stress, cholestasis, and necrosis and, when persistent, may progress to fibrosis and liver failure (Katarey & Verma, 2023; Xiao *et al.*, 2025).

Hepatotoxicity, particularly drug-induced liver injury (DILI), is an important cause of liver dysfunction

worldwide (Li *et al.*, 2023). Its incidence varies across populations and regions. A systematic review estimated the global incidence of DILI at 4.94 cases per 100,000 person-years, with higher rates reported in Asia (17.82 per 100,000 person-years) than in North America (1.72 per 100,000 person-years) (Li *et al.*, 2023). In sub-Saharan Africa, data on hepatotoxicity remain limited, but available studies indicate a considerable burden. A systematic review reported a pooled hepatotoxicity prevalence of 25.45% among HIV-infected patients in Ethiopia (Mohammed *et al.*, 2022). In Nigeria, hepatotoxicity has been reported in different regions, including 18.2% among patients receiving anti-tuberculosis therapy in Jos, Plateau State, and 17.9% among patients receiving highly active antiretroviral therapy in Benin City, Edo State (Isa *et al.*, 2016; Ugiagbe *et al.*, 2012).

Liver diseases constitute a substantial global health burden, accounting for approximately two million deaths annually, with cirrhosis and hepatocellular carcinoma contributing substantially to mortality (Moon *et al.*, 2023). Drug- and chemical-induced liver injury is also an important clinical problem because severe cases may progress to acute liver failure and death (Ma *et al.*, 2023). Clinical manifestations of hepatotoxicity vary according to the severity and pattern of hepatic injury. Mild injury may be asymptomatic and detected only through elevated serum liver enzymes, whereas symptomatic cases may present with fatigue, weakness, nausea, vomiting, abdominal discomfort, anorexia, dark urine, and jaundice. Severe hepatic injury may result in coagulopathy, hepatic encephalopathy, acute liver failure, and, in extreme cases, death (Teschke & Danan, 2023; Ozturk *et al.*, 2025).

Carbon tetrachloride (CCl₄) is a well-established experimental hepatotoxicant widely used to investigate mechanisms of liver injury and evaluate the hepatoprotective potential of natural products (Ezhilarasan *et al.*, 2025). Following hepatic

biotransformation, CCl₄ generates reactive metabolites that initiate oxidative stress and lipid peroxidation, resulting in disruption of cellular membranes and hepatocellular injury (Fareed *et al.*, 2024). Consequently, CCl₄ exposure is associated with increased liver enzymes, depletion of antioxidant defenses, inflammatory responses, apoptosis, and histological alterations (Algefare *et al.*, 2024; Munakarmi *et al.*, 2024).

Treatment of hepatotoxicity primarily involves discontinuation of the offending drug or toxic agent, followed by supportive care and close monitoring of liver function. N-acetylcysteine (NAC) is the established antidote for acetaminophen-induced liver injury and may also be considered in selected patients with acute liver failure (Fontana *et al.*, 2023). Corticosteroids are not routinely recommended but may be considered in selected cases of immune-mediated or autoimmune hepatitis-like DILI. Patients who develop severe acute liver failure may require referral to a specialist centre and consideration of liver transplantation (Fontana *et al.*, 2023). Despite the availability of conventional management strategies, the limited specific pharmacological options for many forms of liver injury have stimulated interest in medicinal plants as potential sources of hepatoprotective agents (Shawon *et al.*, 2024).

Alstonia boonei is a medicinal tree belonging to the Apocynaceae family that is traditionally used for the management of various ailments and is widely distributed in tropical and subtropical regions of Africa, Australia, Southeast Asia, and Central America (Mollica *et al.*, 2022). It is a large deciduous tree that can attain approximately 45 m in height, with a deeply fluted trunk reaching about 1.2 m in diameter and producing copious milky latex. The leaves are simple, whorled, typically occurring in groups of 3–6 per node, lanceolate to elliptic in shape, 10–25 cm long and 4–10 cm wide, with a glossy green and leathery appearance (Mollica *et al.*, 2022).



***Alstonia boonei* Plant (Okpashi *et al.*, 2022)**

In ethnomedicine, *A. boonei* has been used in the management of malaria, sore throat, cough, toothache, snake bites, ulcers, jaundice, skin conditions, arthritis, rheumatism, and hypertension, as well as for its anthelmintic properties (Mollica *et al.*, 2022). The wide range of traditional applications has stimulated scientific investigations into the biological and pharmacological activities of the plant. Recent phytochemical investigations have identified phenolic acids, flavonoids, alkaloids, tannins, saponins, triterpenes, and other bioactive constituents in different parts of *A. boonei* (Njoya *et al.*, 2022). These phytochemicals have been associated with antioxidant and anti-inflammatory activities, suggesting potential therapeutic value against oxidative and inflammatory tissue injury (Njoya *et al.*, 2022). Recent chemical characterization of *A. boonei* leaves has also identified several indole alkaloids, including a newly characterized ulean-type alkaloid, further demonstrating the phytochemical richness of the plant (Grimm *et al.*, 2024). In addition, a recent experimental study demonstrated that *A. boonei* stem-bark extract and its ethyl acetate fraction improved liver function indices, antioxidant enzyme activities, and liver histoarchitecture in isoniazid-induced hepatotoxicity in Wistar rats, supporting its potential hepatoprotective activity (Adesina *et al.*, 2025). Also, Okpashi *et al.*, (2022) reported hepatoprotective Effects of *A. boonei* stem-bark extract in CCl₄ -induced liver injury. However, limited information exists regarding the histomorphological Effects of *A. boonei* on CCl₄ -induced hepatotoxicity specifically in adult male mice (Okpashi *et al.*, 2022; Adesina *et al.*, 2025). Therefore, this study investigates the role of *Alstonia boonei* on CCl₄ -induced hepatotoxicity in adult male Wistar rats.

