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Research Article
Acute Toxicity of Hydroethanolic Extracts from the Leaves of Three Medicinal Plants: *Justicia secunda* Vahl., *Sorghum bicolor* (L.) Moench and *Theobroma cacao* L.

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Abstract

Background and Objective: Medicinal plant extracts are wrongly considered to be harmless to health. However, numerous studies have demonstrated the contrary. This study evaluated the acute toxicity of hydro-ethanolic extracts from the leaves of *Justicia secunda*, *Sorghum bicolor*, and *Theobroma cacao* according to OECD Guideline No. 423. **Materials and Methods:** The preparations were obtained by hydro-ethanolic maceration (50%) of dried leaf powder. Four groups (n = 3) of rats, including a control group, received distilled water and 2,000 mg/kg of hydroethanolic extract of *Justicia secunda* (HEJs), *Sorghum bicolor* (HESb), and *Theobroma cacao* (HETc), respectively. For 14 days, all clinical signs of distress were noted. At the end of the study, all rats were sacrificed under anesthesia, and the heart, liver, pancreas, spleen, kidneys, lungs, and blood were collected for biological analysis. **Results:** No signs of suffering or organ damage were observed. However, the relative mass of the spleen in the HEJs and HETc groups was significantly higher ($p<0.01$) than that of the control group, suggesting an adaptive response to treatment. However, ASAT levels in the treated groups were significantly lower ($p<0.0001$) than in the control group. **Conclusion:** Hydro-ethanolic extracts of *J. secunda*, *S. bicolor*, and *T. cacao* at a single dose of 2,000 mg/kg administered orally are relatively safe. These extracts were well tolerated as they had no serious toxic effects. According to the OECD Globally Harmonized System of Classification (GHS), these extracts are classified as substances with an $LD_{50} \geq 5.000$ mg/kg.

Key words: Acute toxicity, Wistar rat, *Justicia secunda*, *Sorghum bicolor*, *Theobroma cacao*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of plants for therapeutic purposes is an ancient practice dating back to the earliest human civilizations. Relegated to the background in the face of significant advances in modern medicine, herbal medicine has seen a resurgence of interest in recent years, both in developing countries, where it is the main therapeutic alternative, and in industrialized countries, where it is perceived as a "gentle and natural" alternative to synthetic molecules[1,2]. This interest in phytotherapy is fueled by recent discoveries that tend to show that chemically-derived drugs sometimes cause serious side effects that are often only discovered several years after their use. In addition, certain drug residues have the potential to contaminate the environment because they are difficult to eliminate once released into nature. On the other hand, numerous studies have shown that medicinal plants, in addition to being biodegradable, have therapeutic and organ-protective properties. For example, glycosides extracted from the leaves of *Justicia secunda* are thought to have antimicrobial and antiparasitic properties[3]. *Sorghum bicolor* is believed to contain bioactive compounds with anti-radical, anti-diabetic, anti-obesity, anti-inflammatory, anti-cancer, and anti-benign prostatic hyperplasia properties[4-7]. The leaves of *Theobroma cacao* are rich in theobromine, a molecule that improves lipid profile, blood pressure, blood sugar levels, and gut microbiota, reduces tumor formation, and acts as a neuroprotective agent[8].

However, because of their natural origin, medicinal plants are perceived in the popular consciousness as harmless, devoid of any harmful or toxic effects. Nevertheless, it must be acknowledged that the toxic effects of medicinal plants do indeed exist and can occur after a single or repeated dose. The severity of the toxic effects can be moderate, severe, or lethal: *Digitalis purpurea*, from which digitalis, a cardiotonic glycoside used to treat heart failure, is extracted, can cause nausea, vomiting, visual disturbances, bradycardia, and even death if ingested in excessive amounts. *Datura stramonium*, used in herbal medicine for the sedative, tranquilizing, anti-asthmatic, and anti-inflammatory effects of its tropane alkaloids, can cause dry mouth, tachycardia, photophobia, migraine, tremors, hallucinations, and even coma when used in high doses[9-11]. *Crocus sativus*, *Vernonia bipontini*, and *Schefflera barteri* are believed to cause severe nephrotoxic effects through irreversible destruction of the renal parenchyma[12-14]. These examples show that while it is essential to evaluate the safety and tolerance of modern treatments before they are placed on the market, it is equally important to evaluate the toxicity of medicinal plants before they are used in herbal medicine.

