Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 1609-1614
Received: 01-11-2020
Accepted: 05-12-2020

Gnahoué Goueh
Biochemical microbiological laboratory of SVT, higher teacher school of Côte d'Ivoire, PO Box, 10 Abidjan, Côte d'Ivoire

Koné Allassane
Laboratory of biology and health, UFR Biosciences, Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire

Kouadio Koffi John
Laboratory of biology and health, UFR Biosciences, Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire

Kouakou Koffi
Laboratory of biology and health, UFR Biosciences, Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire

Corresponding Author:
Koné Allassane
Laboratory of biology and health, UFR Biosciences, Felix Houphouet-Boigny University, Abidjan, Côte d'Ivoire

Assessment of the acute, subacute and Subchronic toxicity of a total aqueous extract of leaves of *Alchornea cordifolia* (Schumach and Thonn) Müll Arg (Euphorbiaceae) on rats Wistar

Gnahoué Goueh, Koné Allassane, Kouadio Koffi John and Kouakou Koffi

DOI: https://doi.org/10.22271/phyto.2021.v10.i1w.13576

Abstract
The leaves of *Alchornea cordifolia* are traditionally used by African populations for the treatment of several diseases. The acute toxicity study concluded that the total aqueous extract of *Alchornea cordifolia* (ETAAC) is not toxic by single administration and that its LD_{50} is greater than 5000 mg/kg of body weight (bw). Tests carried out by daily administration of ETAAC did not result in any significant change in the body weight of the animals and the weight of vital organs. After 28 days of treatment, no significant difference was observed in the figurative elements of the blood and in the biochemical parameters. Significant decreases were observed in urea and alanine-amino transferase after 60 days of treatment. *Alchornea cordifolia* does not affect the functioning of the kidneys, liver and heart. It is therefore not toxic at doses of 100 mg/kg of bw, 200 mg/kg of bw and 400 mg/kg of bw in rats.

Keywords: Toxicity, acute, subacute, subchronic, biochemical, haematological

Introduction
Traditional medicine remains the main remedy for a large majority of populations to resolve their health problems [1]. According to estimates by the World Health Organization [2], more than 80% of the population in Africa still use this medicine to meet their health care needs. Plant resources therefore occupy a large place in human life [3] and medicinal plants remain the primary reservoir of new drugs [4]. They are an inexhaustible source of nutrients and active ingredients useful for maintaining human balance. According to [5], medicinal plants have been and continue to be the subject of various research studies which generally favor ethnobotanical, pharmacological and phytochemical studies. *Alchornea cordifolia* is a plant that belongs to the Euphorbiaceae family. It is frequently used in traditional African medicine for the treatment of various diseases [6]. It is also reported to possess a multiplicity of biological effects. It is antibacterial [7], spasmolytic [8], anti-inflammatory [9], anti-diarrheal [10], antioxidant [11] and antimicrobial agent [12]. If the pharmacological effects of many plants have been proven in various laboratories, their toxicity is generally unknown. Therefore, assessing the toxicity of herbal preparations is important in determining the safety of these remedies [13]. And in view of the frequent use of *Alchornea cordifolia* in traditional medicine, it makes sense to broaden its safety profile. This is how this study was initiated with the aim of securing the therapeutic use of *Alchornea cordifolia.* The general objective is therefore to study the toxicity of the total aqueous extract of *Alchornea cordifolia* on rats. More specific, it is a question of determining first the acute toxicity, then the subacute toxicity before coming to the subchronic toxicity of the aqueous extract of *Alchornea cordifolia.*

Material and Methods
Equipment
The plant material consisted of fresh leaves of *Alchornea cordifolia* collected in the town of Azaguié in the region of Agneby-Tiassa (Côte d'Ivoire). These leaves have been identified at the National Floristic Center (C.N.F.) of Felix Houphouet-Boigny University (Cote d'Ivoire). The animal material consisted of nulliparous and not pregnant male and female rats of the Wistar strain weighing between 95 and 130 g, reared in the animal house of the upper normal school (ENS) in Abidjan. They were kept in cages lined with wood chips. And had free access to water and food. Animals were marked and put under laboratory conditions for at least five days before each experiment.

