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Quantitative Screening and Study of the *in vivo* Subchronic Toxicity of Ethanolic Extract from the Stem Bark of *Canarium schweinfurthii* Engl. (Burseraceae) in Wistar Rats

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Abstract

Many plant species have their own toxicity, direct or indirect. On this point, the perfect knowledge of the metabolite constituents of the plants as well as the toxicity related to them is necessary for an adequate use in the formulation of the Improved Traditional Medicines and all other products related to the plants. Our study aims to carry out the quantitave screening and the study of the subchronic toxicity of ethanolic extract of the stem bark of *Canarium schweinfurthii*. Harvested in Bamendjou in the West Cameroon region, the stem bark of *C. schweinfurthii* was extracted with ethanol at 70^0. The estimation of total polyphenols, total flavonoids, tannins, saponins and alkaloids has been evaluated by different methods described in the literature. The subchronic toxicity assessment was performed over a 90-day period, with 4 batches of 10 rats (5 males and 5 female's albino Wistar rats) following OECD 408 guidelines. The determination of biochemical parameters, and hematological parameters was done in serum and histological sections on organ. Among the quantified compounds, saponins were the most abundant followed by polyphenols, alkaloids, then flavonoids and finally tannins. On repeated dosing for 90 days, the extract contributed to non-significant weight growth in rats at all dose levels in both male and female rats. Analysis of biochemical, hematological and histological parameters and histological sections did not show any serious signs of toxicity in the treated groups. Finally, the ethanolic extract of the stem bark of *C. schweinfurthii* even rich in secondary metabolites appears to conserve an acceptable safety for the formulation of improved traditional medicines.

Keywords: *Canarium schweinfurtii*, Burseraceae, Quantitative Screening, Subchronic Toxicity, Wistar rats, Medicinal plant.

INTRODUCTION

The preservation and regulation of traditional medicine is the subject of great debate among political authorities, health professionals and populations on issues relating to the safety, efficacy, quality and availability of herbal medicines. Medicinal species do not receive adequate attention in global discussions related to health and the potential toxicity of the majority is not well stablished or simply unknown [1]. Extremely toxic substances like strychnine, the digitoxines, cyanogenic glycosides, among others, are extracted from plants. Therefore, we can only assure that the use of particular specie is secure after a careful investigation [2]. For this purpose, the perfect knowledge of the metabolite constituents of plants as well as the toxicity related to them is necessary for adequate use in the formulation of Enhanced Traditional Medicines and all other plant-related products.To make our contribution to this vast program, we have chosen to conduct our dissertation work on *Canarium schweinfurthii* Engl.

The choice of this plant was motivated by the fact that a literature review revealed that it has several interesting pharmacological activities that have already been studied including anticancer, antihelminthic, antimalarial, nephroprotective, analgesic, antidiabetic, antibacterial, antioxidant [3]. Our recent work on the acute and subacute toxicity of stem bark has shown to have low toxicity [4]. However, subchronic toxicity has not yet been studied to our knowledge. In order to make our contribution to the enhancement of floristic biodiversity and reassure us that it presents an acceptable safety for users at doses long repeated, we proposed to study the subchronic toxicity of the ethanoic extract of the stem bark of *Canarium schweinfurthii*.

MATERIALS AND METHODS

Plant material

Plant material consists of the stem bark of *Canarium schweinfurthii* which was collected on November 24, 2020 from the village of Bamendjou in the department of Hauts-plateaux, West region of Cameroon (latitude: 5023'23" North; longitude 10019'48" East; altitude above sea level: 1604 m). The harvested plant was identified at the Cameroon National Herbarium in comparison to the specimen registered at number 40804/HNC.

Animal material

The experiments were carried out on 20 Male Wistar albino rats and 20 non-pregnant nulliparous females weighing an average of 130 ± 35 g. The rats were acclimatized for 2 weeks. The experimental animals were housed in standard plastic cages and provided access to food and water ad-libitum. The animals were fed a daily diet consisting of maize meal (43%), roasted soybean meal (26%), bone meal (1%), palm kernel cake (6%), wheat meal (10%), table salt (0.8%), table oil (0.1%), fish meal (13%), vitamins (0.1%) and drinking water.

METHODOLOGY

Preparation of extracts

The stem bark of *C. schweinfurthii* was cut, dried, and then ground into fine fibers. 3500 g of the fine powder were macerated with 7 000 ml of ethanol 70° for 72 hours at room temperature (25°C), stirred using a magnetic stirrer. Then the mixture was filter three times on cotton and Wattman 3 mm filter paper, then the filtrate evaporated at reduced pressure at 40°C using a rotary evaporator. The crude extract obtained was used to carry out the various tests.

Quantitative phytochemical screening

The determination of the bioactive compounds in our sample was made through estimates of the contents of total polyphenols, flavonoids, tannins, saponins, and alkaloids as described by the above protocols.

❖ **Total polyphenols**

The total polyphenol content of the sample of interest was determined by the method of Singleton and Rossi (1965) using the reagent of Folin–Ciocalteu [5]. Briefly, an aliquot of 0.1 ml of the extract (4 mg/ml) was added to 0.75 ml of Folin-ciocalteu reagent (diluted 10 times). The entire mixture was incubated at room temperature (25 ± 1°C). After 5 min, 0.75 ml of sodium carbonate solution (Na_2CO_3, 6%) was added. The mixture was homogenized and incubated at room temperature (in the dark) for 90 min and the absorbance was read at the length of 725 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu-Japan) against a reactive white. A gallic acid standard (0-1000 µg/ml) was used. The total polyphenol content of the extract was calculated using a calibration curve (r^2 = 0.97) and expressed as micrograms of catechin equivalent per gram of dry matter (µg GaE/g DM).

❖ **Total flavonoids**

The estimation of the flavonoid content in the extract was performed by the method of Aiyegoro and Okoh (2010) [6]. An aliquot of 0.5 ml of the extract (4 mg/ml) was added to 1.5 ml of methanol solution. Then, 0.1 ml of aluminum chloride solution ($AlCl_3$, 10%), 0.1 ml of potassium acetate (CH_3COOK, 1M) and 2.8 ml of distilled water were added. The whole mixture was well homogenized and incubated for 30 min at room temperature (25 ± 1°C) and the absorbance was read at 415 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu Japan) against the reactive white. Quercetin at different concentrations (0-1000 µg/ml) was used as standard. The flavonoid content was calculated using a standard curve (r^2 = 0.99) and expressed in micrograms of quercetin equivalent per gram of dry matter (µg QE/g DM).

❖ **Tannins**

The quantification of tannins in the extract is carried out by the method of Sun *et al*., (1998) [7]. A volume of 0.3 ml of chloridric acid (HCl, 1N) is added to 0.6 ml of vanillin dissolved in ethanol (4% v/v) and 0.1 ml of extract (4 mg/ml). The solution is homogenized and incubated at room temperature (25 ± 1°C) for 15 min. The absorbance is read at the wavelength of 500 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu Japan) against white. Tannic acid is used as a standard at different concentrations (0-1000 µg/ml) to establish the calibration range. The results are expressed in micrograms of tannic acid equivalent per gram of extract (µg TAE/g DM).

❖ **Total saponin content**

The determination of saponins in plant extract is carried out by the method described by Hiai *et al*., (1976) [8]. 200 µl of extract were introduced into a test tube; 200 µl of vanillin, prepared by dissolving vanillin in ethanol (80%), then 2000 µl of a solution of sulfuric acid (72%) were added. The mixture was homogenized

and placed in a water bath at 60°C for 10 minutes. The absorbance of this prepared solution was read after incubation at a wavelength of 535 nm relative to the blank. Saponins have been used as standard at different concentrations (0-1 mg/ml) to establish the calibration range. The results are expressed in milligrams of saponin equivalent per gram of dry matter of the sample (mg SE/g DM). A total of three repetitions are performed for each.

❖ **Alkaloids**

The determination of alkaloids in the extract was carried out by the method described by Diouf *et al*., (2014) [9] with some modifications. 100 mg of extract powder are extracted in 10 ml of 80% ethanol, then filtered and centrifuged at 5000 rpm (Rotofix 32 A, Hettich Zentrifugen, Germany) for 10 min. In the supernatant obtained, 1 ml was taken from which is added 1 ml of $FeCl_3$ (0.025 M) + HCl (0.5 M) and 1 ml of 1.10 phenanthroline (0.05 M) in ethanol. The mixture obtained was incubated in a water bath for 30 minutes with the temperature maintained at 70 ± 2°C. The absorbance of the red coloration of the formed complex was read at the wavelength of 510 nm (UVmini-1240, UV-Vis Spectrophotometer, Shimadzu-Japan) against white. Quinine has been used as a standard at different concentrations (0-1000 µg/ml) to establish the calibration range. Results are expressed in micrograms of quinine equivalent per gram of dry matter (µg QiE/g DM).

Subchronic toxicity

Subchronic toxicity was assessed according to OECD Test Guideline 408 [10]. Male and females Wistar albino rats were divided into four experimental batches of ten animals each:

- Batch 1: control received distilled water (DW) at a dose of 1 ml/100 g body weight;
- Batch 2: ethanolic extract of the stem bark of *C. schweinfurthii* at a dose of 100 mg/kg body weight;
- Batch 3: ethanolic extract from the stem bark of *C. schweinfurthii* at a dose of 200 mg/kg body weight;
- Batch 4: ethanolic extract from the stem bark of *C. schweinfurthii* at a dose of 400 mg/kg body weight.

The rats were force-fed daily for 90 days and external signs of toxicity were noted. Body mass was reported weekly during the study period.

