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ORIGINAL RESEARCH ARTICLE

Neurobehavioral and Toxicological Evaluation of Guiera senegalensis J.F. Gmel. Methanolic Leaf Extract in Wistar Rats

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Abstract

Background Guiera senegalensis (G. senegalensis) is widely used in African ethnomedicine for treating neurological and inflammatory disorders, yet its safety and neuropharmacological profile remain incompletely defined. This study evaluated the phytochemical composition, neurobehavioral effects, and toxicity profile of the methanolic leaf extract of G. senegalensis in Wistar rats.

Methods Phytochemical screening was conducted using standard protocols. Acute toxicity was assessed in accordance with the Organisation for Economic Co-operation and Development (OECD) 423 guidelines at 2000 mg/kg, while subacute toxicity was evaluated through 28-day oral administration (125–500 mg/kg) with hematological, biochemical, and histopathological assessments. Neurobehavioral effects were investigated using ketamine-induced psychosis models (open field and Y-maze tests).

Results Phytochemical analysis revealed tannins, saponins, flavonoids, glycosides, and steroids. Acute toxicity testing showed no mortality up to 2000 mg/kg. In the open field test, methanolic leaf extract of G. senegalensis modestly attenuated ketamine-induced hyperlocomotion, with 800 mg/kg exerting the strongest antipsychotic-like effect. In the Y-maze, intermediate doses (200–400 mg/kg) preserved working memory, while 800 mg/kg caused severe cognitive impairment. Subacute administration induced variable hematological and biochemical alterations, including elevated platelet indices, increased Aspartate Aminotransferase, and hyperbilirubinemia at 250–500 mg/kg. Histopathology revealed progressive hepatocellular degeneration and renal tubular necrosis.

Conclusion Methanolic leaf extract of G. senegalensis exhibits antipsychotic-like and cognition-modulating properties, but produces progressive hepatotoxicity and nephrotoxicity upon subacute exposure. The most consistent evidence of toxicity was derived from histopathology, whereas hematological and biochemical markers showed non-monotonic responses. These findings suggest a narrow therapeutic margin for the methanolic leaf extract of G. senegalensis and highlight the need for quantitative phytochemical profiling, mechanistic studies, and dose–response bridging to define its pharmacological potential and safety.

Keywords Antipsychotic, Guiera senegalensis, Hepatotoxicity, Nephrotoxicity, Neurobehavior, Phytochemistry

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1 Introduction

Medicinal plants remain a cornerstone of traditional healthcare, especially in sub-Saharan Africa, where more than 70% of the population relies on herbal remedies for primary health needs.[1] Guiera senegalensis, commonly known as “Sabara,” is a multipurpose shrub distributed across the Sahelian region and widely employed in ethnomedicine for the management of fever, epilepsy, psychosis, microbial infections, and inflammatory disorders.[2] Its leaves and roots are traditionally decocted or macerated and used as remedies for neurological and gastrointestinal ailments, reflecting its pharmacological versatility.[3] Phytochemical investigations of G. senegalensis have identified flavonoids, tannins, alkaloids, saponins, and steroids, which possess antioxidant, anti-inflammatory, and neuroprotective activities.[2,4,5] Recent experimental evidence has suggested that G. senegalensis extracts may modulate cholinergic transmission, attenuate oxidative stress, and improve memory performance in cognitive deficit models, supporting its ethnomedicinal application in neuropsychiatric disorders.[5,6] Despite these promising reports, concerns remain regarding its toxicological safety.

Neurobehavioral disturbances such as psychosis and cognitive impairment are frequently modeled in rodents using ketamine, an NMDA receptor antagonist that reproduces schizophrenia-like hyperlocomotion and working memory deficits. Such models allow the evaluation of candidate agents for antipsychotic and cognition-modulating potential.[7] Concurrently, standardized acute and subacute toxicity testing provides critical insights into the safety margin of phytomedicines, ensuring translational relevance. This study, therefore, aimed to comprehensively evaluate the phytochemical constituents, acute and subacute toxicity, and neurobehavioral effects of the methanolic leaf extract of G. senegalensis (GSME) in Wistar rats. By integrating phytochemical profiling, behavioral assays, hematological and biochemical analyses, and histopathological evaluations, this work provides a mechanistic and safety-oriented appraisal of GSME’s pharmacological potential.

2 Methods

Experimental Animals

Animals weighing 150-180 g were purchased from the animal housing facility of the Department of Pharmacology, Sa’adu Zungur University, Bauchi State. The animals were maintained under standard laboratory conditions in accordance with the approved protocol of the Faculty of Basic Medical Sciences Research and Ethics Committee. The animals were fed a commercial diet (Vital Feed) and water ad libitum.

Plant Material Collection and Identification

The fresh leaves of G. senegalensis were collected from Gadau village in Itas/Gadau LGA of Bauchi State, and identified at the Herbarium Unit of the Department of Biological Sciences, Faculty of Science, Sa’adu Zungur University, Bauchi State (voucher number 00157).

Plant Material Preparation and Extraction

The leaves were thoroughly washed, air-dried, and ground into a coarse powder. The coarse powder (150 g) was macerated in 1 L of 70% methanol (v/v) for 72 hours. The mixture was stirred every 12 hours and filtered after 72 hours using the Whatman filter paper. The filtrate was kept in an oven at 45-50 °C, and the resulting extract was labelled and stored in a container for further use.

