Original Article

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Fractionated *Cleome gynandra* Extract Attenuates Acetaminophen-Induced Hepatic Dysfunction in Wistar Rats

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Abstract

This study examined the protective effect of methanol leaf extract of *Cleome gynandra* against acetaminophen-induced liver damage in Wistar rats. Forty male rats were divided into eight groups and administered the extract or its fractions orally for eight days. Liver toxicity was induced with the use of acetaminophen on day nine. Serum levels of liver enzymes were determined and microscopic examination of the liver tissues conducted. Enzyme activity was significantly increased by acetaminophen compared with controls. Pre-exposure with the ethyl acetate and n-butanol fractions at the dose of 300mg/kg body weight reduced the levels of enzymes and maintained liver morphology in the normal state. Surprisingly, the ethyl acetate fraction (95.19%) and the n-butanol fraction (94.80%) afforded the highest percentage of hepatoprotection, comparable to the reference standard, silymarin (77.91%). Histopathological analysis revealed that EAF 300 and NBF 300 preserved normal hepatic architecture. The hepatoprotective effects of the ethyl acetate and n-butanol fractions were statistically significant ($p < 0.05$) compared with both the acetaminophen group and the silymarin group. These findings show that C. gynandra has the potential to serve as natural remedy for liver injury.

Keywords: Acetaminophen, Hepatotoxicity; Hepatoprotection; *Cleome gynandra*; Liver enzymes

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1. Introduction

The most common cause of acute liver failure (ALF) in the industrialised world is drug-induced liver injury (DILI), which is liver damage brought on by medications and xenobiotics (1). DILI has emerged as a public health menace accounting for over 50% cases of ALF, with antimicrobials, statins, non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen being the most common culprit agents (2). There is a dearth of information on clinical instances of drug/herb-induced liver damage in some underdeveloped nations, including Nigeria, because the majority of cases are either not reported to medical professionals or hospital reports are not made public (3). Conservative estimates indicate close to a thousand marketed drugs have hepatotoxic potential either directly due to innate toxicity or idiosyncratic patient reactions (4). This has led to huge financial repercussions for the pharmaceutical industry while severely limiting therapy choices for associated medical conditions.

The over-the-counter (OTC) analgesics, acetaminophen (paracetamol) overdose is a global emergency resulting in profound morbidity and considerable mortality attributed to severe liver necrosis (4). Acetaminophen was found to be associated with a threefold higher risk of liver transplantation registra-

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tion due to acute liver injury at non-overdose levels, escalating to a sevenfold higher risk at overdose levels of exposure (5). Acetaminophen (N-acetyl-para-aminophenol) or APAP is ubiquitously employed as an accessible antipyretic and analgesic remedy, but acute overdose instigates substantial mortality from fulminant liver disappointment (6). APAP largely undergoes safe conjugative metabolism via sulfation and glucuronidation pathways which get saturated at supratherapeutic doses, consequently surplus APAP gets bioactivated in liver by CYP450 mixed function oxidases especially CYP2E1 into N-acetyl-p-benzoquinone imine (NAPQI), an electrophilic metabolite toxic to tissues (7).

Cleome gynandra L., also known as *Gynandropsis gynandra*, belongs to the Capparaceae family and occurs widely as a tropical medicinal food plant (8). It is known by many common names including Shona cabbage, African cabbage, spiderwisp, cat's whiskers and stinkweed. This annual wildflower is native to Africa but has become widespread in many tropical and sub-tropical parts of the world (9). The leaves and flowers are both edible, with the leaves having a strong bitter, sometimes peppery flavour similar to mustard greens. It is considered a rich natural source of essential nutrients such as vitamins, minerals and proteins (8). Ethnomedical texts recognize use of *C. gynandra* against liver diseases in folk practices of Asia and Africa. It is known to contain a wide range of phytochemicals, including flavonoids (quercetin derivatives), phenolic acids, alkaloids, glucosinolates and terpenoids. These constituents have been previously shown to possess antioxidant, anti-inflammatory and cytoprotective activities, which are relevant in preventing oxidative hepatic injury. Earlier studies have reported strong free-radical scavenging activity of *C. gynandra* extracts, suggesting the presence of bioactive compounds capable of modulating redox imbalance during toxic liver injury (10). However, there is a dearth of focused research assessing its bioactivity against acetaminophen-induced liver injury, which represents a significant clinical burden.