Methods

Preparation of the extract

The harvested leaves were dried at room temperature at 26 to 30± 2°C for 2 weeks. They were then pulverized and fifty grams of the powder were extracted by grinding in one liter of distilled water in a mixer three times for three minutes each. The homogenate obtained was drained three times on a square cloth and then filtered successively four times through cotton wool and once through wattman paper (3 mm). The resulting filtrate was lyophilized to obtain the aqueous total extract of *Alchornea cordifolia* leaves (ETAAC).

Acute toxicity

The experiment was conducted according to the Organization for Economic Cooperation and Development's Test Guideline 423 [14]. Two dose levels were tested: 2000 mg/kg of bw and 5000 mg/kg of bw. A total of 12 rats divided into groups of 3 rats were used for the experiment. The animals were fasted overnight 24 hours before the experiment. They were weighed before administration of the substance. Administration is by gavage (intra-esophageal route) at a rate of 1 ml per 100 g of body weight in a sequential process in which three animals are used at each step. They were then observed for five hours after administration and for 14 days for signs of acute poisoning.

Subacute toxicity

The study of subacute oral toxicity, with a view to looking for clinical and / or biological signs of intoxication, was carried out in accordance with OECD guidelines 407 [15] which recommend the use at least 10 rodents (5 females and 5 males) for each dose assessed. The experiment was carried out on 40 young rats. The animals were divided into 4 groups of 10 homogeneous rats according to weight. Each batch consisted of two cages (5 females and 5 males). Lot 1 received distilled water at a rate of 1 ml per 100 g of body weight. Lots 2, 3 and 4 received 100 mg/kg of bw, 200 mg/kg of bw and 400 mg/kg of bw of the total aqueous extract, respectively, for 28 days. Twenty-four (24) hours after the administration of the last dose, the animals were anesthetized with ether and sacrificed by decapitation. Blood was collected in EDTA (Ethylene Diamine Tetra Acetate) tubes and dry tubes for respective hematological and biochemical analyzes. The rats were then dissected and the following organs: heart, liver, lungs and kidneys were removed, weighed.

Subchronic toxicity

For the subchronic oral toxicity study, we replicated the above protocol used to assess subacute toxicity, with a treatment duration of 60 days. At the end of the test, the liver and the right kidney of each animal were preserved in 10% formalin for carrying out the histopathological examinations.

Relative weight of organs removed

The relative weight of the organs removed is obtained according to the following formula:

$$Pr(g) = \frac{Po\ (g)}{Pc\ (g)} \times 100\ (g)$$

With: Pr: Relative weight, Pc: Body weight; Po: Body weight

Determination of haematological and biochemical parameters

Haematological analysis was performed using an automatic hematological system (Sysmex KX21N).Biochemical analysis of the blood was performed after centrifugation at 3000 rotations per minute for 10 min. Serum was collected. The parameters were determined using a biochemical machine (Roche Hitachi 902, Germany) with the Spinreact biochemical kit (Spain).

Histopathological Examination

For histopathological examination, the organs were subjected to a series of dehydration in ethanol baths, and embedding in paraffin. 5µm sections were made with a microtome and then stained with Hematoxylin and Eosin (H&E) and observed under an optical microscope (Olympus CKX41, Germany). As for organ weights, there was no significant difference between the relative weights of different organs in rats treated at different doses, compared to control rats (Table I).

Results

Acute toxicity

Oral administration of ETAAC at doses of 2000 and 5000 mg/kg of bw did not cause death in rats. Observations revealed no sign of intoxication during the experimental period. The lethal dose 50 (LD_{50}) is therefore greater than 5000 mg/kg of bw.

Subacute and subchronic toxicity

Body weight and organ weight

After 28 days of treatment, no significant difference was observed between the change in body weight of animals treated with the plant extract compared to control animals (Figure 1). The same is true for the 60 day treatment (Figure 2), no significant difference between the treated rats and the controls. As for organ weights, there was no significant difference between the relative weights of different organs in rats treated at different doses, compared to control rats (Table 1).

