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CYTOTOXICITY, ACUTE TOXICITY AND SUBACUTE TOXICITY OF AN AQUEOUS EXTRACT OF SORGHUM BICOLOR (POACEAE), A PLANT USED IN THE TREATMENT OF ANEMIA IN IVORY COAST

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ABSTRACT

Sorghum bicolor is a medicinal plant used by oral administration. This plant is known in traditional medicine for the treatment of several conditions including anemia. The objective of this work is to evaluate the cytotoxicity on blood cells and acute and subacute toxicities of an aqueous extract of dry leaves of *Sorghum bicolor* (AESb) administered orally in mammals. The study of the acute toxicity of AESb in *Mus musculus* (Muridae) – *mice* – shows that, for up to the maximum dose of 5000 mg / kg BW, this extract does not cause death in treated animals by oral administration. Similarly, the study of the subacute toxicity the AESb shows that this extract administered by gavage for 28 days causes no death in treated rats. In addition, the monitoring of the change in weight during the 28 days of the experiment showed no loss of body mass and no significant difference in the increase in body mass of the rats treated with AESb at doses of 1000, 1500 and 2000 mg / kg BW, compared to controls. In addition, after 28 days of treatment of the rats with AESb at doses of 1500 and 2000 mg / kg BW, no significant change in the relative mass of certain vital internal organs (liver and kidneys) of these treated animals was recorded. Finally, the study of the cytotoxicity of AESb shows that this extract has a protective effect on the plasma membrane of red blood cells in rats. The results obtained in this study suggest that the administration of AESb would not constitute a risk in the pharmacological use of this extract in the treatment of anemia.

KEYWORDS: *Sorghum bicolor*, Anemia, Toxicity, Cytotoxicity, Antianemic.

INTRODUCTION

Herbal medicine, which essentially means "healing with plants", is presented as one of the challenges in responding to the fundamental concerns of public health. Thus, herbal medicine is originally considered as whole, natural, powerful and complex medicine. What interests the physiotherapist is therefore the "why" and "how" of the therapeutic effect of particular plants on particular diseases and not on others, in order to provide a scientific basis for the use of these plants in traditional medicine. It is therefore important to identify medicinal species and examine their biological activity in scientific research. To this end, the duty of everyone, at all times, to be up and doing and above all to share their knowledge, research and experiences with others. [1] It is in this context that this present work aims at contributing to the enhancement of active ingredients contained particularly in the species *Sorghum bicolor* of the family Poaceae, known under the name of *red sorghum*.

Thus, this section bearing on the study of aqueous Sorghum bicolor (AESb) aims to justify scientifically the use by oral administration of this plant in traditional medicine against anemia.

I-MATERIALS AND METHODS

I-1. Equipment

I-1-1. Plant material

The plant material is composed of leaves of *Sorghum bicolor* (Poaceae). The dried leaves of this plant are bought at the large market of Bingerville (Ivory Coast). The dried leaves come mostly from Burkina Faso or northern Côte d'Ivoire. They were identified in the National Floristic Center, located in an area of the Félix Houphouët- Boigny University which is administratively attached to the Faculty of Biosciences. These leaves are spread to dry on an aluminum foil, at a temperature of about 28 °C. The drying time is one to two days, in very sunny weather. The crisp leaves are ground in a porcelain mortar. The powder obtained is used for the

preparation of the aqueous extract of *Sorghum bicolor* (AESb).

I-1-2. Animal material

In this study, *Mus musculus* (Muridae), Swiss-strain *mice*, weighing between 40 and 50g, are used. They were reared in the animal facility of the Biosciences Training and Research Unit (UFR), at room temperature, in daylight and darkness (at night). They are fed with food (granules) supplied by the IVOGRAIN® company in Abidjan, Côte d'Ivoire, and have free access to water.

Besides the mice, rats of the *Ratus norvegecus* species (Muridae) are also used. They weigh between 150 and 200 g. They were also reared at the UFR Biosciences animal facility under the same conditions as the mice.

