



Research Article

## TOXICOLOGICAL EFFECT OF AQUEOUS EXTRACTS OF *Croton lobatus* L. AND *Schrankia leptocarpa* L. IN RATS MODEL

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### ABSTRACT

In a preliminary evaluation of ethnobotanically selected Beninese medicinal plants for their pharmacological activity, *Croton lobatus* L. (Euphorbiaceae) and *Schrankia leptocarpa* (Mimosaceae) showed interesting activities against Plasmodium and microbial strains. Considering these biological activities and the extensive use of these plants in traditional medicine, the toxicological profile of their aqueous extracts was assessed using oral acute toxicity in the rat model. Acute toxicity of aqueous extracts of tested plants was assessed at a dose of 2000 mg/kg as per Organization for Economic Cooperation and Development, guideline n° 423. The rats were observed for signs of toxicity or death after administration of extracts. Distilled water was used for control rats. Blood samples were collected and used to evaluate biochemistry and hematology parameters. Histopathological evaluation was also done on liver and kidney. The results revealed that all tested animals were physically active during the test. No signs of toxicity or morbidity in rats treated with extracts were observed. There were no significant variations in the biochemical and hematological parameters between rats treated with aqueous extract of *Schrankia leptocarpa* and those received distilled water. A significant decrease of serum alanine aminotransferase and Creatinine ( $P = 0.01$ ) level was observed in rats treated with aqueous extract of *Croton lobatus* while a significantly increased level of RBC ( $P = 0.04$ ) and Hematocrit ( $P = 0.01$ ). Histopathological examination of liver and kidney sections of rats treated with 2000 mg/kg body weight of aqueous extracts of *Croton lobatus*, *Schrankia leptocarpa* did not show any changes when compare to control rats. These results indicate that the oral administration of aqueous extracts (decoction) of *Croton lobatus* and *Schrankia leptocarpa* did not produce any significant toxic effect in rats.

**KEYWORDS:** Biochemical, heamatological, histopathology, toxicity, *Croton lobatus*, *Schrankia leptocarpa*,

### INTRODUCTION

The use of medicinal plants is still relevant and demand has increased in recent years. These plants are involved in the preparation of first-line drugs for the primary health care of population. The WHO Traditional Medicine (TM) Strategy 2014–2023 stated that traditional treatments, traditional practitioners and herbal medicines are the main source of

health care, if not the only source, for many millions of people [1]. It was also reported that approximately 80 % of the population still rely on traditional medicine, mainly based on herbal medicine products, for their primary health care [2]. However, for proper and documented herbal medicinal products, the toxicity should be explored as in the case with conventional orthodox drugs that are properly researched and developed; the toxicity of

traditional herbal medications is not often assessed [3]. As such, the users often look at the medicinal benefit of the herbal drugs and neglect their toxic effects to various organs. In our previous study, using ethnopharmacological approach, the antiparasitic and antimicrobial activities of extracts and compounds isolated from *Croton lobatus* L. (Euphorbiaceae) and *Schrankia leptocarpa* (Mimosaceae) have been assessed. These plants are traditionally used to treat different ailments. In Benin, a decoction of the leaves and flowers or roots of *Croton lobatus* is taken to treat fever, malaria and antispasmodic [4]. *Schrankia leptocarpa* is traditionally used against eruptive fevers and hypertension [5]. Many studies reported the biological activities of extracts and isolated compounds of these medicinal plants [6][7][8].

Despite their biological properties the toxicological profile has not been clearly established. Therefore, the evaluation of the toxicity remains a priority in order to bring more confidence to their use. Thus, the acute toxicity of aqueous extracts of *Croton lobatus* and *Schrankia leptocarpa* was evaluated in rat models.

## MATERIALS AND METHODS

### Collection of studied plants

*Croton lobatus* L. Euphorbiaceae (Figure 1) was collected from township of Abomey Calavi, Atlantic region (Southern Benin) and *Schrankia leptocarpa* DC. Mimosaceae

Figure 2 from township of Adjarra in Ouïmï region (Southeastern Benin), in March 2013. The plants were identified by botanist from National Herbarium of University of Abomey-Calavi where the voucher specimens were deposited Table 1.

