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# Phytochemical and toxicity study of *Excoecaria grahamii* Stapf aqueous extract on female mice

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**Abstract**
*Excoecaria grahamii* Stapf is an *Euphorbiaceae* used in traditional medicine in Burkina Faso and many other parts of the world. Phytochemical compounds as total phenolics, flavonoids and alkaloids have been measured. Acute and subacute toxicity of the plant have been studied on NMRI female mice.
The results showed that the extract contained total polyphenols and flavonoids but not alkaloids. In the acute toxicity the $DL_{50}$ could be greater than 5000mg/kg bw. The extract had no significant difference on organs weight and biochemical parameters during 72h after single oral administration.
During the subacute treatment by oral administration no animal was dead. There was significant difference in body weight at 75mg/kg bw compared to the control mice that received distilled water. There was significant difference in food consumption. According to relative organ weights, water consumption and biochemical parameters, there was no significant difference. Histological study of important organs such as kidney, heart and spleen showed no damage when compared to the control. However, liver and lungs presented signs of damage.
Aqueous extract of *Excoecaria grahamii* has no toxic effect at the organ level in acute toxicity but regarding the subacute effect, the plant has a toxic effect on some organs such as liver and lungs.

**Keywords:** *excoecaria grahamii*, toxicity, phytochemical contents

## 1. Introduction
Many plants are used in traditional medicine through the world. According to the World Health Organization (WHO) about 80 % of the population around the world depends on traditional medicine, mostly herbal remedies, for their primary health care needs (WHO) [1]. The biological activities of these plants use in traditional medicine are often based on their secondary metabolites such as phenolics, alkaloids and terpenes.
*Excoecaria grahamii* Stapf (synonym *Sapium grahamii*) is a species of *Euphorbiaceae* family used in traditional medicine in Burkina Faso. All parts of *E. grahamii* are used in several therapeutic formulations (decoction, pounded, burnt leaves or roots) to treat a broad range of affections and diseases. Roots and leaves are used against skin diseases, ascites, leprosy, constipation, dropsy, rectal prolapse, dysentery and other gastro-intestinal diseases [2, 3]. The plant is also used as diuretic, drastic purgative, uterotonic and abortive, antiseptic. Roots in fumigation are hallucinogenic and used as arrow poison ingredient. The leaf dried powder is used as crop protection insecticide. Latex is used in skin diseases, filariasis, for ritual scarifications and tattooing [4].
Recently Traoré et *al.* [5] highlighted scientifically the anthelminthic effects of the plant showing that it could be used as a drug to combat some gastro-intestinal diseases. Furthermore the muscarinic activity of this plant on rabbit blood pressure has been demonstrated by Ouédraogo et *al.* [6].
Despite the wide use of the plant in traditional medicine, very few investigations have been published in the literature about its toxicological and phytochemical profile. Therefore, the purpose of this study was to determine: i) total phenolics, flavonoids and alkaloids ii) acute and subacute oral toxicity on female mice.

## 2. Materials and Methods
### Plant collection and extract preparation
The plant sample was collected from a natural habitat of Kombissiri locality, Bazèga province, in the South East region of Burkina Faso located at about 42 km from Ouagadougou (position 11° 55' 33.8'' North; 01°17'10'' East) in dry season and identified by Biodiversity Center Herbarium of the University Ouaga I Pr Joseph KI-ZERBO, where the voucher specimen (n° ID: 16703 sample n°: 6786) has been deposited.
Aqueous extract was prepared from the shade dried leaves of *E. grahamii*. Leaves powder (100 g) were macerated in 1 L of deionized water with agitation for 24 h at room temperature and then filtered through whatman n°2 and freeze-dried. This aqueous extract of the plant was stored at -4°C and used for the different tests [6].

### Animals
Naval Medical Research Institute (NMRI) adult female mice (25 to 32 g) were used in these experiments. Mice were fed with standard diet and were kept in our animal house at 22 ± 5 °C, 60 ± 10% humidity and submitted to a 12 h light/dark cycle with food and tap water *ad libitum*. All animals' procedures were strictly within respect for the ethics of scientific research, the treatments of laboratory animals' standards described in the "Guide for the Care and Use of Laboratory Animals" of the

National Academy of Sciences of the United States.