MATERIALS AND METHODS

1. Plant Material and Extract Preparation

Collection and Identification: Fresh stem bark of *Alstonia boonei* is collected from Ife East Government Reserve and authenticated by a plant taxonomist in the department of Botany Obafemi Awolowo University, Ile-Ife. A voucher specimen was deposited at the herbarium for reference purpose.

Preparation of Extract: The collected stem bark of *Alstonia boonei* was cleaned and air-dried under shade to preserve phytochemicals, until a constant weight was achieved to ensure dryness, pulverized into fine powder, using electric blender and the powdered extract was subjected to Soxhlet extraction using ethanol (70%) as the solvent. The mixture was macerated for 72 hours with intermittent shaking and agitation, filtered, and concentrated under reduced pressure using a rotary evaporator. The ethanol extract is suspended in distilled water and partitioned sequentially with ethyl acetate as solvent, which was collected separately after repeated partitioning. The ethyl acetate was concentrated under reduced pressure to reduce residual solvent. The dried fraction was stored in airtight containers at 4°C until use.

2. Experimental Animals

Animal Model: Forty-two (42) adults male Wistar rats weighing 190-210 g were procured from animal house of College of Health Sciences, Obafemi Awolowo University, Ile-Ife.

Housing Conditions: Rats were housed in plastic cages under natural environmental conditions; natural night and day cycle, room temperature and relative humidity. They were fed standard rat chow and had free access to water. All experimental procedures were conducted in accordance with institutional guidelines for animal care and use.

3. Experimental Design

The animals were randomly divided into six groups (n = 7 per group):

- A. Normal control (received olive oil only)
- B. Toxic control (CCl₄ only, 1 ml/kg, i.p.)
- C. CCl₄ (1 ml/kg, i.p.) + EAFAB (100 mg/kg, p.o)
- D. CCl₄ (1 ml/kg, i.p.) + EAFAB (200 mg/kg, p.o)
- E. CCl₄ (1 ml/kg, i.p.) + EAFAB (400 mg/kg, p.o)
- F. CCl₄ (1 ml/kg, i.p.) + Silymarin (100 mg/kg, oral)

***EAFAB** - ethyl-acetate fraction of *Alstonia boonei*

Induction of Hepatotoxicity: Hepatic injury was induced by intraperitoneal administration of carbon tetrachloride (CCl₄) diluted in olive oil (1:1 v/v) at a dose of 1 ml/kg body weight, twice weekly for 2 weeks.

Treatment Regimen: Rats in treatment groups received graded doses of EAFAB (100, 200, and 400 mg/kg) orally once daily for 14 days, alongside CCl₄ administration. Control groups received either olive oil or CCl₄ only.

4. Sample Collection

At the end of the experimental period, rats were fasted overnight and sacrificed with cervical dislocation. Blood samples were collected via cardiac puncture into plain tubes, allowed to clot, and centrifuged at 3000 rpm for 10 minutes to obtain serum. Liver tissues were excised, rinsed in ice-cold saline, blotted dry, and divided for biochemical assays and histopathological examination.

5. Biochemical Analysis

Serum Liver Function Tests: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total protein were measured using standard diagnostic kits. **Oxidative Stress Markers:** Malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activities were determined in liver homogenates using established protocols (Ohkawa *et al.*, 1979; Ellman, 1959; Marklund & Marklund, 1974; Aebi, 1984).

6. Histopathological Examination

Liver samples were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned at 5 µm and stained. Hematoxylin and Eosin (H&E) was employed to

assess the general histoarchitecture of the liver and Reticulin stain was employed to check for the structural network (collagen) of the liver. Sections were examined under a light microscope for histological alterations.

7. Statistical Analysis

Data were expressed as mean $\pm$ standard error of mean (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. GraphPad prism version 9 was employed for data analysis.

RESULTS

BODY WEIGHT ASSESSMENT

Figures 1a and 1b reveal the Effects of EAFAB on body weight pre CCl_4 treatment and post CCl_4 treatment plus interventions respectively. Result showed that before CCl_4 treatment, all rats were within the same weight range with no significant differences among groups (1a), however after the administration of CCl_4 to rats in group B, there was a significant weight loss in these animals relative to group A indicating a toxicity in these animals (1b).