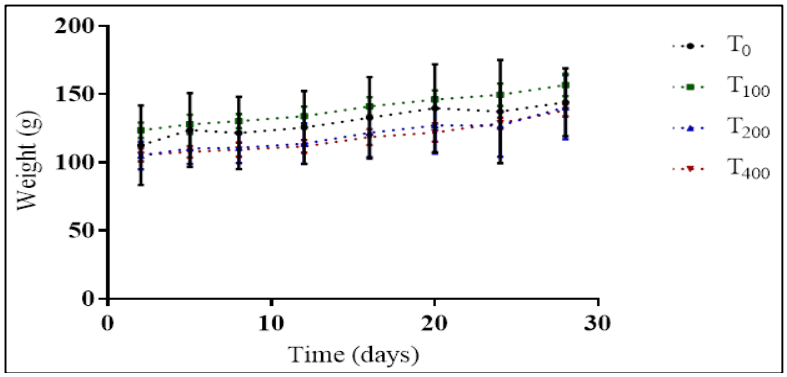

Fig 1: Evolution of the body weight of the rats during 28 days of treatment
T_0: distilled water; T_{100}:100 g/kg of bw; T_{200}:200 mg/kg of bw; T_{400}:400 mg/kg of bw

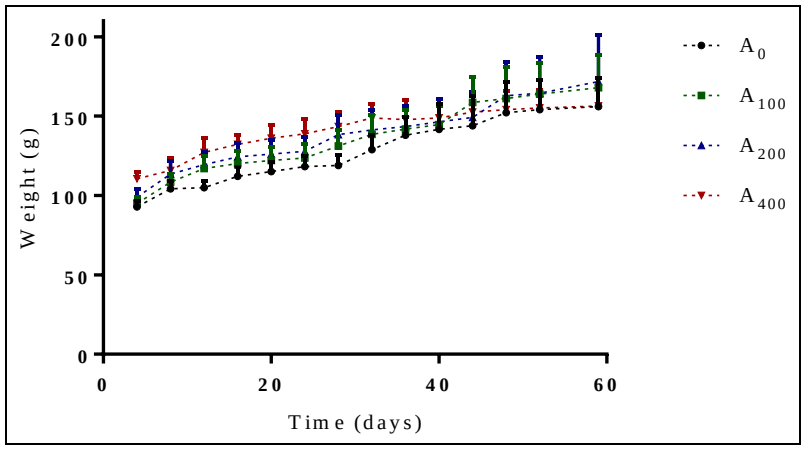

Fig 2: Evolution of the body weight of the rats during 28 days of treatment
A_0: distilled water; A_{100}: 100 mg/kg of bw; A_{200}: 200 mg/kg of bw; A_{400}: 400 mg/kg of bw.

Table 1: Relative weights of organs removed

Time	28 days				60 days			
Doses / Organs	T_0	T_{100}	T_{200}	T_{400}	A_0	A_{100}	A_{200}	A_{400}
Heart	0,28±0,01	0,3±0,01	0,28±0,01	0,27±0,01	0,35±0,02	0,32±0,01	0,35±0,01	0,37±0,01
Liver	3,17±0,09	3,46±0,14	3,19±0,14	3,44±0,13	3,81±0,17	3,85±0,09	3,52±0,08	3,42±0,07
Kidneys	0,49±0,02	0,51±0,01	0,45±0,01	0,5±0,01	0,55±0,02	0,52±0,01	0,55±0,01	0,54±0,01
Lungs	0,65±0,02	0,56±0,03	0,68±0,04	0,57±0,04	0,75±0,04	0,74±0,02	0,68±0,03	0,71±0,02

Values represent mean ± error of mean (ESM). Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. *Subacute toxicity*: T_0: distilled water; T_{100}: 100 mg/kg of bw; T_{200}: 200 mg/kg of bw; T_{400}: mg/kg of bw. *Subchronic toxicity*: A_0: distilled water; A_{100}: 100 mg/kg of bw; A_{200}: 200 mg/kg of bw; A_{400}: mg/kg of bw.

Effect on haematological parameters

The hematological examination carried out on the blood of rats treated daily with ETAAC over the periods of 28 days and 60 days at the doses 100 mg/kg of bw, 200 mg/kg of bw and 400 mg/kg of bw did not no significant modification in the hematopoiesis line was shown. The values are reported in Table 2.