**Phytochemical study**

- ***Total phenolic compounds*:** Folin-Ciocalteu method was used [7]. The aqueous extract of the plant was dissolved in methanol to obtain 1 mg/mL. This methanolic solution (25 µL) was mixed with Folin-Ciocalteu Reagent (125 µL; 0.2 N). After 5min incubation at room temperature, 100 µL of $Na_2CO_3$ (75g/L) was added to the mixture. After one hour incubation, the absorbance was measured with the spectrophotometer at 760nm. A standard calibration curve was plotted using gallic acid. The experiments were carried out in triplicate and the results were expressed as mg of gallic acid equivalent/100 g of dry mass.
- ***Total flavonoids*:** Total flavonoids content was measured by an adapted method [8]. The plant aqueous extract was dissolved in methanol to obtain 1mg/mL. This solution (100 µL) was mixed with a solution of aluminium chloride ($AlCl_3$) in methanol (2%). The absorbance was read at 415 nm after 15 min incubation against blank sample (100 µL of methanol and 100 µL of extract, without $AlCl_3$). A standard calibration curve was plotted using quercetin and results were expressed as mg of quercetin equivalent (QE)/100 g of extract.
- ***Total alkaloids*:** The total alkaloids of extract were determined by Shamsa *et al*. [9] method. The plant materials (100g) were ground and then extracted with methanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45 °C to dryness. The plant extract was dissolved in 2 N HCl and then filtered. One mL of this solution was transferred to a separatory funnel and washed with 10 mL chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 mL of bromocresol green (BCG) solution and 5 mL of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. A standard calibration curve was plotted using atropine and the absorbance of the complex in chloroform was measured at 470 nm.

**Acute toxicity**

Forty-two (42) NMRI female mice were divided into seven (7) groups of six mice (female mice are more sensitive than male (OCDE, 2001)) and put on a diet for 24 hours [10, 5]. Extracts were administrated by oral route. The extract was administered at 600, 1000, 2000, 3000 and 5000 mg/kg body weight then the control group received distilled water. Animals once treated were observed during the hour which follows extract administration and then they were fed. They were observed again after 24 h, 48 h and 72 h. Animal's intoxication symptoms such as piloerection, changes in exploratory behavior were noted. Each animal was weighted before an extract administration and 72 h later. When the animal was then pithed, liver, lung, heart, kidney and spleen were carefully removed and weighted.

Before the animals were pithed, they were deprived of food for 15 h and anesthetized by ketamine (87 mg/mL) and xylazin (13 mg/mL, ip). Blood samples were then collected in anti-coagulating (EDTA) tubes by cardiac puncture for biochemical parameters measure [11]. Blood samples were centrifuged at 3000 rpm for 5 min to obtain plasma for biochemical analyses. Plasma was used to determine biochemical parameters values, performed with spectrophotometer RMS BCA 201.

**Subacute toxicity**

In this part, we present body and organs weight, food and water consumption, biochemical parameters analysis and histological studies.

***Body and organs weight***

Thirty-two (32) female NMRI mice were divided into four (4) groups of eight (8) mice. The first group of mice received distilled water and is considered as control. The second, third and fourth groups of mice received orally and daily doses of aqueous extract of *E. grahamii* respectively 10, 50, 75 mg/kg bw during 28 days. Body weight was taken before first administration and weekly (7 days) after the extract administrations began [10]. Animals were daily observed to detect abnormality signs during the period of study.

***Food and water consumption***

The amounts of food and water consumed were measured weekly and every three days respectively from the quantity of food and water supplied and the remaining.

***Biochemical parameter studies***

After 28 days of treatment, animals were deprived of food for 15h and then, blood samples and organs were collected and taken as described above [11].

***Histological studies***

Liver, lung, heart, kidney and spleen tissues were removed and fixed immediately with 10% neutral buffered formaldehyde solution (pH 7.0). The tissues were dehydrated in ascending grades of ethanol (70–100°), cleared in xylene and embedded in paraffin. Then, sections of 5µm thickness were cut and mounted on clean glass slides which had been smeared with a drop of Mayer's egg albumin. It was then dried on a hotplate at about 50°C for 30 min, stained with hematoxylin eosin, and examined under a light microscope and photographed [12].

