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## Triphytochemistry and toxicology of the total aqueous extract of *Clerodendrum splendens* (G. Don) leaves (Lamiaceae) in the Wistar rat

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**Abstract**

*Clerodendrum splendens* (Lamiaceae) is a plant with therapeutic properties, traditionally used in several countries for the treatment of several diseases. The aim of this study was to evaluate the toxicity of the total aqueous extract obtained from the leaves of *C. splendens* in order to contribute to a better knowledge of the therapeutic effects of this plant. It consisted of a phytochemical study which was carried out according to the classical method used in natural substance chemistry for the search of secondary metabolites. It revealed the presence of polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponosides. Acute toxicity, which was carried out by oral administration of ETAC to rats, showed that the LD50 was greater than 5000 mg/kg bw. For subacute toxicity, there was no significant difference in the body weights of treated rats compared to controls. Histological sections showed no structural or functional abnormalities in the thymus, spleen and kidney.

**Keywords:** *Clerodendrum splendens*, triphytochemistry, toxicity

**Introduction**

According to the World Health Organisation (WHO), medicinal plants are the best sources of medicines. Indeed, nearly 60% of the world's population uses herbal medicines as pharmaceutical remedies. In addition, 80% of the population in Africa still uses traditional medicine to meet their health care needs. This shows that plants continue to play an important role in the maintenance of human health since ancient times [1]. In addition, medicinal plants produce a wide range of different bioactive molecules that are involved in the development of new drugs. Therefore, such plants should be studied to better understand their properties and efficacy [2]. The study of plant chemistry is still relevant despite its long history. This is mainly due to the fact that the plant kingdom represents an important source of basic medicines. The Ivorian territory, due to its geographical position, presents a wide range of rich vegetation and various bioclimatic stages of plants used as condiments, natural foods and for therapeutic purposes. Among these medicinal plants, those belonging to the genus *Clerodendrum* (Lamiaceae), are widely used in the treatment of various diseases, chronic and acute disorders. Extracts obtained from the roots, leaves and bark of *Clerodendrum splendens* (Lamiaceae) are used to treat malaria, coughs, venereal infections including gonorrhoea and syphilis, skin diseases, ulcers, rheumatism, asthma, and uterine fibroids [3, 4, 5]. Most of the plant species that grow all over the world have therapeutic properties, as they contain active ingredients that act directly on the body. However, the geographical location of a plant can affect its active component. A plant growing wild in one country may not necessarily have the same components as the same plant in another country, and their biological activity may not be similar. This is why we undertook the study of the triphytochemistry and toxicity of *C. splendens* from the lagoon region. Considering the immunomodulatory effect of some *Clerodendrum* species, the antibacterial effect of *C. splendens* extract [6, 7], as well as the rich content of this plant in secondary metabolites and its anti-inflammatory effect [3, 4], we oriented our study to verify a possible toxic effect of the total aqueous extract of *Clerodendrum splendens* leaves with a view to contributing to the valorisation of this species.

**Material and Methods****Material****Plant Material**

The plant material consists of *Clerodendrum splendens* (Lamiaceae) leaves collected in June, in the SICOI 1 district, on the Bingerville-Abidjan axis (Côte d'Ivoire).

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### Animal Material

White albino male and female Wistar rats were used for the acute and sub-acute toxicity study. These animals came from the animal house of the Life and Earth Sciences Laboratory of the Ecole Normale Supérieure (ENS) in Abidjan. They were maintained under standard lighting conditions (12 hours light, 12 hours dark) at an ambient temperature of  $25 \pm 1$  °C. These animals had free access to standard food and tap water.

### Methods

#### Preparation of the extract

The harvested leaves of *Clerodendrum splendens* were cut into small pieces and dried at room temperature for two (2) weeks. After drying, they were ground to obtain a fine vegetable powder. The total aqueous extract was prepared according to the method described by Guédé-Guina<sup>[8]</sup> with slight modifications. Thus, 50 grams of plant powder were dissolved in one litre of distilled water. The aqueous mixture was stirred with a Blender-type mixer for three minutes and the operation was repeated three times. The homogenate obtained was filtered three times on square cloth. The filtrate obtained was purified four times on cotton wool and once on Whatman paper (3 mm). The new filtrate was evaporated at 50 °C using an oven (Memmert, Germany) to obtain the total aqueous extract of *C. splendens* (ETAC).

#### Qualitative analysis of the total aqueous extract of *C. splendens* (ETAC)

The main chemical groups were searched using characterisation techniques<sup>[9]</sup>. This consisted of searching for alkaloids, saponosides, flavonoids, quinone substances, polyphenols, polyterpenes, sterols and tannins.

