Journal of Bioscience and Biotechnology Discovery
Volume 11(2), pages 45-54, April 2026
Article Number: 629C4F6A3
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2026.251
https://integrityresjournals.org/journal/JBBD
Full Length Research

Bioassay-driven fractionation and *in vivo* evaluation of hepatoprotective potentials of *Gymnema sylvestre* methanolic leaf extract in Wistar albino rat

Kabiru Usman[1]*, Umar Adamu[1], A.B. Hamza[1], Muttaka Auwalu[1], Mansur Usman[2], A.A. Naibi[1], N. I. Danmaliki[1], Nafiu Bello[1], Abubakar Yusuf[1], Aliyu Garba[1] and Sambo A. Sambo[1]

[1]Department of Biochemistry, Federal University Gusau, Zamfara State, Nigeria.
[2]Department of Microbiology, Federal University Gusau, Zamfara State, Nigeria.

*Corresponding author. Email: usmankabir@fugusau.edu.ng/kabiruusman002@gmail.com; Tel: +2348065247435.

Received Date: 5 February 2026 | Accepted Date: 10 March 2026 | Published Date: 30 April 2026

ABSTRACT: This study was conducted to assess the hepatoprotective potential of *Gymnema sylvestre* methanolic leaf extract through bioactivity-guided fractionation with Wistar albino rats induced with Carbon tetrachloride (CCl_4). Study of qualitative phytochemical analysis performed on the extract revealed the presence of flavonoids, saponins, alkaloids, steroids, tannins, and triterpenoids, while quantitative analysis indicated flavonoids (28.57±0.30 mg/g) as the most abundant constituent, followed by saponins and alkaloids. The extract also contained high levels of antioxidant Vitamins A, C, and E and phenolic compounds, suggesting strong antioxidant potential. CCl_4 administration significantly elevated serum Alanine Transferase (ALT), Aspartate Transferase (AST), Total Bilirubin (TB), and Direct Bilirubin (DB) while reducing Total Protein (TP) and Albumin (ALB), confirming severe hepatic injury ($p<0.05$). Treatment with Silymarin and graded doses of thyl acetate fraction (100–400 mg/kg) significantly restored these biochemical parameters in a dose-dependent manner, with the 400 mg/kg dose showing the highest therapeutic reversal comparable to Silymarin. Antioxidant enzyme assays revealed marked reductions in SOD, CAT, GPx, and GSH levels and elevated MDA in the CCl_4 group, indicating oxidative stress. Administration of the extract fraction, particularly at 400 mg/kg, significantly improved antioxidant enzyme activities and reduced lipid peroxidation. Histopathological observations further supported these biochemical findings, showing severe necrosis, inflammation, and fatty degeneration in the CCl_4 group, while treated groups demonstrated progressive restoration of normal hepatic architecture, with the 400 mg/kg group exhibiting near-normal liver histology.

Keywords: Bioactivity-guided fractionation, carbon tetrachloride, *Gymnema Sylvestre,* hepatoprotective potential, histopathological study.

INTRODUCTION

Medicinal plants continue to gain increasing attention as promising sources of hepatoprotective agents. It is estimated that approximately 25–30% of conventional drugs are either directly derived from plant-based compounds or are synthetic analogues of natural products (Newman and Cragg, 2020). Traditional medicine is also recognised by the World Health Organisation (WHO) as an essential component of global healthcare systems, particularly in developing countries where a large proportion of the population depends on herbal remedies for primary healthcare needs (WHO, 2023). Naturally occurring plant products exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, analgesic, antipyretic, antimicrobial, antiviral, and cardioprotective activities.

These properties are largely attributed to bioactive phytochemicals such as phenolics, flavonoids, alkaloids, terpenoids, glycosides, coumarins, and saponins (Chen *et al.,* 2025).

Bioactive phytochemicals exert hepatoprotective effects through mechanisms such as scavenging reactive oxygen species (ROS), inhibition of lipid peroxidation, modulation of inflammatory pathways, and enhancement of endogenous antioxidant defence systems (Li *et al.*, 2015). The liver is one of the largest and most vital organs in the human body, playing a central role in maintaining physiological homeostasis. It functions as the primary metabolic hub, regulating carbohydrate, protein, and lipid metabolism, as well as synthesising and storing essential biomolecules such as glycogen, vitamins, and plasma proteins (Trefts *et al.,* 2017).

However, the liver is susceptible to various pathological conditions, including cirrhosis, alcoholic liver disease, and viral hepatitis. These liver diseases represent a major global health burden, contributing significantly to morbidity and mortality worldwide. Recent estimates indicate that liver diseases account for more than two million deaths annually (Devarbhavi *et al*., 2023). This burden is particularly pronounced in low- and middle-income countries, where healthcare systems face significant challenges (Devarbhavi *et al*., 2023).

The use of herbal medicine for the treatment of liver disorders has a long history across various traditional systems such as Ayurveda, Traditional Chinese Medicine, and African ethnomedicine. A substantial proportion of the global population continues to rely on medicinal plants for the management of hepatic ailments (WHO, 2023). Several plants have demonstrated hepatoprotective effects against toxin-, drug-, and alcohol-induced liver damage in experimental models. Examples include *Eclipta alba*, *Solanum nigrum*, *Balanites aegyptiaca*, and *Khaya senegalensis* (Rani *et al.,* 2024).

Gymnema sylvestre is an important medicinal plant belonging to the family Apocynaceae, widely distributed across Africa, India, China, and Australia. It has been traditionally used for the management of diabetes, obesity, asthma, cardiovascular disorders, and other chronic diseases. The plant contains key bioactive constituents such as gymnemic acids, saponins, flavonoids, and phenolic compounds, which contribute to its diverse pharmacological activities. Recent studies have demonstrated that *Gymnema sylvestre* exhibits antidiabetic, antioxidant, anti-inflammatory, antimicrobial, anti-obesity, and anticancer properties (Tiwari *et al.,* 2022).