**Statistical analysis**

The data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS for Windows version 20.0 (SPSS Inc. Chicago, IL, USA). A Student pair *t*-test was used to compare the mean differences between two groups and One-way analysis of variance (ANOVA) followed by Dunnett's *t*-test to compare mean of effect among different groups to that of control groups. P values of less than 0.05 ($p<0.05$) were considered as statistical significance. The relative organ weight was calculated for each animal as follows:

$$\text{Relative organ weight} = \frac{absolute\ organ\ weight\ of\ the\ organ}{Body\ weight\ of\ the\ animal}\ x\ 100.$$

**3. Results**

**Phytochemical compounds**

Total phenolics, flavonoids, and alkaloids were determined from respective standard calibration curves: phenolics (y =

0.005x + 0.0961; $R^2$ = 0.999), flavonoids (y = 16.819x + 0.0898; $R^2$ = 0.995) and alkaloids (y = 0.0008x + 0.057; $R^2$ = 0.994). Results are presented in table 1.

**Table 2:** Phytochemical compounds measurement

| Total phenolics (mg GAE/100 g dry sample) | Total flavonoids (mg QE/100 g dry sample) | Total alkaloids (mgAE/100 g dry sample) |
|---|---|---|
| 195.41 ± 34.34 | 5.59 ± 0.06 | 0 |

- mg GAE/100 g : mg Gallic Acid Equivalent per 100 g of dried extracts;
- mg QE/100 g : mg of Quercetin Equivalent per 100 g of dried extracts;
- mg AE/100 g : mg of Atropine Equivalent per 100 g of dried extracts.
- Values are means ± standard deviation (n = 3)

**Acute toxicity**

During the 72 hours which followed the administration of extracts no mouse was dead. An increase of the motricity activity was observed just some minutes later and one hour after the administration a decrease of motricity activity was observed. The plant extract was tolerated by the animals up to the highest dose of 5 g/kg, bw.

Results about relative organ weight are presented in table 2. These results showed significant decrease in relative organ weight for liver and kidney at dose of 3000 mg/kg, bw.

**Table 2:** Effect of plant aqueous extract on relative organ weight change on mice during 72 h.

| Doses (mg/kg, bw) | Spleen | Liver | Kidney | Lung | Heart |
|---|---|---|---|---|---|
| 0 | 0.43±0.02 | 5.25±0.16 | 1.28±0.02 | 0.62±0.04 | 0.49±0.02 |
| 200 | 0.34±0.03 | 5.04±0.10 | 1.21±0.06 | 0.56±0.03 | 0.47±0.01 |
| 600 | 0.4±0.04 | 4.66±0.30 | 1.13±0.12 | 0.58±0.06 | 0.44±0.02 |
| 1000 | 0.32±0.07 | 5.29±0.28 | 1.17±0.07 | 0.79±0.22 | 0.53±0.07 |
| 2000 | 0.41±0.02 | 5.06±0.26 | 1.23±0.04 | 0.64±0.03 | 0.46±0.02 |
| 3000 | 0.44±0.10 | 4.07±0.13* | 0.97±0.08* | 0.53±0.02 | 0.41±0.01 |
| 5000 | 0.45±0.04 | 5±0.19 | 1.17±0.03 | 0.6±0.04 | 0.44±0.01 |

Values are means ± standard deviation (n = 6) compared to control group at dose 0 mg/kg bw; ANOVA follow by Dunnett's *t*-test, *p<0.05.

Biochemical parameter measurement showed no significant changes for the different parameters ALT, AST and creatinine. Blood Urea nitrogen showed significant increase at doses 2000, 3000 and 5000 mg/kg. The results have shown in table 3.