### Plants extraction

The collected plants were air-dried in laboratory (22°C±3) for 3 weeks and grounded into powder using an electric grinder (MARLEX Electroline Excella). Hundred and fifty grams (150 g) of each species were extracted three times with 500 mL of distilled water at 60°C. The filtrates were concentrated under reduced pressure using a rotary evaporator. The obtained extracts were stored at 4 ° C before assay.

### Animals

Female wistar rats with a body weight of 180 ± 20 g and obtained from the laboratoire de Biologie Humaine, Faculty of Health Sciences, University of Abomey-Calavi were used in this study. All animals were nulliparous and nonpregnant. They were acclimatized with laboratory conditions for two weeks at 22 ± 3°C, constant 12 h light/dark, and relative humidity between 30-70%. They were fed with laboratory diets given water *ad libitum*.

The acute toxicity of plants was assessed according to guidelines n° 423 of the Organization for Economic Cooperation and Development [9]. All experiments have been approved by the laboratory ethics committee. Animals were divided into three groups of three rats each and extracts were tested at a dose of 2000 mg/kg body weight. *Croton lobatus* extract was administered to group 1, *Schrankia leptocarpa* extract to group 2 and group 3 (control) received distilled water. Monitoring of animals during experiments was performed according to the method previously described [10].

### Haematological and Biochemical parameters analyses

Haematological and biochemical parameters were determined according to method described previously [11]. Briefly, blood was collected from anesthetized rats (thiopental, 0.5 ml/Kg bw), in both heparinized and non-heparinized tubes to determine haematological and biochemical parameters respectively. Haematological parameters including Hematocrit (Ht), RBC, WBC, Hemoglobin concentration (Hc), Mean Corpuscular Hemoglobin Concentration (MCHC), etc were determined using an automatic hematological analyzer (Sysmex, XP-300, Japan). The nonheparinised blood was allowed to coagulated, centrifuged 10 min at 3500 rpm, and the obtained serum was assayed for biochemical parameters such as Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), Creatinine (CREA), Glucose (GLU), and Cholesterol (CHOL). These biochemical parameters were determined for all groups using an autoanalyzer (ErbaChem 7, Germany).

### Histopathological examination

The histopathological study was done according to the methods described previously [10]. Kidney and liver were isolated from treated and untreated (control) rats for histological examination.

### Statistical analysis

Data collected from the biochemical and hematological analyses were expressed as mean ± SEM. One-way ANOVA was used to test the means. Results were considered significant when P value less than 0.05. All results were represented as mean ± SEM (n = 3).

## RESULTS

### Oral acute toxicity of tested extracts

This study showed that *croton lobatus* and *Schrankia leptocarpa* had no visible adverse effects on animals after oral administration of the aqueous extracts at 2000 mg/kg body weight. General observation during the experiment did not display any changes in behavioral pattern. No mortality and no visible symptoms of acute toxicity were observed. These results suggested that the Lethal dose 50 (DL<sub>50</sub>) of aqueous extracts of these plants were higher than 2000 mg/kg body weight.

### Effect of plants extracts on body and organs weight

### Oral Acute toxicity

The body weight of treated rats gradually increased but not statistically significant during the experiment when compared to controls Figure 3. The relative weight of kidney and liver slightly increased during the experiment. However, the difference is non-significant when compare to controls Figure 4. Effect of plants extracts on Biochemical and haematological parameters

The results obtained for biochemical parameters are summarized in Table 2. The tested biochemical parameters where not significantly changed after oral administration of aqueous extracts of *S. leptocarpa* ( $P > 0.05$ ). In contrary, significant change on some biochemical parameters was observed with aqueous extract of *Croton lobatus*. The level of creatinine (CREA) and alanine aminotransferase (ALT) was significantly increased when compare to control ( $P = 0.01$  for both parameters). The results of haematological

evaluation are summarized in Table 3. Aqueous extract of *S. leptocarpa* did not cause significant change when compare to control ( $P > 0.05$ ). In contrary, a significant increase was observed for RBC ( $P = 0.04$ ), Hematocrit ( $P = 0.01$ ) and Monocytes ( $P = 0.04$ ) whereas a significant decrease was observed for Mean Corpuscular Hemoglobin ( $P = 0.03$ ) after administration of *C. lobatus* aqueous extract.