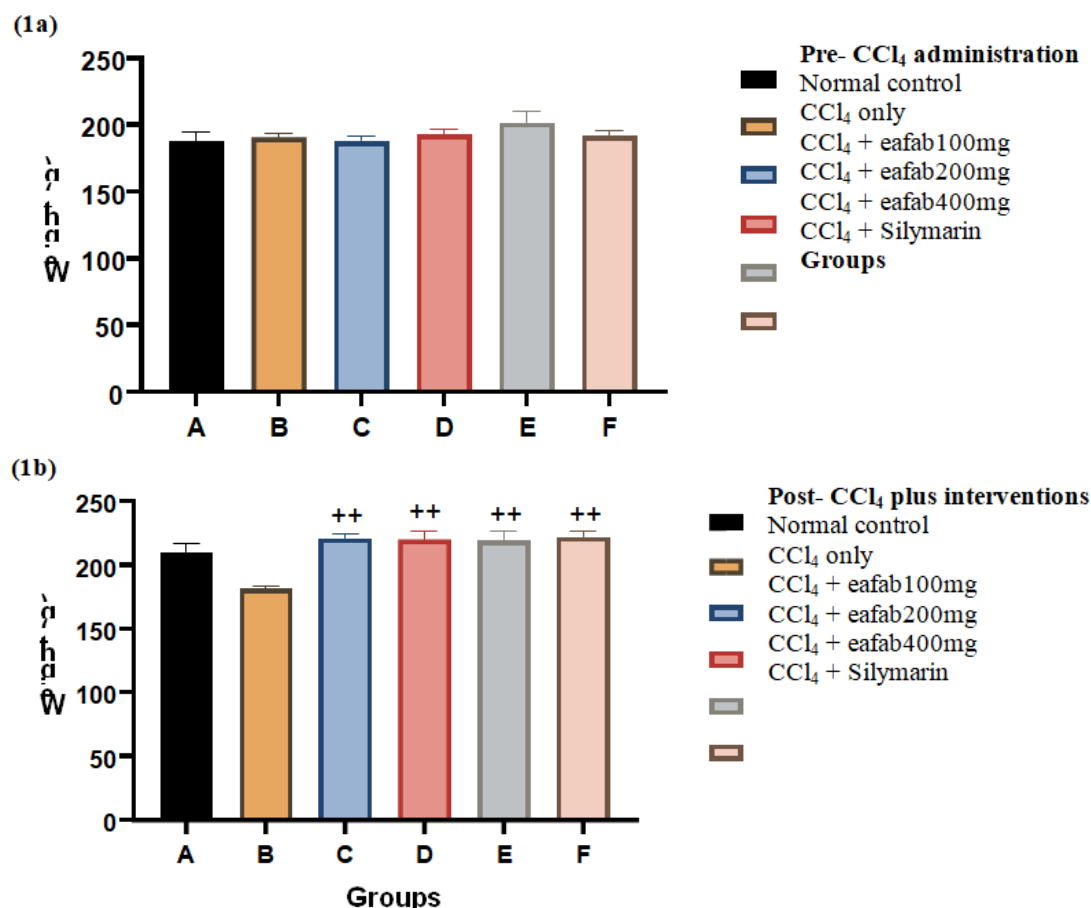


Figure 1: Effects of ethyl-acetate fraction of *Alstonia boonei* on the body weight in adult male rat pre (a) and post CCl_4 -induced hepatotoxicity (b). Data were presented as mean $\pm$ SEM. ++ = $p < 0.05$ against group A, using one way ANOVA, Tukey's test.

BIOCHEMICAL ASSESSMENT

As shown in figure 2, the administration of CCl_4 led to a significant reduction of liver total protein (TP) in group B when compared with group A ($p = 0.0024$). The administration of silymarin the standard drug, caused a significant increase in TP levels relative to group B, C and D indicating TP level restoration ($p = 0.0057$, $p = 0.0012$ and $p = 0.0079$ respectively). Nevertheless, the administration of EAFAB mitigated the toxicity by causing an increase in TP level, with the highest dose (400 mg/kg) demonstrating the highest efficacy when compared with group B ($p = 0.0279$).

Shown in figure 3, is the Effects of EAFAB on superoxide dismutase activity. There was a significant reduction of SOD activity in rats treated with CCl_4 (B) when compared with group A, the normal control ($p = 0.0254$), nevertheless the interventions provided (EAFAB and Silymarin) improved SOD activities however, based on statistics these differences were found not significant ($p > 0.05$).

Illustrated in figure 4 is the Effects of EAFAB on malondialdehyde concentration. The administration of CCl_4 resulted in an elevation of lipid peroxidation

evident by the marked increase in MDA concentration as seen in groups B-F when compared with group A ($p =$

0.0011, $p = 0.0003$, $p = 0.0006$, $p = 0.0238$ and $p = 0.0184$ respectively). The administration of EAFAB 100, 200, 400 mg/kg and Silymarin caused a significant reduction in lipid peroxidation when compared with group B ($p = 0.0436$, $p = 0.0358$ and $p = 0.0011$ respectively), with the highest dose of EAFAB and Silymarin demonstrating the best antioxidant capabilities.

Figure 5 shows the Effects of EAFAB on ALT activity. A marked elevation of the liver protein activity ALT, was seen in rats treated with CCl_4 (B-F) when compared with group A, the normal control ($p = 0.0025$, $p = 0.0410$, $p = 0.0125$, $p = 0.0687$ and $p = 0.0022$ respectively). Meanwhile an ameliorative Effects was observed in the intervention groups indicated by a reduction of ALT activity, with the best Effects seen in group D, E and F, when compared with group B.

Illustrated in figure 6 is the Effects of EAFAB on AST activity. Result reveals a significant rise in AST activity in rat treated with CCl_4 when compared with group A ($p = 0.0018$). Meanwhile an ameliorative Effects was observed in the intervention groups indicated by a reduction of AST activity, with the best Effects seen in group D, E and F, when compared with group B ($p = 0.0034$, $p = 0.0009$ and $p = 0.0001$ respectively).