Therefore, the objective of this study is to assess the hepatoprotective potential of *Gymnema sylvestre* methanolic leaf extract through bioactivity-guided fractionation with Wistar albino rats induced with Carbon tetrachloride (CCl_4).

MATERIALS AND METHODS

Chemicals and reagents

Solvents for extraction and partitioning of the plant materials included methanol, ethyl acetate and n-butanol (saturated with water) (BDH Chemical Limited, Pooled, England). These solvents and all other chemicals are of analytical grade.

Experimental animal

A total of sixty (60) Wistar albino rats (*Rattus norvegicus* L.) weighing 150-200 g of both sexes were used for the studies. The rats were obtained from the animal house of the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto. The rats were housed in cleaned and disinfected cages and were allowed to acclimatise for seven (7) days before the experiment. The rats were fed on a standard rat chow and allowed to drink water at room temperature, increasing in growth until ready for use. All experiments were carried out in accordance with the (WHO, 2023) guidelines for the use of experimental animals.

Plants collection and identification

Fresh leaves of *Gymnema sylvestre* were collected from Mada Town, Gusau Local Government of Zamfara State, Nigeria. The plant was authenticated by a botanist from the Department of Biological Sciences, Federal University Gusau and a voucher specimen number (FUG/BIO/HEB/2026/250) was obtained.

Preparation of plant extracts

The leaves of the plant collected were cleaned, air-dried at room temperature, and then ground into powder using a mortar and pestle. About 100 g was macerated using 1 L of 95% methanol for 4 hours (twice) and filtered with Whatman No.1 filter paper, and the filtrate was concentrated in a rotary evaporator at 45°C. The dried methanol extract and fractions were screened for hepatoprotective activity in induced rats with CCl_4. Further fractionation with butanol and ethyl acetate was conducted for significant activity.

Fractionation of the methanol extract

Sixty grams (60 g) of the methanol extract was subjected to fractionation in a separator funnel and sequential

partitioning with ethyl acetate (3×400 mL) and Butanol (saturated with water, 3×400 mL). Each of the fractions was evaporated to dryness and screened for hepatoprotective activity.

Acute oral toxicity study (determination of LD_{50})

The acute oral toxicity study was conducted according to the method of Organization for Economic and Co-operation and Development for testing of chemicals (OECD, 2022). A total of five (5) animals were randomly selected and used for the experiment. The extract was administered to each animal in a single oral limit test dose of 5 g/kg body weight (2 mL/200 g). After the administration, food was withheld for a further 3-4 hours. The animals were observed individually at least once during the first 30 minutes after dosing, periodically at 8, 14, 24, and 48 hours intervals (with special attention during the first 4 hours) (OECD, 2022). The observations included hair loss, loss of appetite, drowsiness, salivation, tremors, fatigue, and convulsion. The animals were observed for a period of 14 days for any signs of delayed toxicity. The LD_{50} is less than 5 g/kg if three (3) or more animals died within 48 hours; however, the LD_{50} is greater than 5 g/kg if one, two or none died within 48 hours (OECD, 2022). The median lethal dose (LD_{50}) shows no sign of toxicity from methanol extract and fractions at the highest dose (500 mg/kg).

Experimental design for hepato-protective activity

Methanol extract and other fractions

Induction of hepatotoxicity was done according to OECD (2022), and the experimental groups were divided into (6) groups of five rats each. Group I (Control): Rats in this group were treated with a daily dose of liquid paraffin (1 mL/kg body weight, per mouth). Group II: Rats in this group were treated with 30% solution of carbon tetrachloride in liquid paraffin (1 mL/kg). Group III: Rats in this group were treated with silymarin, a known hepatoprotective drug (100 mg/kg). Group IV: Rats in this group were treated with 200 mg/kg body weight of methanol extract. Group V: Rats in this group were treated with 200 mg/kg body weight of ethyl acetate fraction. Group VI: Animals in this group were treated with 200 mg/kg of butanol fraction. The treatment duration of the extract and silymarin was once daily in aqueous suspension for 14 days. Carbon tetrachloride was administered in 30% solution of liquid paraffin every 7 days (OECD, 2022). The rats were sacrificed 48 hours after the last injection of CCl_4. Blood samples were collected in an empty sample container and allowed to clot naturally to obtain serum. The serum was used for estimations of biochemical parameters (AST, ALT, ALP, Bilirubin, Total Protein and Albumin).

Experimental design for the mechanism of action of the most active fraction

This experimental design was carried out on the most active fraction, which is ethyl acetate. Induction of hepatotoxicity was done according to the method of OECD (2022). The rats were divided into six (6) groups of five (5) rats each. Group I (Control): Rats in this group received daily food and water only. Group II: Treated with 30% solution of Carbon tetrachloride in liquid paraffin (1 mL/kg body weight intraperitoneal). Group III: Treated with silymarin at a dose induction of 100 mg/kg body weight. Group IV: Treated with 100 mg/kg body weight of the most active fraction. Group V: Treated with 200 mg/kg of the most active fraction. Group VI: Treated with 400 mg/kg of the most active fraction. Treatment duration of the most active fraction and silymarin was once daily in aqueous suspension for 28 days. Carbon tetrachloride was administered in 30% solution of liquid paraffin every 7 days (OECD, 2022). The rats were sacrificed 48 hours after the last injection of CCl_4, after a light anaesthesia with isoflurane. A blood sample was collected and allowed to clot naturally to obtain the serum. The serum was used for estimations of biochemical parameters, and the liver was removed and used for histopathological examination.

Phytochemical screening

Phytochemical screening to test for the presence of Alkaloids, Anthraquinones, Anthraquinone Glycosides, Cardiac Glycosides, Flavonoids, Glycosides, Phenols, Saponins, Saponin Glycosides, Steroids, Tannis, Terpenoids, Volatile oils and resins was conducted qualitatively using standard methods as described by Tiwari *et al*. (2022).

