Acute and subacute toxicity of the aqueous aerial parts extract of *Oxalis barrelieri* (Oxalidaceae)

ABSTRACT

Aims: The present study was carried out to investigate the possible (eventual) toxic effects of the aqueous aerial parts extract of *Oxalis barrelieri* and also to evaluate the acceptable safety level of this extract.

Place and Duration of Study: Department of Biological Sciences (Animal Physiology Laboratory), Higher Teachers' Training College, University of Yaoundé I. Between April 2017 and June 2018.

Materials and methods: Acute toxicity using a single dose of 2000 mg/kg was administered to mice and effects were observed for 14 days. In sub-acute toxicity, the experimental rats (males and females) received the aqueous extract of *Oxalis barrelieri* at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg daily for 28 days while the control and satellite control groups received distilled water and satellite test group received extract at the dose of 800 mg/kg. The physical parameters were evaluated throughout the treatment, while the haematological, biochemical and histological parameters were evaluated at the end of the treatment.

Results: The results obtained show no death and no significant variation ($p>0.05$) in the behavioral and morphological parameters. In sub-acute toxicity assay, few modifications were observed in biochemical parameters. At the higher dose of extract (800 mg/kg), there were a significant increase ($P<0.001$) in ASAT activity in male and female rats two weeks after extract administration, and a reversible significant increase ($P<0.05$) in triglyceride level in male rats only. Histopathology showed a reversible slight dose dependent structural alteration of the kidney and reversible vascular congestion in liver.

Conclusion: The aqueous aerial parts extract of *Oxalis barrelieri* could possess moderate toxicity at high doses and adequate caution should be exercised in its use in ethnomedicine.

Keywords: *Oxalis barrelieri*, aqueous extract, acute toxicity, sub-acute toxicity, Oxalidaceae.

1. INTRODUCTION

Oxalis is a cosmopolitan genus of more than 800 species, but major centers of diversity are in South America and South Africa. *Oxalis barrelieri* (synonym: *O. sepium*) is native to tropical South America, but has naturalized in many areas. It was first observed in Java in 1888. In South-East Asia it is common in Indonesia (Sumatra, Bangka, Java, Irian Jaya), Peninsular Malaysia, and Papua New Guinea [1]. *O. barrelieri* is erect, branched herb or shrub, up to 1.5 m tall, pubescent, without bulbs or rhizomes. Leaves subopposite, pinnately 3-foliolate,without stipules; petiole 2-9 cm long, canaliculate, ascendent; petiolule fleshy, about 1 mm long; leaflet elliptical to oblong, 1-5.5 cm x 0.5-2.5 cm, terminal one largest, base cuneate to emarginate, margin ciliate (especially at base), apex obtuse to rounded, discolorous, glaucous above. Inflorescence cymose, up toll(-30)-flowered; peduncle up to 6.5 cm long, bifid with branches up to 3 cm long, pubescent; bracts opposite the pedicels, pilose; pedicel up to 3 mm long with appressed bracteoles; sepals ovate lanceolate, 2-4 mm x 0.5-1.5 mm, light green, sometimes reddish veined; petals obovate-lanceolate, 6-9 mm x 2-2.5 mm, pink but lower half greenish with yellow spots, rolling inwards after anthesis; outer stamens up to 2 mm long, inner ones up to 3 mm long bearing a dorsal tooth; pistil 3.5-4 mm long, carpels 3-4-ovuled, styles 1-1.5 mm long, pubescent. Capsule ovoid, 5-10 mm x 3-5 mm, 5-angular, base and apex 5-lobed, glabrous. Seeds usually 3 per carpel flatten edovoid, about 1.5-2 mm x 1 mm, 8-ribbed in zigzag, deeply transversely

striate, brownish [1]. *O. barrelieri* is known as “belimbing tanah” in Malaysia, as “Tetele owono bekon” in South Cameroon. *O. barrelieri* has been claimed to have effect on antifungal and free radical scavenging activities [2]. Enoch et al. [3] reported that administration of 500 mg/kg and 1000 mg/kg aqueous and ethanolic extracts of *O. barrelieri* on Sprague-dawley rats produced significant reductions of glycemia in both non-diabetic and diabetic rats. A decoction of the entire plant is used for the treatment of diarrhea [4, 5]. *O. barrelieri* is rich in phenols, flavonoïds, tannins, alkaloids and saponins [6]. However, the toxicity of *Oxalis barrelieri* has not been intensively studied in order to ascertain the limits of it application. The aim of this study was to investigate the acute and sub-acute toxicity effects of the aqueous aerial parts extract of this plant.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Extract Preparation

The leaves of *Oxalis barrelieri* were harvested in April 2017 at Yaoundé, in the Center Region of Cameroon. Botanical identification was done in the National Herbarium, Yaounde, by Paul MEZILI, by comparing with existing herbarium specimen no. 24509. The aerial parts of *Oxalis barrelieri* were dried at room temperature. The dried ground aerial parts of *Oxalis barrelieri* were extracted in distilled water by boiling 168 g in 4.18 L of water for 15 minutes and the extract solution was filtered using Wattman filter paper no 3. The filtrate was lyophilized and the resulting solid was used for the toxicity tests. The resulting material weighed 34.44 g, giving a percentage yield of 20.52% with respect to the powder. The extract re-dissolved readily in distilled water which was used as the vehicle.