#### Acute toxicity

Acute toxicity was performed according to guideline 423<sup>[10]</sup>. Nine (9) healthy, nulliparous, non-pregnant adult rats aged 8 to 12 weeks and weighing on average 150 to 200 grams were used. These rats were divided into three (3) batches of three (3) rats each. They were then acclimatised to laboratory conditions for one week before the experiment. Referring to the OECD guidelines, the dose of 2000 mg/kg bw was used. The rats in lot 1 (control lot) were each given 1 mL of distilled water orally via a gavage cannula. The animals in batch 2 were each given 1 mL of ETAC at a dose of 2000 mg/kg bw orally (gavage) via cannula in a single dose. After the administrations, the six (6) rats were again deprived of food for 3 to 4 hours. The rats were observed individually for the first thirty (30) minutes and then regularly for 24 hours. Thereafter, observations were made daily for a period of 14 days. After treatment, observations were made on fur, eye colour, salivation, lethargy, sleep, coma, tremor, diarrhoea, morbidity, mortality, heart rate, food and water intake. The number of deaths and/or moribund rats determined the continuation of the test. After 14 days of observation, if there are one or two deaths, the test is repeated with the lower dose. If there are no deaths and/or moribund rats then a confirmation test is performed with the same dose before repeating the test with the higher dose (5000 mg/kg bw). The confirmatory test consists of administering the same dose (2000 mg/kg bw) to batch 3 rats, which will be treated under the same conditions as before and then observed regularly for 14 days.

#### Subacute toxicity

The OECD (2008) guideline 407 for the testing of chemicals, with some modifications, was used for this study. Thirty-two (32) rats aged eight (8) to twelve (12) weeks with weights ranging from 150 to 200 grams on average were used. The rats were randomly divided into four (4) batches of eight (8) rats each according to their weight. Each batch consisted of four (4) male and four (4) female animals divided into different bins according to sex. In each batch, the number and sex of the animals were marked on the bins. Rats in lot T (control lot) were each given 1 ml of distilled water by gavage daily during the 28 days of treatment. The three (3) test batches (1; 2 and 3) received 125 mg/kg bw, 250 mg/kg bw and 500 mg/kg bw of ETAC daily by gavage for 28 days. The volume of ETAC administered daily as a single dose was 1 mL. During the 28 days of treatment, animals were observed daily for clinical signs and symptoms of toxicity before, immediately and three (3) hours after ETAC administration.

On the 29th day, the day after the last day of treatment, all rats were sacrificed using the decapitation technique. In this technique, after anaesthetising the rats with ether, their necks were cut with sterile cutting scissors. After each section, the blood was immediately collected in dry tubes and EDTA (ethylene diamine tetra acetic acid) tubes for the following respective analyses: biochemical and haematological. Organs such as liver, kidney, heart, thymus, lung and spleen were removed, cleaned with saline and weighed. The liver and kidneys were preserved in 10% formalin for histological sections.

#### Relative weight of organs removed

The relative weight of the organs (Rw) removed is obtained using the following formula:

$$Rw(g) = \frac{Aw(g)}{Bw(g)} \times 100g$$

With: Bw: Body weight of the animal; Aw: Absolute weight of the organ

#### Determination of haematological parameters

Haematological analysis was performed on the blood contained in EDTA tubes using an automatic analyser (URIT®-2900 PLUS) which gave direct values for the following erythrocyte parameters: The number of red blood cells (RBCs), haemoglobin (Hb), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT) were determined.

#### Determination of biochemical parameters

The blood in the dry tubes was centrifuged using a centrifuge at 3000 rpm for 5 minutes. The serum obtained was collected and stored at -20°C for serum marker analysis using the ROBONIK® PRIETES TOUCH analyser.

#### Histopathology examination

For histopathology examination, organs removed and fixed in formalin underwent a series of dehydration in ethanol baths followed by a series of thinning in toluene baths. After these steps, the organs were impregnated in paraffin baths followed by block formation. Sections of 5µm were made with a Leica

RM2125 RTS microtome (Germany) and then stained with Haematoxylin and Eosin (H&E) and observed under a light microscope (Olympus CKX41, Germany) <sup>[11]</sup>.

### Statistical analysis

Statistical analysis and graphical representations were carried out using Graph Pad Prism version 8.02. The values are presented as mean  $\pm$  standard deviation. The comparison of means and variances was carried out by the ANOVA test followed by the Tukey test.

