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*Research article*

# Hepatoprotective and antioxidant potential of *Chamaecrista mimosoides* leaf methanol extract on paracetamol-induced hepatotoxicity in albino rats

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**Abstract:** Liver diseases comprise a spectrum of conditions that impair hepatic function and may be life-threatening. Medicinal plants represent an important source of bioactive compounds with therapeutic potential and comparatively fewer adverse effects than synthetic drugs. This study evaluated the hepatoprotective and antioxidant effects of the methanolic leaf extract of *Chamaecrista mimosoides* in paracetamol-induced hepatotoxicity in albino rats. Qualitative and quantitative phytochemical analyses were performed using standard laboratory methods. Hepatic injury was induced by paracetamol administration, followed by treatment with graded doses of the plant extract (100, 200, and 400 mg/kg) and a standard hepatoprotective drug. Serum liver function biomarkers and antioxidant parameters were assessed using established protocols. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycosides, phenols, and terpenoids, with tannins showing the highest concentration (91.98 ± 0.81). Paracetamol administration resulted in significant elevations ($P<0.05$) of serum AST, ALT, ALP, total bilirubin, and direct bilirubin in the induced control group compared with the normal control. Treatment with the extract, particularly at 400 mg/kg, significantly ameliorated these alterations, with AST and ALT levels comparable ($P>0.05$) to the normal control. Similarly, antioxidant enzyme activities, including glutathione, catalase, and superoxide dismutase, were significantly restored in extract-treated groups relative to the induced control. These findings demonstrate that *C. mimosoides* leaf methanol extract exhibits significant hepatoprotective and antioxidant activities, supporting its traditional use in the management of liver disorders.

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## Introduction

Liver is the most important organ in xenobiotic metabolism/detoxification, in which exogenous lipophilic xenobiotics or drugs are converted to hydrophilic compounds via biochemical processes catalyzed by the p450 enzyme system [1]. Recently, more than 1000 drugs exhibit direct toxicity or produce toxic (reactive) metabolites, which can lead to changes in protein conformation, DNA mutations, or lipid peroxidation, subsequently resulting in hypersensitivity reactions or drug-induced liver injury (DILI) [2]. Liver diseases are a group of conditions that prevent the liver from functioning properly and can be caused by viruses (like hepatitis virus), alcohol, obesity, inherited disorders, or toxins. Common types include non-



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alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and viral hepatitis (A, B, and C) [3].

Medicinal plants offer advantages for treating diseases by providing natural compounds with diverse beneficial properties, often exhibiting fewer side effects than synthetic drugs and being more affordable and accessible in many regions [4-6]. They have anti-inflammatory, antioxidant, and antimicrobial effects [7]. They can be particularly valuable for chronic and infectious diseases, with some having synergistic effects that make them more potent than isolated compounds [8, 9]. Medicinal plants are significant in the management of liver diseases because they contain bioactive compounds that confer hepatoprotective effects, including antioxidant, anti-inflammatory, and anti-fibrotic properties [10, 11]. They are used as an adjunct treatment to help protect the liver, reduce inflammation, and support recovery by targeting various pathological steps in diseases like viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD) [12].

*Chamaecrista mimosoides* is an annual or occasionally a short-lived perennial plant with a more or less woody base, a flowering plant in the plant family Fabaceae used traditionally to treat various ailments, including inflammatory diseases, microbial related diseases, digestive issues like diarrhea and stomach spasms, as well as for wounds and sores, and has been explored for potential anti-epileptic effects [13].

## Material and methods

### *Sample collection and storage*

*C. mimosoides* leaves were collected in Aliero Local Government, Kebbi State, Nigeria. The plant was presented to the Department of Plant and Biotechnology at Abdullahi Fodio University of Science and Technology, Aliero, for identification. The specimen was identified, and the voucher number was assigned. Specimen number was subsequently deposited at the herbarium of the Department, for reference purposes.