**Histological examination**

The histopathological examination of the liver and kidney sections of treated rats did not show any changes when compare to control (figure 5). There is no difference in the shape of the central vein, seize of hepatic sinusoids and hepathocytes.

**Table 1:** Selected plants for evaluation of acute toxicity

Botanical name	Family	Local name	Part use	Voucher n°
<i>Croton lobatus</i>	Euphorbiaceae	Aloviaton (G,F), Erougali (Y)	AP	HP565a
<i>Schrankia leptocarpa</i>	Mimosaceae	Kpatanman olokun (Y,N), Ahossibassa (G) Danhunkan (F)	EP	Hougnon 954b

Y: yoruba; G: goun; F: fon; N: nago; AP: aerial part; R: root; EP: entire plant

**Table 2:** Effects of aqueous extracts of *Croton lobatus* and *Schrankia leptocarpa* on biochemical parameters

Biochemical parameters	Control	Experimentals (2000 mg/kg b w)			
		<i>C. lobatus</i>	P	<i>S. leptocarpa</i>	P
GLU	1.06 ± 0.02	0.86 ± 0.09	0.10	1.24 ± 0.26	0.32
CREA	8.73 ± 0.26	7.33 ± 0.52	0.01	8.87 ± 1.46	0.85
CHOL	0.91 ± 0.07	1.02 ± 0.25	0.33	0.74 ± 0.05	0.38
AST	189.33 ± 4.16	208 ± 21.65	0.21	192.66 ± 5.51	0.11
ALT	81.33 ± 9.01	73.66 ± 10.06	0.01	74.33 ± 2.51	0.38



ALT alanine aminotransferase, AST aspartate transaminase, CREA Creatinine, CHOL cholesterol, GLU Glucose,. Values are mean  $\pm$  SEM (n = 3 per group), differences were considered significant when p-values were less than 0.05 (p< 0.05). **a**: values non-significantly different; **b**: values significantly different

**Table 3:** Effect of aqueous extracts of *Croton lobatus* and *Schrankia leptocarpa* on haematological parameters

Haematological parameters	Control	<i>C. lobatus</i>	<i>p value</i>	<i>S. leptocarpa</i>	<i>p value</i>
Hc (g/dl)	13.73 $\pm$ 0.11	15 $\pm$ 0.62	0.08	13.26 $\pm$ 0.35	0.08
Ht (%)	41 $\pm$ 1	47 $\pm$ 2	0.01	41.66 $\pm$ 2.51	0.53
MCV (fl.)	68.6 $\pm$ 1.44	64 $\pm$ 1.73	0.11	66 $\pm$ 1	0.10
MCH (Pg)	23.38 $\pm$ 0.54	20.33 $\pm$ 0.5	0.03	21 $\pm$ 1	0.11
MCHC (%)	33.72 $\pm$ 1.11	31.66 $\pm$ 0.57	0.06	31.9 $\pm$ 1.82	0.05
Rbc (T/L)	5.95 $\pm$ 0.07	7.31 $\pm$ 0.43	0.04	6.31 $\pm$ 0.42	0.27
Wbc (G/L)	4.05 $\pm$ 0.44	4.14 $\pm$ 1.33	0.89	3.51 $\pm$ 0.62	0.46
Nc (%)	2.66 $\pm$ 1.15	6.66 $\pm$ 3.21	0.15	4 $\pm$ 2	0.18
L (%)	85.66 $\pm$ 2.08	75.66 $\pm$ 3.51	0.08	82.66 $\pm$ 5.77	0.57
M (%)	6.33 $\pm$ 1.53	10.66 $\pm$ 0.58	0.04	7.66 $\pm$ 3.05	0.63