2.2 Experimental animals

Female Swiss mice weighing 23 ± 3 g (10 ± 2 weeks) were used for acute toxicity ,and male and female young Wistar rats weighing 78 -120 g (6 to 8 weeks) for sub acute toxicity. These animals were raised in the Animal house of the Higher Teachers’ Training College, University of Yaoundé I. They were fed a standard laboratory died (NAAPCAM Sarl, Yaoundé, Cameroon) and given fresh water *ad libitum*. Before the experiments (acute toxicity), they were starved for 12 h in wire mesh bottom cages to prevent coprophagy but allowed free access to water. Prior authorization for the use of Laboratory Animals was obtained from the Cameroon National Ethics Committee (Reg. N° FWAIRB00001954). The use, handling and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8 and 9.

2.3 Acute toxicity

The acute toxicity was performed according to the sequential method of OECD (Organization for Economic Co-operation and Development). Using a stomach tube, the *O. barreleri* extract was administered to three female mice (20 – 26g) with a single dose (2000 mg / kg). The control group received vehicle. The same method and the same dose were repeated 48 hours later, on 3 additional animals. Thereafter, all animals were observed carefully for 14 days during which mortality, body weights and gross behavioral change were noted daily [7].

2.4 Sub acute toxicity

Young Wistar rats (78-120 g) in six groups of 12 animals (6 males and 6 females) for each dose level of *Oxalis barrelieri* were used in these tests. Sub acute toxicity was evaluated after single daily administration of extract at 200, 400 and 800 mg / kg orally for a period of 4 weeks. The satellite group was also treated with the extract of *Oxalis barrelieri* (800 mg/kg) for 4 weeks but these animals were sacrificed 2 weeks after stopping treatment. The satellite control and control groups received vehicle, satellite control and satellite group were sacrificed 2 weeks after treatment. All rats were maintained under identical conditions with food and water *ad libitum* for the entire period with close observation. Toxicity was evaluated in terms of corporal and organ weights (heart, kidney, liver, spleen, lungs, ovaries and testicles), gross behavior, gross and histological appearance of detoxification organs (kidney and liver). The plasma from EDTA blood prepared was carefully collected for blood chemistry and enzyme analysis (total protein, AST, ALT, creatinine, urea, total cholesterols and triglycerides) using Commercial kits (Fortress) and glycaemia using a glucometer (One Touch Ultra). The Haematological parameters (white blood cell count, red blood cell count, platelet count, hemoglobin, haematocrit, Medium Globular Volume (MGV), Average Corpuscle Concentration in Hemoglobin(ACCH), Average Volume of Platelets (AVP), Thrombocritis (THT), Average Corpuscle Content in Hemoglobin (ACCh) and Red Blood Cell Distribution Index (RDI) were evaluated using a Coulter counter [7, 8, 9].

2.5 Statistical analysis

The results were reported as mean ± SEM. The statistical significance was determined by using one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values less than 0.05 were considered as significant.

3. RESULTS

3.1 Acute toxicity

Administration of a single dose of aqueous extract of *Oxalis barrelieri* (2000 mg/kg) in mice did not result in any deaths in the first stage. 48 hours later, carrying out a second test did not result in any deaths. After 14 days of observation, no changes were observed in mice regarding: coat color, appearance, saddles, reflexes, alertness, heart rate, respiratory rate, sensitivity to noise, sensitivity to touch and body weight (Tables 1 and 2). The aqueous extract is *Oxalis barrelieri* categorized 5 which includes substances with LD_{50} is greater than 2000 mg/kg according to OECD guideline 423, 2001.

Table 1. Behavioral parameters observed in mice dosed with *O. barrelieri* aqueous extract

Treatment	Sex	Convul-sions	Reac tions weird	Agressivity	pilo-erection	sensitivity noise	sensitivity touch	Change coat	Number of deaths	Stool appearance
Distilled water	Female 1	-	-	-	-	+	+	-	-	Normal
	Female 2	-	-	-	-	+	+	-	-	Normal
	Femelle 3	-	-	-	-	+	+	-	-	Normal
O.b 2000 mg/kg	Femelle 1	-	-	-	-	+	+	-	-	Normal
	Femelle 2	-	-	-	-	+	+	-	-	Normal
	Femelle 3	-	-	-	-	+	+	-	-	Normal

-Parameter absent; + Parameter present (Each group contains 3 females)

Mice given the aqueous extract of single dose O. barrelieri leaves showed nonsignificant and non-dose-dependent changes in body weight (Table 2).