Determination of liver biochemical parameters

At the end of the experimental period, the rats were subjected to an overnight fast for 12 hours and subsequently anaesthetised with isoflurane, and later, a blood sample was collected in a sterile container. Serum was separated from the blood of each rat sacrificed and stored at -20°C until the time for biochemical determinations of Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Total Bilirubin, Total Protein and Albumin. Determinations were done spectrophotometrically using Randox analytical kits according to the standard procedure of the manufacturer's protocols (Reitman and Frankel, 1957).

Determination of Alanine Aminotransferase (ALT) activity

Serum Alanine Aminotransferase (ALT) activity was

determined using the kinetic UV method described by Reitman and Frankel (1957), following the procedure outlined in the Randox Practical Manual.

Determination of Aspartate Aminotransferase (AST) Activity

Serum Aspartate Aminotransferase (AST) activity was determined using the colourimetric method described by Reitman and Frankel (1957), in accordance with the procedure outlined in the Randox Practical Manual.

Determination of total bilirubin activity

Total Bilirubin concentration was determined using the colourimetric method of Jendrassik and Grof (1938), as described in the Randox practical manual.

Determination of total protein assay

Total protein concentration in serum was determined using the method of Lowry (1951), as described in the assay kit manual.

Serum albumin assay

Serum Albumin concentration was determined using the bromocresol green (BCG) dye-binding method, as described in the Randox assay kit manual (Doumas *et al.,* 1971; Shukla *et al.,* 2023).

Preparation of tissue lysate for antioxidant enzymes parameters

About 200 g of liver tissue was removed and transferred into a tube containing 2 mL of Tris buffer (homogenization buffer). The tissue was homogenised at 10,000 rpm for 2 minutes using a homogeniser. The resulting homogenate was centrifuged at 12,000 rpm for 20 minutes to pellet unhomogenized debris. The clear supernatant obtained was collected and used for the determination of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR), and Malondialdehyde (MDA) levels (Ercan and Koçkaya, 2017).

Determination of Superoxide Dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined according to the Randox practical manual, using a colorimetric method based on the enzyme's ability to inhibit the formation of formazan dye, as described by Kakkar *et al*. (1984).

Measurement of reduced glutathione

The reduced glutathione was estimated by the method of Rahman *et al*. (2007). Reduced glutathione (GSH) was determined based on its reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow chromophore measurable at 412 nm. The intensity of the colour formed is proportional to the concentration of GSH present in the sample.

GSH + DTNB → TNB (yellow chromophore) + GS–TNB

Measurement of Vitamin E

Vitamin E was estimated by the method of Jargar *et al*. (2012). Vitamin E (α-tocopherol) was determined based on its ability to reduce ferric ions to ferrous ions, which subsequently form a colored complex measurable spectrophotometrically. The absorbance of the resulting complex is directly proportional to the concentration of vitamin E in the sample.

Histological examination of the liver tissue

Histological examination of the liver tissue was carried out in rats within both phases of the experiment. A 2 g portion of the liver tissue was removed and placed in 10% formaldehyde solution for histological study. The pieces of the liver were then processed and embedded in paraffin wax, and sections were made about 4-6 µm in thickness. After staining with haematoxylin and eosin (H&E), slides were then examined under a microscope (Olympus, Japan) for histological changes (Mouleeswaran *et al.,* 2023).

Statistical analysis

The data was analysed by one-way ANOVA using the SPSS 26 Version statistical software. Significant means were expressed using Turkey's post-hoc test at a $p<0.05$ significance level. Experimental results were expressed as mean ± standard error of the mean (SEM).

RESULTS

The acute oral toxicity test of methanol leaf extract and solvent fractions of *Gymnema sylvestre* indicated that neither the methanol extract nor the solvent fractions

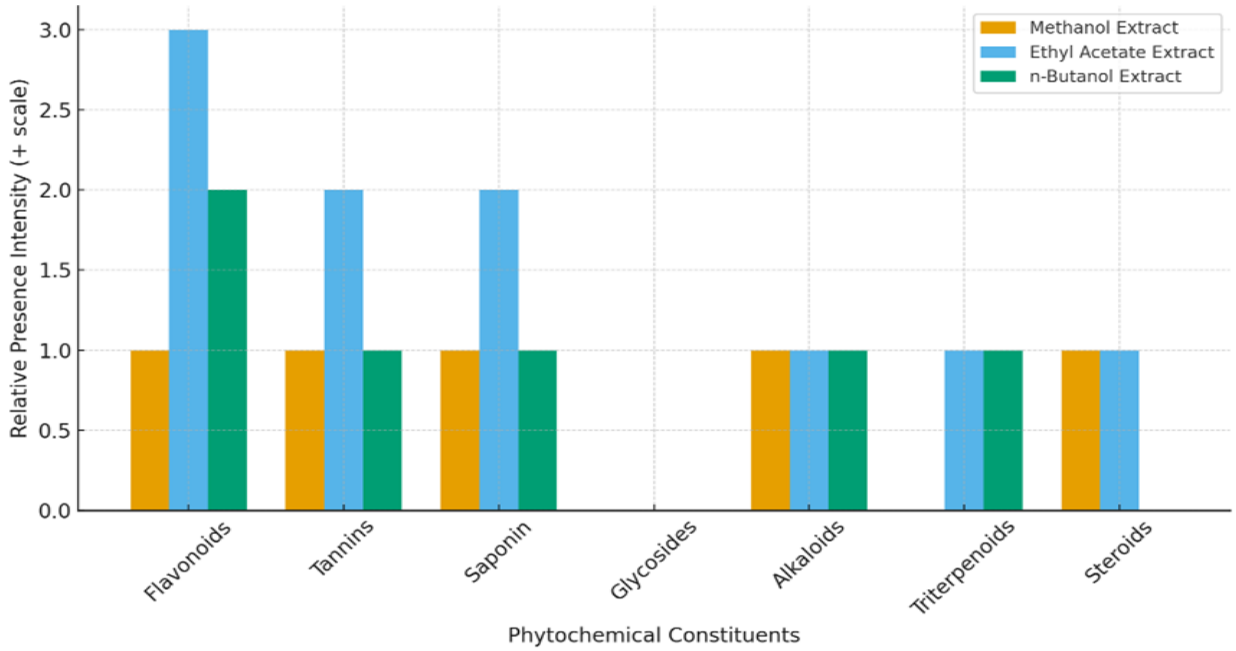

Figure 1. Qualitative phytochemical analysis of methanol extract and fractions of *Gymnema sylvestre.*

caused gross behavioural changes and mortality within 24 hours as well as in the next 14 days, indicating that the median lethal oral dose of the methanol extract and fractions were greater than 5000 mg/kg in Wister albino rats.

