Phytochemical Profiling and Hepatoprotective Activity of *Caesalpinia bonduc* Leaf Extract Against Ampicillin-Induced Hepatic Damage

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Abstract

The present study was conducted to evaluate the extractive value, phytochemical composition, safety, and hepatoprotective efficacy of Caesalpinia bonduc ethanolic extract. The extractive value obtained using ethanol as solvent was 26.5%, yielding a dark brown residue, indicative of a rich presence of ethanol-soluble phytoconstituents. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, glycosides, carbohydrates, and proteins, while phenols and phytosterols were absent. Acute oral toxicity studies, carried out in accordance with OECD guideline 423, revealed no signs of toxicity or mortality up to 400 mg/kg, demonstrating a high margin of safety. Behavioral tests, including actophotometer, rotarod, and open field tests, showed that ampicillin-induced hepatotoxic rats exhibited marked impairments in locomotion and neuromuscular coordination, which were significantly reversed by C. bonduc treatment in a dose-dependent manner. Biochemical analyses demonstrated that ampicillin administration caused substantial elevations in liver enzyme markers (AST, ALT, ALP), indicative of hepatocellular damage. Co-treatment with C. bonduc extract, particularly at 400 mg/kg, significantly normalized these parameters, though less effectively than the standard hepatoprotective agent Liv.52. Histopathological evaluation further corroborated these findings, as C. bonduc treatment preserved liver architecture and reduced degenerative changes compared to the toxic group. These results collectively suggest that C. bonduc ethanolic extract possesses hepatoprotective potential, likely mediated through its antioxidant and anti-inflammatory phytoconstituents, with efficacy observed in both biochemical and histological outcomes.

Keywords: *Caesalpinia bonduc, extractive value, phytochemical screening, hepatoprotective activity, ampicillin-induced toxicity.*

1. Introduction

Drug-induced liver injury (DILI) is one of the most significant causes of acute liver failure and represents a major clinical challenge worldwide. Among antibiotics, ampicillin, a widely prescribed β-lactam antibiotic, has been associated with hepatotoxic effects including oxidative stress, mitochondrial dysfunction, and hepatocellular necrosis (1). The mechanisms underlying ampicillin-induced hepatotoxicity are multifactorial, but oxidative stress, free radical generation, and depletion of endogenous antioxidants are considered central events (2). Given the limitations and adverse effects of conventional hepatoprotective drugs, attention has shifted toward natural products and medicinal plants as safer alternatives for hepatoprotection.

Caesalpinia bonduc (L.) Roxb., a climbing shrub belonging to the family Fabaceae, is traditionally used in Ayurveda and folk medicine for the treatment of fever, inflammation, gastrointestinal disorders, and hepatic ailments (3, 4). Phytochemical investigations of *C.*

bonduc have revealed the presence of flavonoids, alkaloids, saponins, tannins, and terpenoids, which are known to contribute to antioxidant and hepatoprotective activities (5). Flavonoids and phenolic compounds in particular are potent free radical scavengers that enhance antioxidant defense mechanisms such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), thereby protecting hepatocytes from oxidative insult (6).

Several studies have demonstrated the hepatoprotective potential of medicinal plant extracts against antibiotic-induced liver injury. For instance, herbal extracts enriched with flavonoids and phenolic compounds have shown significant efficacy in lowering serum biomarkers of hepatic injury such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin, while improving histopathological features of liver tissue (7, 8). However, limited data exist on the protective role of *C. bonduc* against antibiotic-triggered hepatic dysfunction.

Therefore, the present study aims to evaluate the phytochemical profile and hepatoprotective activity of *Caesalpinia bonduc* leaf extract in a rat model of ampicillin-induced hepatic damage. By integrating biochemical, antioxidant, and histopathological parameters, this study seeks to provide scientific validation for the traditional use of *C. bonduc* and establish its potential as a natural hepatoprotective agent.

2. Materials and Methods

2.1 Collection and Authentication of Plant Materials

The medicinal plant *Caesalpinia bonduc* leaves were collected from the hilly regions near Koria district, Chhattisgarh. The plant material was identified and authenticated by the Department of Botany, Guru Ghasidas Central University, Bilaspur, Chhattisgarh (Ref. No.: Bot/GGV/2025/137). The leaves were dried, powdered, and stored in airtight containers for further studies (9 – 11).

2.2 Preparation of Ethanolic extract of leaves of Caesalpinia bonduc

Leaves of *Caesalpinia bonduc* were carefully washed with tap water, shade-dried, and powdered. The powdered material was packed into a Soxhlet apparatus and extracted with ethanol (68–78 °C) for 24 hours. The obtained extract was concentrated and dried at room temperature, and the ethanolic leaf extract was used for further studies.

For large-scale extraction, about 300 g of powdered *Caesalpinia bonduc* leaves was extracted with 1 L of ethanol using a Soxhlet apparatus for 24 hours at 68–78 °C. The extract was then concentrated to one-fourth of its original volume by distillation, allowing recovery and reuse of the solvent for subsequent extraction (4, 5, 12).

