Available online on 15.07.2026 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Toxicological assessment of the hydroethanolic extract of Sterculia setigera leaves', a neuroactive medicinal plant of Togolese flora

Yendubé T. Kantati [1]*, Akomagni D.C. Donko [1], Komlan M. Dossou-Yovo [1], Essotolom Badjabaissi [2], Sabrina C.J. Sanvee [2], Aboudoulatif Diallo [2], Kossi Metowogo [1], Povi Lawson-Evi [1], Amegnona Agbonon [1], Magloire K. Kodjo [1], Aklesso Mouzou [1], Kwashie Eklu-Gadegbeku [1]

[1] Physiopathology Bioactive Substances and Innocuity Research Unit (PSBI), Department of Animal Physiology, University of Lomé, Togo.

[2] Department of Pharmaceutical Sciences, University of Lomé, Togo.

Article Info:

Article History:

Received 20 April 2026
Reviewed 03 June 2026
Accepted 27 June 2026
Published 15 July 2026

Cite this article as:

Kantati YT, Donko ADC, Dossou-Yovo KM, Badjabaissi E, Sanvee SCJ, Diallo A, Metowogo K, Lawson-Evi P, Agbonon A, Kodjo MK, Mouzou A, Eklu-Gadegbeku K, Toxicological assessment of the hydroethanolic extract of Sterculia setigera leaves', a neuroactive medicinal plant of Togolese flora, Journal of Drug Delivery and Therapeutics. 2026; 16(7):116-123 DOI: *https://doi.org/10.22270/jddt.v16i7.7866*

For Correspondence:

Yendubé T. Kantati, Physiopathology Bioactive Substances and Innocuity Research Unit (PSBI), Department of Animal Physiology, University of Lomé, Togo.

Abstract

Background: *Sterculia setigera* is a plant used in traditional medicine for many purposes. We previously reported that its dry hydroethanolic leaf extract (SSE) protected cerebellar granule neurons and PC12 cells against H_2O_2, 6-OHDA, and serum deprivation-induced cell death, and mitigated ethanol neurotoxicity in the cerebellar cortex of neonatal Wistar rats while preserving motor coordination. This study aims to establish the toxicological profile of *Sterculia setigera* leaves' hydroethanolic extract, using appropriate *in vitro* and *in vivo* models.

Methods : The brine shrimp (*Artemia salina*) lethality assay served as a cytotoxicity test (*in vitro* model). An acute oral toxicity test was conducted according to OECD TG 423 (2000 mg/kg). The repeated dose oral toxicity of 28 days was performed following the OECD TG 407 (100, 200, and 400 mg/kg) on Wistar rats.

Results: The 50% lethal concentration (LC50) was 1.84 mg/mL. Based on acute oral toxicity, the LD50 value of the extract was up to 2000 mg/kg of body weight. The 28-day repeated oral administration of *Sterculia setigera* leaves hydroethanolic extract did not provoke negative effects on rats, as they were safe based on body and organ weight, hematological, biochemical, and histopathological results.

Conclusion : These results indicate that the dry hydroethanolic extract of *S. setigera* leaves did not provoke adverse effects that can lead to death or internal histological lesions in Wistar rats after 28-day oral administration.

Keywords: *Sterculia setigera*; cytotoxicity; *Artemia salina*; toxicological profile

INTRODUCTION

Sterculia setigera is one of the plants of the Sterculiaceae family, used traditionally in African countries like Togo to cure many diseases[1]. The plant is, therefore, used for the treatment of various ailments such as leprosy, malaria, coughs, bronchitis, snake bites, and syphilis[2]. The use of plants for health purposes is not a recent practice. Plants have been traditionally used since antiquity for their medicinal properties to heal many diseases around the world[3]. In developed countries, a renewed interest in plant usage is spreading among the population due to the search for alternatives to conventional medicine. Conventional medicine is associated with side effects that discourage patients and motivate their need for alternative solutions[4]. Another reason, mainly for pharmaceutical companies, is the fact that plants remain the main source of bioactive compounds[5]. Thus, several new drugs are sourced from medicinal plant sources[6]. In developing countries, where over 80% of people refer to traditional medicine for health care, the use of plants as medicine remains and is spreading due to many factors[7]. Among those factors, there are lack of money, usages, lack of health care infrastructure, and fear of side effects[8]. *Sterculia setigera* in previous studies has shown some pharmacological properties. Thus, its leaves have neuroprotective properties[9]. It also has antioxidant and anti-inflammatory properties[10] and anti-plasmodial properties[11]. Despite its scientifically proven properties and its widespread use, studies should be carried out to ensure its safety. This is because certain plants' molecules are commonly