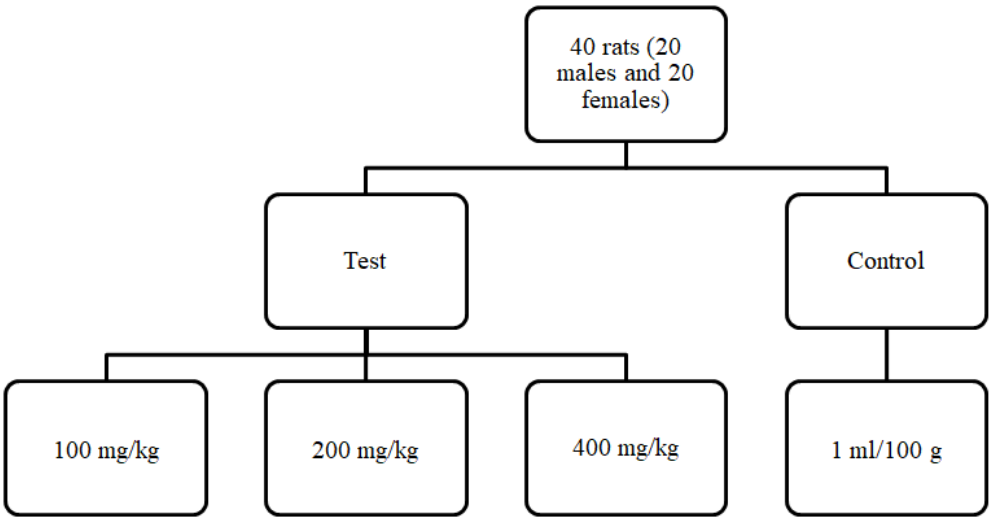


Fig-1: Batches Distribution

Blood samples

The animals were anaesthetized by intraperitoneal injection of ketamine acepromazine (1.8 mg/190 g body weight). The blood of each animal was taken by orbital puncture using a hematocrit tube, transferred into the dry tubes, centrifuged at 3000 rpm for 5 minutes. The serum required was stored at 4°C for biochemical analyses (Transaminase, CT, PT, Triglyceride and HDL, Urea and Creatinine). EDTA tube for hematological tests (RBC, WBC, Hematocrit, Blood platelet, Hemoglobin).

Organ harvesting

After sacrifice, the organs (kidneys, liver, lungs, and heart) were isolated and rinsed in a physiological solution of 0.9% NaCl and weighed *in situ* [3]. Organs were preserved in formalin 10% according to the paraffin inclusion technique for histological analyses.

Analysis of biochemical parameters

Several biochemical parameters were determined including serum urea by the Urease-GLDH UV kinetic method, using the UREA UV SGMitalia kit; creatinine by the Jaffé colorimetric method, using the CREATININE LR SGMitalia kit; analine

aminotransferase by the optimized UV IFCC kinetic method, using the GSMitalia GPT-ALT LR kit and aspartate aminotransferase by the optimized UV IFCC kinetic method, using the GSMitalia GOT-AST LR kit. The total cholesterol, HDL, triglyceride assay protocol was provided by the LABKIT kit, and the determination of total proteins was carried out according to the Biuret method [11].

Hematological examinations

The hematological parameters for the blood count formula (SNF) were : white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) count, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin Concentration (MCHC), platelet (PLT) count. These parameters were carried out by Medonic (Beckman Coulter, USA).

Histological analysis

Histological analysis is the technique of preparing tissues and/or organs for observation under a microscope. It includes different stages including fixing, trimming, dehydration, inclusion, cutting, coloring, assembly and observation.

Statistical Analyzes

Data were expressed as a mean ± Standard error on Mean (ESM). The statistical analysis of the data was done using Graphpad Instat software version 8.0.1. The analysis of the relative weight of the organs and the biochemical parameters was done with ANOVA "one-way" followed by the post-test of TUKEY. The ANOVA test "two way with repeated measures" followed by the DUNNETT post-test was used for the processing of data on hemodynamic parameters and body weight

RESULTS

Quantitative phytochemical screening

Quantitative phytochemical analysis revealed the presence of all classes of metabolites measured, namely polyphenols, flavonoids, tannins, alkaloids and saponins. Among these compounds, saponins were the most abundant followed by polyphenols, alkaloids followed by flavonoids and finally tannins.

Table-I: Contents of a few classes of secondary metabolites of the ethanolic extract of the stem bark of *Canarium schweinfurthii*

Levels of secondary metabolites	Polyphenols (µg GaE/g MS)	Flavonoids (µg QE/g MS)	Tannins (µg TAE/g MS)	Alkaloids (µg QiE/g MS)	Saponins (µg SE/g DM)
EESB	38.90 ± 3.56	26.00 ± 1.99	2.85 ± 1.99	26.79 ± 4.67	125.00 ± 12.25

EESB: Ethanol extract of the stem bark of *Canarium schweinfurthii*; **GaE:** Galic acid equivalent; **QE:** Quercetin Equivalent; **TAE:** Tannic Acid Equivalent; **SE:** Saponin Equivalent; **EQi:** Quinine Equivalent; **g**: Gram; **DM:** Dry matter.

Subchronic toxicity

Effect of repeated doses of *C. schweinfurthii* extract on behavioural parameters in rats

The animals were observed after the first 30 minutes and then at 1 h, 2 h, 4 h, 8 h, and then every 24 h for 90 days and there was no variation in behavioural parameters for all rats tested (male and female) for either the test or control batch (Table-II).

Table-II: Clinical parameters of rats tested for subchronic oral toxicity

Parameters	Distilled water 1 ml/100 g	C S 100 mg/kg	C S 200 mg/kg	C S 400 mg/kg
Number of rats	10	10	10	10
Mortality	0	0	0	0
Coat modification	A	A	A	A
Eye modification	A	A	A	A
Reactivity	N	N	N	N
Excessive agitation	A	A	A	A
Tremor	A	A	A	A
Convulsion	A	A	A	A
Appearance of stool	N	N	N	N
Reaction to noise	N	N	N	N
Intense thirst	A	A	A	A
Salivation	A	A	A	A
Gait disturbance	A	A	A	A

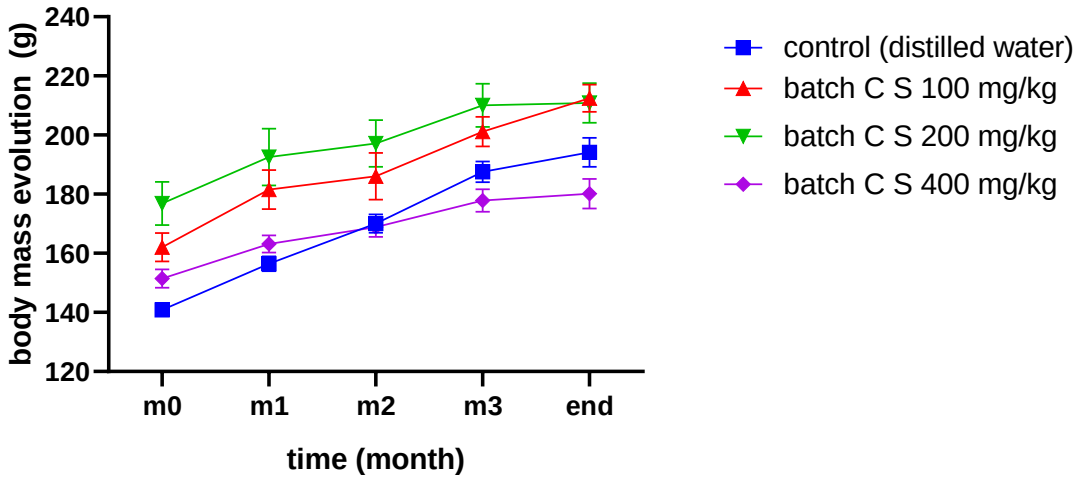
Caption: C S: *Canarium schweinfurthii*; A: Absent; N: Normal

Effect of repeated doses of the extract on rat weight change

During the 90 days of the study, the body mass of each rat was taken using a sensitive KERN brand balance (EMB 600-2) which allowed us to determine the weight evolution of the rats used.

• Female rats

The weight evolution of the treated female rats is shown in Figure 2. The analysis of this figure shows a weight gain in all animals throughout the period of the experiment with a gain of about 35 g for each batch between the beginning and the end of the study.

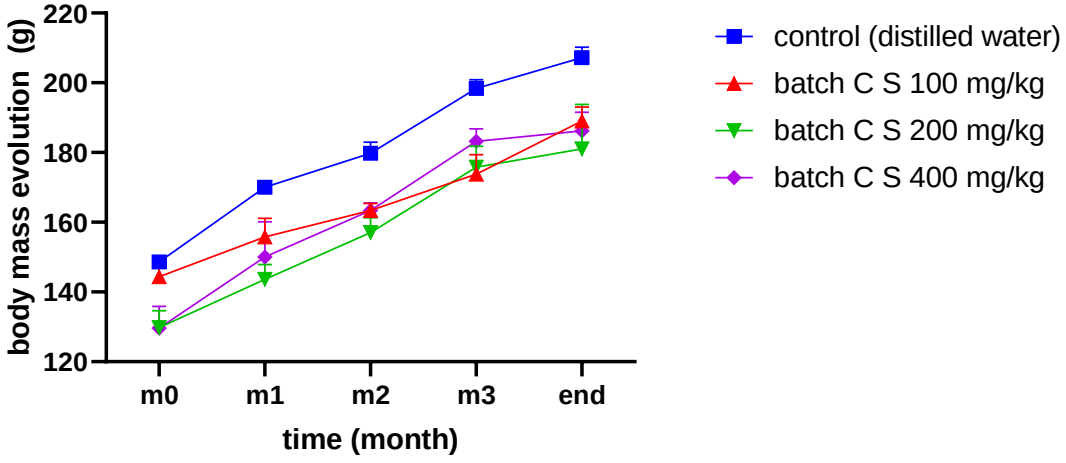


C S: *Canarium schweinfurthii*

Fig-2: Body mass evolution of female rats

• Male rats

Figure 3 illustrates the weight evolution of male rats over the 90 study days. Weight monitoring of male rats tested daily demonstrates similar growth between test rats and control rats with a gain of about 50 g for each batch between the beginning and end of the study.