I- 2. Study methods

I- 2 -1. Preparation of the aqueous extract of *Sorghum bicolor* (Poaceae)

The method of extraction used is decoction. Five hundred grams (500 g) of dried and powdered leaves of *Sorghum bicolor* are boiled in two liters (2 L) of distilled water for 10 to 15 minutes. The decoction obtained is cooled. It is filtered first through cotton wool to retain large-sized impurities, and then through filter paper to remove smaller-sized impurities, according to the method revised by [2] Abo *et al.* (2015). The aqueous filtrate is concentrated in a BUCCHI rotavapor (France) and then left to dry in a VENTICELL MEDCENTER brand oven (France) at 40 °C.

After evaporation, a dry and compact pellet is obtained. This pellet is collected, Then crushed in a porcelain mortar. At the end of this process, the water-soluble powder that is obtained is the aqueous extract of a *Sorghum bicolor* (AESb).

I-2-2. AESb acute toxicity study

The study of the acute toxicity of the aqueous extract of *Sorghum bicolor* (Poaceae) (AESb) is carried out on the mice by gavage. This study is conducted according to the guidelines of the Organization of Economic Cooperation and Development (OECD) no. 423.[3]

I-2-2-1. Experimental protocol for acute oral toxicity in mice

The study of acute oral toxicity was performed on 18 mice of weight between 40 and 50 g divided into 6 batches of 3 mice each. Thus, each mouse receives 1 ml single dose, measured in mg / kg body weight (mg / kg BW) of the substance, with the predefined 50 doses are also 300, 500, 2000 and 5000 mg / kg PC for 5 test batches. The mice in the control batch each receive 1 ml of distilled water. The experiment is carried out on an equitable basis, batch by batch. The first batch of mice receive a single dose of 50 mg / kg BW. The mice are placed under observation, with particular attention paid during the first 4 hours of the day sequel to force-feeding, and this is done within 24 hours. The effects on the behavior of the treated animals are observed and symptomatic disorders are noted.

The number of dead mice, if any, is counted 24 hours after administration of the substance. The absence or manifestation of mortality, related to the dose of the substance in the batch which received it at a given stage, determines the next stage, that is to say:

– Stop the test if there is death of mice;
– Administration of the immediately higher dose (300 mg / kg BW) to the 3 mice of the second batch, and so on until the last batch which receives the limit dose of 5000 mg / kg BW.

I-2-2-2. Acute toxicity assessment

The number of dead mice is counted 24 hours after administration of the substance. The absence of mortality observed with the animals of the first batch, makes it possible to administer the higher dose, which is 300 mg / kg BW, to the second batch. The same observations are made on the animals of this batch, and so on until the last batch which receives the limit dose of 5000 mg / kg BW.

I-2-3. Study of the sub- acute toxicity of AESb in rats

This study makes it possible to look for possible toxic effects of AESb on the rats divided into different test batches, after 28 days of oral administration, using a gastric tube connected to a syringe.

I-2- 3-1. Experimental Protocol for determining subacute oral toxicity in rats

This test is carried out according to the standards of the OECD 407.[4] Forty (40) rats with body weights of between 150 and 200 g are grouped into 4 batches of 10 rats each. The rats in the control group each received distilled water. The *Sorghum bicolor* extract is dissolved in distilled water and administered orally in different doses to the rats of the three test batches as follows:

- Batch 1 (control batch): the rats receive 2 ml of distilled water,
- Batches 2, 3, and 4 receive 1 ml of AESb in respective doses of 1000, 1500 and 2000 mg / kg BW.

The extract is administered to each test batch in a single daily dose for 28 days; distilled water is also administered daily to the control for 28 days. During the experiment, the day before the tests (D_0), and after every 7 days (7^{th}, 14^{th}, 21^{st} and 28^{th} day), the body weights of the rats are measured and the behavior of the animals, together with the external signs of toxicity, are also noted during the experiment.