Therefore, this study aimed to investigate the hepatoprotective potential of the methanolic extract and solvent fractions of *C. gynandra* against acetaminophen-induced liver injury in Wistar rats. We hypothesized that the ethyl acetate and n-butanol fractions, being richer in antioxidant phytoconstituents, would demonstrate superior protective effects compared to the crude extract and might show efficacy comparable to silymarin.

2. Materials and Methods

2.1. Plant Material and Extraction

Fresh leaves of *C. gynandra* were collected from wild sources (10°16'29.348"N, 13°18'52.293"E) Barama, Mubi North, Adamawa State in a paper bag. The plant was identified by an expert in the Department of Plant Science, Modibbo Adama University, Yola, Adamawa State. The collected leaves were cleaned, shade-dried, powdered and stored in an airtight container.

2.2. Preparation of Extract and Fractions

Air-dried leaves of *C. gynandra* were mechanically powdered and 200 g of the powder was subjected to cold maceration extraction using 1.5 L of methanol for 72 hours with intermittent shaking (11). Methanol was selected as the extraction solvent because of its high efficiency in extracting polar and semi-polar bioactive constituents such as polyphenols, flavonoids and phenolic acids. The crude extract was then filtered sequentially through muslin cloth and Whatman No. 1 filter paper and concentrated under reduced pressure in a rotary evaporator at 40 °C. Subsequently, 40 g of the crude extract was dissolved in 200 mL of distilled water and successively partitioned with 150 mL each of ethyl acetate and n-butanol. After vigorous shaking and phase separation, the fractions were concentrated under vacuum at 40 °C and stored at 4 °C for further analysis (12).

2.3. Drugs and Chemicals

Acetaminophen (from McNeil Product Ltd, SL6, UK) was used to induce hepatotoxicity by a single intraperitoneal injection dose

of 1 g/kg body weight (13). Methanol, Ethyl acetate, Butanol (Sigma-Aldrich, USA); Test kits for total protein, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, albumin, and bilirubin were sourced from Randox Laboratories Ltd. (UK).

2.4. Animals

In this study, forty (40) male Wistar rats, weighing 120±10 g were procured from National Veterinary Research Institute, Vom, Plateau State. The animals were housed under standard room temperature and 12 hours of light/dark cycle and were fed with commercial pellet diet and water ad libitum for two weeks to acclimatise to the environment before the commencement of the study. The study protocol adhered to ethical guidelines for laboratory animal use, approved by Modibbo Adama University Animal Care Committee, Approval No. UAEC/YMAU/YL/0855.

2.5. Experimental Design

The rats were randomly divided into eight (8) groups, each containing five animals (n=5). The treatment regimen for each group is shown in Figure 1. The selected doses (100 and 300 mg/kg) were based on previous hepatoprotective studies on Cleome species, where similar dose ranges demonstrated safety and therapeutic efficacy. Pilot observations in our laboratory also indicated that these doses were well tolerated without signs of toxicity. All extracts and silymarin were administered orally once daily for eight days prior to acetaminophen exposure, indicating a prophylactic treatment design. The 8-day pretreatment period was chosen to allow adequate systemic accumulation of phytoconstituents before toxic APAP challenge, based on standard hepatoprotection protocols (25).

2.6. Sample Collection

On day 10, 24 hours post-acetaminophen intoxication, animals were anesthetized with diethyl ether and sacrificed by cervical dislocation following the method described by Parvez, Al-Dosari (14). Blood samples were then carefully collected via cardiac puncture in centrifuge tubes without anticoagulant, allowed to clot for 30 minutes at room temperature, and the serum was separated by centrifugation at 3000 rpm for 15 minutes. The liver tissue was excised and perfused with cold 0.15% Tris-KCl buffer (pH 7.4) to remove residual blood and clots. A 10% (w/v)

