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Acute and sub-chronic toxicity study of the mixture of *Calotropis procera* (Ait). R. Br. (Apocynaceae) and *Zanthozylum zanthozyloïdes* Lam. (Rutaceae) roots bark powder

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ABSTRACT

In Burkina Faso, sickle cell syndromes affect 8.42% of hospital patients. For the management of sickle cell anemia, a phytomedicine called FACA® capsules based on a mixture of roots barks powdered from *Calotropis procera* and *Zanthozylum zanthozyloïdes* has been developed. The objective of this study was to evaluate the acute and sub-chronic toxicity of this mixture. Toxicity studies were conducted according to OECD guidelines on Wistar rats. The acute toxicity test estimated the LD_{50} to be 5000 mg/kg body weight. The sub-chronic toxicity study showed a significant dose-dependent decrease in food consumption; and the body weight gain of the treated rats. Biochemical analysis showed a significant decrease in the level of serum total protein and cholesterol and an increase of ALT and blood sugar levels at 500 and 1000 mg/kg. A significant decrease in the number of red blood cells and hemoglobin have been observed at 1000 mg/kg. Histopathological examination of the organs shows atrophy of the muscle wall and destruction of the epithelial surface of the stomach at 1000 mg/kg body weight. Based on these results, this mixture does not exhibit acute oral toxicity and could be well tolerated at therapeutic doses (17.5 mg/kg).

Keywords: *Calotropis procera*, *Zanthoxylum zanthozyloïdes*, acute toxicity, sub-chronic toxicity, sickle cell anemia, FACA®.

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INTRODUCTION

Sickle cell disease is a genetic disease due to an abnormality of hemoglobin. It affects about 50 million people worldwide and is particularly common among people from sub-Saharan Africa, India and Mediterranean countries (Belala et al., 2016). In Burkina Faso, the major sickle cell syndromes have a fairly high prevalence because they affect 8.42% of patients in the hospital environment (Ouédraogo-yugbaré et al., 2014).

Traditional medicine has an important place in the treatment of sickle cell anemia in Africa. Many plants are used in traditional treatment of sickle cell anemia in Africa. In Burkina Faso, *Calotropis procera (C. procera)* and *Zanthoxylum zanthozyloïdes (Z. zanthoxyloïdes)* are among the many plants used in traditional treatment of sickle cell disease (Zerbo et al., 2008). An improved traditional medicine called FACA® based on these two medicinal plants was developed by the Research Institute of Health Science of Burkina Faso. FACA® is used for the symptomatic treatment of sickle cell crisis and preventive treatment of the morbid events related to sickle cell disease.

Previous studies have demonstrated the anti-sickling, anti-inflammatory, analgesic, antipyretic and muscle relaxant properties of these plants and FACA® (Ouedraogo et al., 2020). The acute toxicity of the mixture of *Z. zanthozyloïdes* and *C. procera* was studied on mice and permit to classify this mixture among the low toxic substances with an oral LD_{50} greater than 2000 mg/kg (Ouedraogo, 2015). However, there are no data on the toxicity associated with chronic use of these plants. But the treatment of sickle cell disease is a long-term treatment. Thus this study was aimed at assessing the sub-chronic toxicity of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders called FACA® capsule for its better use for the management of sickle cell disease.

MATERIALS AND METHODS

Plant material

The plant material consisted of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* (FACA® powder). It was, supplied by U-PHARMA laboratory of "Institut de Recherche en Sciences de la Santé (IRSS)" was mixed with distilled water and the resulting suspension was administered orally to the rats.

Animals

Wistar male and female rats weighing 182.3 ± 8.64 g and 159.2 ± 4.85 g respectively were used for this study. These animals were procured from the IRSS pet shop and were reared at an ambient temperature (23-25°C) and 40-60% of humidity. They were fed with 29% protein enriched wheat cake and tap water. These animals were subjected to 12 hours of illumination and 12 hours of darkness. The experiments using animals were carried out in accordance with protocols already validated by the Research Institute of Health Sciences (IRSS, Burkina Faso) and that meet international standards guiding line set by the European Union on the protection of animals (CEC Council 86/609).

Acute toxicity test

Acute toxicity test was conducted using OECD N°423 guideline, the acute toxicity class method for the testing of chemicals (OCDE, 2001).This test was carried out on two groups of three healthy females Wistar rats (one control and one test group). The rats were fasted four hours before testing and then, a single dose of 2000 mg/kg b.w. of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders was administered orally using a gastric tube to the test group. After administration, the animals were observed every 30 minutes for 2 hours. After the two hours observation, the animals were fed and then observed daily for 14 days. Mortality and any behavioral change such as changes in skin and fur, eyes, mucus membranes, convulsion, salivation, diarrhea, lethargy, sleep and coma were recorded during the observation period.

Sub-chronic toxicity test

The study of sub-chronic toxicity was performed according to the OECD 408

guideline for chemicals testing (OCDE, 1998) on Wistar rats.

The test was conducted on five groups of 20 rats each (10 females and 10 males) including control group and four test groups. All groups were homogeneous and sex-specific. Females were nulliparous and nonpregnant. Distilled water (solvent) was administered to the control group. The four test groups received respectively 100, 250, 500, and 1000 mg/kg of the mixture. All of these substances were administered to animals by gavage once daily at the same time for 90 days using a gastric tube.

Clinical observation

All animals were observed twice daily, at the same time, for symptoms of morbidity and mortality.

Body weight and relative organ weights

All animals were weighed on the day prior to administration of the extract, once a week throughout the trial period and the last day before the sacrifice. Organs such as the liver, kidney, spleen, heart, lungs and stomach were removed and weighed and preserved in formalin buffer.

The relative weight of each organ was calculated according to the following formula:

$$\text{Relative organ weight (\%)} = \frac{\text{absolute weight of organe (g)}}{\text{body weight of animal (g)}} \text{ X } 100$$

Water and Food intake

Water consumption was measured daily at the same time during the trial period and food consumption was measured once a week during the 90 days of treatment.

