

Subchronic toxicity study of the extract of *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae) in wistar rats.

Abstract

Introduction: *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae) is a medicinal plant used in the treatment of Buruli ulcer in Ivory Coast. To ensure its effect over a long period of use, the subchronic toxicity of the total aqueous extract of *S. gabonensis* stem bark (ETASg) in rats was evaluated.

Methods: 80 rats were homogeneously distributed in 4 lots of 20 rats each, including 10 males and 10 females. ETASg was administered daily orally for 90 days for 2 mL/100 g body weight (b.w.) rats at doses of 3.5; 35 and 350 mg/kg b.w. The control group received distilled water. A venous blood sample is taken every 30 days for 90 days to determine serum biochemical parameters.

Results: The study showed that ETASg did not influence serum biochemical markers at the therapeutic dose of 3.5 mg/kg b. w. significant increases in ASAT serum activity, ALT, PAL, and LDH in rats tested at 35 and 350 mg/kg b. w. were found on the 60th and 90th days. Administration of ETASg did not affect most of the metabolites and electrolytes studied at doses of 3.5; 35 and 350 mg/kg b. w. After 30 days of discontinuing oral administration of ETASg, the increase observed at the 350 mg/kg b. w. is moderate and reversible.

Conclusion: This study revealed that ETASg is nontoxic for biochemical parameters, at doses of 3.5; 35 and 350 mg/kg b. w. during 90 days of administration in rats.

Key words: *Sacoglottis gabonensis*, subchronic toxicity, serum biochemical parameters, Buruli ulcer

1- Introduction

Medicinal plants, the main therapeutic sources in Africa, are used by more than 80% of populations [1]. Their empirical use exposes patients to risks of intoxication that can sometimes be fatal [2]. Studies have shown that 35% of cases of acute renal failure and liver damage are associated with the use of herbal medicines [3, 4]. *Sacoglottis gabonensis* (Baille) Urban is a medicinal plant found in both West and Central Africa [5]. It is used in traditional medicine in the treatment of various pathologies such as leprosy, hemorrhoids, abdominal pain, dental problems, infectious diseases and Buruli ulcer [6, 7, 8]. In Ivory Coast, Vangah et al. [9] revealed that the decoction of *S. gabonensis* stem bark is taken as a drink and locally applied in the treatment of Buruli ulcer. *S. gabonensis* has been the subject of several pharmacological studies. Koné et al. [10] showed the anti-mycobacterial activity on the *in vitro* growth of *Mycobacterium ulcerans* of the total aqueous extract of *S. gabonensis* stem bark (ETASg). In addition, ETASg has anthelmintic activity against *Heligmosomoides polygyrus* [11], and acute and subacute toxicity tests in mice and rats, respectively, have shown that ETASg has an LD₅₀ greater than 5000 mg/kg b. w. These tests also showed that it does not influence the biochemical parameters in rats for 28 days of administration [12].

Therefore, in order to ensure its safety over a long period of use and recommend it as an alternative way of treating Buruli ulcer in Ivory Coast, this research work aims to evaluate the subchronic toxicity of ETASg on serum biochemical parameters in albino wistar rats for 90 days.

2- Material and Methods

2-1- Plant material

The stem barks of *Sacoglottis gabonensis* were harvested in November 2016, in Ingrakon, in the department of Alépé (Ivory Coast), located 45 km from the city of Abidjan. A sample was identified and kept at the herbarium of the National Floristic Center of Abidjan (Ivory Coast), under the number 131.

2-2- Animal material

The experiments were conducted on albino rats of the species *Ratus norvegicus* strain Wistar. The rats were six to eight weeks old and weighed between 73 and 103 grams. They were raised in the animal house of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Nangui Agrogoua University according to good laboratory practice [13]. The rats were acclimated to a temperature of 22 ± 2 °C and an alternation of 12 hours of light and 12 hours of darkness. They were fed daily with IVOGRAIN® pellets and had access to water at will in their bottles.

