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Toxicological Study of the Aqueous Extract of the Stem Bark of *Rauvolfia vomitoria* (Apocynaceae)

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ABSTRACT

Background and Objective: *Rauvolfia vomitoria* is a plant used in traditional medicine in Togo. This study aims to determine the chemical composition of this plant and then assess its toxicity to larvae of *Artemia salina* and Wistar rats, respectively. **Materials and Methods:** Cytotoxicity was studied in larvae of *Artemia salina*. Acute and subchronic toxicity tests were performed orally on female Wistar rats. The acute toxicity study was carried out with a concentration of 5000 mg/kg of aqueous extract and the subchronic toxicity study which lasted for 28 days was carried out with doses of 75, 150 and 300 mg/kg. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and saponins. Larval cytotoxicity revealed a lethal concentration LC_{50} of 1.205 mg/mL. Acute toxicity at 5000 mg/kg of extract in rats caused apathy, drowsiness and difficulty moving without causing death. The lethal oral dose LD_{50} in rats is therefore greater than 5000 mg/kg of body weight. Exposure of rats to doses of 75, 150 and 300 mg/kg of extract resulted in a significant increase in relative liver weight without an increase in body weight. Hematological and biochemical tests carried out at the end of 28 days showed no significant variation. Histological analysis of the liver of treated rats confirmed the presence of liver lesions. **Conclusion:** *Rauvolfia vomitoria* is toxic at 300 mg/kg in chronic administration in Wistar rats. It causes damage to the liver among other things.

KEYWORDS

Rauvolfia vomitoria, phytochemical screening, cytotoxicity, *Artemia salina*-acute-subacute-toxicity, histological sections

INTRODUCTION

People around the world used plants to heal themselves and various illnesses before the advent of modern medicine[1]. The art of healing with plants has been known and practised in Africa for a long time. According to the World Health Organization (WHO), approximately 80% of populations in developing countries resort to traditional medicine and in particular herbal medicine for their health care needs due to cultural tradition or lack of other alternatives[2]. Some studies have revealed cases of poisoning following the use of medicinal plants[1,3]. Several studies have revealed the use in West and Central Africa of *Rauvolfia vomitoria* in the treatment of a wide variety of illnesses, ranging from simple indigestion to

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mental disorders[2,4]. A decoction of the leaves or roots taken orally is used in the treatment of mental illnesses[5,6]; the macerated leaf, for the treatment of hypertension and fever; the decoction against gonorrhea, rheumatism, growth retardation, liver disorders and skin diseases[7]; root decoction to treat hemorrhoids[8]. Rauwolfia is reported to contain indole alkaloids which include yohimbine, reserpine, rescinnamine, raucaffricine, ajmaline and ajmalicine. These may be responsible for the different properties exhibited by the plant[9].

Several studies have shown that *Rauvolfia vomitoria* is not toxic when administered orally to Wistar rats, including Bonheur *et al.*[10] work which has shown that this plant, up to a dose of 900 mg/kg of body weight, is not toxic[11]. However, other work and observations, as well as the chemical composition of *Rauvolfia vomitoria*, point towards a potential toxic risk. Indeed, *Rauvolfia vomitoria* contains indole alkaloids including ajmaline, yohimbine and reserpine which are substances likely to be dangerous when the doses are not well chosen[12].

In Togo, *R. vomitoria* is well-known and widely used. The present work aims to study the larval toxicity, the acute and the 28-day subchronic toxicity of *R. vomitoria* in Wistar rats.

MATERIALS AND METHODS

Study setting: The study took place in the Pharmaceutical Sciences Laboratory and the Animal Physiology Laboratory of the University of Lomé (Togo) and the Anatomical Pathology Laboratory of the University Teaching Hospital of Lomé, Lomé, Togo from 6 January to 30 December, 2020.

Study materials: The plant material was the root bark (1200 g) of *Rauvolfia vomitoria* which was collected from the Hahotoé site, a locality situated 35 km Northeast of Lomé (Latitude: 6.37649731, Longitude: 1.42448298, UTM: 325740.223E 705089.831N 31N and Altitude: 33 m). The roots were identified and authenticated at the Botany and Plant Ecology Laboratory of the Faculty of Sciences of the University of Lomé under the registration number TOGO15675.