C S: *Canarium schweinfurthii*

Fig-3: Body mass evolution of male rats

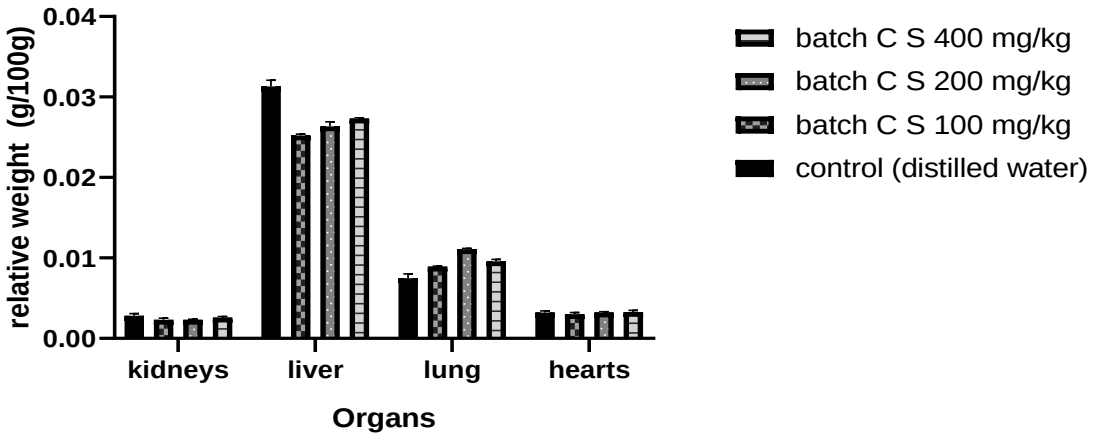
Effect of repeated doses of the extract on the relative weight of the main detoxification organs

At the end of the experiment, the rats were sacrificed and organs such as kidney, lung, heart and liver were removed and the relative weight was calculated from the total rat weight from which each organ was removed.

• In male rats

Monitoring of the relative weight of rats tested daily shows a slight increase in lung mass and a slight decrease in liver mass in the test batches compared to control rats with a non-significant difference because $p > 0.05$ (Figure 4).

The kidneys and hearts have substantially the same relative weights for all test batches as well as the control lot.



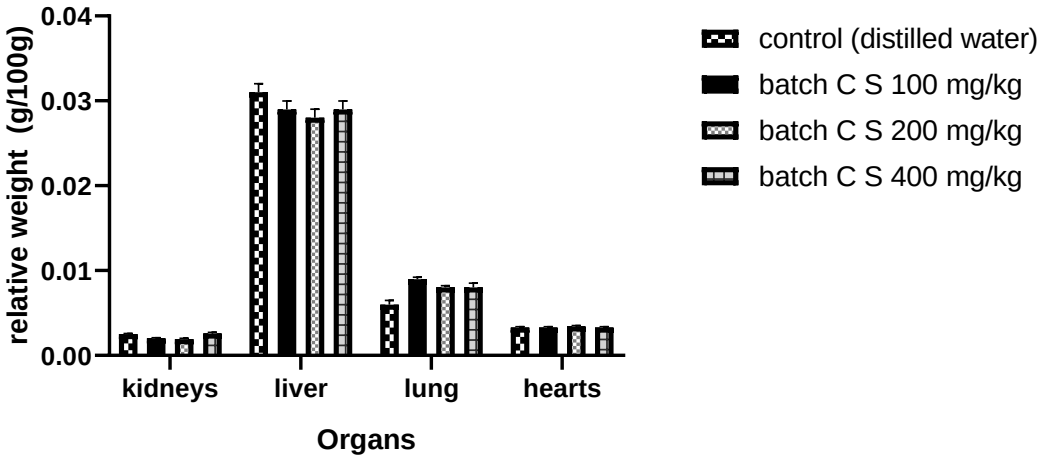
Caption: C S: *Canarium schweinfurthii*

Fig-4: Comparison of the relative organ weights of the male rats tested compared to the control batch

• In female rats

Figure 5 illustrates the relative weight of female rats over the 90 study days. Monitoring of the relative weight of female rats tested daily shows a slight increase in relative lung weights and a slight decrease in livers of the batches that received the C S extract compared to control rats but this difference remains non-significant because $p > 0.05$.

However, the kidneys and hearts of the batches that received the C S extract at different doses have substantially the same relative weights.



Caption: C S: *Canarium schweinfurthii*

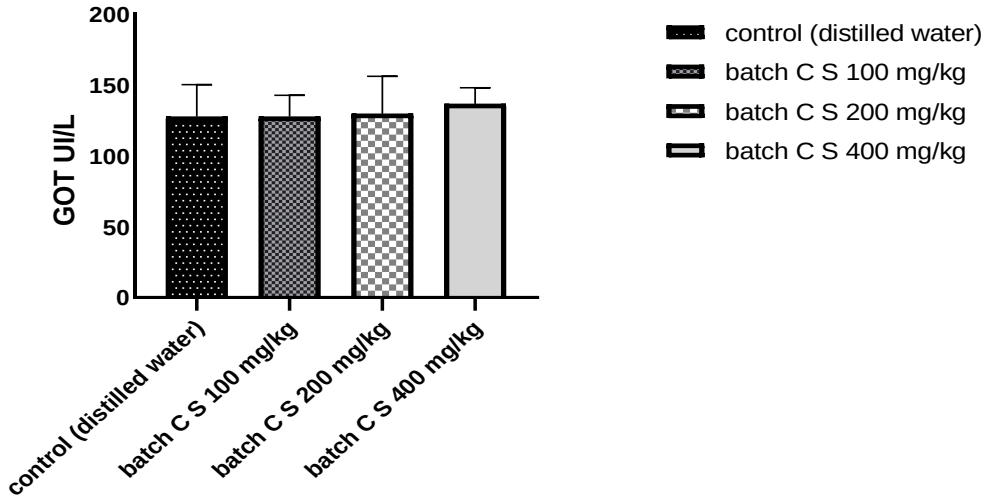
Fig-5: Comparison of the relative organ weights of the female rats tested compared to the control batch

Effect of repeated doses of the extract on some biochemical parameters

Effect of repeated doses of the extract on rat GOT (Glutamate Oxolo-acetate Transaminase) levels

• In male rats

Exploration of biochemical markers of liver function for GOT shows that only the GOT activity of male rats received the ethanolic extract of C S at 400 mg/kg is slightly higher than that of the control batch with a non-significant difference $p > 0.05$ whereas the GOT activity of batches 100 and 200 mg/kg is substantially close to the control batch with a statistical analysis performed which reveals this non-significant difference because $p > 0.05$ (Figure 6).

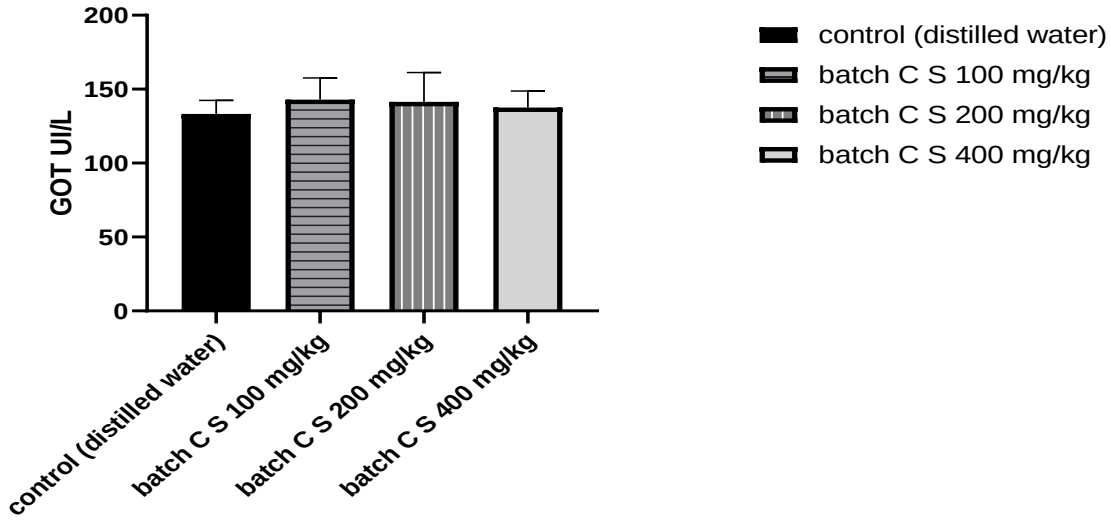


Caption: C S: *Canarium schweinfurthii*

Fig-6: GOT (Glutamate Oxolo-acetate Transaminase) activity of male rats subjected to the subchronic toxicity test for the different samples compared to the control batch

• In female rats

Exploration of this biochemical marker of liver function shows a slight non-significant increase in GOT activity in female rats given the ethanolic extract at different doses compared to the control batch with a non-significant difference because $p > 0.05$ (Figure 7).



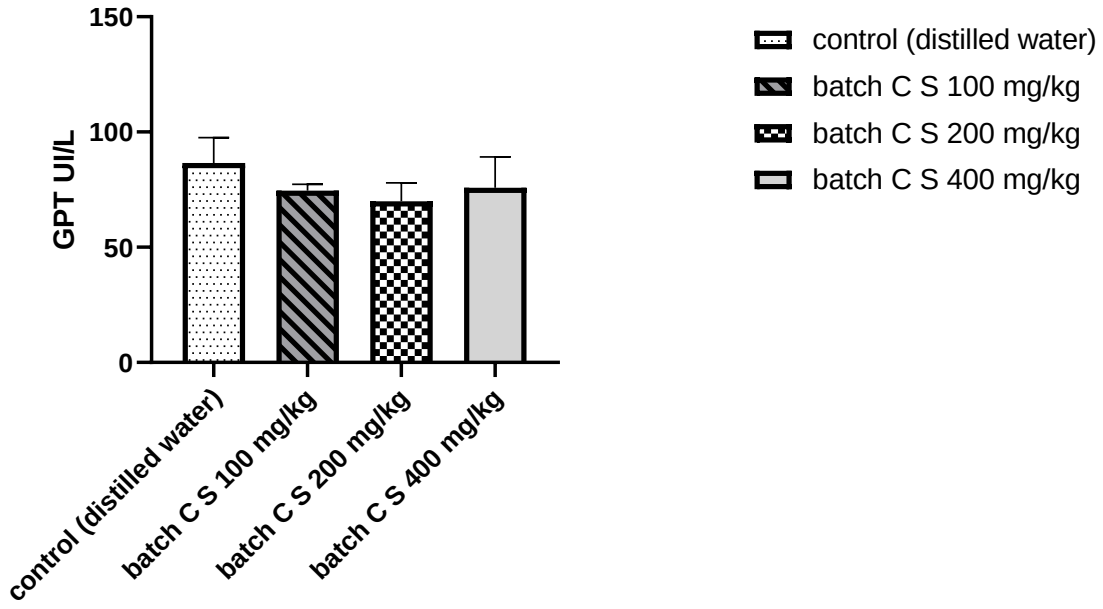
Caption: C S: *Canarium schweinfurthii*

Fig-7: Comparison of the GOT (Glutamate Oxolo-acetate Transaminase) activity of female rats subjected to the subchronic toxicity test for the different samples with the control batch

Effect of repeated doses of the extract on rat GPT (Glutamate Pyruvate Transaminase) activitie

• In male rats

Exploration of biochemical markers of liver function shows that the GPT activity of male rats given the ethanolic extract of C S at 100 mg/kg and 400 mg/kg is relatively low compared to those of the control batch $p > 0.05$ ($P = 0.2622$; 0.0802 and 0.3476 respectively) (Figure 8).