2-3- Preparation of the stem barks total aqueous extract of *Sacoglottis gabonensis*

The total aqueous extract of *S. gabonensis* stem barks (ETASg) was obtained according to the method described by [12]. The fresh stem barks of *S. gabonensis* harvested were carefully cleaned and then dried in the laboratory for three weeks at room temperature of 25 ± 2 °C. After drying, these barks were reduced to a fine powder by means of an electric grinder (Culatti, France). Four hundred grams (400 g) of stem bark powder was put in 2 L of distilled water and the whole was boiled at 100 °C for 30 minutes. The decoction obtained was double-filtered on hydrophilic cotton and Whatman filter paper (n°1). The filtrates were evaporated and oven dried at 50 °C for 48 hours. A quantity of 31 g of black-brown powder with a yield of 7.75 % was obtained and stored in a refrigerator at 7 °C. until use.

2-4- Subchronic toxicity

It has been determined according to OECD Guideline 408 [13]. Eighty (80) rats were homogeneously distributed in 4 lots of 20 rats each. Each lot consisted of ten males and ten females. A volume of solution of 2 mL/100 g b. w. was administered to the rats orally. The control group (A) received distilled water. Batches B, C and D respectively received 3.5; 35 and 350 mg/kg b. w. ETASg for 90 days. The dose of 3.5 mg/kg b. w. was considered the therapeutic dose of the traditional practitioner. Additional satellite batches of ten rats (ten males and ten females) in the control group and ten rats in batch D were included in order to possibly observe the reversibility, persistence or late onset of toxic effects 30 days after stopping the administration.

2-5- Blood samples

Rats were fasted for 10 hours and then anesthetized with ether. 0.5 mL to 2 mL of blood was taken from the retro-orbital sinus of the eye using a sterile Pasteur pipette before the administration of either the extract or distilled water to rats [14]. Blood samples were taken every 30 days for 90 days

2-6- Biochemical tests

The venous blood contained in the dry tubes was centrifuged at 3000 rpm for 10 minutes using a centrifuge (HERAEUS SEPATECH). The serum was stored at -20 °C until the determination of the following biochemical parameters: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Urea, Creatinine, Glucose, total cholesterol, HDL-cholesterol, triglyceride, total protein, calcium, potassium, sodium and chlorine. The assay of blood biochemical parameters was performed with a ROPONIK® brand automaton and reagents from the BioSystems Costa Brava Laboratory (Spain).

2-7- Statistical Analysis

The results were expressed as averages followed by the standard error on means ($M \pm ESM$). The repeated two-way ANOVA variance analysis test with the mixed model associated with Bonferroni's post-hoc test was used to determine the statistical significance of the results. The different statistical tests were considered significant for a probability level p less than 0.05 ($p < 0.05$). Statistical analysis of the values and graphical representation of the data were performed using the GraphPad Prism software version 5.01 for Windows (GraphPad Software Inc., San Diego, MO, California / USA, 2007).

3- RESULTS

In this study, the effects of the extract on the various parameters did not vary according to the sex of the rats ($p > 0.05$).

3-1- Effects of total aqueous extract of *Sacoglottis gabonensis* stem bark on serum enzyme markers

3-1-1 Alanine Aminotransferase (ALT)

Figure 1 shows the influence of ETASg on serum activity of ALT. At day 30, ETASg did not influence serum ALT activity in rats administered at doses of 3.5; 35 and 350 mg/kg of b. w. compared to that of the control rats. In contrast, a significant increase ($p < 0.05$) was seen from day 60 in rats administered orally with ETASg at 350 mg/kg b. w. At day 90, a significant increase in ALT serum activity was observed in rats tested by ETASg at 35 mg/kg b. w. ($p < 0.05$) and 350 mg/kg b. w. ($p < 0.01$).