Preliminary Phytochemical Analysis

The phytoconstituents present in GSME were identified using established qualitative phytochemical tests, as described by Trease and Evans.[8]

- **Alkaloids**

The extract (2 mL) was acidified with 1% hydrochloric acid (HCl) and filtered. The filtrate was treated separately with Dragendorff’s reagent and Mayer’s reagent. Formation of an orange-red precipitate (Dragendorff’s) or a creamy-white precipitate (Mayer’s) indicated the presence of alkaloids.

- **Tannins**

Extract solution (2 mL) was treated with 1% ferric chloride solution. A blue-black or greenish-black coloration was taken as evidence of hydrolysable or condensed tannins, respectively.

- **Saponins**

The frothing test was employed. About 5 mL of extract solution was vigorously shaken with 10 mL of distilled water in a test tube. Persistent froth (≥ 1 cm height for > 15 min) after standing was considered positive for saponins.

- **Flavonoids**

The extract (2 mL) was treated with 1 mL of 10% sodium hydroxide (NaOH). An intense yellow color that disappeared on the addition of dilute HCl indicated the presence of flavonoids.

- **Glycosides**

For the Keller–Killiani test, 2 mL of extract was mixed with 1 mL of glacial acetic acid containing a trace of $FeCl_3$. Concentrated H_2SO_4 was carefully added down the side of the test tube. Formation of a brown ring at the interface suggested the presence of cardiac glycosides.

- **Steroids**

Liebermann–Burchard test was used. 2 mL of extract was mixed with 2 mL of chloroform, followed by the addition

of 2 mL acetic anhydride and 1 mL concentrated H_2SO_4. A blue-green or violet coloration at the interface indicated the presence of steroids.

Acute Toxicity Studies

Six animals were used for the acute toxicity studies in accordance with the guidelines outlined by Co-operation and Development (OECD) 423.[9] The animals were randomly divided into two groups (n = 3). Group 1 served as the control and received normal saline (5 mL/kg), whereas Group 2 was administered GSME at 2000 mg/kg body weight. The animals were observed over 14 days for signs of toxicity or notable behavioral changes.

Antipsychotic Evaluation

Open Field Test (OFT)

The OFT was employed to evaluate the locomotor and exploratory behavior following ketamine administration. The OFT apparatus consisted of a square wooden arena measuring 75 × 75 cm, with 40 cm-high walls; the floor was divided into 16 equal squares, with a designated central zone.[10]

Eighteen animals were randomly allocated into six groups (n = 3). Group I received normal saline (5 mL/kg, p.o.) and served as the control, while Group II was administered ketamine (25 mg/kg, i.p.) only. Group III received chlorpromazine (3 mg/kg, p.o.) as the standard antipsychotic. At the same time, Groups IV, V, and VI were treated orally with GSME at doses of 200, 400, and 800 mg/kg, respectively, one hour before ketamine injection. Chlorpromazine was administered 30 minutes before the ketamine injection. Each rat was placed individually in the center square and observed for a 5-minute session under uniform lighting conditions. Behavioral activity was recorded using a video camera mounted above the arena and subsequently analyzed. The number of line crossings, frequency of center-square entries, and time spent in the center, rearing frequency, and freezing time were recorded.[11]

Y-maze Spontaneous Alternation

The same grouping and treatment protocol were applied as described for the OFT, with six groups (n = 3) receiving saline, ketamine, chlorpromazine, or GSME (200, 400, or 800 mg/kg). The Y-maze spontaneous alternation task was used to assess spatial working memory and cognitive flexibility. The apparatus consisted of three identical arms, each measuring 40 × 10 × 15 cm, arranged at 120° angles from each other. At the start of the test, each rat was placed at the center arm and allowed to explore the maze freely for 5 minutes. Arm entries were defined as all four paws entering an arm, and both the total number of entries and the sequence of entries were recorded. Spontaneous alternation behavior was calculated as the percentage of successive entries into all three arms without repetition, using the formula:

$$\%SA = \frac{Number\ of\ alternations}{Total\ arm\ entries - 2} \times 100$$

Subacute Toxicity Studies

The subacute toxicity of GSME was studied for 28 days in female Wistar rats. The body weight of the experimental animals was recorded before the study and at 7-day intervals thereafter. Hematological parameters and histology studies were respectively conducted at the Laboratories of the Department of Physiology and Anatomy, Sa'adu Zungur University, Bauchi State, while the liver and kidney function parameters were evaluated at the Department of Chemical Pathology, Federal University of Health Sciences Teaching Hospital, Azare, Bauchi State.

Determination of Serum Direct Bilirubin

Serum direct bilirubin concentration was determined using the Jendrassik and Grof colorimetric method.[12] This assay is based on the reaction between conjugated (direct) bilirubin and a diazotized sulfanilic acid reagent, forming a stable blue-green azobilirubin complex. The intensity of the color produced, which is directly proportional to the concentration of direct bilirubin, was measured spectrophotometrically at 546 nm.