The animal material consisted of 27 female Wistar rats with an average weight of 140.3±11.4 g. These rats were acclimatized in the animal room of the Faculty of Sciences (24-25°C, alternating 12 hrs of light and 12 hrs of darkness) for two weeks. They were kept in cages where they had free access to water and food. The 528 Brine shrimp eggs were used and were provided by the Toxicology Department of Pharmaceuticals Sciences of the University of Lomé (Togo).

Preparation of the extract: The roots were washed, peeled and then dried under air conditioning at 20°C away from solar radiation. After drying, the bark of *R. vomitoria* was ground into a coarse powder in a mill. The powder (1050 g) obtained was macerated in 10.5 L of distilled water. The mixture was subjected to mechanical stirring for 72 hrs. The macerate was filtered through hydrophilic cotton and then through Whatman filter paper (110 mm). The filtrate was evaporated under vacuum at a temperature of 45°C using a Rotavapor R-210 Buchi® until a dry extract of 96 g was obtained (R = 9.14%). The collected extract was stored in a freezer at -18°C in a tightly closed box until use. Before each use, the extract was solubilized in distilled water with 1% Tween 80. Ethical approval was obtained from the Committee on Institutional Ethics in Education and Research under issue (Ref. CNCB-CEER 2801/2010).

Phytochemical screening: The major chemical families (alkaloids, flavonoids, tannins, saponins, cardiac glycosides and anthracenes) were screened for identification of major groups[13].

Toxicological study: Larval cytotoxicity was carried out according to the method of Michael *et al.*[14]. The degree of toxicity of the root bark was evaluated based on the correspondence table available in the publication of Assih *et al.*[15]. Animal care and handling was consistent with accepted guidelines[16,17].

Acute toxicity was carried out according to Diallo *et al.*[16] protocol. Three female rats were treated at a dose of 5000 mg/kg. Each rat was observed for 14 days and signs were noted.

For subchronic toxicity, the test was carried out as described by Diallo *et al.*[17]. The test was conducted as described. Doses of 75, 150 and 300 mg/kg were chosen based on preliminary tests where doses of 500 and 1000 mg/kg caused death of the rats in less than ten days.

The rats were grouped into 4 groups of 6 distributed as follows. A control batch received distilled water containing 1% Tween 80 and three other batches were treated with aqueous extract of *R. vomitoria*, respectively at a dose of 75; 150 and 300 mg/kg. The *R. vomitoria* extract was dissolved in distilled water with 1% Tween 80.

Distilled water and extracts were administered orally every day for 28 consecutive days to each rat depending on the batch. The extracts were prepared daily just before administration to the rats. Rats received 10 mL/kg of distilled water or the aqueous extract of *R. vomitoria*. On the 29th day, after 12 hrs of fasting, the rats were anesthetized with ether.

A blood sample was collected using a capillary tube from the retro-orbital sinus in a dry tube for biochemical tests (urea, blood sugar, creatinine, calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), sodium, potassium, chlorine) on the Roche Cobas c311 analyzer and a tube containing EDTA for hematological tests (Red Blood Cells (RBC) count, Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC) count, neutrophils (ANC), lymphocytes, monocytes and platelets) on an automated hematology analyzer (BC 6800, Mindray, China). Animals were then sacrificed and certain organs (heart, kidney, spleen, liver and ovaries) were isolated and weighed and their relative weights were calculated using the formula:

$$RW = \frac{\text{Organ weight}}{\text{Rat weight}} \times 100^{17}$$

After fixation with 10% formalin, the organs were dehydrated by successive baths of increasing concentrations of alcohol. The organs were then cleared in toluene and packed in paraffin. They were then cut into fine slices (five micrometers thick) using a microtome. The sections were stained with Hematoxylin and Eosin (H&E). Photographs of histological sections were taken.