I-2-3-2. Removal of organs

At the end of the treatment period (that is, on the 28^{th} day), the rats were weighed and slaughtered. After the autopsy of the animals, the liver and kidneys are isolated, weighed and stored in 10% formalin. The

relative weights of the organs are determined with respect to the average weights of the rats in each batch.

Histological sections of the removed organs are made and observed under an optical microscope in order to assess the histotoxic effect of AESb.

I -2-4. Study of the cytotoxicity of the AESb

The in vitro cytotoxicity carried out in this work aims to assess the direct action of AESb on red blood cells. To do this, we put the red blood cells in contact with the AESb at different concentrations.

I-2- 4-1. Principle

The protocol followed to study the cytotoxicity of the extracts is that of Okoko and Ere.[5] The extract is tested at concentrations ranging from 100 µg / ml to 1000 µg / ml. The evaluation of cytotoxicity of the extract, vis-à-vis the red blood cells, is achieved by measuring the percentage of hemolysis and observing the microscope. The results are expressed as a percentage with respect to the hemolysis in the control samples. The samples are treated with the extract as follows:

% hemolysis = [AE \ AC] x100

AE = Sample absorbance

AC = Absorbance of positive control (hypotonic solution)

I-2-4-2. Demonstration of AESb cytotoxicity

This demonstration begins by washing the cells with physiological conditions. Then, it proceeds to a suspension of 2% of the cells in physiological saline (20 µl of cells against 980µl of distilled water). The subsequent steps are as follows:

- Preparation of the different concentrations of the extract: 1000 µg / ml and 100 µg / ml (This will serve as a blank for each extract);
- Preparation of the controls: negative (20 µl of the 2% cell suspension for 980 µl of distilled water) and positive (20 µl of the 2% cell suspension for 980 µl of a hypotonic solution);
- Preparation of the mixtures to be assayed: the cells in each concentration of extract (20 µl of the cell suspension with 2% to 980 µl of extract) incubated at 37 °C for 30 minutes; the extracted blank controls of the samples to be assayed;
- Centrifugation for 10 minutes at 3000 rpm;
- Finally, reading of the supernatant at 460 nm and the ODs are noted.

The% hemolysis is determined as follows:

% hemolysis = [AE \ AC] x100

AE = Sample absorbance

AC = Absorbance of the positive control (hypotonic solution),

with **AE = A $_{read}$ – AB**

A $_{read}$ = Absorbance read

AB = Blank Absorbance,

$$\% \text{ Hemolysis} = [(A_{read} - AB) \setminus AC] \times 100$$

I-2-4-3. Demonstration of the anti-hemolytic activity of AESb

When red blood cells are subjected to oxidative stress, osmotic resistance forces lead to membrane alterations and therefore hemolysis.[6] Thus, this test aims to evaluate the protective effect of AESb on cell integrity, which is essentially linked to the membranes of red blood cells. The hemolysis rate is evaluated quantitatively by a spectrophotometric assay of the hemoglobin level in the supernatant at 545 nm. Then we calculate the percentages of inhibition of hemolysis.

% Inhibition of hemolysis = [1 – (AE / AC)] x100

AE = Sample absorbance

AC = Absorbance of the positive control (complete hemolysis) ,

with **AE = A $_{read}$ – AB**

A $_{read}$ = Absorbance read

AB = Absorbance white, therefore

$$\% \text{ Inhibition of hemolysis} = [1 - ((A_{read} - AB) \setminus AC)] \times 100$$

I- 3. Processing of results

I- 3 -1. Statistical analyses

The results are analyzed by the ANOVA variance of the *GraphPad Prism 8* software. The Turkey-Kramer multiple comparison test gives the difference between the two values. P <0.05 is considered significant with *: P <0.05 (significant), **: P <0.01 (very significant), ***: P <0.001 (significant). Values are presented as the mean ± standard error of the mean (M ± ESM). **G**raphs are plotted using *GraphPad Prism 8* software (San Diego CA, USA).

