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TOXICITY STUDIES ON THE LEAVES OF SENNA ALATA, A MEDICINAL PLANT FROM BURKINA FASO, IN MICE AND RATS

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ABSTRACT

The aim of the work is to study the acute and subacute toxicity of the aqueous extract of the leaves of *Senna alata* (ED-SA). Extracts doses of 500, 1000 and 2000 mg/kg of body weight (bw) were administered to the mice for acute toxicity study. The administration volume was 1 ml/100g. A limit test has been carried out to determine the DL_{50}. For subacute toxicity, rats received *Senna alata* extracts orally for 28 days. The first group to constitute the control received distilled water (solvent for diluting the extracts). Groups 2, 3 and 4 received extracts of *S. alata* at the respective doses of 500, 1000 and 2000 mg/kg of body weight; satellite groups 5 and 6 received, respectively, distilled water (satellite control) and extract at the maximum dose of 2000 mg/kg (satellite). The satellite groups were observed 14 days after stopping treatment to assess reversibility to toxicity. The collected serum was used for biochemical assays (ALAT, ASAT, creatinine, total cholesterol, and triglycerides). Plasma has been used to assess the effects of the extract on hematological parameters such as blood cells, red blood cells, hematocrit, hemoglobin. The acute toxicity assessment of the aqueous extract of *Senna alata* has shown that the lethal dose 50 (DL_{50}) is greater than 2000 mg/kg, suggesting that the extract would be practically non-toxic at this dose. In subacute toxicity, no major lesion was observed after histological analysis of the liver and kidneys. These results suggest that the aqueous extract of *Senna alata* does not affect liver and kidney. In conclusion, this study shows that ED-SA is of low toxicity.

KEYWORDS: Acute toxicity; subacute toxicity; rats, mice, *Senna alata.*

INTRODUCTION

Toxicity would be defined as a functioning disorder of a living organism following exposure to a toxic substance. The definition of toxicity must take into account the nature (physical or chemical) and the dose of the substance absorbed. To this must be added the circumstances of the exposure (route, duration, frequency), the type of individual (the species and the strain, body mass, age, sex, degree of maturity, physiological condition), the type and degree of damage caused.[1] The purpose of toxicity is to establish the risk incurred by humans, before any contact (oral or blood administration, exposure of the skin or respiratory tract), whether it is a drug, a chemical, a pollutant or a pesticide. Depending on the dose administered and the time of exposure of the organism to xenobiotic, it can be distinguished acute, subacute, subchronic and chronic toxicity. Foreign substances that are never used by body cells to produce energy or synthesize biological molecules are called xenobiotics. Toxicology assesses the risks associated with xenobiotic exposure. Chemicals can cause morphological and functional damage to certain organs in animals. Toxicology is concerned with these lesions. It allows and identifies and studies the mechanisms of these alterations. Toxicity makes it possible to recognize the target organs for toxicity and to define the characteristics of dose response relationships.[2]

I. MATERIALS AND METHODS

I.1. Rats and Mice

The rats and mice used were supplied by the pet store of the Faculty of Sciences of the University of Yaounde I. The male and female *Wistar* rats weighed between 100 and 120 g. *NMRI* mice weighed 20-30 g. The animals were distributed by sex in cages (at the rate of five rats or mice per cage). They were fed water and food every day and kept under ambient temperature, sufficient ventilation, and natural light cycle.

I.2. Plant

The leaves of *Senna alata* were harvested in Ouagadougou. They were dried under ventilation in the laboratory and protected from light. These dry leaves have been powdered. The botanical identification was made at the Plant Biology and Ecology Laboratory of University Joseph KI-ZERBO by comparison with an authentic specimen deposited in the herbarium of the Plant Biology and Ecology Department of University Joseph KI-ZERBO. The plant has been identified under ID number 15965.

I.3. Toxic effects of the aqueous extract of the leaves of Senna alata

Studies have shown that the DL_{50} of *Senna alata* is between 1850 and 2000 mg/kg of body weight.[3,4,5] The doses tested in our study were 500, 1000 and 2000 mg/kg/bw.