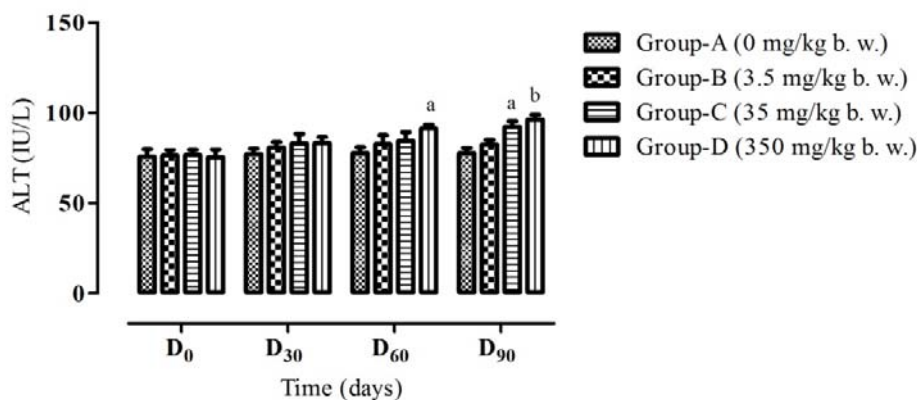


Figure 1: Effect of ETASg on serum ALT activity of rats as a function of time

Each bar represents the mean $\pm$ standard deviation; D₀: before the administration; D₃₀: 30 days of administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration; n = 20 rats/group; a = $p < 0.05$; b = $p < 0.01$.

3-1-2- Aspartate Aminotransferase (AST)

Oral repeated administration of ETASg in rats did not alter ($p > 0.05$) serum AST activity at all doses studied at day 30 of experimentation. However, rats receiving ETASg at 35 and 350 mg/kg b. w., showed significant increases at day 60 and day 90 compared with the control rats (Figure 2).

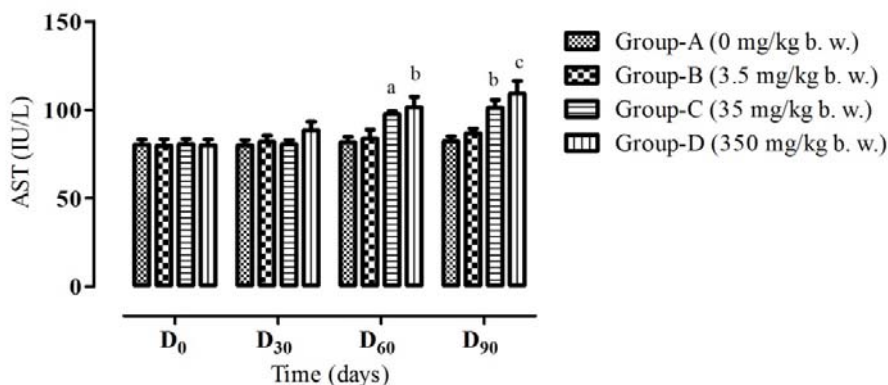


Figure 2: Effect of ETASg on serum AST activity of rats as a function of time

Each bar represents the mean $\pm$ standard deviation; J₀: before the administration; D₃₀: 30 days administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration; n = 20 rats/group; a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$

3-1-3- Alkaline phosphatase (ALP)

ETASg did not influence serum PAL activity at Day 30 and Day 60 in the rats tested with all dose levels compared to control rats (Figure 3). However, at day 90, ETASg has significantly ($p < 0.05$) induced the increase of serum PAL activity in rats administered at 350 mg/kg b. w.

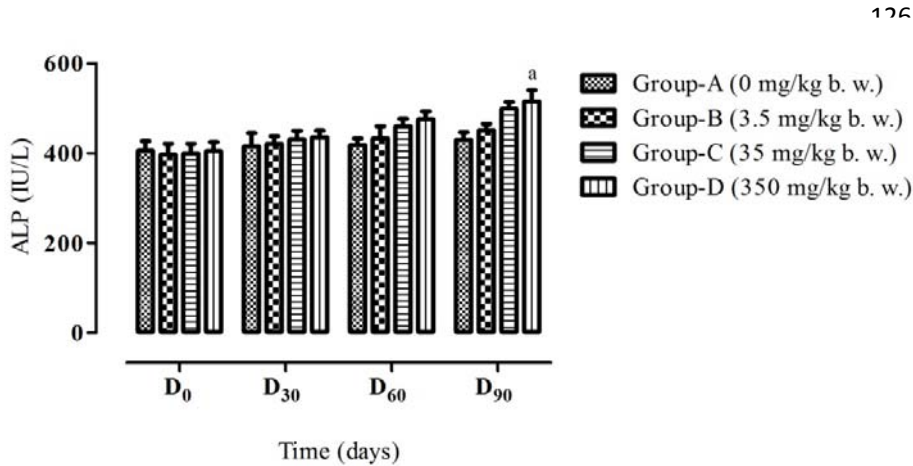


Figure 3: Effect of ETASg on serum activity of rat ALP as a function of time. Each bar represents the mean $\pm$ standard deviation; D₀: before the administration; D₃₀: 30 days of administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration; n = 20 rats/group; a = $p < 0.05$.