implicated in causes of poisoning[12]. Concerning *Sterculia setigera*, if the plant is thought to be promising for its pharmaceutical properties[1], just a little toxicological data is available on its safety. This study is then conducted to assess the safety of the hydroethanolic extract of *Sterculia setigera* leaves. The finding will be useful to strengthen the use of *Sterculia setigera* leaves for health care purposes.

MATERIALS AND METHODS

Collection and extraction of plant materials

S. setigera leaves were collected in Nano (District of Dapaong, Togo) in September 2025. A voucher specimen holding the number 08655TG was generated and deposited in the herbarium of the Laboratory of Botany and Plant Ecology, Faculty of Sciences, University of Lomé (Togo). Following this identification procedure, leaves were cleaned and dried at 20°C in the Animal Physiology Laboratory at the University of Lomé's Faculty of Sciences. After pulverizing the dried leaves, 400 grams of *S. setigera* leaf powder were macerated for 72 hours in 4 liters of an ethanol–water mixture (80:20, v/v). A Rotavapor (Buchi R-134) was then used to evaporate the filtrate under vacuum at 40°C. Before being used, the concentrated hydroethanolic extract of *S. setigera* (SSE) was freeze-dried, weighed, and kept in a tightly sealed bottle at -20°C.

Animals

Brine shrimp eggs provided by the Toxicology laboratory of Pharmaceutical sciences Department, University of Lomé (Togo), were used for cytotoxicity tests when Wistar rats (both sexes) weighing 120-200 g were used for the in vivo toxicity tests. Rats were provided by the Animal Physiology Department and were acclimated at least one week prior to the debut of the manipulations. They were fed with rodent standard diets and water ad libitum and kept before and during the study at 22°C ± 2°C with a relative humidity level of 40%. The photoperiod was 12-h light and 12-h dark. Animal care and handling conformed to accepted guidelines[13].

Experimental design

Cytotoxicity test

This test was conducted following the Brine shrimp lethality test method as described by Dossou-Yovo et al. [14]. Artemia salina's eggs (500 mg) were incubated in 500 mL of seawater under automatic agitation for 48 hours. Ten tubes of different concentrations (25–0.05 mg/mL) containing, each, 16 larvae in 2 mL of solution were placed under automatic agitation for 24 hours, with an 11th tube containing also 16 larvae in 2 mL of seawater serving as a control. Tubes were observed to determine larvae mortality, which was defined as larvae immobility for 30 seconds. The test was valid if the mortality level in control was under 10%.

Acute toxicity test

A limit test was conducted on three female Wistar rats. Animals were administered sequentially a single dose of 2000 mg/kg of SSE at intervals of 48 h according to the Organization of Economic Cooperation and Development (OECD) test guidelines 423[15]. The animals were observed individually to check acute toxicity signs or behavioral changes after 30 minutes, 1 hour, and 4 hours postdosing during the first 24 h. The rats were observed thereafter at least once daily for 14 days.

Subacute toxicity test

The test was performed according to OECD guidelines 407[13]. Animals were grouped into 4 batches of six rats each. The first group (group 1) received distilled water and served as the control group. The second (group 2), third (group 3), and fourth (group 4) groups received the extract of S. setigera at 100, 200, and 400 mg/kg body weight, respectively. The extract was administered daily for 28 days at the same time. The animals were observed at least twice daily for morbidity and mortality. Their body weights were recorded every day. On the 29th day, the rats were anesthetized by ether after 12 hours of fasting. Blood samples were collected from the retroorbital sinus in dry tubes for biochemical analyses and in ethylene diamine tetraacetic acid (EDTA) tubes for hematological analyses. Biochemical parameters such as Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), creatinine, urea, glucose, Total bilirubin (TB), Direct bilirubin (DB), and Lactate dehydrogenase (LDH) were performed. Hematological parameters achieved were white blood cell count (WBC), red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MHC), platelet count, and mean corpuscular volume (MCV). For organ relative weight, the liver, the brain, the heart, and the kidney were collected. Histological sections were prepared from the brain, liver, and kidney.

