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# Acute and Subchronic Toxicity Assessments of Hydro Alcoholic Extract of Roots of *Anogeissus leiocarpus* (Combretaceae)

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**ABSTRACT**

Genus of the family of Combretaceae, *Anogeissus leiocarpus* is a tropical plant widely used to treat many diseases in traditional medicines. This study was designed to assess the acute and subchronic toxicity of the hydroalcoholic extract of roots of *Anogeissus leiocarpus*. In the acute test, the limit test dose of 5000 mg.kg$^{-1}$ was administered orally to three Sprague Dawley female rats and then observed individually one hour post-dosing, and at least twice daily for 14 days. Subchronic toxicity was conducted by daily oral administration at doses of 500 and 1000 mg.kg$^{-1}$ to both sexes of rats during 28 days. At the end of experimentation, general clinical signs, mortality, haematological, biochemical and histopathological assessments were carried out. The limit dose of 5000 mg.kg$^{-1}$did not cause any mortality in the rats tested during the observation period. This suggests that the median Lethal Dose 50 (LD50) is higher than 5000 mg.kg$^{-1}$ by oral administration in rats. In subchronic toxicity study, no abnormalities in body weight, food consumption, clinical signs, serum biochemistry, electrolytes, hematology, organ weight, and histopathological examination were revealed in both sexes of rats treated with roots of *Anogeissus leiocarpus* at the doses of 500 and 1000 mg.kg$^{-1}$. Based on these results, we concluded that roots of *Anogeissus leiocarpus* are safe to be used as a medicinal plant.

## INTRODUCTION

In developing countries, over eighty percent of the population use plants for primary health care due to the lack of health facilities; linked to socio-economic and socio-cultural factors[1]. Composed by multitude of secondary metabolites, plants represent and serve as track in drug discovery. Apart from the beneficial aspect of the plants, its possible adverse side might not be also neglected. Plants actually contain several active ingredients whose mechanisms of action are little or poorly known, which increases the risk of toxicity. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used clinically or preclinically, is a crucial part of its assessment of potential toxic effects[2].

*Anogeissus leiocarpus* (Combretaceae) is one of the tropical plants widely used to treat many diseases in traditional medicine. The plant has been shown to have various pharmacological actions as antimicrobial, antiproliferative, antioxidant and hepatoprotective activities[3,4,5,6]. Our previous study revealed the antihyperglycemic property of the roots of *A. leiocarpus*[7]. Phytochemically, *A. leiocarpus* contains many secondary metabolites including flavonoids, tannins, phenolic acids, and triterpenes[8].

Currently, no scientific evidence displaying the toxicological aspect of roots of the plant is available. Hence, for the safe use of this plant, the aim of this study was to assess potential toxicity as oral acute and subchronic toxicity of the roots of *Anogeissus leiocarpus*.

## MATERIAL

### Plant material

Roots of *Anogeissus leiocarpus* were collected from Tsevie, Zio (TOGO) in July 2018. A voucher specimen was identified and was deposited in the herbarium of Laboratory of Botany and Plant Ecology under the number TOGO 15483.

### Animals

Female and male Sprague Dawley rats (130 ±150 g) were housed in standard environmental conditions (temperature 24–25 °C, relative humidity and a 12t/12 h light-dark cycle) and fed with standard rat diet and water *ad libitum*. The animals were deprived of both food and water before fasting. Principles of laboratory animal care as described in institutional guidelines and ethics of Laboratory of Physiology/Pharmacology of University of Lome-Togo (ref: 001/2012/ CB-FDS-UL) were followed.

## METHODS

### Hydroalcoholic extraction

About 400g of roots of *Anogeissus leiocarpus* were extracted in water/ethanol (5:5) for 72 hours. The crude extract was filtered on Whatman paper and evaporated in vacuum at 45°C using a rotary evaporator (Buchi R120). The yield of the dry extract was 5.68 % and was stored at 4°C [7].

### Acute oral toxicity test

This test was performed with the limit test procedure according to OECD test guidelines, 420[9]. Hydroalcoholic extract of roots of *A. leiocarpus* at the dose of 5000 mg.kg $^{-1}$ was administrated orally to three (3) female rats. All rats were then observed for mortality, signs of gross toxicity or behavioral changes (excitability, convulsions, lethargy, and sleep) at least once daily for 14 days.