Ht hematocrit, Rbc red blood cells, Hc hemoglobin concentration, MCHC mean corpuscular hemoglobin concentration, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin levels, Wbc white blood cells, Nc Neutrophil count, L lymphocytes, M monocytes. Values are mean  $\pm$  SEM (n = 3 per group), differences were considered significant when p-values were less than 0.05 (p<0.05) a: values non-significantly different; b: values significantly different

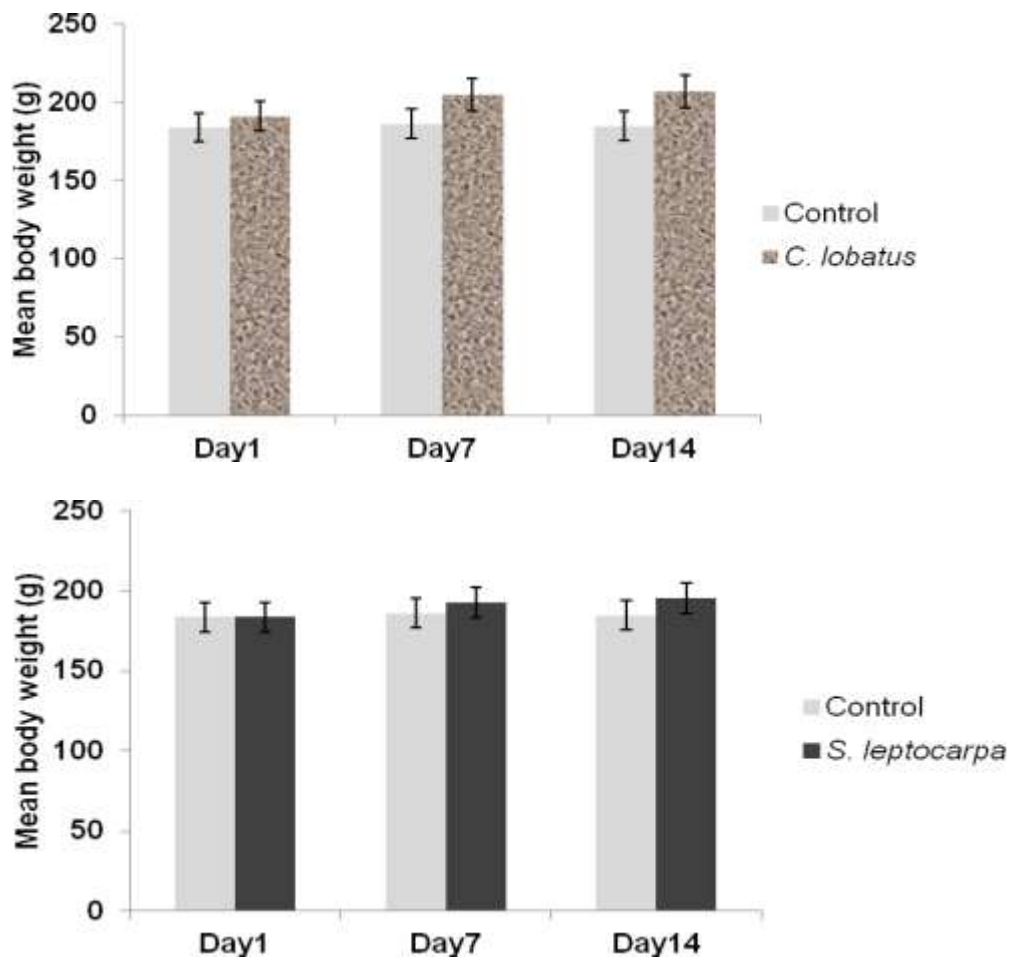
**Figure 1:** Leaves and flowers of *Croton lobatus* ( Latifou LAGNIKA, 2016)