### *Plant preparation and extraction*

*The C. mimosoides* leaf was washed with clean water and shadow-dried under the canopy, after which it was pulverized into semi-powdered form using a pestle and mortar. One hundred and fifty grams (150 g) of the semi-powder sample was soaked in 1 liter of 99.8% methanol for 72 hours, with stirring every morning using a spatula and resealing the container with masking tape to prevent methanol from escaping. The sample was subsequently filtered through muslin. The filtrate was concentrated in a rotary evaporator, after which the concentrated crude was exposed to allow the remaining methanol to evaporate. The solid extract was stored in a fridge until needed [14].

### *Experimental animals*

The albino rats used in this study were bought from Animal House, Usman Danfodiyo University, Sokoto, in February 2024. They were brought to Animal House, Faculty of Life Sciences, Abdullahi Fodio University of Science and Technology, Aliero, in well-ventilated cages. Before the trial started, the rats were kept in a clean cage and given fourteen days to acclimatize. The rats were fed a typical rat diet and given unlimited access to water.

### *Qualitative and quantitative phytochemical analysis*

The extract was subjected to both qualitative and quantitative phytochemical screening using the standard procedures [15, 16].

### *Hepatoprotective studies*

Rats were randomly divided into six groups of six animals each as detailed in **Table 1**.

**Table 1.** Rats grouping and treatment.

| Group | Treatment |
|---|---|
| Group I | Received distilled water (10 ml/kg b.w) once daily for 14 days, and served as a normal control |
| Group II | Received paracetamol (600 mg/kg b.w) once daily for 14 days and served as disease control. |
| Group III | Received standard drug silymarin (100 mg/kg b.w) once daily for 14 days and served as a positive control. |
| Group IV | Received methanol extract of *C. mimosoides* leaf, 100 mg/kg b.w, once daily for 14 days, served as the treatment groups. |
| Group V | Received methanol extract of *C. mimosoides* leaf 200 mg/kg b.w once daily for 14 days served as treatment groups. |
| Group VI | Received methanol extract of *C. mimosoides* leaf, 300 mg/kg b.w, once daily for 14 days, served as the treatment groups. |

All groups except group I were administered paracetamol (600 mg/kg b.w.) once daily, followed by the respective treatment according to the method reported in previous study [17], with slight modifications. The animals were anesthetized with chloroform 24 hours after the last administration (14$^{th}$

day). Estimation of average rat body weight and biochemical and antioxidant parameters was carried out.

### Measurement of liver function

The Sood technique was used to estimate alkaline phosphatase activity. Reitman and Frankel's approach was used to measure the catalytic activity of aspartate aminotransferase and alanine aminotransferase. The bromocresol green method from a previous study [18] was used to measure albumin. The Biuret reaction method to determine total protein was employed [19]. Jendrassik and Grof's calorimetric method was used to measure total and direct bilirubin [20] .

### Antioxidant assay

Lipid peroxidation was determined by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) [21]. SOD) Activity was determined using the method of Xin *et al*. (1991). The activity of catalase was assayed according to the method of [22], glutathione (GSH) and Vitamin A concentration was determined using the technique from previous study [23], and vitamin E was determined as described by Bahrami et al 2020 [23].

### *Data analysis*

Data were presented as mean ± standard error of the mean and statistically examined using one-way analysis of variance (ANOVA). Means were grouped using the Duncan multiple-comparison test with the aid of the Statistical Package for the Social Sciences (SPSS) version 20. $P<0.05$ is considered significant.

## Results

### *Qualitative phytochemical composition*

The qualitative phytochemical screening of *C. mimosoides* leaf methanol extract is presented in **Table 2**.

### *Quantitative phytochemical composition*

The quantitative phytochemical composition of *C. mimosoides* leaf methanol extract is presented in **Table 3**. The result revealed a high concentration of tannins (91.98 ± 0.81) and minimal concentrations of alkaloids, flavonoids, glycosides, and phenols (0.218 ± 0.01, 1.52 ± 0.6, 2.49 ± 0.40, and 4.15 ± 0.50, respectively).