## Results and Discussion

### Results

#### Qualitative analysis of the total aqueous extract of *C. splendens* (ETAC)

The phytochemical study of the total aqueous extract of the leaves of *Clerodendrum splendens* (ETAC) revealed the presence of polyterpenes, polyphenols, flavonoids, catechic tannins, quinone substances, alkaloids and saponosides. On the other hand, it revealed an absence of gall tannins.

#### Acute toxicity

Following the test with the 2000 mg/kg bw dose, which did not cause death or any signs of toxicity, the 5000 mg/kg bw dose of ETAC was used and administered as a single oral dose to Wistar rats. The results indicate that the administration of ETAC to female rats at single doses of 2000 and 5000 mg/kg bw did not result in death during the 14 days observation. The treated animals showed no signs of mortality and no apparent toxicity during the experimental study. They showed no clinical signs of abnormality during their macroscopic examination of the skin, coat, eyes and mucous membranes. Signs of tremors, salivation, diarrhoea, coma, coughing and drowsiness were not recorded either. All rats survived the 14-day observation period. Therefore, the LD50 is estimated to be greater than 5000 mg/kg bw (LD50 > 5000 mg/kg bw).

### Subacute toxicity

#### Effect of ETAC on the body weight of rats and their organs

Figure 1 shows the rate of change in body weight of animals treated for 28 days with ETAC at daily doses of 125, 250 and 500 mg/kg bw. During the 28 days of treatment, there was an increase in body weight in both treated and untreated animals. From day 1 to day 28, the increase in growth rate of the experimental batches was not significant compared to that of the control animals. The organ weights (heart, lungs, liver, kidneys, thymus and spleen) of animals from the control and treated batches taken on the day following day 28 of treatment are shown in Table I. No significant ( $p > 0.05$ ) changes in weight were observed compared to the organ weights of the control animals. The same is true for the relative organ weights.

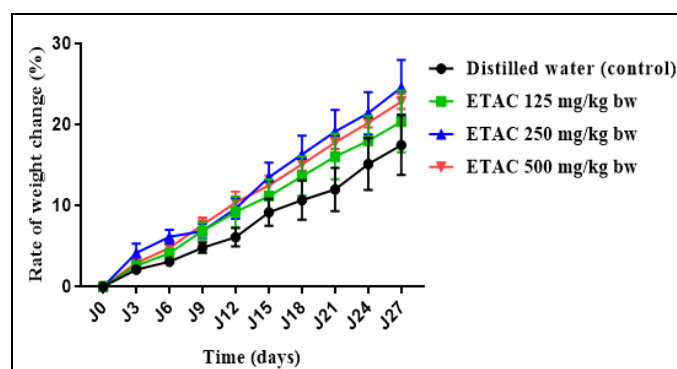


Fig 1: Effect of total aqueous leaf extract of *C. splendens* on the growth rate of rats during 28 days of oral treatment.

Values are means  $\pm$  standard errors, each batch consists of 8 animals ( $n=8$ /batch), ETAC: Total aqueous leaf extract of *Clerodendrum splendens*

Table I: Effect of ETAC on organ weights in rats after 28 days of oral treatment

Organs	Organs weight (g)				Relative weight of organs (g)			
	Distilled water (control)	ETAC 125 (mg/kg bw)	ETAC 250 (mg/kg bw)	ETAC 500 (mg/kg bw)	Distilled water (control)	ETAC 125 (mg/kg bw)	ETAC 250 (mg/kg bw)	ETAC 500 (mg/kg bw)
Heart	0,71 $\pm$ 0,08	0,59 $\pm$ 0,05ns	0,83 $\pm$ 0,04ns	0,83 $\pm$ 0,03ns	0,37 $\pm$ 0,01	0,37 $\pm$ 0,01ns	0,42 $\pm$ 0,01 ns	0,42 $\pm$ 0,01 ns
Lungs	1,70 $\pm$ 0,11	1,46 $\pm$ 0,11ns	1,86 $\pm$ 0,04ns	1,88 $\pm$ 0,02ns	0,92 $\pm$ 0,03	0,94 $\pm$ 0,02ns	0,93 $\pm$ 0,01ns	0,95 $\pm$ 0,01 ns
Liver	6,63 $\pm$ 0,76	5,07 $\pm$ 0,75ns	7,88 $\pm$ 0,33ns	8,04 $\pm$ 0,26ns	3,48 $\pm$ 0,14	3,17 $\pm$ 0,28ns	3,92 $\pm$ 0,06 ns	4,03 $\pm$ 0,11 ns
Spleen	0,68 $\pm$ 0,08	0,52 $\pm$ 0,07ns	0,73 $\pm$ 0,04ns	0,74 $\pm$ 0,03ns	0,35 $\pm$ 0,03	0,33 $\pm$ 0,03ns	0,36 $\pm$ 0,01 ns	0,37 $\pm$ 0,01 ns
Thymus	0,47 $\pm$ 0,05	0,34 $\pm$ 0,02ns	0,47 $\pm$ 0,04ns	0,48 $\pm$ 0,01ns	0,25 $\pm$ 0,01	0,22 $\pm$ 0,24ns	0,23 $\pm$ 0,02 ns	0,24 $\pm$ 0,00 ns
A. gland	0,05 $\pm$ 0,01	0,04 $\pm$ 0,01ns	0,06 $\pm$ 0,00ns	0,05 $\pm$ 0,00ns	0,03 $\pm$ 0,00	0,03 $\pm$ 0,00ns	0,03 $\pm$ 0,00 ns	0,03 $\pm$ 0,00ns
Kidneys	0,96 $\pm$ 0,09	0,80 $\pm$ 0,07ns	1,05 $\pm$ 0,03ns	0,94 $\pm$ 0,02 ns	0,51 $\pm$ 0,02	0,51 $\pm$ 0,02ns	0,52 $\pm$ 0,01 ns	0,47 $\pm$ 0,01 ns