Analysis of hematological parameters

At the end of the test period, the animals were fasted for 12 h and then sacrificed. Blood samples were collected into anticoagulant (EDTA) tubes for hematological analysis. The levels of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hematocrit (HCT), hemoglobin (HGB), platelets (PCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), Platelet Distribution Width (PDW) index were determined using a semiautomatic cell counter (Hospitex Diagnostic, model: Hema screen 13, Italy).

Biochemical parameters Analysis

Blood samples collected in anticoagulant-free tubes were centrifuged at 3000 rpm for 10 min. The collected sera stored at -20°C were used for assaying the biochemical parameters such us glucose, creatinine, total cholesterol, total protein, AST, ALT, Cl^-, $PO4^{2-}$, Ca^{2+},Na^+ and K^+ using a semi-automatic biochemical analyzer (Mindray, BA-88A/WR-84028454).

Histopathological study

The histopathological study was conducted using the method described by Lam et al. (Lam et al., 2007). Organs previously stored in 10% formol were paraffin treated and cut (5 µm thick) using a microtome. The sections were then stained with hematoxyline-eosin, and then observed under the optical microscope.

Statistical analysis

The results were presented as mean ± standard deviation. Data were calculated separately for males and females. The statistical analysis of the results was carried out using one-way analysis (ANOVA) of GraphPad Prism software. 5 (GraphPad software, San Diego, California, U.S.A.) followed by Dunett's multiple comparison tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Oral acute toxicity study

Oral administration at a single dose of this mixture did not caused death of the Wistar rats during the study period Also, no toxic symptoms in terms of behavioral changes, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss were observed in any animals, up to 14 days after the administration of extracts.

The LD_{50} of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders was estimated to be 5000 mg/kg body weight, according to the OECD test guideline.

Sub-chronic toxicity test

During the sub-chronic (90-day) toxicity study period, daily administration of the mixture to rats resulted in no mortality of rats. However, during the clinical observation of rats exposed to the extract, there were cases of diarrhea in both sex at 500 and 1000 mg/kg b.w. during the first week of treatment.

Body weight change

Changes in body weight of control rats and those treated daily with the mixture for 90 days are presented in Figures 1 and 2. These results globally showed an increase in body weight of control and treated rats during the treatment period. At 500 and 1000 mg/kg b.w. there was a significant decrease in body weight of females' rats during the 1st month of treatment. At the same dose, male rats showed a slight decrease in body weight during the first month of treatment. However, unlike females, this body weight decrease was not significant. During the 2nd and 3rd month of treatment, statistical analysis of the results showed a significant difference in body weight between control groups and male rats treated with 500 and 1000 mg/kg. In females' rats, there was a significant difference in body weight between control and all the treated rats during the 2nd and 3rd month of treatment.

Relative weight of organ

The relative organ weights of control rats and those treated with different doses of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders are presented in Table 1. These results showed that there were no significant differences in relative organ weights such as liver, kidney, heart, spleen, and lungs in all treated rats (males as well as females) compared with controls. However, the relative weights of the stomach of the rats treated with 500 and 1000 mg/kg were significantly increased.

Water consumption

Daily administration of the mixture resulted in a decrease in daily water consumption in all treated rats throughout the treatment period compared to the control. Statistical analysis of the results (Table 2) showed that the decrease in water consumption was significant ($p<0.05$) in males treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg compared with control rats. In females, however, the difference was not statistically significant.

Food intake

The results of the mean food consumption of control rats and those treated with the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders are presented in Table 3. These results showed a decrease in food consumption in all rats treated at 500 and 1000 mg/kg body weight compared with controls throughout the treatment period. Statistical analysis of the results revealed a significant difference in food consumption among males treated at 500 and 1000 mg/kg during the first two months of treatment. In females' rats, the decrease in food consumption was statistically significant at 1000 mg/kg during the first month of treatment and at 500 and 1000 mg/kg during the second month of treatment. In contrast, in the third month, the decrease in food consumption was not statistically significant ($p>0.05$) in both males and females treated groups compared with controls groups.

Hematological parameters

Table 4 presents the results of the effect of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders on hematological parameters in rats. Based on the results of this table, daily administration of this mixture resulted in a significant increase of the levels of PLT of all rats treated at 1000 mg/kg and PCT level in females treated at 500 mg/kg and in males treated at 1000 mg/kg b.w. compared with controls. The administration of this phytomedicine also resulted in a significant ($p<0.05$) decrease in red blood cell in males rats treated at 500 mg/kg and 1000 mg/kg body weight, in hemoglobin at 1000 mg/kg in males and HCT blood levels in all rats (males and females) treated at 100, 500 and 1000 mg/kg b.w. compared to controls.

Biochemical parameters

Table 5 presents the results of biochemical analysis of serum from control rats and daily treated rats with FACA® for 90 days. These results show that daily administration of the mixture from *Calotropis*

procera and *Zanthoxylum zanthoxyloïdes* root bark powders resulted in decreased levels of some biochemical parameters such as creatinine, AST, cholesterol, total proteins, chlorine, and PO_4^{2-} ions. Statistical analysis of the results showed that this decrease was statistically significant ($p<0.05$) for creatinine in all treated rats (males and females). Decreased cholesterol levels were significant at 500 mg/kg in males and 1000 mg/kg in both males and females. The decrease in total proteins was significant in all rats (males as well as females) treated with 1000 mg/kg body weight. With respect to the level of AST, there was a significant decrease at 250 mg/kg in males, 500 mg/kg in females, and all rats treated with 1000 mg/kg body weight. For Cl^- and PO_4^{2-}, this decrease was significant ($p<0.05$) in males at 250 mg/kg for both ions and at 500 mg/kg for PO_4^{2-} ions in males. At 1000 mg/kg b.w., the increase in Cl^- was significant in all treated rats, whereas for PO_4^{2-} this decrease was significant only in males.