Statistical analysis: GraphPad Prism 8.0 software (Graph Pad software, Inc. USA) was used to analyze current study results which were then expressed as a mean value accompanied by the standard error of the mean (Mean±SEM). The number of values (n) was specified in each case. Analysis of Variance (ANOVA) was used to compare multiple groups. The difference between the two groups was determined using the Tukey's test. The significance threshold was set at $p<0.05$.

RESULTS

Phytochemical screening: Alkaloids, flavonoids, tannins and saponins were present in the aqueous extract of *R. vomitoria*, with an absence of cardiac glycosides and anthracenes.

Larval cytotoxicity: The aqueous extract of *R. vomitoria* caused the death of the larvae of *Artemia salina* indicating that the samples are biologically active. The degree of toxicity of the aqueous extract of *R. vomitoria* root bark was evaluated.

Acute toxicity: During the 14 days of monitoring for acute toxicity, apathy, drowsiness and difficulty moving were noted in the rats during the first 3 hrs. No mortality was recorded during the experiment, which implies that the lethal dose is greater than 5000 mg/kg.

28-day subchronic toxicity: The subchronic toxicity study in rats showed that the aqueous extract of *R. vomitoria* administered orally at doses of 150 and 300 mg/kg caused drowsiness compared to control rats. Mortality is 86% at a dose of 300 mg/kg (Table 1).

The aqueous extract of *R. vomitoria* did not significantly ($p<0.05$) modify the body weight of rats throughout the study period.

Rauvolfia vomitoria extract had no significant effect ($p<0.05$) on the relative weight of the heart, spleen, kidneys and ovaries; but it caused a significant increase in the relative weight of the liver irrespective of the dose of the extract (Table 2).

After 28 days of administration of *R. vomitoria* at doses of 75, 150 and 300 mg/kg, no statistically significant difference was observed in the hematological parameters studied (Table 3). The aqueous extract had no significant effect on biochemical parameters (Table 4).

Histological sections: After administration of the aqueous extract of *R. vomitoria* for 28 days, no organ lesions were observed on histological sections of the heart, spleen and kidneys, respectively, at doses of 75, 150 and 300 mg/kg compared to the control batch (Fig. 1-4). However, in the liver, the aqueous extract of *R. vomitoria* caused edema at doses of 75 and 150 mg/kg. At a dose of 300 mg/kg, the liver showed edema, lymphoplasmacytic cells (LPC) and fibrosis (Table 5).

Table 1: Clinical signs of toxicity observed in rats depending on the concentration of the extract (n = 6)

		Aqueous extract of *Rauvolfia vomitoria*		
Parameter	Control	75 mg/kg	150 mg/kg	300 mg/kg
Apathy	-	-	-	+
Excitation	-	-	-	-
Breathing disorders	-	-	-	-
Reduction in food intake	-	-	-	+
Bleeding from mouth and nose	-	-	-	-
Drowsiness	-	-	+	+
Coma	-	-	-	-
Diarrhoea	-	-	-	+
Nasal congestion	-	-	-	+
Tremors	-	-	-	+
Inability to move	-	-	-	+
Ocular irritation	-	-	-	+
Convulsion	-	-	-	-
Mortality (%)	1 (14%)	3 (43%)	1 (14%)	6 (86%)

+: Presence and -: Absence

Table 2: Effect of the root bark extract of *Rauvolfia vomitoria* on the relative weight of rat organs

		Aqueous extract of *Rauvolfia vomitoria*		
Parameter	Control (n = 6)	75 mg/kg (n = 4)	150 mg/kg (n = 6)	300 mg/kg (n = 1)
Heart	0.45±0.03	0.48±0.02	0.47±0.03	0.51±0.00
Liver	2.82±0.08	3.12±0.11**	3.25±0.14***	3.48±0.00***
Kidneys	0.65±0.02	0.62±0.03	0.65±0.03	0.73±0.00
Spleen	0.21±0.00	0.28±0.02	0.28±0.03	0.29±0.00
Ovaries	0.04±0.01	0.05±0.00	0.04±0.01	0.03±0.00