### Subchronic oral toxicity test

Three groups of 10 rats of the 2 sexes were treated daily during 28 days, according to OECD test guidelines, 408 [10]. Group 1 as control received distilled water. Groups 2 and 3 received respectively 500 mg.kg$^{-1}$ and 1000 mg.kg$^{-1}$ of total extract.

Animals were observed at least twice daily for morbidity and mortality. During the experiment, body weight and food intake were monitored every day. Blood glucose level was measured at vein tail of the rats by glucometer Accu- Check Active (Germany) at J0, J7, J14, J21 and J28 after 12 hours of fasting. At the term of the treatment (28 days), all animals were fasted and anesthetized. Blood was collected via retro-orbital sinus. Rats were euthanized and internal organs as liver, kidney, spleen, lungs, heart, brain, testis and ovaries were removed, weighted and examined macroscopically. The relative organ weight was calculated.

### Blood glucose level

Blood glucose level was measured at vein tail of the rats by glucometer Accu- Check Active (Germany) at J0, J7, J14, J21, and J28 after 12 hours of fasting.

**Hematological parameters**

This analysis was performed using an automatic hematology analyzer (AJ-2400 Auto). Blood was collected in EDTA tubes for the estimation of Red Blood Cells (RBC), White Blood Cells (WBC), Platelets (Plt), Hemoglobin (Hb), Hematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC).

**Biochemical parameters and dosage of electrolytes**

Blood samples collected in anticoagulant-free tubes were centrifuged at 3000 rpm for 10 min. The obtained serum was analyzed for Triglyceride (TG), Total Cholesterol (T-Chol), lipoproteins (HDL, LDL, VLDL), Creatinine, Urea, Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Creatine Kinase (CK) using an automatic analyzer (Rayto Chemray-120).

The electrolytes including natrium ($Na^+$), chloride ion ($Cl^-$) and potassium ion ($K^+$) were estimated using an electrolyte analyzer (ST-200 PRO).

**Relative organ weight**

The Relative Organ Weight (ROW) of the rats was calculated as follows:

ROW = Organ weight / Bodyweight of rat on sacrifice day

**Histological assessments**

Principal vital organs (liver, kidney, and spleen) were fixed solution of buffered formalin 10% and examined microscopically[11].

**Statistical analysis**

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by analysis of variance (ANOVA) followed by Turkey's test to evaluate significant differences between groups. The level of significance was set at $p < 0.05$ and statistical analysis were carried out using Graph Pad Prism 7.0.

## RESULTS

### Acute oral toxicity study of total extract of roots of *A. leiocarpus*

No signs of toxicity: mortality, behavioral changes, were observed in female and male rats up to 5000 mg.kg$^{-1}$ bodyweights. At the end of the experiment, no signs of gross pathology were observed in the organs. According to the OECD limit test[8], the 50% lethal dose of the extract is higher than 5000 mg .kg$^{-1}$ by oral administration in rats.

### Subchronic oral toxicity test of total extract of *A. leiocarpus*

### Effect on Bodyweight

The body weigth of all animals did not reveal any change as compared to controls (Figures 1.A and 1.B). Food and water intake were also found to be normal and similar between control and treated groups (data not showed).