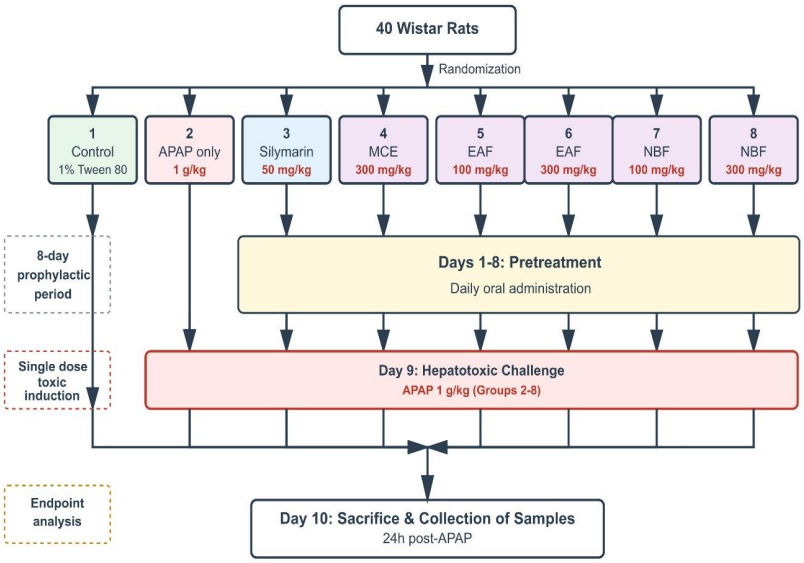

Figure 1. Experimental Design Showing Groupings, Treatment and Duration. MCE= Methanolic crude extract; EAF= Ethyl acetate fraction; NBF= n-Butanol fraction; APAP= Acetaminophen.

liver homogenate was subsequently prepared by homogenizing 2 g of liver tissue in 8 ml of the same buffer and stored at −80 °C, as detailed by Owumi, Akomolafe (15).

2.7. Assessment of Hepatoprotective Activity

Liver function markers were evaluated using standard methodologies: Aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured via oxaloacetate hydrazone formation (AST) and NADH consumption (ALT) at 546 nm and 340 nm, respectively, using methods by Reitman and Frankel (16) as modified by Schmidt (17). Gamma-glutamyl transferase (GGT) activity was determined kinetically using L-γ-glutamyl-3-carboxy-4-nitroanilide and glycylglycine, monitoring p-nitroaniline formation at 405 nm (18). Total protein (TP) concentration was quantified via the biuret reaction at 546 nm (19), while albumin (AL) levels were assessed using bromocresol green (BCG) binding at 578 nm (20). Serum bilirubin (total and direct) was measured via diazotized sulphanilic acid reaction in alkaline medium, with caffeine as an accelerator for total bilirubin, and absorbance read at 550 nm (21).

2.8. Histological Assessment of the Liver

The histopathological analysis of liver tissues was carried out as described by Choji, Ngokere (22). The liver from each rat was surgically extracted, rinsed with normal saline and preserved in 10% formalin.

Liver samples were fixed in 10% neutral buffered formalin for 24 hours. Following fixation, tissues were dehydrated through graded alcohol (50-90%), cleared in xylene, embedded in paraffin, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined for histopathological alterations.

2.9. Statistical Analysis

Data was evaluated using SPSS software (version 28.0 SPSS Inc. Chicago, IL, USA) using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. Data was expressed as mean ± SEM and P <0.05. Percentage hepatoprotection against acetaminophen-induced liver damage in biochemical markers was estimated using the formula (Equation 1):

$$\% \text{ Protection} = \frac{\text{Acetaminophen-Treatment}}{\text{Acetaminophen-Normal Control}} \times 100 \quad \text{(Eq. 1)}$$

3. Results

3.1. Effects of C. gynandra Extract on Liver Function Biomarkers

Liver enzyme biomarkers were significantly altered in response to acetaminophen-induced hepatotoxicity, with notable restorative effects observed following treatment with *Cleome gynandra* extract and its fractions (Figure 2). AST levels increased significantly ($p<0.05$) in the acetaminophen group (148.29±11.38 U/L) compared to the normal control (60.23±2.45 U/L). Treatment with silymarin resulted in a significant reduction ($p<0.05$) to 82.48±5.46 U/L, while the EAF 300 and NBF 300 groups showing greater reductions (77.52±7.41 U/L and 77.32±3.28 U/L, respectively, $p<0.05$). A similar trend was observed in ALT levels, which were significantly elevated (50.26±4.21 U/L, $p<0.05$) in the acetaminophen group compared to the normal control (22.72 ± 0.82 U/L). The silymarin group exhibited a significant reduction to 30.49±1.87 U/L, while the EAF 300 and NBF 300 groups showing further declines to 23.84±0.95 U/L and 17.11±0.82 U/L, respectively. Additionally, GGT levels were markedly elevated in the acetaminophen group (9.56±0.44 U/L) compared to the normal control (5.60±0.33 U/L), with all treatment groups demonstrating significant reductions ($p<0.05$).