Table 2: Effect of ETAAC on haematological parameters in rats

Time	28 Jours				60 Jours			
Treatment / Parameters	T_0	T_{100}	T_{200}	T_{400}	A_0	A_{100}	A_{200}	A_{400}
WBC×10^3/ml	15,44±0,67	14,73±0,66	16,7±0,69	15,77±0,66	21,01±0,93	19,42±0,44	22,18±0,9	19,12±0,66
RBC×10^6/ml	7,63±0,22	7,22±0,12	7,33±0,15	7,39±0,12	7,63±0,17	7,25±0,17	7,29±0,11	7,89±0,27
NEU%	2,03±0,06	1,77±0,08	1,84±0,11	2,12±0,06	3,76±0,3	3,88±0,23	3,95±0,24	3,47±0,32
EOS %	0,305±0,02	0,25±0,02	0,3±0,02	0,25±0,02	0,49±0,03	0,4±0,03	0,39±0,04	0,39±0,02
LYM %	8,75±0,31	7,77±0,38	9,812±0,4	8,6±0,49	11,69±0,65	10,87±0,21	11,81±0,37	10,47±0,29
MON%	1,26±0,05	1,4±0,02	1,38±0,032	1,39 ±0,031	4,32±0,32	3,65±0,3	4,2±0,2	3,54±0,2
HCT %	45,18±1,06	43,3±0,24	43,12±0,42	43,07±0,75	45,08±0,76	44,03±1,17	44,21±0,5	45,23±1,13
HGB ×g/dl	13,28±0,48	12,75±0,11	12,83±0,24	13,18±0,2	12,82±0,28	12,67±0,35	12,87±0,18	13,42±0,37
PLQ	566±53,27	558,2±44,6	568,5±28,5	557,8±37,7	575,3±32,1	577±29,49	553,9±35,1	555,5±30,41

Values represent mean ± error of mean (ESM). n = 10 for each lot; Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. WBC: white blood cell; RBC: red blood cells; NEU: neutrophils EOS: eosinophils; LYM: Lymphocytes; MON: monocytes; HGB: hemoglobins; HCT: hematocrits; PLT: platelets.

Effect of ETAAC on biochemical parameters

The treatment of the rats at the different doses did not cause a significant difference between the biochemical parameters of the treated rats compared to the controls after 28 days of treatment (Table III). As for the 60-day treatment, renal workup (uric acid, creatinine and urea) revealed a significant decrease in urea at a dose of 400 mg/kg of bw. At the level of the hepatic assessment (ASAT and ALAT), a dose dependent decrease marked by a significant difference at the dose of 400 mg/kg of bw. As for the cardiac assessment (total cholesterol and HDL cholesterol), no significant difference was observed in the controls compared to the treated (Table 3).

Table 3: Effect of *Alchornea cordifolia* on biochemical parameters in rats

Treatment / Parameters	28 days				60 days			
	T_0	T_{100}	T_{200}	T_{400}	A_0	A_{100}	A_{200}	A_{400}
GLY (g/l)	1,26±0,01	1,13±0,03	1,17±0,05	1,07±0,04	1,34±0,11	1,30±0,07	1,25±0,11	1,46±0,18
URE (g/l)	0,37±0,01	0,34±0,01	0,33±0,01	0,34±0,01	0,35±0,02	0,32±0,01	0,32±0,01	0,27±0,03*
CREA(mg/l)	5,17±0,31	4,33±0,21	4,83±0,31	5±0,258	5±0,26	4±0,31	4,57±0,3	5,33±0,33
A Uric (g/l)	16,33±0,67	18,17±0,54	18,17±0,48	18,4±0,75	18,75±1,44	19,43±0,43	20,29±0,75	22,5±1,61
ASAT (g/l)	186,5±3,31	179±3,67	192±5,61	184,3±4,79	188,8±6,15	186,3±2,85	179,8±2,61	179±3,14
ALAT (UI/l)	62,17±1,82	55,67±2,14	69,33±2,39	67,83±1,65	67±2,07	66±1	64,25±2,2	59, 2±1,5*
CHOL (g/l)	0,62±0,02	0,65±0,02	0,66±0,02	0,68±0,01	0,64±0,02	0,63±0,01	0,69±0,02	0,65±0,03
HDL (g/l)	0,13±0,01	0,14±0,01	0,14±0,01	0,15±0,01	0,13±0,01	0,15±0,01	0,16±0,02	0,14±0,01