Table 2. Body weight change of mice during acute toxicity study of aqueous aerial parts extract of *Oxalis barrelieri*

Traitment / Weight	Distilled water (1 ml/100g)	*Oxalis barrelieri* extract	
		Test group	Confirmation group
Initial weight(g)	21.00±1.11	22.67±1.33	22.35±3.18
Final weight (g)	23.67±0.87	25.33±0.88	25.33±2.40
Body weight variation (%)	+12.71	+11.73	+13.33

n = 3 animals in each group; Values are expressed as mean ± SEM

3.2 Sub acute toxicity

Body weight change of male rats during sub acute toxicity study of aqueous aerial parts extract of *Oxalis barrelieri*

All rats (male and female) treated with O. barrelieri extract showed a body weight gain similar to that of control rats. No loss of body weight was observed. Male rats had higher weight gain than female rats (Tables 3 and 4).

Table3: Body weight change of male rats during sub acute toxicity study of aqueous aerial parts extract of *Oxalis barrelieri*

	Weight body variation of male rats (%).					
	7^{th} day	14^{th} day	21^{st} day	28^{th} day	35^{th} day	42^{th} day
Control	+29,45	+46,16	+77,57	+83,36		
O.b. 200mg/kg	+30,19	+57,39	+60,17	+70,30		
O.b. 400mg/kg	+20,99	+31,08	+51,33	+64,37		
O.b. 800mg/kg	+22,91	+40,03	+64,49	+73,24		
Satellite test	+18,22	+46,48	+67,05	+72,42	+100,64	+113,13
Satellite control	+25,71	+43,55	+56,67	+69,07	+92,70	+107,83

Table4: Body weight change of male rats during sub acute toxicity study of aqueous aerial parts extract of *Oxalis barrelieri*

	Weight body variation of female rats (%).					
	7th day	14th day	21st day	28th day	35th day	42th day
Control	+24,71	+37,22	+55,13	+63,55		
O.b. 200mg/kg	+19,01	+29,41	+50,30	+60,22		
O.b. 400mg/kg	+11,69	+27,25	+40,37	+54,41		
O.b. 800mg/kg	+16,85	+33,76	+51,92	+64,11		
Satellite test	+18,30	+37,79	+49,50	+56,40	+72,55	+71,12
Satellite control	+20,45	+34,65	+48,99	+61,28	+74,30	+80,05

Table 5 shows that the extract of *O. barrelieri* did not cause any significant variation in the weight of the vital organs compared to the control group. However, a significant decrease in spleen weights was observed in satellite male rats ($p < 0.01$). A significant ($p < 0.001$) non-dose-dependent increase in heart weight was observed in male rats treated with the 200 mg / kg extract dose.

Table 5: Effect of the aqueous aerial parts extract of *Oxalis barrelieri* on rat organs weights (values expressed as the percentage of organ weight over the body weight)

Organs	Control	O. b. 200mg/kg	O. b. 400mg/kg	O. b. 800mg/kg	Satellite test	Sat. C.
Males						
Liver	3.62±0.41	3.15±0.07	3.09±0.07	2.89±0.15	2.81±0.10	2.35±0.21
Right kidney	0.30±0.05	0.35±0.01	0.39±0.07	0.33±0.01	0.34±0.05	0.35±0.03
Left kidney	0.31±0.05	0.34±0.02	0.37±0.05	0.33±0.02	0.33±0.05	0.33±0.02
Lungs	0.81±0.04	0.99±0.07	0.93±0.04	0.82±0.04	0.94±0.21	0.81±0.03
Spleen	0.55±0.05	0.46±0.01	0.45±0.03	0.49±0.03	0.36±0.01**	0.58±0.03
Heart	0.38±0.01	0.51±0.03 ***	0.46±0.01	0.40±0.02	0.37±0.00	0.39±0.01
Right testicle	0.66±0.04	0.61±0.04	0.59±0.04	0.62±0.02	0.60±0.05	0.71±0.03
Left testicle	0.66±0.05	0.62±0.03	0.61±0.04	0.60±0.03	0.66±0.05	0.70±0.02
Female						
Liver	2.89±0.06	2.87±0.06	2.96±0.14	2.92±0.18	2.85±0.07	3.11±0.08
Right kidney	0.39±0.03	0.40±0.05	0.37±0.04	0.36±0.03	0.37±0.02	0.44±0.04
Left kidney	0.36±0.02	0.38±0.04	0.36±0.03	0.36±0.03	0.39±0.03	0.43±0.04
Lungs	0.75±0.07	0.90±0.11	0.82±0.04	1.01±0.07	0.81±0.07	0.96±0.05
Spleen	0.51±0.07	0.43±0.03	0.56±0.07	0.61±0.09	0.42±0.06	0.49±0.02
Heart	0.39±0.04	0.49±0.03	0.50±0.04	0.44±0.02	0.42±0.03	0.48±0.02
Right ovary	0.05±0.01	0.04±0.00	0.05±0.01	0.06±0.01	0.05±0.00	0.06±0.01
Left ovary	0.06±0.01	0.05±0.01	0.07±0.01	0.07±0.01	0.05±0.01	0.06±0.00

O. b: aqueous aerial parts extract of *Oxalis barrelieri ;* Sat. C: Satellite control
N = 5 animals in each group; Values are expressed as mean ± SEM
**p<0.01: statistically significant compared to control; ***p<0.001: statistically significant compared to control

The extract of *O. barrelieri* did not cause any significant dose-dependent variation on the hematological parameters. However, female rats treated at the extract dose of 200 mg / kg showed a significant ($p < 0.05$) non-dose-dependent increase (Table 6).