I.3.1. Acute toxicity

Twenty female mice of eight-week-old were divided into five groups of four. They were fasted four hours before tests: extracts in doses of 500, 1000 and 2000 mg/kg/bw were orally administered to the mice; administration volume was 1 ml/100g for each dose. Mice were then observed for fourteen (14) days. Observations focused on touch sensitivity, mobility, restlessness, and mortality. To locate (find) the DL_{50}, a limit test was carried out: a mouse received orally one milliliter of the experimental dose of 2000 mg/kg/bw. The trial was stopped if the mouse died within seventy-two (72 hours) after administration. The DL_{50} was estimated as below. On the other hand, if the mouse survives, the same operation was repeated with three other mice. The trial was terminated in the absence of the death of the three mice within seventy-two (72) hours. The DL_{50} has been estimated:

- lower than the test dose (2000 mg/kg/bw), if at least three animal deaths have been observed;
- higher than the test dose if at least three animals have survived.[6]

I.3.2. Subacute toxicity

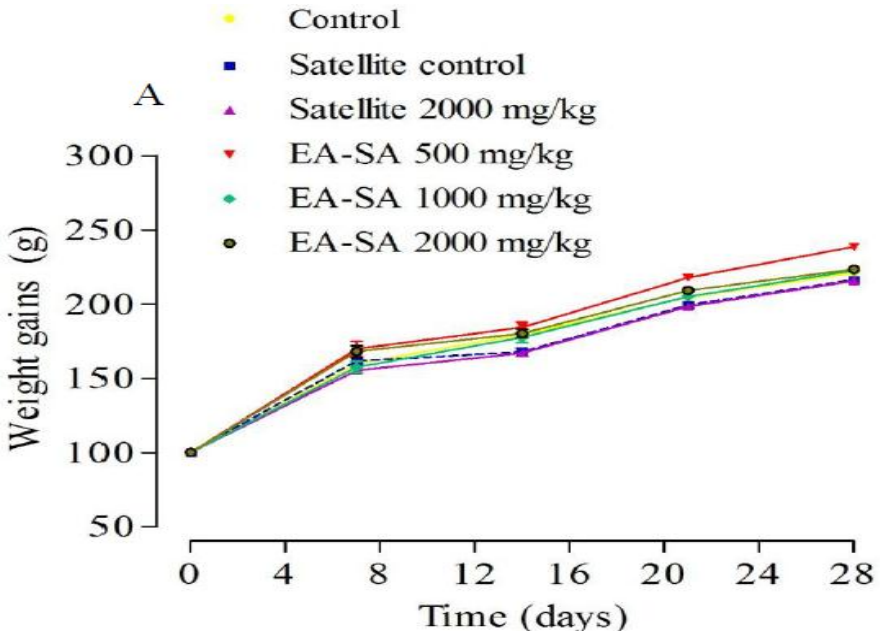
Thirty (30) males and 30 females rats were divided into six groups of ten (five males and five females). Once randomized, the rats received the extracts of *Senna alata* orally for 28 days. Group 1 was a control one and received distilled water (used solvent for diluting the extracts). Groups 2, 3 and 4 received extracts of *S. alata* respectively at the doses of 500, 1000 and 2000 mg/kg of body weight; satellite groups, 5 and 6, received respectively distilled water (satellite control) and extract at 2000 mg/kg/bw (satellite). These groups were observed fourteen days after stopping treatment to assess toxicity reversibility. During the experimental period, body weight, food and water consumption were evaluated twice a week. At the end of the experiments all animals of all groups were sacrificed and this, after blood sampling. Thee blood samples were collected in two types of tubes: with an anticoagulant (EDTA) for hematological analyzes and without anticoagulant (dry tubes) for the analysis of biochemical parameters. The blood collected in dry tubes was left to stand for sedimentation at room temperature for fifteen minutes. Blood was then centrifuged at 3600 rpm for fifteen minutes. The collected serum was stored at -20 ° C until different biochemical dosages (ALAT,

ASAT, creatinine, total cholesterol, and triglycerides). Enzyme kits and codes used are among others Cypress diagnostics kits code HBE07 (ALAT), Cypress diagnostics kits code HBE06 (ASAT), Fortress diagnostics kits code BXC0193 (TB) and Fortress diagnostics kits code BXC0184 (ALP). The plasma obtained from an anticoagulant was used to assess the effects of the extract on a few hematological parameters (white blood cells, red blood cells, hematocrit, hemoglobin).[6] The analysis of the various hematological parameters was carried out in the laboratory of the central hospital of Yaounde using the device: "Ham screen 18 Hematology Analyzer". The histological examination was carried out on the liver and kidneys of rats treated with the highest dose of plant extract (2000 mg/kg).[7] The organs removed for examination were kept in formalin (10%).