Statistical analysis

Data are presented as mean ± SEM. For subacute toxicity test, groups (n = 6) were compared using analysis of variance (one-way ANOVA) and Tukey post-tests. The program used was GraphPad Prism 10.2.3 (2024, San Diego, USA). $P<0.05$ values were considered significant.

RESULTS

Brine shrimp lethality test (*in vitro* cytotoxicity)

From the number of dead larvae, a logarithmic trend curve was designed, and the LC50 was determined graphically (figure 1). Its value is 1.84 mg/mL.

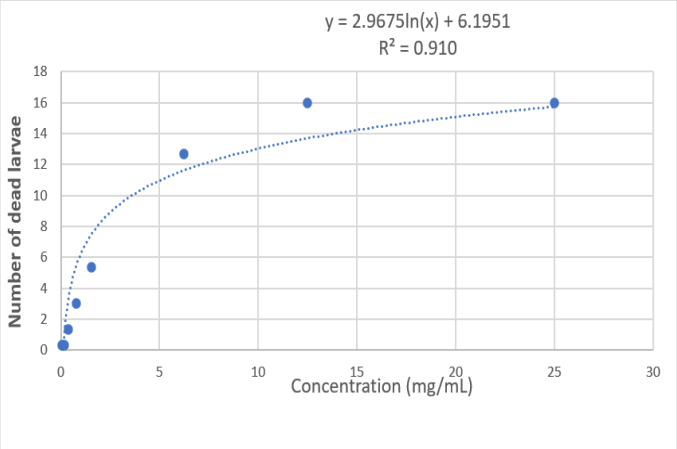

Figure 1: Trend curve expressing the number of dead larvae as function of concentration of *S. setigera* leaves dry hydroethanolic extract

Acute toxicity

No behavioral signs of toxicity, such as abnormal respiration, salivation, sleep disturbances, tremors, convulsions, aggressiveness, or mortality, were observed during the observation periods (table 1).

Table 1: Time Course observation of female rats following *S. setigera* leaves dry hydroethanolic extract administration

Parameters	Time	*S. setigera* 2000 mg/kg (p.o.)		
		Rat 1	Rat 2	Rat 3
Observations	30 min	-	-	-
	1 hour	-	-	-
	4 hours	-	-	-
	24 hours	-	-	-

- : no symptoms observed.

Additionally, at necropsy, no macroscopic abnormalities were observed in the heart, lungs, or visceral organs. As shown in figure 2, the animals' body weights did not decrease at J14, compared to J1.

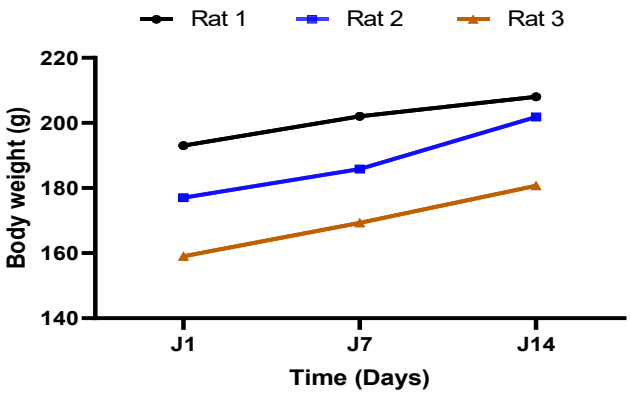

Figure 2: Effects of *S. setigera* leaves dry hydroethanolic extract on the body weight of female rats after 14 days.

Data are shown as individual body weights (g) of each rat at J1, J7, and J14.

Sub-acute toxicity

Body and organs weight

Through the 28-day experiment, no significant differences were observed when comparing the control animal's body weight to the treated ones (Figure 3).