3-1-4- Lactate dehydrogenase (LDH)

Serum LDH activity did not change at day 30 and day 60 in rats tested with all dose levels compared to control rats. In contrast, EATSG at 350 mg/kg b. w. resulted in a significant increase ($p < 0.05$) of serum LDH activity at day 90 compared to control rats (Figure 4).

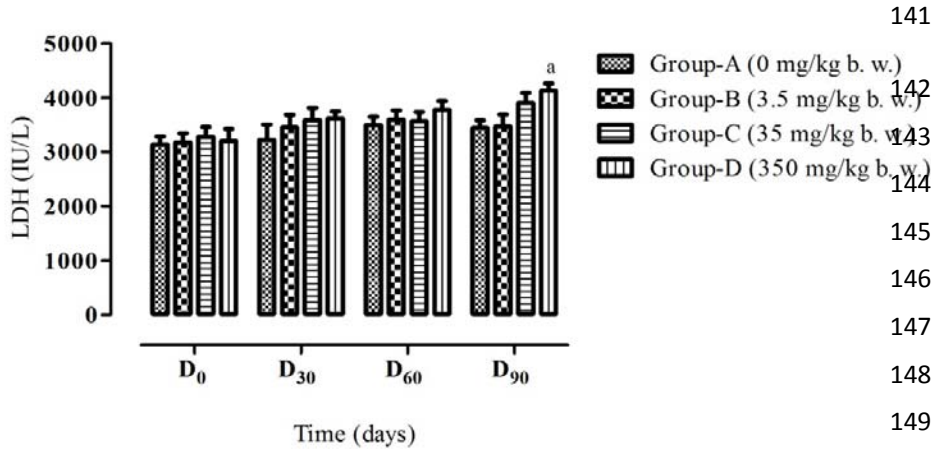


Figure 4: Effect of ETASg on serum LDH activity of rats as a function of time

Each bar represents the mean $\pm$ standard deviation; D₀: before the administration; D₃₀: 30 days of administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration; n = 20 rats/group; a = p < 0.05.

3-2- Effects of ETASg on plasma metabolites of rats

Repeated administration of ETASg by the oral route had no significant influence (p > 0.05) on urea, creatinine, glucose, total cholesterol, HDL-cholesterol, Triglycerides and total proteins at all dose levels tested in ETASg-tested rats compared to control rats during 90 days of testing (Table 1).