II. RESULTS

II.1. Effects of the aqueous extract of Senna alata on weight gain and the relative weight of organs.

In both sexes, general signs of intoxication such as mortality, behavior change have not been observed. On the other hand, weight gain has been observed. These observations did not differ significantly from one group of rats to another ($p > 0.05$) (Fig. 1). The relative organ weights did not show a significant difference between the groups of rats (Fig. 2). The autopsy of the internal organs (liver and kidneys) revealed no atrophy, nor hypertrophy.



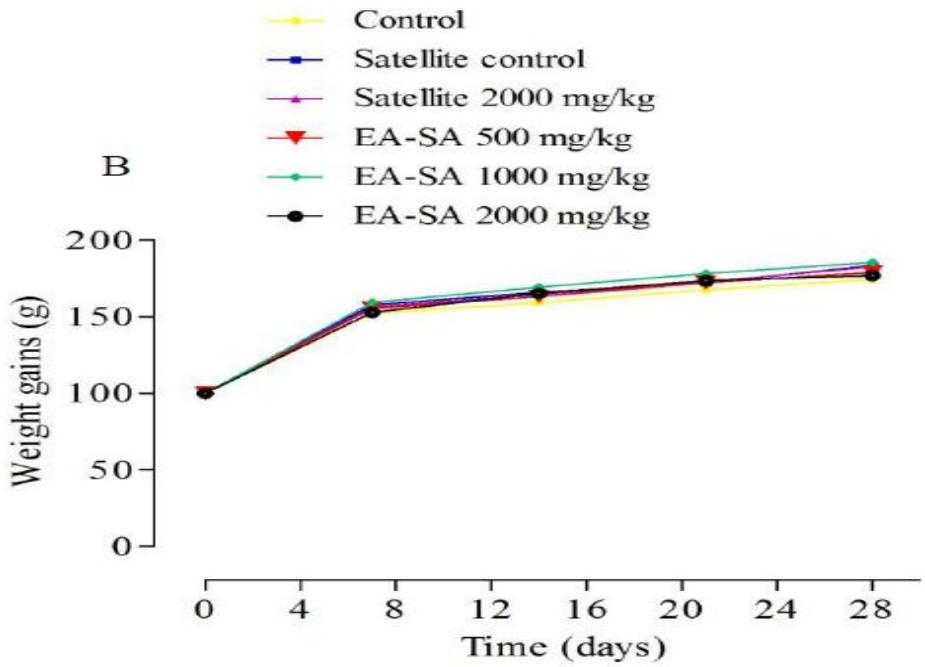
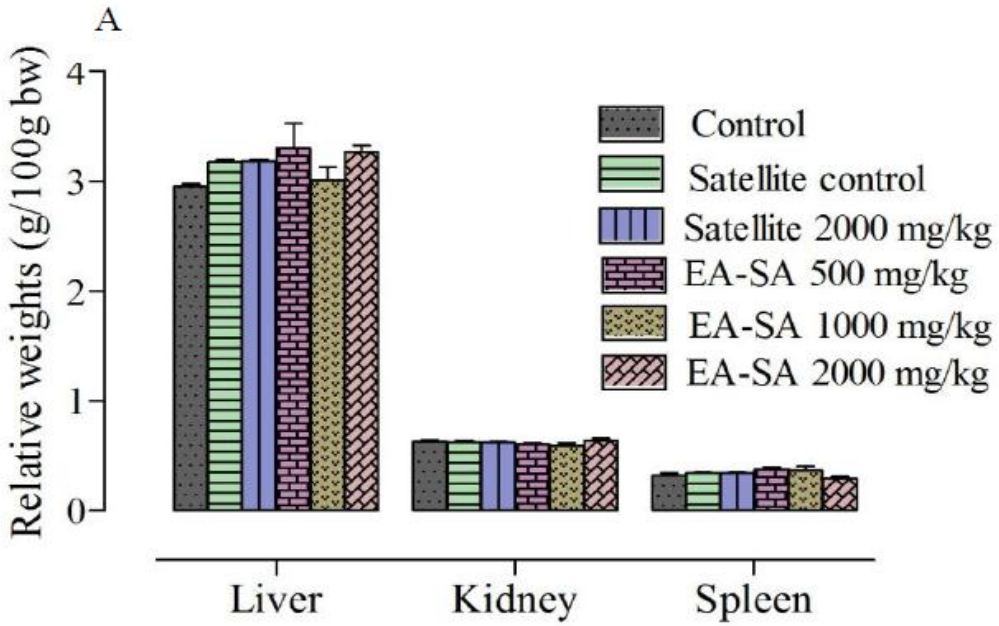


