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EFFECT OF CHRONIC ADMINISTRATION OF THETOTAL AQUEOUSEXTRACT OF SACOGLOTTISGABONENSIS STEM BARK ON SERUM MARKERS AND SOMEORGANS IN WISTAR RATS

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Abstract

Sacoglottisgabonensis is a plant used in the treatment of Buruli ulcer in Côte d'Ivoire. The aim of this study was to verify the safety of the total aqueous extract of Sacoglottisgabonensis stem bark (TAESg) on serum and organ markers in Wistar rats over a six-month period. 80 rats were divided into four groups, namely A, B, C and D of 20 rats/group. These groups respectively received distilled water and TAESg at doses of 3.5, 17.5 and 35 mg/kg bw orally at 1 mL/100 g bw. At the end of the experiment, venous blood from the retroorbital sinus was collected in dry tubes to determine the chronic effect of TAESg on biochemical parameters and anatomo-histology. This study showed that daily oral administration of TAESg for six months had no effect on serum enzyme and metabolite levels. However, a highly significant ($p< 0.0001$) increase in calcium and sodium levels was observed in group D rats compared to group A rats. As for anatomo-histology, TAESg administration did not alter the morphology and tissue integrity of the vital organs liver, kidneys and heart. In short, TAESg is non-toxic to biochemical parameters and organs. Formulations are needed to make it available to the general public.

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Introduction

The liver, kidneys and heart are organs that perform many complex functions in the body. The liver, for example, is involved in the metabolism and storage of nutrients supplied by digestion [1]. It plays a crucial role in detoxifying substances that are harmful to the body, notably xenobiotics, and prevents them from entering the general circulation [2-3]. As for the kidneys, their main function is to purify the blood via the glomeruli. The kidneys eliminate metabolic end products such as urea, protons and creatinine [4]. They also eliminate a large number of exogenous substances such as certain drugs, toxins, food additives and electrolytes [5]. As far as the heart is concerned, its main function is to circulate blood throughout the body [6]. However, these organs remain a prime target for drug toxicity [3]. In modern medicine, the prolonged use of certain drugs such as antibiotics in the treatment of Buruli ulcer has led to liver and kidney damage in children [7]. Consequently, the search for new, more effective drugs that are less prone to organ side-effects is essential. The use of natural resources, particularly medicinal plants, is becoming an important avenue of exploration. Indeed, many patients very often turn to traditional medicine, and more specifically medicinal plants, as a first-line treatment

for Buruli ulcer [8-9]. However, the empirical use of these medicinal plants exposes patients to risks of intoxication that can sometimes prove fatal [10]. In addition, studies have shown that 35% of cases of acute renal failure and liver damage are associated with the use of herbal medicines [11]. In Côte d'Ivoire, *Sacoglottisgabonensis* is a plant used orally and dermally to treat Buruli ulcer [12]. The aqueous extract of this plant had an inhibitory effect on the growth of various strains of *Mycobacterium ulcerans* [13]. The acute toxicity test carried out in mice showed that the extract has an LD_{50} greater than 5000 mg/kg bw. Subacute oral and dermal toxicity tests [14] and subchronic oral toxicity tests [15] showed that the extract was non-toxic at the practitioner's therapeutic dose on the various biological parameters studied. Other studies have shown the extract to possess healing activity [16-17], haemostatic potential [18] and anti-inflammatory activity [19]. However, no chronic study of the effect of this extract has been carried out. The objective of this study is to verify the safety of the total aqueous extract of *Sacoglottisgabonensis* stem bark (TAESg) on serum markers and on some organs for six months.

Materials and methods

Plant Material

The plant material consists of *Sacoglottisgabonensis* stem barks. The bark was harvested in March 2021 at Ingrakon in the Alépé region, a town about 45 km from the Abidjan district. A sample was identified at the National Floristic Center (CNF) for confirmation on the one kept under number 1154 dated June 16, 1965.

Animal Material

The experiments were carried out on male and female albino rats of the species *Rattus norvegicus* of the *Wistar* strain. They were six to eight weeks old. The animals were kept at a temperature of 22 ± 2°C with a 12-hour photoperiod. Rats were fed daily with IVOGRAIN® pellets and tap water without interruption in feeding bottles. The experimental protocol and animal handling procedures were carried out in accordance with Good Laboratory Practice [20].