2.3 Phytochemical testing of Ethanolic extract of leaves of Caesalpinia bonduc

The ethanolic extract of *Caesalpinia bonduc* leaves was subjected to preliminary phytochemical screening to detect the presence of different classes of bioactive compounds. Alkaloids were confirmed by Mayer's test (cream-white precipitate), Dragendorff's test (orange-red color), and Wagner's test (brown precipitate). Carbohydrates were indicated by a violet ring in Molisch's test, while glycosides were detected by Keller–Killiani test, producing a reddish-brown ring at the interface. Flavonoids were confirmed by the Shinoda test with the formation of a magenta color, and phytosterols were detected by Salkowski's test, showing red or golden-yellow coloration. Phenolic compounds were identified by ferric chloride test (bluish-black

coloration), and proteins were confirmed by the Biuret test with the appearance of a purplish-violet color. These results indicated that the extract contains diverse secondary metabolites responsible for its pharmacological activities (10, 11).

2.4 Animal Selection, Housing, and Preparation

Healthy Wistar albino rats (150–250 g) were used for the study. The animals were procured from a CPCSEA-registered supplier (Reg. No. 1275/PO/Re/S/09/CPCSEA) and housed in the animal facility of the School of Pharmacy, Chouksey Group of Colleges, Bilaspur, under standard laboratory conditions (22–25 °C, 12 h light/dark cycle). They were provided with a standard pellet diet and water ad libitum. The animals were acclimatized for 7 days prior to the commencement of the study and were randomly selected, marked for individual identification, and maintained in cages for at least 5 days before dosing to ensure adaptation to laboratory conditions. Care was taken to ensure the use of animals of appropriate size and age throughout the experimental protocol. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the School of Pharmacy, Bilaspur (13 – 15).

Table no. 2.4 Experimental Grouping of animals for present study

S. No.	Animal (either sex)	Weight	Treatment	Duration	Animal/ Groups
1	Wistar albino Rats	150-250g	Normal Saline (0.9%)	28 days	6
2	Wistar albino Rats	150-250g	Ampicillin 200mg/kg	1-10 days	6
3	Wistar albino Rats	150-250g	Ampicillin +CBE	11-28 days	6
4	Wistar albino Rats	150-250g	Ampicillin +CBE	11-28 days	6
5	Wistar albino Rats	150-250g	Ampicillin + Liv52 Polyherbal Syrup	11-28 days	6
	Total				30

2.5 Acute Oral Toxicity Study

The study was conducted in accordance with OECD guideline No. 423. Animals were divided into five groups, with six animals in each group. Group I served as the normal control, while Groups II, III, and IV received ampicillin (200 mg/kg body weight, intraperitoneally) for 11 consecutive days. Group II received only ampicillin, whereas Groups III and IV were treated with the plant extracts at two different dose levels. Drug treatment with extracts began five days prior to ampicillin administration and continued until day 15. After 48 hours of the final ampicillin dose, the animals were sacrificed under ether anesthesia. Blood samples were collected by the retro-orbital plexus method, and serum was separated for biochemical analysis. The livers were immediately excised; small portions were fixed in 10% formalin and preserved for histopathological examination (16 – 18).

Table no. 2.5 Experimental Grouping of animals for Acute Oral Toxicity Study

S. No.	NAME	DOSING (mg/kg)	ROUTE
1.	Normal Saline (0.9%)	2ml/kg	oral
2.	Ampicillin (Inducing Agent)	200mg/kg	Intraperitoneal
3.	Liv52 polyherbal (standard Drug)	1ml/kg	oral
4.	Caesalpina Bonduc (Test drug)	200mg/kg	oral
5.	*Caesalpinia bonduc* (Test drug)	400mg/kg	oral

2.6 Study of Behavioral Parameters

Behavioral parameters were assessed to evaluate the neuropharmacological effects of the treatment in experimental animals. Standard behavioral models, including Actophotometer for locomotor activity, RotaRod for motor coordination and muscle relaxation, and Open Field Test for exploratory behavior and anxiety-related responses, were employed. These models are widely accepted in neurobehavioral research to provide reliable insights into locomotion, motor performance, and emotional states of animals under different treatment conditions.

2.6.1 Actophotometer Test

The Actophotometer is a standard behavioral apparatus used to measure locomotor activity in rodents, which is often influenced by CNS-acting drugs. It consists of a chamber (30 × 30 × 30 cm) equipped with photoelectric cells and light beams arranged horizontally across the walls, with infrared filters to minimize external light effects. As the animal moves within the chamber, interruptions of the light beams are detected by photocells, and the activity counts are digitally recorded, providing an index of locomotor activity. Each rat was individually placed in the apparatus for 10 minutes after weighing and numbering, and their basal activity scores were recorded. Animals were first acclimatized to the apparatus for 10–30 minutes to reduce stress, after which locomotor activity was measured. The data were analyzed based on the total counts, representing horizontal and vertical movements, thereby reflecting overall motor activity and behavioral response to treatment (19 – 20).