**Table 3:** Biochemical parameter measurements

| Doses (mg/kg, bw) | Creatinine (µmol/L) | Urea nitrogen (mmol/L) | ALT (U/L) | AST (U/L) |
|---|---|---|---|---|
| 0 | 13.79±3.06 | 5.22±0.24 | 98.72±33.34 | 154.4±41.29 |
| 200 | 11.91±5.34 | 5.04±0.36 | 149.88±30.52 | 201.75±53.85 |
| 600 | 15.35±2.05 | 5.64±0.43 | 94.4±35.99 | 122.78±19.28 |
| 1000 | 28.98±5.41 | 6.05±0.44 | 176.6±29.00 | 132.5±14.04 |
| 2000 | 31.59±7.52 | 7.29±0.48* | 151.78±28.07 | 115.85±33.67 |
| 3000 | 17.94±5.07 | 9.47±0.67* | 110.52±17.01 | 142.13±54.6 |
| 5000 | 34.73±8.58 | 11.17±0.89* | 120.11±18.79 | 254.55±61.57 |

Values are means ± standard deviation (n = 6) compared to control group at dose 0 mg/kg bw; ANOVA follow by Dunnett's *t*-test, *p<0.05.

**Subacute toxicity**

***Body and organ weight***

The results of body weight were summarized in figure 1. In the treated groups, animal body weight decreased gradually from dose 10 to 75mg/kg body weight (bw). There was no significant difference for doses 10 and 50 mg/kg bw compared to control group (0 mg/kg bw) but significant difference was observed for dose 75 mg/kg bw from 14th day compared to control group. Only one group (0mg/kg bw) showed significant increase at the end of treatment period (28 days) compared to the first weight. In addition, we recorded that animals in treated groups had slightly food intake (figure 2). Water consumption had not changed significantly (figure 3).

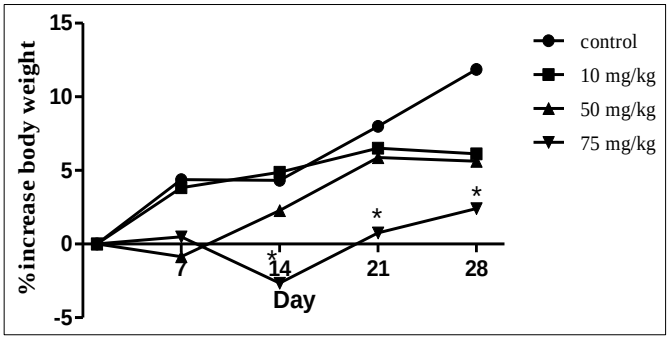


**Fig 1:** Animal body weights increase with treatment time during four weeks, *p<0.05.

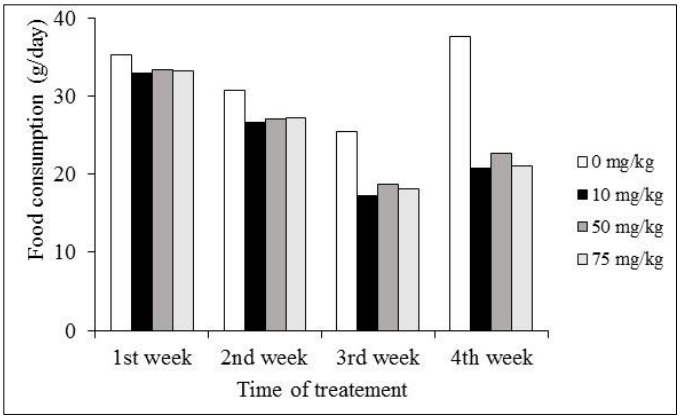


**Fig 2:** Mice food consumption. Control (0 mg/kg) received distilled water, treated mice received extract at different doses (10, 50, 75 mg/kg bw).

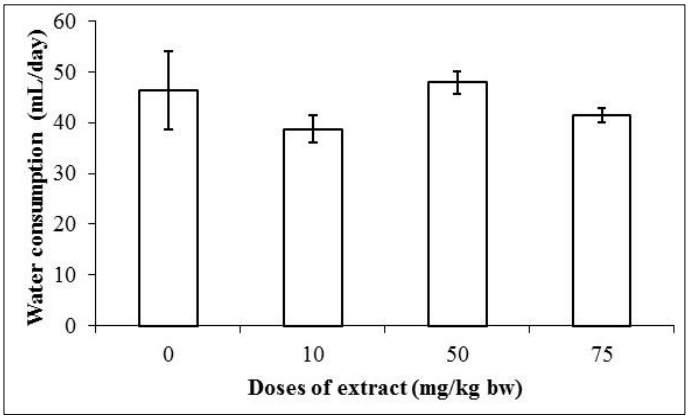


**Fig 3:** Mice water consumption. Control (0 mg/kg) received distilled water, treated mice received extract at different doses (10, 50, 75 mg/kg bw).