Technical Equipment

All the equipment consisted of a grinder (Retsch SM 100, Hann, Germany), a Denver S-234 electronic balance (Belgium), a Selecta® drying oven (Spain) and an Ovan MCG05E magnetic stirrer (Europe). The small equipment consisted of a funnel, absorbent cotton and Wattman N°1 filter paper; a 500 mL graduated cylinder; a gastric tube for force-feeding the animals. Dry tubes and a semi-automated counter-analyzer (Prietest Touch Robonik, India) were used to measure biochemical parameters. An AXEL JOHNSON LABSYSTEM kerosene tray, cassettes and rotary microtome (SHANDON AS 325, Switzerland) were used for histological sections. A camera (Samsung Galaxy A50, Korea) and an Olympus BX41 electron microscope (Olympus, Tokyo, Japan) were used to observe the various sections.

Chemicals and Reagents

Chemicals and reagents consisted of diethyl ether (Cooper); Hematoxylin; Eosin; kerosene; toluene; ethanol 100%, 95% and 85%; Eukitt® balsam.

Methods

1. Preparation of ETASg

The preparation of the total aqueous extract is done according to the preparation method described by **Kouassi** [15]. Four hundred grams (400 g) of *Sacoglottisgabonensis* stem bark powder is dissolved in two liters (2L) of distilled water and boiled for 30 minutes. After cooling, the decoctate is filtered, first on absorbent cotton and then on Wattman N°1 paper. The filtrates are oven-dried at 50°C for 48 hours. A dry powder, TAESg, was obtained. This powder, which was used to prepare different concentrations of ETASg, was kept refrigerated at -5°C until the days of manipulation.

2. Oral safety assessment

This study is conducted in accordance with the **OCED 452** protocol [21]. It consists of daily oral administration of the test substance to test groups and distilled water to a control group, at increasing doses, over a period of at least six months or 12 months or more.

2.1. Composition of animal groups and administration of TAESg

Eighty six- to eight-week-old rats were homogeneously divided into four groups of 20 rats (10 male and 10 female), with one control group (A receiving distilled water) and three test groups (B, C and D receiving TAESg at doses of 3.5, 17.5 and 35 mg/kg bw respectively). Two other groups (E receives distilled water and F receives TAESg at a dose of 35 mg/kg bw) called satellite groups, consisting of 10 rats including five male and five female rats, were used to assess reversibility, persistence or late onset of toxic effects. At the end of the six months of experimentation, the animals in satellite groups E and F did not receive any substance for 60 days.

2.2. Biochemical test

Blood samples were taken from the retroorbital sinus of the rat eye using a sterile Pasteur pipette [22]. Blood collected in gray tubes containing NAF was used to determine blood glucose levels. Blood collected in dry tubes was centrifuged at 3,000 rpm for 5 min using a centrifuge (HERAEUS SEPATECH). The serum obtained was stored in a freezer until the biochemical parameters, to know serum enzymes, serum metabolites and serum electrolytes, were determined.

2.3 Anatomical-Histological Testing

At the end of the oral experiments, with the exception of satellite groups, all animals were euthanized with ethyl ether for anatomical-histological study. After euthanasia, organs such as the liver, kidneys and heart, were removed during dissection. After macroscopic examination, these

organs are preserved in 10% formalin for histological study. This operation will be repeated on satellite groups at the end of the 60-day observation period without administration, in order to assess the persistence or reversibility of toxic effects. The macroscopic study consisted in observing the organs with the naked eye, and assessing any morphological alterations indicating the nature of the toxicity. Microscopic examination involved histological sections to assess the condition of the various organ tissues sampled. These steps were carried out according to the protocol indicated by **Hould** [23], which is the kerosene embedding technique. These harvested organs were weighed fresh using a precision balance (NEO-TECH SA, Belgium) to determine their relative mass according to the following formula [24]:

$$\text{Relative mass (\%)} = \frac{\text{Absolute mass of the organ}}{\text{Animal body weight on day of euthanasia}} \times 100$$

Statistical Analysis

Data are analyzed using Graph Pad Prism 8.0.1 (San Diego, CA, USA). Results are expressed as the mean followed by the standard error of the mean (M ± SEM). Statistical significance is determined by ANOVA 1 followed by the Turkey test. These tests will give us the degree of significance for $p < 0.05$. In the presentation of results, symbols (*, **, ***, ****/ #, ##, ###, ####) will indicate significant decreases and increases compared with controls.

Results

1. Effect of TAESg on biochemical parameters

1.1 Effect of TAESg on serum enzymes

Analysis of the results shows that TAESg did not induce any significant variation ($p > 0.05$) in the activity of transaminases (ASAT and ALAT), alkaline phosphatase (PAL) and lactate dehydrogenase (LDH) in treated rats compared with control rats (Table 1). Serum levels of ALAT, ASAT, PAL and LDH in control rats were 69.45 ± 3.67; 228.8 ± 17.34; 506.6 ± 42.32 and 1400 ± 229 IU/L respectively, compared with 3.5; 17.5 and 35 mg/kg bw. which are respectively 79.15 ± 7.40; 82.18 ± 4.28 and 78.42 ± 5.41 IU/L for ALT, 239.3 ± 15.73; 223.4 ± 17.77 and 219.9 ± 16.14 for AST, 696.3 ± 33.75; 664.1 ± 40.46 and 614.8 ± 26.75 IU/L for PAL, 1418 ± 199.65; 1522 ± 271.1 and 1743 ± 274.7 IU/L for LDH.