Values represent Mean ± error of mean (ESM). n = 10 for each lot; Treated groups were compared to control group using one-way ANOVA followed by Turkey test was used to compare against the control. GLY: blood sugar; URE=urea; CREA= creatinine; A Uric: Uric Acid; ASAT: Aspartate aminotransferase; ALAT: Alanine-Aminotransferase; CHOL: Cholesterol; HDL: Higth desity lipoprotein; *: significant difference $P<0,05$. *Subacute toxicity*: T_0: distilled water; T_{100}: 100 mg/kg of bw; T_{200}: 200 mg/kg of bw; T_{400}: mg/kg of bw. *Subchronic toxicity*: A_0: distilled water; A_{100}: 100 mg/kg of bw; A_{200}: 200 mg/kg of bw; A_{400}: mg/kg of bw.

Histological Examination

Observation of histological sections of the liver (Figure 3) and kidney (Figure 4) revealed no structural difference in the treated rats compared to the controls after 60 days of treatment.

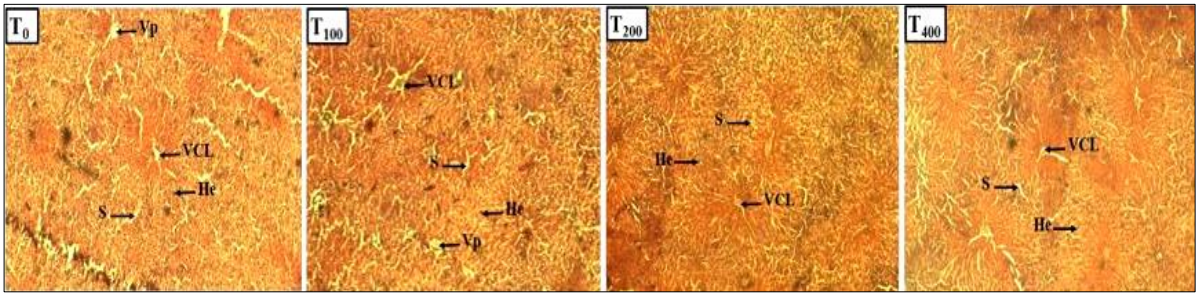

Fig 3: Micrograph of the liver of control rats and rats treated with the different doses of *Alchornea cordifolia* for 60 days
He: Hepatocyte; Vp: portal vein; S: Sinusoid; VCL: Centrilobular Vein; T_0: distilled water; T_{100}: 100 mg/kg of bw; T_{200}: 200 mg/kg of bw; T_{400}: mg/kg of bw.. Magnification: × 40; Stain: Hematoxylin eosin

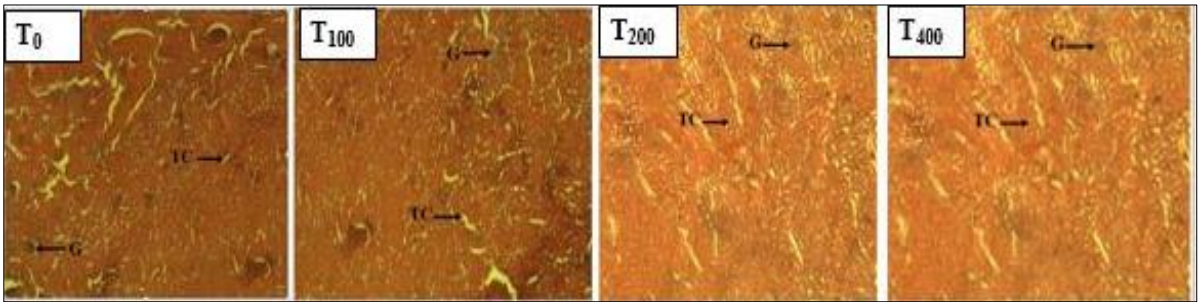

Fig 4: Micrograph of the kidney of control rats and rats treated with the different doses of *Alchornea cordifolia*
G: Glomerulus, TC: Bypassed tube, T_0: distilled water; T_{100}: 100 mg/kg of bw; T_{200}: 200 mg/kg of bw; T_{400}: 400 mg/kg of bw. Magnification: × 40; Stain: Hematoxylin eosin