Table 6: Effect of the aqueous aerial parts extract of *Oxalis barrelieri* on hematological parameters in rat.

Parameters	Control	O.b. 200mg/kg	O.b. 400mg/kg	O.b. 800mg/kg	Satellite test	C. Sat.
Males						
RBC(10^6/mm^3)	8.14±0.34	8.07±0.34	6.87±0.29	7.07±0.17	6.44±0.29	7.01±0.56
Haematocrit (%)	45.24±3.54	48.34±3.14	37.30±4.03	39.12±7.62	39.50±5.62	42.62±4.48
Haemoglobin(g/dl)	15.30±0.39	16.24±0.76	14.94±0.56	14.76±0.90	15.22±1.25	14.82±0.20
Platelet(10^3/mm^3)	672.80±51.12	699.60±23.03	628.80±39.75	614.60±27.54	605.60±15.11	508.60±65.73
WBC (10^3/mm^3)	7.04±0.64	8.72±0.88	7.42±0.74	7.89±1.34	7.82±1.86	7.11±1.35
MGV (fL)	57.80±0.80	62.60±1.03	61.60±0.93	62.60±0.81	60.80±2.13	62.20±0.86

ACCH (g/dL)	29.74±0.66	32.62±2.71	34.64±1.12	32.10±1.69	33.98±2.05	31.82±2.87
VMP (fL)	9.72±0.40	10.16±0.25	8.74±0.27	9.30±0.41	9.98±0.67	9.36±0.39
THT (%)	0.66±0.04	0.69±0.03	0.57±0.04	0.65±0.07	0.62±0.08	0.52±0.09
ACCh (pg)	17.66±0.26	21.90±0.99	21.64±0.96	20.12±1.19	20.90±1.98	19.88±1.51
RDI (%)	13.32±0.49	13.94±0.51	13.20±0.94	13.84±0.76	13.96±0.54	14.52±0.83
Females						
RBC(10^6/mm^3)	7.52±0.28	9.44±0.52*	8.03±0.34	8.31±0.23	7.30±0.52	8.05±0.21
Haematocrit (%)	45.16±1.27	57.26±2.59	44.38±6.50	49.44±1.54	43.38±3.83	50.36±1.15
Haemoglobin(g/dl)	13.76±0.39	16.32±1.27	13.92±0.25	14.28±0.47	13.66±1.12	14.42±0.39
Platelet(10^3/mm^3)	624.40±43.72	748.40±88.77	818.60±66.49	732.60±29.84	618.00±70.13	554.80±31.81
WBC (10^3/mm^3)	8.31±0.51	7.27±0.46	9.02±0.72	7.83±1.14	7.23±0.12	8.05±0.42
MGV (fL)	59.60±0.87	61.00±1.52	60.80±0.58	59.40±1.69	59.40±2.42	61.60±0.81
ACCH (g/dL)	30.10±1.37	28.36±0.99	2 7.88±0.76	28.86±0.50	31.76±1.49	29.50±0.35
AVP (fL)	9.80±0.40	9.62±0.35	8.88±0.38	9.60±0.55	9.16±0.52	9.48±0.06
THT (%)	0.55±0.05	0.72±0.06	0.69±0.09	0.70±0.04	0.52±0.09	0.59±0.02
ACCh (pg)	18.88±1.83	17.22±0.61	17.00±0.51	17.18±0.28	18.90±1.50	18.06±0.41
RDI (%)	13.80±0.66	14.84±0.53	14.78±0.0 0	14.92±0.33	14.28±0.41	13.16±0.35

ACCH:Average Corpuscle Concentration in Hemoglobin; ACCh :Average Corpuscle Content in Hemoglobin;MGV: Medium Globular Volume;AVP: Average Volume of Platelets;IDR: Red Blood Cell Distribution Index;THT: Thrombocritis;WBC: white blood cells; RBC: red blood cells.

Table 7 shows that the extract of O. barrelieri resulted in a significant increase ($p < 0.001$) of the activity of aspartate aminotransferase (AST) two weeks after discontinuation of the extract treatment (Satellite test). in male and female rats. In female rats, the urea level increased significantly ($p < 0.05$) two weeks after stopping treatment (Satellite test). However, the significant ($p < 0.05$) increase in triglyceride levels observed in males treated with the extract (800 mg / kg) was corrected two weeks after stopping treatment (Satellite test). The other plasma parameters (glycemia, ALT, total proteins, cholesterol, creatinine) did not show any significant variation.