2.6.2 Rota-Rod Test

The Rota-Rod test is a widely employed behavioral assay used to evaluate motor coordination, balance, muscle strength, and fatigue in rodents. The principle is based on the ability of animals to remain on a rotating rod for a specified period, where a shorter latency to fall reflects motor impairment, muscle weakness, or central nervous system dysfunction, while a prolonged retention time indicates improved neuromuscular function or therapeutic efficacy of test compounds. Rodents are initially trained to stay on the rotating rod (usually at constant or accelerating speeds), followed by testing after treatment, during which the latency to fall is recorded. This method is particularly useful in assessing motor deficits associated with neurodegeneration, drug-induced toxicity, and hepatotoxic conditions where impaired energy metabolism and muscular weakness are secondary consequences of liver dysfunction. Thus, the Rota-Rod serves as a sensitive indicator of drug effects on motor performance and coordination in preclinical studies (21 – 23).

2.6.3 Open Field Test (OFT)

The Open Field Test (OFT) is a widely used behavioural paradigm for assessing locomotor activity, anxiety-like behaviour, and exploratory tendencies in rodents. The test

involves placing animals, usually mice or rats, in a large, enclosed arena (square or circular), where their movements are recorded using sensors or video tracking systems. Key parameters include the total distance travelled, time spent in the central versus peripheral zones, and frequency of behaviours such as grooming or rearing. Greater activity in the center is interpreted as reduced anxiety, while peripheral preference reflects thigmotaxis, a sign of heightened anxiety. The OFT not only provides insight into exploratory behaviour and emotional responsiveness but has also been applied to study drug-induced alterations, such as ampicillin-induced hepatotoxicity, which may affect locomotor activity and overall behavioural responses. Data are typically analyzed by comparing locomotor indices and zone preference, with higher center activity indicating anxiolytic-like effects and decreased activity suggesting anxiety or motor impairment (24 – 25).

2.7 Biochemical Parameters

The administration of ampicillin (200 mg/kg, i.p. for 11 days) resulted in a significant elevation of serum biomarkers of hepatotoxicity, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), compared to the normal control group, confirming liver injury. Pretreatment with *Caesalpinia bonduc* ethanolic extract (CB, 200 and 400 mg/kg, orally) markedly attenuated these biochemical alterations, indicating restoration of hepatic integrity. The hepatoprotective potential of CB was found to be dose-dependent, with CB at 400 mg/kg showing a significantly greater protective effect than both CB at 200 mg/kg and the standard reference drug LIV-52 (1 mL/kg, orally). These findings suggest that the hepatoprotective activity of *C. bonduc* may be attributed to its phytoconstituents, such as flavonoids, alkaloids, and saponins, which possess strong antioxidant and free radical scavenging properties that stabilize cellular membranes and prevent enzyme leakage (26 – 28).

2.8 Histopathological Analysis

The liver was carefully excised without causing mechanical injury after opening the abdominal cavity. The collected tissue was rinsed with ice-cold normal saline to remove blood and debris, and subsequently fixed in 10% neutral buffered formalin. Paraffin-embedded sections of 5 µm thickness were prepared using a microtome and processed through graded alcohol and xylene series. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological alterations, including hepatocellular degeneration, necrosis, and other morphological changes (29 – 31).

3. Results and Discussion

3.1 Extractive Value

The extractive value of *Caesalpinia bonduc* seeds was determined using ethanol as the solvent. The ethanolic extract yielded a dark brown residue with an extractive value of 26.5% (Table 3.1).

Table no. 3.1 Extractive Value

S. No.	DRUG	SOLVENT	COLOUR	% Extractive
1.	Caesalpinia bonduc	Ethanol	Dark brown	26.5%

Extractive values serve as important parameters to assess the quality, purity, and chemical constituents of crude drugs. The percentage yield of 26.5% for the ethanolic extract of *Caesalpinia bonduc* indicates a substantial presence of ethanol-soluble phytoconstituents such as alkaloids, flavonoids, glycosides, tannins, and phenolic compounds.

The dark brown coloration of the extract suggests the presence of phenolic compounds and tannins, which are commonly soluble in ethanol. A higher extractive value generally indicates the richness of phytoconstituents, which can be correlated to the pharmacological potential of the plant.

Comparable studies on *Caesalpinia bonduc* have reported significant extractive yields with polar solvents like ethanol and methanol, attributed to their efficiency in extracting bioactive secondary metabolites. The obtained extractive value falls within the expected range, supporting the suitability of ethanol for further phytochemical and pharmacological evaluations.

3.2 Phytochemical Screening

The phytochemical screening of *Caesalpinia bonduc* ethanolic extract revealed the presence of major secondary metabolites including alkaloids (Mayer's and Dragendorff's tests positive), glycosides (Keller-Killiani test positive), flavonoids (Shinoda test positive), carbohydrates (Molisch's test positive), and proteins (Biuret test positive). However, phenols (Ferric chloride test) and phytosterols (Salkowski's test) were absent in the extract.

Table no. 3.2 Phytochemical Screening

S. No.	TEST	INFERENCES
1.	ALKALOIDS	+
	Mayers Test	+
	Wagner's Test	-
	Dragendroffs Tes	+
2.	GLYCOSIDE	+
	Killer killani Test	+
3.	FLAVONOIDES	+
	Shinoda Test	+
4.	CARBOHYDRATES	+
	Molisch Test	+
5.	PROTIENS	+
	Biuret Test	+
6.	PHENOLS	-
	Ferric chloride Test	-
7.	PHYTOSTEROLS	-
	Salkowski's Test	-

(+) Indicate presence while (-) stand for absence.