Discussion

The assessment of the acute toxicity of the extract obtained from the leaves of *Alchornea cordifolia* to consist of measuring and recording the various adverse effects that appeared after administration of the tested substance [16]. In fact, in this study, the animals which received the limit doses of 2000 mg/kg of bw and 5000 mg/kg of bw from ETAAC did not show any change in behavior or signs of intoxication immediately after administration of the doses and during the observation period. Also, no case of death was recorded. At the end of the acute toxicity assessment test, the LD_{50} is therefore greater than 5000 mg/kg of bw orally. According to the OECD Globally Harmonized Classification System (GHS), ETAAC is classified as non-toxic orally [14]. This same method was used by [17] who showed that the LD_{50} of ETAAC is greater than 5000 mg/kg of bw. Evaluating the signs of clinical and / or biological intoxication linked to repeated use of medicinal plants is judicious, especially since the acute use of medicinal plants is rather rare in traditional medicine [18]. Thus after daily administration of ETAAC at doses of 100 mg/kg of bw, 200 mg/kg of bw and 400 mg/kg of bw for 28 days and 60 days, the comparison of the values was made between the treated rats and the controls. The absence of a significant difference between the evolution of the body weight of the rats shows that the ETAAC has no considerable effect on the weight gain of the treated animals, which is in line with the results of [19]. The relative organ weight which is considered to be a relatively sensitive indicator in toxicity studies [20] has not seen any significant change in the heart, liver, kidneys and lungs. ETAAC did not cause damage in the form of organ swelling, atrophy or hypertrophy in any of the organs removed from these treated

animals. These results corroborate with those of [21] and [22]. The hematopoietic system is one of the most sensitive targets for toxic substances. It represents an important marker of the physiological and pathological state of humans and animals [23, 24]. Thus, any change in haematological parameters has a predictive value for human intoxication, when the data is translated from studies performed on animals [25, 26]. In our study, no significant difference was observed in haematological parameters after 28 and 60 days of treatment with ETAAC. These results differ from those of [27] who observed a significant increase in the neutrophil count. This could be explained by the fact that these authors used a high dose (1000 mg/kg of bw) of a hydro-alcoholic extract for their study in mice. Analysis of the biochemical parameters did not show any significant difference between the treated rats and the control rats after 28 days of treatment. This shows that the extract was tolerated by the body. ETAAC would therefore not be toxic at the different doses studied after 28 days of treatment. These results are similar to those obtained by [22]. As for the biochemical analysis, after 60 days of treatment, some differences were significantly different. Indeed, the analysis of the function of the liver and the kidney is very important in the evaluation of the toxicity of drugs and plant extracts because they are necessary for the survival of an organism / Renal dysfunction can be assessed by measurements of urea, creatinine and uric acid [28-30]. In the present study, a dose-dependent increase in uric acid and creatinine levels was observed but no significant difference. As for urea, a dose-dependence reduction marked by a significant difference at the dose of 400 mg/kg of bw was observed. In view of these different analyzes, it appears that the aqueous extract of *Alchornea cordifolia* does not adversely affect the functioning of the kidneys. Nevertheless, urea is considered to be the main marker of nephrotoxicity, it is the more reliable predictor of renal function than creatinine and uric acid [31]. The decrease in this parameter shows that the kidneys were not affected. And this was confirmed by the observation of the histological structures of the kidneys of the different batches, which showed no sign of intoxication. As for transaminases (ALAT and ASAT), these are the main enzymes for assessing the state of liver function [32]. In general, ASAT and ALAT are enzymes of mitochondrial and cytoplasmic origin. And any cell necrosis, destruction of the hepatic parenchyma or an increase in the membrane permeability of hepatocytes leads to the flow of these enzymes into the bloodstream and therefore to an increase in their serum levels [28-30, 33]. In this study, a dose-dependent decrease was observed with a significant difference at the 400 mg/kg of bw dose in the ALT level. This would mean that the liver was not damaged. The extract therefore did not cause any abnormalities in liver functions, especially since observation of histological sections did not show any alteration in liver structure. These results are consistent with those reporting the hepatoprotective effect of *Alchornea cordifolia* [28-30, 34-36]. In addition to the liver and kidneys, which are the main organs for assessing toxicity, the heart can be observed. Cholesterols (total and HDL) are metabolites whose activities and concentrations make it possible to qualify the vitality of the heart [37]. In view of the absence of a significant difference in cholesterol levels, the extract of *Alchornea cordifolia* would be tolerated by the heart.