Figure 1: Variations in body weights of rats treated orally with the aqueous extract of the leaves of *Senna alata* for 28 consecutive days. A and B: weight gains of male and female rats, respectively. Values are expressed as mean ± SEM; n = 10.



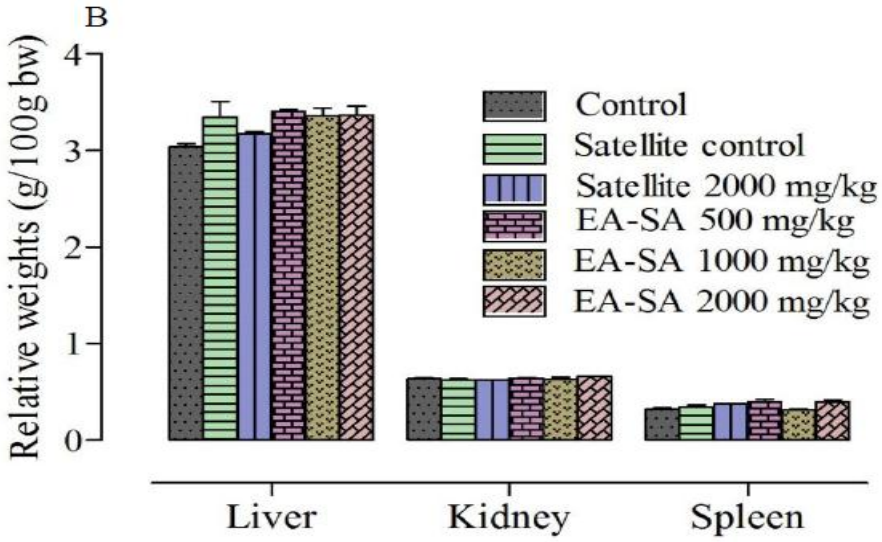


Figure 2: Variations in the relative weights of the liver, kidneys and spleen of rats treated orally with the aqueous extract of the leaves of *Senna alata* for 28 consecutive days. A and B: relative weights of these organs in male and female rats, respectively. Values are expressed as mean ± SEM; n = 10.

Weight gain and the relative organ's weight did not differ between the control rats and the rats treated with the extract 2000 mg/kg ($p > 0.05$).

II.2. Effects of the aqueous extract of Senna alata on hematological parameters.

The hematological parameters such as the number of white and red blood cells, the hematocrit, and the hemoglobin did not vary significantly between the control rats and those treated with the extract ($p > 0.05$) (Table I).

Table I: Effects of massive doses of the aqueous extract of *Senna alata* on the hematological parameters of different groups of male and female rats.

