



## Study of biological tolerance of aqueous extract of *Sorghum bicolor*

Sènou M<sup>1,2\*</sup>, Tchogou AP<sup>1</sup>, Assogba F<sup>3</sup>, Agossadou A<sup>1</sup>, Dougnon TV<sup>1</sup>, Agbangnan DCP<sup>4</sup>, Lalèyè A<sup>5</sup> & Loko F<sup>1</sup>

<sup>1</sup>Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Cotonou, R. Benin

<sup>2</sup>Experimental and Clinic Biology Unit, Faculty of Sciences and Techniques of Dassa, University of Science, Technology, Engineering and Mathematics (USTM). Dassa-Zoumé, R. Benin

<sup>3</sup>Laboratory of Pharmacognosy and Essentials Oils, Institute of Applied Bio-medical Sciences, University of Abomey-Calavi, Cotonou, R. Benin

<sup>4</sup>Laboratory of Study and Research in Applied Chemistry / Polytechnic School of Abomey-Calavi / University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin

<sup>5</sup>Human Biology Unit, Faculty of Health Science, Cotonou, R. Benin

\* Adresse pour correspondance : [senouxim@yahoo.fr](mailto:senouxim@yahoo.fr)

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### RESUME

**Objectif:** *Sorghum bicolor* est l'une des principales céréales utilisées dans la nutrition animale humaine en Afrique et en Inde. Ces feuilles sont utilisées pour soigner l'anémie dans ces régions. Ce travail visait à étudier la tolérance biologique des feuilles.

**Méthodologie et Résultats :** Sur l'extrait aqueux des feuilles de la plante, a été effectué le test de toxicité larvaire et celui de la toxicité orale aiguë suivant la ligne directrice 423 de l'OCDE. Des rats Wistar injectés avec 2000 mg d'extrait/Kg de poids corporel ont été suivis pendant 14 jours. Aux J0 et J14, des bilans sanguins ont été effectués de même que l'histologie du foie, des reins et de la rate. *In vitro*, la CL<sub>50</sub> est de 7,9 mg/ml. Le poids des rats, l'urémie, la créatininémie, les transaminases et le nombre des leucocytes n'ont pas significativement changé à J14, suggérant l'absence de toxicité rénale, hépatique et immunologique confirmée par l'histologie.

**Conclusion:** L'extrait aqueux de *Sorghum bicolor* n'a pas révélé de toxicité *in vitro* aux larves. *In vivo*, il n'a pas induit de cytolysé hépatique, les transaminases étant légèrement abaissées. Il n'a pas non plus altéré la fonction rénale, l'urémie et créatininémie étant normales. La structure de ces deux organes semble être préservée à l'observation histologique. Aussi, l'extrait semble ne pas affecter la fonction immunitaire avec la numération normale des globules blancs sanguins. Le parenchyme de la rate, organe lymphoïde a gardé son architecture typique. L'étude de la tolérance biologique mérite d'être poursuivie par les tests de toxicité chronique et des essais cliniques appropriés en vue d'une transformation en Médicament Traditionnel Amélioré (MTA).

**Mots clés:** *Sorghum bicolor*, tolérance biologique.

## ABSTRACT

**Objectives:** *Sorghum bicolor* is a major grain crop for human and animal nutrition in Africa and India. Its leaves are used to treat anaemia. This work aimed to study its biological tolerance.

**Methodology and Results:** In the aqueous extract of the leaves of the plant were conducted larval toxicity test and the acute oral toxicity according to guideline 423 of the OECD. Wistar rats were injected with 2000 mg of extract / kg body weight was followed for 14 days. The D0 and D14, blood tests were performed as well as liver histology, kidney and spleen. *In vitro*, the LC<sub>50</sub> was 7.9 mg / ml. The weight of the rats, blood urea, creatinine, transaminases and the leukocyte count did not change significantly on day 14, suggesting the absence of renal, hepatic and immunological toxicity, confirmed by

**Conclusion and application of results:** *Sorghum bicolor* aqueous extract showed no toxicity *in vitro* to the larvae. *In vivo*, it did not induce hepatic cytolysis, liver enzyme AST and ALT slightly decreased. It did not alter renal function, blood urea and creatinine levels were normal. The structure of these two organs seems to be preserved at histological observation. In addition, the extract did not appear to affect immune function since the count of white blood cells was normal. The parenchyma of the spleen, lymphoid organ kept its typical architecture. The study of biological tolerance should be continued by chronic toxicity tests and appropriate clinical trials for a transformation into Improved Traditional Medicine (ITM).