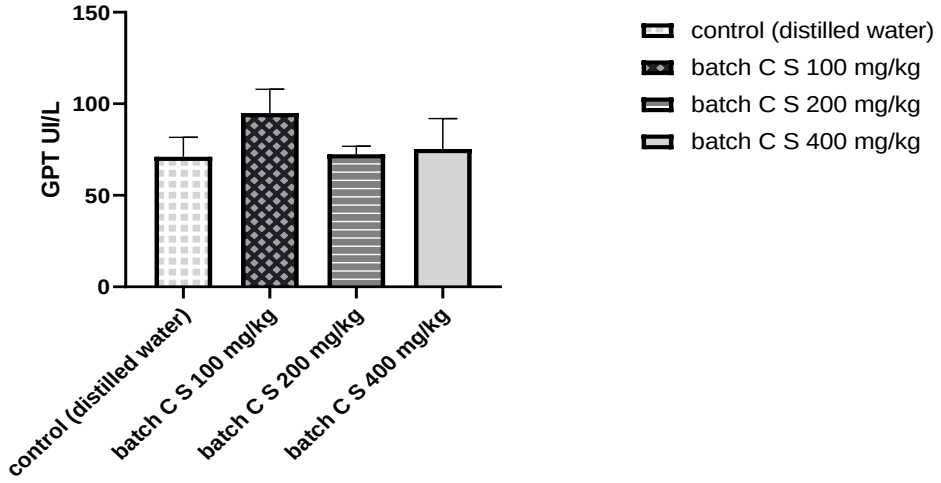


Caption: C S: *Canarium schweinfurthii*

Fig-8: GPT (Glutamate Pyruvate Transaminase) activity of male rats subjected to the subchronic toxicity test for the different samples compared to the control batch

• In female rats

For female rats, the GPT activity of rats given the ethanolic extract of C S at a dose of 100 mg/kg increased but not significantly according to statistical analysis. On the other hand, at doses 200 and 400 mg/kg, this rate is substantially close to the control lot with non-significant statistical analysis $p > 0.05$ (Figure 9).



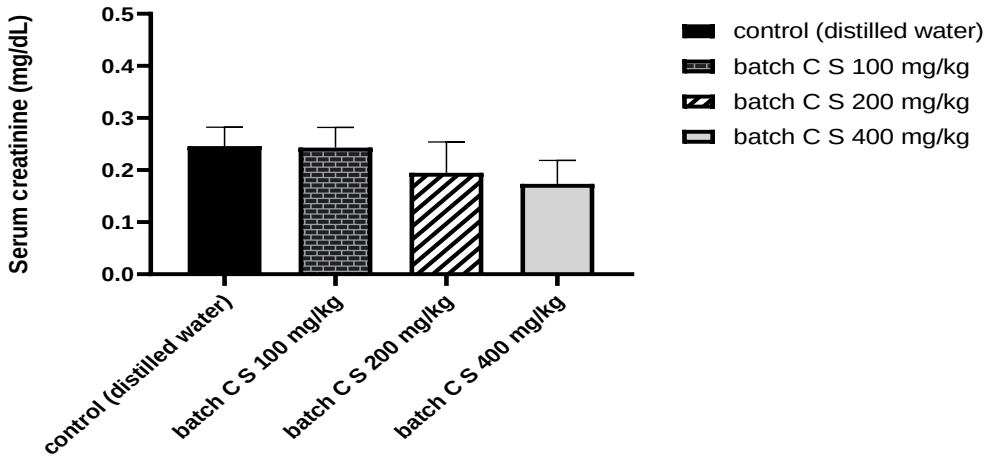
Caption: C S: *Canarium schweinfurthii*

Fig-9: GPT (Glutamate Pyruvate Transaminase) activity of female rats subjected to the subchronic toxicity test for the different samples compared to the control batch

Effect of repeated doses of the extract on serum creatinine levels in rats

• In male rats

Exploration of the serum creatinine of male rats shows a creatinine level significantly close in the test batch that received the extract at 100 mg/kg and control batch with a difference remains insignificant because the p values are 0.9997. Contrary, batches 200 and 400 mg/kg were non-significant decrease ($p = 0.3999$ and 0.1524 respectively) So $p > 0.05$. (Figure 10).

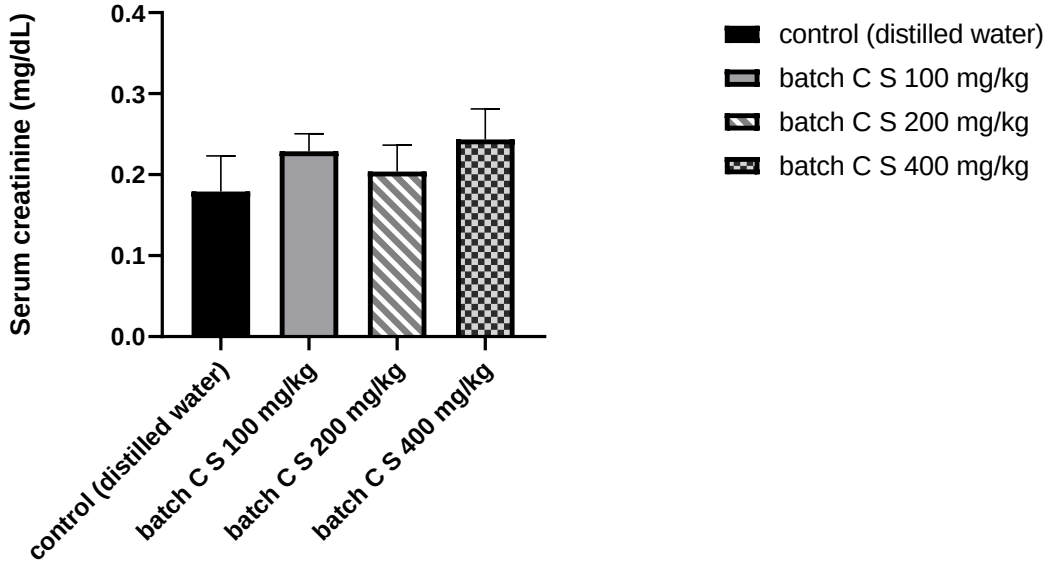


Caption: C S: *Canarium schweinfurthii*

Fig-10: Creatinine in male rats subjected to the subchronic toxicity test for the different samples compared to the control batch

• In female rats

Exploration of the serum creatinine of female rats reveals that the creatinine level of the batches receiving the C S extract is slightly higher than that of the control batch but the difference remains non-significant: $p > 0.05$ (Figure 11).



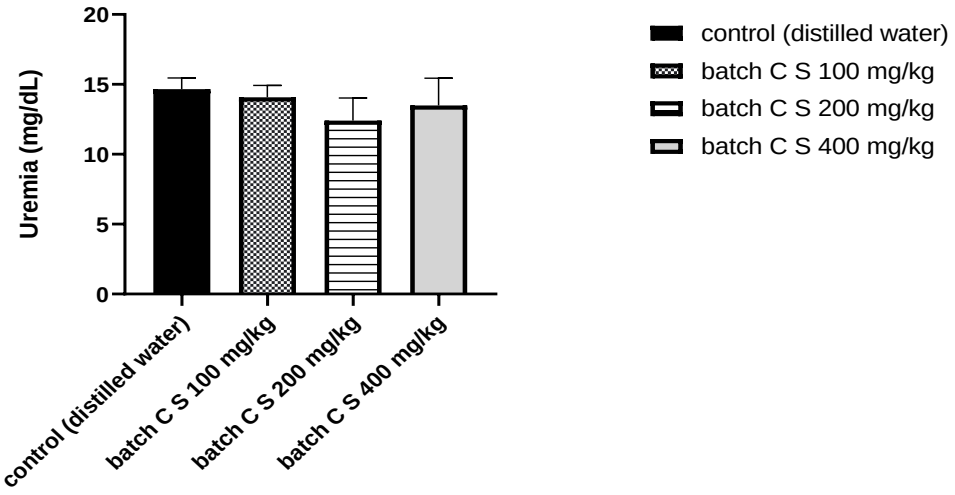
Caption: C S: *Canarium schweinfurthii*

Fig-11: Creatinine in female rats subjected to the subchronic toxicity test for the different samples compared to the control batch

Effect of repeated doses of the extract on urea levels in rats

• In male rats

Exploration of uremia in male rats revealed a urea level in the test batches that received the extract at doses 100 and 400 mg/kg substantially close to the control batch with $p > 0.05$. The urea content of the batch that received the extract of the stem bark of C S at 200 mg/kg did not significantly decrease compared to that of the control lot with p value = 0.0662; So, $p > 0.05$ (Figure 12).