The ethanolic extract of *Caesalpinia bonduc* was found to be rich in diverse phytoconstituents. The presence of alkaloids suggests potential pharmacological properties, as these compounds are often associated with analgesic, antimicrobial, and anti-inflammatory effects. The detection of glycosides indicates possible cardioprotective

and antioxidant activity. Flavonoids, which were strongly positive, are well-known for their neuroprotective, antioxidant, and anti-inflammatory roles, making them important bioactive constituents. The presence of carbohydrates and proteins reflects the nutritive value of the extract, which may also contribute to its biological activities. On the other hand, the absence of phenols and phytosterols suggests that ethanol may not be an efficient solvent for extracting these compounds from C. bonduc.

Overall, the phytochemical profile of the ethanolic extract highlights the therapeutic potential of C. bonduc, aligning with its traditional use in various ailments. Particularly, the abundance of flavonoids and alkaloids provides a scientific basis for its antioxidant and anti-inflammatory applications, which are crucial in managing chronic and degenerative diseases.

3.3 Acute Oral Toxicity Study

Administration of *Caesalpinia bonduc* ethanolic extract at doses of 100, 200, and 400 mg/kg in rats produced no mortality or clinical signs of toxicity during the initial 24 hours and throughout the 14-day observation period, as per OECD guideline 423. No significant changes in body weight, behavioral patterns, or macroscopic abnormalities in vital organs were observed at the end of the study.

The findings indicate that the ethanolic extract of *C. bonduc* is safe up to 400 mg/kg by oral route, suggesting a high margin of safety. The absence of toxic symptoms such as tremors, convulsions, diarrhea, or lethargy confirms the non-toxic nature of the extract. Hence, doses of 100 and 200 mg/kg were selected for further pharmacological evaluation in experimental models. These results are consistent with previous reports highlighting the safety of *C. bonduc* extracts in rodents.

3.4 Assessment of Behavioral Outcomes

Various behavioral tests related to particular motor functions were also conducted because AD is a motor condition characterized by rigidity, resting tremor, postural instabilities, and slowness of movement. Weekly behavioral tests, as detailed below, were administered to the animals in various groups.

3.3.1 Actophotometer Test

Table 3.3.1 shows the effect of *Caesalpinia bonduc* leaf extract on locomotor activity in mice using an actophotometer. The normal group exhibited significantly higher locomotor activity (348 ± 7.5), while ampicillin treatment drastically reduced the activity (210 ± 6.9; ***$p < 0.001$ vs. normal). Co-treatment with *Caesalpinia bonduc* extract at 200 and 400 mg/kg significantly restored locomotor activity (260 ± 6.4 and 295 ± 5.8, respectively; ***$p < 0.001$ vs. toxic), in a dose-dependent manner. The standard (Liv52 syrup) group also showed significant recovery (312 ± 6.0; ***$p < 0.001$ vs. toxic).

Ampicillin-induced toxicity markedly impaired locomotor performance, suggesting central nervous system depression and reduced neuromuscular coordination. Co-administration of *Caesalpinia bonduc* extract significantly improved locomotor activity, with the higher dose (400 mg/kg) showing near-comparable efficacy to the standard Liv52. This suggests that the neuroprotective and adaptogenic phytoconstituents of *C. bonduc* (such as flavonoids and diterpenoids) may counteract ampicillin-induced toxicity and enhance motor activity..

Table no. 3.3.1 Effect of *Caesalpinia bonduc* leaf Extract on locometer activity score in Actophometer

Animal groups (n=6)	Group/ Treatment	Mean ± SEM
1.	Normal	348 ± 7.5
2.	Ampicillin	210 ± 6.9***a
3.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p + 200mg/kg p.o)	260 ± 6.4***b
4.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p + 400mg/kg p.o)	295 ± 5.8***b
5.	Ampicillin + Liv52 syrup (standard)	312 ± 6.0***b

Superscripts: a – comparison with normal control; b – comparison with toxic control. Statistical analysis was performed using one-way ANOVA followed by Tukey–Kramer's post hoc test. ns = $p > 0.05$; $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***). Comparisons: (a) Group I (Normal) vs. Group II (Toxic); (b) Group II (Toxic) vs. Groups III, IV, V.