**Key words:** *Sorghum bicolor*, biological tolerance.

## INTRODUCTION

Medicinal plants have been very helpful throughout the ages in the treatment of various diseases and the preservation of human and animal health (Yakubu *et al.*, 2009). They contain inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.*, 2008). The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (Schmincke, 2003). The renewed interest in medicinal plants is linked both to the inaccessibility of expensive pharmacy drugs to poor people, and secondly to the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). Most people in rural areas utilize these plants to bring about cure and relief to disease conditions with little or no knowledge about the safety and toxicity of such plants (Oduola *et al.*, 2007).

However, the literature has documented several toxicity resulting from the use of herbs on many occasions (Jou-fang, 1994; O'Hara *et al.*, 1998). *Sorghum bicolor* is one of the major grain crops for human food throughout the drier areas of Africa and India and its grain is extensively used for animal feeding, (Dalziel, 1948; Ogwumike, 2002). In West Africa, dye can be extracted from the plant to colour leather, cloths, calabashes and as body pigment (Cobley and Steele, 1976). Recently focus has been on the leaf sheath of *S. bicolor* being used as a herbal remedy for anaemia and having a boosting effect on blood hemoglobin and red cells concentration (Ogwumike, 2002; Friday *et al.*, 2010 and Sènou *et al.*, 2016). The present study aimed to test the biological tolerance of *S. bicolor* leaf sheath used to treat anaemia.

## MATERIAL AND METHODS

### Identification and preparation of plant material:

Leaves of *Sorghum bicolor* were collected from Tchaourou in Benin during March 2013. The collected samples were identified and authenticated at the National Herbarium of Benin (HNB) at the University of Abomey Calavi. The samples were dried at moderate temperatures (20-25° C), protected from moisture for four weeks.

They were then crushed powder and stored in suitable containers at room temperature. Fifty (50) g of the powder was boiled in 500 ml of distilled water contained in a 1000 ml flask for 30 minutes. After cooling, the filtrate collected was evaporated in a rotary evaporator between 50° C and 60° C. The extract was dried in an oven at 50° C. The dry

residue obtained was powdered and kept in the fridge in a black bottle.

**Larvae toxicity test:** This test is based on the survival of shrimp larvae in sea water in the presence of the test solution.

*Animal material:* The brine shrimp larvae (*Artemia salina*) were used. There is a correlation between the sensitivity of larvae and that of human cells (lung cells and enteric cells) to cytotoxic substances. (McLaughkin *et al.*, 1998; Pelka *et al.*, 2000).

*Incubation of brine shrimp eggs and larvae obtaining:* Eggs of *Artemia salina* summers were incubated at laboratory temperature (28 - 30°C) in a conical flask 1000 ml containing sea water collected in the Atlantic Ocean. The whole was placed on a shaker for forty eight (48) hours with gentle agitation. The eggs hatch into young active shrimp larvae, which were isolated from eggshells using a light source. They were then collected with a pipette and transferred into another flask still containing seawater.

*Preparation of extracts solutions:* Stock solutions of aqueous extracts were prepared in sea water at a concentration of 50 mg / ml. In ten (10) test tubes, were made a range of decreasing concentrations of the extract by making a dilution, in a geometric series with common ratio 2, of the stock solution.

*Achieving Linear Concentration 50 (LC50) extracts:* Into test tubes containing a range of decreasing concentrations of each extract (25 mg / ml in the first tube and  $4.88 \cdot 10^{-2}$  mg / ml in the last), we inoculated sixteen (16) larvae contained in 1 ml of Water Sea. The whole was incubated for 24 hours at the laboratory temperature (28-30°C).