Hematological parameters	**Traitements (mg/kg/bw)**					
	Control	**Sat. control**	**Sat. 2000**	**EA-SA 500**	**EA-SA 1000**	**EA-SA 2000**
Males						
WBC (10^3/µl)	11.8 ± 0.7	11.3 ± 0.6	11.1 ± 0.1	11.3 ± 0.7	10.5 ± 0.4	10.8 ± 2.1
RBC (10^6/µl)	7.56 ± 0.57	8.23 ± 0.51	8.11 ± 0.15	7.61 ± 0.22	8.66 ± 0.28	7.95 ± 0.43
Ht (%)	46.3 ± 4.1	46.3 ± 1.3	47.0 ± 0.6	46.0 ± 1.0	51.7 ± 0.9	50.3 ± 3.7
Hb en g/dl	16.2 ± 0.8	15.8 ± 0.3	15.8 ± 0.1	16.5 ± 0.5	16.8 ± 0.9	16.6 ± 1.0
Females						
WBC (10^3/µl)	11.2 ± 1.7	11.3 ± 1.5	10.8 ± 0.7	10.5 ± 1.2	10.0 ± 0.6	11.6 ± 0.6
RBC (10^6/µl)	8.35 ± 0.25	7.87 ± 0.19	7.90 ± 0.11	8.70 ± 0.23	8.30 ± 0.26	8.90 ± 0.33
Ht (%)	50.3 ± 1.9	49.0 ± 2.1	49.4 ± 0.6	53.3 ± 0.3	51.0 ± 1.0	53.0 ± 1.5
Hb en g/dl	19.7 ± 2.4	15.6 ± 0.7	15.8 ± 0.2	18.0 ± 0.2	17.4 ± 0.2	17.5 ± 0.4

WBC: White blood cells, RBC: Red blood cells, Ht: Hematocrit, Hb: Hemoglobin, Sat. control: Satellite control, Sat. 2000: Satellite 2000.

II.3. Effects of the aqueous extract on biochemical parameters

The biochemical profiles of the rats treated with the aqueous extract of the leaves of *Senna alata* and of the control rats are presented in Table II. No significant difference ($p > 0.05$) was observed after the rats were treated with the extract. In addition, the extract has no effect on liver and kidney enzymes such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP), bilirubin total (TB), and other parameters such as creatinin, total protein, triglycerides (TG) and cholesterol. In the satellite group there is no late effect of toxicity. No reversibility of any lesion was observed.

Table II: Effects of the aqueous extract of *Senna alata* on the biochemical parameters of the different groups of male and female rats.

Biochemical parameters	Traitements (mg/kg/bw)					
	Control	Sat. control	Sat. 2000	EA-SA 500	EA-SA 1000	EA-SA 2000
	Males					
ALAT (U/l)	28,44 ± 1,49	34,67 ± 3,21	34,56 ± 1,49	31,06 ± 3,06	34,67 ± 3,84	35,88 ± 2,99
ASAT (U/l)	48,12 ± 8,74	51,19 ± 3,81	55,13 ± 6,76	51,62 ± 7,05	53,38 ± 7,20	56,00 ± 4,29
TB (µmol/l)	9,23 ± 0,51	9,66 ± 0,18	9,68 ± 0,51	9,74 ± 0,44	9,94 ± 0,4	9,69 ± 0,55
ALP (U/l)	53,99 ± 9,58	52,18 ± 4,60	53,07 ± 0,78	52,67 ± 0,49	53,07 ± 0,78	55,14 ± 15,88
CREA (µmol/l)	177,85 ± 5,81	174,59 ± 8,2	189,48 ± 7,5	178,63 ± 6,78	179,27 ± 5,40	198,56 ± 50,45
Proteins (mg/dl)	17,72 ± 0,62	16,55 ± 0,49	16,77 ± 0,36	17,08 ± 1,34	16,44 ± 0,96	17,38 ± 0,97
TG (mg/dl)	75,00 ± 4,42	76,37 ± 1,73	76,06 ± 2,08	76,52 ± 1,55	77,38 ± 1,54	76,25 ± 3,59
Cholesterols (mg/dl)	53,15 ± 3,35	54,07 ± 3,21	64,26 ± 1,84	55,03 ± 5,48	53,35 ± 1,84	52,83 ± 1,86
	Females					
ALAT (U/l)	31,77 ± 3,72	36,43 ± 1,48	35,66 ± 3,73	32,38 ± 1,68	34,02 ± 2,58	36,42 ± 2,03
ASAT (U/l)	53,59 ± 1,57	56,88 ± 1,82	56,88 ± 8,13	54,69 ± 1,49	56 ± 1,43	56,88 ± 1,13
TB (µmol/l)	8,58 ± 0,4	8,68 ± 0,10	8,44 ± 0,26	9,19 ± 0,79	8,32 ± 0,74	9,36 ± 0,75
ALP (U/l)	52 ± 0,96	51,95 ± 0,62	51 ± 0,56	53,77 ± 1,69	54,62 ± 2,94	54,11 ± 2,83
CREA (µmol/l)	173,60 ± 9,33	196,22 ± 5,64	188,20 ± 11,14	180,40 ± 5,03	187,21 ± 8,16	193,74 ± 11,71
Proteins (mg/dl)	20,78 ± 0,60	21,01 ± 0,30	21,53 ± 0,35	20,99 ± 0,43	21,97 ± 0,82	21,51 ± 0,12
TG (mg/dl)	73,35 ± 2,79	71,95 ± 1,12	73,19 ± 2,59	74,44 ± 3,50	74,23 ± 2,8	75 ± 1,99
Cholesterols (mg/dl)	55,12 ± 4,81	51,53 ± 2,83	53,79 ± 3,06	51,10 ± 4,24	51,69 ± 3,41	51,19 ± 4,25