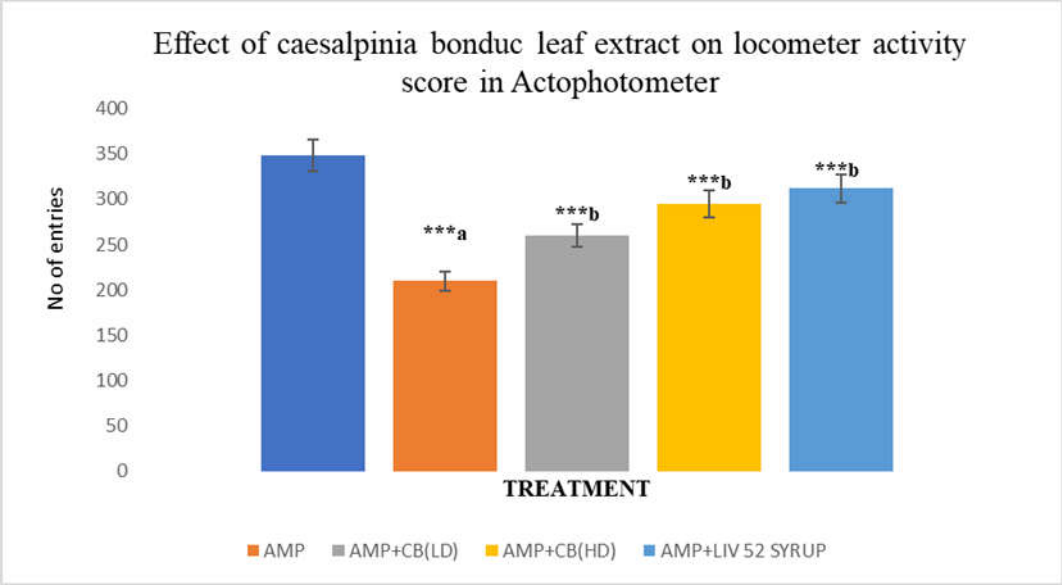


Fig. no. 3.3.1 Effect of *Caesalpinia bonduc* leaf Extract on locometer activity score in Actophometer

3.3.2 Rota-Rod Test

Ampicillin administration significantly reduced the retention time of rats on the Rotarod compared to the normal group, indicating impaired motor coordination and muscle weakness due to hepatotoxicity ($p < 0.001$). Treatment with *Caesalpinia bonduc* leaf extract at both 200 mg/kg and 400 mg/kg doses significantly improved Rotarod performance compared to the toxic control, suggesting a protective effect against Ampicillin-induced motor deficits. Among the treated groups, the higher dose of the extract (400 mg/kg) demonstrated a greater improvement, though slightly lower than the standard Liv52 group. These findings suggest that *C. bonduc* possesses dose-dependent hepatoprotective and neuromuscular restorative effects, consistent with its role in improving coordination and reducing fatigue associated with hepatic injury.

Table no. 3.3.2 Effect of *Caesalpinia bonduc* treatment on Rotarod Activity in Ampicillin Treated Rats

Animal group (n=6)	Group/ Treatment	Mean ± SEM
1.	Normal	184.5 ± 1.93
2.	Ampicillin	86.87±1.6***a
3.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p + 200mg/kg p.o)	159.9±6.4***b
4.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p + 400mg/kg p.o)	139.1±2.6***b
5.	Ampicillin+Liv52 syrup (standard)	147.26±1.3***b

Values are expressed as Mean ± SEM (n = 6). a = statistically compared with the normal group. b = statistically compared with the toxic control group. Analysis was performed using one-way ANOVA followed by Tukey–Kramer's post hoc test. ns > 0.05; *p < 0.05, **p < 0.01, ***p < 0.001. Comparisons: (a) Group I (Normal) vs. Group II (Toxic); (b) Group II (Toxic) vs. Groups III, IV, and V.

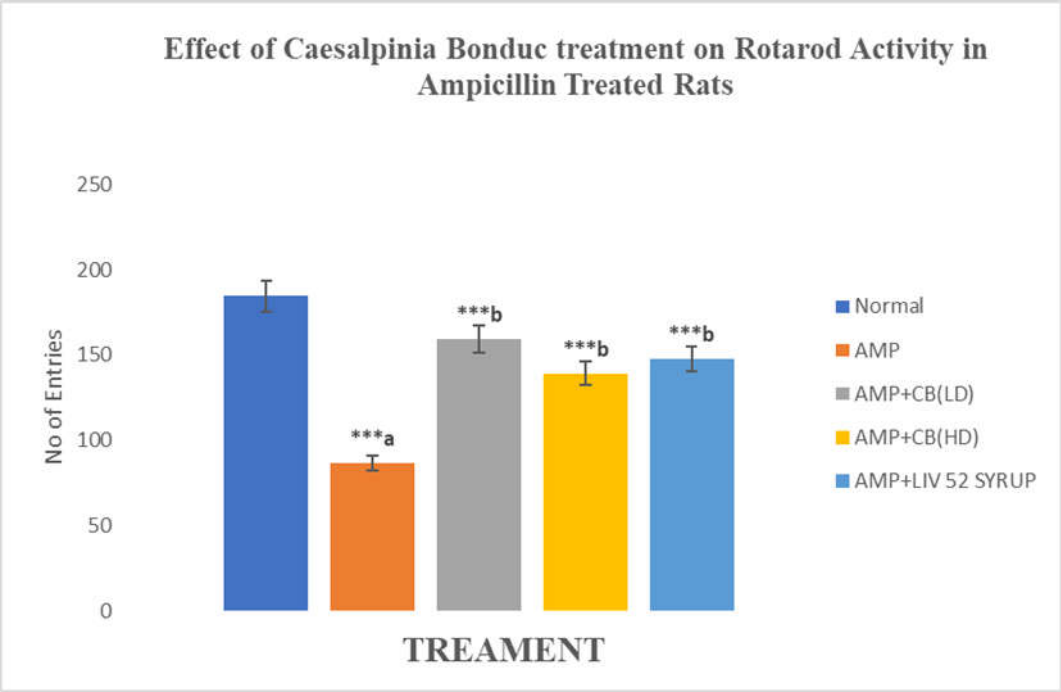


Fig. No. 3.3.2 Effect of *Caesalpinia bonduc* treatment on Rotarod Activity in Ampicillin Treated Rats