In contrast, glucose and ALT levels were increased in all treated rats compared to controls. Statistical analysis of the results showed that the increase in glucose was significant ($p<0.05$) for males treated at 100 mg/kg body weight and 1000 mg/kg in all treated rats. For ALT, the difference from controls was significant in male and female rats treated at 500 mg/kg and only in males treated at 1000 mg/kg body weight.

In addition, calcium ion levels were significantly decreased in males treated at 1000 mg/kg, whereas in females treated at 100 and 250 mg/kg, calcium ion levels were significantly increased. The same was true for K^+, which increased significantly in females treated at 250 mg/kg but decreased significantly in males treated at 500 and 1000 mg/kg body weight.

Histological evaluation

Histopathological examination of the organs (Figures 3 and 4) shows that this mixture caused an atrophy of the muscular wall and a destruction of the epithelium surface of the stomach in rats treated with 1000 mg/kg b.w. No abnormalities were observed in the other organs of the treated rats when compared to the controls.

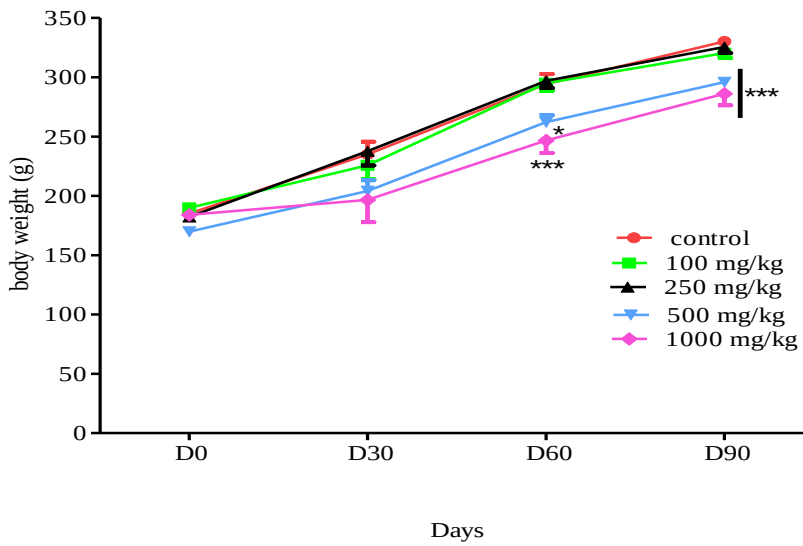

Figure 1: Body weight evolution in male rats.

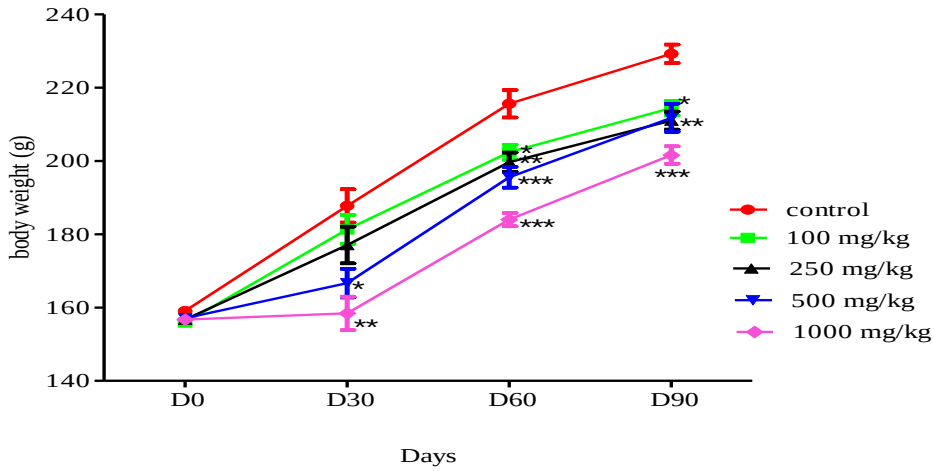

Figure 2: Body weight evolution in female rats.

Table 1: Relative weight of organs.

Organs	Sex	control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Liver	M	2.57±0.25	2.64±0.22	2.42±0.15	2.45±0.17	2.42±0.14
	F	2.59±0.24	2.76±0.15	2.73±0.16	2.78±0.20	2.77±0.17
Kidneys	M	0.61±0.02	0.65±0.04	0.59±0.03	0.60±0.06	0.58±0.03
	F	0.61±0.06	0.58±0.05	0.64±0.06	0.62±0.06	0.65±0.04
Heart	M	0.30±0.02	0.29±0.02	0.31±0.02	0.31±0.03	0.29±0.03
	F	0.34±0.04	0.34±0.03	0.33±0.03	0.32±0.03	0.32±0.03
Lungs	M	0.44±0.05	0.49±0.02	0.44±0.04	0.46±0.05	0.43±0.04
	F	0.50±0.05	0.53±0.06	0.55±0.08	0.53±0.10	0.55±0.12
Stomach	M	0.89±0.11	1.07±0.42	1.31±0.23	2.68±0.90***	4.37±1.75***
	F	1.02±0.26	1.53±0.77	1.98±0.64	4.18±1.40***	6.80±2.44***
Spleen	M	0.22±0.04	0.19±0.03	0.21±0.03	0.21±0.03	0.22±0.05
	F	0.25±0.03	0.24±0.02	0.24±0.04	0.23±0.02	0.25±0.04

n = 10; values are presented as mean ± standard deviation (S.D.) ***: P <0.001. M = Male; F = Female.

Table 2: Average water consumption (mL/rate/day).

Months	Sex	Control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
1st month	M	49.14±6.83	41.40±2.95	32.81±3.06***	35.17±2.61***	30.13±3.04***
	F	36.91±2.90	32.95±1.25	32.52±2.29	32.41±2.57	34.78±2.56
2nd month	M	58.39±10.36	53.57±8.475	39.63±2.394**	40.56±4.219**	35.73±5.003***
	F	36.47±2.00	32.38±2.75	34.34±1.98	33.45±3.15	35.97±3.06
3rd month	M	68.25±10.24	63.76±10.00	47.82±7.36**	50.26±5.90**	49.21±3.49**
	F	39.38±3.65	34.37±4.23	34.86±5.46	36.84±3.78	39.09±4.61

n = 10; values are presented as mean ± standard deviation (S.D.); **: P<0.01; ***: P <0.001. M = Male; F = Female.