1.2 Effect of TAESg on serum metabolites

With regard to the serum metabolites urea, creatinine, glucose, total cholesterol, HDL-cholesterol, triglycerides and total protein, analysis of the results showed no significant variation in all rats in groups treated at all doses with TAESg compared with rats in the control group (Table 2).

Table 01. Effects of TAESg on serum enzyme marker concentrations in all rats

Groups/Doses				
Serum enzyme markers (IU/L)	**Group A (0 mg/kg bw)**	**Group B (3.5 mg/kg bw)**	**Group C (17.5 mg/kg bw)**	**Group D (35 mg/kg bw)**
ALAT	69.45 ± 3.67	79.15 ± 7.40	82.18 ± 4.28	78.42 ± 5.41
ASAT	228.8 ± 17.34	239.3 ± 15.73	223.4 ± 17.77	219.9 ± 16.14
PAL	506.6 ± 42.32	696.3 ± 33.75	664.1 ± 40.46	614.8 ± 26.75
LDH	1400 ± 229	1418 ± 199.65	1522 ± 271.1	1743 ± 274.7

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group A, the control, and groups B, C and D treated with 3.5, 17.5 and 35 mg/kg bw respectively.

Table 02. Effects of TAESg on serum metabolic levels in all rats

Groups/Doses				
Serum metabolites (mg/dL)	**Group A (0 mg/kg bw)**	**Group B (3.5 mg/kg bw)**	**Group C (17.5 mg/kg bw)**	**Group D (35 mg/kg bw)**
Urea	0.28 ± 0.02	0.25 ± 0.01	0.23 ± 0.06	0.26 ± 0.01
Creatinine	11.01 ± 0.69	7.34 ± 7.28	9.28 ± 0.48	10.30 ± 0.48
Glucose	0.90 ± 0.03	0.90 ± 0.05	0.92 ± 0.04	0.96 ± 0.02
Total cholesterol	1.09 ± 0.06	1.03 ± 0.40	1.37 ± 0.06	1.37 ± 0.08
HDL-cholesterol	0.13 ± 0.01	0.14 ± 0.02	0.16 ± 0.02	0.13 ± 0.01
Triglycerides	1.53 ± 0.13	1.28 ± 0.23	1.93 ± 0.53	1.30 ± 0.11
Total protein	7.07 ± 0.35	7.36 ± 0.30	8.11 ± 0.26	8.07 ± 0.31

*Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group A, the control, and groups B, C and D treated with 3.5, 17.5 and 35 mg/kg bw respectively.*These levels were 0.28 ± 0.02; 0.25 ± 0.01; 0.23 ± 0.06; 0.26 ± 0.01 mg/dL respectively for groups A ; B ; C and D for urea and 11.01 ± 0.69; 7.34 ± 7.28; 9.28 ± 0.48 and 10.30 ± 0.48 mg/dL respectively for groups A ; B ; C and D for creatinine. Glucose, total cholesterol, HDL-cholesterol and triglyceride levels ranged from 0.90 ± 0.03 ; 1.09 ± 0.06 ; 0.13 ± 0.01 and 1.53 ± 0.13 mg/dL respectively for group 1, to 0.90 ± 0.05 ; 1.03 ± 0.40; 0.14 ± 0.02 and 1.28 ± 0.23 mg/dL for group B, 0.92 ± 0.04; 1.37 ± 0.06; 0.16 ±

0.02 and 1.93 ± 0.53 mg/dL for group C and 0.96 ± 0.02; 1.37 ± 0.08; 0.13 ± 0.01 and 1.30 ± 0.11 mg/dL for group D. Total protein levels were 7.07 ± 0.35 for control rats and 7.36 ± 0.30, 8.11 ± 0.26 and 8.07 ± 0.31 mg/dL for TAESg-treated groups at doses of 3.5, 17.5 and 35 mg/kg bw.

1.3 Effect of TAESg on some serum electrolytes

With regard to serum electrolytes, the results showed no significant difference (p> 0.05) for potassium, chlorine and magnesium in rats treated with all doses of TAESg compared with control rats (Table 3). Their levels were 5.33 ± 0.12; 4.11 ± 0.33 and 6.17 ± 0.38 mmol/L for potassium, 154.9 ± 2.17; 155 ± 2.43 and 156.4 ± 3.64 for calcium and 2.45 ± 0.22 ; 2.43 ± 0.12 and 2.75 ± 0.08 mmol/L for magnesium, compared with control group, which was 5.29 ± 0.47; 147.8 ± 5.13 and 2.26 ± 0.11 mmol/L for potassium, chlorine and magnesium respectively.