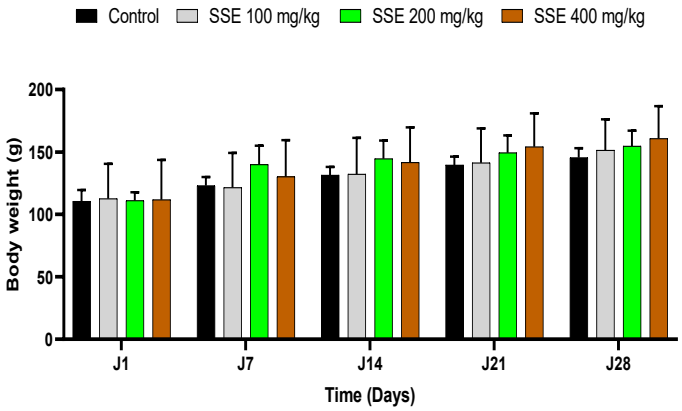

Figure 3: Effects of *S. setigera* leaves dry hydroethanolic extract on the body weight of rats after 28 days

The animals received SSE at doses of 100, 200, and 400 mg/kg/day for 28 days. Data are shown as mean ± SEM (n = 6). No significant differences were found.

The organ relative weight when compared to dosed groups to control did not show significant differences (Figure 4).

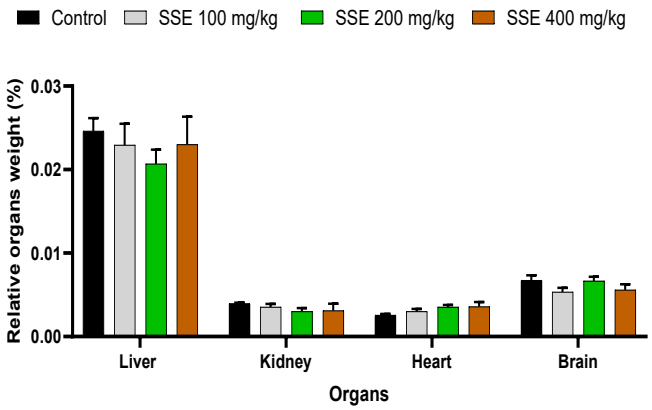

Figure 4: Effects of SSE on the relative organ weight of rats after 28 days

Data are shown as mean ± SEM (n = 6). No significant differences were found.

Hematological parameters

A significant decrease of hematocrit, mean corpuscular volume, and platelet count was observed at 100 mg/kg. The mean corpuscular volume also decreased at 200 mg/kg (Table 2).

Table 2: Effects of SSE on hematological parameters of rats in sub-acute toxicity test.

Blood parameters	Control	Dose of *S. setigera* extract		
		100 mg/kg	200 mg/kg	400 mg/kg
WBC (10^3/µL)	6.70 ± 0.57	6.38 ± 0.32	7.96 ± 0.63	7.50 ± 0.91
RBC (10^6/µL)	6.87 ± 0.27	7.63 ± 0.15	7.18 ± 0.29	6.72 ± 0.85
HGB (g/dL)	13.60 ± 0.40	14.44 ± 0.17	13.66 ± 0.38	13.05± 1.17
HCT (%)	39.86 ± 1.66	42.32 ± 0.69*	40.02 ± 1.39	39.47 ± 4.00
MCV (fL)	58.10 ± 1.00	55.58 ± 0.65*	55.90 ± 0.98*	59.77 ± 4.47
MHC (pg)	19.80 ± 0.52	18.90 ± 0.24	18.98 ± 0.34	19.70 ± 1.09
MCHC (g/dL)	34.16 ± 0.66	34.08 ± 0.20	34.12 ± 0.23	33.20 ± 0.94
Platelets (10^4/µL)	861.20 ± 73.51	775.20 ± 72.10*	854.60 ± 70.51	866.33 ± 23.51

The animals received SSE at doses of 100, 200, and 400 mg/kg/day for 28 days. At the end of the study, blood was collected by retro-orbital method for hematological analysis. Data are shown as mean ± SEM (n=6). *P < 0.05: vs Control group. Abbreviations: WBC = White Blood Cell, RBC = Red Blood Cell, HGB = Hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

Biochemical Markers

No significant difference was observed except a decrease in total bilirubin and lactate dehydrogenase at 400 mg/kg (table 3).