Table 3: Daily mean food consumption (g/day/rate).

Days	Sex	control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
1st month	M	25.06±2.15	27.11±1.77	22.89±0.38	21.18±1.85*	20.30±2.28**
	F	19.44±0.86	22.36±0.30	20.26±0.69	18.83±2.65	14.25±3.01**
2nd month	M	25.94±1.40	25.04±1.12	24.42±0.53	20.41±2.04***	18.87±2.38***
	F	20.06±1.04	19.74±0.12	21.46±1.03	17.52±1.93*	14.46±0.95***
3rd month	M	23.44±2.32	25.13±1.35	23.49±2.10	20.38±3.44	20.01±1.42
	F	17.82±1.44	20.99±1.03	19.83±3.86	16.65±2.90	16.01±3.01

n = 10; values are presented as mean ± standard deviation (S.D.); *: p<0.05; **: P<0.01 ***: P <0.001. M = Male; F = Female.

Table 4: Values of hematological parameters in control and treated rats following administration of FACA® capsule.

Parameters	sex	control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
WBC x (10^9)/L	M	7.46±4.91	4.78±2.79	8.70±5.60	4.25±2.20	4.78±1.98
	F	8.43±4.19	4.76±1.95	9.22±2.33	10.73±6.47	6.02±3.59
RBC x (10^{12})/L	M	6.75±0.43	6.44±0.35	6.50±0.31	6.01±0.25***	6.32±0.27*
	F	5.93±0.34	5.57±0.43	6.24±0.33	6.15±0.17	5.84±0.52
HGB (g/L)	M	151.40±11.10	144.80±6.43	144.90±6.89	132.00±4.90***	136.60±8.11**
	F	133.60±8.90	124.70±11.03	136.80±9.45	138.00±9.29	128.90±13.31
HCT (%)	M	42.19±2.81	36.83±1.61***	40.29±1.47	32.85±1.41***	34.30±1.84***
	F	38.64±1.97	31.82±3.12***	38.64±2.23	34.01±1.51***	32.14±2.89***
MCV (fl)	M	60.75±1.49	55.50±1.77***	60.33±2.35	53.00±2.00***	52.38±1.30***
	F	63.30±2.16	55.40±2.01***	60.20±3.36*	53.56±1.42***	53.38±1.30***
MCH (pg)	M	22.52±1.04	22.59±0.73	22.52±0.95	22.12±0.83	21.70±0.58
	F	22.64±0.89	22.58±0.77	22.06±1.35	22.54±1.05	22.18±0.75
MCHC (g/L)	M	366.40±8.75	402.30±10.73***	368.80±12.72	412.30±7.60***	407.50±4.96***
	F	353.40±10.93	403.40±15.74***	362.10±8.03	415.70±12.65***	410.10±6.58***
RDWCV (%)	M	15.88±0.83	16.38±1.60	15.78±1.09	17.78±2.11*	17.88±1.36*
	F	13.70±0.95	15.30±1.64	15.80±2.39*	17.22±1.20***	16.88±1.13***
RDWSD (fL)	M	82.29±0.97	84.37±1.39*	82.52±1.55	85.55±1.67***	86.04±1.07***
	F	78.83±1.77	83.58±1.36***	82.00±2.87**	85.27±1.25***	84.58±1.09***
PLT (10^9)/L	M	472.90±53.53	535.40±55.45	452.60±75.22	538.80±68.29	564.80±45.18*
	F	517.00±77.78	569.60±67.21	562.50±65.83	599.30±67.70	631.40±60.33**
MPV (fL)	M	10.64±0.17	11.34±2.01	10.57±0.52	10.16±0.28	9.91±0.38
	F	9.94±0.48	9.82±0.46	9.92±0.38	9.76±0.28	10.08±0.39
PDW (fL)	M	15.10±0.24	15.15±0.78	15.24±0.60	14.80±0.22	14.85±0.18
	F	14.80±0.13	14.84±0.28	14.94±0.25	14.82±0.19	14.78±0.20
PCT (%)	M	0.49±0.05	0.56±0.07	0.47±0.07	0.56±0.08	0.55±0.04
	F	0.50±0.06	0.55±0.06	0.55±0.06	0.64±0.13**	0.63±0.09**

n = 10; values are presented as mean ± standard deviation (S.D.); *: p<0.05; **: P<0.01; ***: P <0.001. M = Male; F = Female.

Table 5: Values of biochemical parameters.