Table7. Effect of the stem bark the aqueous aerial parts extract of *Oxalis barrelieri* on blood biochemical parameters in rats

Parameters	Control	O.b. 200mg/kg	O.b. 400mg/kg	O.b. 800mg/kg	Satellite test	C. Sat.
Males						
Glycaemia(mg/dl)	58.40±2.50	54.40±3.11	62.40±1.50	52.40±2.14	61.20±2.44	59.00±0.84
AST (UI/l)	105.85±6.49	86.45±5.52	128.72±16.39	114.16±17.61	264.84±29.56 ***	87.07±5.03
ALT (UI/l)	62.70±6.88	64.88±7.01	68.88±3.53	66.24±1.13	55.13±6.52	51.00±3.42
Total protein(mg/dl)	96.79±5.86	94.36±5.35	80.44±5.06	91.98±4.31	80.59±4.08	84.82±2.08
Cholesterol (mg/dl)	43.30±0.32	65.91±2.67	67.39±6.73	60.73±9.05	51.33±3.42	66.07±7.12
Triglyceride (mg/dl)	76.50±8.51	78.18±7.31	87.08±10.62	121.31±10.94*	77.26±10.17	94.23±3.19
Creatinine (mg/dl)	0.51±0.04	0.63±0.03	0.58±0.06	0.53±0.03	0.69±0.02	0.61±0.05
Urea (mg/dl)	40.11±3.23	61.83±6.93	40.76±7.41	43.91±5.69	43.66±2.32	42.98±2.52
Females						
Glycaemia(mg/dl)	66.00±1.00	61.00±2.74	63.00±2.51	68.00±3.56	67.20±0.58	66.00±5.41
AST (UI/l)	98.62 ±10.43	104.51±5.66	94.36±4.38	162.76±19.92 *	217.79±21.55 ***	78.61±1.90
ALT (UI/l)	47.62±7.71	42.38±3.00	51.99±2.25	39.89±3.31	45.43±7.97	39.75±8.69
Total protein (mg/dl)	84.84±6.23	83.34±3.69	72.17±7.02	72.96±1.71	92.02±2.61	87.99±3.79
Cholesterol (mg/dl)	40.44±3.99	59.06±10.58	51.44±6.46	38.97±4.09	42.78±4.45	45.42±0.78
Triglyceride (mg/dl)	57.68±5.66	74.24±4.27	55.03±5.98	64.91±11.80	77.94±9.23	66.71±8.67
Creatinine (mg/dl)	0.43±0.01	0.60±0.06	0.52±0.03	0.49±0.03	0.56±0.03	0.54±0.05
Urea (mg/dl)	31.67±4.51	33.56±2.23	33.49±2.41	29.60±3.04	59.87±8.94 *	35.87±3.61

* P<0,05 significant difference compared to the control group; *** P<0.001 significant difference compared to the control group; Sat. C: Satellite control;

After four weeks of administration of the aqueous extract of Oxalis barrelieri, the rats of the lots receiving doses 200; 400 and 800 mg / kg presented vascular congestions compared to the control.

These increased as the dose increased with cell destruction that mixed with the Centro-lobular vein; virtually closes the latter. Observation of liver tissue from the test cells fourteen days later showed complete repair of these congestions (Figure 1). Histological section analysis revealed renal tissue damage including enlargement of the glomerular chamber and destruction of nephrons. This alteration is dose-dependent and appears to have worsened two weeks after stopping treatment (Figure 2). The analysis also showed that the said alteration is more pronounced in males than in females