The aim of this study was to evaluate the acute toxicity of hydroethanolic extracts from the leaves of *J. secunda, S. bicolor*, and *T. cacao* according to OECD guidelines[15] in a rat model. As shown above, these three medicinal plants are commonly used in ethnomedicine, either in combination with other plants or alone, to treat various pathologies[3,8,16]. Particular attention was paid to possible liver and kidney damage due to their physiological functions of neutralizing and eliminating toxic substances from the body.

MATERIALS AND METHODS

Study area: This study was conducted between August-October 2025 at the animal facility of the medical sciences training and research unit at Alassane Ouattara University in Bouaké, Côte d'Ivoire.

Materials

Biochemical analysis equipment: Biochemical parameters were measured using the Cobas C311® automated analyzer (Roche Diagnostics, Switzerland) with the appropriate reagent kits. Blood glucose, conjugated and total bilirubin (C-Billi, T-Billi), triglycerides (TG), total cholesterol (T-Chol), transaminases (ASAT and ALAT), creatinine, Blood Urea Nitrogen (BUN), uric acid (UA), Phosphokinases (CPK), and Alkaline Phosphatase (AlkP) were determined. Serum electrolyte concentrations were determined using the Balio EX300® analyzer (Balio Diagnostics, France).

Animal model: Healthy, nulliparous, non-pregnant female Wistar rats (n = 12) aged 3 to 4 months, weighing between 170-240 g were supplied by the animal facility of the Medical Sciences Training and Research Unit at Alassane Ouattara University (Bouaké, Côte d'Ivoire). The experiment was also conducted on these premises. These animals were acclimatized for one month in the experimental room before the start of the trials. The temperature was 23°C with a regular 12 hrs light/dark cycle. They were placed in polypropylene cages with free access to water (*ad libitum*) and food.

Plant material: We used the leaves of *J. secunda* (Acanthaceae), *S. bicolor* (Poaceae), *T. cacao* (Malvaceae), three unprotected medicinal plants. The leaves of *J. secunda* and *T. cacao* were collected in the locality of Sran Bondossou, a village near the town of Sakassou in central Côte d'Ivoire. The leaves of *S. bicolor* were purchased from medicinal plant traders at the market in the commune of Marcory in Abidjan (Côte d'Ivoire). These plants were authenticated at the

National Center for Floristics of Côte d'Ivoire. According to the latest classification of angiosperm phylogenetic groups for orders and families of flowering plants, *J. secunda*, *S. bicolor*, and *T. cacao* belong to the Acanthaceae, Poaceae, and Malvaceae families, respectively[17].

Methods

Preparation of hydroethanolic extracts: The leaves of *J. secunda* and *T. cacao* were picked at dawn, washed with drinking water, and then dried for two weeks in shade and open air. The leaves of *S. bicolor* were purchased at the market already dried and tied in small bundles. These dried leaves were first cut into small pieces with scissors and then finely ground using a Nasco® blender to obtain a fine leaf powder. These powders were stored separately in jars away from moisture and light. Fifty grams (50 g) of each leaf powder was cold macerated in 500 mL of hydroethanolic solution (50%) for 48 hrs with stirring. The solutions obtained were filtered using a funnel and cotton wool, and the filtrates were placed in an oven (Panacea®, Italy) at below 40°C to evaporate the solvents. The three hydroethanolic extracts obtained were collected and stored at between 5°C and 4°C for further experimentation: Hydroethanolic extract of *J. secunda leaves* (HEJs), *S. bicolor* (HESb), *T. cacao* (HETc). The yields were 14.95%, 15.73%, and 13.64%, respectively.