Total Bilirubin Determination

The total serum bilirubin was estimated using the classical colorimetric method. The method is based on the formation of a blue-green azobilirubin complex following the reaction of bilirubin with diazotized sulfanilic acid in the presence of an accelerator. The complex is stable and measurable by spectrophotometry, enabling accurate quantification of total bilirubin in serum.[13]

Determination of Serum Aspartate Aminotransferase (AST)

Serum AST was estimated using the colorimetric endpoint method.[13] This method involves the enzymatic conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate, catalyzed by AST. The oxaloacetate formed reacts with reagents to form a colored complex, whose intensity is measured spectrophotometrically and is directly proportional to AST activity.

Determination of Serum Alkaline Phosphatase (ALP)

The determination of serum ALP activity was carried out using the p-nitrophenol colourimetric endpoint method with Teco reagents.[14] This method is based on the hydrolysis of p-nitrophenyl phosphate by ALP, releasing p-nitrophenol, which forms a yellow-colored complex

under alkaline conditions. The intensity of the color produced is directly proportional to the ALP activity in the sample and is measured spectrophotometrically at 580 nm.

Determination of Alanine Aminotransferase (ALT)

The activity of serum ALT was determined using the reagent blank method. This colorimetric assay is based on ALT catalyzing its substrate, producing pyruvate, which reacts with specific reagents to form a colored complex. The color intensity is directly proportional to enzyme activity and is measured spectrophotometrically.[15]

Determination of Serum Albumin

Serum albumin concentration was determined using the bromocresol green (BCG) dye-binding method. In this assay, albumin binds specifically to BCG at acidic pH, forming a green-colored complex whose intensity is directly proportional to the albumin concentration in the sample.[16]

Determination of Total Protein

Serum total protein concentration was determined using the Biuret colorimetric method. The principle is based on the reaction of peptide bonds with copper(II) ions in alkaline solution, which forms a violet-colored complex measurable at 546 nm.[17]

Determination of Serum Bicarbonate

Serum bicarbonate concentration was determined by titrimetric analysis. The principle is based on the neutralization of bicarbonate by a known excess of HCl, followed by back-titration of the residual HCl with NaOH.[18]

Determination of Serum Potassium

Potassium was determined by the colourimetric endpoint method (Teco reagents). The principle is based on the formation of a colored complex between potassium ions and a chromogenic reagent, measurable at 500 nm.[19]

Determination of Blood Urea Nitrogen (BUN)

Serum urea concentration was determined by the enzymatic (urease) method using Biosystem reagents. Urea is hydrolyzed by urease to form ammonia and carbon dioxide, and the ammonia reacts with a chromogenic reagent to produce a colored complex measurable at 630 nm.

Determination of Serum Creatinine

Serum creatinine concentration was determined using the Jaffe's colorimetric method. This method is based on the principle that creatinine reacts with alkaline picrate solution to form an orange-red complex. The intensity of this color, which develops at alkaline pH, is directly proportional to the concentration of creatinine in the sample and is measured spectrophotometrically.[20]

Determination of Serum Chloride

Chloride ion concentration in serum was determined using the colorimetric endpoint method with Teco reagent. This method involves the formation of a colored complex between chloride ions and mercury(II) thiocyanate in the reagent, which is then measured spectrophotometrically. The color intensity is directly proportional to the chloride concentration in the sample.[21]

Determination of Serum Sodium

Serum sodium concentration was measured using an Ion-Selective Electrode (ISE) analyser, a widely adopted method in clinical laboratories due to its accuracy, speed, and minimal sample preparation. The ISE method relies on a sodium-selective membrane that responds specifically to sodium ions in solution, generating an electrical potential proportional to their concentration. This potential is interpreted by the analyzer and displayed as a quantitative sodium value.[22]

Histopathological Examination

Liver and kidney tissue histopathological slides were prepared at the Histology Laboratory, Department of Anatomy, Sa'adu Zungur University, Bauchi State. The tissues were processed using standard histological techniques as outlined by Kiernan.[23] The tissues were processed for hematoxylin and eosin staining, and tissue analysis was conducted qualitatively, emphasizing morphology, architecture, and cytological structures.

Statistics

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons post-hoc test to compare each treatment group against the control. using SPSS v25. Data are presented as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

3 Results

Percentage Yield

A crystalline black powdered extract was obtained. The extraction of 150 g of leaves of G. senegalensis produced 20.44 g of the extract with a percentage yield of 13.63%.

Qualitative Phytochemical Analysis

Phytochemical screening of the GSME revealed the presence of tannins, saponins, flavonoids, glycosides, and steroids, while alkaloids were absent, as shown in Table 1.

Table 1 Phytoconstituents of GSME

Phytoconstituents	Observation	Inference
Alkaloids	–	Not detected
Tannins	+	Detected
Saponins	+	Detected
Flavonoids	+	Detected
Glycosides	+	Detected
Steroids	+	Detected

Acute Toxicity Study

Oral administration of 2000 mg/kg of GSME did not result in mortality during the 14-day observation period. No quantifiable signs of toxicity were recorded. A transient reduction in food intake was qualitatively observed on day 1, but this did not persist, and all animals maintained normal activity and appearance throughout the period.