CREA: Creatinine, TG: triglyceride, ALP: alkaline phosphate, TB: total bilirubin, ASAT: Aspartate Transaminase, ALAT: Alanine Transaminase, Sat. control: Satellite control, Sat. 2000: Satellite 2000. The hematological and biochemical parameters (Tables I and II) did not present significant variations ($p > 0.05$) in the different groups.

II.4. Effects of the aqueous extract on the structure of the liver and kidney

Histological examination of the liver and kidneys of rats treated with the aqueous extract of *Senna alata* 2000 mg/kg/bw showed a normal architecture. However, liver histology showed

steatosis and slight edema of the hepatocytes in both the control rats and those treated with the 2000 mg/kg/bw extract. On the other hand, the signs of hepatotoxicity such as necrosis, infiltration, edema were not observed (Fig. 3).

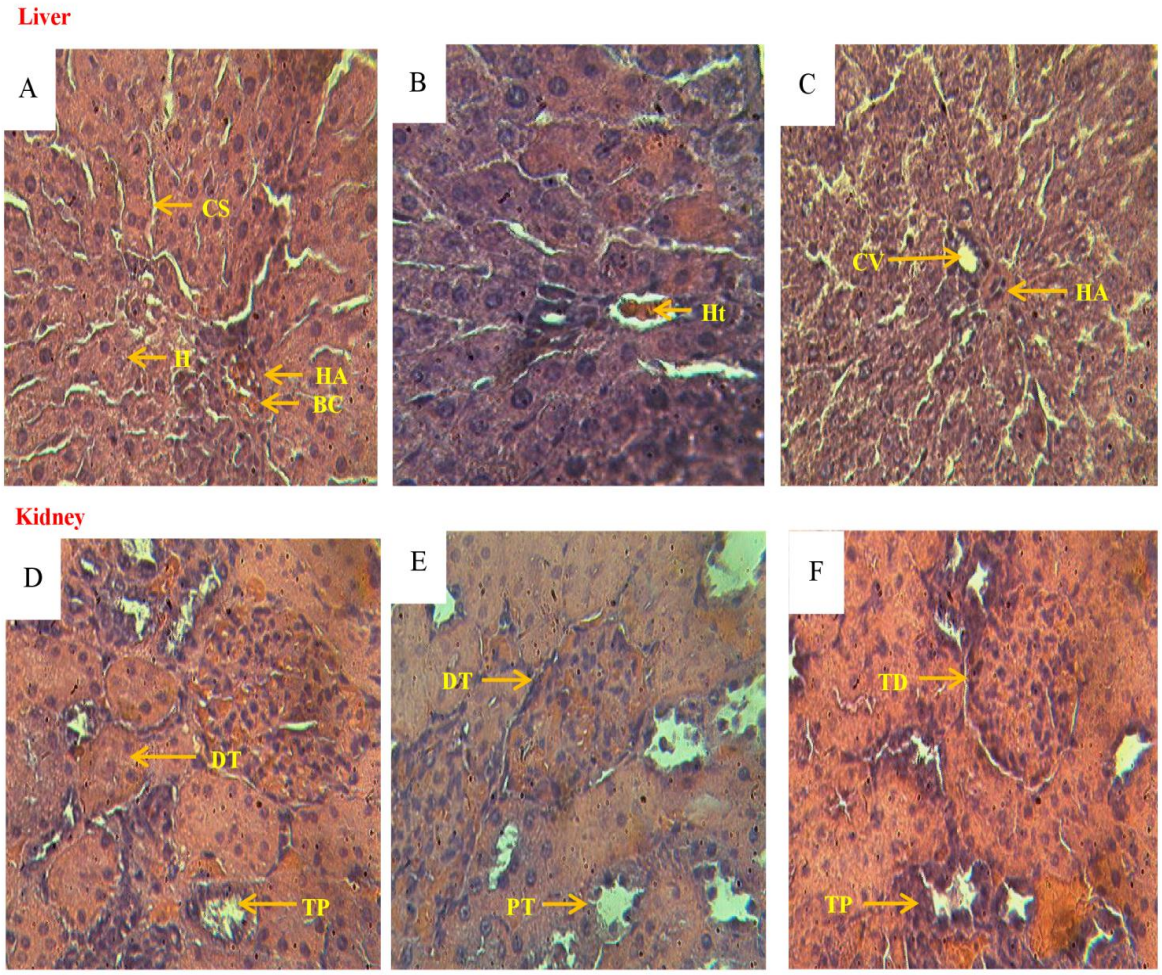


Figure 3: Liver and kidney sections of rats from different groups (H&E x 400). A: control rat liver, B: male rat liver treated with EA-SA 2000 mg/kg, C: female rat liver treated with EA-SA 2000 mg/kg, D: control rat kidney, E: male rat treated with EA-SA 2000 mg/kg, F: kidney rat female treated with EA-SA 2000 mg/kg. HA: hepatic artery, BC: biliary canaliculus, CV: central vein, H: hepatocytes, Ht: hematia, CS: canaliculus, PT: proximal tube, DT: distal tube.

III. DISCUSSION

The extract did not cause any change in the behavior of mice exposed to doses of 500; 1000 and 2000 mg/kg. No mortality was observed fourteen (14) days after the oral administration of the extract. A research team has proposed a classification of toxicity levels according to DL_{50}.[8] This classification stipulates that substances whose DL_{50} is between 5000 and 15000

mg/kg are considered to be practically non-toxic. It is concluded that the aqueous extract of *Senna alata* is practically non-toxic. Toxicological reports indicate that reduced body and internal organ weights are considered to be indices of toxicity after exposure to a toxic substance.