Table 3: Effects of SSE on biochemical parameters of rats in sub-acute toxicity test

Markers	Control	Dose of *S. setigera* extract		
		100 mg/kg	200 mg/kg	400 mg/kg
Urea (g/L)	0.414 ± 0.033	0.414 ± 0.014	0.394 ± 0.013	0.376 ± 0.018
Creat (mg/L)	6.96 ± 0.52	6.52 ± 0.35	6.22 ± 0.30	6.82 ± 0.45
AST (UI/L)	128.400 ± 4.045	136.333 ± 3.180	136.000 ± 8.727	137.000 ± 6.083
ALT (UI/L)	56.50 ± 3.59	58.00 ± 4.58	63.33 ± 2.02	64.40 ± 8.55
TB (mg/L)	3.00 ± 0.00	2.80 ± 0.13	2.86 ± 0.04	2.62 ± 0.15*
DB (mg/L	0.28 ± 0.02	0.36 ± 0.05	0.42 ± 0.04	0.38 ± 0.02
LDH (U/L)	858.100 ± 74.783	832.220 ± 81.796	797.920 ± 87.184	735.450 ± 9.250*
Glycemia (g/L)	0.684 ± 0.081	0.707 ± 0.064	0.666 ± 0.075	0.690 ± 0.031

The animals were treated orally with SSE at doses of 100, 200, and 400 mg/kg/day for 28 days. At the end of the study, blood was collected by retro-orbital method for biochemical analysis. Data are shown as mean ± SEM (n=6). *P < 0.05: vs Control group. Abbreviations: Creat = Creatinine, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, TB = Total bilirubin, DB = Direct bilirubin, LDH = Lactate dehydrogenase.

Histopathological sections

No architectural or morphological abnormality was observed on the organ's histopathological section (figures 5, 6, and 7).