Acute oral toxicity study: The acute toxicity study was conducted in accordance with the OECD guidelines for testing chemicals-acute toxicity class method in accordance with international ethical standards. These guidelines made it possible to reduce the number of animals used and to alleviate their suffering and improve their living conditions during the study[15,18]. Given that there is very little data in the scientific literature attesting to the harmfulness of the leaves of these three medicinal plants when taken orally, we chose to conduct a limit test at a dose of 2,000 mg/kg.

The female Wistar rats were divided into four groups of three animals (n = 3) by random selection. After fasting for 16 hrs, the rats received the following treatments:

- **Control group:** Rats treated with distilled water (10 ml/kg)
- **HEJs group:** Rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg)
- **HESb group:** Rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg)
- **HETc group:** Rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg)

Immediately after this treatment, clinical signs of distress were observed continuously for 2 hrs, then every 4 hrs, and finally once a day for 14 days. The following clinical signs of distress were monitored: Tremors, salivation, diarrhea or urination, lethargy, excitement, piloerection, bleeding, hyperventilation or bradypnea, and mortality. In accordance with OECD Guideline No. 423, this test was repeated a second time due to the absence of mortality. At the end of the observation period for the second test, all rats were euthanized under anesthesia at the end of the 14th day after a 16 hrs fast. Blood was collected by cardiac puncture, centrifuged, and serum was then taken for biochemical testing. To conduct this study, we first obtained authorization No. 16/2024/MESRS/UAO/UFRSM/OK from the ethics committee of the medical sciences training unit at Alassane Ouattara University (Côte d'Ivoire).

Statistical data analysis: Two-way ANOVA with multiple comparisons test was performed at a significance level of $p<0.05$ using GraphPad Prism®, version 8.4.3. Results are presented as mean±SEM. Percentages of change were calculated using the following formula[19]:

$$\text{Variation (\%)} = \frac{\text{Essai value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS

Change in body weight: At the end of the 14-day observation period, the body weight of the animals in the groups treated with the various plant extracts increased by an average of 4.29%, which is twice as much as that of the Control group, which increased by 2.32%. On day 12, this difference in weight gain was significantly higher for the HESb and HETc groups compared to the Control group ($p<0.01$). But there was no significant difference between the three treated groups (Fig. 1).

Clinical signs of distress: The rats in the HEJs, HESb, and HETc groups showed moderate excitement during the first 30 minutes after a single oral dose of 2,000 mg/kg. After this period, no clinical signs of distress were observed and all rats survived at the end of the 14-day observation period (Table 1).

Comparison of relative organ mass: Female rats treated with HESb and HETc had spleen-to-body weight ratios of 0.32±0.02 and 0.31±0.01, respectively, compared to

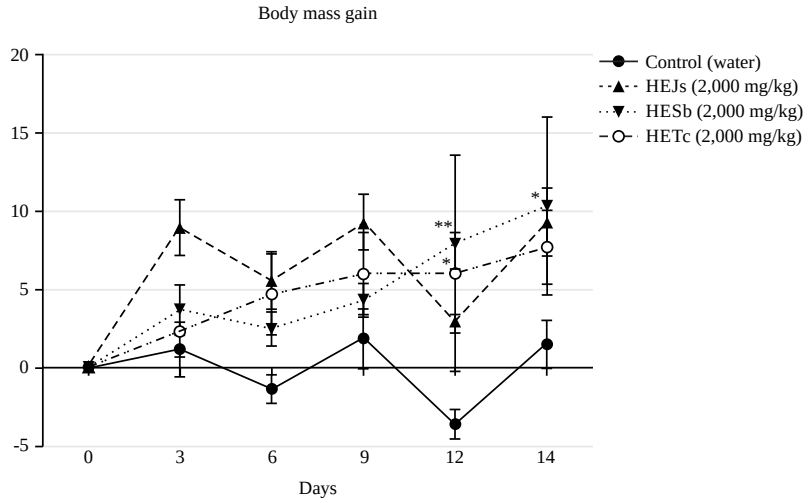

Fig. 1: Change in body weight of groups of female rats after administration of a single oral dose of 2,000 mg/kg (bw) of hydroethanolic extracts of *J. secunda*, *S. bicolor*, and *T. cacao* leaves over a period of 14 days