Each value represents the Mean±SEM, (n) is the number of animals per batch, **$p<0.01$, ***$p<0.001$: Significant difference at doses of 75, 150 and 300 mg/kg compared to the control

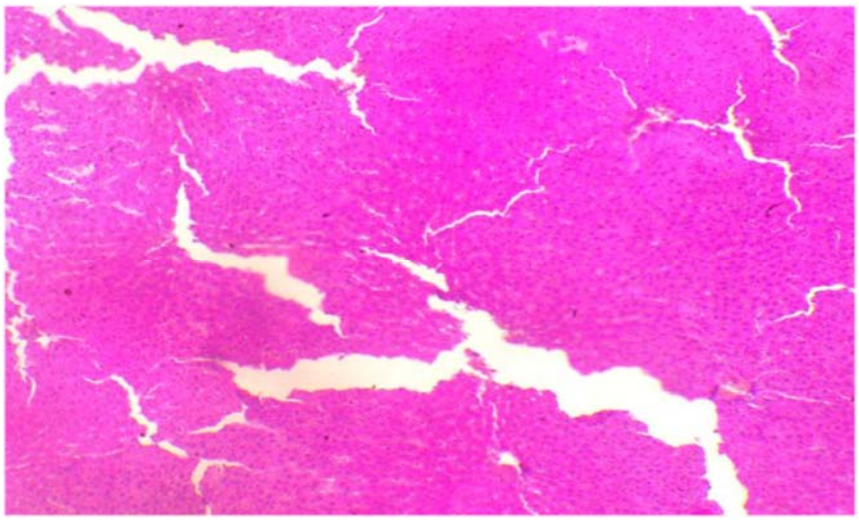

Fig. 1: Histological section of control rat liver stained with H&E (Magnification: 10×) on day 29

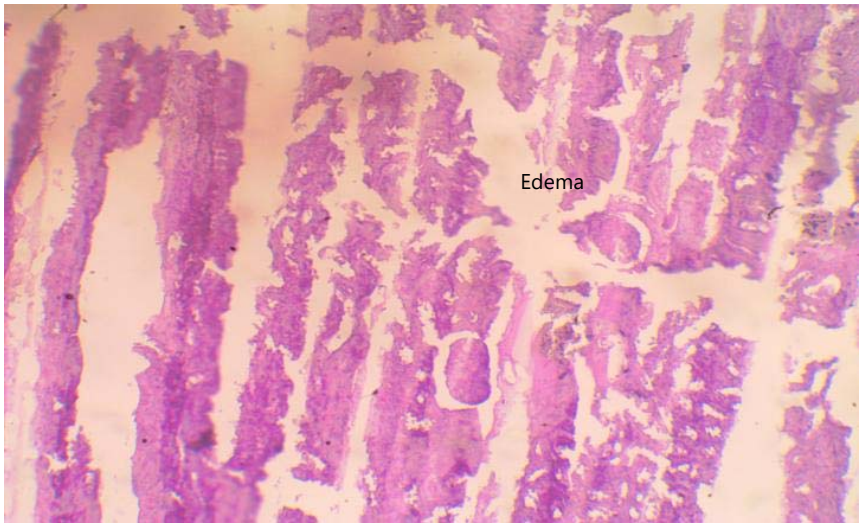

Fig. 2: Histological section of rat liver exposed to a 75 mg/kg concentration of extract, stained with H&E (Magnification: 10×) on day 29, Edema was noted

Table 3: Effect of the root bark extract of *Rauvolfia vomitoria* on hematological parameters