II- RESULTS

II -1. Acute toxicity, subacute toxicity and cytotoxicity of the aqueous extract of *Sorghum bicolor* (AESb)

II -1-1. Acute oral toxicity of AESb administered to mice

The study of acute toxicity, carried out in accordance with the recommendations of the OECD 423, makes it possible, on the one hand, to observe the behavior of mice treated with AESb and, on the other hand, to determine the mortality of these animals which receive this extract.

II-1-1-1. Behavior of mice after oral administration of AESb

The administration of AESb in the predefined and successive doses of 50, 300 and 500 and 2000 mg / kg BW did not alter the behavior of the treated mice. On the other hand, the dose of 5000 mg / kg BW of AESb causes, compared to the control mice, a slow movement of the treated mice which, in addition, skirt the corners of the cage during the first 30 minutes after force-

feeding. For about 20 minutes after the administration, the hairs of the mice stand on end. After this time, the animals move normally and their condition becomes normal.

II -1-1-2. Mortality of mice after oral administration of AESb

The administration of AESb at successive doses of 50, 300, 500, 2000 and 5000 mg / kg BW does not cause any death in mice, up to the limit dose of 5000 mg / kg BW, within 48 hours after force-feeding.

II-1 -2. Sub-acute toxicity of AESb orally administered to healthy rats

II-1-2-1. Mortality and effects of AESb on the behavior of healthy rats treated for 28 days

During 28 days of oral administration of the AESb in the dose of 2000 mg / kg BW to rats, no death is recorded.

At the behavioral level, one observes of the rats that received the dose of 2000 mg / kg BW of AESb, during the first 5 days, a curling of hair and a slight slowing down in movement during the first 5 to 10 minutes after each force-feeding. These signs disappear from the 6th day of administration.

II-1-2-2. Effect of the aqueous extract of Sorghum bicolor on the body mass of healthy rats treated for 28 days

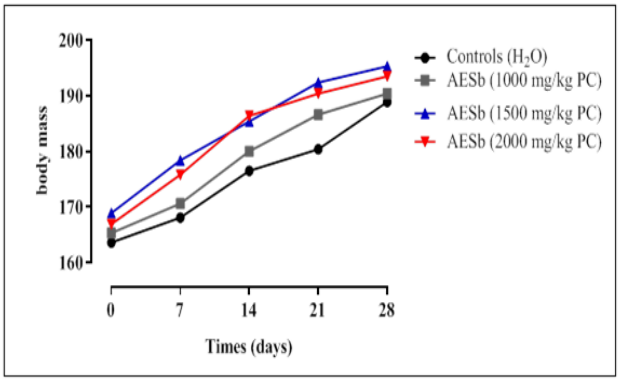
During the experiment, the body masses of control rats and rats treated with AESb gradually increased. In the control rats, the body mass goes from 163.6 ± 4.5 g, at the start of the experiment, to 188.9 ± 6.4 g after 28 days; or an increase in body mass of 15.46%. In rats treated with AESb at doses of 1000, 1500 and 2000 mg / kg BW, no weight loss was recorded, and there were no significant differences ($p > 0.05$) in increases in body mass, compared to the evolution of the body mass of the control rats during the 28 days of the experiment. Indeed, after 28 days of treatment of the rats with AESb at doses of 1000, 1500 and 2000 mg / kg BW, the increases in body mass in these animals are 15.18%, 15.63% and 15.94 % ($p > 0.05$) respectively.

The weight change of the animals treated with the AESb is presented in the **Fig 1**, compared with that of control rats.

II-2-2-3. Effects of AESb on the relative weight of the livers and kidneys of healthy rats treated for 28 days

The study of the effects of AESb on these organs shows that the treatment of rats over 28 days with this extract at a dose of 2000 mg / kg BW does not cause any significant variation ($p > 0.05$) in the relative weight of these different organs compared to those of the control rats.