Antipsychotic Evaluation of GSME Using OFT

In the OFT, ketamine markedly increased locomotor activity and reduced freezing time compared to the control, reflecting psychosis-like hyperactivity. Chlorpromazine attenuated these effects, confirming its antipsychotic action. GSME reduced ketamine-induced hyperactivity, with the 800 mg/kg dose showing the strongest attenuation. GSME at 200–400 mg/kg reduced ketamine-induced hyperlocomotion; however, the effects were characterized by greater within-group variability, resulting in a less consistent overall response than the 800 mg/kg dose (Table 2).

Antipsychotic Evaluation of GSME Using Y-maze

In the Y-maze, ketamine significantly impaired working memory, as indicated by reduced alternations and lower spontaneous alternation percentage. Chlorpromazine improved memory performance despite reduced exploratory entries. GSME at 200–400 mg/kg produced modest increases in alternation counts relative to the ketamine-only group, but values remained substantially lower than control. The percent spontaneous alternation values for the GSME groups were highly variable and did not provide clear evidence of restoration to control performance. Importantly, arm entries were reduced in the GSME groups (significantly for GSME 200 vs control), which likely confounds interpretation of alternation measures. Overall, GSME at 200–400 mg/kg produced inconclusive, modest effects on working memory in the Y-maze, rather than a clear preservation of performance at control levels (Table 3).

Table 3 Cognitive effects of GSME in the Y-maze test of ketamine-treated Wistar rats

Treatment group	No. of arm entries	No. of alternations	Spontaneous alternation (%)
NS (Control)	14.00 ± 0.00	8.67 ± 0.88	72.20 ± 7.35
Ketamine only	7.67 ± 1.76	3.00 ± 0.58	57.03 ± 6.61
Ketamine + CPZ	7.67 ± 2.03	4.33 ± 1.45	81.50 ± 9.79
GSME 200	5.33 ± 1.45*	4.33 ± 1.45	77.67 ± 39.99
GSME 400	6.00 ± 2.31	4.00 ± 2.31	66.67 ± 33.33
GSME 800	1.67 ± 0.33*	0.00	0.00

Values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test to compare each treatment group to the control. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract; CPZ: Chlorpromazine.

Subacute Toxicity Study

Body Weight Changes

Body weights of the experimental animals were recorded before treatment and subsequently at weekly intervals for 28 days. All treatment groups showed an overall increase in body weight by the end of the study period, as shown in Table 4. No mortality was recorded during the 28-day study in any of the treatment or control groups.

Hematological Parameters

Administration of the GSME resulted in dose-dependent variations at 125 and 250 mg/kg in several hematological parameters (Table 5). Total white blood cell (WBC) count showed no significant change across groups, though an elevation was observed at the 250 mg/kg dose. Lymphocyte (LYM) counts decreased notably at 250 and 125 mg/kg doses, while granulocyte (GRA) counts were elevated at 125 mg/kg. A significant reduction in the MID (monocyte and other rare cell types) count was recorded in the 500 mg/kg group. Among red cell indices, hemoglobin (HGB) concentration increased significantly in the 500 mg/kg group; however, mean corpuscular

Table 2 Behavioral effects of GSME in the OFT of ketamine-treated Wistar rats

Treatment group	Line crossing	Center square entry	Time spent in center (s)	Rearing	Freezing (s)
NS (Control)	3.00 ± 0.56	0.00	0.00	0.67 ± 0.33	164.00 ± 37.53
Ketamine only	72.00 ± 2.31*	1.67 ± 0.88	1.78 ± 0.88	3.00 ± 0.56	7.00 ± 0.56*
Ketamine + CPZ	36.33 ± 1.45	0.00	0.00	2.00 ± 0.58	35.67 ± 3.18*
GSME 200	49.00 ± 13.86*	0.00	0.00	2.00 ± 0.58	76.33 ± 1.45*
GSME 400	65.33 ± 11.26*	0.67 ± 0.33	2.00 ± 1.16	4.00 ± 1.16	47.33 ± 7.80*
GSME 800	58.00 ± 1.73*	0.00	0.00	2.33 ± 0.88	68.00 ± 6.93*

Values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test to compare each treatment group to the control. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract; CPZ: Chlorpromazine.

Table 4 Weekly body weight (g) of rats treated with GSME

Time Point	GSME 500	GSME 250	GSME 125	NS (Control)
Day 1	132.80 ± 9.87	142.60 ± 8.05	151.40 ± 0.93	137.00 ± 7.09
Day 7	141.40 ± 6.24	144.40 ± 15.04	157.40 ± 3.41	156.80 ± 14.15
Day 14	149.60 ± 5.99	134.80 ± 9.04*	157.60 ± 7.26	182.20 ± 16.36
Day 21	147.80 ± 5.96	141.60 ± 8.57	153.20 ± 4.22	170.40 ± 14.62
Day 28	157.60 ± 5.52	147.60 ± 8.96*	161.20 ± 4.35	189.40 ± 17.21

Values are expressed as mean ± SEM. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract.