Illustrated in figure 7 is the Effects of EAFAB on ALP concentration. There was a significant increase of ALP activity in rats treated with CCl_4 (B-F) when compared with group A, the normal control ($p = 0.0288$, $p = 0.0183$, $p = 0.0020$, $p < 0.0001$ and $p = 0.0429$ respectively). Meanwhile a reduction of ALP activity was seen in the groups treated with EAFAB (C-E) and Silymarin relative to group B, nevertheless these differences were statistically found not significant.

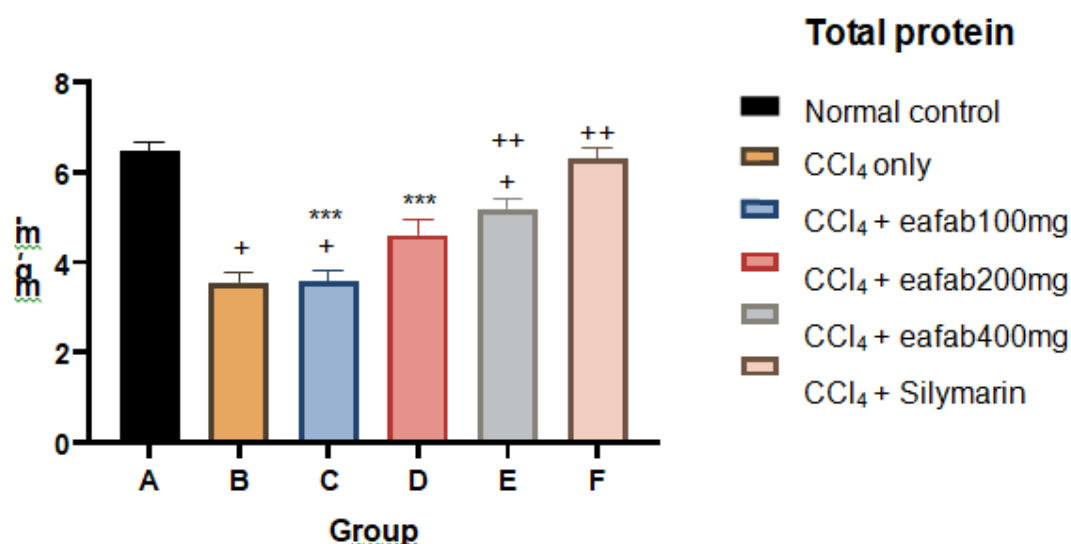


Figure 2: Effects of ethyl-acetate fraction of *Alstonia boonei* on the total protein concentration in adult male rat with CCl_4 -induced hepatotoxicity. Data are presented as mean $\pm$ SEM. + = $p < 0.05$ against group A, ++ = $p < 0.05$ against group B, and *** = $p < 0.05$ against group F, using one way ANOVA, Tukey's test.

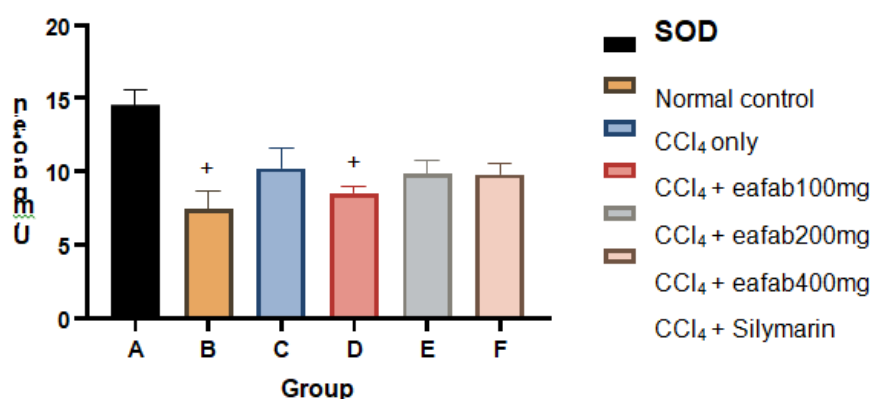


Figure 3: Effects of ethyl-acetate fraction of *Alstonia boonei* on the superoxide dismutase activity in adult male rat with CCl_4 -induced hepatotoxicity. Data are presented as mean $\pm$ SEM. + = $p < 0.05$ against group A, using one way ANOVA,

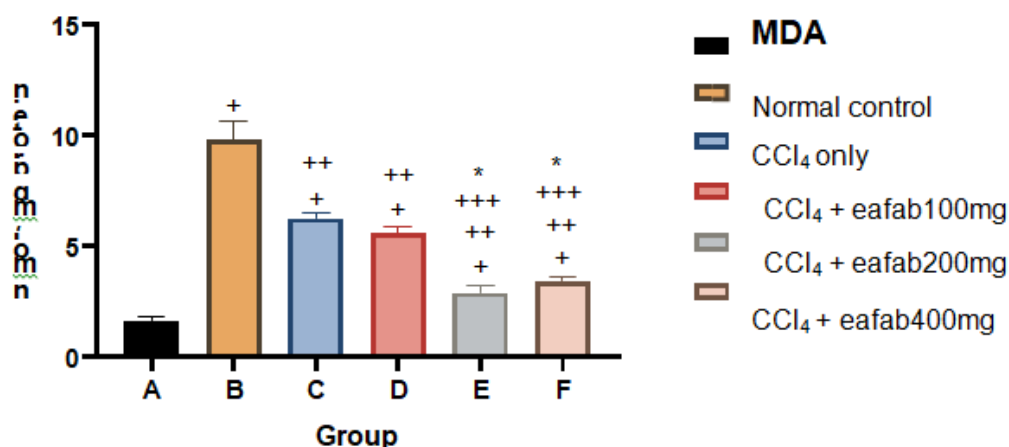


Figure 4: Effects of ethyl-acetate fraction of *Alstonia boonei* on the malondialdehyde concentration in adult male rat with CCl₄-induced hepatotoxicity. Data are presented as mean $\pm$ SEM. + = $p < 0.05$ against group A, ++ = $p < 0.05$ against group B, +++ = $p < 0.05$ against group C, and * = $p < 0.05$ against group D, using one way ANOVA, Tukey's test.