Parameters	Sex	control	100 mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Glucose (mmol/L)	M	3.44±0.83	4.55±1.18	4.31±0.34	4.83±0.36*	4.76±0.73**
	F	3.69±0.46	4.42±0.37	4.16±0.58	4.18±0.66	4.76±0.82**
Craatinine (µmol/L)	M	85.34±9.08	68.25±8.07***	69.46±5.09***	60.89±3.16***	54.65±4.66***
	F	80.94±7.69	69.84±7.61**	66.09±4.63***	62.53±7.52***	56.35±6.52***
Total cholesterol (mmol/L)	M	1.94±0.31	1.78±0.45	1.69±0.28	1.39±0.41*	1.08±0.36***
	F	1.55±0.29	1.33±0.41	1.51±0.41	1.49±0.25	1.08±0.45*
Total Protides (g/L)	M	58.90±2.90	54.79±5.12	59.21±3.64	56.84±4.12	46.31±10.00***
	F	61.65±4.29	63.03±3.92	60.50±2.22	59.54±13.02	45.06±9.46***
AST (UI/L)	M	168.70±49.01	155.40±21.49	129.30±16.89*	132.00±16.83	112.40±43.25**
	F	164.10±33.49	127.10±28.09	133.60±34.14	118.70±28.81*	103.50±40.57**
ALT **(**UI/L)	M	52.17±9.17	64.57±10.63	50.33±10.34	79.44±10.49**	73.38±24.74*
	F	49.88±12.48	52.11±10.15	65.70±18.10	76.44±25.01*	62.88±21.46
Cl^{-} **(**mmol/L)	M	111.50±9.33	112.90±7.53	104.40±2.33	108.10±4.22	89.75±4.53***
	F	106.90±20.33	109.60±4.09	101.60±5.46	103.90±6.51	92.75±1.75*
PO_4^{2-} (mmol/L)	M	4.83±1.44	3.97±0.85	3.50±0.42**	3.80±0.33*	2.59±0.63***
	F	2.87±1.10	3.67±0.60	2.88±0.32	2.50±0.71	2.33±0.52
Ca^{2+} (mmol/L)	M	2.42±0.63	2.11±0.30	2.78±0.17	2.68±0.21	1.86±0.43*
	F	2.10±0.51	2.69±0.28**	2.70±0.29**	1.81±0.42	1.80±0.33
Na^{+} (mmol/L)	M	158.10±8.43	157.50±5.68	159.70±3.20	162.80±16.46	158.40±11.15
	F	156.50±7.04	158.60±8.85	157.40±6.98	160.40±9.81	165.80±3.97
K^{+} (mmol/L)	M	7.66±1.20	6.48±1.26	7.29±1.33	5.82±0.69**	5.84±0.65**
	F	6.52±1.33	6.17±0.41	7.86±0.53*	5.57±1.17	6.54±1.34

n = 10; values are presented as mean ± standard deviation (S.D.); *: p<0.05; **: P<0.01 ***: P <0.001. M = Male; F = Female.

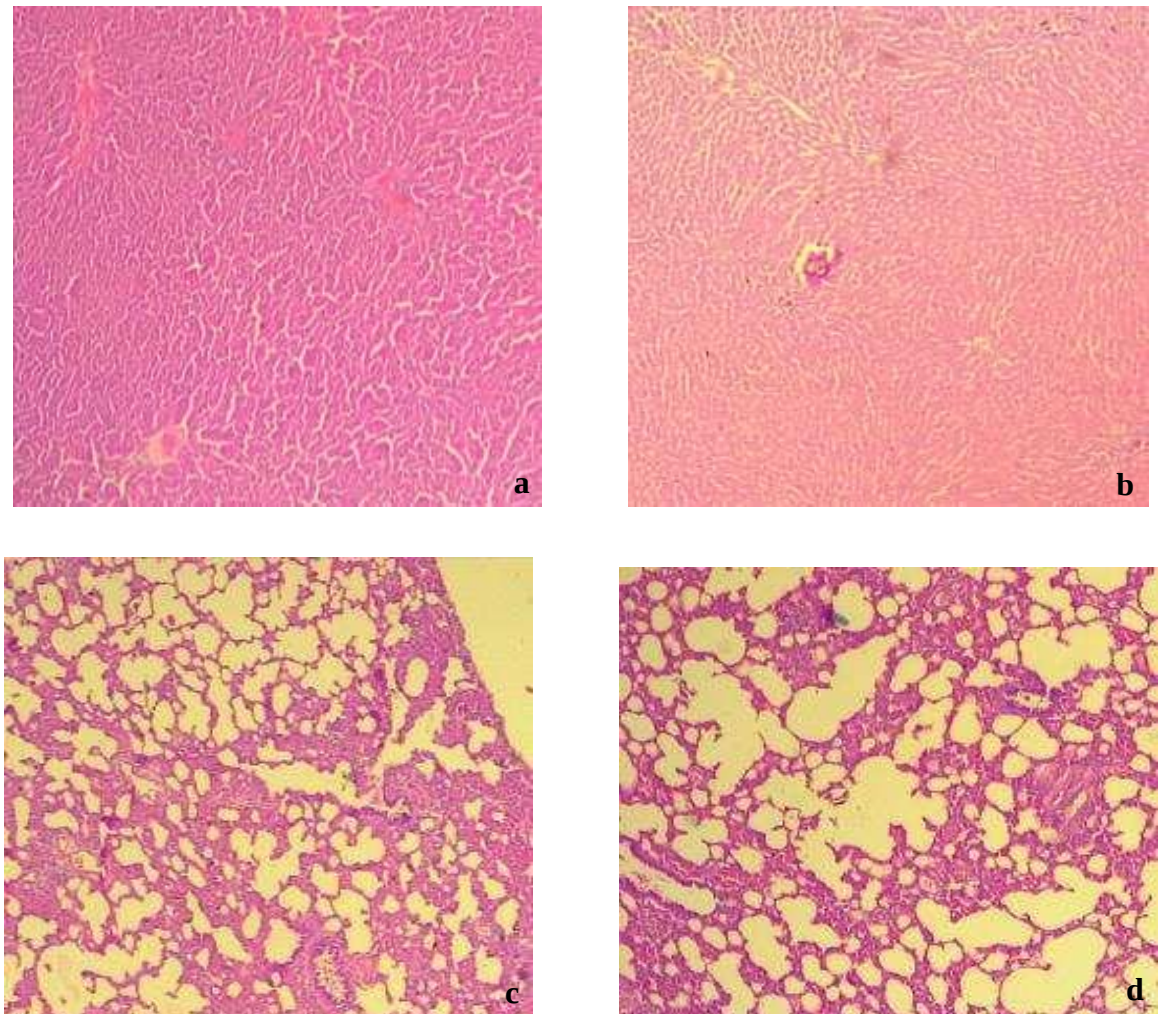

Figure 3: post-treatment liver and lung photomicrograph (GX40).
a: normal liver architecture; **b**: High dose treated liver; **c**: normal lung architecture; **d**: high dose treated lung. No obvious in lung and liver architecture.