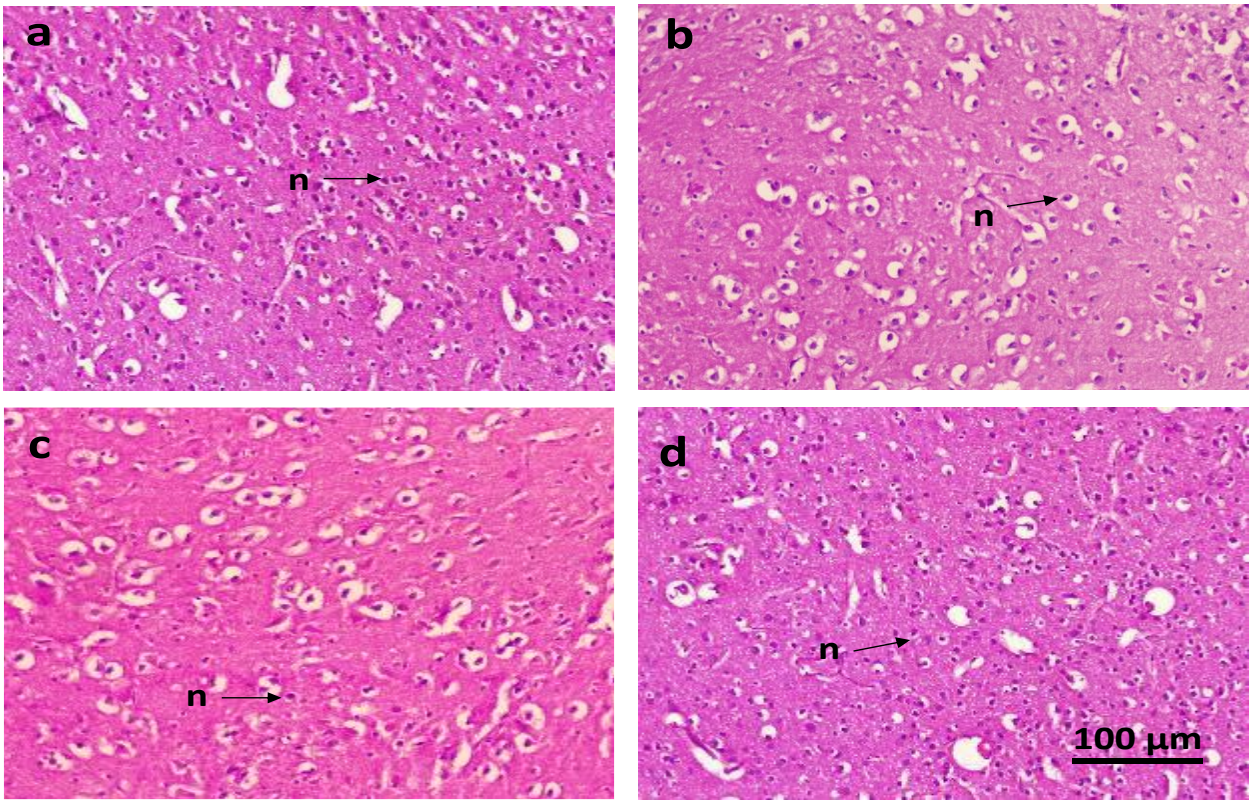

Figure 5 : Histopathological section of brain

a= Control; b = SSE 100 mg/kg; c = SSE 200 mg/kg; d = SSE 400 mg/kg. Abbreviations: n = neuron. H&E stain, x 100.

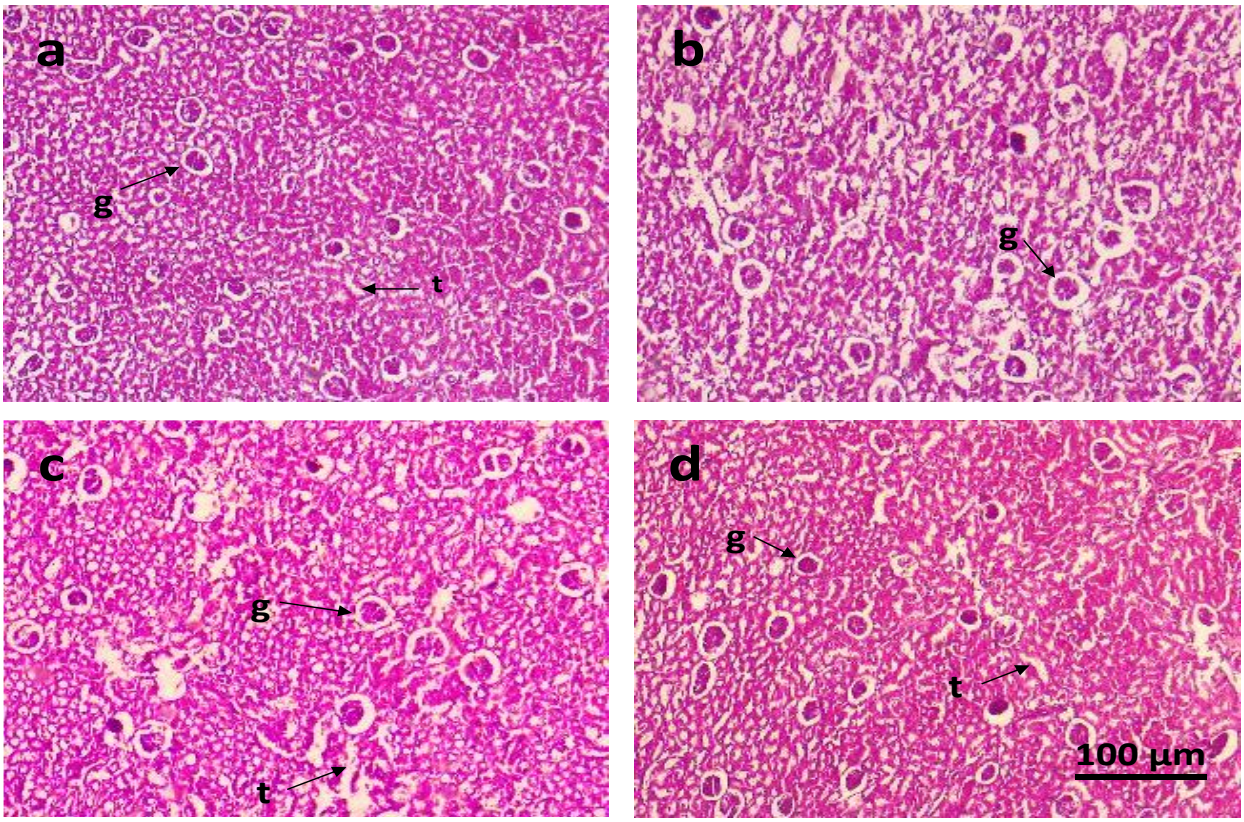

Figure 6: Histopathological section of kidney

a= Control; b = SSE 100 mg/kg; c = SSE 200 mg/kg; d = SSE 400 mg/kg. Abbreviations: g = glomerulus; t = tubule. H&E stain, x 100.