In addition to enzyme markers, *Cleome gynandra* extract and its fractions modulated biochemical liver function indices (Figure 2). Acetaminophen significantly reduced TP levels ($p<0.05$) to 40.06±0.94 g/L

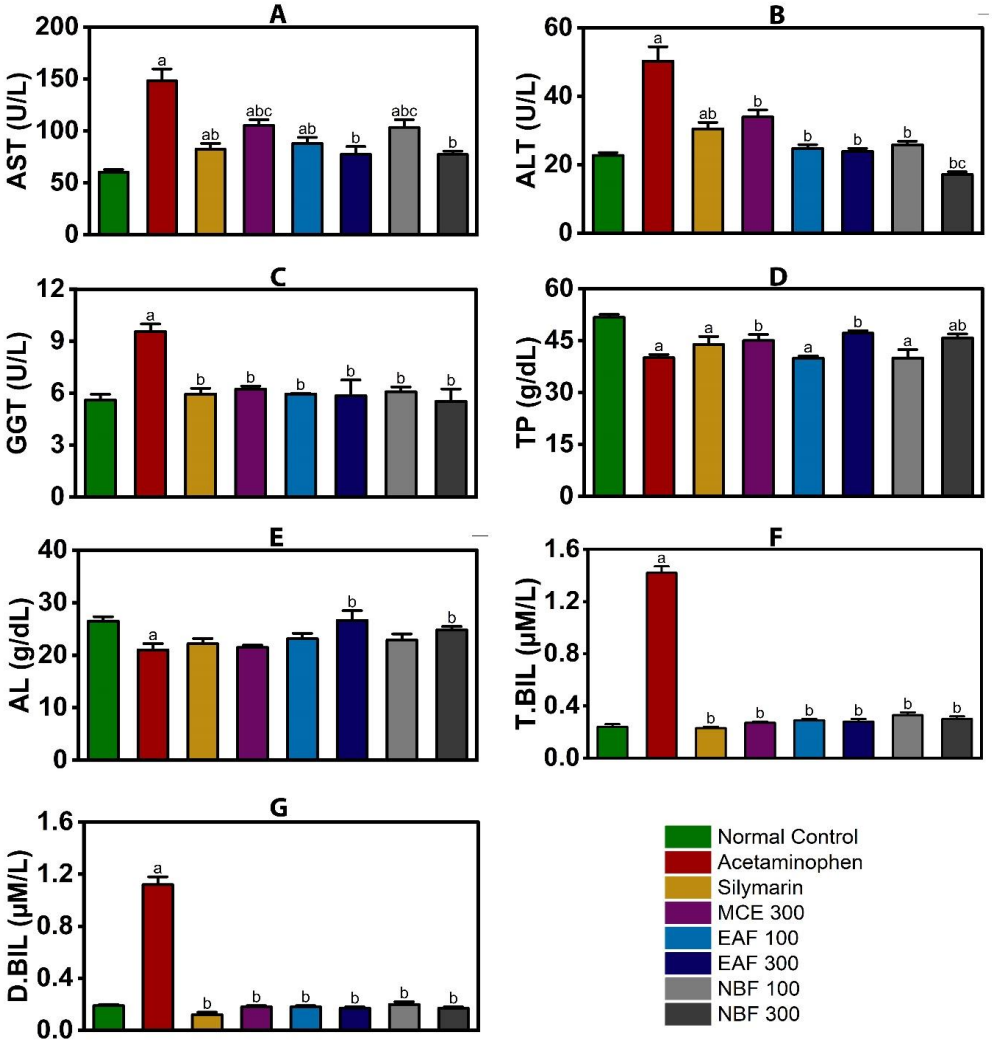

Figure 2. Effects of *C. gynandra* Extracts and Fractions on Biochemical Markers in Acetaminophen-Induced Liver Injury. All data expressed as mean ± SEM. Statistical analysis performed using one-way ANOVA followed by Tukey's test. a $p<0.05$ vs. normal control; b $p<0.05$ vs. acetaminophen-treated group; c $p<0.05$ vs. silymarin-treated group.