**Table 2.** Qualitative Phytochemical Constituents of *C. mimosoides* Leaf Methanol Extract.

| **Phytochemicals** | **Observation** |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Tannins | + |
| Saponin | + |
| Glycoside | + |
| Cardiac glycoside | + |
| Anthraquinones | - |
| Phenols | + |
| Terpenoids | + |

KEY: + = Present, - = Not detected

**Table 3.** Quantitative Phytochemical Constituents of *C. mimosoides* Leaf Methanol Extract.

| **Phytochemicals** | **Concentrations** |
|---|---|
| Alkaloids mg% | 0.218 ± 0.01 |
| Flavonoids mg% | 1.52 ±0.6 |
| Tannins mg/100g | 91.98±0.81 |
| Glycosides mg% | 2.49±0.40 |
| Phenols GAE / gm | 4.15±0.50 |

Values are expressed as Mean ± SD, n = 3, which is the triplicate of each result.

### *Hepatoprotective studies*

The hepatoprotective effect of *C. mimosoides* leaf methanol extract on paracetamol-induced liver-damaged rats is presented in **Table 4**. Significant ($P<0.05$) increases in AST, ALT, ALP, TB, and DB were observed in induced controls compared with normal controls, positive controls, and all extract-treated groups. However, only the AST and ALT of groups treated with extract 400 mg/kg was comparable ($P>0.05$) with normal control. Meanwhile, only ALP and TB of groups treated with standard drugs and extract 400 mg/kg were comparable ($P>0.05$) to normal control. Similarly, the serum DB of groups treated with standard drug, extract 200 and 400 mg/kg were not significantly different ($P>0.05$) compared to normal control. A significant ($P<0.05$) decreases in Albumin and TP were observed in induced control compared to normal control, positive control and all extract treatment groups. However, both positive control and all extract treatment groups significantly ($P<0.05$) decreases compared to normal control. There is no significant ($P>0.05$) difference between normal control and extract treated group 400mg/kg, however standard drug, extract treatment groups 100 and 200 mg/kg significantly decreases compared to normal control.

**Table 4.** Hepatoprotective Effect of *C. mimosoides* Leaf Methanol Extract on Paracetamol induced Liver Damaged Rats.

| Parameter | Normal Control (10 m D. $H_2O$) | Negative control (600 mg/kg Paracetamol) | Positive control (100 mg/kg Silymarin) | Extract (100 mg/kg) | Extract (200 mg/kg) | Extract (400 mg/kg) |
|---|---|---|---|---|---|---|
| AST (U/L) | 44.62±2.00$^{a}$ | 76.40±2.24$^{d}$ | 51.57±0.91$^{b}$ | 73.37±2.74$^{d}$ | 60.37±0.64$^{c}$ | 49.57±0.89$^{ab}$ |
| ALT (U/L) | 31.22±1.15$^{a}$ | 67.30±2.13$^{d}$ | 40.84±1.19$^{b}$ | 67.84±1.81$^{d}$ | 55.38±1.62$^{c}$ | 36.32±3.09$^{ab}$ |
| ALP (U/L) | 75.24±2.56$^{a}$ | 105.26±4.26$^{c}$ | 84.09±3.09$^{ab}$ | 98.81±1.07$^{c}$ | 88.53±0.88$^{b}$ | 81.53±5.05$^{ab}$ |
| ALB (g/L) | 4.92±0.02$^{d}$ | 1.72±0.08$^{a}$ | 3.23±0.36$^{b}$ | 2.11±0.06$^{a}$ | 3.45±0.05$^{b}$ | 4.01±0.07$^{c}$ |
| TB (mg/dL) | 1.02±0.04$^{a}$ | 2.00±0.08$^{c}$ | 1.15±0.02$^{a}$ | 1.48±0.03$^{b}$ | 1.45±0.04$^{b}$ | 1.04±0.04$^{a}$ |
| DB (mg/dL) | 0.52±0.01$^{a}$ | 0.64±0.02$^{c}$ | 0.54±0.02$^{a}$ | 0.60±0.01$^{bc}$ | 0.56±0.02$^{ab}$ | 0.51±0.01$^{a}$ |
| TP (g/L) | 6.24±0.32$^{d}$ | 2.76±0.35$^{a}$ | 5.26±0.20$^{c}$ | 2.58±0.11$^{a}$ | 3.82±0.30$^{b}$ | 5.74±0.30$^{cd}$ |

Values are presented as mean ± SEM (n = 3). Values with similar superscripts are not significantly different (P > 0.05), analyzed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. AST-Aspartate Amino Transferase, ALT- Alanine Amino Transferase, ALP- Alkaline Phosphatase, DB- Direct Bilirubin, TB- Total Bilirubin, ALB- Albumin, and TP- Total Protein.