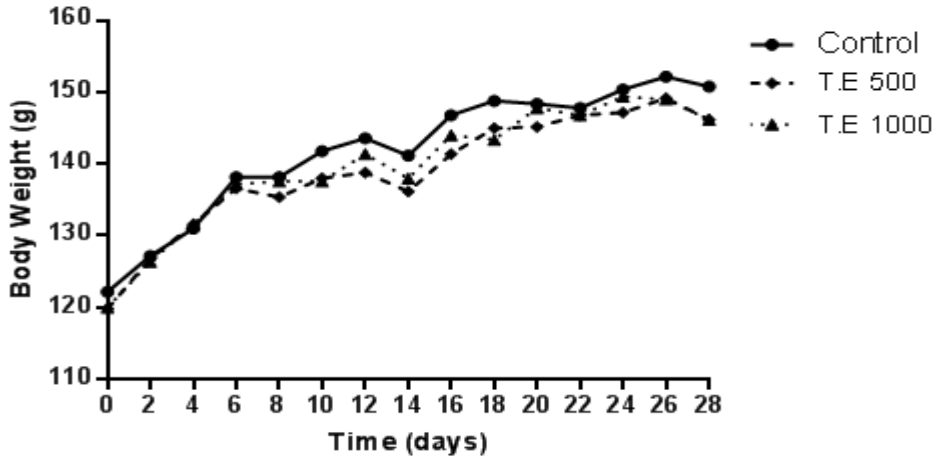


**Figure No. 1A: Effect of the total extract on body weight of female rats**

Bodyweight was measured each day before the treatment. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 mg.kg$^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5. Results were expressed as the mean±ESM.

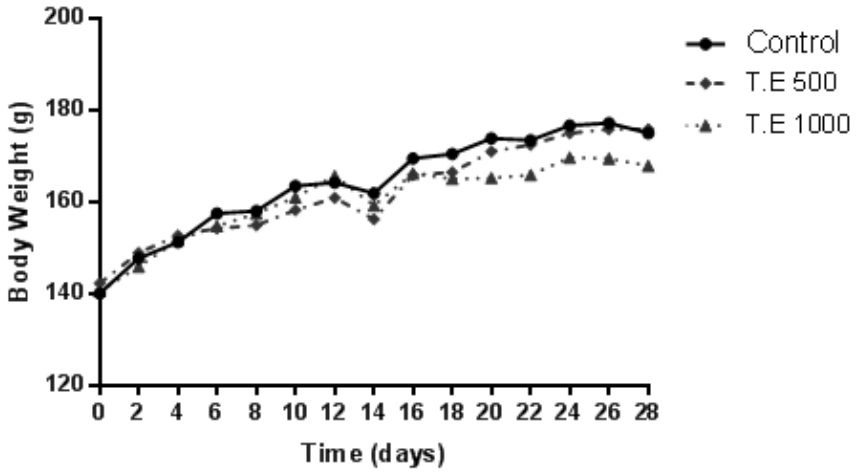


**Figure No. 1B: Effect of the total extract on body weight of male rats**

Bodyweight was taking each day before the treatment. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5. Results were expressed as the mean±ESM.

**Effect on basal blood glucose level**

Compared to the control group, no significant variation of the basal blood glucose level was revealed in rats of both sexes treated with the extract (Table No**.** 1.A and No**.** 1.B).

**Table No. 1.A: Effect of the total extract on basal blood glucose level of female rats**

| Glycaemia ($mg.dL^{-1}$) | | | |
|---|---|---|---|
| Days | Controls | T.E 500 | T.E 1000 |
| 0 | 80.0 ± 2.38 | 76.40 ± 3.74 | 78.40 ± 1.46 |
| 7 | 79.80 ± 3.96 | 77.80 ± 2.01 | 75.00 ± 5.31 |
| 14 | 80.00 ± 1.14 | 75.80 ± 2.65 | 78.80 ± 1.65 |
| 21 | 81.60 ± 2.37 | 80.60 ± 2.76 | 79.40 ± 3.32 |
| 28 | 82.40 ± 3.17 | 82.20 ± 2.67 | 81.40 ± 2.61 |

Glycaemia was measured every $7^{th}$ day on 14 hours -fasted animals. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5. Results were expressed as the mean±ESM.

**Table No. 1.B: Effect of the total extract on basal glucose level of male rats**

| Glycaemia ($mg.dL^{-1}$) | | | |
|---|---|---|---|
| Days | Controls | T.E 500 | T.E 1000 |
| 0 | 79.40 ± 3.28 | 81.60 ± 2.42 | 75.60 ± 3.66 |
| 7 | 78.60 ± 2.89 | 75.40 ± 2.11 | 76.20 ± 2.47 |
| 14 | 82.20 ± 4.07 | 78.00 ± 3.53 | 76.40 ± 1.28 |
| 21 | 84.00 ± 2.02 | 78.60 ± 3.90 | 81.80 ± 2.57 |
| 28 | 86.00 ± 2.28 | 84.20 ± 4.21 | 83.40 ± 2.22 |

Glycaemia was measured every $7^{th}$ day on 14 hours - fasted animals. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5. Results were expressed as the mean±ESM.