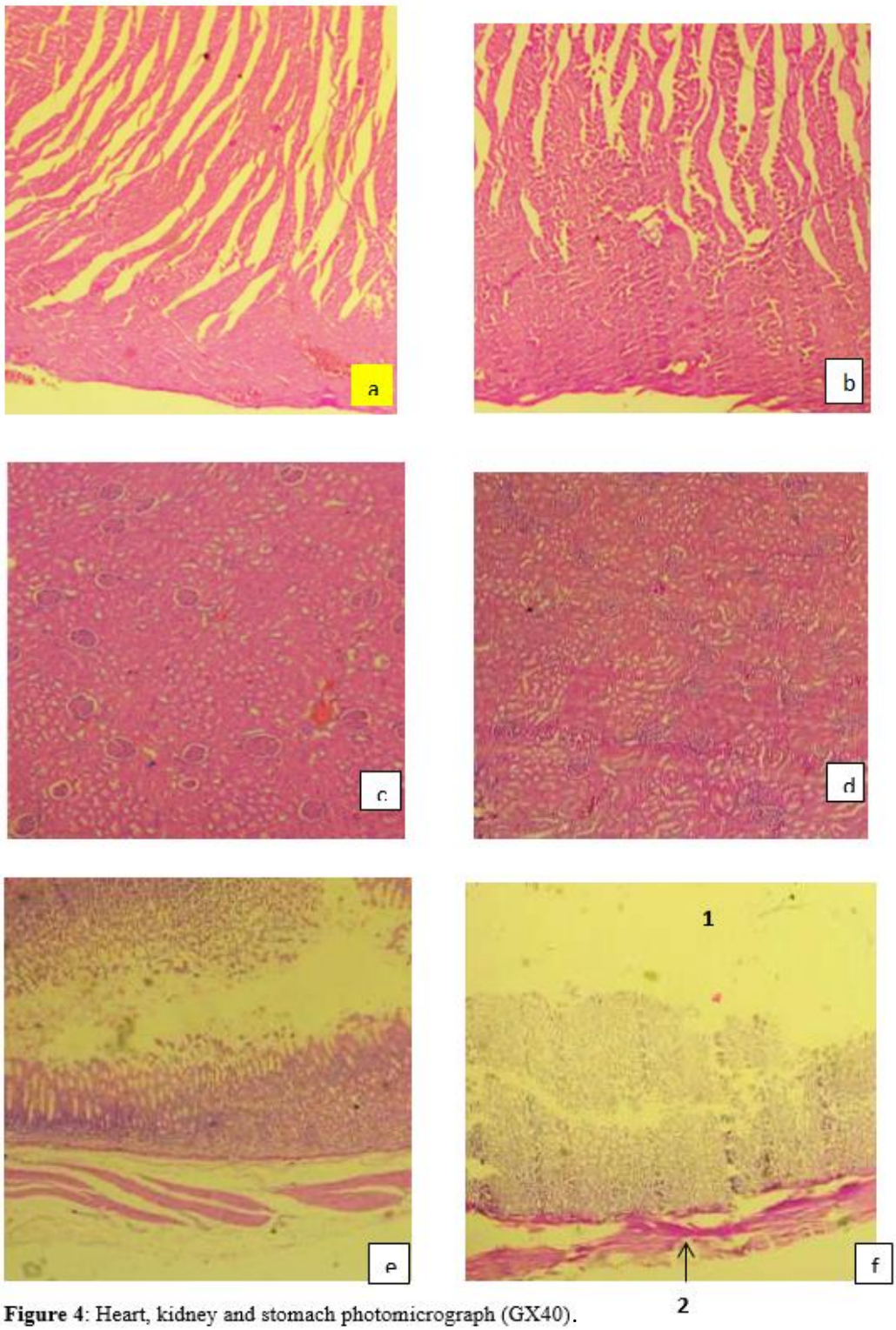

Figure 4: Heart, kidney and stomach photomicrograph (GX40).

a: narmal heart architecture; **b**: high dose treated heart architecture (No obvious change in heart architecture); **c**: Normal kidney architecture; **d**: high dose treated kidney architecture (No obvious change in kidney architecture); **e:** normal stomach architecture; **f**: high dose treated stomach architecture (destruction of epithelium surface at the stomach (1) and atrophy of the stomah muscular wallf (2).

DISCUSSION

Plants are well known for their many uses and medicinal efficiencies. However, the lack of data from toxicological studies is often a major barrier to the acceptance of drugs of plant origin. This is exacerbated by the fact that plants, apart from their therapeutic properties, can be toxic. *C. procera* and *Z. zanthoxyloïdes* are known for their many pharmacological properties. These plants are widely used in traditional medicine for the treatment of various diseases including sickle cell disease (Zahoui et al., 2016). The purpose of this study was to evaluate the toxicity of the mixture of *C. procera* and *Z. xanthoxyloides* root bark powders called FACA® capsule, to allow the safe use of this phytomedicine in the treatment of sickle cell disease.

Acute toxicity study did not show any sign of toxicity and any mortality at 2000 mg/kg body weight in rats. The estimated oral LD_{50} of this phytomedicine was 5000 mg/kg. According to the United Nations (2019) and the OECD Globally Harmonized System of Classification (OCDE, 2001), this phytomedicine may be classified as Category 5 of substances without acute toxicity effects. These results are similar to those of Ouédraogo (2015) who reported that the FACA® capsule may be classified as Category 5 of substances without acute toxicity effects with an LD_{50} superior at 2000 mg/kg body weight. Ouedraogo et al. (2020) also reported that the FACA® syrup has an estimated LD_{50} of 5000 mg/kg b.w. in Wistar rats.

In sub-chronic toxicity study, no extract-related mortality was observed during the three months of administration. A significant decrease of food consumption in rats treated with 500 and 1000 mg/kg b.w. was observed during the first two months of treatment. In males rats treated with doses of 250, 500, and 1000 mg/kg, there was also a significant decrease in water consumption throughout the treatment period. This decrease in water and food consumption may be explained by the fact that at high doses, the amount of plant material administered is significant. This could reduce the stomach's capacity, leading to a feeling of satiety. In addition, this mixture may slow metabolism in high-dose rats. Previous studies have shown that alkaloids delay gastric emptying and slow intestinal motility, while tannins reduce digestibility (Bouziri et al., 2011). However, several authors have reported the presence of alkaloids and tannins in both plants (Kouri, 2004; Khan and Akhtar, 2012). The presence of these chemical compounds may partly explain the decrease in food and water consumption in rats in this study.