*Reading and counting of dead larvae:* After 24 hours, the test tubes were examined. The number of survivors in each tube was counted and the number of dead larvae was recorded. Larvae were considered dead if they did not exhibit internal or external movement for a few seconds observation. The results were summarized in a table. The larvae did not receive food. To ensure that death observed in the trials was attributed solely to the extracts, not hunger, the test tubes were compared with control tubes containing larval solutions only. The brine shrimp larvae can survive up to 48 hours without food because they feed on their yolk sac (Michael *et al.*, 1956). The results obtained were expressed as dose-response. Dose-response data were transformed by logarithms, and the LC50 was determined by linear regression. According to Clarkson *et al.*, 2004 and Krishna Raju *et al.*, 2005, we note the following correspondence between the values of LC50 and toxicity of extracts:

**Table1:** Correspondence between extract concentration and toxicity

LC <sub>50</sub>	Toxicity
LC <sub>50</sub> ≥ 1 mg / ml	- (nontoxic)
1 mg / ml > LC <sub>50</sub> ≥ 0.5 mg / ml	+ (weak)
0.5 mg / ml > LC <sub>50</sub> ≥ 0.1 mg / ml	++ (moderate)
LC <sub>50</sub> < 0.1 mg / ml	+++ (strong)

**Evaluation of acute oral toxicity**

*Animal material:* It consisted of *Wistar* albino rats of average body weight 185 g. They had free access to water and food and acclimated to farming conditions from the pet of the Research Laboratory in Applied Biology (LARBA) located in the Polytechnic School of the University of Abomey Calavi (EPAC) in Benin Republic. Breeding was done in a well ventilated room, with a day-night rhythm of 12 h. The animals were kept in wire mesh cages with metal feeders and drinking

troughs. Their daily diet was made from a mixture of food in the form of croquettes and marketed by Vet Services (Benin). The enclosure was regularly cleaned to ensure optimal development of the animals avoid infection.

*Protocol:* The toxicity test was carried out as recommended by the guideline 423 of the Organization for Economic Cooperation and Development for the testing of chemicals (OECD, 2002). The substance was tested in a sequential process in which three animals

including multiparous females and no pregnant aged 8 to 12 weeks are used at each stage. The absence or the manifestation of substance related mortality in a group dosed at a step would determine the next step. The initial dose was selected from the following four doses: 5, 50, 300 and 2000 mg / kg body weight. We administered by gavage to animals 2000 mg of extract/kg body weight. The animals were observed carefully during the four (4) hours and then daily for 14 days. They were weighed and blood was collected by orbital puncture at the start of the experiment and then after 14 days.

**Blood tests:** Biochemical parameters such as urea and serum creatinine were doses to explore renal function. Transaminases AST and ALT were assayed for liver function. The WBC count was performed as hematological parameter.

**Histology:** At the end of the experiment, the animals were dissected. The liver, the kidney and the spleen

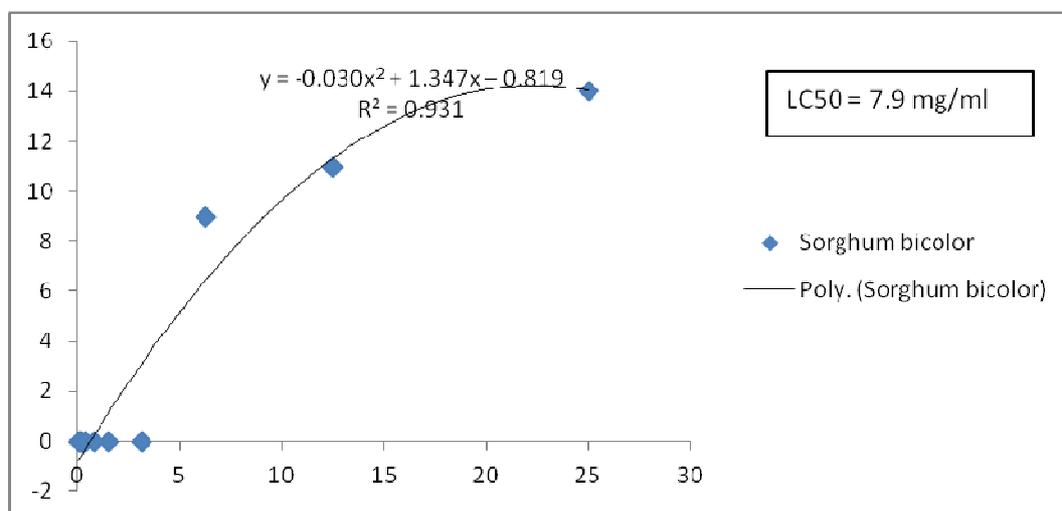
were removed, fixed in Bouin solution and embedded in paraffin. The specimens sections (5  $\mu$ m) were mounted on glass slides, deparaffinated, and hydrated. For histological analysis, sections were stained with hematoxylin and eosin (H&E), following a standard protocol (Sènou *et al.*, 2009). The pictures were taken at 400X magnification.