3.3.3 Open Field Test (OFT)

Ampicillin administration (Group II) significantly reduced the number of crossings (48 ± 2.8) and rearing (14 ± 1.2) compared to the normal control group (82 ± 3.5 and 25 ± 1.4, respectively; ***p < 0.001), indicating impaired locomotor and exploratory behavior due to hepatotoxicity. Pretreatment with *Caesalpinia bonduc* extract at both 200 mg/kg and 400 mg/kg (Groups III and IV) significantly improved the number of crossings (60 ± 3.0 and 72 ± 2.9) and rearing (17 ± 1.3 and 21 ± 1.1) compared to the toxic group, demonstrating a dose-dependent protective effect. The higher dose (400 mg/kg) showed results comparable to the standard Liv52 syrup group (crossings: 76 ± 3.2; rearing: 23 ± 1.2), suggesting that *Caesalpinia bonduc* effectively restored locomotor and exploratory activities in ampicillin-induced hepatotoxic rats.

Table no. 3.3.3 Effect of *Caesalpinia bonduc* treatment on Open field Test Activity In Ampicillin Treated Rats

Values are expressed as Mean ± SEM (n = 6). a = comparison with normal control (Group

Animal group (n=6)	Group/ Treatment	Number of Crossings Mean ± SEM	Number of Rearing Mean ± SEM
1.	Normal	82 ± 3.5	25 ±1.4
2.	Ampicillin	48 ± 2.8***a	14±1.2***a
3.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p+ 200mg/kg p.o)	60 ± 3.0***b	17 ±1.3***b
4.	Ampicillin + Caesalpinia Bonduc (200mg/kg i.p+ 400mg/kg p.o)	72 ± 2.9***b	21 ±1.1***b
5.	Ampicillin+Liv52Syrup(standard)	76 ± 3.2***b	23 ±1.2***b

I) b = comparison with toxic control (Group II) Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer's post hoc test. ns > 0.05 (non-significant), *p < 0.05, **p < 0.01, ***p < 0.001. Comparisons: (a) Group I (Normal) vs Group II (Toxic); (b) Group II (Toxic) vs Groups III, IV, V.

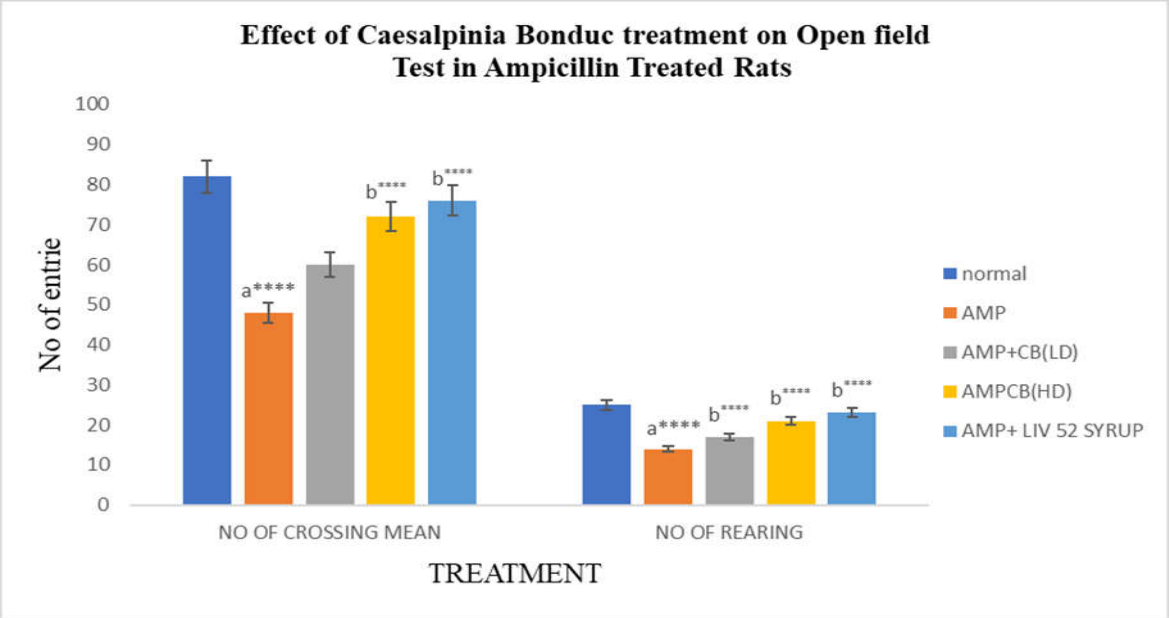


Fig. no. 3.3.3 Effect of *Caesalpinia bonduc* treatment on Open field Test in AmpicillinTreated