**Effects on biochemical and hematological parameters**

The hematological analysis revealed no significant change of the different parameters in control and extract- treated rats of both sexes (Table No. 2). Likewise, there was no significant change observed in the biochemical parameters nor the dosage of electrolytes between the control and extract treated groups (Table No. 3).

**Table No. 2: Effect of the total extract on hematological parameters**

| Parameters | Controls | T.E500 | T.E1000 |
|---|---|---|---|
| Female | | | |
| WBC($10^3$/µL) | 1.88± 0.26 | 2.12± 0.22 | 2.90 ± 1.04 |
| RBC($10^6$/µL) | 6.23± 0.03 | 6.65± 0.13 | 6.10± 0.08 |
| Plt($10^6$/µL) | 826.6 ± 25.6 | 794.8 ± 53.7 | 850.4 ± 42.0 |
| MCV(fl) | 53.22± 0.75 | 53.84± 0.78 | 53.36± 0.08 |
| MCHC (g/dL) | 19.98± 0.16 | 20.36 ± 0.24 | 20.56± 0.16 |
| Ht(%) | 33.18± 0.43 | 35.86± 1.12 | 32.18± 0.64 |
| Hb(g/dL) | 12.480± 0.11 | 13.32± 0.22 | 12.62± 0.17 |
| Male | | | |
| WBC($10^3$/µL) | 1.92± 0.21 | 2.21± 0.25 | 2.38± 0.13 |
| RBC($10^6$/µL) | 6.64± 0.11 | 6.78± 0.06 | 6.67± 0.05 |
| Plt($10^6$/µL) | 724.0 ± 25.7 | 702.4 ± 16.8 | 746.0± 24.3 |
| MCV(fl) | 53.02± 1.01 | 53.02± 0.96 | 52.50± 0.5 |
| MCHC (g/dL) | 19.70± 0.33 | 20.26± 0.21 | 20.18± 0.05 |
| Ht(%) | 36.16± 0.36 | 35.82± 0.85 | 34.94± 0.65 |
| Hb(g/dL) | 13.44± 0.33 | 13.76± 0.24 | 13.52± 0.12 |

Total blood was collected on 14 hours -fasted animals. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 mg.kg$^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5 per sex. Results were expressed as the mean±ESM.

**Table No. 3: Effect of the total extract on serum biochemical parameters**

| Parameters | Controls | T.E500 | T.E1000 |
|---|---|---|---|
| Female | | | |
| GGT (UI/L) | 9.27±0.49 | 12.55±0.63 | 11.71±0.6 |
| ASAT (UI/L) | 203.0±19.1 | 134.5±8.3 | 160.3±5.0 |
| ALAT (UI/L) | 101.0±20.0 | 88.40±8.56 | 87.61±8.25 |
| Urea (g/l) | 0.59±0.04 | 0.57±0.05 | 0.56±0.02 |
| Creat(mg/l) | 7.71±0.29 | 7.58±0.33 | 7.98±0.19 |
| TG (g/l) | 0.74±0.11 | 0.76±0.13 | 0.75±0.08 |
| T-Chol(g/l) | 0.75±0.06 | 0.81±0.04 | 0.72±0.09 |
| HDL (g/l) | 0.39±0.02 | 0.48±0.01 | 0.49±0.03 |
| LDL(g/l) | 0.29±0.03 | 0.17±0.05 | 0.18±0.08 |
| CK (UI/L) | 1948.6±199.7 | 1789.2±170.1 | 2074.0±144.7 |
| $K^+$(mmol/l) | 5.67±0.22 | 5.60±0.10 | 5.65±0.11 |
| $Na^+$(mmol/l) | 149.76± 2.31 | 151.73± 3.86 | 145.50± 0.32 |
| $Cl^-$ (mmol/l) | 107.16±3.79 | 106.16± 2.76 | 101.53 ±0.23 |
| Male | | | |
| GGT (UI/L) | 9.36±0.67 | 9.70±0.93 | 9.36±0,89 |
| ASAT (UI/L) | 184.1±18 | 159.19±20.24 | 152.20±8,82 |
| ALAT (UI/L) | 83.59±9.37 | 80.36±8.25 | 94.56±2.72 |
| Urea (g/l) | 0.44±0.05 | 0.44±0.05 | 0.49±0.05 |
| Creat(mg/l) | 6.84±0.28 | 7.22±0.25 | 7.74±0.09 |
| TG (g/l) | 0.56±0.07 | 0.55±0.07 | 0.48±0.07 |
| T-Chol (g/l) | 0.80±0.09 | 0.84±0.04 | 0.78±0.09 |
| HDL(g/l) | 0.49±0.02 | 0.57±0.02 | 0.51±0.03 |
| LDL(g/l) | 0.22±0.08 | 0.16±0.05 | 0.23±0.07 |
| CK (UI/L) | 2031.8±108.5 | 1966.4±117.0 | 1947.2±109 |
| $K^+$(mmol/l) | 5.88±0.03 | 5.61±0.07 | 5.44±0.10 |
| $Na^+$ (mmol/l) | 147.30± 1.33 | 147.83± 0.91 | 146.30± 1.22 |
| $Cl^-$ (mmol/l) | 105.26±3.79 | 102.90± 2.76 | 103.33 ±0.68 |