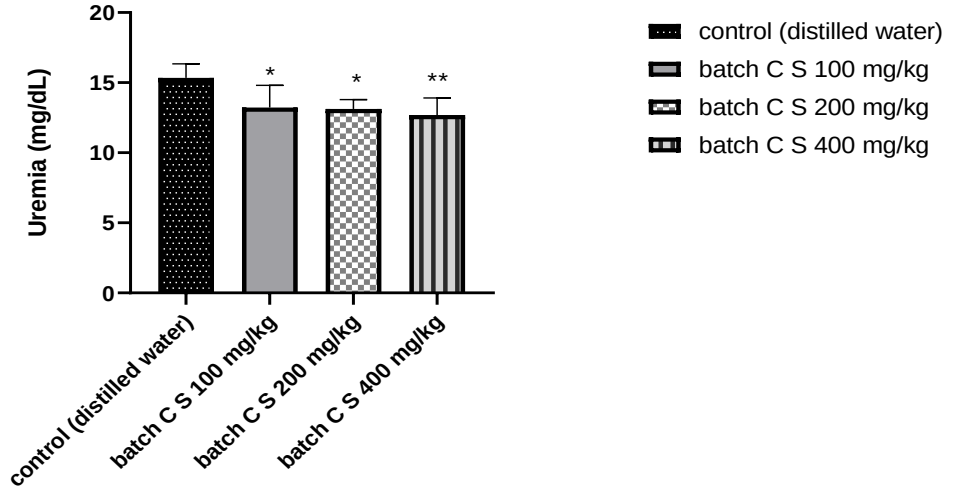


Caption: C S: *Canarium schweinfurthii*

Fig-12: Urea concentration for each male rats for the different samples compared to the control batch

• In female rats

Exploration of uremia in female rats reveals that urea levels in the batches that received the CS extract are significantly lower than in the control batch. (p values are 0,0207; 0.0158 and 0.0045 respectively for batches 100, 200 and 400 mg/kg) (Figure 13).



Caption: C S: *Canarium schweinfurthii*

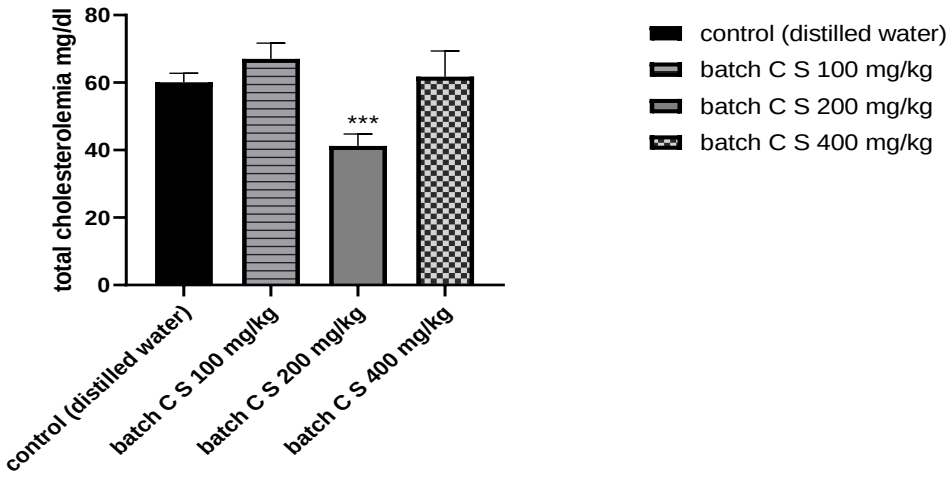
p < 0.05 (*) = not very significant; P < 0.01 (**)= significant;

Fig-13: Urea concentration for each female rats for the different samples compared to the control batch

Effect of repeated doses of the extract on total cholesterol levels in rats

• In male rats

Exploration of male rat's cholesterol reveals a slight non-significant increase in total cholesterol levels in male rats in the 100 and 400 mg/kg batch compared to control group with p > 005. On the other hand, there was a significant decrease in total cholesterol levels in the test batches that received the extract at doses 200 mg/kg compared to the control batch p = 0.0004 (Figure 14).



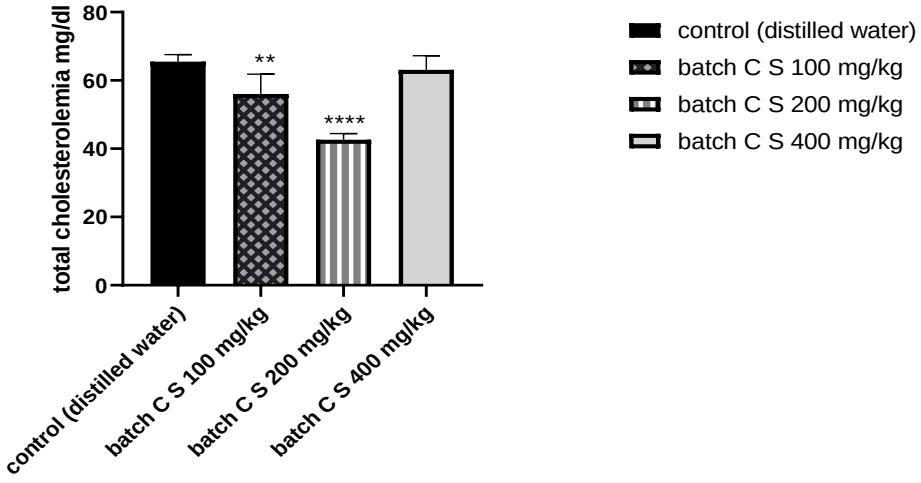
Caption: C S: *Canarium schweinfurthii*

p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant;

Fig-14: Total cholesterol concentration for each male rats for the different samples compared to the control batch

• In female rats

Exploration of the cholesterolemia of female rats reveals a significant decrease in total cholesterol levels in the test rats of the batches that received the extract at 100 and 200 mg/kg compared to the control batch ($p = 0.0017$ and $p < 0.0001$ respectively). On the other hand, in lot 400 mg/kg, this rate is substantially close to that by the control lot $p > 0.05$ (Figure 15).



Caption: C S: *Canarium schweinfurthii*

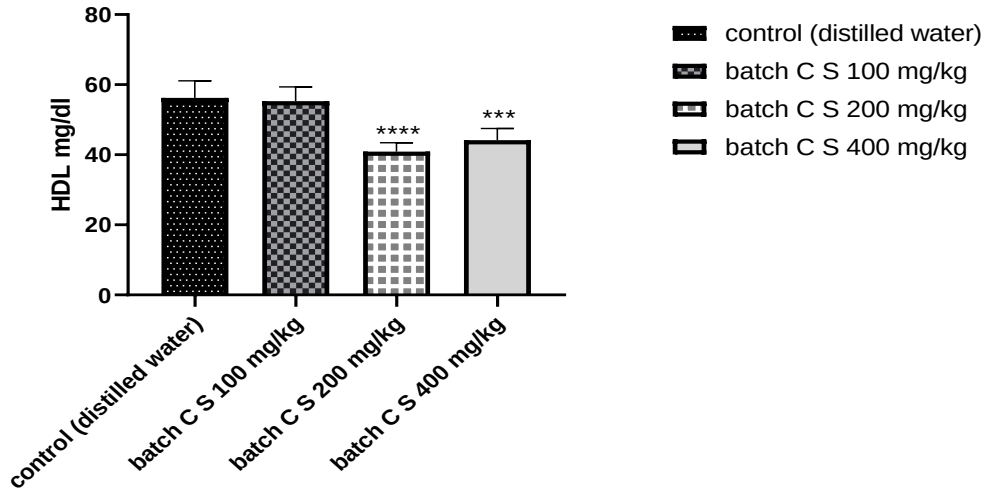
p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant; P < 0.0001 (****) = extremely significant.

Fig-15: Total cholesterol concentration for each female rats for the different samples compared to the control batch

Effect of repeated doses of the extract on HDL (High-Density Lipoproteins) levels in rats

• In male rats

Exploration of the HDL level of male rats reveals an HDL level in male rats in the batch that received the extract at 100 mg/kg substantially close to that of the control batch. On the other hand, there was a significant decrease in HDL levels in the test batches that received the extract at doses 200 and 400 mg/kg compared to the control batch ($p < 0.0001$ and $p = 0.0008$ respectively) (Figure 16).

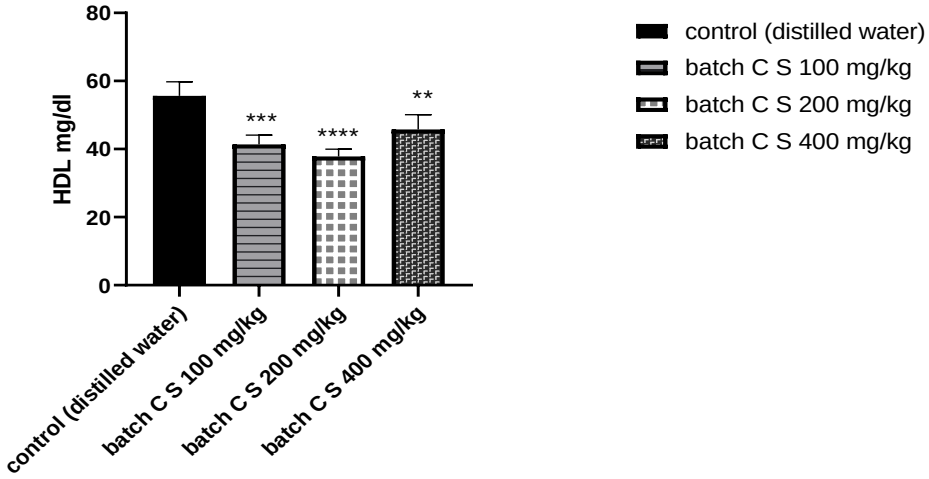


Caption: C S: *Canarium schweinfurthii*
p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant; P < 0.0001 (****) = extremely significant.

Fig-16: HDL (High-Density Lipoproteins) concentration for each male rats for the different samples compared to the control batch

• In female rats

Exploration of HDL levels in female rats revealed a significant decrease in HDL levels in rats in all test batches that received the extract compared to the control batch (p = 0.0002; p < 0.0001 and p = 0.0051 respectively) (Figure 17).