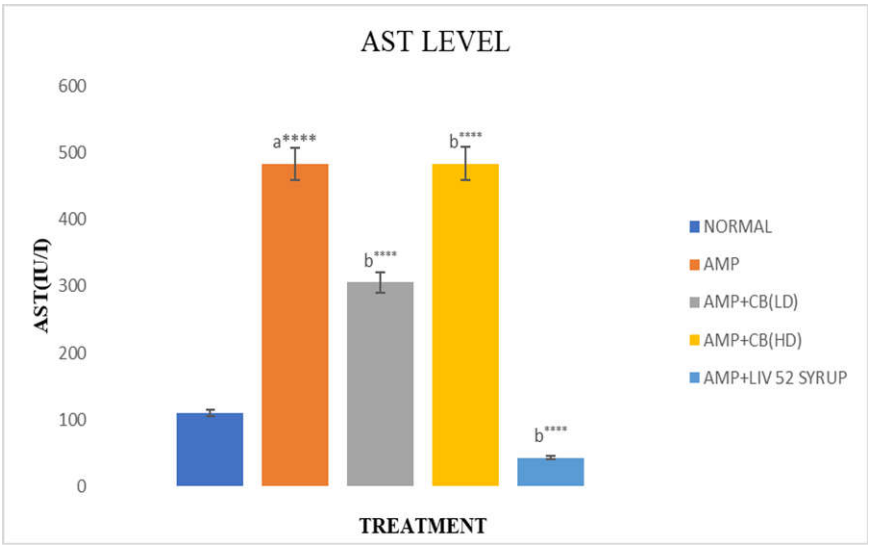
3.4 Evaluation of Biochemical Parameters

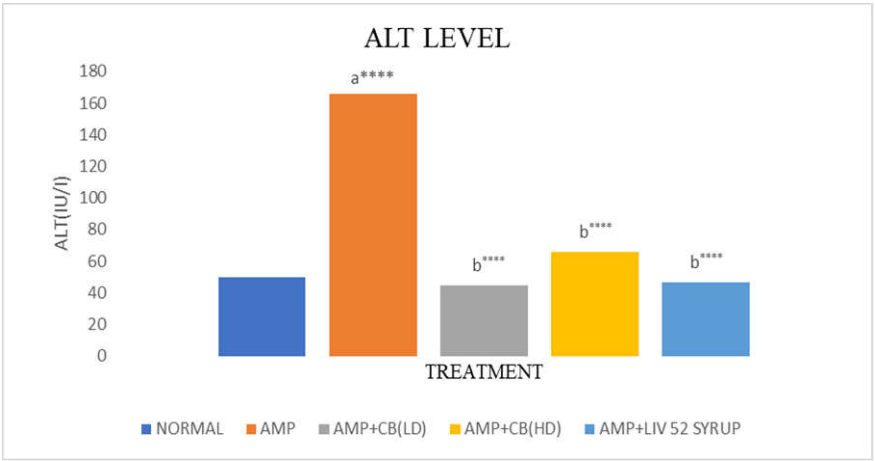
Ampicillin (200 mg/kg) administration produced a marked elevation in serum AST, ALT, and ALP levels compared to the normal group ($p < 0.0001$), confirming hepatotoxicity. Pretreatment with *Caesalpinia bonduc* ethanolic extract (400 mg/kg) significantly reduced these elevated liver enzyme levels ($p < 0.0001$), indicating hepatoprotective activity. The higher dose of CB (400 mg/kg) showed better restoration of AST, ALT, and ALP than the lower dose, though the effect was still less pronounced compared to the standard drug Liv.52, which exhibited the strongest hepatoprotective effect by normalizing biochemical parameters. These findings suggest that CB exerts dose-dependent hepatoprotection against AMP-induced hepatic injury, likely due to its antioxidant and hepatorestorative phytoconstituents.

Table no. 3.4 Effect of CB treatment on different biological parameters in AMP induced Hepatotoxicity in rats

Group	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
I	Normal	110.12 ± 0.021	49.35 ± 0.03	9.54 ± 0.04
II	AMP (200mg/kg)	483.82 ± 4.065 a****	166.14 ± 0.02 a****	4.24 ± 0.03 a**
III	AMP (200mg/kg) + CB (400mg/kg)	305.64 ± 0.03 b****	44.69 ± 0.018 b****	6.65 ±0.01 b****
IV	AMP (400mg/kg) +CB (400mg/kg)	483.78± 0.11b****	98.99±0.82 b****	8.01 ± 0.8 b**
V	AMP (200mg/kg) + Liv 52syrup(1ml/kg)	43.24 ± 0.007 a****	6.42 ±0.02 b****	3.94± 0.01 b****

Results were expressed as Mean ± S.E.M., n = 6. Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, ns (not significant) when p > 0.05. Comparisons: a: statistically compared with Normal (Group I vs. Group II – Toxic) b: statistically compared with AMP (Group II vs. Group III) c: statistically compared with AMP + LIV 52 (Group II vs. Group IV) d: statistically compared with AMP + CB (Group II vs. Group V).