Table 1: Effects of ETASg on plasma metabolites

Serum metabolites	Doses (mg/kg b. w.)	Administration time (Days)			
		D ₀	D ₃₀	D ₆₀	D ₉₀
Urea (mg/dL) (NS)	0	33.51 $\pm$ 2.92	38.83 $\pm$ 1.86	40.92 $\pm$ 2.18	39.84 $\pm$ 2.43
	3.5	38.34 $\pm$ 2.02	41.39 $\pm$ 2.17	40.23 $\pm$ 1.91	45.97 $\pm$ 2.19
	35	34.85 $\pm$ 1.73	44.96 $\pm$ 2.06	45.43 $\pm$ 1.63	43.73 $\pm$ 2.92
	350	30.64 $\pm$ 1.37	45.84 $\pm$ 3.03	46.07 $\pm$ 2.21	45.56 $\pm$ 2.10
Creatinine (mg/dL) (NS)	0	0.71 $\pm$ 0.08	0.93 $\pm$ 0.03	0.94 $\pm$ 0.06	0.99 $\pm$ 0.06
	3.5	0.75 $\pm$ 0.05	0.85 $\pm$ 0.03	0.97 $\pm$ 0.04	0.99 $\pm$ 0.03
	35	0.74 $\pm$ 0.06	0.83 $\pm$ 0.03	1.09 $\pm$ 0.05	1.02 $\pm$ 0.03
	350	0.68 $\pm$ 0.06	0.95 $\pm$ 0.03	1.12 $\pm$ 0.09	0.99 $\pm$ 0.07
Glucose (mg/dL) (NS)	0	0.62 $\pm$ 0.41	0.54 $\pm$ 0.35	0.68 $\pm$ 0.03	0.96 $\pm$ 0.07
	3.5	0.54 $\pm$ 0.42	0.35 $\pm$ 0.24	0.69 $\pm$ 0.05	0.80 $\pm$ 0.07
	35	0.45 $\pm$ 0.24	0.52 $\pm$ 0.42	0.98 $\pm$ 0.20	0.86 $\pm$ 0.06
	350	0.34 $\pm$ 0.24	0.52 $\pm$ 0.37	0.84 $\pm$ 0.11	0.85 $\pm$ 0.07
Total cholesterol (mg/dL) (NS)	0	58.19 $\pm$ 3.95	58.34 $\pm$ 3.33	49.94 $\pm$ 4.27	61.39 $\pm$ 2.93
	3.5	55.67 $\pm$ 2.61	64.80 $\pm$ 4.02	57.67 $\pm$ 1.37	61.92 $\pm$ 2.72
	35	58.85 $\pm$ 2.87	55.52 $\pm$ 4.40	55.04 $\pm$ 3.03	59.82 $\pm$ 2.95
	350	53.55 $\pm$ 3.21	62.73 $\pm$ 3.56	52.18 $\pm$ 5.25	64.85 $\pm$ 2.84
HDL-Cholesterol (mg/dL) (NS)	0	55.46 $\pm$ 2.30	58.22 $\pm$ 2.88	65.25 $\pm$ 3.78	62.80 $\pm$ 1.63
	3.5	51.89 $\pm$ 2.51	69.32 $\pm$ 2.87	63.05 $\pm$ 4.77	64.28 $\pm$ 3.61
	35	54.13 $\pm$ 2.99	59.97 $\pm$ 2.74	64.83 $\pm$ 4.98	65.03 $\pm$ 2.44
	350	56.69 $\pm$ 2.51	61.22 $\pm$ 2.81	65.61 $\pm$ 3.09	64.38 $\pm$ 5.14
Triglycerides (g/L) (NS)	0	86.47 $\pm$ 2.11	85.59 $\pm$ 2.68	85.92 $\pm$ 2.58	85.73 $\pm$ 1.74
	3.5	88.59 $\pm$ 1.65	88.70 $\pm$ 1.91	87.01 $\pm$ 2.76	82.96 $\pm$ 2.17
	35	88.92 $\pm$ 1.83	85.63 $\pm$ 2.65	78.54 $\pm$ 3.91	83.85 $\pm$ 2.77
	350	87.72 $\pm$ 2.01	87.58 $\pm$ 2.25	84.96 $\pm$ 2.79	87.18 $\pm$ 1.89
Totals proteins (mg/dL) (NS)	0	6.56 $\pm$ 0.29	6.76 $\pm$ 0.19	6.33 $\pm$ 0.23	6.72 $\pm$ 0.21
	3.5	7.33 $\pm$ 0.25	7.36 $\pm$ 0.24	6.93 $\pm$ 0.33	6.64 $\pm$ 0.21
	35	7.00 $\pm$ 0.22	7.49 $\pm$ 0.19	6.99 $\pm$ 0.29	6.74 $\pm$ 0.20
	350	6.66 $\pm$ 0.27	7.58 $\pm$ 0.43	6.71 $\pm$ 0.26	7.50 $\pm$ 0.24

The values are expressed as averages followed by the standard error on the mean; n = 20 rats; NS = statistical difference not significant (p > 0.05); D₀: day before the administration; D₃₀: 30 days of administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration.

3-3- Effects of ETASg on plasma electrolytes

The calcium, potassium, sodium and chlorine levels showed no significant variation ($p > 0.05$) at the different doses studied during the whole experimentation period in rats tested with ETASg compared with rats controls (Table 2).