The body weight changes is an important endpoint in the toxicity study that can provide preliminary information on the test substance (Soeharto et al., 2018). In the study, all rats (control and treated) gained weight during the treatment. These results indicate that the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders did not prevent weight gain in rats. These results are similar to those of Ouedraogo et al. (2013) who found an increase in body weight in rats treated with 20 mg/kg of *C. procera* extract for 3 to 6 weeks. Aloke et al. (2012) also found weight gain in rats fed with diets containing 10-20% leaves of *Z. zanthoxyloïdes*. However, during the last two months of treatment, there was a significant decrease in body weight in all the treated females rats compared to the controls groups and the males treated with 250, 500 and 1000 mg/kg. This decrease in weight gain may be related to the decrease in food consumption observed during this study. These results are consistent with those of Aloke et al. (2012), who observed a significant decrease in the weight of rats fed with diets containing 10-20% *Z. zanthoxyloïdes* leaves. Macroscopic observations of the organs of treated animals (liver, heart, lung, kidney, spleen, and stomach) showed no abnormalities of color or texture compared with the control group. The heart,

liver, kidneys, spleen and lungs are the first organs affected by the metabolic reaction induced by the toxicant (Jothy et al., 2011). In this study, daily administration of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders resulted in a significant increase in the relative stomach weight of rats treated at 500 and 1000 mg/kg compared with controls. In addition, the stomachs of the rats treated with 500 and 1000 mg/kg b.w. were loaded with food, implying food indigestion due to the amount of plant material administered or the presence of alkaloids in *Z. xanthoxyloides* reported by several authors (Khan and Akhtar, 2012). Indeed, previous studies have shown that alkaloids have anticholinergic effects resulting in decreased tone, amplitude and frequency of intestinal peristaltic contractions, resulting in delayed gastric emptying (Bouziri et al., 2011). These results are corroborated by those of Zahoui et al. (2016) who have shown that the root bark of *C. procera* and *Z. zanthoxyloïdes*, as well as FACA®, have myorelaxant properties.

Biochemical analysis of serum showed a significant decrease in the level of AST at 250 mg/kg in males, 500 mg/kg in females and all the rats treated with 1000 mg/kg body weight. ALT and AST are enzymes with significant metabolic activity within cells, used to detect chronic liver disease (Shokoohinia et al., 2017). The results of this study are similar to those of other authors who have shown that *C. procera* and *Z. zanthoxyloîdes* have hepatoprotective properties related to the presence of flavonoids, triterpenes and tannins (Olaleye et al., 2010; Ogunbolude et al., 2014). In contrast, ALT levels were significantly increased in all rats treated with 500 mg/kg and in males treated with 1000 mg/kg body weight. Ouedraogo et al. (2013) showed that daily administration of aqueous extract of *C. procera* at 20 mg/kg increased ALT levels after six (06) weeks of treatment. Renal function can be assessed by determining serum levels of urea, uric acid, creatinine, potassium, and sodium (Mukinda and Syce, 2007). Creatinine level in particular is a key indicator of potential toxicity. The kidney is normally able to filter large amounts of creatinine. Therefore, increased blood creatinine is a reliable indicator of a negative impact on renal function (i.e., renal lesions or altered glomerular filtration) (Shokoohinia et al., 2017).

The mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders resulted in a significant decrease in creatinine levels in all treated rats (males and females) compared with controls. Therefore, this medicine did not alter renal function. This lack of nephrotoxicity is confirmed by normal relative kidney weights and results of histopathological analysis of the organs of treated rats. The liver is the major site of plasma protein synthesis. Alterations in serum proteins may reflect liver dysfunction (Blomme et al., 2009; Tothova et al., 2016). Serum proteins such as albumin can also be used as a criterion for assessing liver synthesis capacity; since almost all are synthesized in hepatocytes. A reduction in serum protein therefore tends to reflect chronic damage in hepatocytes (Tothova et al., 2016). In this current study, a significant decrease in the level of serum total protein was observed in the rats treated with 1000 mg/kg body weight. In contrast, no significant differences were observed between treated and control rats at 100, 250 and 500 mg/kg. These results suggest that at high doses, this phytomedicine may result in increased degradation of the protein components, or in impairment of liver function leading to a decrease in its protein synthesis capacity for protein synthesis. The results of this study also showed a significant decrease in cholesterol levels in rats treated with 500 and 1000 mg/kg body weight. This decrease may be due to the presence of hypolipidemic agents in this medicine. These results are comparable to those of Aloke et al. (2012) who showed that supplemental feeding of *Z. zanthoxyloïdes*

leaves significantly decreased total cholesterol and LDL levels in diabetic rats. According to Millar et al. (2017), flavonoids have a cholesterol-lowering action that appears in the long term. Thus, the decrease in cholesterol levels in this study may be due to flavonoids present in both plants (Kouri, 2004; Khan and Akhtar, 2012).

A significant increase in blood glucose was observed in males treated at 500 and 1000 mg/kg and in females treated at 1000 mg/kg. This increase in blood glucose showed that at high doses, this mixture disrupted glucose metabolism. The hyperglycemic activity of this phytomedicine may be related to the presence of alkaloids that have hyperglycemic effects in rats and mice (Ojiako et al., 2016). This may be explained by the ability of these metabolites to decrease insulin release or to inhibit insulin activity. This mixture may also exercise hyperglycemic activity by deactivating AMP-kinase or inhibiting GLP113 secretion, an intestinal hormone that also stimulates insulin secretion (Aloke et al., 2012).

After three months administration of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders, there were some changes in serum ion composition. A significant decrease in Cl^- concentration was observed in all rats treated with 1000 mg/kg and PO_4^{2-} in male rats treated at 250, 500 and 1000 mg/kg body weight. In addition, the concentrations of Ca^{2+} and K^+ ions were significantly decreased in males treated at 500 and 1000 mg/kg, whereas in females treated at 100 and 250 mg/kg, concentrations were significantly increased.