Control: Group of rats treated with distilled water (10 mL/kg), HEJs: Group of rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg), HESb: Group of rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg), HETc: Group of rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg). Results are presented as mean±SEM (n = 3; OECD 423, 2002). Two-way ANOVA, multiple comparisons test: *p<0.05, ** p<0.01

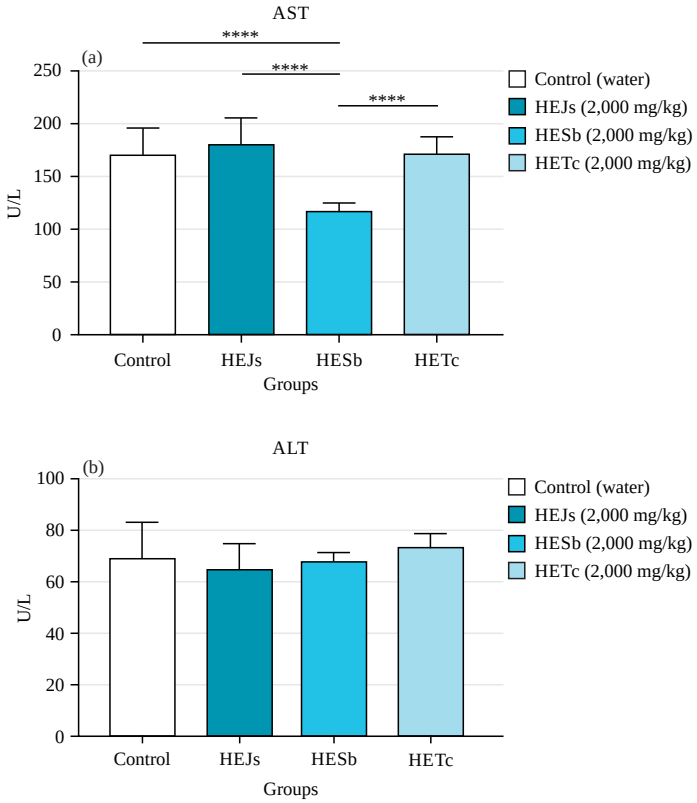

Fig. 2(a-b): Effects of a single oral dose (2,000 mg/kg) of HEJs, HESb, and HETc extracts on liver function in rats after 14 days, (a) Serum aspartate aminotransferase concentrations and (b) Serum alanine aminotransferase concentrations

Control (n = 3): group of rats treated with distilled water (10 ml/kg), HEJs (n = 3): group of rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg), HESb (n = 3): group of rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg), HETc (n = 3): group of rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg) and results are presented as mean±SEM (n = 3; OEDC 423, 2002). 2-way ANOVA, multiple comparisons test: ****p<0.0001

Table 1: Observation of clinical signs of distress in groups of rats after administration of hydroethanolic extracts for 14 days

Clinical signs of distress	Control (water, 10 mL/kg)	HEJs (2,000 mg/kg)	HESb (2,000 mg/kg)	HETc (2,000 mg/kg)
Tremors	0	0	0	0
Salivation	0	0	0	0
Defecation/urination	0	0	0	0
Lethargy	0	0	0	0
Excitement	0	1	1	1
Piloerection	0	0	0	0
Bleeding	0	0	0	0
Hyperventilation or bradypnea	0	0	0	0
Death	0	0	0	0

Absence: 0, presence: 1, Control: Group of rats treated with distilled water (10 mL/kg), HEJs: Group of rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg), HESb: Group of rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg) and HETc: Group of rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg)