**Table 5.** Anti-oxidant Potentials of *C. mimosoides* Leaf Methanol Extract on Paracetamol-induced Liver Damaged Rats.

| Treatment | GSH (mg/dl) | MDA (nmol/L) | CAT (U/ml enzyme) | SOD (U/ml enzyme) |
|---|---|---|---|---|
| Normal Control (Distilled $H_2O$ 10 ml/kg b. wt) | 281.20±2.06$^{f}$ | 120.72±4.41$^{a}$ | 1.14±0.06$^{b}$ | 0.58±0.10$^{c}$ |
| Negative Control (600 mg/kg Paracetamol) | 113.63±0.37$^{a}$ | 335.68±2.88$^{d}$ | 0.43±0.09$^{a}$ | 0.16±0.01$^{a}$ |
| Positive Control (100 mg/kg Silymarin) | 169.84±0.70$^{c}$ | 139.87±12.39$^{b}$ | 1.29±0.15$^{b}$ | 0.19±0.02$^{a}$ |
| Extract (100 mg/kg) | 262.92±0.32$^{d}$ | 141.02±0.74$^{b}$ | 1.55±0.10$^{b}$ | 0.36±0.07$^{b}$ |
| Extract (200 mg/kg) | 273.02±0.60$^{e}$ | 136.53±4.27$^{ab}$ | 1.20±0.12$^{b}$ | 0.48±0.01$^{bc}$ |
| Extract (400 mg/kg) | 133.68±4.49$^{b}$ | 215.81±1.13$^{c}$ | 1.56±0.10$^{b}$ | 0.52±0.02$^{bc}$ |

Values are expressed as mean ± standard error of mean. Mean values having common superscript letters in a column are not significantly different (P<0.05) (one-way ANOVA followed by Duncan's multiple range test). CAT = Catalase, MDA = Malondialdehyde, GSH = Glutathione, SOD = Superoxide dismutase.) PCF18- pooled chromatographic fraction 18.

### *Antioxidant potentials*

The antioxidant potential of the *C. mimosoides* leaf methanol extract in paracetamol-induced liver-damaged rats is presented in **Table 5**. A decrease in glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) activities was observed in the induced control compared to the normal control, positive control, and all extract-treated groups. However, all GSH levels in groups treated with the standard drug and all extract-treated groups were significantly (P<0.05) lower than in the normal control. All extract-treated groups and the standard drug control showed comparable (P>0.05) CAT activities compared with the normal control. Meanwhile, only the groups treated with extracts at 200 and 400 mg/kg did not differ significantly (P>0.05) in SOD from the normal control. A significant increase in malondialdehyde was observed in the induced control compared with the normal control, positive control, and all extract-treated groups. Only the treated groups at 200 and 400 mg/kg were comparable (P>0.05) with the normal control.

### *Histopathological effect of C. mimosoides leaf methanol effect on paracetamol-induced of liver damaged rats*

The effect of *C. mimosoides* leaf methanol extract on liver histology of paracetamol induced of liver damaged rats A-E **(Figure 1)**. Plate A showed liver tissue obtained from a normal control showing normal vacuoles (red arrow), normal central vein (yellow arrow), and normal hepatocytes (black arrow). Plate B showed liver tissue obtained from an induced control, showing oedema (black arrow), steatosis and ballooning (yellow arrow), and an increased number of phagocytic Kupffer cells