		Aqueous extract of *Rauvolfia vomitoria*		
Parameter (unit)	Control (n = 6)	75 mg/kg (n = 4)	150 mg/kg (n = 6)	300 mg/kg (n = 1)
WBC (×10^9/L)	10.21±1.33	8.13±1.32	10.12±0.77	9.42
ANC (×10^9/L)	3.09±0.35	3.63±0.65	3.60±0.76	3.38
Lymphocytes (×10^9/L)	6.28±0.94	3.79±0.53	5.56±0.36	5.51
Monocytes (×10^9/L)	0.67±0.10	0.57±0.12	0.70±0.09	0.35
RBC (×10^{12}/L)	7.73±0.27	7.97±0.31	7.28±0.22	7.86
Hb (g/dL)	13.60±0.34	13.70±0.32	13.02±0.42	13.70
HCT (%)	42.34±1.11	42.42±0.97	40.85±1.30	43.20
MGV (fl)	54.84±0.79	53.37±0.89	56.08±0.88	55.00
MCH (pg)	17.64±0.34	17.20±0.26	17.85±0.29	17.50
MCHC (%)	32.18±0.16	32.22±0.06	31.85±0.10	31.80
Platelets (×10^9/L)	978.40±88.56	909.00±56.14	909.33±86.91	1129.00

Each value represents the Mean±SEM, (n) is the number of animals per batch, p>0.05, WBC: White blood cells, RBC: Red blood cells, Hb: Haemoglobin, HCT: hematocrit, MGV: Mean globular volume, MCH: Mean corpuscular haemoglobin and MCHC: Mean corpuscular haemoglobin concentration

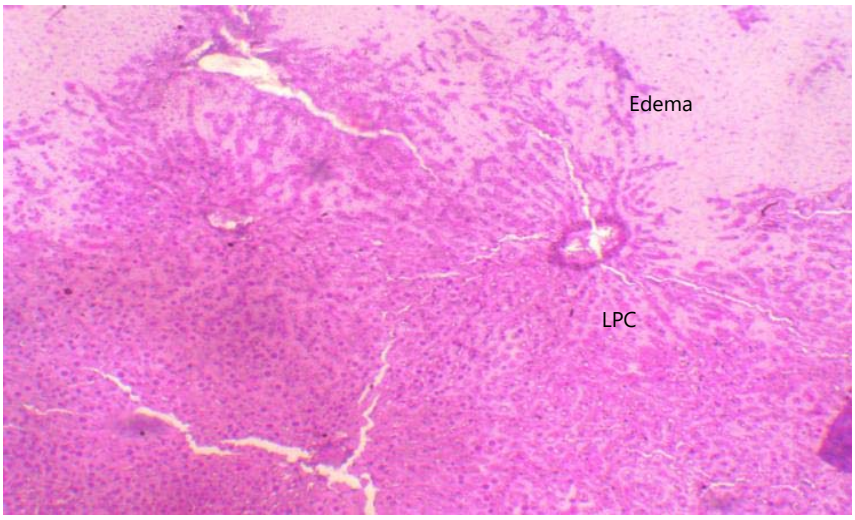

Fig. 3: Histological section of rat liver exposed to a 150 mg/kg concentration of extract, stained with H&E (Magnification: 10×) on day 29. Edema and lymphoplasmacytic cells (LPC) were noted

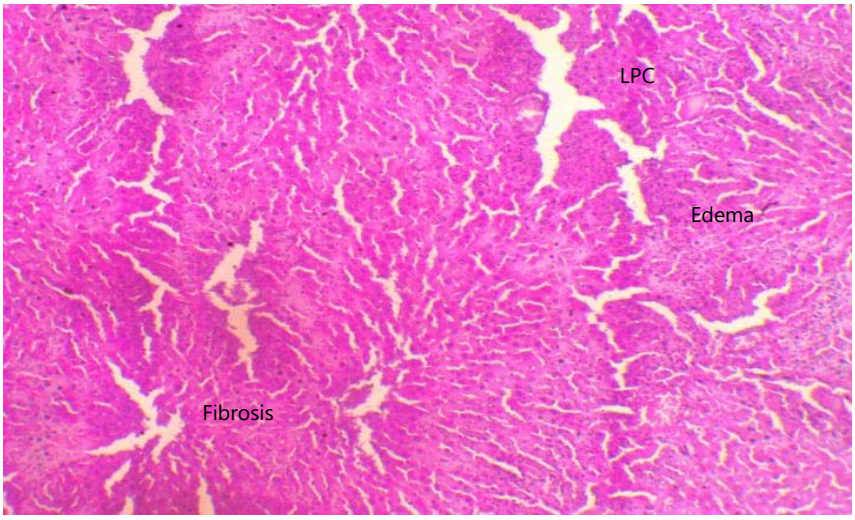

Fig. 4: Histological section of rat liver exposed to 300 mg/kg concentration of extract, stained with H&E (Magnification: 10×) on day 29. Edema, lymphoplasmacytic cells (LPC) and fibrosis were noted