Table 2: Organs to body weight ratio comparation after 14 days of observation

Body weight and organs ratio (%)	Control (water, 10 mL/kg)	HEJs (2,000 mg/kg)	HESb (2,000 mg/kg)	HETc (2,000 mg/kg)
Heart/b.w.	0.35±0.03	0.33±0.01	0.36±0.02	0.35±0.01
Liver/b.w.	2.03±0.04	2.27±0.11	2.22±0.05	2.26±0.05
Kidneys/b.w.	0.46±0.02	0.48±0.01	0.47±0.00	0.45±0.01
Spleen/b.w.	0.20±0.01	0.23±0.01	0.32±0.02*	0.31±0.01**
Lungs/b.w.	0.75±0.05	0.71±0.06	1.07±0.17	0.72±0.06
Pancreas/b.w.	0.42±0.08	0.46±0.04	0.52±0.03	0.49±0.02

Control: Group of rats treated with distilled water (10 mL/kg), HEJs: Group of rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg), HESb: Group of rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg), HETc: Group of rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg) and results are presented as mean±SEM (n = 3; OEDC 423, 2002). 2-way ANOVA, multiple comparisons test: *p<0.05; ** p<0.01

Table 3: Biochemical profile of rats 14 days after administration of the three hydroethanolic extracts

Biochemical indicator	Control (Water, 10 mL/kg)	HEJs (*Justicia secunda*)	Variation (%)	HESb (*Sorghum bicolor*)	Variation (%)	HETc (*Theobroma cacao*)	Variation (%)
Glycemia (g/L)	1.00±0.060	0.98±0.090	-1.7	0.81±0.072	-18.7	0.97±0.073	-3.3
CPK (U/L)	994.67±4.48	999.67±11.17	+0.5	950.33±31.75	-4.46	1005.00±5.69	+1.04
AlkP (U/L)	95.00±3.06	88.00±1.53	-7.4	91.33±2.03	-3.9	88.00±4.36	-7.4
C-Billi (U/L)	0.85±0.084	0.65±0.041	-23.6	0.67±0.074	-20.5	0.78±0.044	-7.5
T-Billi (U/L)	7.00±0.577	5.67±0.333	-19.0	7.00±1.53	0.0	5.67±1.20	-19.0
TG (g/L)	0.54±0.065	0.79±0.110	+44.8	0.73±0.141	+33.7	0.69±0.031	+27.0
T-CHOL (g/L)	0.51±0.025	0.58±0.018	+13.1	0.52±0.061	+2.0	0.50±0.029	-2.6
UA (mg/L)	16.33±1.86	13.33±2.03	-18.4	11.33±0.33	-30.6	13.67±1.21	-16.3
Ca^2 (mmol/L)	89.67±0.33	90.00±0.58	+0.4	83.00±6.51	-7.4	87.33±5.49	-2.6
Mg^2 (mmol/L)	19.67±0.88	17.67±0.67	-10.2	17.33±0.67	-11.9	19.67±0.88	0.0
Na (mmol/L)	129.33±1.86	132.67±11.17	+2.6	134.00±2.08	+3.6	134.67±4.33	+4.1
K (mmol/L)	3.96±0.14	3.76±0.52	-5.1	3.43±0.22	-13.5	3.97±0.12	+0.1
Cl (mmol/L)	84.00±1.73	88.67±12.20	+5.6	86.67±1.33	+3.2	87.67±8.84	+4.4

The values are expressed as mean±S.E.M., (n = 3), Glycemia: Level of glucose in the blood, CPK: Creatine phosphokinase, AlkP: Alkaline phosphatase, C-Billi: Conjugated bilirubin, T-Billi: Total bilirubin, TG: triglycerides, T-CHOL: Total cholesterol, BUN: Blood urea nitrogen, UA: Uric acid, Ca^{2+}: Calcium ion, Mg^{2+}: Magnesium ion, Na^+: Sodium ion, K^+: Potassium ion, Cl^-: Chloride ion, Control: Rats treated with vehicle, HEJs: Rats treated with hydroethanolique extract of *J. secunda* leaves, 2,000 mg/kg, HESb: Rats treated hydroethanolique extract of *S. bicolor* leaves, 2,000 mg/kg, HETc: Rats treated with hydroethanolique extract of *T. cacao* leaves, 2,000 mg/kg. ANOVA 2ways, multiple comparisons test: p<0.05. Variation (%) represents the percent change relative to the control group: "+" indicates an increase and "–" indicates a decrease compared to the control