Results obtained from the phytochemical screening of both the extract and its solvent fractions are presented in Figure 1. The screening revealed the presence of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, triterpenoids, and steroids, with variations in their occurrence among the extract and the fractions.

Results for the quantitative phytochemical compositions of both the extract and its solvent fractions are presented in Figure 2. The concentrations of the measured phytochemicals differed across the extract and fractions as indicated in Figure 2.

Figure 3 shows the presence of a significant amount of antioxidant vitamins in *G. sylvestre.* Both the extract and the fractions contained measurable levels of antioxidant vitamins as indicated in Figure 3.

Table 1 shows the effect of the ethyl acetate fraction of G. *sylvestre* on serum liver function parameters in Wistar albino rats. Significant changes were observed in Alanine Aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), direct bilirubin (DB), and total bilirubin (TB) following Carbon tetrachloride (CCl_4) administration when compared with the control group.

Table 2 shows the effect of different doses of the ethyl acetate fraction of *G. sylvestre* on serum liver function parameters. Variations in the measured parameters were observed across the treatment groups, as shown in Table 2.

Table 3 shows the effect of the ethyl acetate fraction of *G. sylvestre* on hepatic antioxidant enzymes, Superoxide Dismutase (SOD) and Catalase (CAT) activities in Wister albino rats.

Table 4 shows the effect of ethyl acetate fraction on Glutathione Peroxidase, Malondialdehyde, and Reduced Glutathione on the tissue harvested from the liver of Wister albino rats.

Histopathology

Histology of the liver from the ethyl acetate fraction of *Gymnema sylvestre* leaves of the Wister albino rat is shown in Plate 1. Plate 1.1 (control group 1) shows normal hepatic architecture with intact hepatocytes and central veins. Plate1. 2 (control group 2, CCl_4) reveals extensive hepatic injury marked by inflammatory infiltration, ballooning degeneration, and disrupted cell morphology. Plate 1.3 (control group 3, CCl_4 + Silymarin) presents notable regenerative features, suggesting partial recovery and hepatocyte normalisation. Plate 1.4 (treated groups 1.4–1.6) displays dose-dependent restoration, with group 1.6 exhibiting a near-complete recovery resembling the control group, while lower doses (100 mg and 200 mg) show intermediate improvement.

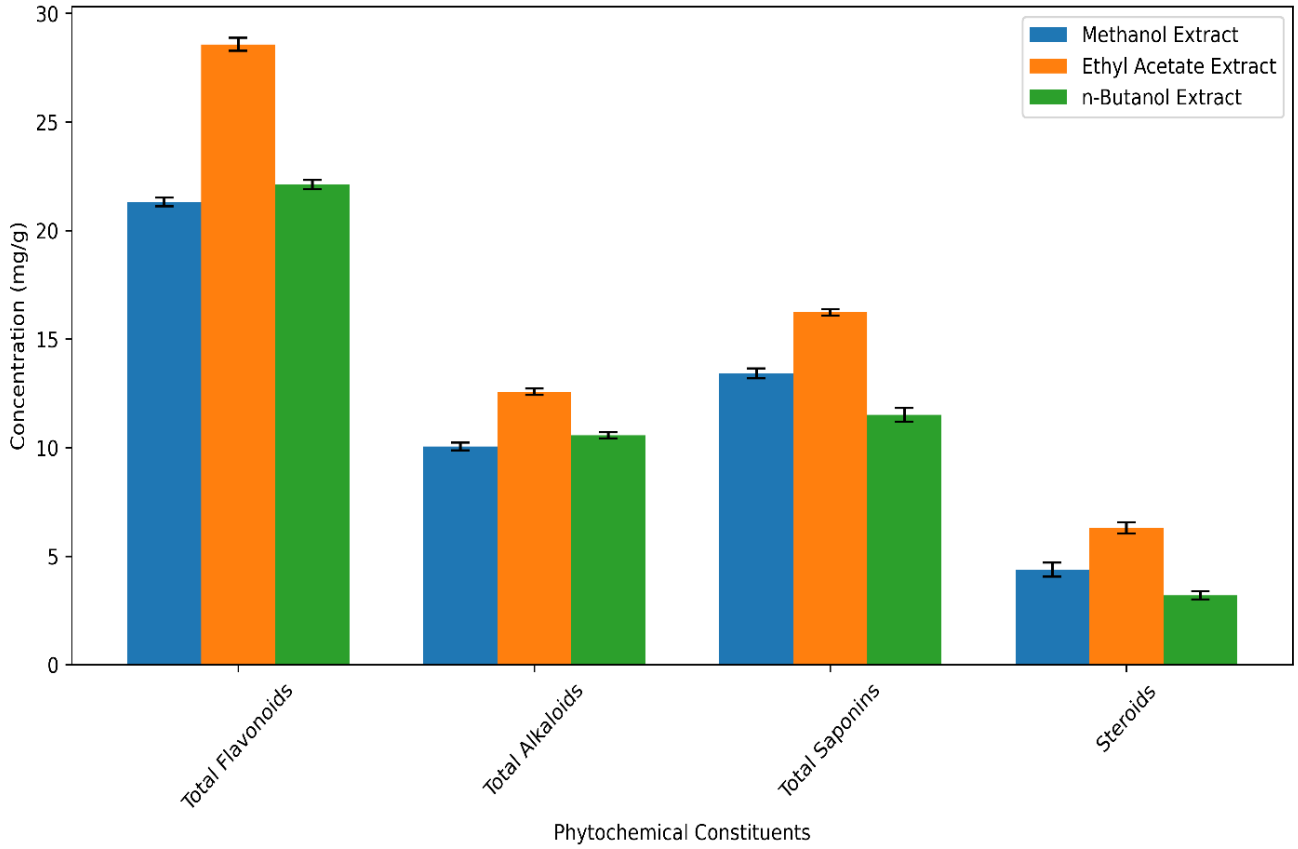

Figure 2. Quantitative phytochemicals of methanol extract and fractions of *G. sylvester*.