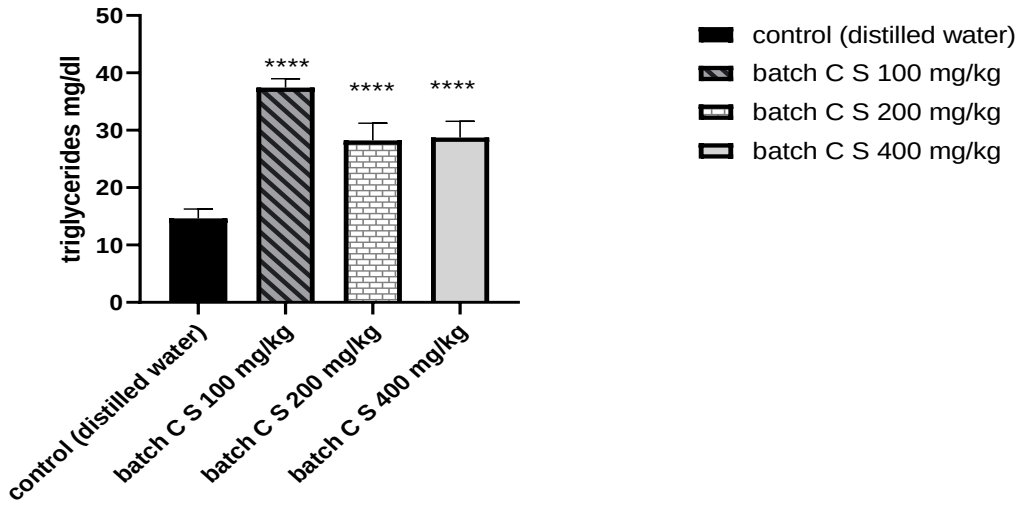
Caption: C S: *Canarium schweinfurthii*
p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant; P < 0.0001 (****) = extremely significant.

Fig-17: HDL (High-Density Lipoproteins) concentration for each female rats for the different samples compared to the control batch

Effect of repeated doses of the extract on triglyceride (TG) levels in rats

• In male rats

Exploration of the TG level of male rats reveals a significant increase in TG levels in male rats in the batches that received the extract compared to control batch p < 0.0001 (Figure 18).



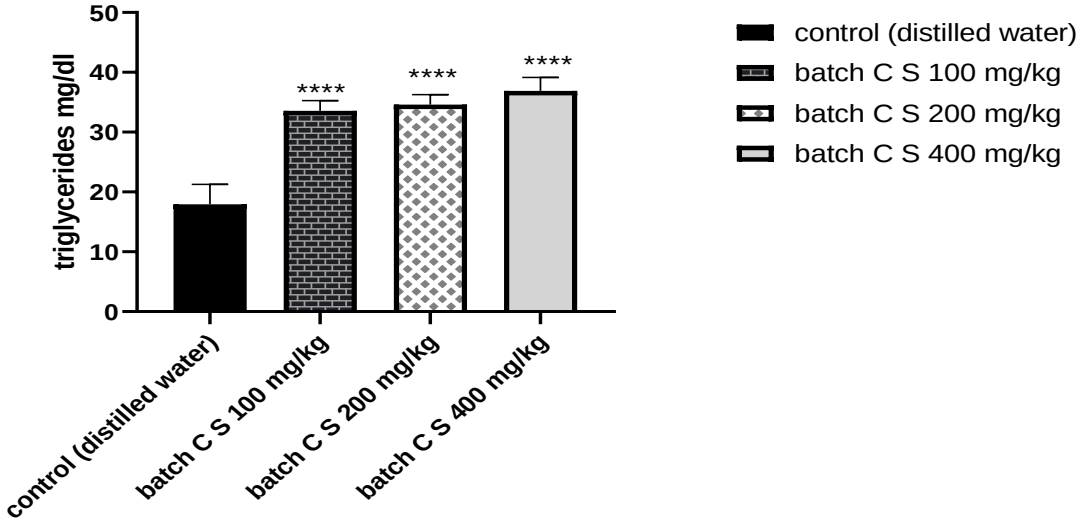
Caption: C S: *Canarium schweinfurthii*

p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant; P < 0.0001 (****) = extremely significant.

Fig-18: TG (triglycéride) concentration for each male rats for the different samples compared to the control batch

• In female rats

Exploration of TG levels in female rats revealed a significant increase in TG levels in rats in all test batches that received the extract compared to control batch p < 0.0001 (Figure 19).



Caption: C S: *Canarium schweinfurthii*

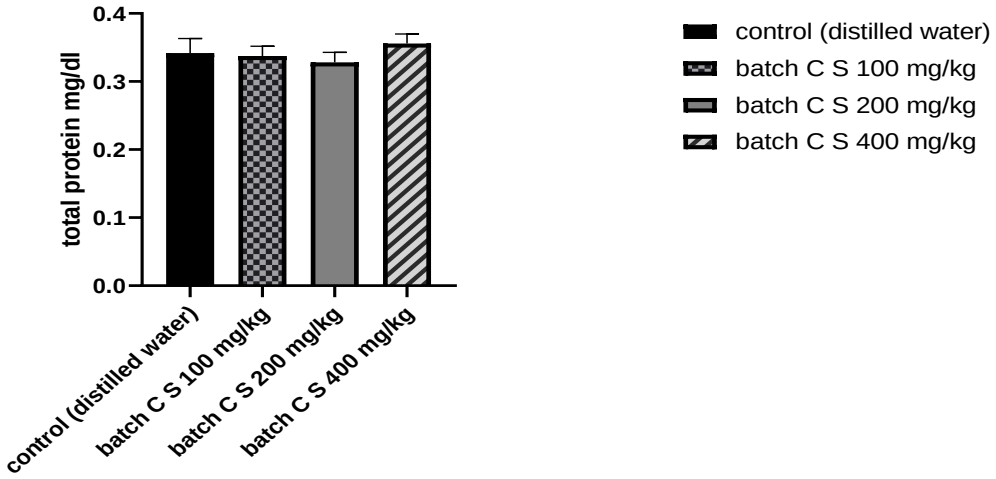
p < 0.05 (*) = not very significant; P < 0.01 (**)= significant; p < 0.001 (***) = very significant; P < 0.0001 (****) = extremely significant.

Fig-19: TG concentration for each female rats for the different samples compared to the control batch

Effect of repeated doses of the extract on total protein (PT) levels in rats

• In male rats

Exploration of the PT level of male rats reveals a PT level in male rats in the batches that received the extract at doses of 100 and 200 mg/kg substantially close to the control batch. Conversely, for lot 400 mg/kg there is a slight increase compared to the control batch but this increase remains insignificant p > 0.05 (Figure 20).

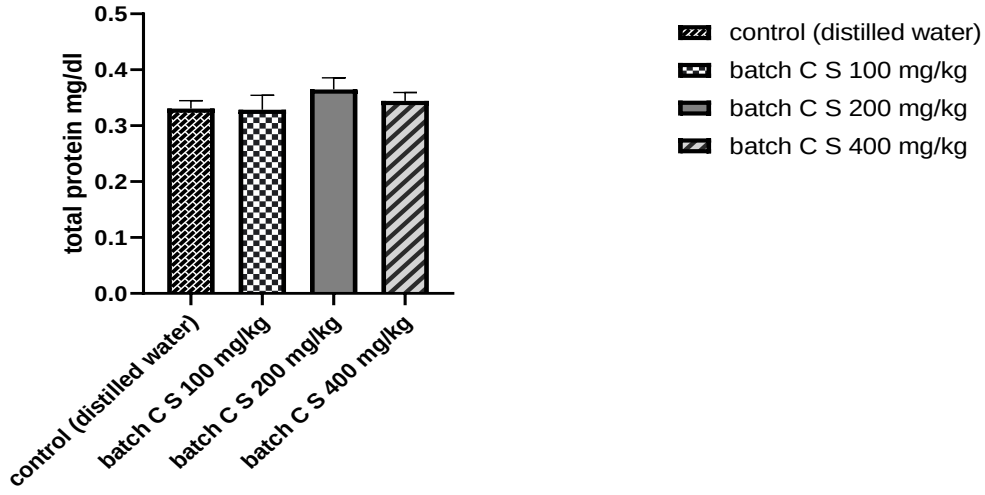


Caption: C S: *Canarium schweinfurthii*

Fig-20: Total protein concentration for each male rats for the different samples compared to the control batch

• In female rats

Exploration of the PT level of female rats reveals a slight non-significant increase in the level of PT in rats in all test batches that received the extract at 200 mg/kg compared to the control batch $p > 0.05$. On the other hand, in the test batches that received the extract at doses 100 and 400 mg/kg, this rate is substantially close to that by the control batch $p > 0.05$ (Figure 21).



Caption: C S: *Canarium schweinfurthii*

Fig-21: Total protein concentration for each female rats for the different samples compared to the control batch

Effect of repeated doses of ethanolic extract of *Canarium schweinfurthii* on blood count

• In female rats

It appears in Table-III that there is no change in the level of red blood cells (RBCs), white blood cells (WBC), hemoglobin concentration (Hb), and lymphocyte content (LYM) between animals treated with plant extract and control animals. However, a significant difference was observed in mean corpuscular volume (MCV) and hematocrit level (HCT) between the different groups receiving the extract at 100 mg/kg and 400 mg/kg and the control lot. The mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin content (MCH) varied significantly between the 200 mg/kg extract group and the control group. Similarly, platelet counts (PLTs) varied significantly between animals tested at doses of 200 and 400 mg/kg compared to controls.