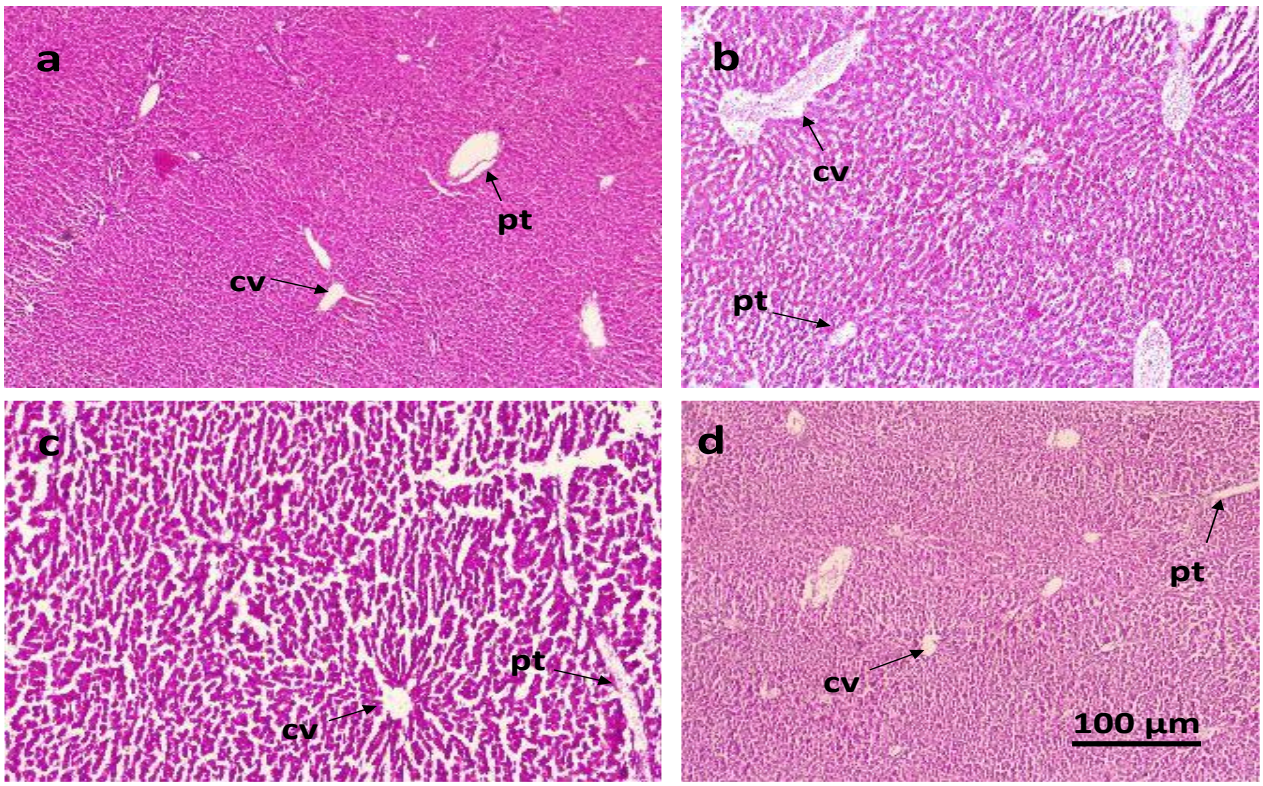

Figure 7: Histopathological section of liver

a= Control; b = SSE 100 mg/kg; c = SSE 200 mg/kg; d = SSE 400 mg/kg. Abbreviations: cv = central vein; pt = portal tract. H&E stain, x 100.

DISCUSSION

This study is a toxicological assessment of *Sterculia setigera*. It was carried out by using *in vitro* and *in vivo* toxicity tests.