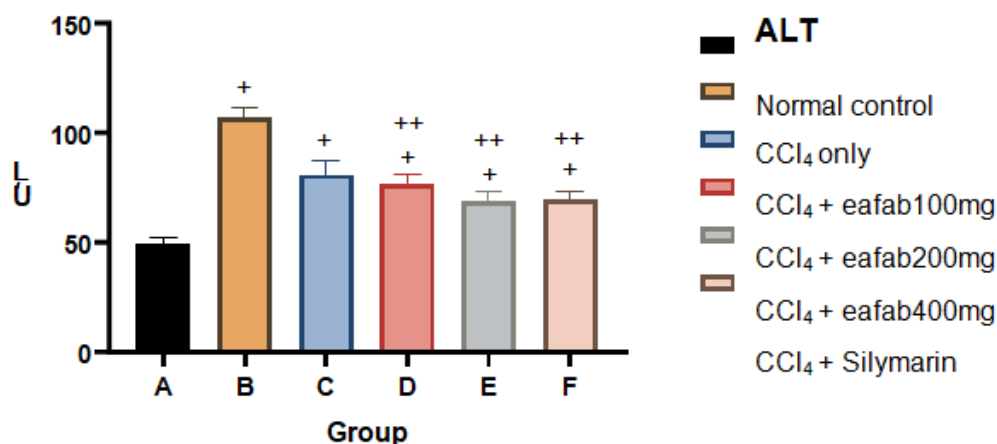


Figure 5: Effects of ethyl-acetate fraction of *Alstonia boonei* on the Alanine aminotransferase (ALT) activity in adult male rat with CCl₄-induced hepatotoxicity. Data are presented as mean $\pm$ SEM. + = $p < 0.05$ against group A, ++ = $p < 0.05$ against group B, using one way ANOVA, Tukey's test.

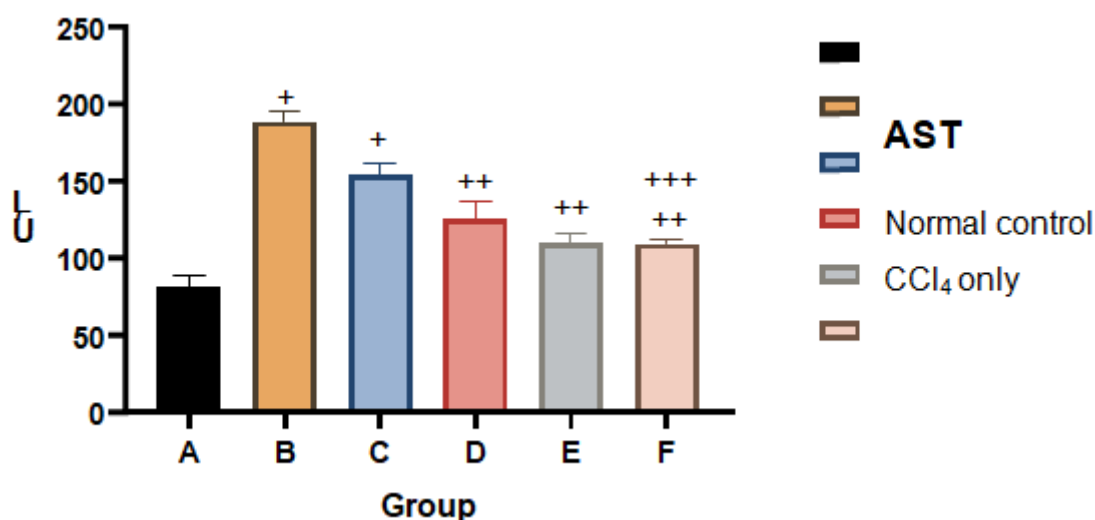


Figure 6: Effects of ethyl-acetate fraction of *Alstonia boonei* on the Aspartate aminotransferase (AST) activity in adult male rat with CCl₄-induced hepatotoxicity. Data are presented as mean $\pm$ SEM. + = $p < 0.05$ against group A, ++ = $p < 0.05$ against group B and +++ = $p < 0.05$ against group C, using one way ANOVA, Tukey's test.