from 51.72±0.84 g/L in controls. Silymarin improved TP to 43.88±2.3 g/L, while MCE 300, EAF 300, and NBF 300 increased it further to 45.03±1.74 g/L, 45.03±1.74 g/L, and 45.82±1.10 g/L ($p<0.05$). Albumin levels decreased ($p<0.05$) from 26.50±0.83 g/L in controls to 20.99±1.21 g/L in the acetaminophen group, with EAF 300 and NBF 300 restoring levels to 26.63±1.84 g/L and 24.79±0.67 g/L, respectively. T.BIL increased ($p<0.05$) from 0.24±0.02 µM/L in controls to 1.42±0.05 µM/L, while D.BIL rose ($p<0.05$) from 0.19±0.01 µM/L to 1.12±0.06 µM/L. All treatments significantly reduced bilirubin levels ($p<0.05$). The observed improvements in liver function biomarkers indicate a potential hepatoprotective role of *C. gynandra*, comparable to or exceeding that of silymarin in mitigating acetaminophen-induced hepatic injury.

3.2. Percentage Hepatoprotection of C. gynandra Extracts and Fractions

The percentage protection demonstrated by *C. gynandra* extracts and fractions against acetaminophen-induced hepatotoxicity is shown in Figure 3. Of the treatment groups, EAF 300 showed the highest protection (95.19%), closely followed by NBF 300

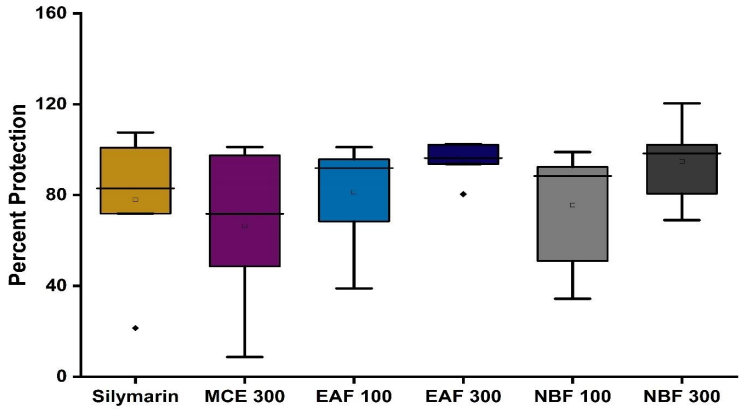

Figure 3. Percentage Protection of the Extract and Fractions of *C. gynandra* and Silymarin Against Acetaminophen-Induced Toxicity.

(94.80%), both of which exceeded the reference standard, silymarin (77.91%). EAF 100 and NBF 100 showed moderate protection at 81.30% and 75.54%, respectively, while MCE 300 showed the lowest protection (66.54%) with more variability. These results indicate that EAF 300 and NBF 300 provide the most significant hepatoprotective effects, comparable to the effect of silymarin in this model, although further validation is required.

3.3. Histopathological Effects of C. gynandra Extract

The liver histology of rats under various conditions was examined. Normal liver tissue (Figure 4A) showed well-preserved ar-