Values are means  $\pm$  standard errors, each batch consists of 8 animals ( $n=8$ /batch). Statistical analyses are done per column compared to the control lot. ETAC: Total aqueous leaf extract of *C. splendens*, A. gland: Adrenal gland, ns: There is no significant difference at  $p>0.05$

#### Effect of ETAC on platelets and erythrocyte parameters

Platelets and erythrocyte parameters such as red blood cells (RBCs), haemoglobins, haematocrit, mean corpuscular haemoglobin volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and procalcitonin (PCT) were determined. The effect of total aqueous extract of

*Clerodendrum splendens* (ETAC) on these parameters in treated and untreated rats is presented in Table II. These results showed that daily administration of 125, 250 and 500 mg/kg bw for the 28 days did not significantly alter erythrocyte and platelet parameters compared to the control lot ( $p > 0.05$ ).

**Table II:** Hematological profile of rats treated with different doses of ETAC orally for 28 days

	Batches			
	Distilled water (control)	ETAC 125 (mg/kg bw)	ETAC 250 (mg/kg bw)	ETAC 500 (mg/kg bw)
Red blood cells ( $\times 10^6/\mu\text{L}$ )	7,02 $\pm$ 0,18	6,95 $\pm$ 0,21 ns	7,19 $\pm$ 0,13 ns	7,10 $\pm$ 0,17 ns
Platelets ( $\times 10^3/\mu\text{L}$ )	847,0 $\pm$ 39,82	853,2 $\pm$ 29,05 ns	904,0 $\pm$ 38,98 ns	864,2 $\pm$ 27,22 ns
Haemoglobin (g/dL)	12,43 $\pm$ 0,28	12,44 $\pm$ 0,19 ns	12,59 $\pm$ 0,18 ns	12,55 $\pm$ 0,28 ns
Haematocrit (%)	44,39 $\pm$ 0,27	44,43 $\pm$ 0,33 ns	44,69 $\pm$ 0,38 ns	45,55 $\pm$ 0,83 ns
MGV (fL)	56,15 $\pm$ 0,94	56,37 $\pm$ 2,15 ns	60,05 $\pm$ 2,99 ns	62,35 $\pm$ 1,49 ns
MCHT (pg)	17,41 $\pm$ 0,32	17,39 $\pm$ 0,12 ns	17,54 $\pm$ 0,34 ns	17,60 $\pm$ 0,39 ns
MCHC (g/dL)	26,88 $\pm$ 0,13	26,84 $\pm$ 0,15 ns	27,01 $\pm$ 0,14 ns	26,90 $\pm$ 0,08 ns
PCT (ng/mL)	0,68 $\pm$ 0,01	0,68 $\pm$ 0,01 ns	0,69 $\pm$ 0,01 ns	0,71 $\pm$ 0,02 ns

Values are means  $\pm$  standard errors, each batch consists of 8 animals (n=8/batch). Statistical analyses are done by column compared to the control lot. ns: There is no significant difference at  $p > 0.05$ , ETAC: Total aqueous extract of *Clerodendrum splendens* leaves, MGV: Mean corpuscular volume, MCHT: Mean corpuscular haemoglobin content, MCHC: Mean corpuscular haemoglobin concentration, PCT: Procalcitonin

### Effect of ETAC on the biochemical profile of healthy rats

The biochemical parameters assessed after 28 days of oral treatment were the liver profile (total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase), the kidney profile (urea and creatinine), the lipid profile (total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) as well as the blood glucose level (table

III). The results show that daily administration of 125, 250 and 500 mg/kg bw for the 28 days did not significantly alter the liver, kidney and lipid profiles compared to the control lot ( $p > 0.05$ ). Blood glucose levels in rats treated with 125, 250 and 500 mg/kg bw were increased, but this increase was not significant compared to the control lot ( $p > 0.05$ ).