Serum was obtained on total blood of 14 hours -fasted animals. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 mg.kg$^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5 per sex. Results were expressed as the mean±ESM.

**Effect of the total extract on relative organ weights**

No significant difference was observed in relative organ weights of both sexes compared to the control (Table No**.** 4). The macroscopic examinations of the organs did not show any change compared with control group.

**Table No. 4: Effects of the total extract on relative weight of organs**

| Relative organ weights (g) | | | |
|---|---|---|---|
| Organs | Controls | T.E 500 | T.E1000 |
| Female | | | |
| Liver | 3.39±0.07 | 3.42±0.12 | 3.47±0.11 |
| Kidney | 0.57±0.02 | 0.62±0.02 | 0.63±0.03 |
| Heart | 0.38±0.02 | 0.44±0.02 | 0.38±0.01 |
| Lungs | 0.73±0.05 | 0.76±0.05 | 0.75±0.05 |
| Spleen | 0.39±0.05 | 0.30±0.01 | 0.40±0.02 |
| Brain | 0.90±0.03 | 1.02±0.02 | 0.98±0.03 |
| Ovaries | 0.07±0.01 | 0.09±0.01 | 0.09± 0.01 |
| Male | | | |
| Liver | 3.30±0.08 | 3.05±0.15 | 3.26±0.08 |
| Kidney | 0.66±0.04 | 0.55±0.05 | 0.62±0.03 |
| Heart | 0.41±0.02 | 0.39±0.03 | 0.34±0.01 |
| Lungs | 0.74±0.07 | 0.84±0.15 | 0.61±0.01 |
| Spleen | 0.38±0.05 | 0.35±0.03 | 0.39±0.03 |
| Brain | 0.93±0.14 | 0.96±0.06 | 0.84±0.04 |
| Testis | 1.29±0.13 | 1.21±0.06 | 1.20±0.03 |

Organs were removed and weighted on the last day of the test. Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus.* N=5 per sex. Results were expressed as the mean±ESM.

**Histological examination**

The liver of the three groups presented a normal morphological appearance, with hepatocyte spans forming lobules delimiting door spaces. There was an absence of hepatocyte necrosis, inflammatory process and neoplastic tumor proliferation (Figures No. 2.A and No. 3.A).

The histological aspect of the spleen had showed the lymphoid follicles formed by lymphocytes of normal architecture in the different groups. There was an absence of sinusoidal congestion and an absence of tumor process (Figure No. 2.B and Figure No. 3.B).

The histological section showed glomeruli and renal tubes of normal structure and morphology. The histological appearance of the kidneys was normal in the control and treated groups (Figure No. 2.C and Figure No. 3.C).