Table 2: Effects of ETASg on Plasma Electrolytes

Serum electrolytes	Doses (mg/kg b. w.)	Administration time(Days)			
		D ₀	D ₃₀	D ₆₀	D ₉₀
Calcium (mg/dL) (NS)	0	9.46 ± 0.58	8.74 ± 0.57	9.68 ± 0.18	9.01 ± 0.35
	3.5	10.00 ± 0.43	9.34 ± 0.25	9.19 ± 0.37	10.25 ± 0.47
	35	9.61 ± 0.32	9.45 ± 0.22	8.93 ± 0.37	10.31 ± 0.31
	350	10.16 ± 0.90	9.56 ± 0.56	10.07 ± 0.47	9.97 ± 0.54
Potassium (mmol/L) (NS)	0	5.03 ± 0.20	4.79 ± 0.14	4.82 ± 0.16	4.62 ± 0.30
	3.5	4.96 ± 0.17	4.78 ± 0.15	4.97 ± 0.11	4.57 ± 0.22
	35	4.91 ± 0.15	4.81 ± 0.12	5.08 ± 0.20	4.67 ± 0.11
	350	5.30 ± 0.32	4.94 ± 0.18	5.65 ± 0.54	4.65 ± 0.33
Sodium (mmol/L) (NS)	0	144.1 ± 1.04	142.4 ± 3.26	144.0 ± 1.59	143.6 ± 1.53
	3.5	145.1 ± 2.61	140.8 ± 2.91	143.9 ± 1.33	144.6 ± 2.37
	35	143.8 ± 3.46	141.0 ± 2.60	143.8 ± 1.58	142.9 ± 3.04
	350	145.7 ± 2.28	142.9 ± 2.05	144.6 ± 2.89	143.7 ± 3.61
Chlore (mmol/L) (NS)	0	101.6 ± 1.55	99.68 ± 2.48	101.4 ± 2.39	99.05 ± 2.16
	3.5	104.2 ± 1.56	102.6 ± 0.75	105.2 ± 1.23	100.3 ± 2.43
	35	100.8 ± 2.70	101.9 ± 1.12	104.1 ± 0.79	101.6 ± 3.41
	350	102.0 ± 1.24	98.56 ± 3.84	103.6 ± 1.99	101.4 ± 3.87

The values are expressed as averages followed by the standard error on the mean; n = 20 rats; NS = statistical difference not significant ($p > 0.05$); D₀: day before the administration; D₃₀: 30 days of administration; D₆₀: 60 days of administration; D₉₀: 90 days of administration.

3-4- Reversible and delayed effects of ETASg on the biochemical parameters of rats

The enzymatic disturbances observed during the administration of ETASg at a dose of 350 mg/kg b. w., normalize after stopping the tests. Moreover, no change in urea, creatinine, glucose, total cholesterol, HDL-cholesterol, triglyceride, total protein, calcium, potassium, sodium and chlorine levels is recorded after stopping the administration of ETASg at a dose of 350 mg/kg b. w. (Table 3).

Table 3: Reversible and delayed effects of ETASg on biochemical parameters

Biochemical parameters	Doses (mg/kg b. w.)	Time	
		D ₉₀	D ₁₂₀
ALT (IU/L)	0	77.57 ± 2.90	77.56 ± 4.58
	350	96.19 ± 2.70 ^b	81.56 ± 3.51
AST (IU/L)	0	82.21 ± 2.73	80.32 ± 4.04
	350	109.3 ± 7.00 ^c	103.8 ± 11.62
ALP (IU/L)	0	414.6 ± 21.96	415.5 ± 19.26
	350	515.3 ± 24.87 ^b	457.9 ± 12.06
LDH (IU/L)	0	3141 ± 100.1	3218 ± 212.5
	350	3926 ± 147.7 ^a	3352 ± 118.8
Urea (mg/dL)	0	39.84 ± 2.43	42.40 ± 1.26
	350	45.56 ± 2.10	45.70 ± 2.67
Creatinine (mg/dL)	0	0.99 ± 0.06 ^V	0.90 ± 0.03
	350	0.99 ± 0.07	0.84 ± 0.04
Glucose (mg/dL)	0	0.96 ± 0.07	0.84 ± 0.04
	350	0.85 ± 0.07	0.89 ± 0.04
Total cholesterol (mg/dL)	0	61.39 ± 2.93	61.10 ± 1.71
	350	64.85 ± 2.84	60.87 ± 1.91
HDL-Cholesterol (mg/dL)	0	62.80 ± 1.63	57.02 ± 3.08
	350	64.38 ± 5.14	60.50 ± 4.02
Triglycerides (g/L)	0	85.73 ± 1.74	89.67 ± 2.53
	350	87.18 ± 1.89	88.72 ± 2.58
Totals proteins (mg/dL)	0	6.72 ± 0.21	6.54 ± 0.22
	350	7.50 ± 0.24	7.12 ± 0.22
Calcium (mg/dL)	0	9.01 ± 0.35	8.67 ± 0.23
	350	9.97 ± 0.54	8.97 ± 0.24
Potassium (mmol/L)	0	4.62 ± 0.30	5.04 ± 0.29
	350	4.65 ± 0.33	5.36 ± 0.30
Sodium (mmol/L)	0	143.6 ± 1.53	142.6 ± 1.54
	350	143.7 ± 3.61	143.2 ± 2.65
Chlore (mmol/L)	0	99.05 ± 2.16	98.85 ± 1.65
	350	101.4 ± 3.89	100.5 ± 0.77