Table 5 Effects of GSME on hematological parameters in rats

Parameter	NS	GSME 500	GSME 250	GSME 125
WBC ($\times 10^9$/L)	5.38 ± 0.63	5.24 ± 1.10	6.86 ± 1.56	3.93 ± 0.18
LYM ($\times 10^9$/L)	4.68 ± 0.59	4.79 ± 0.95	2.37 ± 0.49	3.24 ± 0.15
MID ($\times 10^9$/L)	0.49 ± 0.12	0.20 ± 0.00*	0.69 ± 0.06	0.48 ± 0.01
GRA ($\times 10^9$/L)	0.25 ± 0.02	0.09 ± 0.00	0.35 ± 0.00	0.63 ± 0.08*
LYM%	87.03 ± 2.82	91.90 ± 1.08	83.30 ± 3.04	83.60 ± 7.62
MID%	7.00 ± 0.06	5.53 ± 0.83	8.85 ± 0.32	11.27 ± 0.20*
GRA%	3.15 ± 0.09	2.58 ± 0.28	7.25 ± 0.55*	1.57 ± 0.15*
RBC ($\times 10^{12}$/L)	5.83 ± 0.16	6.23 ± 0.54	5.72 ± 0.65	6.58 ± 0.03
HGB (g/L)	125.67 ± 3.18	148.00 ± 1.73*	136.67 ± 9.53	127.67 ± 1.45
HCT (%)	32.17 ± 0.66	33.90 ± 2.85	31.90 ± 3.34	35.60 ± 0.35
MCV (fL)	55.57 ± 0.58	54.43 ± 0.27	55.90 ± 0.93	54.00 ± 0.35
MCH (pg)	21.30 ± 0.15	21.77 ± 0.23	21.67 ± 0.38	18.80 ± 0.52*
MCHC (g/L)	383.67 ± 6.17	400.33 ± 3.38	383.33 ± 11.29	367.00 ± 4.04
RDW-SD (fL)	33.50 ± 0.95	33.40 ± 0.87	33.73 ± 0.57	32.20 ± 0.52
RDW-CV (%)	13.13 ± 0.41	13.40 ± 0.44	13.10 ± 0.20	13.10 ± 0.10
PLT ($\times 10^9$/L)	394.33 ± 7.22	731.33 ± 32.04*	295.67 ± 3.76*	624.00 ± 5.20*
MPV (fL)	7.07 ± 0.18	7.10 ± 0.06	7.23 ± 0.32	6.57 ± 0.18
PDW (%)	12.63 ± 0.41	12.97 ± 0.35	13.97 ± 0.94	11.87 ± 0.66
PCT (%)	0.28 ± 0.01	0.52 ± 0.03*	0.22 ± 0.00	0.59 ± 0.09*
P-LCR (%)	12.70 ± 0.69	14.60 ± 0.64	19.70 ± 1.56*	18.27 ± 0.84*

Values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test to compare each treatment group to the control. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract.

hemoglobin (MCH) was significantly reduced in the 125 mg/kg group. Platelet indices showed marked changes: platelet count (PLT), plateletcrit (PCT), and platelet large cell ratio (P-LCR) were significantly increased in both 500 and 125 mg/kg groups. Mean platelet volume (MPV) and platelet distribution width (PDW) remained relatively stable across groups.

Liver Function Test

The liver function test results revealed dose-dependent alterations, although not all parameters followed a strictly monotonic pattern following oral administration of the GSME for 28 days (Table 6). A significant increase in AST was observed in the GSME 500 mg/kg group compared with the control group; however, ALT levels remained within the normal range across all treatment groups. ALP showed a slight, non-significant increase at 500 mg/kg but decreased at lower doses. Total and direct bilirubin levels were significantly elevated in the GSME 250 mg/kg group. In comparison, bilirubin levels at 125 mg/kg and 500 mg/kg were lower than or comparable to those of the control group. Total protein and albumin levels were significantly increased in the 500 mg/kg and 125 mg/kg groups.

Kidney Function Test

Blood samples were collected for the evaluation of renal function parameters, including serum urea, creatinine, and electrolytes (Na^+, K^+, Cl^-, and HCO_3^-). The results are presented in Table 7. Serum levels of urea, creatinine, and electrolytes remained within normal limits across all treatment groups. Slight but statistically significant changes in sodium levels were observed at 500 and 125 mg/kg doses of the extract.

Table 6 Effects of GSME on liver function parameters in rats

Parameter	NS 5 mL/kg (control)	GSME 500 mg/kg	GSME 250 mg/kg	GSME 125 mg/kg
AST (U/L)	96.50 ± 8.37	143.00 ± 17.89*	86.00 ± 6.93	94.00 ± 4.62
ALT (U/L)	21.50 ± 0.29	17.00 ± 1.73	24.00 ± 3.46	29.00 ± 3.46
ALP (U/L)	17.00 ± 2.31	23.00 ± 0.58	12.00 ± 1.15	13.00 ± 1.73
Total bilirubin (mg/dL)	9.80 ± 1.50	7.65 ± 0.89	15.55 ± 0.89*	6.45 ± 0.95
Direct bilirubin (mg/dL)	4.80 ± 0.98	3.50 ± 0.58	8.80 ± 0.98*	3.25 ± 0.26
Total protein (g/L)	55.00 ± 0.58	73.50 ± 4.33*	58.00 ± 1.15	65.00 ± 1.15*
Albumin (g/L)	31.50 ± 0.87	37.00 ± 1.73*	34.50 ± 0.87	36.00 ± 0.00*

Values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test to compare each treatment group to the control. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract.