Table-III: Mean ± ESM of hematological parameters of different of female rats for the different samples compared to the control batch

Code	Control (distilled water)	batch C S 100 mg/kg	batch C S 200 mg/kg	batch C S 400 mg/kg
RBCs	4.19 ± 0.45	3.88 ± 0.45	5.28 ± 0.38	4.59 ± 0.43
WBC	5.83 ± 0.75	3.15 ± 0.40	5.26 ± 0.31	6.26 ± 0.64
Hb	11.10 ± 0.04	12.07 ± 0.34	11.80 ± 0.18	12.01 ± 0.46
HCT	60.00 ± 4.65	45.62 ± 2.83^{b}	59.00 ± 2.00	45.94 ± 1.46^{b}
MCV	69.60 ± 3.34	96.25 ± 3.43^{d}	65.60 ± 5.16	86.80 ± 1.96^{c}
MCHC	28.60 ± 1.44	24.58 ± 10.27	46.00 ± 5.98^{c}	28.50 ± 1.03
MCHD	31.34 ± 1.18	38.75 ± 6.51	42.20 ± 7.09^{a}	30.02 ± 2.92
PLTs	158.40 ± 7.48	148.30 ± 1.34	145.42 ± 7.17^{d}	147.00 ± 2.92^{a}
LYM	1.61 ± 0.28	0.97 ± 0.06	0.44 ± 0.11	1.84 ± 0.39

Caption: *C S: Canarium schweinfurthii; RBCs = Red blood cells (106/mm^3); WBC = White blood cells/(mm^3); Hb = Hemoglobin (g/l); HCT = Hematocrit (%); MCV = mean globular volume in ftl; MCHD = mean corpuscular hemoglobin content in Pg; MCHC = mean corpuscular hemoglobin concentration in (g/dl); PLTs = Platelets (103 mm^3); LYM = lymphocyte (%); pg = pictogram; ftl = fentoliter.*

The letters a, b, c and d make it possible to deduce significant variations in the values on the same column having the same superscript letter between them do not differ significantly ($p > 0.05$).

• In male rats

Table IV shows that there is no change in red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb) concentration, lymphocyte content (LYM) and hematocrit level (HCT) between animals treated with plant extract and controlled animals. However, a significant difference was observed in mean corpuscular volume (MCV) between the different groups receiving the extract at 200 mg/kg and 400 mg/kg and the control group. The mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin content (MCHD) varied significantly between the 100 mg/kg extract group and the control group. Similarly, platelet counts (PLTs) varied significantly between all batches of animals tested compared to controls.

Table-IV: Mean ± ESM of hematological parameters of different male rats for the different samples compared to the control batch

Code	Control (distilled water)	batch C S 100 mg/kg	batch C S 200 mg/kg	batch C S 400 mg/kg
RBCs	5.23 ± 0.08	4.92 ± 0.27	4.94 ± 0.23	4.38 ± 0.13
WBC	6.41 ± 0.40	4.57 ± 0.36	5.74 ± 0.68	12.65 ± 5.01
Hb	11.32 ± 0.28	10.68 ± 0.48	12.52 ± 0.68	11.86 ± 0.65
HCT	53.72 ± 0.85	52.72 ± 2.25	49.53 ± 2.27	54.80 ± 5.36
MCV	58.20 ± 2.04	54.20 ± 2.71	83.60 ± 7.78^{d}	83.60 ± 2.21^{d}
MCHC	32.80 ± 0.20	46.80 ± 4.25^{b}	34.62 ± 4.14	28.20 ± 0.26
MCHD	31.21 ± 0.27	42.82 ± 5.59^{a}	35.94 ± 4.56	36.60 ± 1.57
PLTs	131.20 ± 3.76	149.00 ± 3.42^{d}	163.50 ± 9.18^{d}	152.80 ± 2.01^{d}
LYM	1.41 ± 0.40	0.51 ± 0.04	1.33 ± 0.51	1.65 ± 0.42

Caption: *C S: Canarium schweinfurthii; RBCs = Red blood cells (106/mm^3); WBC = White blood cells/(mm^3); Hb = Hemoglobin (g/l); HCT = Hematocrit (%); MCV = mean globular volume in ftl; MCHD = mean corpuscular hemoglobin content in Pg; MCHC = mean corpuscular hemoglobin concentration in (g/dl); PLTs = Platelets (103 mm^3); LYM = lymphocyte (%); pg = pictogram; ftl = fentoliter.*

The letters a, b, c and d make it possible to deduce significant variations in the values on the same column having the same superscript letter between them do not differ significantly ($p > 0.05$).

Effect of repeated doses of ethanolic extract of *C. schweinfurthii* on histological sections

• In female rats

Figure 22 shows the structure of the liver, kidney, heart and lung of female rats in the groups treated with ethanolic extract of *C schweinfurthii* at doses of 100, 200 and 400 mg/kg respectively for 90 days. Liver microarchitecture was normal (hepatic parenchyma with centrolobular vein, and distinct hepatocytes), kidney (normal parenchyma with distinct glomerulus and urinary space), heart (distinct nuclei and muscle fibers) and lung (pulmonary epithelium, alveolar sac). No structural alteration in its various organs was observed in the groups receiving the plant extract at different doses.

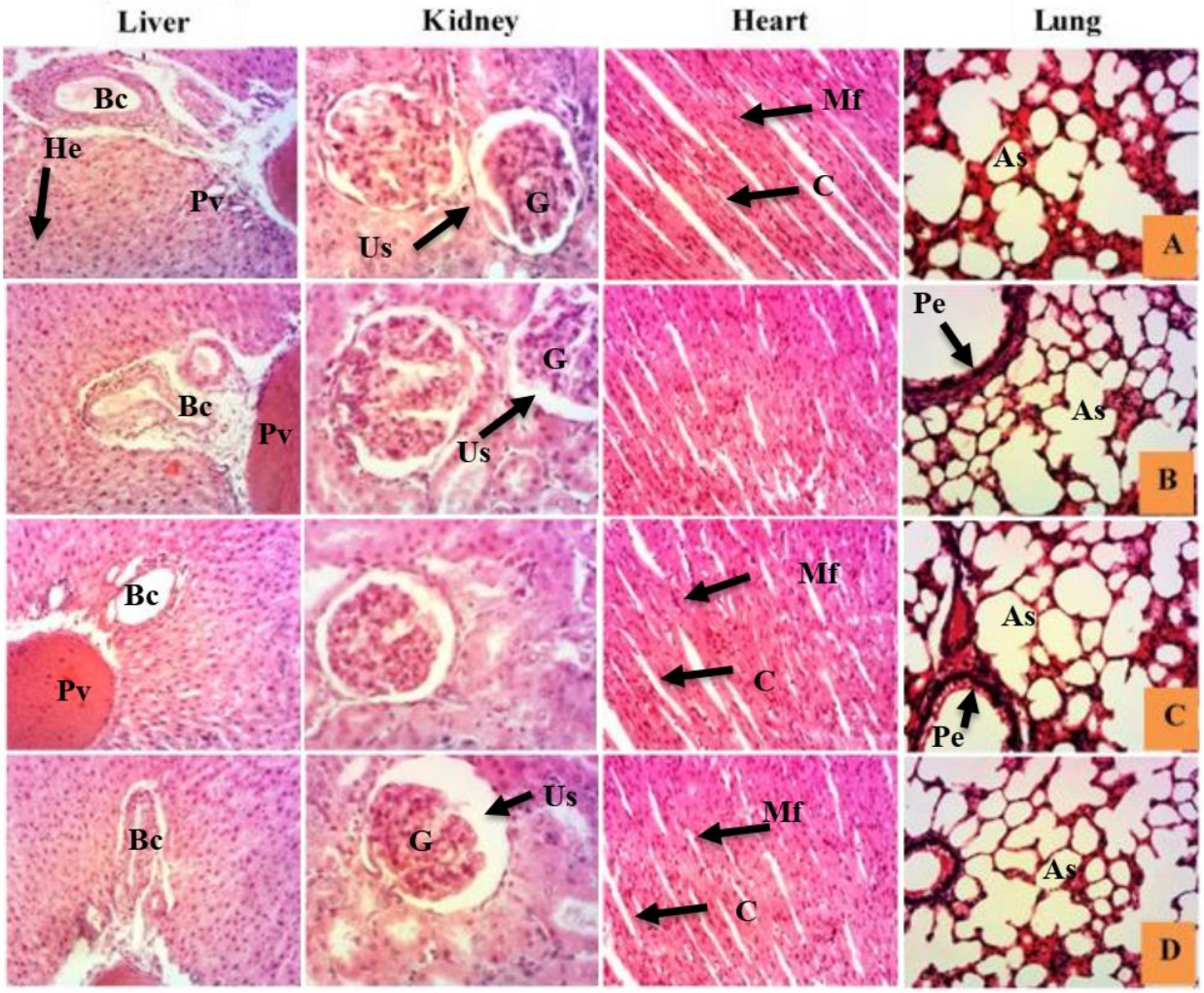


Fig-22: Effects of *Canarium schweinfurthii* extract on liver (100X), kidney (200X), heart (100X) and lung (200X) structure in female rats for the different samples compared to the control batch (EO)

Liver: Pv = portal vein, He = hepatocyte, Bc = biliary canalicule; Kidney: G = Glomerulus, Us = Urinary space, Heart: C = Core, Mf = Muscle fiber; Lung: Pe = Pulmonary epithelium; Sa = alveolar sac; A = Control (distilled water) ; B = batch C S 100 mg/kg; C = batch C S 200 mg/kg; D = batch C S 400 mg/kg.

• In male rats

Figure 23 shows the structure of the liver, kidney, heart and lung of male rats in the groups treated with ethanolic extract of *C. schweinfurthii* at doses of 100, 200 and 400 mg/kg respectively for 90 days. Normal architecture of the liver (hepatic parenchyma with centro-lobular vein, and distinct hepatocytes), kidney (normal parenchyma with a distinct glomerulus and urinary space), heart (distinct nuclei and muscle fibers) and lung (pulmonary epithelium, alveolar sac) were observed. No evidence of structural alterations in its organs was observed in the groups receiving the plant extract at different doses.