[9,10] The evaluation of the subacute toxicity of *S. alata* at the doses of 500, 1000 and 2000 mg/kg per day for twenty-eight (28) days did not reveal a body weight gain difference. Behavioral changes were not observed. Blood cells are produced in the red bone marrow. Erythropoietin is the main stimulator of erythropoiesis. This hormone is mainly synthesized by the kidneys, liver cells and in the bone marrow.[11] Hematological variations such as anemia or cytopenia are often due to intoxication of the bone marrow.[12] However, no anemia was observed after subacute treatment of male and female rats with *S. alata* extract. The aqueous extract of the leaves of *Senna alata* would not cause lysis of blood cells. These results are comparable to the results of some authors.[13,14] The liver and kidneys play a major role in metabolic processes. The liver detoxifies the body of its toxic substances. The kidneys help the body to maintain homeostasis by reabsorbing vital substances and excreting waste.[15] The lack of significant variation in the levels of ALAT, ASAT, creatinine, TB, and ALP are indicators of healthy liver and kidney function.[9] This suggests that the aqueous extract of *S. alata* did not damage rat hepatocytes and glomeruli.[13,16] The cholesterol and triglyceride levels were not changed by the high-dose extract. The tannins found in the leaves of *Senna alata* protect against high cholesterol and variations in blood count. The almost normal architecture of the organs does not suggest morphological disturbances. However, slight modifications of the glomeruli should be noted. These are the tubular clarifications and the proliferation of mesangial cells.

CONCLUSION

The results of the toxicity tests show that the aqueous extract of the leaves of *Senna alata* is practically non-toxic in acute treatment. After 28 days of treatment at a dose of 2000 mg/kg/bw, it induces steatosis and slight bloating of the hepatocytes without modifying the biochemical parameters in both sexes. *Senna alata* has a protective effect on hepatocytes and improves the architecture of the liver. Its prolonged use deserves vigilance. These results justify its use in traditional medicine in Burkina Faso in the treatment of many diseases.

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