Concentrations of many ions in blood plasma, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, bicarbonate, phosphate, and sulfate, are regulated by the kidneys (Shokoohinia et al., 2017). The serum electrolytes such as sodium, potassium, bicarbonate and chloride ions are biochemical indicators for the renal damages in addition to serum urea and creatinine. In a normal kidney, urea and creatinine are excreted out of the body whereas reabsorption of electrolytes takes place in the renal tubules. But, in the renal cellular failure and nephrotoxicity, the excretion of urea and creatinine is impaired accompanied by less reabsorption of electrolytes in the renal tubules (Rahim et al., 2014). The decrease in the concentration of these ions in rats treated with high doses of the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders shows that this medicine decreases the ion reabsorption capacity at the tubule level. The hematopoietic system is one of the main targets of toxic compounds. It is also an important marker of physiological and pathological status in humans and animals (Mukinda and Syce, 2007). The blood profile not only provides important information about the body's response to damage or stress, but also provides a prediction of hematological toxicity in humans (Mukinda and Syce, 2007; Soeharto et al., 2018). Daily administration of the mixture resulted in an increase of the level of PLT and PCT in all treated rats compared with controls. In addition, decreases in red blood cell (RBC), hemoglobin (HGB), HCT and MCV blood levels were observed in all treated rats compared with controls. This decrease was significant at 500 mg/kg and 1000 mg/kg body weight for red blood cell and hemoglobin levels and in rats treated with 100, 500 and 1000 mg/kg for HCT and MCV. This decrease in red blood cells, hemoglobin, hematocrit and MCV may be due to increased destruction of red blood cells by the alkaloids and flavonoids (Mapfunde et al., 2016). It is also possible that this mixture, at high doses, inhibit the synthesis of hematopoiesis regulatory elements such as CSF (Colony tissue factor), EPO (Erythropoietin), TPO (Thrombopoietin), which provide an environment conducive to the development of the immune system (Ojo et al., 2014). The increased levels of PLT and PCT showed that FACA® stimulated the production of blood platelets. Platelet formation occurs from mega-

karyocytes and is modulated by thrombopoietin (TPO) (Zhang et al., 2012). It is suggested that this phytomedicine may stimulate blood platelet production by activating TPO production. In addition, it may act through other mechanisms such as activation of mega-karyopoiesis regulatory cytokines (interleukin-3, Mega-CSA and megacaryocyte-stimulating factor (MSF)) (Zhang et al., 2012). These results are consistent with those of Kouri (2004), who showed that aqueous and organic extracts of *Z. zanthoxyloïdes* reduce the blood clotting time by half.

Numerous pathological organ lesions can be detected by macroscopic examination. However, histopathological examinations assess pathological changes in tissues and organs and may reveal the site, extent and morphological appearance of these lesions. Histopathological examination of the organs shows an atrophy of the muscular wall and a destruction of the epithelium surface of the stomach at 1000 mg/kg b.w.

The gastric mucosa includes four types of secretory cells including mucus cells, main cells, parietal cells and G cells. The mucus secreted by mucus cells plays a role of lubrication and protective barrier preventing the destruction of the stomach wall by harmful agents. This mucus inhibits the growth of microorganisms and facilitates the movement of food in the gastrointestinal tract. Its secretion is induced by prostaglandin E2 (PGE2) (Rahim et al., 2014). Prostaglandin is a major component of protective factors that maintain the integrity of the gastrointestinal mucosa and microcirculation. It increases the mucus layer and viscosity, maintains the pH gradient, and also inhibits the movement of acid and pepsin in the mucus layer (Hoshino et al., 2003). Apart from gastric mucus, superoxide dismutase (SOD) is an enzyme that protects cells in the intestine and stomach from the irritable action of reactive oxygen species (ROS). This enzyme is essential for the body to evacuate harmful ROS from the cellular environment such as superoxide which is capable of causing micro-vascular damage as well as damage to the gastric mucosa (Bae et al., 2011). In high doses, the mixture from *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders could cause the destruction of the gastric mucosa either by inhibiting the secretion of mucus by mucus cells, or by inhibiting the secretion or action of prostaglandin or by inhibiting the activity of superoxide dismutase. Inactivity of gastric mucus and SOD exposes the gastric mucosa to hydrochloric acid secreted by G cells and ROS, which causes its destruction. However, a thorough study would clarify the mechanism of action of this medicine on the gastric mucosa.

The dosages required for FACA® are 4 to 6 capsules of 175 mg per day; therefore, in an adult male, the amount of substance to be administered per day would be 1050 mg. If this adult male weights 60 kg, the administered dose is 17.5 mg/kg (Lompo et al., 1998). Thus, the doses of 500 and 1000 mg/kg that induce the toxic effects observed in this study are 28 to 57 times higher than the therapeutic dose of FACA® capsule.

Conclusion

Acute toxicity studies show that FACA® have a low toxicity when administrated orally. The sub-chronic toxicity study showed that at high doses, FACA® resulted in decreased of food and water consumption in rats. This decrease in food consumption results in a decrease in weight gain in these animals. This phytomedicine have hyperglycemic activity and stimulate the production of high-dose PLT, and PCT. The administration of FACA® resulting in decreased levels of red blood cells, hemoglobin and hematocrit at high doses. Macroscopic and histopathological examination of the organs of treated animals showed that FACA® induced at high dose, food indigestion and lead to an atrophy of the muscular wall and a destruction

of the epithelium surface of the stomach. FACA® resulted in hyperglycemia, an increase of AST level and decrease of the level of total proteins. It do not alter renal function. These results show that the use of FACA® in the management of sickle cell seizures is well tolerated and does not present a risk of acute toxicity to patients.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

SDG, OGG, IS and SO designed and conducted experimental work. ON and MO have supervised the study. OS collected plant sample and corrected the first draft. LSA Conducted histopathological examination. KFB performed literature searches and corrected the first draft. IP validated the results of the study

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