0.20±0.01 for the Control group. These ratios were significantly higher than those of the Control group at significance thresholds of p<0.05 and p<0.01, respectively. However, the relative masses of the heart, kidneys, lungs, and pancreas of the treated rats were not significantly different from those of the Control group (Table 2).

Comparison of biochemical indicators: In the HEJs group, the concentration of C-billi decreased by 23.6% (0.647±0.041 U/L) while the TG level increased by 44.8% (0.79±0.110 g/L) compared to the Control group. As for UA, its concentration decreased by 30.6% (11.33±0.333 mg/L) in the HESb group compared to the Control group. However, there was no significant difference between these biochemical indicators (Table 3).

Effects of hydroethanolic extracts on the liver 14 days after treatment

Aspartate Aminotransferase (AST): The animals in the Control, HEJs, and HETc groups had comparable mean AST concentrations of 170.67±25.208, 180.67±28.109, and 172.33±15.320 U/L, respectively. But the HESb group had a significantly (p<0.0001) lower ASAT blood concentration of 117±9.292 U/L than the other groups (Fig. 2a).

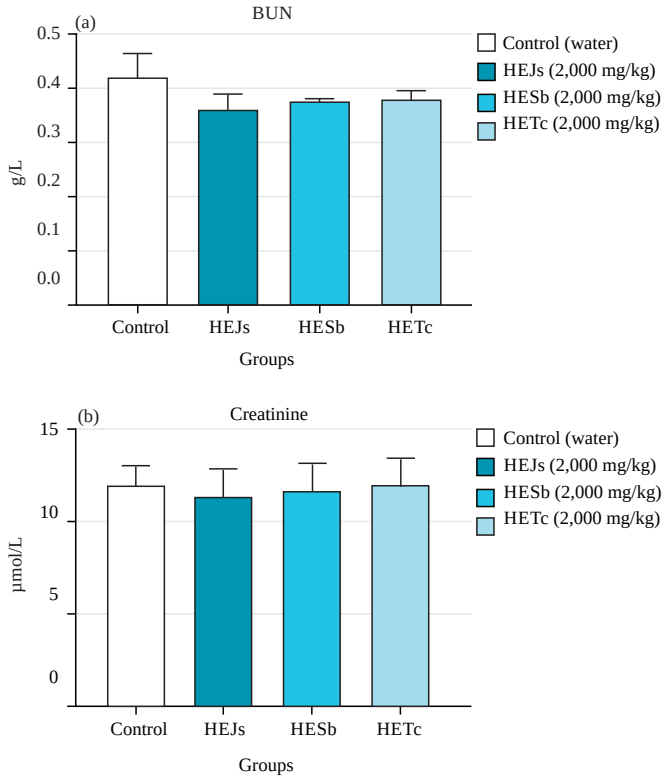

Fig. 3(a-b): Effects of a single oral dose (2,000 mg/kg) of HEJs, HESb, and HETc extracts on renal function in rats after 14 days, (a) Blood urea nitrogen (BUN) concentrations and (b) Serum creatinine concentrations

Control (n = 3): Group of rats treated with distilled water (10 mL/kg), HEJs (n = 3): Group of rats treated with hydroethanolic extract of *J. secunda* leaves (2,000 mg/kg), HESb (n = 3): Group of rats treated with hydroethanolic extract of *S. bicolor* leaves (2,000 mg/kg), HETc (n = 3): Group of rats treated with hydroethanolic extract of *T. cacao* leaves (2,000 mg/kg) and results are presented as mean±SEM (n = 3; OEDC 423, 2002). 2-way ANOVA, multiple comparisons test: *p<0.05

Alanine Aminotransferase (ALT): Serum ALT concentrations were 69±14.526, 64.67±10.171, 68±2.517 and 73.33±5.548 U/L for the Control, HEJs, HESb and HETc groups, respectively. These serum ALT concentrations did not differ significantly from each other (Fig. 2b).