Table 7 Effects of GSME on kidney function parameters in rats

Treatment (mg/kg)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
NS (control)	139.00 ± 0.58	3.90 ± 0.58	104.00 ± 0.00	27.00 ± 0.58	6.25 ± 0.03	47.00 ± 0.00
GSME 500	140.50 ± 0.29*	4.05 ± 0.03	100.00 ± 2.31	26.00 ± 0.58	7.40 ± 0.06	47.50 ± 0.29
GSME 250	139.50 ± 0.29	3.80 ± 0.58	101.00 ± 2.31	25.00 ± 0.58	6.75 ± 0.49	46.50 ± 0.29
GSME 125	141.00 ± 0.00*	3.85 ± 0.20	101.50 ± 0.87	26.00 ± 0.58	6.55 ± 0.43	47.00 ± 0.00

Values are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Dunnett's post-hoc test to compare each treatment group to the control. *Significant difference compared to control ($p < 0.05$). NS: Normal saline; GSME: Guiera senegalensis Methanolic Leaf Extract.

Histopathological Findings

To corroborate the biochemical findings and to provide direct evidence of tissue-level toxicity, histopathological examination of the liver and kidney was performed following 28 days of oral administration of GSME. Representative photomicrographs illustrating dose-related morphological alterations in renal and hepatic tissues are presented below. Figure 1 presents representative hematoxylin and eosin–stained photomicrographs of kidney sections from control and GSME-treated rats, illustrating dose-dependent renal structural alterations following subacute exposure.

Control kidney sections (A) show normal renal architecture with intact tubules (T) lined by cuboidal epithelial cells (CE). At 125 mg/kg GSME (B), severe toxic damage is observed, with widespread coagulative necrosis (CN) and degeneration around the tubules. At 250 mg/kg GSME (C), renal tissue demonstrates marked disruption of tubular epithelium (TE), destruction of tubular architecture, and extensive necrosis (N), with absence of visible glomeruli. At 500 mg/kg GSME (D), the overall histological architecture is distorted with indistinct corpuscles, disrupted tubules, and large areas of necrosis, indicating progressive, dose-dependent nephrotoxic damage.

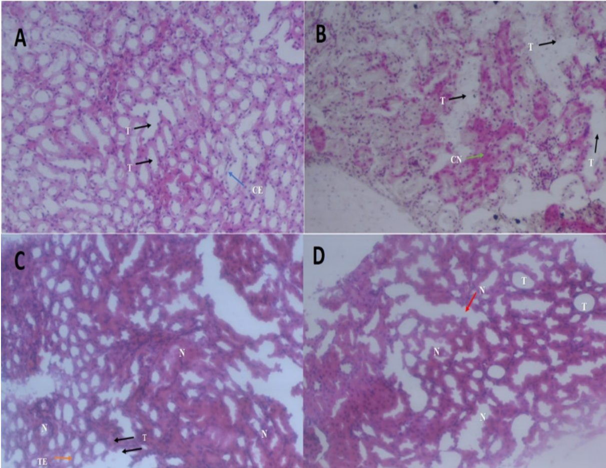

Figure 1 Histological photomicrographs of kidney tissue (Control and GSME-treated groups, H&E stain, ×400)

Figure 2 below illustrates representative hematoxylin and eosin–stained liver sections from control and GSME-treated rats, highlighting progressive hepatocellular damage and architectural distortion with increasing extract dose.

Control liver sections (A) show preserved hepatic architecture with central vein (CV), hepatocytes (H), and sinusoids (S). At 125 mg/kg GSME (B), moderate hepatotoxicity is evident with necrosis (N), ballooning degeneration, swollen hepatocytes (sH), and inflammatory infiltration. At 250 mg/kg GSME (C), severe hepatotoxicity is observed, characterized by diffuse necrosis, collapse of architecture, congestion, and inflammation. At 500 mg/kg GSME (D), hepatocytes are arranged in cords around central veins with centrally placed nuclei. Still, cellular boundaries are indistinct, sinusoids (S) appear dilated, and dark shrunken nuclei (P) suggest pyknosis/apoptosis.

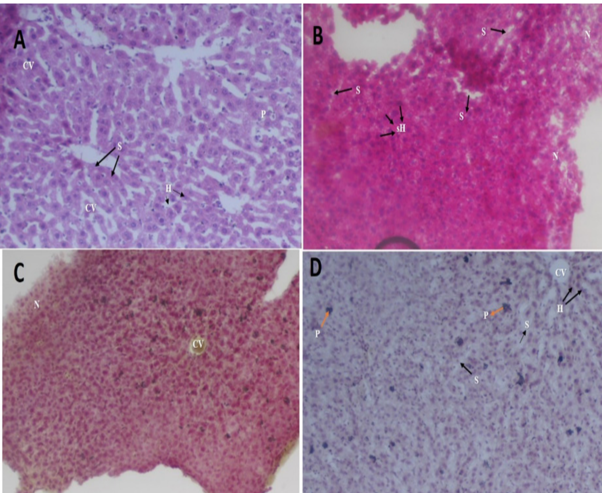

Figure 2 Histological photomicrographs of liver tissue (Control and GSME-treated groups, H&E stain, ×400)

4 Discussion

The present study evaluated the phytochemical profile and the acute and subacute toxicity of GSME in Wistar rats over 28 days, focusing on hematological, biochemical, and histopathological parameters. Phytochemical screening of the methanolic leaf extract of Guiera senegalensis revealed the presence of tannins, saponins, flavonoids, glycosides, and steroids, while alkaloids were absent. The acute toxicity study was conducted in accordance with OECD 423 guidelines. Oral administration of 2000 mg/kg of the methanolic extract to Wistar rats did not cause any mortality during the 14-day observation period. The acute lethal dose is therefore considered to be greater than 2000 mg/kg, placing the extract in the "relatively safe" category in terms of acute exposure.