**Table III:** Biochemical profile of rats treated with different doses of ETAC orally for 28 days

Biochemical parameters	Batches				
	Distilled water (control)	ETAC 125 (mg/kg bw)	ETAC 250 (mg/kg bw)	ETAC 500 (mg/kg bw)	
TP (g/L)	63,17 $\pm$ 1,08	63,00 $\pm$ 1,65 ns	64,50 $\pm$ 0,65 ns	64,00 $\pm$ 1,08 ns	
ASAT (UI/L)	187,7 $\pm$ 7,07	195,5 $\pm$ 4,36 ns	188,5 $\pm$ 6,44 ns	187,3 $\pm$ 6,90 ns	
Liver profile	ALAT (UI/L)	44,33 $\pm$ 1,80	45,83 $\pm$ 1,20 ns	44,25 $\pm$ 2,32 ns	44,50 $\pm$ 0,65 ns
	PAL (UI/L)	301,7 $\pm$ 5,55	302,5 $\pm$ 24,55 ns	292,0 $\pm$ 5,40 ns	306,5 $\pm$ 4,57 ns
	Creatinine (mg/L)	3,50 $\pm$ 0,22	3,83 $\pm$ 0,48 ns	5,00 $\pm$ 1,23 ns	4,75 $\pm$ 0,63 ns
Renal profile	Urea (mg/L)	0,23 $\pm$ 0,00	0,24 $\pm$ 0,00 ns	0,24 $\pm$ 0,00 ns	0,25 $\pm$ 0,01 ns
	TC (g/L)	0,62 $\pm$ 0,03	0,65 $\pm$ 0,03 ns	0,68 $\pm$ 0,04 ns	0,62 $\pm$ 0,02 ns
Lipid profile	TG (g/L)	0,66 $\pm$ 0,05	0,74 $\pm$ 0,02 ns	0,74 $\pm$ 0,03 ns	0,66 $\pm$ 0,01 ns
	HDL-c (g/L)	0,16 $\pm$ 0,01	0,17 $\pm$ 0,01 ns	0,18 $\pm$ 0,01 ns	0,16 $\pm$ 0,01 ns
	LDL-c (g/L)	0,25 $\pm$ 0,01	0,25 $\pm$ 0,02 ns	0,24 $\pm$ 0,018 ns	0,25 $\pm$ 0,02 ns
Blood glucose	Glucose (g/L)	1,33 $\pm$ 0,08	1,37 $\pm$ 0,09 ns	1,52 $\pm$ 0,04 ns	1,56 $\pm$ 0,07 ns

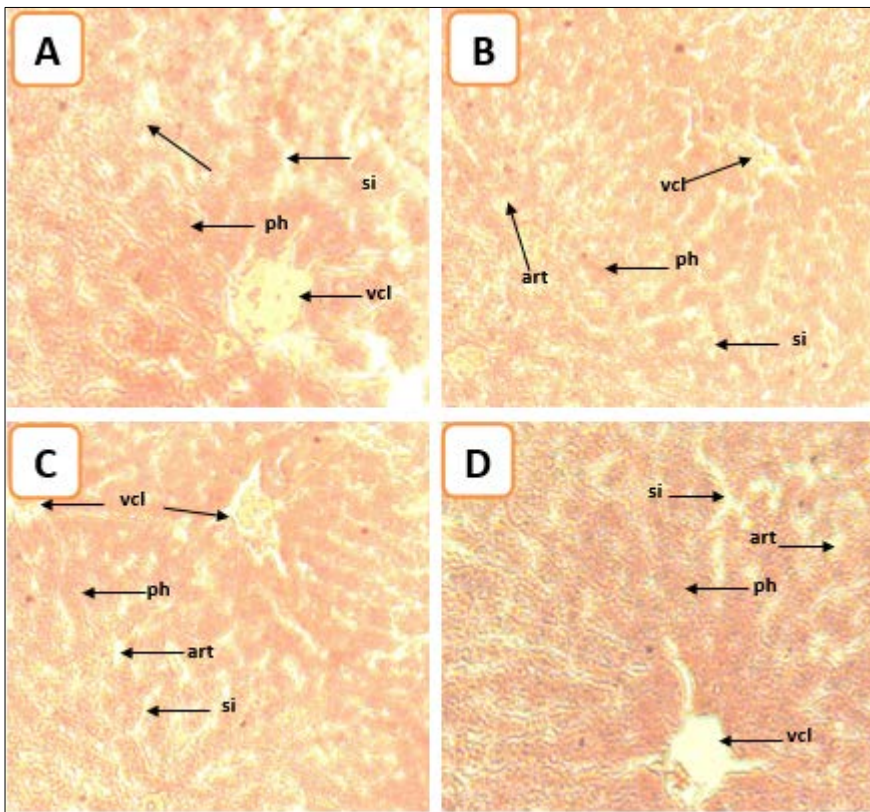
Values are means  $\pm$  standard errors, each batch consists of 8 animals (n=8/batch). Statistical analyses are done by column compared to the control lot. ns: There is no significant difference at  $p > 0.05$ , TP: total protein, ASAT: aspartate aminotransferase, ALAT: alanine aminotransferase, PAL: alkaline phosphatase, TC: total cholesterol, TG: triglycerides, HDL-c: HDL-cholesterol, LDL-c: LDL-cholesterol, ETAC: Total aqueous leaf extract of *Clerodendrum splendens*