Table I shows the values of the relative masses of the livers and kidneys of rats treated over 28 days with the AESb.



n = 10; p> 0.05 compared to controls

Fig. 1: Evolution of the body mass of healthy rats treated for 28 days with the aqueous extract of *Sorghum bicolor* (AESb).

Table I: Weights of the livers and kidneys of healthy rats treated for 28 days with the AESb.

Lots	Relative weights of organs (g / kg BW)	
	Liver	Kidneys
Control rats (H_2O)	44.96 ± 0.71	5.12 ± 0.14
Rats treated with AESb 1500 mg / kg BW	44.94 ± 0.70	5.13 ± 0.15
Rats treated with AESb 2000 mg / kg BW	44.97 ± 0.71	5.11 ± 0.13

n = 10; p> 0.05 compared to controls

II-1-3. Effects of AESb on the histological structure of the liver and kidneys of healthy rats treated for 28 days.

II- 1-3-1. Effects of AESb on the histological structure of the liver of healthy rats treated for 28 days.

Microscopic observation of the liver of control rats shows normal architecture with well differentiated hepatocytes. The cells are sinusoidal. The cytoplasm is homogeneous and the numerous nuclei are frosted **(Fig 2 -A).** The healthy rats treated over 28 days with the aqueous extract of *Sorghum bicolor* in respective doses of 1500 mg / kg BW and 2000 mg/kg BW have a histological structure of the liver very close to normal **(Fig 2 -B and Fig 2 -C).**

II-1-3-2. Effects of AESb on the histological structure of the kidneys of healthy rats treated for 28 days

Microscopic observation of the kidney of control rats shows normal structure, with normal renal parenchyma **(Fig 3 -A).** The healthy rats treated with the aqueous extract of *Sorghum bicolor* in respective doses of 1500 and 2000 mg / kg BW exhibit a normal renal structure with the glomerulus as well as the normal renal tubules **(Fig 3 -B and Fi g 3 -C).**

II-2. Cytotoxicity of the AESb on red cells of healthy rats

The cytotoxicity of an aqueous solution of the extract of *Sorghum bicolor*, evaluated by the hemolysis of blood cells under the impregnation of this extract, shows that

the rate of hemolysis of blood cells is low in the presence of this solution of the AESb. Indeed, when the red blood cells are introduced into media containing the AESb in aqueous medium at concentrations of 100 and 1000 µg / ml, hemolysis percentages of 2.35 and 4.85% ($p > 0.05$) are obtained respectively, while the hemolysis is at 100 % when the red blood cells are in the aqueous medium (H_2O) alone, and at 0 % in the saline medium (0.9% NaCl).

Thus, for these concentrations of AESb, the hemolysis inhibition rates are 95.15% and 97.65% ($p < 0.001$) respectively, while it is 0% in the aqueous medium (H_2O) alone and 100% in physiological conditions (0.9% NaCl) **(Table II)**.