Table 4: Effect of *Rauvolfia vomitoria* root bark extract on biochemical parameters

		Aqueous extract of *Rauvolfia vomitoria*		
Parameter (unit)	Control (n = 6)	75 mg/kg (n = 4)	150 mg/kg (n = 6)	300 mg/kg (n = 1)
Urea (mg/dL)	45.74±3.37	56.75±4.27	42.67±5.05	38.00
Blood sugar (mg/dL)	51.90±7.96	48.00±6.92	65.00±11.15	41.00
Creatinine (mg/dL)	0.48±0.02	0.47±0.02	0.47±0.02	0.40
AST (U/L)	154.80±6.27	220.25±53.45	170.00±17.83	117.00
ALT (U/L)	36.60±1.75	72.00±18.53	57.33±11.55	44.00
Calcium (mg/dL)	10.30±0.13	10.35±0.25	10.23±0.12	9.90
GGT (U/L)	2.80±0.58	4.75±2.43	2.67±0.80	1.00
ALP (U/L)	79.80±9.65	66.00±10.68	89.17±12.78	53.00
Sodium (mmol/L)	142.40±0.60	143.00±1.68	139.83±0.60	140.00
Potassium (mmol/L)	4.78±0.12	5.12±0.54	4.58±0.13	4.20
Chlorine (mmol/L)	102.40±0.60	101.75±0.48	99.50±0.85	99.00

Each value represents the Mean±SEM, (n) is the number of animals per batch, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase and ALP: Alkaline phosphatase

Table 5: Histological assessment of rat organs

		Aqueous extract of *Rauvolfia vomitoria*		
Organs	Control	75 mg/kg	150 mg/kg	300 mg/kg
Heart	N	N	N	N
Spleen	N	N	N	N
Kidneys	N	N	N	N
Liver	N	Edema+	LPC+, Edema++	Edema+ LPC++, Fibrosis+

N: Normal, +: Mild, ++: Moderate and LPC: Lymphoplasmacytic cells

DISCUSSION

The phytochemical screening of the aqueous extract of *R. vomitoria* root bark revealed the presence of alkaloids, tannins, flavonoids and saponins. These results corroborated those of Akanji *et al.*[13] in Nigeria. Also in Nigeria, Ezeani *et al.*[18] found in addition to present study data, cardiac glycosides, terpenoids and carotenoids. Several factors could explain these variations as its geographical location, the organ collected, the period, the time of collection and the storage conditions. some authors have also observed this variation in the chemical composition of *R. vomitoria*[19,20].

The larval cytotoxicity test results showed that the LC_{50} was 1.205 mg/mL. Referring to the Mousseux correspondence table on LC_{50} and toxicity[15], the LC_{50} is greater than 0.1 mg/mL. It can be concluded that the aqueous extract of the root bark of *R. vomitoria* is not cytotoxic.

Evaluation of the acute toxicity of the aqueous extract of root bark of *R. vomitoria* in Wistar rats showed that the LD_{50} is greater than 5000 mg/kg. Based on OECD recommendations, the extract can therefore be classified as non-toxic[12]. These results are comparable to those obtained by several authors[21-26].

The evaluation of the subchronic toxicity of the aqueous extract of *R. vomitoria* made it possible to observe and record the various adverse effects which appeared after its administration. In this study, animals which received doses of 300 mg/kg of body weight showed changes in behavior and signs of intoxication of varying severity (apathy, difficulty feeding, nasal congestion, eye irritation, difficulty moving, pasty fecal matter, death).