lining bile and duct channels, indicating cholestasis. Severe reactive changes: Plate C shows liver tissue obtained from the group administered the standard drug, showing oedema (black arrow), vacuoles (yellow arrow), and an increased population of phagocytic Kupfer cells (red arrow) lining ducts and channels. Plate D showed liver tissue obtained from a group administered 100 mg/kg, showing hepatocyte nests and cords (yellow arrow), separated by bile and sinusoid channels lined by numerous phagocytic inflammatory Kupfer cells cholestatic effect. Plate E showed liver tissue obtained from the group administered 200 mg/kg, showing feathering (blue arrows) and necrosis (black arrows) of hepatocytes with very few noticeable ducts severe degenerative changes. Plate F showed liver tissue obtained from a group administered 400 mg/kg, showing feathering (blue arrows), apoptosis (black arrows), and necrotic areas (yellow arrows), indicating degenerative changes.

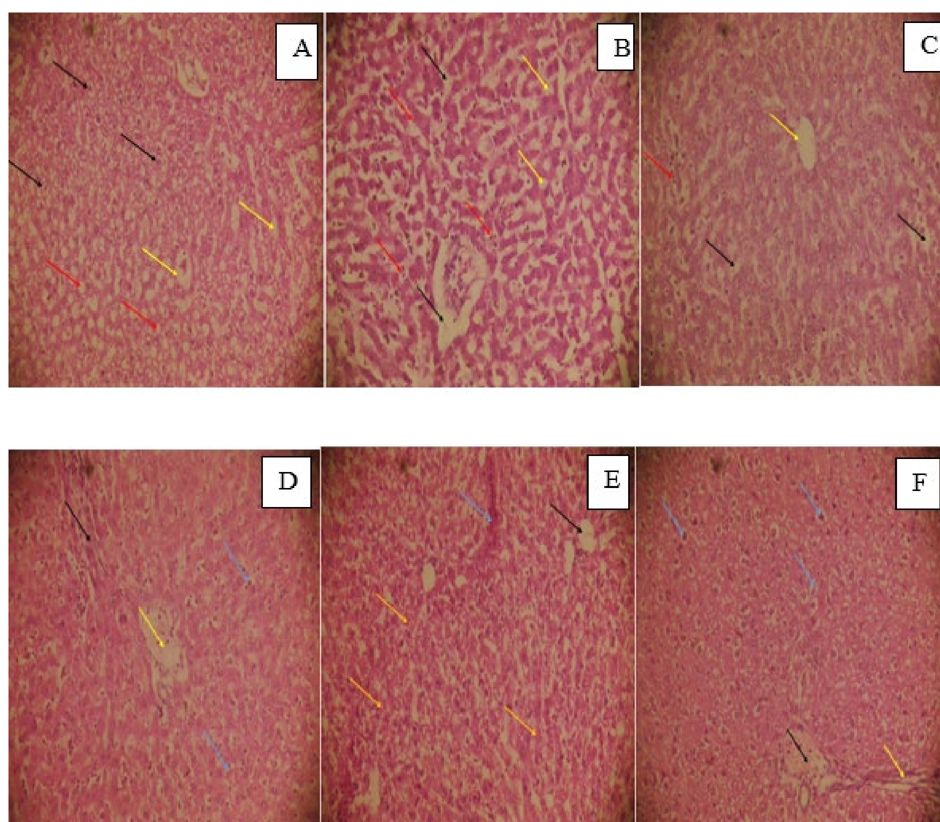


**Figure 1.** Liver histology of Paracetamol induced liver damaged Rats. **Plate A:** Photomicrograph of Rat’s Liver (Normal Control)**. Plate B:** Photomicrograph of Rat’s Liver (Negative control) Paracetamol 600 mg/kg. **Plate C:** Photomicrograph of Rat’s Liver (Positive Control), silymarin 5 mg/kg. **Plate D:** Photomicrograph of Rat’s Liver (100 mg/kg b. wt) Extract. **Plate E:** Photomicrograph of Rat’s Liver (200 mg/kg b. wt) Extract. **Plate F:** Photomicrograph of Rat’s Liver (400 mg/kg b. wt) Extract.