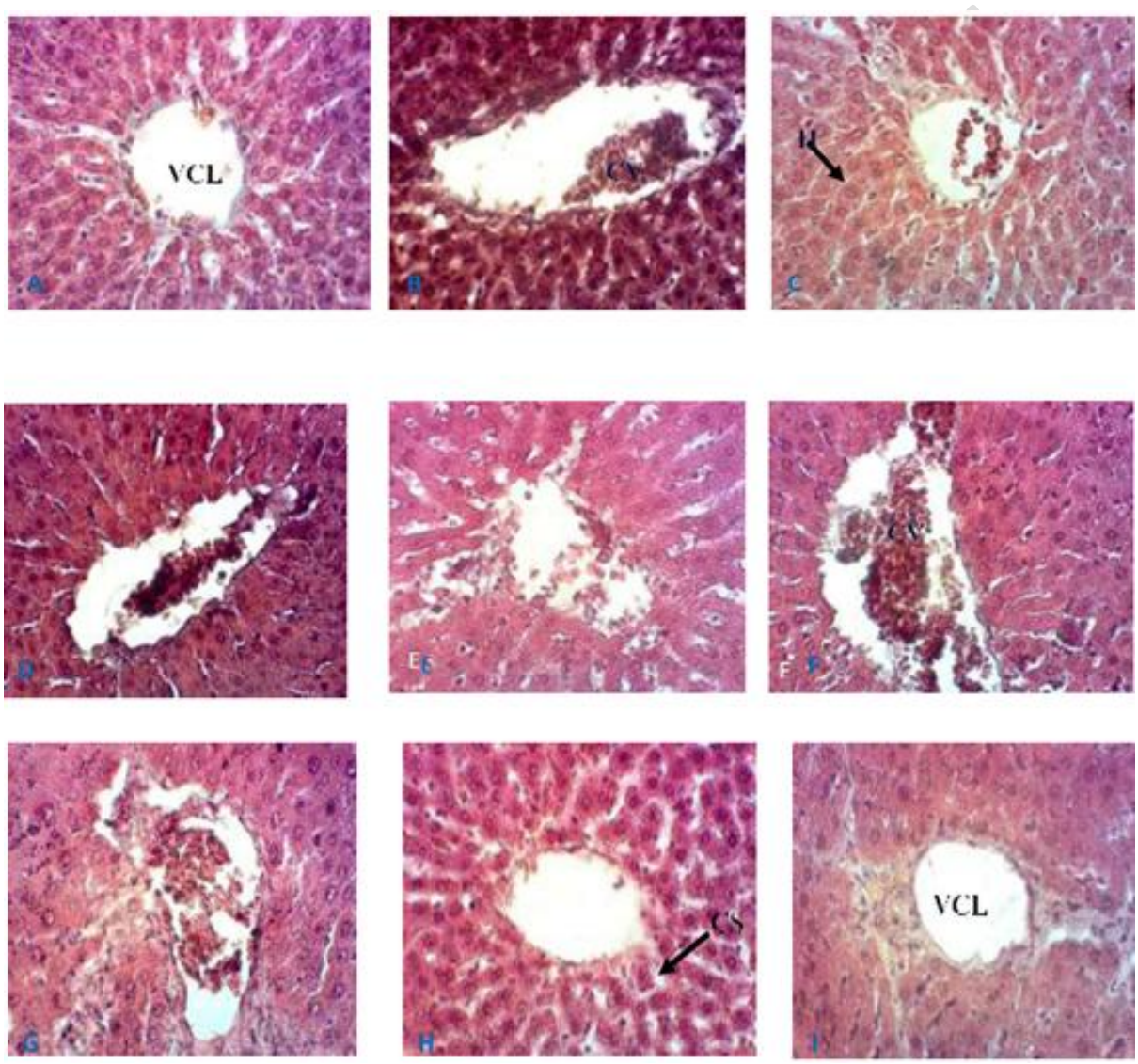

Figure 1. Effect of the aqueous extract of *Oxalis barrelieri* on liver tissues of rats (HE x 400)
CS = sinusoidal capillary; CV = Vascular congestion; H = hepatocyte; VCL = Centro-lobular vein; A = control (male and female); B = 0.b 200 mg / kg (female); C = O.b 200 mg / kg (male); D = O.b 400 mg / kg (female); E = O.b 400 mg / kg (male); F = O.b 800 mg / kg (female); G = O.b 800 mg / kg (male); H = Satellite test; I = control satellite.