The organ weights were shown in table 4. In all cases, there was no significant difference of organ weights compared to control group.

**Table 4:** Effect of aqueous extract on organ weight change on female mice during four weeks

| Doses (mg/kg) | 0 | 10 | 50 | 75 |
|---|---|---|---|---|
| Liver | 4.39±0;08 | 4.68±0.14 | 4.04±0.21 | 4.75±0.25 |
| Spleen | 0.42±0.01 | 0.42±0.04 | 0.44±0.06 | 0.45±0.045 |
| Lung | 0.57±0.04 | 0.57±0.01 | 0.69±0.07 | 0.57±0.01 |
| Kidney | 1.05±0.02 | 1.1±0.01 | 1±0.01 | 1±0.03 |
| Heart | 0.48±0.03 | 0.54±0.02 | 0.49±0.02 | 0.53±0.03 |

Values are means ± standard deviation (n = 8) compared to the dose 0mg/kg bw for each organ; ANOVA follow by Dunnett's *t*-test.

***Biochemical parameter studies***

Biochemical parameters presented in table 5 indicate that there was no significant difference for AST, ALT, urea nitrogen, and plasma creatinine rates.

**Table 5:** Effect of plant aqueous extract on biochemical parameters during 28 days.

| Doses (mg/kg) | 0 | 10 | 50 | 75 |
|---|---|---|---|---|
| Urea nitrogen (mmol/L) | 11.01±0.90 | 13.17±1.11 | 12.87±0.65 | 10.86±0.77 |
| Creatinine (µmol/L) | 30.85±1.45 | 30.45±0.44 | 32.84±1.38 | 30.96±3.79 |
| ALT (U/L) | 96.08±7.51 | 94.5±18.36 | 87.9±11 | 106.93±22.33 |
| AST (U/L) | 115.9±23.47 | 165.46±28 | 251.5±45 | 198.6±5.80 |

***Histological studies***

Histological examination of organs showed that there was no damage of the different tissues such as kidney, heart and spleen. However, liver and lungs showed signs of damage. Liver examination indicated a mild inflammation at the dose 75mg/kg. Regarding lungs, severe inflammation was observed along with a hemorrhage.

Results are presented in figures 4, 5, 6, 7 and 8.

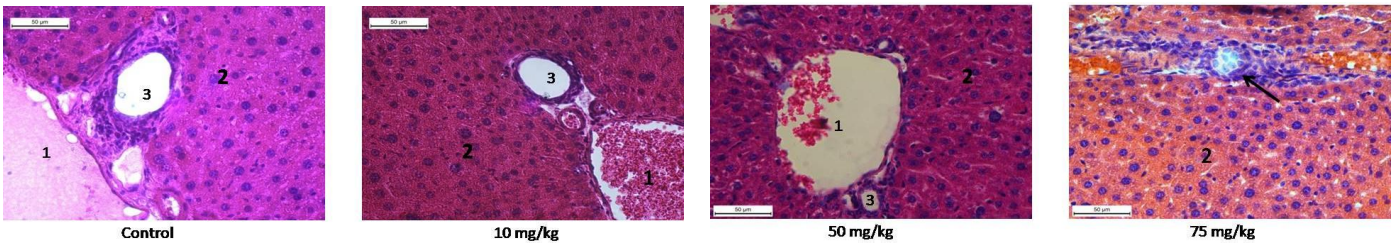


**Fig 4:** Liver histology of control mice and those exposed to *Excoecaria grahamii* aqueous extract various doses for 28 days. 1 (portal vein), 2 (hepatocytes), 3 (bile duct) the arrow shows slight inflammation. (HEx400)