Conclusion

Oral administration of aqueous extracts of *Alchornea cordifolia* did not show any signs of acute, subacute or subchronic toxicity in rats at the doses studied. No significant difference was observed in the hematopoietic line, body weight and the weight of vital organs during the different treatments. These results indicate that the aqueous extract of *Alchornea cordifolia* is relatively non-toxic. It is accepted by the heart and does not cause any structural abnormalities in the liver and kidneys. This plant is therefore tolerated by the body, which explains its use by traditional healers. However, it would be wise to study the chronic toxicity of this extract, at higher doses in order to certify its safety.

References

1. Carillon E. La phytothérapie face à l'évolution médicale. Phytochimie 2000;1:10-15.
2. OMS. Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. Genève, Belgique 2002,65.
3. Handa SS, Rakesh DD, Vasisht K. Compendium of medicinal and aromatic plants. Asia. ics unido asia 2006;2:305
4. Boussahel S. Étude biochimique et histologique de l'effet de quelques extraits des plantes toxiques dans la région de Sétif Université Ferhat Abbes, Sétif, Algérie 2011,102.
5. Gnahoué G. Evaluation de l'activité physiologique et biochimique de Landalphia hirsute et de Trema guineesis sur le système cardio-vasculaire et sur l'activité contractile du duodenum de mammifères. Université de Cocody, Abidjan, Côte d'Ivoire 2009,160.
6. Kouakou K, Igor AS, Yapi A, Liliiya NK, Jutila MA, Quinn MT. Immunomodulator activity of polysaccharides isolated from Alchornea cordifolia. Journal Ethnopharmacology 2013;146(1):232-242.
7. Ajali U. Antibacterial activity of Alchornea cordifolia stem bark. Fitoterapia 2000;71:436-438.
8. Ogungbamila FO, Samuelsson G. Smooth muscle relaxing flavonoids from Alchornea cordifolia (leaves). Acta Pharmaceutica Nordica 1990;2:421-422.
9. Marva-Manga H, Brkic D, Marie DEP, Quetin-Leclercq J *In vivo* antiinflammatory activity of Alchornea cordifolia (Schumach & Thonn.) Mull. Arg. (Euphorbiaceae). J Ethnopharmacol 2004;92:209-214.
10. Agbor GA, Leopold T, Jeanne NY. The antidiarrhoeal activity of Alchornea cordifolia leaf extract. Phytotherapy Research 2004;18:873-876.
11. Olaley MT, Rocha JB. Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. Experimental Toxicology and Pathology 2008;59:319-327.
12. Ebi GC. Antimicrobial activities of Alchornea cordifolia. Fitoterapia 2000;72:69-72.
13. Atsamo AD, Nguelefack TB, Datté JY, Kamanyi A. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of Erythrina senegalensis DC (Fabaceae) in rodents. Journal of Ethnopharmacology 2011;134(1):697–702.
14. OCDE. Ligne directrice de l'OCDE pour les essais de produits chimiques: Toxicité orale aiguë - Méthode par classe de toxicité aiguë 2001;N°423:14.
15. OCDE. Repeated Dose Oral Toxicity Test Method. Guidelines for testing of chemicals, Paris 2008,327.
16. Leblanc GA, Acute toxicity, in A Textbook of Modern Toxicology, 4th ed. John Wiley & Sons. Inc (Hoboken NJ, Editor 2010,125-236p,
17. N'ga EN, Yinyang J, Bidias EBà, Etame-Loe G, Dibong SD. Étude phytochimique et pharmacologique