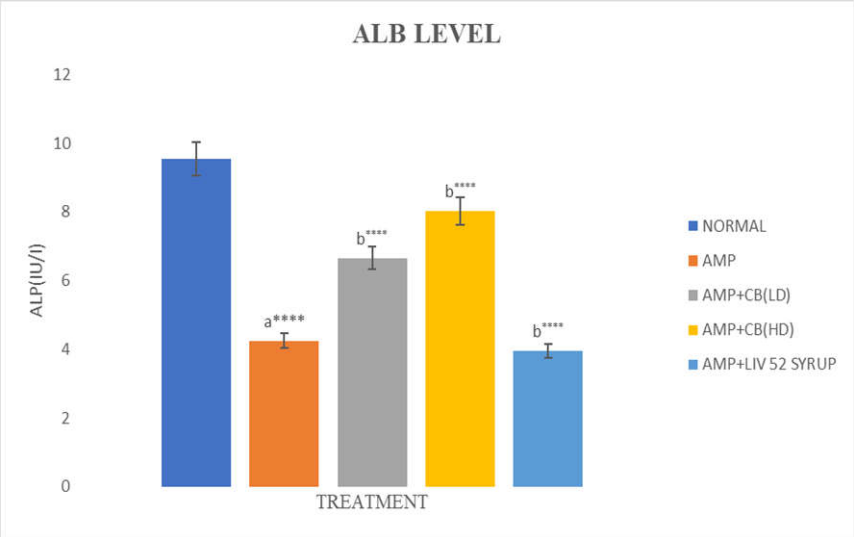


Fig. no. 3.4 Effect of CB treatment on different biological parameters in AMP induced Hepatotoxicity in rats

3.5 Histopathological Analysis

Histopathological analysis was performed on liver tissues stained with Hematoxylin and Eosin (H&E, 400X). The sections revealed the structural differences among groups, including the normal control, ampicillin-treated (toxic) group, and treatment groups administered with *C. bonduc* at doses of 200 mg and 400 mg along with ampicillin. The normal control group displayed well-preserved hepatic architecture with clear cellular morphology, while the ampicillin-treated group showed marked hepatic damage characterized by degeneration and disruption of liver architecture. In contrast, the groups treated with *C. bonduc* in combination with ampicillin exhibited significant improvement in hepatic histology, with the 400 mg treatment group showing nearly restored hepatic structure compared to the 200 mg group, indicating a dose-dependent hepatoprotective effect.

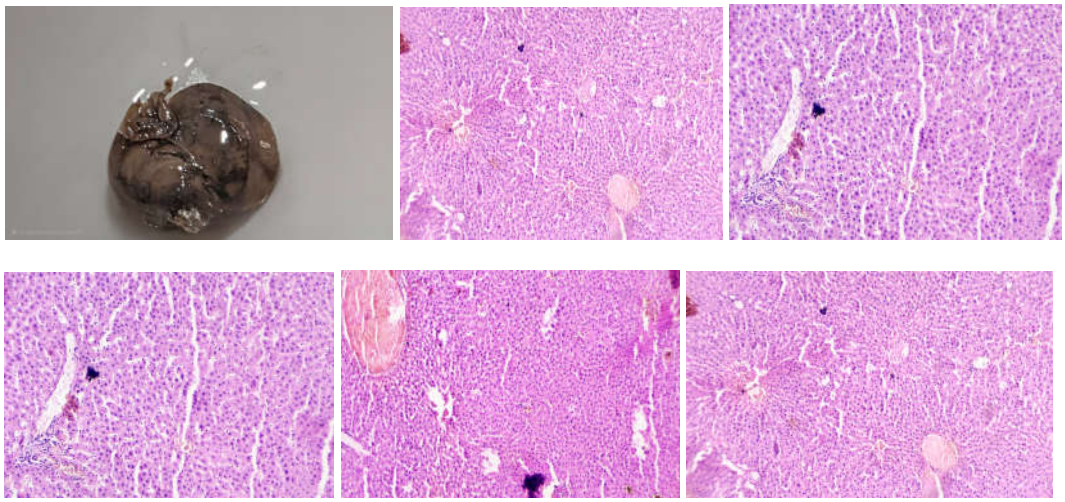


Fig. no. 3.5 Sections stained with Hematoxylin and Eosin (H&E 400X) displaying the Liver Tissue of Rats treated with Normal, Ampicillin Groups + *C. bonduc* 200mg, Ampicillin + *C. bonduc* 400mg

4. Conclusion

The findings of this study highlight the therapeutic potential of *Caesalpinia bonduc* ethanolic extract against ampicillin-induced hepatotoxicity. The extract demonstrated a high extractive value and a diverse phytochemical profile rich in alkaloids, flavonoids, and glycosides, which may underlie its pharmacological properties. Acute oral toxicity assessment confirmed its safety up to 400 mg/kg, supporting its use in experimental models. Behavioral studies revealed improvements in locomotor, exploratory, and motor coordination activities, while biochemical analysis demonstrated significant restoration of liver enzyme levels. Histopathological evaluation further validated its hepatoprotective effect, showing preserved hepatic architecture, particularly at higher doses. Although the efficacy of *C. bonduc* was slightly lower than that of the standard drug Liv.52, its dose-dependent protective effects indicate promising therapeutic potential. Overall, these results provide scientific evidence supporting the traditional use of *C. bonduc* in liver disorders and encourage further investigations into its bioactive constituents and mechanisms of action.

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