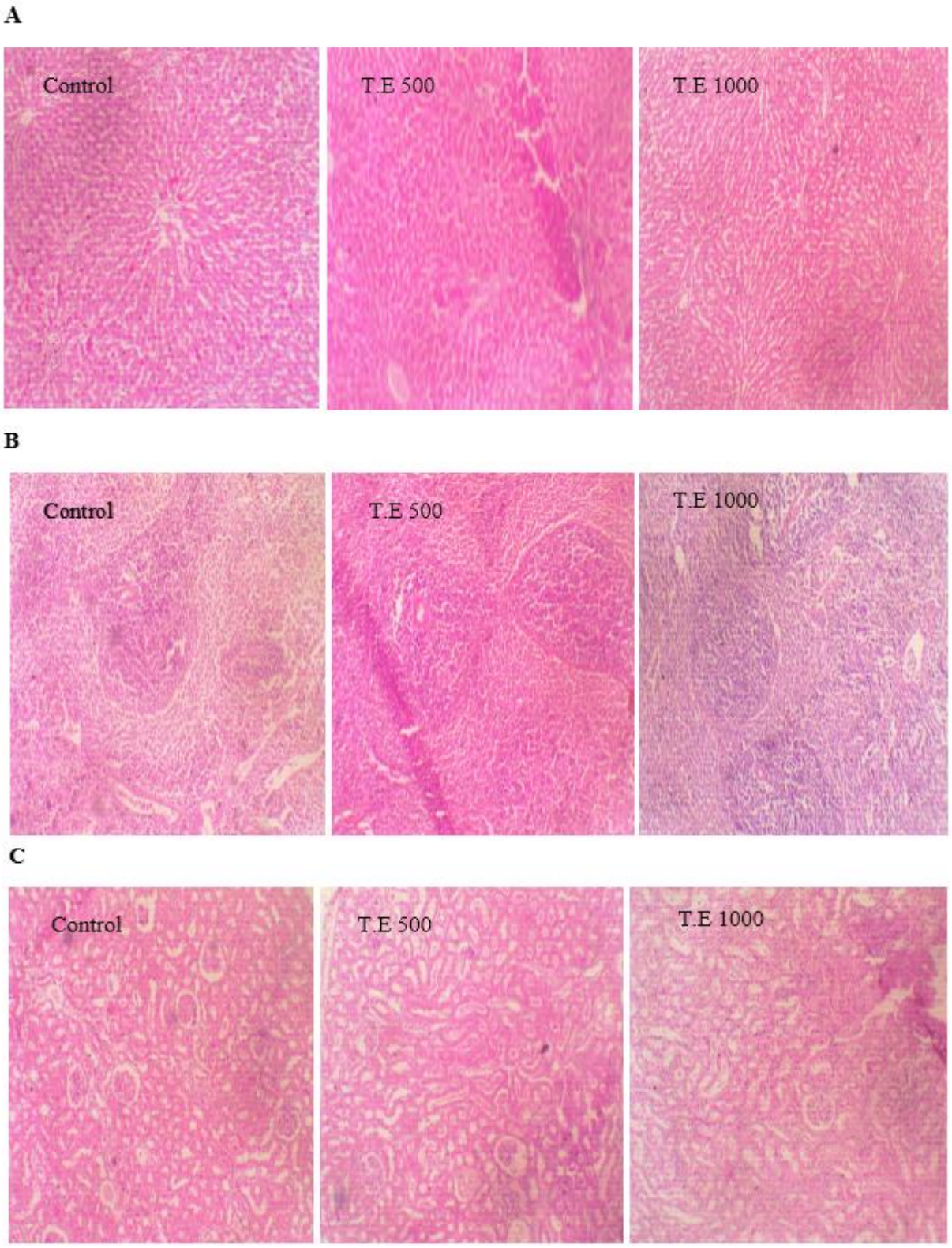


**Figure No. 2: Histological study of liver (A), spleen (B) and kidney**(C) **of male rats**

Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus*. At the end of the treatment, liver, kidney,

and spleen were preserved in fixation medium of 10% solution of buffered formalin for histopathological study. (x100)