190 The values are expressed as averages followed by the standard error on the mean; n = 10 rats; a = p
 191 <0.05; b = p <0.01; c = p <0.001; D₉₀: 90 days of treatment; D₁₂₀: 30 days after stopping treatment.

192 4-Discussion

193 Oral administration of ETASg revealed no significant influence of serum ALT, AST, ALP, and LDH
 194 at the therapeutic dose of 3.5 mg/kg b. w. throughout the duration of the study. ALT, AST, ALP and
 195 LDH are enzymatic markers that reflect the tissue integrity of the liver, kidneys and heart [15]. These
 196 results would indicate good hepatic, renal and cardiac tolerance of ETASg at a dose of 3.5 mg/kg b.
 197 w. during 90 days of administration in rats. However, at doses of 35 and 350 mg/kg b. w., ETASg

resulted in a significant increase in serum activity of these markers at day 60 and day 90. Studies have shown that a lesion of the liver, kidneys or heart is capable of inducing an increase in the activities of these different markers following their release in large quantities in the plasma [15]. Thus, the increase in aminotransferase activity (ALT and AST), observed in this work, is due to an action of ETASg on hepatic and cardiac tissue releasing these enzymes into the bloodstream [16]. ALT is a cytoplasmic enzyme predominantly present in the liver, and an increase in this specific enzyme suggests alterations of the hepatocyte membrane or liver cell damage. AST, the cytoplasmic enzyme more specific to the heart, provides information on the state of the myocardium [17]. Its strong presence in the bloodstream could cause necrosis of the heart tissue that can extend to myocardial infarction [16].

In addition, Desjarlais [18] has shown that tissue damage is characterized by an increase in enzymatic activity greater than 20 or 100 times the initial value. Increases in serum aminotransferase activity observed in this study at doses of 35 and 350 mg/kg b. w. are less than double their initial values. These increases would be moderate and, therefore, would not be due to pathological aggression of the liver and heart tissues. Our results are similar to those observed in the study of the biotolerance of aqueous extracts of *Terminalia superba* stem bark in rats. In the course of their study, the researchers indicated that the administration of this extract had no effect on serum aminotransferase activities at doses of 125, 250 and 500 mg/kg b. w. [19]. Our results are contrary to those obtained by Etame *et al.* [20]. They reported that the ethanolic extract of the stem bark of *Harungana madagascariensis* caused a decrease in aminotransferases.

With regard to the serum activity of the ALP, a serum elevation of its activity is observed at the dose of 350 mg/kg b. w. ALP is usually elevated in the bloodstream in cases of liver and kidney damage [21]. In this study, its elevation is less than twice the initial value. Therefore, it would be moderate and transient and then indicate no liver and kidney damage. These results are similar to those obtained by Zaza *et al.* [22]. These authors showed no change in serum ALP activity after oral administration of the *Terminalia ivorensis* X42 fraction in rats at doses of 250, 500 and 1000 mg/kg b. w. The appearance of LDH in the plasma is a sign of the destruction of hepatocytes or important disorders of cell permeability [23]. Moreover, according to Rasekh *et al.* [24], LDH is a less specific marker of the heart. The myocardial cells, containing the LDH are damaged, the cell membrane becomes permeable, resulting in its leakage to the intercellular space hence its elevation serum. In our study, the serum LDH level experienced significant variation at the high dose of 350 mg/kg b. w. at the 90th day of experimentation. This variation is less than twice its initial value and would be temporary. As a result, ETASg would not result in cardiac injury. Our results are similar to those obtained by Olatunde *et al.* [25]. These authors showed no change in LDH activity in the rat liver after administration of the aqueous extract of *Mangifera Indica* stem bark. The liver is involved in the metabolism and regulation of glucose, lipids and total proteins [26]. The influence of ETASg was evaluated on hepatic metabolism by following the evolution of serum glucose levels, total cholesterol, HDL-cholesterol,