**Statistical Analysis:** For larvae toxicity test, adjustment points presenting the results were first made. After watching the homogeneity and normality of variables, were performed comparing the variables of the extract and the variable of the control group. The equation of the adjustment curve was used to determine the LC<sub>50</sub> using STATISTICA Statsoft software version 5.5 and Microsoft Excel 2007 software on Windows Vista. For the acute oral toxicity, the means were compared using Mann-Whitney test. The significance level was set at 5%.

## RESULTS

The *Sorghum bicolor* aqueous extract was not toxic *in vitro*. Figure 1 showed the evolution of the number of dead larvae based on the concentration of the *Sorghum bicolor* extract. The LC<sub>50</sub> indicate the concentration of

the extract able to kill half of the larvae present. Its value was 7.9mg/ml greater than 1mg /ml indicating the extract was non-toxic.



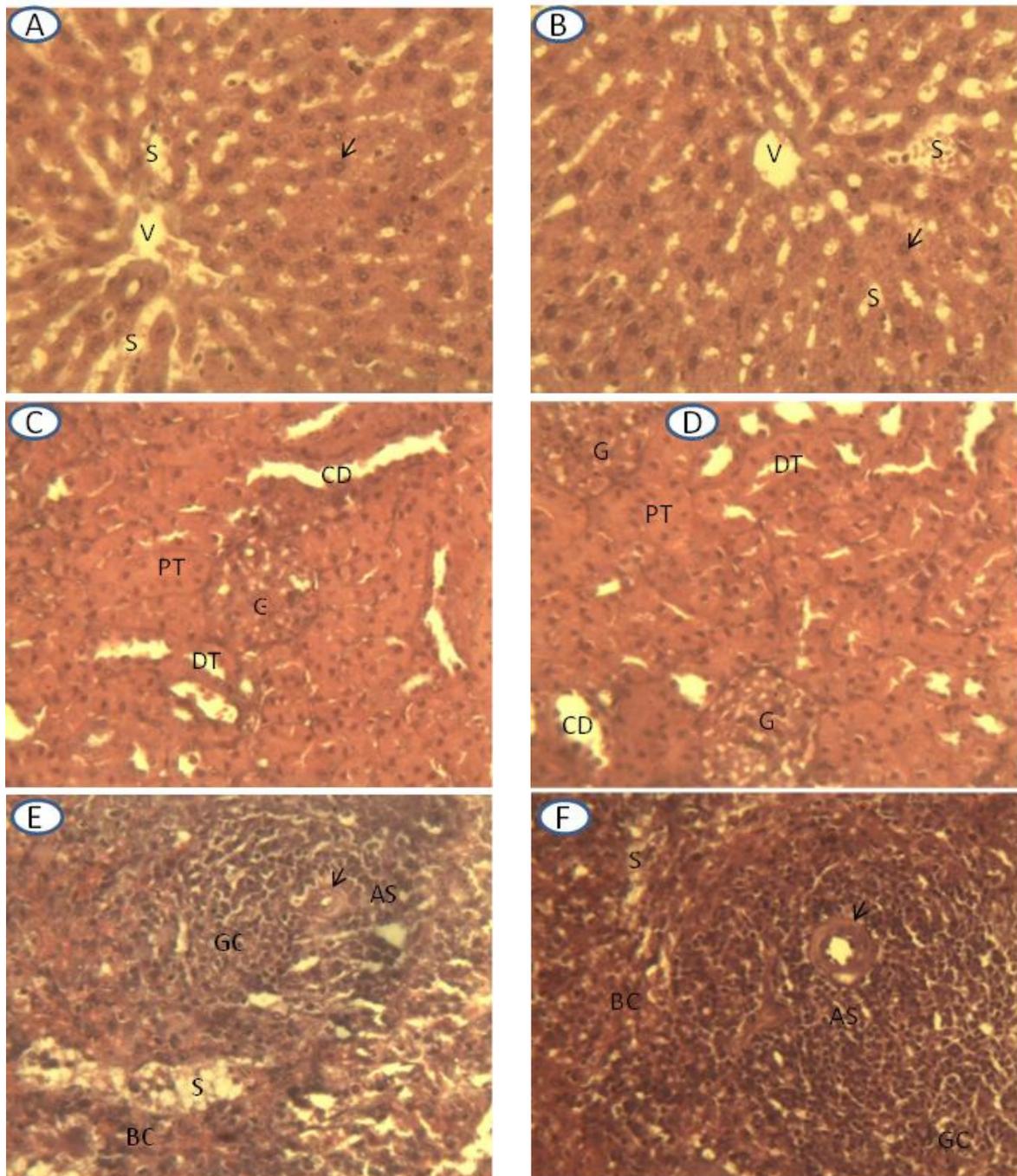
**Figure 1:** Evolution of the number of dead larvae based on the concentration of the *Sorghum bicolor* extract.