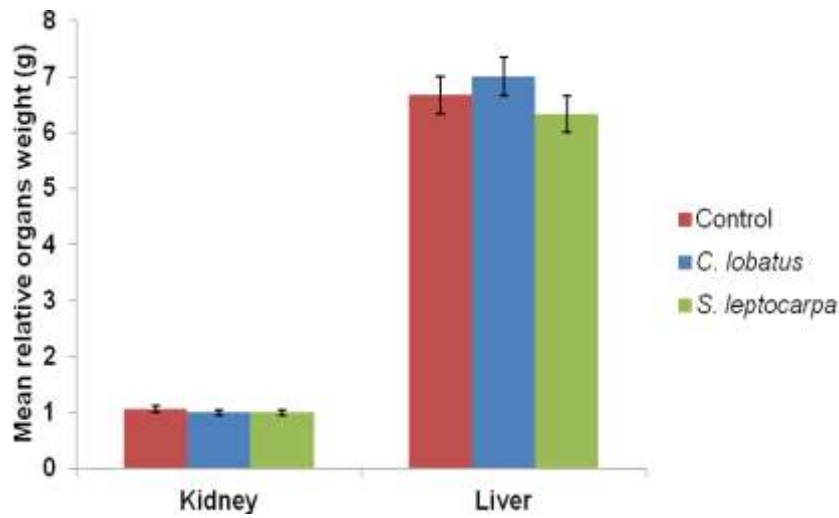
Figure 2: Leaves and flowers of *Schrankia leptocarpa* (Latifou LAGNIKA, 2016)



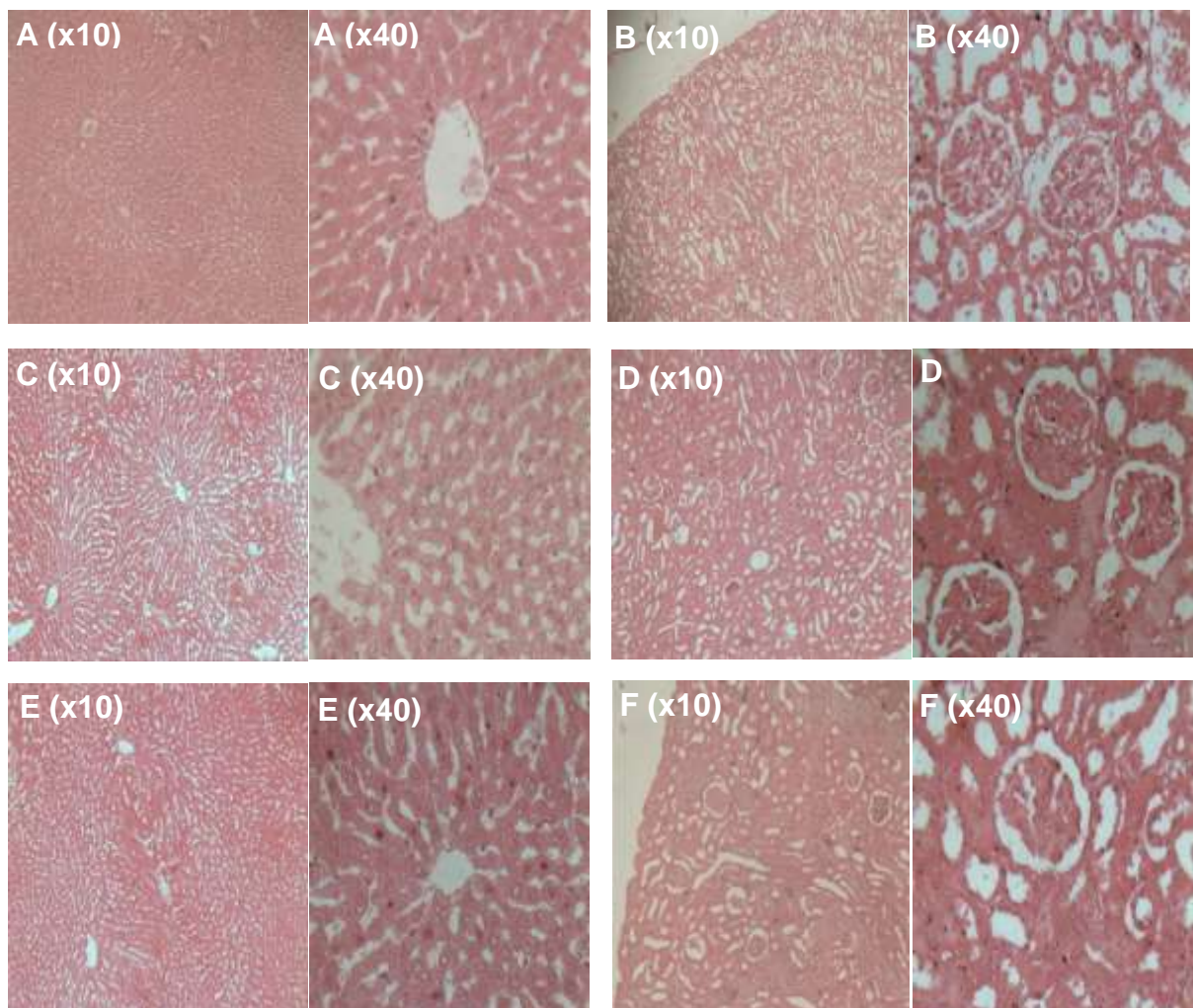
Figure 3: Effects of aqueous extracts of *C. lobatus* and *S. leptocarpa* on treated rats body weight during experiment