d'Alchornea cordifolia (Schum. & Thonn.) Mull. Arg. et de Mangifera indica dans le traitement traditionnel de la maladie hémorroïdaire J. Appl. Bio Sci 2017;109:10649-10661
18. Irie-n'guessan AG, Effo KE, Koua KBD, Kouakou SL, Djadji ATL, Adepo AA *et al*. La toxicité subaiguë de l'écorce de racines de Dichrostachys cinerea (L.) Wight et Arn. (Fabaceae). Int. J. Biol. Chem. Sci 2019;13(2):836-848.
19. Mohammed RK, Ibrahim S, Atawodi SE, Eze ED, Suleiman JE. Anti-diabetic and haematological effects of n-butanol fraction of Alchornea cordifolia leaf extract in streptozotocin-induced diabetic wistar rats. J Biol Sci 2013;2(3):45-53.
20. Lullmann-Rauch R. Histologie, De Boeck Supérieur, Bruxelles, Belgique 2008,704.
21. Ansah C, Oppong E, Woode E. Subacute oral toxicity assessment of Alchornea cordifolia (Schumach and thonn) mull arg (Euphorbiaceae) extract in rats. Tropical journal of pharmaceutical research 2011;10(5):587-594.
22. Ezeokeke EE, Ene AC, Igwe CU. Sub-acute toxicity studies of Alchornea cordifolia leaf extract in swiss albino rats. Journal of Analytical & Bioanalytical Techniques 2017;8(2):1-6.
23. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. Journal of Ethnopharmacology 2007;112:138-144
24. Li X, Luo Y, Wang L, Li Y, Shi Y, Cui Y, Xue M. Acute and subacute toxicity of ethanol extracts from Salvia przewalskii Maxim in rodents. Journal of Ethnopharmacology 2010;131:110-115.
25. Olson H, Betton G, Robinso D, Thomas K, Monro A, Kolaja G *et al*. Heller A. Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. Regulatory Toxicology and Pharmacology 2000;32:56-67
26. Rhiouani H, El-hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of Herniaria glabra in rodents. Journal of Ethnopharmacology 2008;118:378-386
27. Ansah C, Duwiejua M, Oppong E, Woode E. Toxicity study on Alchornea cordifolia leaf extract in mice. Journal of science and technology 2009;29(1):8-16.
28. Davis MEB ND. Renal methods for toxicity, in Hayes AWC (ed) Principles and Methods of Toxicology, 3rd ed Raven Press, New York, NY, USA 1994.
29. Gregg LV, Voigt DVM. Hematology techniques and concepts for veterinary technicians. USA: Iowa State University Press 2000,95-101.
30. Klaassen CD, Casarett LJ, Doull J. Casarett and Doull's Toxicology: The Basic Science of Poisons, McGraw-Hill Press, New York, NY, USA 2001.
31. Palani S, Raja R, Kumar P, Jayakumar S. Therapeutic efficacy of Pimpinella tirupatiensis (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. International Journal Pharm. Tech 2009;1(3):45-50.
32. Wallace AD, Meyer SA. Hepatotoxicity. In: A Textbook of Modern Toxicology. 4th ed., John Wiley & Sons. Inc (Hoboken, New Jersey) 2010,277-290.
33. Wolf PL, Williams D, Tsudaka T, Acosta L. Methods and Techniques in Clinical Chemistry, John Wiley & Sons, USA 1972,516
34. Arhoghro EM, Ikeh CH, Eboh AS, Angalabiri-Owei B. Liver function of wistar rats fed by the combined ethanolic leaf extract of Alchornea cordifolia and Costus afer in paracetamol-induced toxicity. WJPR 2015;4(5):1-12.
35. Olaleye MT, Kolawole AO, Ajele JO. Antioxidant Properties and Glutathione S Transferases Inhibitory Activity of Alchornea cordifolia Leaf Extract in Acetaminophen Induced Liver Injury. IJPT 2007;6:63-66.
36. Olaleye MT, Adegboye OO, Akindahunsi AA. Alchornea cordifolia extract protects wistar albino rats against acetaminophen-induced liver damage. Afr J Biotechnol 2006;24(5):2439-2445.
37. Coulibaly FA, Coulibaly A, N'Guessan JD, Kouame KG, Djaman AJ, Guina FG. Etudes des paramètres sériques biochimiques: le cas des lapins (Néozelandais – cunistar) de Côte d'Ivoire. Sciences et Nature 2007;4:37-43.