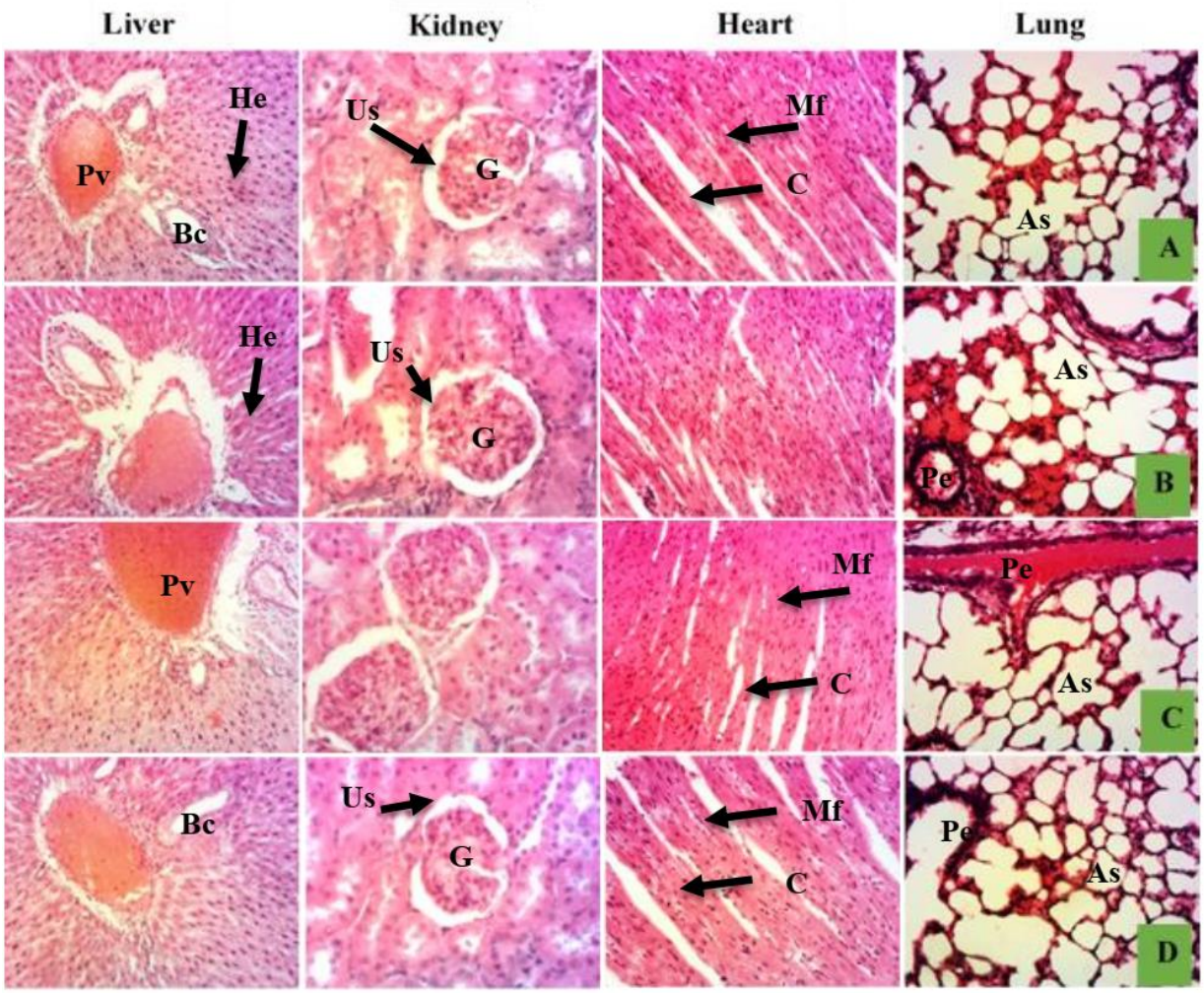


Fig-23: Effects of *Canarium schweinfurthii* extract on liver (100X), kidney (200X), heart (100X) and lung (200X) structure in male rats for the different samples compared to the control batch (EO)

Liver: Pv = portal vein, He = hepatocyte, Bc = biliary canalicule; Kidney: G = Glomerulus, Us = Urinary space, Heart: C = Core, Mf = Muscle fiber; Lung: Pe = Pulmonary epithelium; Sa = alveolar sac; A = Control (distilled water) ; B = batch C S 100 mg/kg; C = batch C S 200 mg/kg; D = batch C S 400 mg/kg.

DISCUSSION

The quantitative phytochemical analysis in Table-I shows the presence of polyphenols, flavonoids, tannins, alkaloids and saponins. These results are not totally in agreement with the work of Mbosso et al. (2022), whose qualitative analysis of the ethanolic extract of the stem bark of the same plant had not shown the presence of saponins [4]. This difference could be explained by the greater precision of quantitative versus qualitative assay. Among these compounds, saponins were the most abundant (125.00 ± 12.25 µg SE/g DM) followed by polyphenols (38.90 ± 3.56 µg GaE/g DM), alkaloids (26.79 ± 4.67 µg QiE/g DM), followed by flavonoids (26.00 ± 1.99 µg QE/g DM), and finally tannins (2.85 ± 1.99 µg TAE/g DM). These results are different from those obtained by Koga et al. (2020) [12], including the quantitative determination of the methanolic extract of the bark of the root of *C. schweinfurthii* which had shown that phenolic compounds were more abundant (130.97 ± 0.25 mg GAE/g DM) and flavonoids less abundant (5.17 ± 0.11 mg RE/g DM) (Rutine equivalent). This difference could be understood since these are two different parts of the same plant, harvested at different times and extracted by two different solvents: Koga et al. worked on the methanolic extract of the root bark harvested in June [12] while we worked on the ethanolic extract of stem bark, harvested in January.

The ethanolic extract from the stem bark of *C. schweinfurthii* administered orally at doses of 100, 200 and 400 mg/kg resulted in no deaths throughout the 90 study days. No signs of clinical toxicity were observed in the days following administration of this extract. In the present study, the results obtained reveal that body mass was not influenced by the ethanolic extract from the stem bark of *Canarium schweinfurthii*. The mean body weight of rats in all batches increased during the observation period. The average mass of rats in batch 1 (distilled water) increased from 148.6 g on day zero to 207.2 g on day 90, an increase of 58.6 g. The average

mass of rats in batch 2 (100 mg/kg) increased from 144.4 g on day zero to 189 g on day 90, an increase of 44.6 g. The average mass of rats in batch 3 (200 mg/kg) increased from 129.8 g on day zero to 181 g on day 90, an increase of 51.2 g. The average mass of rats in bache 4 (400 mg/kg) increased from 129.6 g on day zero to 186.2 g on Day 90, an increase of 56.6 g. The average mass of rats in batch 4 (400 mg/kg) increased from 129.6 g on day zero to 186.2 g on Day 90, an increase of 56.6 g. Comparison of the baseline values of day zero with the final values of day 90 of the test batches compared to the control batches using the ANOVA twoway test revealed no significant difference with a $p > 0.05$.Therefore, the growth observed in rats during this study would not be due to the action of the extract from the stem bark of *C. schweinfurthii* in time, but rather the consequence of the normal process of rat growth [13].

Regarding the relative weight of organs overall, no difference statistically significant ($p < 0.05$) was found between the neutral control and the different doses of the ethanolic extract from the stem bark of *C. schweinfurthii*; This remains consistent with the results observed during the subacute toxicity of the same extract [4].

In this study, no significant differences were observed in GPT and GOT activies in extract-treated animals compared to the control batch. These results are different from those of Etame *et al*., (2017) who observed with the methanolic extract of *Enantia chlorantha* oliv a decrease in the serum GOT activity of male rats in all batches [14]. This could mean that the extract would not have a detrimental effect on liver function on repeated long-term administration (90 days).

We observed a moderate decrease in serum creatinine levels in the extract-treated male batches compared to the control batch and a moderate increase in serum creatinine levels in the extract-treated female batches compared to the control batch but these differences remain non-significant. The difference observed between the two sexes could be explained by hypersensitivity of females. On the other hand, a significant decrease in urea levels was observed in the extract-treated female batches compared to the control group. Low urea levels are usually of little clinical significance but on the other hand this decrease could reflect a deficiency of dietary protein in case of digestive absorption for example, too much water in the blood or serious liver disease except that for the latter, the GPT activity which is the most specific marker of hepatocellular involvement does not provide evidence in favour [15].

A significant decrease in total cholesterol at 200 mg/kg in males and at doses of 100 and 200 mg/kg in female batches was observed, evidence of its protective action against cardiovascular disease. Similar results on cholesterolemia were reported by Mosaddegh *et al*., (2010) with aqueous extracts of *Paliurus spina-christi* [16] and by Sharmila *et al*., (2007) with aqueous extracts of *Trichosanthes dioica* [17]. The analysis of the results highlights a significant decrease in lipid biomarkers with a decrease in HDL-cholesterol levels in male and female rats contrary to triglycerides which are rather high. This result suggests that treatment with ethanolic extract of the stem bark of *Canarium schweinfurthii* does not cause disturbances of lipid parameters at the plasma objectified by hypercholesterolemia. The increase in triglycerides would reflect that the extract would promote the storage of fatty acids in triglyceride. The total protein profile is without any particular sign.

The effects of ethanolic extract from the stem bark of *C. schweinfurthii* on blood cells were evaluated through hematological analyses. These analyses showed overall a non-significant variation in red blood cells (RBCs), white blood cells (WBC), hemoglobins (Hb), lymphocytes (LYM) in both sexes and a significant decrease in HCT (only in females) treated with doses of 100 and 400 mg/kg body weight compared to animals in the control lot. In addition, the results showed that there is a significant variation in MCV, MCHC, MCHD and blood platelets at different doses. A non-significant change in white blood cell count was probably due to a normal foreign body response or stress associated with subchronic toxicity studies [18].

On histological analysis, no evidence of structural alterations in its organs was observed in the groups receiving the plant extract at different doses. This would mean that the plant extract in different doses would not have a toxic effect on the organs. Similar results were obtained by Hechache *et al*., (2013) [19].

CONCLUSION

At the end of the study, quantitative phytochemical screening determined the content of several groups of secondary metabolites, of 38.90 ± 3.56 µg CaE/g DM polyphenols, 26.00 ± 1.99 µg QE/g DM flavonoids, 2.85 ± 1.99 µg TAE/g DM tannin, 26.79 ± 4.67 µg QiE/g DM alkaloids and 125.00 ± 12.25 µg SE/g DM saponins. Weight evolution showed no adverse effect on the metabolism of the animals. Overall, with the analysis of organs and parameters biochemical and hematological studies, it can be concluded that the ethanolic extract from the stem bark of *C. schweinfurthii* would be of low toxicity.

All the observations and results obtained lead to the conclusion that, the acceptable safety observed for this ethanolic extract of the stem bark of *Canarium schweinfurthii* suggests that it is of good quality for the formulation of improved traditional medicines after further studies.

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