### Histology of the liver and kidneys

Observation of the liver sections of the rats from the different batches revealed the presence of a faintly stained inner zone called the centrolobar vein, surrounded by a dense outer layer, the hepatic parenchyma, which surrounds the sinuses and arteries (Figure 2). These different structures are present in both control and treated patients. The different doses of ETAC had no effect on this organ. Observation of kidney sections from rats of different batches revealed the presence

of the glomerulus, which is the site of the initial filtration of blood coming from the afferent arterioles. The glomerulus is covered by a capsule called Bowman's capsule. The tubules (distal and proximal) are the site of control of the concentration and composition of blood returning to the general circulation, as well as the concentration and content of the final urine (Figure 3). These different structures are present in both control and treated patients. The different doses of ETAC did not damage this organ.





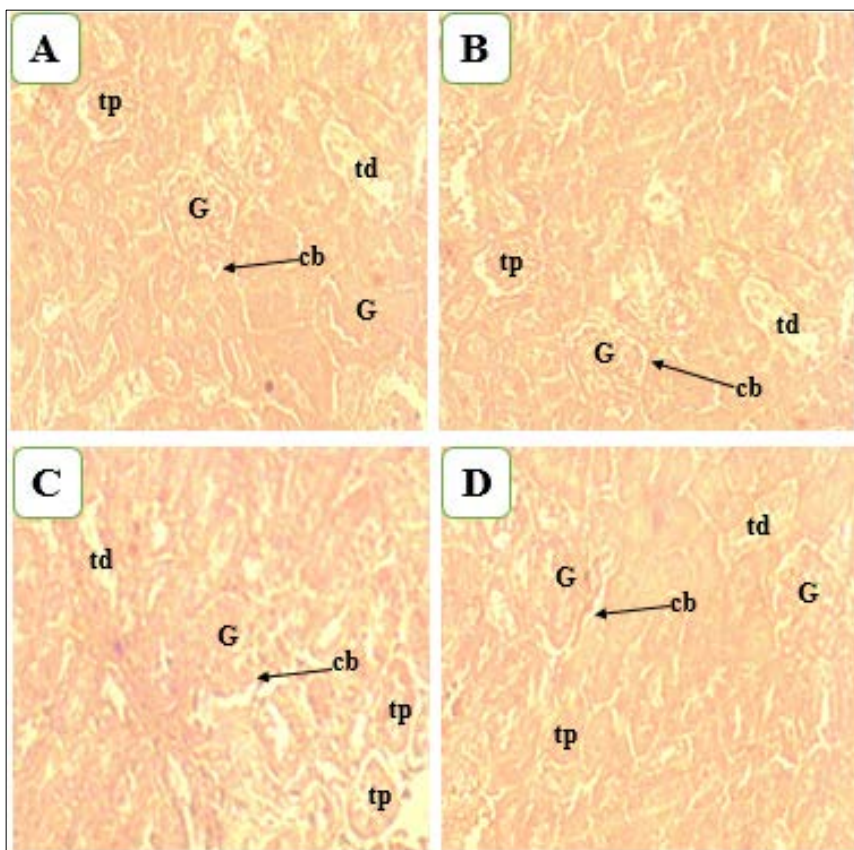
**Fig 2:** Histological sections of liver from treated rats