The results were presented in the form of a point cloud (on the x-axis, were the concentration and on the y-axis the number of dead larvae). The equation of the curve fit was used to determine the LC<sub>50</sub> which was the concentration of the extract able to kill half of the larvae present. The *Sorghum bicolor* aqueous extract did not induce cytotoxicity *in vivo*: The acute oral toxicity of the

extract was assessed by weight and some biochemical, hematological and histological parameters of the rats. Table 2 showed the evolution of the weight, biochemical and hematological parameters. The mean weight of rats was  $182 \pm 26$  g at D0 and no significant change was observed at D14 ( $174 \pm 22$  g). The blood urea and creatinine, respectively  $0.7 \pm 0.02$  g/l and  $6 \pm$

1 mg/l at D0 did not change significantly at D14 ( $0.7 \pm 0.04$  g/l and  $6 \pm 0$  mg/l), indicating an absence of impaired renal function. Transaminases AST and ALT were  $116 \pm 12$  U/l and  $47 \pm 5$  U/l at D0. They recorded a slight respective decrease to  $93 \pm 11$  U/l and  $44 \pm 6$  U/l on day 14, indicating protection of liver function.

However, this decrease was not significant. The number of white blood cells of  $6.1 \pm 1.53$  G/L did not change significantly at D14 ( $7.1 \pm 1.53$  G/L), indicating an absence of disturbance immunity. The *Sorghum bicolor* aqueous extract did not modify hepatic, renal and spleen parenchyma: figure 2



**Figure 2:** Histology of liver, kidney and spleen of treated and control rats.

A: Liver controls rats; B: Liver treated rats

As in control rats, the hepatocytes of treated rats (indicated by arrows) had a typical appearance and were ranged radial around the central vein (V). The cell cords are separated by the sinusoids (S).

C: Kidney of control rats; D: Kidney treated rats

In controls as in the Treated rats, the renal parenchyma was typical with glomeruli (G), proximal tubules (PT), distal tubules (TD) and collecting ducts (CD).

E: Spleen of control rats; F: Spleen of treated rats

In control as in treated rats, the spleen parenchyma did not change. The central arterioles (indicated by arrows), the peri arteriolar sheaths (AS), the germinal center (GC), venous sinusoids (S) and Billroth cords (BC) were typical.

Magnification: 400x

On D14, histology of the liver, kidney and spleen parenchyma of treated rats were not altered. The liver of treated rats (Figure 2B) did not present visible lesions compared with that of control rats (Figure 2A). The hepatocytes exhibited typical appearance and were ranged in approximately radial spans around the centrolobular veins. The hepatocyte blades were separated by visible sinusoids. Similarly, the renal parenchyma of treated rats (Figure 2D) did not differ from that of control rats (Figure 2C). It presented glomeruli, proximal tubules, distal tubules and collecting

ducts with typical appearance. The extract administration did not also change the spleen structure (Figure 2F). Like in controls (Figure 2E), the white pulp did not change. The peri arteriolar sheaths formed by lymphocytes ring around the central arterioles constitute the thymus-dependent area of the spleen. On the outskirts, germinal centers were the bursal-dependent areas. The red pulp comprised sinusoids separated by the Billroth cords containing the spleen siderophages (resident macrophages) and other white blood cells.