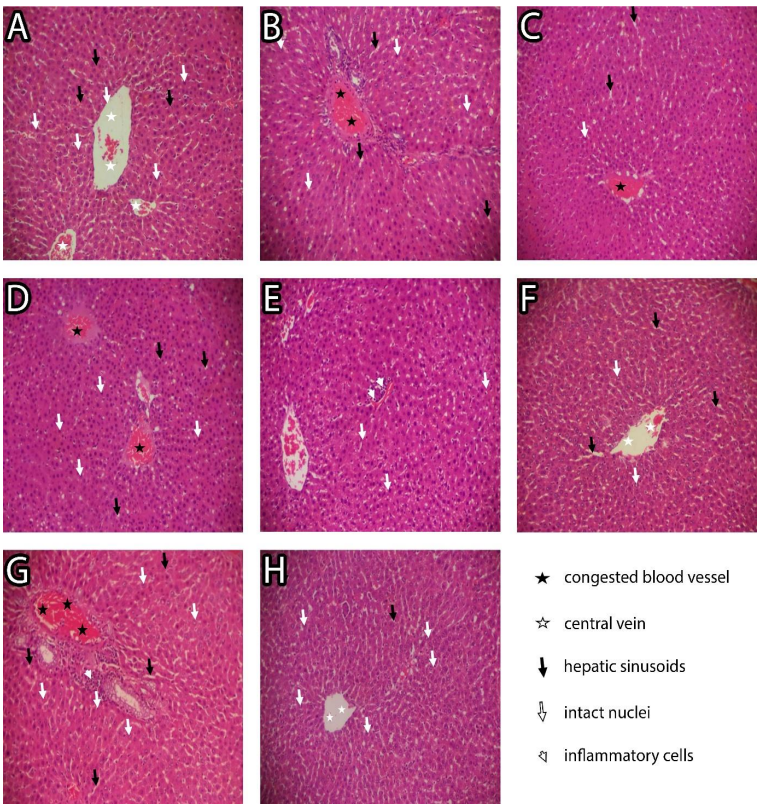

Figure 4. Micrographs of Liver Sections from Different Experimental Groups Showing the effects of *C. gynandra* extracts and fractions on acetaminophen-induced histological changes. (A) Normal Control; (B) Acetaminophen; (C) Silymarin; (D) MCE 300; (E) EAF 100; (F) EAF 300; (G) NBF 100; (H) NBF 300.

chitecture with hepatocytes arranged in cords radiating from the central vein, intact nuclei and clear hepatic sinusoids. Acetaminophen (APAP) administration (1 g/kg) showed severe congestion characterised by coagulation of blood cells within the blood vessels, severe inflammation, and mild necrosis and loss of normal tissue architecture in some areas (Figure 4B). Silymarin pretreatment (50 mg/kg) showed maintained hepatocyte morphology with some residual congestion (Figure 4C). The methanol crude extract (MCE) and n-butanol fraction (NBF) of *C. gynandra* at 300 mg/kg and 100 mg/kg (Figure 4D&G) showed mild congestion and inflammation but maintained hepatocyte integrity. The ethyl acetate fraction (EAF) at 100 mg/kg showed mild inflammation (Figure 4E), while 300 mg/kg showed remarkably preserved normal hepatic architecture (Figure 4F). Similarly, NBF at 300 mg/kg (Figure 4H) showed remarkable preservation of normal liver structure without pathological changes.

4. Discussion

The evaluation of the effect of *C. gynandra* extract and its derived fractions on liver function enzyme markers in the acetaminophen-induced hepatotoxicity model provided valuable insights into the hepatoprotective potential of this plant. The elevation of serum liver enzymes, particularly AST, ALT and GGT, is a well-established indicator of hepatocellular damage (23). In this study, acetaminophen administration led to significant increases in these enzymes, consistent with the known hepatotoxic effects of acetaminophen overdose (24). *C. gynandra* extracts and fractions demonstrated the ability to attenuate these elevations, with some treatments showing effects comparable to or even superior to the standard hepatoprotective agent, silymarin. Particularly significant was the performance of the NBF and EAF at 300 mg/kg, which reduced AST, ALT and GGT levels to near-normal values, outperforming silymarin in some instances.

These findings align with previous studies on other Cleome species. For instance, Gupta and Dixit (25) reported that *Cleome viscosa* extract significantly reduced elevated AST and ALT levels in rats with carbon tetrachloride-induced hepatotoxicity. Similarly, Shaikh, Niazi (26) found that *Cleome felina* extract exhibited hepatoprotective effects against paracetamol-induced liver damage in rats, significantly lowering AST and ALT levels.

GGT is another liver enzyme that is often used as a marker of hepatobiliary dysfunction and cholestatic liver injury (27). The significant increase in GGT levels observed in the acetaminophen group compared to the normal control group in the current study is indicative of the bile duct and liver damage caused by acetaminophen overdose. The effective reduction in the GGT levels to values comparable to the normal control suggests that the plant-derived compounds may have protected the bile ducts and improved the liver function in the acetaminophen-intoxicated animals.