The findings from the open field and Y-maze tests highlight that GSME exerts only a modest antipsychotic-like and cognitive effect in a ketamine-induced Wistar rat model. In the OFT, ketamine significantly increased locomotor activity and reduced freezing time, indicating psychosis-like hyperactivity, which was mitigated by GSME, with 800 mg/kg producing the most pronounced attenuation, approaching the effects of CPZ. Similarly, in the Y-maze, ketamine substantially impaired working memory, evidenced by fewer arm entries and alternations and lower spontaneous alternation percentage. Although the 200–400 mg/kg groups had numerically higher alternation counts than the ketamine-only group, their counts remained below those of the control group, and the data show substantial variability. However, the highest GSME dose (800 mg/kg) severely diminished exploratory behavior and completely abolished alternation, indicating cognitive impairment possibly due to sedative or excessive neuropharmacological effects.

These behavioral trends align with previous research indicating that G. senegalensis exhibits sedative and CNS-depressant properties. For example, Amos and colleagues (2001) reported that the aqueous extract of G. senegalensis reduced spontaneous motor activity, prolonged pentobarbital-induced sleep time, and attenuated amphetamine-induced stereotypy in rodents, indicating CNS-depressant activity.[24] This supports the idea that at high GSME doses, sedation or CNS depression may underlie the observed cognitive impairments, while at lower doses, the extract can exert beneficial modulatory effects.

The biphasic behavioral profile of GSME typically reflects an inverted-U (hormetic) dose–response frequently observed for neuroactive agents. At low to moderate concentrations, bioactive constituents in GSME may engage pro-cognitive mechanisms (e.g., mild cholinergic enhancement, antioxidant and anti-inflammatory effects), whereas higher concentrations may recruit sedative GABAergic activity, induce receptor desensitization, or produce off-target/pro-oxidant effects that overwhelm cognitive benefit.[25] Pharmacokinetic shifts and the complex, multi-constituent nature of the extract also mean that toxic metabolites or components with opposing activity may become pharmacodynamically relevant at high doses.[26] Also, the marked reduction of exploratory motor activity at 800 mg/kg suggests that non-specific sedation substantially contributes to the apparent cognitive impairment.

Beyond behavioral effects, numerous studies have documented the neuroprotective, antioxidant, anticholinesterase, and anti-inflammatory properties of G. senegalensis hydroethanolic extracts, providing mechanistic support for its activity in cognitive and memory models. In scopolamine-induced dementia models, G. senegalensis significantly improved Y-maze performance and spatial memory tasks in Wistar rats, while also restoring acetylcholine (ACh) levels and reducing acetylcholinesterase (AChE) activity.[5,6] G. senegalensis also mitigated oxidative stress by lowering malondialdehyde (MDA) and nitrite levels while enhancing antioxidant enzymes like SOD, CAT, and GSH.[5,6] Importantly, G. senegalensis exhibited anti-inflammatory and neuroplasticity-promoting effects, reducing pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6, IFN-γ), increasing levels of the anti-inflammatory cytokine IL-10 and neurotrophic factor BDNF, and decreasing pathological markers like $A\beta_{1-42}$, phosphorylated Tau, and GFAP in hippocampal tissues.[6] Histological analyses further confirmed the restoration of hippocampal architecture disrupted by scopolamine.[6]

Hematological assessment revealed both stimulatory and suppressive trends. A significant reduction in MCH at 125 mg/kg suggests impaired erythropoiesis or HGB synthesis, consistent with earlier reports

where G. senegalensis extracts altered red cell indices at subacute doses.[27] Conversely, HGB concentration increased significantly at 500 mg/kg, possibly reflecting erythropoietic stimulation or hemoconcentration. The most consistent finding was the elevation of platelet indices (PLT, PCT, P-LCR) in both 125 and 500 mg/kg groups, indicative of enhanced thrombopoiesis or a compensatory response to tissue injury. Similar platelet elevations have been reported in subacute toxicity studies of medicinal plants containing tannins and flavonoids, where pro-oxidant activity may trigger cytokine-driven megakaryopoiesis.[28]

Biochemical assays revealed dose-related hepatocellular perturbations. AST levels increased significantly at 500 mg/kg, while total and direct bilirubin were elevated at 250 mg/kg, suggesting hepatocellular damage and impaired bilirubin clearance. ALT levels, however, remained largely unaltered, reflecting the possibility of a predominant mitochondrial rather than cytosolic injury. Histological sections corroborated these findings. At 125 mg/kg, hepatocytes exhibited ballooning degeneration, necrosis, and inflammatory infiltration. At 250 mg/kg, diffuse necrosis, congestion, and architectural collapse were observed, while the 500 mg/kg dose induced sinusoidal dilatation and pyknotic nuclei, consistent with apoptotic injury. These progressive changes mirror previous report that observed severe necrosis and hepatocellular degeneration following prolonged administration of G. senegalensis.[29] The dichotomy between the hepatoprotective effects of G. senegalensis reported in paracetamol-induced models (where transaminases and oxidative stress markers were reduced)[30] and the hepatotoxicity observed in the current study underscores a context-dependent duality: while antioxidant constituents confer protection in acute injury, prolonged high-dose exposure predisposes to oxidative imbalance and mitochondrial dysfunction.