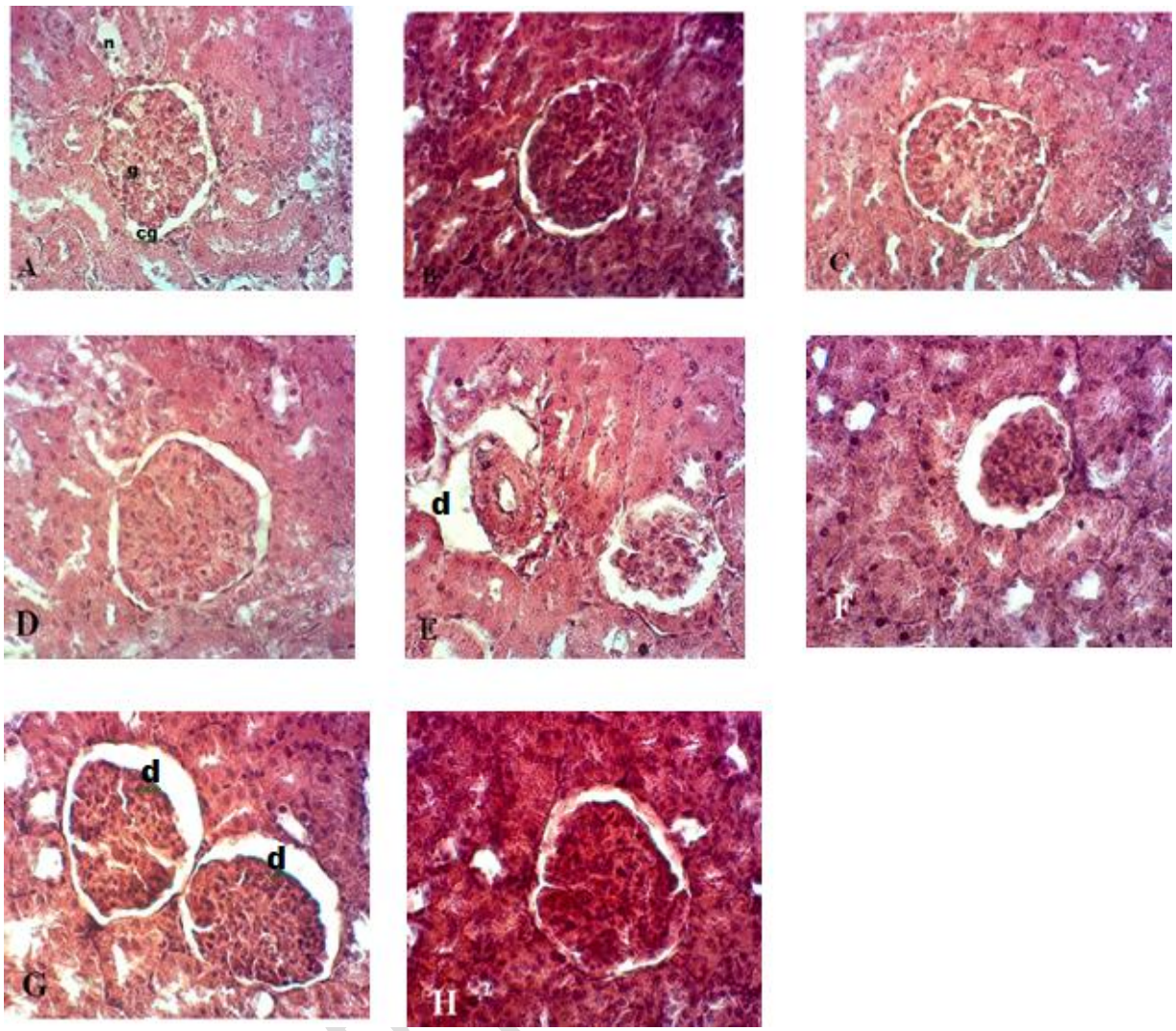

Figure 2. Effect of the aqueous extract of *Oxalis barrelieri* on kidney tissues of rats (HE x 400)

A = control (male and female); B = 0.b 200 mg / kg (female); C = O.b 200 mg / kg (male); D = O.b 400 mg / kg (female); E = O.b 400 mg / kg (male); F = O.b 800 mg / kg (female); G = O.b 800 mg / kg (male); H = Satellite test; n = nephron; g = glomerulus; cg = glomerular capsule; d = enlargement of the glomerular chamber

4. DISCUSSION

Oxalis barrelieri is a medicinal plant used to treat certain pathologies such as diabetes [10] and diarrhea [4,5]. In the course of this study, these studies have made it interesting to evaluate the toxic effects of the plant on biological systems, particularly in the liver and kidneys.

The oral administration of a single dose (2000 mg / kg) of the aqueous extract of the aerial parts of *Oxalis barrelieri* did not cause any significant changes in either the behavior of the animals or the physical condition of these animals. last. The sensitivity to noise and touch, the condition of the coat, the nature of the stool showed no significant variation (Table 1). No deaths were observed during the 14 (fourteen) days of observation. In addition, the body weight of the mice treated with *O. barrelieri* extract did not undergo any significant variation (Table 2). These results suggest that the lethal dose

(LD 50) of the aqueous extract of *O. barrelieri* is greater than 2000 mg / kg. According to OECD Guideline 423, 2001 this extract is slightly toxic [11].

Repeated administration for 28 days (subacute toxicity) of the *O. barrelieri* aqueous extract caused no deaths in the treated animals. At all doses, *O. barrelieri* extract did not cause any significant variation in animal body weight. The growth of animals treated with *O. barrelieri* extract was similar to that of control group rats (Tables 3 and 4). This suggests that *O. barrelieri* extract does not significantly alter animal metabolism as well as growth hormone and cartilage [12]. The relative weight of vital organs showed no significant dose-dependent variation (Table 5). However, O. barrelieri extract at a dose of only 200 mg / kg resulted in a significant increase in the relative weight of the heart. Since this increase in the relative weight of the heart is not dose-dependent, it can not be attributed to the extract. In general, changes in body weight of treated animals, as well as the vital organs (liver, kidneys, lungs, testicles, ovaries, spleen and heart), are indicators of a substance with high toxicity [13, 14]. So this extract would be slightly toxic.

Blood is one of the targets of the body most attacked by toxic substances, it provides important information on the physiology and pathologies of animals [15].Haematological parameters give information on hematopoietic function (evaluation of cells of the myeloid lineage) and the determination of the occurrence of any allergies (white blood cell studies) [16]. Blood parameter analysis in rodents can provide a high predictive index (up to 91% concordance) for risk of toxicity in humans [17]. Our results showed no significant difference in hematological parameters between controls and treated groups with the exception of the rate of red blood cells, female rats treated with O. barrelieri extract at a dose of 200 mg / kg, which showed a significant non-dose-dependent increase (Table 6). These results are similar to those obtained with the aqueous extracts of *A. schweinfurthii* [12], *Eremomastax speciosa* [18] and *Ocimum suave* [19].