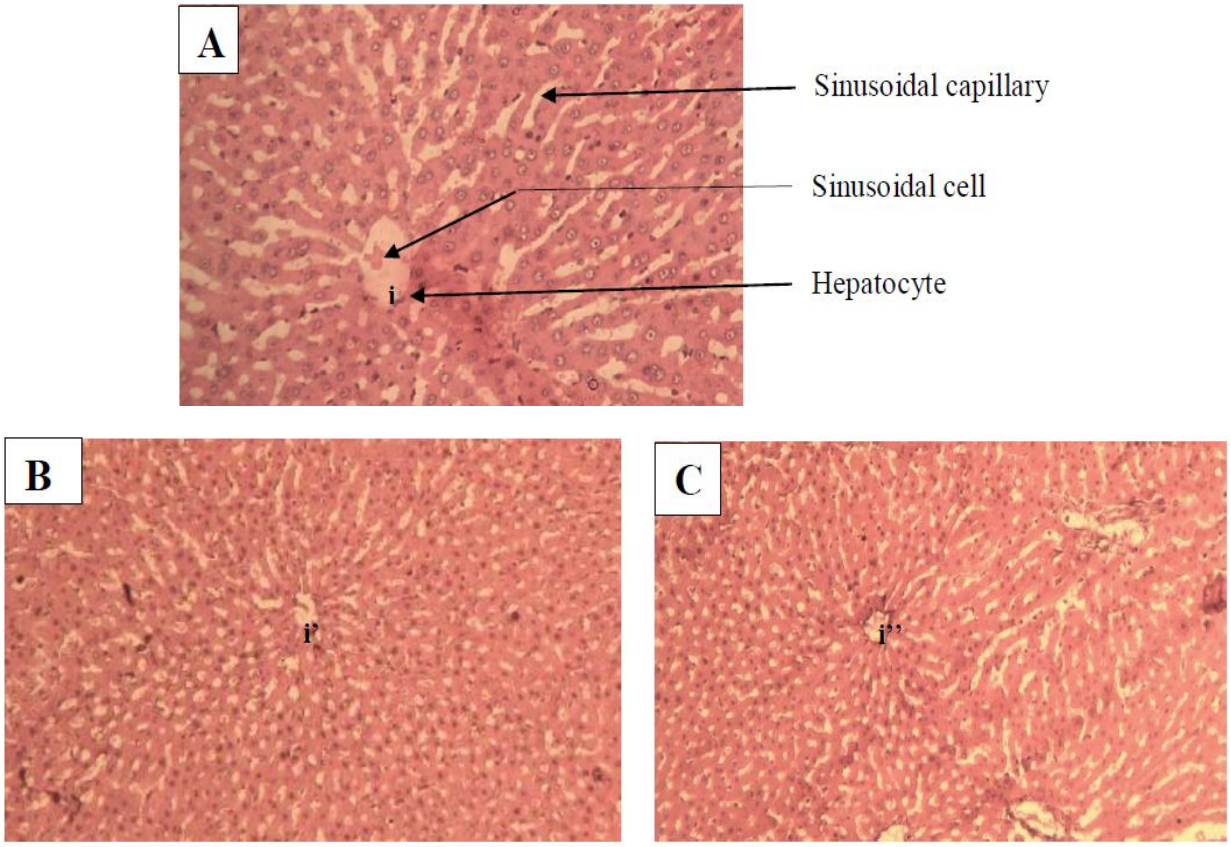


Figure 2: Effects of the aqueous extract of Sorghum bicolor (AESb) on the histological structure of the liver of healthy rats.

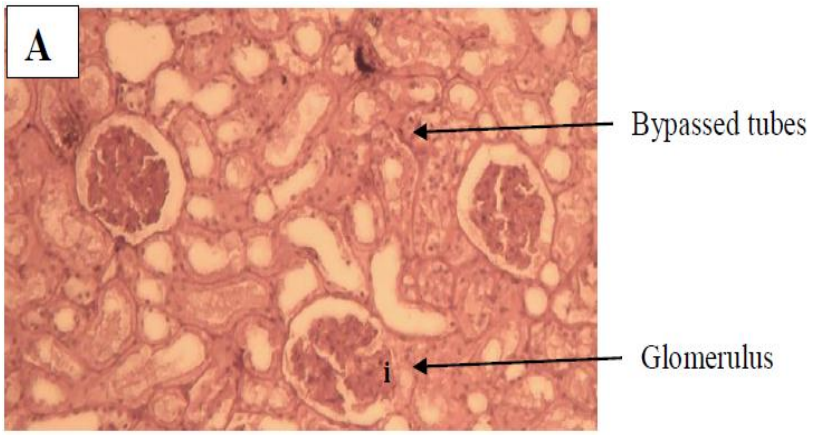
A: Photograph of a control rat liver section (healthy control)