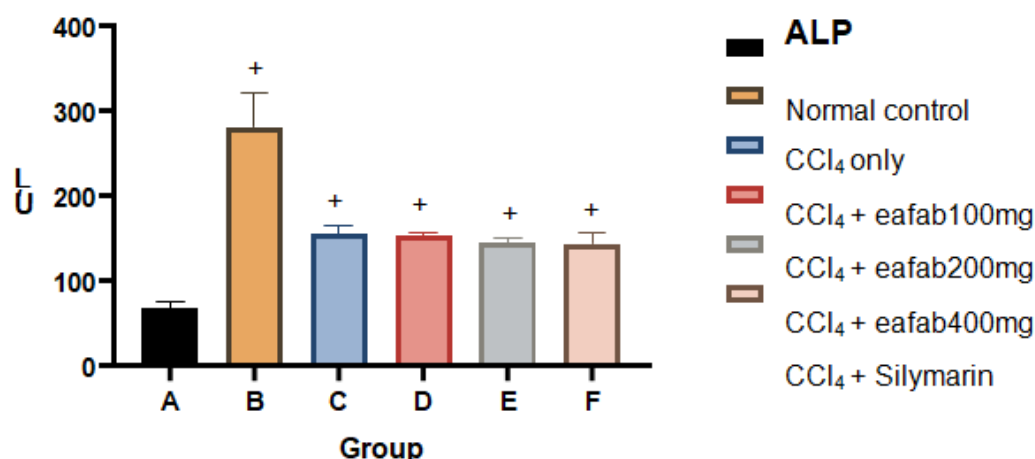


Figure 7: Effects of ethyl-acetate fraction of *Alstonia boonei* on the Alkaline phosphatase (ALP) activity in adult male rat with CCl₄-induced hepatotoxicity. Data are presented as mean $\pm$ SEM. ⁺ = $p < 0.05$ against group A, using one way ANOVA, Tukey's test.

HISTOLOGICAL ASSESSMENT

Shown in figure 8 is the histological examination (H & E) of the rats' liver. Group A shows the normal histoarchitecture of the liver evident by the orderly and well-arranged hepatic cords radiating from the central vein, whereas group B showed dilated sinusoids with disarranged hepatic cords indicating cellular toxicity. The treatment with EAFAB restored liver morphology to almost near normal with the highest dose 400 mg/kg demonstrating the ameliorative Effects as compared with normal control and the Silymarin treated rats.

Figure 9 presents the histological examination (H&E) of the rats' liver tissues. Photomicrograph A shows a well-organized and fine reticulin network with a clear radial arrangement, indicative of preserved hepatic architecture.

In contrast, rats treated with CCl₄ exhibited marked structural damage, characterized by disruption of the normal reticulin pattern. Areas indicated by the blue arrows show fragmented reticulin fibres, with the framework appearing discontinuous rather than forming the relatively uniform network observed in the control group (A). Nevertheless, treatment with the various interventions appeared to promote tissue repair and restoration of the hepatic architecture. Among the treated groups, the highest dose of EAFAB and silymarin demonstrated the most pronounced improvement, with photomicrographs E and F showing a comparatively better-preserved and more organized reticulin network.

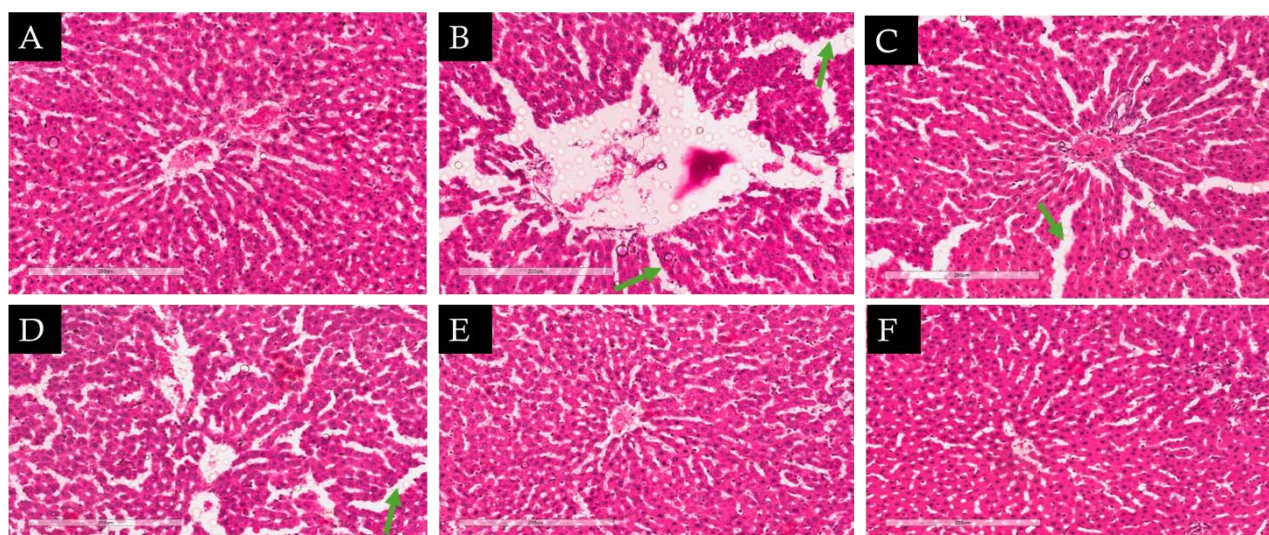


Figure 8: Representative photomicrographs (A - F) showing the Effects of the EAFAB on liver histoarchitecture of rats with CCl₄ induced hepatotoxicity. The green arrow indicates dilated sinusoids. Hematoxylin and eosin x200.