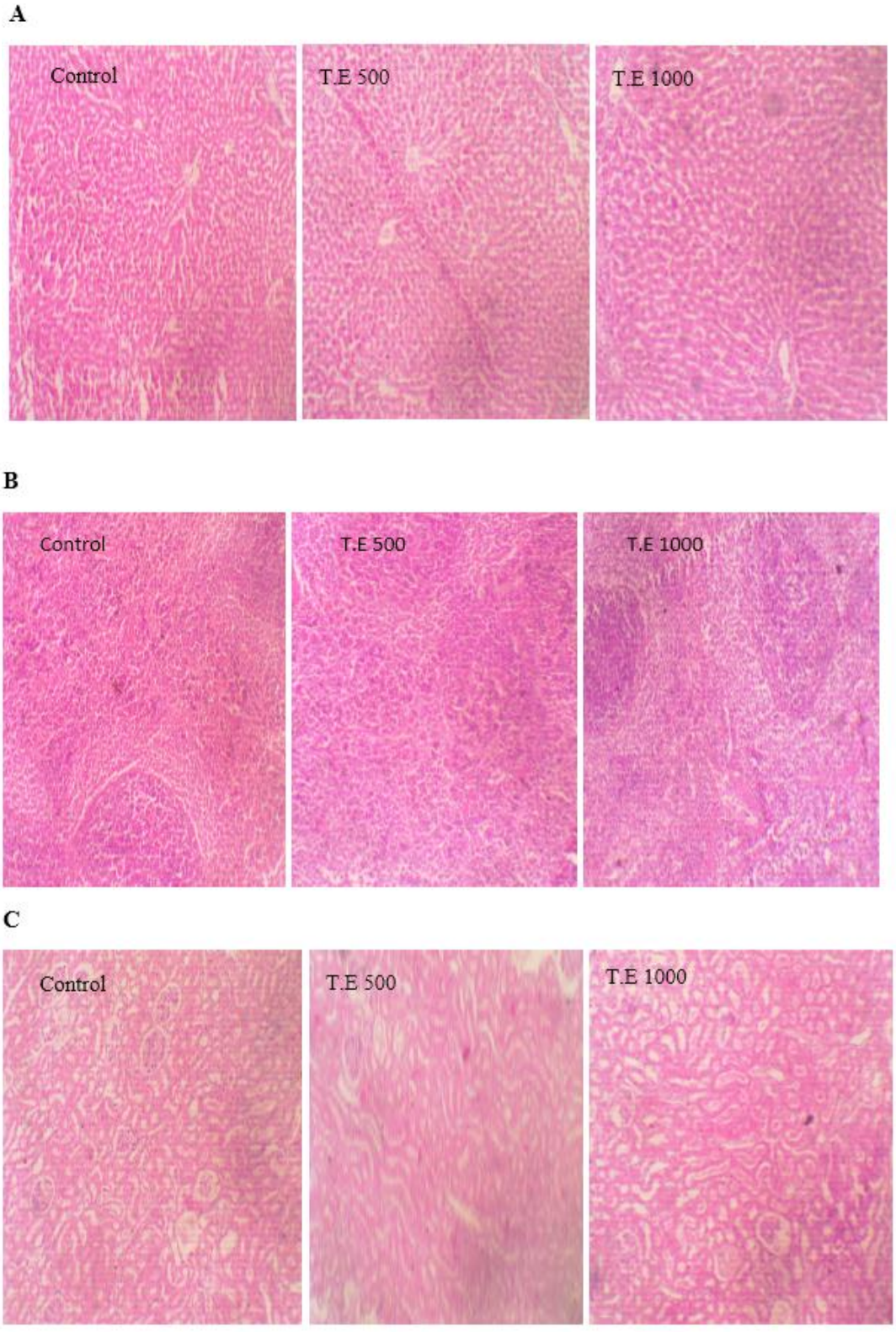


**Figure No. 3: Histological study of liver (A), spleen (B) and kidney** (C) **of female rats**

Control: received distilled water. T.E 500, T.E 1000: respectively treated with 500 and 1000 $mg.kg^{-1}$ of the total extract of roots of *A. leiocarpus*. At the end of the treatment, liver, kidney, and spleen were preserved in fixation medium of 10% solution of buffered formalin for histopathological study. (x100).

## DISCUSSION

Toxicity tests are generally conducted to assess the harmfulness or safety of a substance. In order to standardize toxicity tests at the international level, the Organization for Economic Cooperation and Development (OECD) has established guidelines to classify these tests. The first step is to look for the lethal dose that kills 50% of the animals (LD50) and which consists of a single administration (not exceeding 24 hours) of the dose to be ingested, thus allowing an initial classification of the substance from a toxicological point of view (acute toxicity). And among other measures, to assess the health hazard that may result from long-term exposure (up to 28 days for sub-acute studies) to this substance. This study provides information on dosage, target organ toxicity and identifies observable adverse effects that may affect the average lifespan of animals[10].