Hematoxylin & Eosin; G× 100

A: control rat liver, B: rat liver treated with 125 mg/kg bw ETAC, C: rat liver treated with 250 mg/kg bw ETAC, D: rat

liver treated with 500 mg/kg bw ETAC

ph: liver parenchyma; vcl: centrolobar vein; si: sinus; art: artery



**Figure 3:** Histological sections of kidney from treated rats

Hematoxylin & Eosin; G× 100

A: control rat kidney, B: rat kidney treated with 125 mg/kg bw ETAC, C: rat kidney treated with 250 mg/kg bw ETAC,

D: rat kidney treated with 500 mg/kg bw ETAC. G: Glomerulus; cb: Bowman's capsule tp: Proximal tubule; td: Distal tubule

## Discussion

Phytochemical analysis of the total aqueous leaf extract of *C. splendens* (ETAC) showed the presence of polyterpenes, polyphenols, flavonoids, catechic tannins, quinone substances, alkaloids and saponosides. The evaluation of the phytochemical content of ETAC showed that it is low in gall tannins. These results are in line with those of Donatus and Friday [12]. Indeed, these authors also showed that *C. splendens* contains alkaloids, flavonoids, tannins, saponins and phenols that have antibacterial properties. Earlier work by Scortichini and Pia [13] on the leaves of *C. splendens* collected in Ghana at Asokore, showed the presence of tannins, phytosterols, terpenoids, flavonoids and traces of alkaloids. However, this work did not reveal the presence of polyphenols, quinone substances and saponosides. This difference in chemical composition could be explained by the fact that the harvesting places of the leaves analysed in these studies are different from the place (Abidjan in southern Côte d'Ivoire) where the leaves were harvested for this study. Differences in the agro-ecological characteristics of the soils could therefore justify the difference in chemical composition observed [14].

The acute toxicity study revealed that the single oral doses of 2000 and 5000 mg/kg bw of ETAC did not cause death in rats. In addition, no signs of toxicity were noted during the 14 days of observation. According to the OECD guideline 423 for chemicals, ETAC has a lethal dose 50 (LD50) greater than 5000 mg/kg bw. This OECD guideline does not indicate a precise LD50 value, the death of a portion of the animals remains the main indication, through which it is possible to determine an exposure range where lethality is expected [15]. According to the OECD Globally Harmonised System of Classification [10], ETAC is classified in category 5 and is considered to be a non-toxic substance by the oral route.

Evaluation of the effect of ETAC by daily administration of 125, 250 and 500 mg/kg bw did not result in death or clinical signs of toxicity in treated rats. Body weight is one of the most important parameters for the evaluation of early signs of toxicity [16]. Therefore, body weight monitoring was carried out throughout the study period. It was found that no significant changes in body weight were observed in the treated animals compared to the control lot. The weight growth rate of the animals was not significantly different ( $p > 0.05$ ) from the control. These results suggest that the secondary metabolites contained in ETAC do not have significant impacts on cell multiplication, on water imbibition due to water entry into the cells and also on lipid metabolisms, more precisely on lipid accumulation. Indeed, the increase in body weight of animals is most closely related to fat accumulation [17].

The present study showed that ETAC did not affect the absolute and relative organ weights of animals at doses ranging from 125 to 500 mg/kg bw during the 28 days of treatment. Changes in organ weights are also indicative of the toxicity of a substance administered to animals. There is a possibility that herbal products, when ingested into the body, may be toxic to vital organs such as the heart, lungs, kidneys, liver, spleen, thymus and stomach due to their diverse roles in the human body [18]. Indeed, the liver, kidneys and spleen are essential organs influenced by the metabolic response caused by substances [19]. In general, changes in body weight gain and organ weights reflect toxicity after exposure to toxic substances [20].

The haematopoietic system serves as an important target for substances and chemicals. This system is also a sensitive

index of disease states in both humans and animals [21]. The results of the present study showed that ETAC did not disturb the haematological profile.

Platelets and erythrocyte parameters such as red blood cells (RBCs), haemoglobins, haematocrit, mean corpuscular haemoglobin content (MCHC), mean corpuscular haemoglobin concentration (MCHC) and procalcitonin (PCT), did not vary significantly ( $p > 0.05$ ) compared to controls. Treatment of the animals with different doses of ETAC did not produce any alteration in the values of the erythrocyte parameters. This indicates that ETAC does not have a significant impact on these blood cells. Indeed, the insignificant changes in GMV and MCHF indicate that the morphology and osmotic fragility of red blood cells are not affected [22]. The relatively normal and constant haemoglobin values, haematocrit, MCHT and MCHF during the 28 days of treatment indicate a possible absence of substances with a haemolytic effect in ETAC [23]. This could justify the absence of anaemia in the rats treated during the experiment.