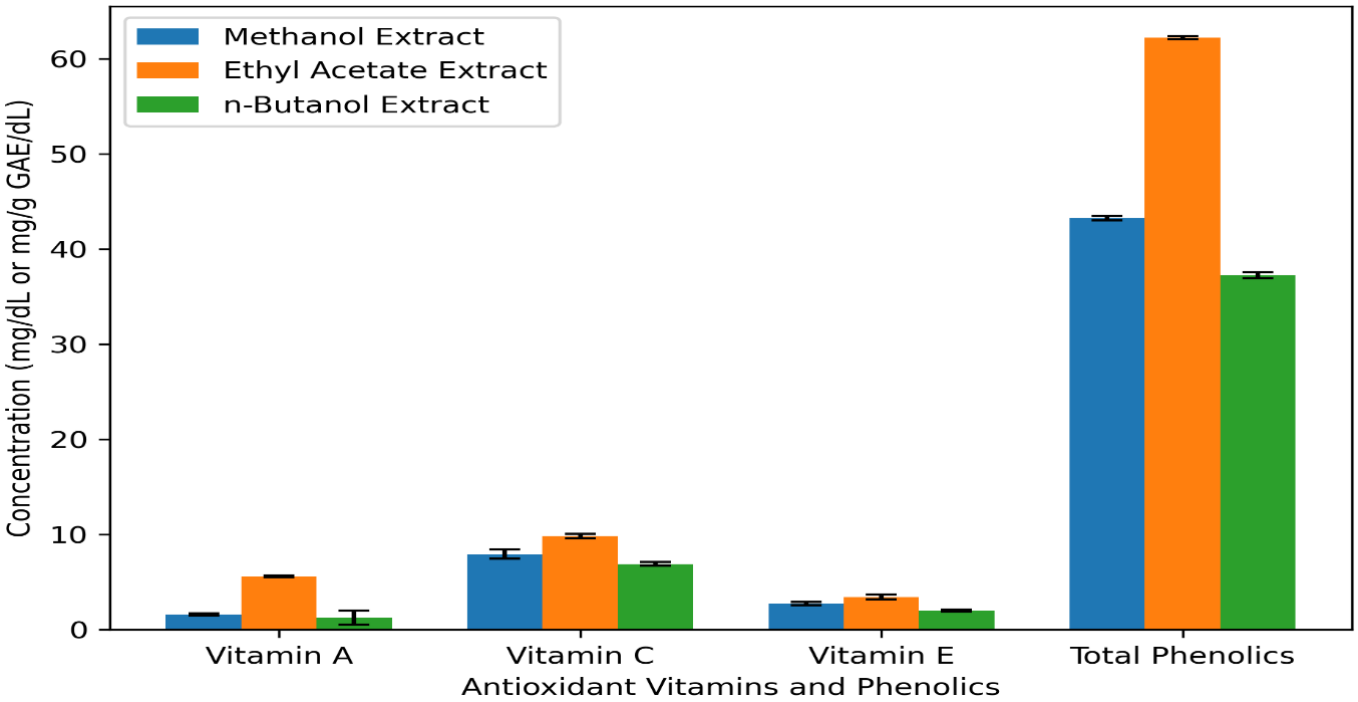

Figure 3. Antioxidant vitamins of methanol extract and fractions of *Gymnema sylvestre*

Table 1. Effect of serum liver function test from ethyl acetate fraction of *G. sylvester* leaf in Wistar albino rats.

Parameter (n=5)	GRP 1 (CTR)	GRP 2 (CCL4)	GRP 3 (Sylimarin)
ALT (IU/L)	33.3 ± 0.55^{a}	142.4 ± 0.21^{e}	58.2 ± 0.10^{b}
AST (IU/L)	81.7 ± 2.05^{a}	224.8 ± 3.90^{e}	154.5 ± 0.35^{b}
TP (g/dL)	6.3 ± 0.10^{a}	11.0 ± 0.02^{e}	8.2 ± 0.10^{b}
ALB (g/dL)	4.2 ± 0.03^{a}	10.4 ± 0.23^{e}	5.7 ± 0.10^{b}
DB (mg/dL)	0.4 ± 0.06^{a}	2.01 ± 0.01^{e}	1.1 ± 0.03^{b}
TB (mg/dL)	0.6 ± 0.08^{a}	2.83 ± 0.64^{e}	2.3 ± 0.25^{b}

Superscripts (a-e) indicate statistical groupings. Values with different superscripts in the same row are significantly different ($p<0.05$) based on Turkey's post-hoc test.

Table 2. Effect of different concentrations of ethyl acetate fraction of *G. sylvestre* leaves on serum liver function parameters in Wistar albino rats.

Parameter (n=5)	GRP 4 (100mg)	GRP 5 (200mg)	GRP 6 (400mg)
ALT (IU/L)	141.6 ± 0.50^{e}	139.8 ± 0.55^{d}	134.6 ± 3.43^{c}
AST (IU/L)	221.1 ± 1.15^{e}	193.0 ± 8.23^{c}	186.8 ± 0.56^{d}
TP (g/dL)	10.6 ± 0.25^{d}	10.1 ± 0.06^{c}	9.9 ± 0.06^{c}
ALB (g/dL)	9.3 ± 0.20^{d}	8.5 ± 0.10^{c}	8.0 ± 0.45^{c}
DB (mg/dL)	1.7 ± 0.10^{d}	1.53 ± 0.29^{c}	1.23 ± 0.16^{c}
TB (mg/dL)	3.1 ± 0.08^{e}	3.1 ± 0.02^{e}	2.67 ± 0.06^{c}

Superscripts (a-e) indicate statistical groupings. Values with different superscripts in the same row are significantly different ($p<0.05$) based on Turkey's post-hoc test.