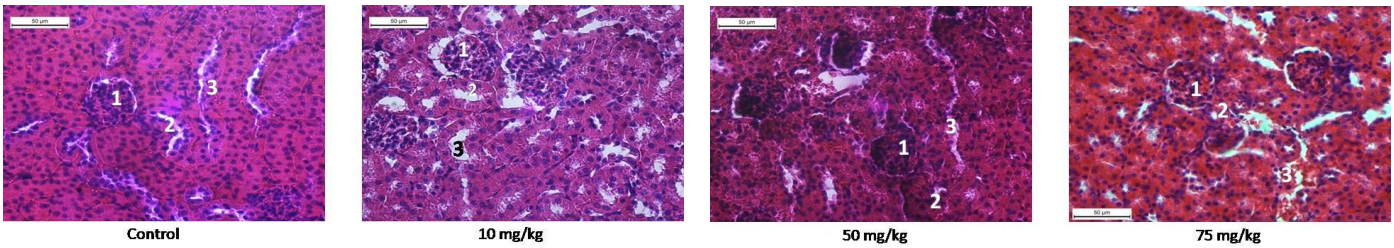


**Fig 5:** Kidney histology of control mice and those exposed to *Excoecaria grahamii* aqueous extract various doses for 28 days. 1 (glomerula), 2 (proximal tube), 3 (distal tube). HEx400

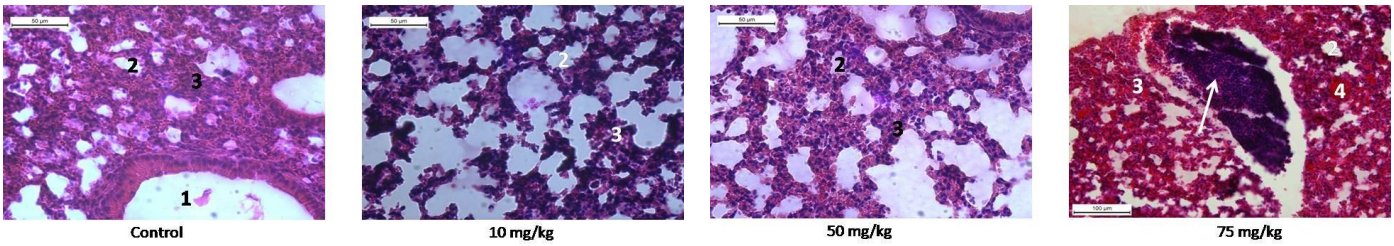


**Fig 6:** Lung histology of control mice and those exposed to *Excoecaria grahamii* aqueous extract various doses for 28 days. 1 (terminal bronchioles), 2 (alveolar cavity), 3 (alveolar cell), 4 (hemorrhage), the arrow showed inflammation site. (HEx400)

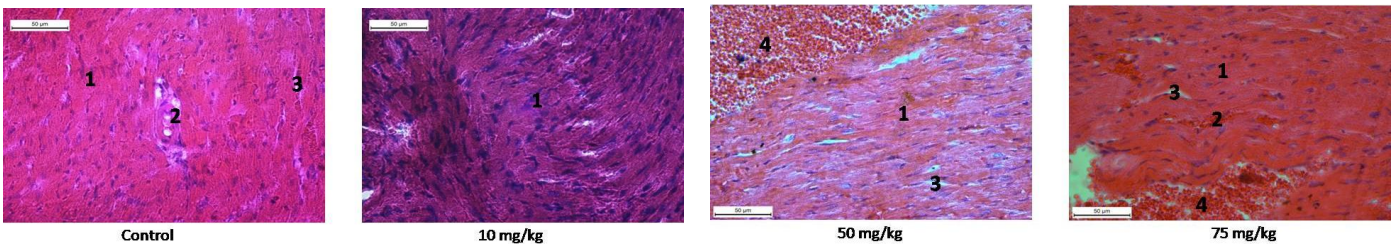


**Fig 7:** Heart histology of control mice and those exposed to *Excoecaria grahamii* aqueous extract various doses for 28 days. 1 (myocytes), 2 (coronary) 3 (interstitial space), 4 (cardiac cavity). (HEx400).