Biochemical analyses were carried out to assess the effect of aqueous total extract of *C. splendens* leaves (ETAC) on blood glucose and to further investigate the organs through serum markers of kidney, liver and heart. Indeed, organs such as the liver and kidney, because of their role in purifying the body [24] and their role in the metabolism of xenobiotics, are the preferred targets of certain toxic compounds in medical plants. Doses of 125, 250 and 500 mg/kg bw for the 28 days did not significantly alter the liver, kidney and lipid profiles compared to the control lot ( $p > 0.05$ ). Blood glucose levels in rats treated with 125, 250 and 500 mg/kg bw were increased, but this increase was not significant compared to the control lot ( $p > 0.05$ ). These results show that serum transaminase levels were not disturbed in this study. This implies that the liver and to a lesser degree the muscles were not affected. Indeed, transaminases (ASAT and ALAT) are good indicators of liver structure and function and biomarkers for predicting drug toxicity [25]. ALAT is a liver-specific cytosolic enzyme secreted in liver cells. It is released into the bloodstream in the event of hepatic cell necrosis [26], which makes it a very sensitive indicator of hepatotoxicity [27]. ASAT is also an indicator of hepatocyte destruction, although in addition to the liver it is found in the heart, skeletal muscle, lungs and kidneys [28]. Indeed, transaminase activities increase in the presence of liver toxicity [29] and under conditions that favour abnormal permeability of the hepatocyte membrane. The results of the study showed that the serum PAL level of the treated group did not significantly differ from the control group. This indicates that ETAC did not cause cholestatic hepatobiliary pathology through bile duct obstruction. Indeed, PAL is an enzymatic marker of the endoplasmic reticulum membrane and is also present in the cells lining the bile duct of the liver [30]. Therefore, increased serum PAL activities may indicate altered plasma membrane permeability and may also be an indicator of the onset of cholestatic diseases [31]. With regard to total protein, the increase in serum levels at the different doses (125, 250 and 500 mg/kg bw) was insignificant compared to the control lot. Indeed, this parameter reflects the nutritional status and is taken into account in the screening and diagnosis of renal pathologies, hepatic etc. Total protein levels are commonly used to report liver damage [18]. The renal profile (urea and creatinine) of rats treated with different doses of ETAC did not change significantly during the 28 days of treatment compared to the control lot. This may reflect the structural and functional integrity of the kidney. Indeed, the state of renal structure and



function is assessed by urea and creatinine levels. The results are in line with those of Mugisha and colleagues [32] who showed that urea and creatinine levels did not change significantly during 28 days of administration of the ethanolic fraction of *Anogeissus leiocarpa* (Combretaceae). Blood glucose levels were also not affected during treatment. This may suggest that ETAC does not have a hyper- or hypoglycaemic effect. Therefore, the blood glucose regulatory system was not affected [33]. The lipid profile (total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) of the animals treated with the different doses of ETAC did not vary significantly during the 28 days of treatment compared to the control lot. The insignificant variation in total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol could account for the good structural and functional state of the cardiovascular system. Indeed, this study is necessary to assess the risk of cardiovascular disease and coronary heart disease in individuals [34]. Elevated serum levels of these parameters predict the risk of cardiovascular disease [35]. Serum levels of total cholesterol, HDL-cholesterol and LDL-cholesterol are used in the assessment of cardiovascular lipid risk and in liver testing. As for triglycerides, their serum concentration is useful to assess the atherothrombotic risk, but also to assess the risk of acute pancreatitis in case of strong increase.

Oral administration of the different doses (125, 250 and 500 mg/kg bw) of ETAC to rats during the 28 days of treatment did not affect the level of lipid parameters. This suggests that lipid metabolism is not affected by ETAC. Indeed, if hepatic synthesis or plasma lipid degradation are not stimulated, there is no observable change in the levels of these parameters [36]. Observation of histological sections of the liver and kidney showed no structural changes in these organs in treated rats compared to controls. This result confirms the non-toxicity of ETAC on the organs studied.

### Conclusion

At the end of this study, the qualitative analysis of the total aqueous extract of *Clerodendron splendens* leaves (ETAC) showed the presence of polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponosides. With regard to acute toxicity, it showed that the total aqueous extract obtained from the leaves of *C. splendens* is not toxic by the oral route.

This allowed *C. splendens* (Lamiaceae) to be classified in category 5 of the OECD 423 guideline with an LD50 greater than 5000 mg/kg bw. With regard to subacute toxicity, it was found that the different doses (125, 250 and 500 mg/kg bw) of ETAC did not result in any significant differences in body mass and organ weights in the treated rats. This subacute toxicity showed that ETAC did not adversely affect the structure and function of the liver and kidney.

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