B: Photograph of a section of rat liver treated with AESb at a dose of 1500 mg / kg BW

C: Photograph of a section of rat liver treated with AESb at a dose of 2000 mg / kg BW

i, i "and i": Hepatocytes

G x 100 Coloration: Haematein-eosin



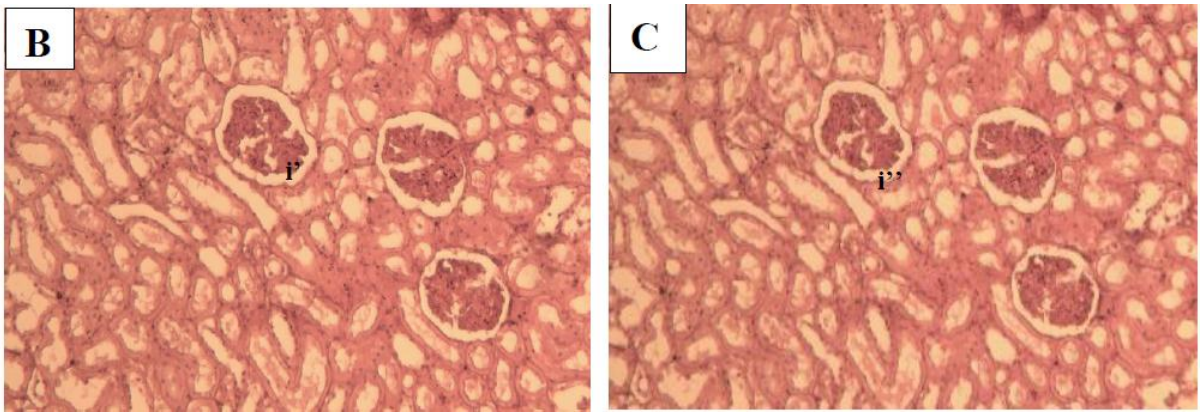


Fig. 3: Effects of the aqueous extract of *Sorghum bicolor* (AESb) on the histological structure of the kidney of healthy rats.

A: Photograph of a kidney section of control rat (healthy control)
B: Photograph of a section of rat liver treated with AESb at 1500 mg / kg BW
C: Photograph of a section of rat liver treated with AESb at 2000 mg / kg BW
i, i' and i'' : Glomerulus
G x 100
Color: Haematein-eosin

Table II: Rate of hemolysis and inhibition of hemolysis induced by the aqueous extract of *Sorghum bicolor* (AESb) on red blood cells of healthy rats.

Middle	H_2O	NaCl 0.9 %	Aqueous solution of AESb at 100 µg / m l	1000 µg / ml aqueous solution of AESb
Hemolysis	100 %	0 %	2.35 %	4.85 %
Inhibition of hemolysis	0 %	100 %	95.15 %	97.65 %

III- DISCUSSION

The acute toxicity study of AESb in mice shows that, up to the maximum dose of 5000 mg / kg BW, this extract does not cause death in treated animals. This indicates that the 50% lethal dose (LD_{50}) of AESb is greater than 5000 mg / kg BW. Similar studies confirm that a plant with an LD_{50} greater than 1000 mg / kg BW is considered non-toxic in animal experiments[7,8] AESb is therefore non-toxic when administered orally.

Likewise, the study of the subacute toxicity of AESb shows that this extract, administered by gavage for 28 days, does not cause any death in the treated rats. In addition, the monitoring of the change in weight during the 28 days of the experiment showed no loss of body mass and no significant difference in the increase in body mass of the rats treated with AESb at doses of 1000, 1500 and 2000 mg / kg BW, compared to controls. Likewise, after 28 days of treatment of the rats with AESb at doses of 1500 and 2000 mg / kg BW, no significant change in the relative mass of certain vital internal organs (livers and kidneys) of these treated animals was recorded.