Serum biochemical parameters are used to evaluate the effects of xenobiotics on liver and kidney function.The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation and its anatomical and physiological structure. The study of fasting blood glucose gives information on the state of functioning of the liver and pancreas. However, the liver provides storage and release while the pancreas information on the availability and deficiency [20]. No significant variation was observed in animals treated with the extract of *O. barrelieri*. This result indicates that this extract does not change the functioning of the liver and pancreas. Generally, analysis of the activities of some basic liver enzymes (such as ALAT and ASAT) in the plasma or serum can be used to indirectly assess the integrity of tissues after being exposed to certain pharmacological agents [21]. Necrosis or membrane damage releases the enzymes into circulation; therefore, it can be measured in the serum. Usually, about 80% of ASAT is found in the mitochondria whereas ALAT is a purely cytosolic enzyme. Therefore, ASAT appears in higher concentrations in a number of tissues (liver, kidneys, heart and pancreas) and is released slowly in comparison to ALAT. But since ALAT is localized primarily in the cytosol of hepatocytes, this enzyme is considered a more sensitive marker of liver inflammation or damage than ASAT and within limits can provide a quantitative assessment of the degree of damage sustained by the liver [22]. *O.*

barrelieri extract at 800 mg / kg resulted in a significant ($p < 0.05$) increase in ASAT activity in female rats. Two weeks after stopping the administration of the extract, ASAT activity significantly ($p < 0.001$) increased (Table 7). This result would suggest that this extract would cause an alteration of the liver or other organs such as the kidney, heart, pancreas, muscles.

The lipid profile is an indicator of lipid metabolism in the liver [23]. The increase in serum triglyceride levels is due to liver dysfunction and may cause cardiovascular problems. Triglycerides increased significantly ($P < 0.01$) in male rats treated with the extract (800 mg/kg), this effect disappear in the two weeks following discontinuation of therapy (table 7). This would suggest that the *O. barrelieri* extract might disturb hepatic lipid metabolism and cause cardiovascular problems, but this effect is reversible. Estimation of total protein is one of the most widely used means of measuring hepatocellular injury. Total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney disease, liver disease, and many other conditions. Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. High total protein levels may be seen with chronic inflammation or liver infections. Total cholesterol test is used to estimate risk of developing a disease (specifically heart disease) and some liver dysfunctions. Increase in the total protein and cholesterol as well would have indicated hepatocyte damage [24]. There were no significant changes in any liver function parameters (such as total cholesterol, total protein) and in serum lipid profile (cholesterol) as compared to the control groups. All these results suggest the absence of major hepatotoxicity and cardiovascular risks factors induced by *O. barrelieri.*

The kidneys are highly susceptible to toxicants for two reasons; a high volume of blood flows through it and its ability to filter large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones. Blood urea nitrogen is derived in the liver protein/amino acid from dietary or tissue sources and is normally excreted in the urine. In renal disease, serum urea accumulates because the rate of serum urea production exceeds the rate of clearance [25]. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet and catabolic state, thus serum urea concentration is often considered the more reliable renal function predictor than serum creatinine [26]. There were no significant changes in the levels of serum creatinine in the treated groups compared with the controls. Extract of *O. barrelieri* (800 mg / kg) resulted in a significant ($p < 0.05$) increase in the urea level that normalized in the two following treatment discontinuation (Table 7). These results would suggest that the extract would have reversible deleterious effects on the kidney.

Liver tissue analysis of animals treated with the *O. barrelieri* aqueous extract suggests the presence of structural abnormalities. Hepatic vein congestion was observed in all rats given the aqueous extract of *O. barrelieri* at all doses. However, within two weeks of stopping the administration of the extract, this anomaly was normalized. This would suggest that hepatic vein congestion induced by the *O. barrelieri* aqueous extract is reversible (Figure 1). This would suggest that the liver has put in

place self-healing mechanisms. Kidney histology revealed nephron destruction and glomerular chamber elevation in the 400 mg / kg and 800 mg / kg dose groups, which were more pronounced in males than in females. These observations suggest that high doses of the extract cause renal tissue damage because the rats treated with the extract (200 mg / kg) showed no structural abnormality on the renal tissue (Figure 2).

5. CONCLUSION

Our study shows that the LD_{50} of the *O. barrelieri* extract is greater than 2000 mg/kg, so this extract is classified as poorly toxic substances. A study with three dose levels (200mg/kg, 400mg/kg and 800mg/kg) administered daily to the animals, for a period of 28 days, did not result in any change in behavior liver function and renal function for the dose 200 mg/kg and 400 mg/kg. Only the dose of 800 mg/kg caused an increase in plasma ASAT level two weeks after treatment.Hepatic vascular congestion was observed in extracted rats, but this congestion disappeared two weeks after treatment. Renal tissue lesions appear to be worsened after treatment. Further investigations need to be done for the complete elucidation of the safety profile of *O. barrelieri*.

6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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