## DISCUSSION

*Sorghum bicolor* is one of the major grain crops for human and animal nutrition throughout the drier areas of Africa and India. Its leaf is also used in traditional medicine to treat anaemia in these regions (Dalziel, 1948; Ogwumike, 2002). It has been previously demonstrated that the aqueous extract of the leaves stimulated erythropoiesis in a specific way and dose-dependent manner (Sènou, 2016). This work focused on the biological tolerance of the extract by targeting its cytotoxicity *in vitro* and *in vivo*. *In vitro*, larvae toxicity test gave an LC50 equal to 7,9 mg/ml which was far greater than 1mg/ml, indicating that the extract was not toxic (Clarkson *et al.*, 2004; Krishnaraju *et al.*, 2005). Similar results were obtained with ethanolic extract of leaf and root of *Alafia barteri*, a plant exhibiting antiplasmodial activity in malaria treatment and with aqueous leaf extracts of *Acmella ciliata*, an anti-inflammatory plant (Mayara, 2016; Lasisi, 2016). *In vivo*, acute oral toxicity was conducted according to the recommendations of the OECD (2002). Investigated organs were liver, kidneys and spleen. The mean weight of rats did not change significantly at the end of the experimental period D14. Similar result was obtained with aqueous suspensions of *Jatropha tanjorensis* leaf, and methanolic extract of *Sphenostylis stenocarpa* bark, which are anti-anemic plants (Idu, 2014; Okonkwo, 2013). Serum urea and creatinine did not increase after fourteen days of

experimentation suggesting no renal defect function. It was confirmed by kidney histology, which exhibited a typical architecture of the parenchyma with apparently normal glomeruli and tubules. Such results were also obtained with ethanolic extract of the plant leaves (Nwinyi, 2009). By contrast, a chronic nephrotoxicity was observed both biochemically (increased blood urea and creatinine) and histologically (hydropic degeneration, tubular atrophy, impaired glomeruli and infiltration of inflammatory cells) with aqueous extracts of *Mentha spicata* and *Smilax sonchifolius*, which are anti-diabetic plants (Akdogan, 2003; Oliveira, 2011). Transaminases AST and ALT have declined slightly on day 14 indicating no liver cell lysis. The finding was confirmed by histology where the hepatic parenchyma kept its typical architecture with normal aspect of hepatocytes organized in cords around the central vein. Between these cords were clearly visible sinusoids. Similar result was obtained with aqueous extract of the plant administered to rats under low or high iron diet (Salawu SO and Adesina YS, 2014) or with ethanolic extract of the plant in chronic administration to rats (Nwinyi, 2009). It was also observed with aqueous extract of leaves of *Landolphia owariensis*, an anti-malarial and anti-microbial plant and with leaves and fruits of *Solanum macrocarpon*, a plant having cholesterol-lowering effect (Nwogu, 2008; Dougnon, 2013). By contrast, a continuous administration during

fourteen days of the extract to Sprague rats showed a slight increase in ALT transaminase without affecting other hepatic parameters (Akande, 2010). In hematology, the number of white blood cells did not significantly increase on day 14, suggesting an absence of disturbance immunity. This was confirmed by histology of the spleen, which presented a typical appearance of the parenchyma. With the white pulp containing periarterial lymphatic sheath and germinal centers and a red pulp having vein sinusoids and Billroth cords. This result was similar to those obtained

with the methanolic extract of the leaf of the plant or with aqueous extract of *Jatropha tanjorensis* leaves, or with methanolic extract from the bark of *Sphenostylis stenocarpa*. (Nwinyi, 2009; Okonkwo, 2013; Idu, 2014). In our previous work, we had detected in the extract of leaves of *Sorghum bicolor*, a presence of antioxidant compounds such as tannins and leucoanthocyanins (Sènou, 2016). The absence of cytotoxicity observed in various organs could be partly due to the antioxidant protection of these chemical groups.

**Table2:** Acute oral toxicity test

Parameters	Means at D0	Means at D14	P value	Difference
Rat weight (g)	182 ± 26	174 ± 22	0.7	no significant
Uremia (g / L)	0.7 ± 0.02	0.7 ± 0.04	0.4	no significant
Creatinine (mg / L)	6 ± 1	6 ± 0	0.7	no significant
Transaminase AST (IU / L)	116 ± 12	93 ± 11	0.2	no significant
Transaminase ALT (IU / L)	47 ± 5	44 ± 6	0.7	no significant
White Blood Cells (G/L)	6.1 ± 1.53	7.1 ± 1.53	0.4	no significant

## CONCLUSION

The *Sorghum bicolor* aqueous extract did not exhibit cytotoxicity *in vitro* in the larvae toxicity test and *in vivo* at acute oral toxicity. Its administration did not affect the liver, kidney and immune function. However,

investigations on its biological tolerance should be pursued by the chronic toxicity test and clinical trials before considering a possible transformation in Improved Traditional Medicine (ITM).

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