triglycerides and total proteins. This study showed no change in serum glucose, total cholesterol, HDL-cholesterol, triglyceride, and total protein levels after administration of ETASg for 90 days. These results show that ETASg does not interfere with carbohydrate, lipid and protein metabolism. These results are comparable to those obtained during the study of biotolerance of the aqueous extract of the stem barks of *Sacoglottis gabonensis*. This study also showed that this extract, dosed at 3.5; 35 and 350 mg/kg b. w. does not interfere with carbohydrate, lipid and protein metabolism for 28 days of study [12].

Regarding the influence of ETASg on the kidneys, the results of this study revealed that the concentrations of the metabolites namely urea and creatinine did not vary significantly during all the periods of experimentation. The kidney is one of the main routes for the elimination of xenobiotics [27]. It produces the urine that contains the metabolic waste that is urea and creatinine. Any substance capable of altering renal function inevitably leads to changes in plasma concentrations of urea and creatinine [28]. ETASg does not influence the renal mechanisms responsible for the regulation of urea and creatinine. These results would be a sign of good kidney function and lack of renal toxicity due to ETASg. Ours differ from those observed in the work of Koné *et al.* [29] where changes in serum urea and creatinine concentrations were observed following administration of dichloromethane extract from *Piliostigma reticulatum* stem bark in rats for 28 days. In addition, at all doses studied, the administration of ETASg had no significant influence on serum concentrations of calcium, potassium, sodium and chlorine. The kidney also plays a vital role in maintaining the balance of water and electrolytes [30]. The lack of significant effect of ETASg on serum concentrations of these electrolytes in rats suggests that the secretory capacity of the kidney and the normal functioning of organs in relation to these parameters were not affected. ETASg would not interfere in maintaining the balance of these electrolytes. These results are in agreement with those obtained by Koné *et al.* [12]. These authors showed that the administration of the aqueous extract of the stem barks of *Sacoglottis gabonensis* had no effect on the serum concentrations of calcium, potassium, sodium and chlorine at doses of 3.5; 35 and 350 mg/kg b. w. in rats for 28 days.

After 30 days of cessation of ETASg administration, the increase in serum AST activities, ALT, ALP, and LDH, evaluated in rats at 350 mg/kg b. w. at day 90 normalize. This increase would therefore be moderate and transitory. The effect of ETASg observed at the high dose on the serum enzymes of the rats tested, would therefore be reversible. These results are comparable to the work done by Koné *et al.* [31]. These authors showed that after stopping the administration of the ethanolic extract of *Piliostigma reticulatum* stem barks at the dose of 1000 mg/kg b. w., the effects were reversible.

Conclusion

The study of the effects of ETASg on the serum biochemical parameters of the rats showed no change at the therapeutic dose of 3.5 mg/kg b. w. Administration of ETASg is non-toxic to the liver, kidneys and heart at a dose of 3.5 mg/kg b. w. Increases in AST, ALT, ALP, and LDH observed in rats after

administration of ETASg at doses of 35 and 350 mg/kg b. w. are moderate and transient. After 30 days of discontinuation of oral administration of ETASg, the increases observed at the high dose of 350 mg/kg b. w. are moderate and reversible.

In total, this study has shown that ETASg is not toxic to biochemical parameters. It would be wise to complete this study with histopathological tests to confirm its non-toxic character.

Ethical approval

The experimental procedures and protocols used in this study were approved by the Ethical Committee of Health Sciences, University Felix Houphouet Boigny. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

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