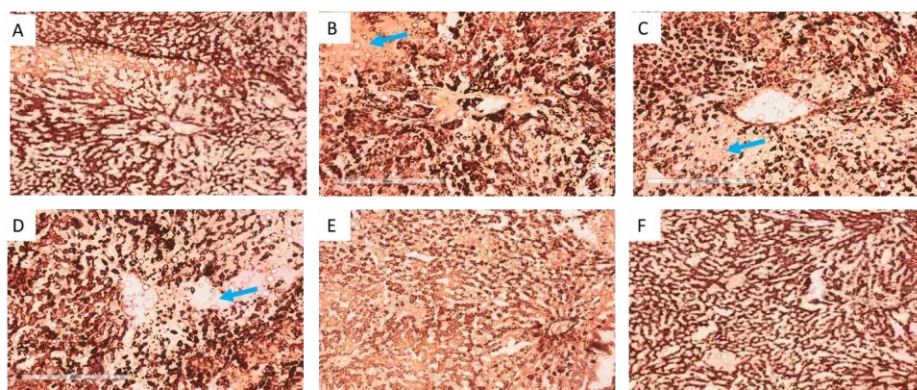


Figure 9: Representative photomicrographs (A – F) showing the Effects of the EAFAB on liver histoarchitecture of rats with CCl₄ induced hepatotoxicity. Blue arrow indicates fragmented network. Reticulin sliver stain x200.

DISCUSSION

Carbon tetrachloride (CCl₄) is a classic hepatotoxicant widely used to investigate hepatic oxidative injury and evaluate hepatoprotective agents. Its toxicity is largely mediated by cytochrome P450-dependent formation of reactive trichloromethyl radicals, which initiate lipid peroxidation, disrupt cellular membranes and Compromised hepatocellular integrity (Zhang *et al.*, 2017). CCl₄ exposure resulted in a significant reduction in body weight, suggesting systemic toxicity, which may be associated with oxidative hepatic damage and impaired metabolic functions. This finding is consistent with Alkreathy *et al.* (2014), who reported significant body-weight reduction following CCl₄ administration in rats, with subsequent improvement following treatment with *Sonchus arvensis* extract. Similarly, the improved body weight observed in the EAFAB-treated groups in the present study suggests that *A. boonei* may help mitigate the systemic Effects of CCl₄ toxicity and support the maintenance or recovery of normal body weight. Thus, the observed reduction in body weight, together with the subsequent biochemical and histological alterations, supports the successful induction of CCl₄-induced hepatic injury in the experimental animals.

The reduction in hepatic total protein following CCl₄ administration suggests impairment of normal hepatic functional capacity. The liver is central to the synthesis of numerous proteins, and the disruption of hepatocellular integrity and metabolic activity can consequently affect protein synthesis and turnover (Trefts *et al.*, 2017). The restoration of total protein following EAFAB administration, particularly at the higher dose, therefore suggests preservation or recovery of hepatic functional integrity following toxic insult. This observation is further strengthened by the response to silymarin, which produced marked restoration of protein levels. Similar Effects have been reported in experimental models of CCl₄-induced liver injury in which hepatotoxic exposure was associated with disturbances in protein metabolism, while silymarin attenuated these changes (Popoola *et al.*, 2022). Therefore, the ability of EAFAB to improve total protein

therefore represent one component of its hepatoprotective action.

The oxidative stress findings provide further evidence for the involvement of redox imbalance in the hepatic injury induced by CCl₄. The reduction in superoxide dismutase (SOD) activity following CCl₄ exposure indicates weakening of an important endogenous antioxidant defense mechanism. SOD constitutes a primary enzymatic defense against superoxide radicals by catalysing their conversion to hydrogen peroxide, thereby limiting the propagation of oxidative damage (Wang *et al.*, 2018). Although the increase in SOD activity following treatment with EAFAB and silymarin was not statistically significant, both interventions showed an observable improvement compared with the CCl₄-treated group. This suggests that EAFAB and silymarin may have enhanced endogenous antioxidant defense by promoting SOD activity, although the magnitude of this Effects was insufficient to reach statistical significance. The observed improvement may nevertheless indicate a potential contribution of SOD-mediated antioxidant protection to the hepatoprotective Effects of EAFAB and silymarin.

Despite the increase of antioxidant activity, the marked reduction in malondialdehyde (MDA) following EAFAB treatment provides stronger evidence of attenuation of oxidative membrane damage. MDA is a commonly used indicator of lipid peroxidation and reflects oxidative degradation of polyunsaturated fatty acids within cellular membranes. The increased MDA associated with CCl₄ administration is therefore compatible with enhanced free-radical mediated lipid peroxidation (Ayala *et al.*, 2014). The ability of EAFAB to significantly reduce MDA suggests that the fraction may interfere with the propagation of lipid peroxidation and thereby preserve cellular membrane integrity. Similar antioxidant and lipid-peroxidation limiting Effects have been demonstrated with hepatoprotective agents in CCl₄ models. Silymarin, for example, has been shown to suppress CCl₄-associated lipid peroxidation through antioxidant and membrane-protective mechanisms. (Baradaran *et al.*, 2019)

The antioxidant response observed with EAFAB can be linked to the phytochemical composition of *A. boonei* (Okoye and Okoye, 2016). Previous phytochemical investigations have identified phenolic compounds, flavonoids, alkaloids, tannins and other bioactive constituents in *A. boonei* extracts and fractions (Mollica *et al.*, 2022). In particular, compounds such as caffeic acid, chlorogenic acid, ferulic acid, quercetin derivatives and other phenolic constituents have been identified in different preparations of the plant and may contribute to its antioxidant and anti-inflammatory activities (Mollica *et al.*, 2022). Hence, the phenolic contents in its ethyl-acetate fraction as reported by Mollica *et al.*, 2016, justifies the antioxidant Effects demonstrated in this study.