Table 3. Effect of antioxidant enzyme activities from ethyl acetate fraction of *Gymnema sylvester* leaf on the liver of the Wistar albino rat.

Groups (n=5)	SOD (U/mg)	CAT (U/mg)
Group 1 (Control)	12.3 ± 0.10^{a}	65.3 ± 0.20^{a}
Group 2 (CCL4)	5.43 ± 0.15^{d}	25.3 ± 0.25^{d}
Group 3 (Syimarin)	11.4 ± 0.25^{b}	24.3 ± 0.06^{d}
Group 4 (100mg/kg)	6.47 ± 0.20^{d}	26.5 ± 0.21^{c}
Group 5 (200mg/kg)	8.70 ± 0.10^{c}	28.4 ± 0.15^{c}
Group 6 (400mg/kg)	10.0 ± 0.10^{b}	32.5 ± 0.47^{b}

Superscripts (a–d) indicate statistical groupings. Values with different superscripts in the same column are significantly different (< 0.05) based on Turkey's post-hoc test.

Table 4. Effect of ethyl acetate fraction of *Gymnema sylvestre* on Glutathione Peroxidase (GPx), Malondialdehyde (MDA), and Reduced Glutathione (GSH) levels in the liver tissue of Wistar albino rats.

Groups (n=5)	GPx (U/mg)	MDA (nmol/mg)	GSH (U/mg)
Group 1 (Control)	28.3 ± 0.55^{a}	2.08 ± 0.03^{a}	23.4 ± 0.26^{a}
Group 2 (CCL4)	12.5 ± 0.40^{d}	6.23 ± 0.15^{d}	9.13 ± 0.15^{d}
Group 3 (Syimarin)	16.2 ± 0.10^{c}	4.17 ± 0.06^{c}	20.2 ± 0.15^{b}
Group 4(100 mg/kg)	12.0 ± 0.01^{d}	5.77 ± 0.15^{c}	10.4 ± 0.15^{c}
Group 5 (200 mg/kg)	13.2 ± 0.25^{c}	5.40 ± 0.10^{c}	10.2 ± 0.06^{c}
Group 6 (400 mg/kg)	15.0 ± 0.80^{b}	4.87 ± 0.06^{b}	17.5 ± 0.90^{b}

Superscripts (a–d) indicate statistical groupings. Values with different superscripts in the same column are significantly different ($p < 0.05$) based on Turkey's post-hoc test.

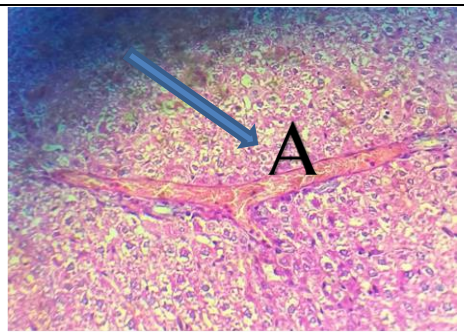

Plate 1.1: Control group I

Plate 1.2: Control group 2 (CCl_4)

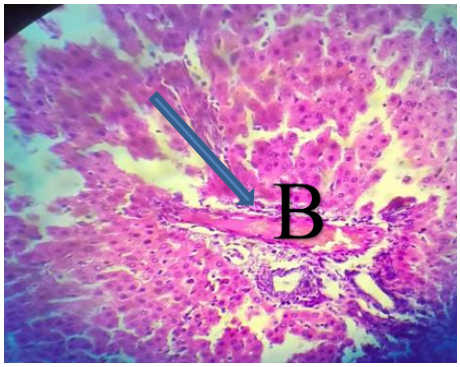

Plate 1.3: Control group 3 (CCl_4 + 100mg/kg Silymarin)

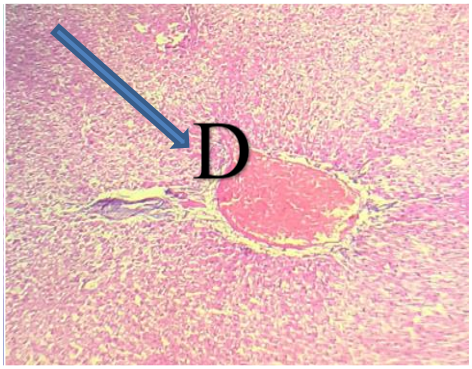

Plate 1.4.1: Treated group 1 **(**(CCl_4 + 100mg/kg ethyl acetate fraction)

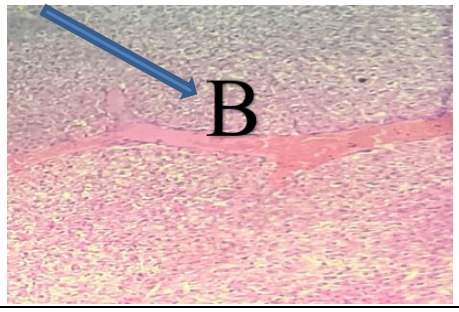

Plate 1.4.2: Treated group 2 (CCl_4 + 200mg/kg ethyl acetate fraction)

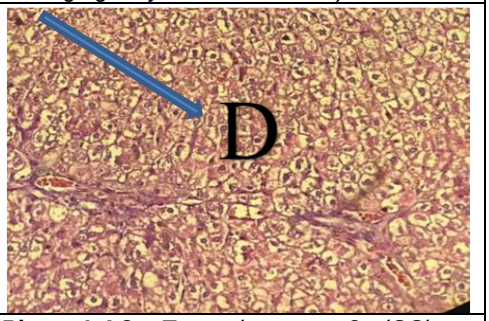

Plate 1.4.3: Treated group 3 (CCl_4 + 400mg/kg ethyl acetate fraction)

Plate 1. Histology of the liver from the ethyl acetate fraction of *Gymnema sylvester* leaves of the Wister albino rat. K**ey**: A = Normal hepatocytes; B = Blood vessels; C = Hepatic inflammation; D = Hepatocyte regeneration.