Similar results were also obtained by authors who respectively showed that the aqueous extracts of *Rauvolfia vomitoria* (Apocynaceae) and *Pseudarthria hookeri* (Fabaceae) have no effects on body weight, nor on the relative weights of the heart, liver and kidneys of treated anemic rats.[9,10]

According to some authors, the change in body weight and / or the weight of internal organs is an index of toxicity after exposure to a toxic substance.[11] Indeed, following the administration of a substance, changes in body mass are an indicator of unwanted side effects, because surviving animals should not lose more than 10% of their initial body mass.[12] Also, there is a very strong possibility that herbal products, when ingested into the body for a shorter or longer period, are toxic to important organs such as the kidneys, liver and heart, due to their various roles in the human body.[13] For example, increased liver weight may be linked to congestion by reserving blood in the liver [14] . The fact that AESb had no effect either on the body weight or on the relative weights of the internal organs of the treated rats confirms that this extract is not toxic when administered orally. Thus, AESb has no adverse effects on body development and the development of internal organs in animals.

The study of the cytotoxicity of AESb shows that this extract has a protective effect on the plasma membrane of red blood cells. In fact, AESb inhibits hemolysis in a dose-dependent manner. This property of AESb could be due to the antioxidant power of this extract.

In fact, it has been shown that free radicals derived from oxygen induce, in lipids and membrane phospholipids, a radical chain reaction with lipid peroxidation.[15] These reactions, often very violent, lead to the loss of membrane integrity or hemolysis in the case of red blood cells. So the antioxidants, by their ability to scavenge

free radicals, play a beneficial role in protecting the integrity of the cells and, in particular, of the membrane. This could explain the anti-anemic potential of the aqueous extract of *Sorghum bicolor* and would justify the use of this plant in the treatment of this pathology.

Furthermore, authors have shown that flavonoids have the property of interacting with the polar groups of membrane phospholipids through hydrogen bonds, thus accumulating on the membrane surface.[1 6,17] which makes it possible to reduce the access of radical molecules to the lipid bilayer. The presence of flavonoids in AESb would also justify the very high protective effect (97%) of this extract on red blood cells.

AESb is therefore not only non-cytotoxic, but also has a protective effect on red blood cells "in vitro". This would justify its use in traditional medicine in the treatment of anemia.

Microscopic observation of the structure of the liver and kidneys shows that AESb, administered to rats for 28 days in doses of 1500 and 2000 mg/kg BW, is not toxic to these organs in treated animals. AESb is therefore neither hepatotoxic nor nephrotoxic.

The liver and kidneys play a very important role in metabolic processes.[18] The function of the liver is to detoxify the body of harmful substances, while the kidney contributes to the maintenance of homeostasis by the reabsorption of vital substances and the excretion of waste[19,20] Several studies show that there is a link between anemia and the functioning of the liver.[21] It has been shown that of 161 cases of cirrhosis of the liver, the end-stage of evolution of most chronic liver disease, the prevalence of anemia was 74.5 %. Likewise, it has been shown that the majority of patients with advanced renal failure also suffer from anemia.[22]

CONCLUSION

At present, the importance of research on traditional pharmacopoeia can no longer be underestimated. Increasingly, the World Health Organization (WHO) allows for the special place of traditional medicine (**WHO, 2003)**. Thus, our study, which aims to contribute to the enhancement of the active ingredients contained in medicinal plants, focused in particular on the toxicity of the aqueous extract of *Sorghum bicolor* (Poaceae) administered orally in Wistar rats.

It emerges from this study that the aqueous extract of *Sorghum bicolor* (AESb) is non-toxic when it is administered orally in mice. The oral route is therefore recommended for the administration of this extract. AESb is also non-cytotoxic on blood cells and shows no signs of toxicity to the liver and kidneys of healthy rats.

The results obtained in this study suggest that the administration of AESb would not constitute a risk in the pharmacological use of this extract in the treatment of anemia.

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