## Discussion

Numerous components from various chemical families of secondary metabolites, including alkaloids, terpenoids, essential oils, glycosides, steroids, phenolic contents, aliphatic compounds, and polysaccharides, have been found in many native plants [24]. Proteins, flavonoids, alkaloids, and glycosides are abundant in the leaves, stems, and roots of most of these plants [25]. These Numerous biological actions, such as antiseptic, anti-inflammatory, anti-cancer, antibacterial, and antidiabetic properties, have been demonstrated by these active substances [26]. Elshafie et al., 2023 [27] stated that plant-derived secondary metabolites are small or large molecules biosynthesized in plants, including steroids, alkaloids, phenolic compounds, lignans, carbohydrates, and glycosides. These compounds exhibit a variety of biological properties that are beneficial to humans, including antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant activities [28]. In the present study, the observed hepatoprotective activity may be due to the presence of these phytoconstituents in the *C. mimosoides* leaf methanol extract.

Enzymatic markers of chronic liver disease include aminotransferases (AST and ALT) and alkaline

phosphatase (ALP), which indicate liver injury. These enzymes are released into the blood when liver cells (hepatocytes) are damaged. Other markers, such as albumin and other proteins, indicate liver function [29]. Since the liver produces albumin and bilirubin, their levels may drop in advanced liver disease when the liver can no longer produce enough protein [30]. In the present study, elevated levels of AST, ALT, and ALP were observed in the induced control, while serum protein levels declined significantly, indicating cellular injury. However, treatments with *C. mimosoides* leaf methanol extract stimulate increases in protein (TP, ALB, DB) levels and also reduce serum enzyme biomarkers of liver toxicity (AST, ALP, ALT). Suggesting hepatoprotective potential conferred by *C. mimosoides* leaf methanol extract

Enzymatic and non-enzymatic antioxidants are created either endogenously or by food [31]. The enzymatic category includes catalase, superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase, tyrodoxin reductase, ariel esterase, and paraoxonase [32]. Decreased levels of these enzymes, superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) in many chronic diseases, are a result of overwhelmed antioxidant defence mechanisms by excessive reactive oxygen species (ROS), as noticed in the induced control [33]. This imbalance leads to oxidative stress, which plays a key role in the progression of various pathological conditions [34]. However, in the present study, the observed increases in SOD, GSH, and CAT in *the C. mimosoides leaf methanol extract-treated groups indicate the possible antioxidant potential of the* methanol leaf extract.

Polyunsaturated fatty acids (PUPA) in the cell membrane degrade to produce malondialdehyde (MDA) [35]. Higher levels of MDA in serum are a marker of oxidative stress and increased lipid peroxidation, which have been linked to chronic liver disease [36]. In the present study, a high level of MDA degradation was observed in induced control animals, indicating induced lipid peroxidation and oxidative stress; however, animals treated with the extract showed lower serum MDA levels. Hence, further supporting the antioxidant activity of *C. mimosoides* methanol leaf extract.

Histological studies of liver tissue involve microscopic examination of liver biopsies to assess its structure and identify disease [37]. These studies are crucial for diagnosing the cellular and architectural changes that occur during disease progression, such as inflammation, necrosis, steatosis (fatty change), and fibrosis (scarring) and also assists in understanding the underlying mechanisms of diseases like nonalcoholic fatty liver disease (NAFLD), autoimmune hepatitis (AIH), and viral hepatitis [38]. In the present study, severe reactive changes, including edema, steatosis, ballooning, and an increase in the number of phagocytic Kupffer cells, were observed in the induced control, although not comprehensively. Still, an obvious tissue regeneration was observed after administration of the *C. mimosoides* leaf methanol extract, further suggesting a tissue-regenerative effect of the plant.

## Conclusion

In conclusion, the present study revealed that *the C. mimosoides* leaf methanol extract contains numerous pharmacologically active phytochemicals and exhibits strong hepatoprotective and antioxidant properties, with minimal liver tissue regeneration. Thus, the present studies validate the traditional utilization of this plant in the management of liver diseases.

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