DISCUSSION

This study examined the hepatoprotective efficacy of *Gymnema sylvestre* leaf extract through bioactivity-guided fractionation using a carbon tetrachloride (CCl_4)-induced liver injury model in albino rats. The median lethal dose (LD_{50}) showed no signs of toxicity from the methanol extract and fractions at the highest dose (500 mg/kg), indicating relative safety in acute exposure studies. Qualitative phytochemical screening of the plant revealed the presence of flavonoids, saponins, alkaloids, steroids, tannins, and triterpenoids, indicating a diverse array of bioactive constituents with known therapeutic importance (Figure 1). Flavonoids were quantified as the most abundant (28.57 ± 0.30 mg/g), followed by saponins (16.23 ± 0.15 mg/g), alkaloids (12.57 ± 0.15 mg/g), and steroids (6.30 ± 0.26 mg/g) (Figure 2). These phytoconstituents are widely reported to possess antioxidant, anti-inflammatory, and membrane-stabilising activities (Syed *et al*., 2023). Flavonoids and triterpenoid saponins are particularly important for their free radical scavenging capacity and modulation of redox signalling, thereby protecting hepatocytes from chemically induced liver damage (Gonfa *et al.,* 2025).

The methanol extract was also rich in antioxidant vitamins such as vitamin A (1.60 ± 0.10 mg/dL), vitamin C (7.93 ± 0.49 mg/dL), and vitamin E (2.73 ± 0.15 mg/dL), alongside a high total phenolic content (43.27 ± 0.21 mg GAE/dL) (Figure 3). These bioactive compounds play significant roles in combating oxidative stress through free radical scavenging and mitigation of oxidative stress–mediated liver injury (Mittal *et al.,* 2025; Abe *et al*., 2021). Vitamins C and E have been shown to enhance endogenous antioxidant defense systems, including superoxide dismutase (SOD) and catalase (CAT), while inhibiting lipid peroxidation during xenobiotic-induced hepatotoxicity (He *et al.,* 2021; Ryan *et al.,* 2010).

Administration of CCl_4 to Wistar albino rats significantly elevated liver enzyme markers (ALT, AST), bilirubin indices (TB, DB), and reduced protein parameters (TP, ALB), reflecting severe hepatic dysfunction due to oxidative stress and membrane destabilisation (Table 1). Such biochemical alterations are well documented in CCl_4 hepatotoxicity models (Moustafa *et al.,* 2021). Treatment with silymarin and graded doses of the ethyl acetate fraction of *G. sylvestre* restored these parameters in a dose-dependent manner ($p<0.005$), with the 400 mg/kg dose showing the most pronounced therapeutic effect (Table 2). This aligns with previous findings demonstrating the hepatoprotective potential of *G. sylvestre* due to its rich phytochemical composition (Oshobu *et al*., 2018). Antioxidant enzyme analysis further revealed that CCl_4 administration markedly suppressed endogenous antioxidant defences, including SOD, CAT, glutathione peroxidase (GPx), and reduced glutathione (GSH), while significantly elevating malondialdehyde (MDA) levels, a key biomarker of lipid peroxidation. These findings are consistent with the established mechanism of CCl_4-induced hepatotoxicity, which involves cytochrome P450-mediated bioactivation to trichloromethyl and trichloromethyl peroxy radicals, leading to oxidative degradation of membrane lipids and cellular macromolecules (Yang *et al.*, 2019; Pulungan *et al.*, 2025). Administration of the ethyl acetate fraction significantly restored antioxidant enzyme activities and reduced MDA levels, particularly at 400 mg/kg, suggesting attenuation of oxidative stress and preservation of hepatocellular membrane integrity (Table 3). This observation is consistent with previous hepatoprotective studies involving flavonoid-rich plant extracts (Kim *et al.,* 2024; Chen *et al.,* 2025). The similarity of these effects to silymarin further suggests synergistic interactions among phytochemicals such as flavonoids, saponins, phenolics, and antioxidant vitamins.

Conclusion

The findings of this study demonstrate that the ethyl acetate fraction of *Gymnema sylvestre* methanol leaf extract possessed significant hepatoprotective activity, effectively mitigating CCl_4-induced hepatic damage. The protective effects were supported by dose-dependent restoration of liver biochemical markers and antioxidant enzyme systems. The 400 mg/kg dose exhibited results comparable to silymarin, validating the therapeutic potential of *Gymnema sylvester* as a natural hepatoprotective agent. These effects are attributed to the presence of flavonoids, saponins, vitamins, and phenolics, which act synergistically to stabilise hepatic membranes, reduce oxidative stress, and enhance endogenous antioxidant defence mechanisms. The histopathological images support the study's hypothesis that *Gymnema sylvestre* has hepatoprotective properties. The extract's efficacy is likely due to its antioxidant and anti-inflammatory phytochemicals (e.g., flavonoids, saponins), which counteract CCl_4-induced oxidative stress and liver injury. These findings align with the biochemical results (reduced ALT, AST, and ALP) and suggest potential therapeutic applications for liver diseases. Overall, the findings of this study demonstrate that the ethyl acetate fraction of *G. sylvestre* leaf possessed substantial hepatoprotective activity, mediated through antioxidant defence enhancement, inhibition of lipid peroxidation, and stabilisation of liver functional architecture.

Recommendations

Further isolation of active compounds**,** additional chromatographic and spectroscopic studies should be conducted to isolate, purify, and structurally characterise the specific bioactive constituents responsible for the observed hepatoprotective activity. Also, molecular and cellular investigations are recommended to identify the precise mechanisms of action, including gene expression pathways, antioxidant signalling systems, and anti-inflammatory cascades influenced by the extract.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abe, R. A. M., Masroor, A., Khorochkov, A., Prieto, J., Singh, K. B., Nnadozie, M. C., Abdal, M., Shrestha, N., & Mohammed, L. (2021). The role of vitamins in non-alcoholic fatty liver disease: a systematic review. *Cureus*, *13*(8), e16855.