Much data have been reported on *A. leiocarpus* in relation to its pharmacological and Phytochemical activities[8,12], but to our knowledge, no data have been reported to date on the *in vivo* toxicity of the root of this plant.

The toxicological study of the total hydro-alcoholic root extract of *A. leiocarpus* was then undertaken through acute and subchronic toxicity tests in order to assess the safety of the plant.

In acute administration, at a dose of 5000 $mg.kg^{-1}$, rats showed no signs of toxicity in their behaviour and no mortality was recorded. This assumes an LD50 greater than 5000 $mg.kg^{-1}$. Repeated administration of the total extract for 28 days at doses of 500 and 1000 $mg.kg^{-1}$ showed no change in the behaviour of the animals, nor did it significantly influence the bodyweight of the animals, food and water consumption and basal blood glucose levels. The values recorded in treated rats in both sex were close to the control values. In toxicity, changes in body weight and food and water consumption are used as well-known indicators of the general condition of the animals. The same applies to the measurement of relative organ weights as well. The results of this study revealed no gross lesions and no significant changes in the relative weight of target organs (liver, kidney, lung, brain, spleen, ovary and testes) of

the treated versus control groups. Changes in blood parameters are considered to be one of the most reliable evidence for the toxicity studies[13]. Hematological parameters used to determine physiological and pathological status revealed no evidence of toxicity of the hematopoietic system. Evaluation of biochemical parameters was performed to identify possible alterations in renal, hepatic or cardiac functions affected by the extract[14]. No significant changes were observed in indicators of hepatic or renal damage (transaminases, Gamma-GT, urea, creatinine), nor in triglycerides and total cholesterol, thus suggested that the total hydroalcoholic extract of *A. leiocarpus* roots had no adverse effects on the liver and kidneys.

Electrolytes are minerals from the blood and other fluids that carry electrical charges. The most commonly used in measuring kidney function include sodium, potassium and chloride. Chloride and sodium are extracellular electrolytes while potassium is intracellular. The concentration of serum chloride increases in renal tubular acidosis and primary hyperparathyroidism and may decrease with the administration of drugs such as thiazides, diuretics and corticosteroids. Sodium serum was measured to assess the water and sodium balance. Its concentration increases in dehydration and decreases in Addison's disease and by diuretic therapy, ascites, kidney failure and excessive consumption of water. The serum potassium concentration is sensitive to changes in the acid-base state; the level is high in case of acidosis, dehydration and renal insufficiency, and increases in response to the administration of certain drugs, such as spironolactone[15,16]. No significant difference in serum electrolytes was observed in this study. The values found are close to those of Osseni *et al.* (2016)[17], Yuet Ping *et al.* (2013).[18] and El Kabbaoui *et al.* (2017).[19]. Contrary to our results, the study of Agaie *et al.* (2007), the aqueous crude extract of leaves of *Anogeissus leiocarpus* during 28 days showed some changes in the biochemical profile of rats[20]. This difference with our results could be explained by the diversity of chemotypes of this plant depending on the sampling site, the harvest season, the organs used and the method of extraction. All these observations concerning the non-toxic effect of the plant, listed above, were confirmed by the evaluation of the histopathological sections of the organs. The liver, spleen and kidneys presented a normal morphological appearance in the groups treated with the extract at doses of 500 and 1000 $mg.kg^{-1}$ compared to the control. Twenty eight days subchronic oral ingestion of the hydroalcoholic extract of *Anogeissus leiocarpus* roots shows the normal architecture of the histological structure, suggesting no detrimental changes and no toxic effect of the plant at the doses used.

## CONCLUSION

Our study has shown that the total hydroalcoholic extract of roots of *A. leiocarpus* is nontoxic in rats following either a single dose or daily repeated doses for 28 days. Further studies will be needed to determine the genotoxicity, mutagenesis and reproduction toxicity.

## CONFLICT OF INTERESTS

The authors have no conflict of interests.

## ACKNOWLEDGMENTS

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