The alterations observed in the hepatic aminotransferases further support the protective activity of EAFAB. ALT, AST and ALP are intracellular enzymes whose increased release into the circulation is commonly associated with hepatocellular membrane disruption and hepatic injury (Robles-Díaz *et al.*, 2016). The marked increase associated with CCl₄ exposure and the subsequent amelioration following EAFAB treatment suggest that the fraction reduced the extent of hepatocellular damage. The more pronounced Effects observed at the higher EAFAB doses are particularly suggestive of a dose-responsive hepatoprotective action. This finding agrees with previous reports demonstrating hepatoprotective activity of *A. boonei* extract. A recent study of *A. boonei* stem bark reported restoration of liver function indices and antioxidant enzymes in an isoniazid-induced hepatotoxicity model, with the ethyl-acetate fraction demonstrating notable protective activity. (Adesina *et al.*, 2024). In addition, Okpashi *et al.* (2022) reported that *A. boonei* stem bark extracts influenced liver marker enzymes in CCl₄ -treated rats, supporting the potential of the plant as a hepatoprotective agent in this experimental model. Taken together, these findings suggest that the hepatoprotective activity of *A. boonei* is not restricted to a single hepatotoxicant and may involve broader antioxidant and cytoprotective properties.

The improvement in AST, ALP and ALT following EAFAB treatment therefore suggests attenuation of the systemic biochemical consequences associated with CCl₄ toxicity. The greater response observed with the higher EAFAB doses, together with the response produced by silymarin, further supports the potential of the fraction to preserve hepatocellular integrity.

The antioxidant findings are particularly important when considered alongside the aminotransferase responses. CCl₄ toxicity involves a sequence in which reactive metabolites initiate oxidative damage, resulting in lipid peroxidation and cellular membrane disruption, followed by leakage of intracellular enzymes (Manibusan *et al.*, 2007; Fareed *et al.*, 2024). Consequently, the reduction in MDA observed with EAFAB treatment, together with the improvement in ALT, ALP and AST, provides a

biologically coherent pattern suggesting that attenuation of oxidative damage may contribute to the preservation of hepatocellular integrity. This interpretation is consistent with studies in which antioxidant interventions reduced CCl₄ -induced lipid peroxidation and simultaneously improved biochemical indicators of hepatic injury (Said *et al.*, 2022).

The comparatively strong response produced by the 400 mg/kg EAFAB treatment is also noteworthy. The highest dose produced the greatest improvement in total protein and demonstrated particularly strong suppression of lipid peroxidation, while the higher EAFAB doses were associated with more pronounced improvement in the aminotransferase profile. This pattern may indicate that increasing the amount of the active constituents available to counteract oxidative injury enhances the protective response. On the other hand, Silymarin served as an appropriate reference treatment because of its established hepatoprotective and antioxidant properties. Previous experimental work has shown that silymarin can protect against CCl₄ -induced hepatic injury through mechanisms involving inhibition of lipid peroxidation, antioxidant activity and preservation of cellular membranes. (Lettéron *et al.*, 1990; Baradaran *et al.*, 2019). The response observed with silymarin in the present study provides a useful benchmark against which the activity of EAFAB can be considered. Notably, the pattern of improvement produced by EAFAB across several parameters suggests that the fraction possesses meaningful hepatoprotective potential, although the magnitude and statistical consistency of its Effects varied according to the biochemical parameter and dose.

Histological findings further support the hepatotoxic Effects of CCl₄, as evidenced by dilated sinusoids, disorganized hepatic cords, and disruption of the reticulin network. Treatment with EAFAB progressively improved these alterations, with the 400 mg/kg dose producing the most pronounced restoration of hepatic architecture, comparable to silymarin.

This restorative Effects is consistent with previous reports of the hepatoprotective activity of *A. boonei* preparations against chemically induced hepatic injury, which may be related to their antioxidant and membrane-protective properties (Okpashi *et al.*, 2022; Adesina *et al.*, 2025).

The biochemical findings were consistent with the observed histological alterations, as CCl₄ induced changes in liver enzyme levels reflected hepatocellular injury, which was further confirmed by disrupted hepatic architecture.

Conversely, the improvement in biochemical parameters following EAFAB treatment corresponded with the restoration of hepatic histoarchitecture. This parallel improvement suggests that the hepatoprotective Effects

of EAFAB was associated with both functional and structural preservation of the liver.

The hepatoprotective activity observed in the present study may therefore result from multiple complementary actions of EAFAB, including attenuation of lipid peroxidation, preservation of hepatocellular membrane integrity, improvement of hepatic protein status and modulation of endogenous antioxidant defense. The phytochemical diversity previously reported in *A. boonei* supports the possibility that several constituents may act synergistically rather than through a single molecular pathway.

CONCLUSION

it can be concluded from this study that EAFAB demonstrated promising hepatoprotective activity against CCl₄-induced hepatic injury, particularly through amelioration of hepatic injury from CCl₄, attenuation of lipid peroxidation and improvement of biochemical and histological alterations.

RECOMMENDATION

The present study did not directly measure inflammatory mediators, CYP2E1 activity, glutathione status, catalase or specific phytochemical constituents of EAFAB. Therefore, these mechanisms remain proposed explanations and should be investigated in future studies.

CONFLICT OF INTEREST: NO CONFLICT OF INTEREST DECLARED.

FUNDING: NO FUNDING WAS RECEIVED FOR THIS WORK.

CONTRIBUTION OF AUTHORS: ALL AUTHORS CONTRIBUTED TO THE WORK.

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