Effects of hydroethanolic extracts on the kidneys (blood urea nitrogen, creatinine) 14 days after treatment

Blood urea nitrogen (BUN): Overall, treatment of female rats with high doses of hydroethanolic extracts (2,000 mg/kg) induced a non-significant decrease in urea concentration. In the HEJs group, this concentration fell to 0.36±0.026 g/L, compared to 0.42±0.044 g/L in the Control group (Fig. 3a).

Creatinine: Creatinine levels in the different groups of female rats treated with hydroethanolic extracts ranged from 11.33±0.882 to 12±0.577 µmol/L, while the Control group had a level of 12±0.577 µmol/L. There was no significant difference between these values (Fig. 3b).

DISCUSSION

The choice of female animals and the number of animals per group (n = 3) was determined in accordance with international ethical standards aimed at reducing suffering and the number of animals used in experimental toxicology studies[15,18]. The administration of an acute dose of 2,000 mg/kg of the various hydroethanolic preparations of *J. secunda*, *S. bicolor*, and *T. cacao* leaves did not cause any major clinical signs of suffering or death in the animals during the 14 days of observation. However, moderate and transient agitation was observed during the first 30 minutes (Table 1). This virtual absence of clinical signs of distress confirms that these leaf extracts are not very toxic or are well tolerated orally at high doses (2,000 mg/kg). This transient agitation could be due to the method of administration of the preparations, which was carried out under restraint by force-feeding with a tube. Indeed, it can be observed that, overall, the body weight of all groups of rats continued to increase during the 14 days

of observation (Fig. 1). This growth ranged from 3.60% to 4.91% for the treated groups, compared to 2.22% for the Control group. However, this difference is not significant and could be related to the small size of the different study groups.

Although few studies have focused on the toxicity of the leaves of these three plants, those that have evaluated this toxicity have confirmed the safety of these leaf extracts when administered orally. For example, Komlaga *et al.*[20] showed that ingestion of the aqueous extract of the *T. cacao* leaf did not cause any symptoms of acute toxicity in female Swiss albino mice at the same dose of 2,000 mg/kg[20]. When administered subacutely at a dose of up to 400 mg/kg for 28 days, the hydromethanolic extract of *S. bicolor* leaves had no significant negative impact on biochemical values in Wistar rats[21]. However, in intraperitoneal administration, the work of Abo[22] showed that the aqueous extract of *J. secunda* leaves was toxic to Swiss albino mice with an estimated LD_{50} of 2.742 mg/kg[22]. Such a high LD_{50} when administered intraperitoneally confirms the good tolerance of aqueous extracts from the leaves of this plant. However, the ethyl acetate extract of *J. secunda* is thought to contain cytotoxic molecules in various human cell models: FaDu and Detroit 562, as well as in VERO green monkey kidney cells, with an LC_{50} between 61.18 and 79.57 µg/mL[23].

Macroscopic analysis of the heart, liver, kidneys, spleen, lungs, and pancreas after the animals were sacrificed under anesthesia revealed no lesions. This observation was corroborated by the relative masses of the organs of the different groups, which were broadly comparable except for the presence of significant hypertrophy ($p<0.01$) of the spleen in the HESb and HETc groups compared to the Control group (Table 2). As the spleen is a blood filtration and defense organ, this hypertrophy could be the result of an adaptive immune response caused by inflammation, allergy, or hemolysis induced by the presence of toxic substances in the blood. Indeed, several clinical cases of oxidative hemolysis have already been reported following the ingestion of plant extracts such as *Acalypha indica*[24,25], a plant used for its anti-inflammatory, antidiabetic, and antihyperlipidemic properties[26]. However, total bilirubin levels in the test groups were close to those in the Control group (Table 3), showing that our extracts did not cause blood hemolysis. But the work of Nwinyi *et al.*[21] showed that, when administered subacutely by mouth for 28 days, the 70% hydro-methanolic extract of *S. bicolor* leaves caused a significant reduction ($p<0.05$) in relative kidney mass without altering renal indicators in Wistar rats at a dose of 200 mg/kg.