The analysis of liver function biochemical markers, including TP, albumin (AL), total bilirubin (T.BIL) and direct bilirubin (D.BIL) are crucial indicators of liver function and integrity. The observed reduction in TP and albumin levels in acetaminophen-treated rats is consistent with previous studies by Stephen and Bello (23) on DILI. This decrease is typically attributed to impaired protein synthesis in damaged hepatocytes and is a hallmark of liver dysfunction (1). The EAF at 300 mg/kg demonstrated remarkable efficacy, restoring protein and albumin levels to near-normal values. This pattern is consistent with findings from plant extracts known to enhance bilirubin clearance, such as those reported for Eucalyptus obliqua (28). The restoration of protein levels suggests that *C. gynandra* may possess compounds that either stimulate protein synthesis in hepatocytes or protect the protein synthesis machinery from acetaminophen-induced damage.

The normalization of bilirubin levels (both total and direct) by *C. gynandra* treatments is another significant finding. Elevated bilirubin is a classic marker of hepatobiliary dysfunction and can indicate impaired bilirubin conjugation or excretion (29). In this study, some fractions (particularly NBF at 300 mg/kg) reduced bilirubin levels to values comparable to the normal control group. This level of efficacy is significant and suggests that *C. gynandra* may contain potent compounds capable of modulating bilirubin metabolism or enhancing its excretion, possibly involving regulation of enzymes like UDP-glucuronosyltransferases (30).

Histological evaluation of liver tissue provided critical insights into the hepatoprotective effects of *C. gynandra* extracts and fractions against acetaminophen-induced liver injury. The normal control group exhibited well-preserved hepatic architecture, serving as a baseline for comparison, while the acetaminophen-treated group showed severe congestion, inflammation, and tissue degeneration, consistent with acetaminophen hepatotoxicity (31). Silymarin treatment significantly mitigated these histological alterations, preserving hepatocyte morphology and reducing inflammatory infiltration, aligning with previous reports on its hepatoprotective properties (32). Among the *C. gynandra* treatments, EAF and NBF at 300 mg/kg demonstrated the most remarkable preservation of hepatic architecture, with histological features closely resembling the normal control. This suggests that these fractions contain potent hepatoprotective compounds, similar to findings on polyphenol- and flavonoid-rich extracts from other medicinal plants (33). The dose-dependent hepatoprotection observed with EAF further supports its pharmacological relevance (23). The significant hepatoprotective effects, in some cases comparable to or exceeding silymarin, suggest that *C. gynandra* extracts, particularly the ethyl acetate and n-butanol fractions, may serve as potential therapeutic agents for acetaminophen-induced liver injury. Their efficacy is likely attributable to bioactive compounds with antioxidant and anti-inflammatory properties, which play a crucial role in mitigating oxidative stress and inflammation in hepatic tissue (34, 35).

Future studies should quantify oxidative stress markers such as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase, as well as isolate phytochemicals of interest through chromatographic profiling (HPLC or LC-MS) to confirm the mechanism of hepatoprotection.

5. Conclusion

The study demonstrates the significant hepatoprotective potential of *Cleome gynandra* against acetaminophen-induced liver injury. The plant extracts and fractions, particularly the ethyl acetate and n-butanol fractions at 300 mg/kg, exhibited remarkable protective effects comparable to or surpassing the standard drug silymarin. These effects were evidenced by amelioration in liver function markers and histopathological findings. The hepatoprotective activity is likely attributable to the rich phytochemical composition of *C. gynandra*, including flavonoids, phenols and terpenoids, which possess antioxidant and anti-inflammatory properties. The dose-dependent effects observed suggest that the bioactive compounds are concentrated in specific fractions. These findings showed the potential of *C. gynandra* as a valuable source of hepatoprotective compounds for managing drug-induced liver injury.

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Authors contributions

Conceptualization: MSN, HS; Data curation, Formal analysis, Methodology, Project administration, Software, Writing-original

draft, Funding acquisition, Investigation, Resources, Supervision, Validation, Visualization, Writing-review & editing: MSN, HS, MIB.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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