**Figure 4:** Effect of aqueous extract of *Croton lobatus* and *Schrankia leptocarpa* (2000 mg/kg) on Mean relative weight of organs of rats after 14 days. Values are expressed as mean  $\pm$  SEM (n = 3). P values > 0.05.



**Figure 5:** Histological examination of liver and kidney sections in rats treated with 2000 mg/kg body weight and rats received distilled water (control). Magnifications X10 and X40 where used. **A** : liver of rats received distilled water (control); **B**: kidney of rats received distilled water (control), **C**: liver of rats treated with aqueous extract *Croton lobatus*; **D**: kidney of rats treated with aqueous extract *Croton lobatus*; **E**: liver of rats treated with aqueous extract *Schrankia leptocarpa*; **F**: kidney of rats treated with aqueous extract *Schrankia leptocarpa*



## DISCUSSION

The first part of this study should determine the lethal dose 50 extracts, based on the observation of clinical signs and mortality in rats. Any clinical toxic effect was observed after oral administration of extracts. No changes in behavior and no deaths were recorded during the experiment. Our results showed that the LD<sub>50</sub> of aqueous extracts of *C. lobatus* and *S. leptocarpa* were higher than 2000 mg/kg. Therefore, these extracts could be considered non-toxic [12]. The body weight of treated rats gradually increased but not statistically significant. This could be due to adverse effects of extracts or body fat accumulation [13] [14] Biochemical parameter as ALT is an enzyme whose increase indicates liver damage [15] No differences was observed in biochemical parameters of rats administrated with aqueous extracts of *S. leptocarpa* whereas significant decrease of ALT and CREA was observed in rats treated with aqueous extract of *C. lobatus*. The decrease of ALT may indicate that liver cells has been preserved which resulted in the reduction of leakage of ALT from liver to the circulate blood [16]. Evaluations of haematological parameters provide useful information on the adverse effects of extracts on blood elements [17] In our study, the non-significant changes observed indicated that *S. leptocarpa* extract does not affect haematological parameters whereas *C. lobatus* extract caused significant change. The significant increase in RBC and Ht indicate that the extract could stimulate erythropoiesis [18] Histological analysis is a very sensitive parameter and crucial in determining cellular changes that may occur in target organs, such as liver and kidney [19] In histopathological examination, no visible injury was observed under the light microscope. These results confirm those obtained previously in biochemical and haematological parameters analyses.

## CONCLUSION

In summary, the LD<sub>50</sub> of aqueous extracts of *Croton lobatus* and *Schrankia leptocarpa* are higher than 2000 mg/kg body weight. These extracts could be considered non-toxic to females rats. However, further studies on sub-chronic toxicity will allow to definitively confirm the toxicity profile of these medicinal plants.

## ETHICAL APPROVAL

Guidelines n° 423 of Organization for Economic Cooperation and Development "for the testing of chemicals acute oral toxicity was used. All authors hereby declare that "Principles of laboratory animal care" were followed. All experiments have been examined and approved by the Laboratory ethics committee.

## ACKNOWLEDGEMENTS

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