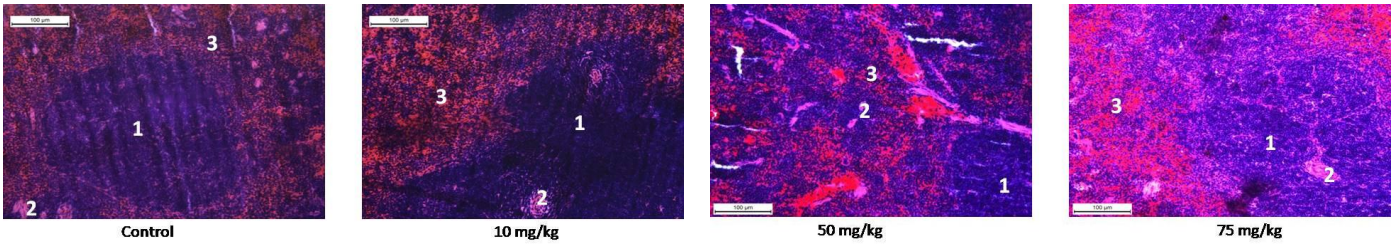


**Fig 8:** Spleen histology of control mice and those exposed to *Excoecaria grahamii* aqueous extract various doses for 28 days. 1 (splenic parenchyma), 2 (red pulp), 3 (splenic artery), (HEx400).

## 4. Discussion

*Excoecaria grahamii* aqueous extract showed important quantities of total phenolics and flavonoids compounds as many other *Euphorbiaceae*. These compounds have bioactive properties such as antioxidant, antihypertensive, anti-inflammatory, antibacterial and antitumor [13, 14, 15, 16, 17]. The presence of total phenolics and flavonoids strengthened the phytochemical composition highlighted by Nacoulma/Ouédraogo [2]. All these bioactive compounds could explain the several pharmacological effects of this plant used in traditional medicine as recalled in introduction. The absence of alkaloids in plant aqueous extract was sustained by Dragendorff reagent. Similar results were reported in some other *Euphorbiaceae* extract such as *Achornea cordifolia* [18] and *Excoecaria* genius plants [19].

Acute toxicity showed that aqueous extract did not cause mortality at 5000mg/kg bw, this suggested that the $LD_{50}$ of this plant by oral route was greater than 5000mg/kg bw. According to Hodge and Sterner [20] scale, this aqueous extract could be classified as slightly toxic. These results are in agreement with those of Traoré *et al*. [5]. During the time of treatment, ALT and AST level had not significantly changed. This result suggests that the extract could not have damage on liver structure. Indeed, ALT and AST are enzymes used in clinical biology to appreciate liver affection [21, 22]. ALT is hepatocytes cytoplasm liver specific enzyme but AST is present in many tissues such as muscle, myocardium, brain, kidney and liver. Increase of these enzymes in blood, especially ALT, is a sign of hepatotoxicity induced by hepatocellular membrane damage. Creatinine and urea nitrogen are used to appreciate kidney affection [11, 23] and the increase of urea nitrogen and creatinine, practically creatinine, is a sign of kidney damage. In our study, significant increase was observed in urea nitrogen level at 2000, 3000 and 5000 mg/kg bw but no significant change was

observed for creatinine. This result shows that extract could not have damage on the kidney structure. The relative organ weight change in the acute toxicity test is not dose dependent. A significant decrease in relative organ weight of the liver and kidneys observed at the dose 3000 mg/kg bw needs to be confirmed by extensive experiments.

The result obtained with subacute toxicity indicated that body weight decrease at the dose 75 mg/kg and suggested that the extract affected the body weight. Our result was similar to that obtained with the methanolic extract of *Pteleopsis hylodendron* stem bark [24]. Slight body weight gain is attested by a little food consumption in treated groups. The decrease in body weight gain indicates the extract possibility effects to decrease appetite [25]. In this study, biochemical parameters and body weight showed no significant difference. However liver and lung histology showed marks of inflammation. Inflammation is a feature of the injury on these organs. Lung injury often showed hypoxemia, alveolar-capillary barrier damage, and pulmonary inflammation, and often associated with multiple organ failure in later stage [26].

The present study did not establish the entire phytochemical profile of the plant. However, it has shown the presence of large quantities of polyphenols and flavonoids. The absence of alkaloids may be related to the assay method used. HPLC method could bring more precision. The toxicity study involved only a small number of parameters that deserve to be expanded. All the parameters that we studied allow us to say that in the acute toxicity the extract did not have toxic effect. But in the subacute toxicity the toxic effects were observed on the liver and the lungs. The rural populations who use this plant in the long term should therefore pay attention on its use in traditional medicine.

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