Monitoring of weight changes showed that the body weight of the treated rats was not statistically different from that of the controls ($p>0.05$). This same observation was made by other authors such as Asoro *et al.*[26] in Nigeria in 2013 with the ethanolic extract of *R. vomitoria* leaves at a dose of 300 mg/kg for 21 days, Oyewole *et al.*[27] in Sierra Leone in 2008 with ethanolic root extract at a dose of 1000 and 2000 mg/kg over 14 days.

Asoro *et al.*[26] and Eluwa *et al.*[28], who used ethanolic extracts of root bark of *R. vomitoria* over 7 and 14 days, respectively revealed a significant decrease in body weight in the treated groups compared to the control group in respective doses of 150-200 and 200-300 mg/kg.

The evaluation of animal organ weights is very important in toxicological studies. The weight of an organ or even more, the relative weight is an important index used in physiology and toxicology[29]. During the present study, the relative weights of the heart, spleen, kidneys and ovaries of the animals receiving the *R. vomitoria* extracts did not significantly vary compared to those of the control rats. On the other hand, the relative weight of the liver had significantly increased.

The increase in the relative weight of the liver reflects hepatic hypertrophy which could be due to inflammation. Histological data from the liver revealed edema at low doses; at intermediate doses,

lymphoplasmacytic cells were noted in addition to edema; at high doses, an increase in lymphoplasmacytic cells with the appearance of fibrosis and regression of edema were observed. Etim *et al*.[30] found a significant increase in the weight of the liver, kidneys and seminal vesicle of rats treated at doses of 25, 50 and 75 mg/kg compared to the control.

The hematopoietic system is one of the most sensitive targets for toxic substances. It represents an important marker of the physiological and pathological state of humans and animals[31]. This is why any change in hematological parameters has a predictive value for human poisoning when the data are translated from studies carried out on animals[32].

Concerning the hematological parameters studied, there are no statistically significant differences between the batches of rats which received the extract and the control batch which received distilled water. No statistically significant differences were found between the different batches having received the extract and the control batch with respect to the biochemical parameters studied. These results were similar to those of Oyewole *et al*.[27] which revealed that there was no significant difference in ALT, AST, alkaline phosphates, urea, creatinine, sodium, or potassium in rats treated at doses of 1000 and 2000 mg/kg with ethanolic extracts of root or leaves of *R. vomitoria* compared to the control batch.

Several authors have shown that *R. vomitoria* contains reserpine which has a toxic effect on the central nervous system[22-25]. Current study results on the behavioral assessment of the animals confirm this. *Rauvolfia vomitoria* due to its composition can then be dangerous. It is therefore recommended that phytomedicines containing this plant be clinically evaluated to confirm their safety before any use by our populations.

CONCLUSION

The present study was carried out on *Rauvolfia vomitoria* toxicity. Phytochemical screening revealed that the aqueous extract of *Rauvolfia vomitoria* root bark contains mainly alkaloids, flavonoids, saponins and tannins. Larval cytotoxicity results revealed that *Rauvolfia vomitoria* root barks are not cytotoxic. Acute toxicity showed an LD_{50}>5000 mg/kg, although associated with behavioral problems. Subchronic toxicity of 28 days showed that aqueous extract of *Rauvolfia vomitoria* causes a significant increase in relative liver weight and 86% death at 300 mg/kg. Histology showed an increase in liver weight. Given the significant increase in the relative weight of the liver and the histological hepatic abnormalities observed, it would be important, through further research, to better elucidate the impact of *Rauvolfia vomitoria* on the liver. Also, future research must be carried out to determine the mechanisms of action of *Rauvolfia vomitoria* on the central nervous system.

SIGNIFICANCE STATEMENT

Rauvolfia vomitoria contains chemical compounds (yohimbine, reserpine, ajmaline and ajmalicine) which, in large doses, can be dangerous to humans. The results of this work showed that at high doses, it causes damage to the liver. This work therefore provides additional proof of the prevalence of *Rauvolfia vomitoria*, a plant widely used in traditional medicine. The use of *Rauvolfia vomitoria* in traditional medicine should be done with caution, especially in cases of liver failure or problems. Phytomedicines containing this plant must therefore be studied clinically before any use by our populations.

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