The study of liver indicators revealed that 14 days after acute treatment (2,000 mg/kg) of the animals, there was a significant decrease ($p<0.0001$) in AST levels in the HESb group compared to the Control group and the treated groups (Fig. 2a). This decrease, estimated at 31.18% compared to the Control group, could be due to hepatoprotective activity. Indeed, several preclinical studies have confirmed the hepatoprotective activity of certain plants. For example, the rinds of *Garcinia mangostana, Khaya senegalensis*, and the fruit of *Adansonia digitata* L. (Baobab) contain antioxidant compounds capable of protecting the liver from damage caused by oxidative stress induced by certain hepatotoxic molecules such as thioacetamide, carbon tetrachloride, and paracetamol[27-29]. As for CPK levels, all concentrations are comparable. This result shows that, overall, these extracts did not induce cell lysis in the viscera, muscles, or brain, with effects lasting up to 14 days after administration of the extracts.

With regard to renal parameters, in line with macroscopic observations that did not reveal the presence of lesions, uremia and creatinine levels were comparable across all groups of female rats, including the Control group. However, there was a non-significant decrease in BUN levels in all treated groups compared to the Control group of 11.35% (Fig. 3). This would suggest an increase in urinary excretion, given that there is no liver damage. Therefore, the administration of a single dose of 2,000 mg/kg of the extracts would have had a non-significant beneficial effect on kidney protection. Indeed, such nephroprotective activity has been observed in Wistar rats with aqueous preparations of *Eurycoma longifolia* root (100-400 mg/kg) against lesions induced by paracetamol at repeated doses of 200 mg/kg for 14 days[30]. This renal tolerance is supported by the absence of electrolyte imbalance between treated animals and those in the Control group (Table 3).

According to the Globally Harmonized System of Classification (GHS), these results lead us to classify these three plant extracts among substances with an $LD_{50} \geq 5.000$ mg/kg[15]. This would mean that these plant extracts are relatively safe. However, determining biochemical indicators 14 days after treatment could allow sufficient time for the rats' bodies to regenerate in the face of moderate or minor toxic effects.

CONCLUSION

A comparative study of the acute toxicity of hydroethanolic extracts from the leaves of *J. secunda*, *S. bicolor*, and *T. cacao*, three medicinal plants, showed that they are only slightly toxic. When administered orally at a dose of 2,000 mg/kg, these extracts did not cause mortality. This study found no toxic effects of these plant extracts at either the clinical or biochemical level when administered acutely.

Chronic toxicity studies should be conducted to evaluate these effects over the long term, as these plants are used to treat both chronic and acute conditions.

SIGNIFICANCE STATEMENT

The leaves of *Justicia secunda*, *Sorghum bicolor*, and *Theobroma cacao* are commonly used in traditional medicine to treat various diseases. However, numerous clinical cases of poisoning have been documented, demonstrating that, like modern medicines, medicinal plants can cause toxic effects of varying severity. Administered at an acute oral dose of 2,000 mg/kg to female Wistar rats, the hydroethanolic extracts of these three plants were well tolerated, apart from a significant increase in the relative mass of the spleen for *S. bicolor* and *T. cacao*, which would suggest an adaptive immune response. According to OECD Guideline No. 423, these extracts would have an $LD_{50} \geq 5.000$ mg/kg. This study showed that the leaves of *J. secunda*, *S. bicolor*, and *T. cacao* are relatively safe, which would justify their common use in traditional medicine by populations. But a chronic toxicity study should be conducted to assess the long-term toxic effects of these plants.

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