Renal biochemical parameters remained largely within physiological limits, with only mild sodium perturbations at 125 and 500 mg/kg. Serum urea and creatinine did not differ significantly across groups, suggesting preserved glomerular function during the subacute period. However, histological analyses revealed progressive tubular degeneration, coagulative necrosis, and architectural distortion at higher doses. This discrepancy between biochemical and histological findings has been previously described in nephrotoxicity models, in which compensatory renal clearance can mask early cellular injury.[31,32] The nephrotoxic potential of G. senegalensis is supported by long-term studies reporting vacuolation and tubular injury at high doses (≥ 1 000 mg/kg).[33] Our findings suggest that histological alterations precede biochemical derangements, emphasizing the importance of histopathology in detecting early toxicity.

The histopathological changes in the liver and kidney provide the most consistent dose-related evidence of GSME toxicity. While biochemical markers such as AST and bilirubin exhibited non-monotonic responses, the microscopic evidence demonstrated a progressive increase in lesion severity with dose. This highlights the importance of correlating biochemical findings with tissue morphology to obtain a reliable assessment of toxicological effects. The discordance between biochemical and histological markers may reflect the complex pharmacokinetics of the extract, the timing of sampling, or compensatory responses that mask serum marker changes despite ongoing tissue injury.

Phytochemical profiling of G. senegalensis revealed the presence of phenolic acids, tannins, flavonoids, saponins, glycosides, and steroids.[2,31,34] These compounds are well-documented for their antioxidant and anti-inflammatory activities, yet at higher doses may exhibit pro-oxidant behavior, generating reactive oxygen species that destabilize membranes and impair mitochondrial function. Tannins and polyphenols in particular can chelate metals and disrupt redox balance, thereby contributing to hepatocellular necrosis and renal tubular degeneration observed in this study.[35, 36] This dualistic activity likely explains why G. senegalensis demonstrates protective properties in acute injury models but exhibits toxic effects under prolonged or high-dose exposure. Thus, the extract's therapeutic potential must be weighed against its toxicological risks, particularly with chronic use.

A limitation of the present study is the absence of brain histopathological assessment. The behavioral experiments (open field and Y-maze) indicate that GSME modulates CNS activity in a dose-dependent manner. Yet, we did not examine brain morphology or cellular markers of neuronal injury in this report. This omission was due to the study's primary focus on subacute peripheral toxicity (liver and kidney) and constraints in histology resources at the time of necropsy.

5 Conclusion

This study demonstrates that GSME possesses neurobehavioral activity with antipsychotic-like effects at lower doses but also exerts toxicological effects upon repeated administration. The most consistent dose-related outcome was a progressive increase in the severity of liver and kidney histopathological lesions, which clearly intensified with higher doses. In contrast, biochemical and hematological parameters exhibited non-monotonic or variable responses: AST and HGB increased only at the highest dose, bilirubin peaked at an intermediate dose, and leukocyte indices fluctuated across groups. These findings suggest that organ-

level injury follows a graded dose–response pattern, whereas circulating markers are influenced by complex interactions among extract constituents, compensatory physiology, and measurement timing. Collectively, GSME exhibits a narrow therapeutic margin: while it confers antipsychotic-like and cognition-modulating effects at lower doses, sustained or higher exposure leads to progressive hepatotoxicity and nephrotoxicity. Future studies incorporating detailed dose–response modeling, time-course biochemical sampling, and mechanistic assays are required to establish safe exposure thresholds and clarify the extract's pharmacological versus toxicological balance.

Declarations

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Artificial Intelligence Disclosure

Grammarly was used solely for language editing, grammar checking, and improvement of clarity and readability of the manuscript. No artificial intelligence tools were used in study design, data collection, data analysis, data interpretation, or the generation of scientific conclusions. The authors take full responsibility for the content, accuracy, and integrity of the work.

Authors' Contributions

Albashir Tahir conceived and designed the study, coordinated the experimental work, performed data interpretation, and drafted the manuscript. Ibrahim Khaleel Muazu contributed to plant extraction, phytochemical analysis, and toxicity studies. Nura Bello participated in neurobehavioral experiments and data analysis. Mubarak Idris, Adamu Abdullahi, and Yasir Idris Saleh conducted biochemical and hematological studies. Abbas Nasidi contributed to the histopathological analyses and critical review of the manuscript. All authors read and approved the final version of the manuscript.

Availability of Data and Materials

All data supporting the findings of this study are available within the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Consent for Publication

Not applicable.

Ethical Considerations

Ethical approval for this study was obtained from the Faculty of Basic Medical Sciences Research and Ethics Committee of Bauchi State University, Gadau, under the Code of Ethics BASUG/FBMS/REC/VOL.08/01049.

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