The Brine shrimp lethality test, an *in vitro* toxicity test, is a rapid preliminary cytotoxicity test. It is used to check the cytotoxicity of bioactive chemicals[16]. The assay has shown that the *Sterculia setigera* hydroalcoholic leaf extract LC50 was over 0.1 mg/mL, the maximum value over which an extract can be considered nontoxic[17]. According to this result, the *Sterculia setigera* hydroalcoholic leaves can be considered non-cytotoxic. Our result is in accordance with Taha and Mudawi's works, which showed that some parts (fruits, leaves, stem barks, and roots) of *Sterculia setigera* are non-cytotoxic to Vero cells[18]. Nevertheless, the fresh stem bark extract of the plant has shown cytotoxicity, according to Maikudi *et al.*[19]. This difference can be due to the plant part used or the type of extract.

In vivo toxicity tests were performed following OECD test guidelines. For the acute toxicity test, a limit test was conducted, as a low toxic effect or no toxic effect is expected. The lethal dose 50 (LD50) is more than 2000 mg/kg, as no toxic effects were observed during the experimentation period. *Sterculia setigera* hydroethanolic leaf extract can be considered safe during an oral acute administration at 2000 mg/kg.

Subacute toxicity of 28 days carried out at 100, 200, and 400 mg/kg did not provoke toxic effects that lead treated rats to death or affect their behavior. Among parameters recorded during the test, we had the body weight. Body weight's variation can be a sensitive and simple index of toxicity, as its reduction (5%) is considered as a predictor of pathological findings[20]. No significant changes in the body weight of the treated group rats compared to control group were observed after 28 days of experiment. The extract; at studied doses did not induce pathological process which can lead to changes in Wistar rat's body weight. The relative weight of organs, an indicator of hypertrophy or atrophy of organs[21], for studied organs such as brain, liver, and kidney has not shown a significant difference between the treated groups and the control.

As blood cells are the first cells exposed to the toxic when it reaches the systemic circulation[22], hematological parameters can give key information on the toxicity process. Thus, a sustained elevation in the mean corpuscular volume can be a predictor of impending hematologic toxicity due to folate depletion [23]. Hematological parameters of treated groups, except hematocrit, platelets, mean corpuscular volume, and platelet count, have not shown a significant difference when compared to the control group. Thus, at 100 mg/kg hematocrit, platelet, and mean corpuscular volume significantly decreased. At 200 mg/kg a significant decrease of mean corpuscular volume was also observed. These results are different from a previous study on stem bark extract, which showed an increase of many blood parameters such as mean corpuscular volume, red blood cell count, hemoglobin,

and platelets [24]. Globally, the extract did not induce toxic effects that affect key hematological parameters.

Results of biochemical parameters showed a decrease in Total Bilirubin and Lactate dehydrogenase at 400 mg/kg. Lactate dehydrogenase (LDH) is an enzyme belonging to oxidoreductase groups. It is an important enzyme of the anaerobic metabolic pathway. Its increase is often associated with troubles such as liver disorders, anemia, heart attack, or cancer. Its low level is considered not to be harmful[25]. The LDH-lowering pattern observed in this study is then in accord with previous antiapoptotic activities recorded with *Sterculia setigera* in vitro in PC12 cells [26]. Total bilirubin is a breakdown product of normal heme catabolism, useful for assessing liver function. Its increase is considered a symptom of liver diseases [27]. In this study, ASAT, ALAT, ALP, and glucose, which are parameters to explore the toxicity [28], did not show significant variation. Urea and creatinine are good markers of renal functions. Changes in urea and creatinine were not significant. It suggests that the extract did not cause renal dysfunction. Histological data have not shown any abnormality suggesting that the hydroethanolic extract of *Sterculia setigera* leaves did not induce toxicity in the liver, brain, and kidney.

CONCLUSION

In this study, we have evaluated cytotoxicity, acute toxicity, and subacute toxicity of the hydroethanolic extract of Sterculia setigera leaves. The results showed that the extract is non-cytotoxic and safe for acute and repeated oral administration to Wistar rats. This marks the initial step in a process that should lead to the formulation of an improved traditional medicine.

Conflict of interest: The authors declared no conflict of interest.

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