Chen, Y., Mei, Y. Q., Hou, L., & Li, K. J. (2025). Therapeutic potential of plant-derived natural products against drug-induced liver injury. *Frontiers in pharmacology*, *16*, 1652860.

Devarbhavi, H., Asrani, S. K., Arab, J. P., Nartey, Y. A., Pose, E., & Kamath, P. S. (2023). Global burden of liver disease: 2023 update. *Journal of Hepatology*, *79*(2), 516-537.

Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971). Albumin

standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, *31*(1), 87-96.

Ercan, N., & Koçkaya, M. (2017). Determination of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels in Kangal dogs with maternal cannibalism. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(12), 1493-1496.

Gonfa, Y. H., Bachheti, A., Semwal, P., Rai, N., Singab, A. N., & Bachheti, R. K. (2025). Hepatoprotective activity of medicinal plants, their phytochemistry, and safety concerns: A systematic review. *Zeitschrift für Naturforschung C*, *80*(3-4), 61-73.

He, L., He, T., Farrar, S., Ji, L., Liu, T., & Ma, X. (2017). Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*, *44*(2), 532-553.

Jargar, J. G., Hattiwale, S. H., Das, S., Dhundasi, S. A., & Das, K. K. (2012). A modified simple method for determination of serum α-tocopherol (vitamin E). *Journal of Basic and Clinical Physiology andPharmacology*, *23*(1), 45-48.

Jendrassik, L. (1938). Vereinfachte photometrische methoden zur bestimmung des blutbilirubins. *Biochem z*, *297*, 81-89.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysis*, *21*(2), 130-132.

Kim, M., Jee, S. C., & Sung, J. S. (2024). Hepatoprotective effects of flavonoids against benzo [a] pyrene-induced oxidative liver damage along its metabolic pathways. *Antioxidants*, *13*(2), 180.

Li, S., Tan, H. Y., Wang, N., Zhang, Z. J., Lao, L., Wong, C. W., & Feng, Y. (2015). The role of oxidative stress and antioxidants in liver diseases. *International journal of molecular sciences*, *16*(11), 26087-26124.

Lowry, O., Rosebrough, N., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265-275.

Mittal, G., Prashanth, A., Dhali, A., Prasad, R., Yogesh, S., Nurani, K. M., & Găman, M. A. (2025). Plant extracts with antioxidant and hepatoprotective benefits for liver health: A bibliometric analysis of drug delivery systems. *World Journal of Gastroenterology*, *31*(18), 105836.

Moustafa, M. A., Ghareeb, D. A., El-Demellawy, M. A., & Elsayed, M. M. (2021). Berberis vulgaris aqueous extract prevention of carbon tetrachloride induced hepatotoxicity and lipopolysaccharides/paracetamol induced hepatitis in rats. *Journal of Medicinal Plants Research*, *15*(5), 206-214.

Mouleeswaran, K. S., Varghese, J., & Reddy, M. S. (2023). *Atlas of Basic Liver Histology for Practicing Clinicians and Pathologists*. Springer. Retrieved from https://doi.org/10.1007/978-981-99-5762-0.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, *83*(3), 770-803.

Organisation for Economic Co-operation and Development (OECD). (2022). *Test No. 425: Acute Oral Toxicity – Up-and-Down Procedure*. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. Retrieved from https://doi.org/10.1787/9789264071049-en.

Oshobu, M. L., Alhassan, A. J., Mansura, A., Ononamadu, C. J., Ibrahim, A. (2018). Hepatoprotective Potential of methanolic extract of *Gymnema sylvestre* leaves on acetaminophen-induced liver damage in Wistar strain albino rats. *Saudi Journal of Biomedical Research*, *3*(1), 1-8.

Pulungan, I. Y., Girsang, E., & Lubis, Y. E. P. (2025). Protective Role of Centella asiatica Extract Against Carbon Tetrachloride-Induced Hepatic Damage: A Biochemical and Ultrasonographic Study. *Pharmacognosy Journal*, *17*(6), 760-769.

Rahman, I., Kode, A., & Biswas, S. K. (2006). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature Protocols*, *1*(6), 3159-3165.

Rani, J., Dhull, S. B., Rose, P. K., & Kidwai, M. K. (2024). Drug-induced liver injury and anti-hepatotoxic effect of herbal compounds: a metabolic mechanism perspective. *Phytomedicine*, *122*, 155142.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

Ryan, M. J., Dudash, H. J., Docherty, M., Geronilla, K. B., Baker, B. A., Haff, G. G., Cutlip, R. G., & Alway, S. E. (2010). Vitamin E and C supplementation reduces oxidative stress, improves antioxidant enzymes and positive muscle work in chronically loaded muscles of aged rats. *Experimental Gerontology, 45*(11), 882-895.

Shukla, P. K., Kumari, N., Fatima, T., & Singh, H. (2023). Evaluation of bio-control agents for the management of guava decline. *Journal of Eco-Friendly Agriculture*, *18*(1), 193-199.

Tiwari, P., Mishra, B. N., & Sangwan, N. S. (2014). Phytochemical and pharmacological properties of Gymnema sylvestre: an important medicinal plant. *BioMed Research International*, *2014*(1), 830285.

Trefts, E., Gannon, M., & Wasserman, D. H. (2017). The liver. *Current Biology*, 27(21), R1147-R1151.Retrieved from https://doi.org/10.1016/j.cub.2017.09.019.

World Health Organisation (WHO) (2023). *World health statistics 2023:* Monitoring health for the Sustainable Development Goals (SDGs). World Health Organisation. Retrieved from https://iris.who.int/handle/10665/367912.

Yang, C. L., Lin, Y. S., Liu, K. F., Peng, W. H., & Hsu, C. M. (2019). Hepatoprotective mechanisms of taxifolin on carbon tetrachloride-induced acute liver injury in mice. *Nutrients, 11*(11), 2655.