Table 03. Effects of TAESg on selected serum electrolyte levels in all rats

	Groups/Doses			
Serum electrolytes (mmol/L)	**Group A (0 mg/kg bw)**	**Group B (3.5 mg/kg bw)**	**Group C (17.5 mg/kg bw)**	**Group D (35 mg/kg bw)**
Calcium	18.37 ± 1.11	21.62 ± 2.12	20.40 ± 0.86	27.34 ± 1.61###
Potassium	5.29 ± 0.47	5.33 ± 0.12	4.11 ± 0.33	6.17 ± 0.38
Sodium	202.8 ± 5.87	208.6 ± 4.01	208.8 ± 3.05	248.4 ± 5.54###
Chlorine	147.8 ± 5.13	154.9± 2.17	155 ± 2.43	156.4 ± 3.64
Magnesium	2.26 ± 0.11	2.45 ± 0.22	2.43 ± 0.12	2.75 ± 0.08

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group A, the control, and groups B, C and D treated with 3.5, 17.5 and 35 mg/kg bw respectively; p< 0.05.

However, a very highly significant (p< 0.0001) increase in calcium and sodium levels was observed in group D rats compared with group A rats. These levels ranged from 27.34 ± 1.61 and 248.4 ± 5.54 mmol/L for calcium and sodium respectively in group 4 rats compared with those in group 1 rats, which were 18.37 ± 1.11 and 202.8 ± 5.87 mmol/L for calcium and sodium.

1.4. Reversible and delayed effects of TAESg on serum biochemical parameters

Two months after stopping TAESg administration, no delayed effects were observed on enzyme markers and serum metabolites in all rats (Table 4). However, the disturbances observed in serum electrolyte levels, namely calcium and sodium, in rats treated with TAESg at a dose of 35 mg/kg bw, after six months' administration, virtually disappeared two months after discontinuation.

Table 04. Effects of TAESg on biochemical parameters in all rats after two months of discontinued administration

		Groups/Doses			
Parameters Serum biochemistry		**Group E (0 mg/kg bw)**		**Group F (35 mg/kg bw)**	
		Month 6	**Month 8**	**Month 6**	**Month 8**
Serum enzyme markers (IU/L)	**ALAT**	69.45 ± 11.67	71.42 ± 2.24	78.42 ± 5.41	74.12 ± 3.14
	ASAT	228.8 ± 17.34	214.55 ± 20.24	219.9 ± 16.14	211.02 ± 18.16
	PAL	506.6 ± 42.32	516.09 ± 31.20	614.8 ± 26.75	588.8 ± 22.57
	LDH	1400 ± 229	1447.25 ± 195.07	1743 ± 274.7	1663 ± 204.7
Serum metabolites (mg/dL)	**Urea**	0.28 ± 0.02	0.27 ± 0.18	0.26 ± 0.01	0.26 ± 0.03
	Creatinine	11.01 ± 0.69	10.70 ± 0.29	10.30 ± 0.48	11.02 ± 0.18
	Glucose	0.90 ± 0.03	0.88 ± 0.11	0.96 ± 0.02	0.92 ± 0.01
	Total cholesterol	1.09 ± 0.06	1.14 ± 0.09	1.37 ± 0.08	1.27 ± 0.03
	HDL-cholesterol	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.02
	Triglycerides	1.53 ± 0.13	1.49 ± 0.15	1.30 ± 0.11	1.33 ± 0.09
	Total protein	7.07 ± 0.35	7.10 ± 0.22	8.07 ± 0.31	7.67 ± 0.24
Electrolytes sériques (mmol/L)	**Calcium**	18.37 ± 1.11	17.22 ± 0.09	27.34 ± 1.61###	17.64 ± 0.61
	Potassium	5.29 ± 0.47	5.12 ± 0.26	6.17 ± 0.38	5.64 ± 0.28
	Sodium	202.8 ± 5.87	201.9 ± 6.16	248.4 ± 5.54###	208.6 ± 4.51
	Chlorine	147.8 ± 5.13	146.9 ± 4.31	156.4 ± 3.64	155.1 ± 2.46
	Magnesium	2.26 ± 0.11	2.22 ± 0.13	2.75 ± 0.08	2.43 ± 0.06

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 10 rats/group. Comparisons are between group E, control and group F treated at 35 mg/kg bw; p<0.05.

2. Effect of TAESg on organ anatomy and histoloy

2.1. Effect of TAESg on the liver

The livers of group A rats given distilled water were light brown in color and normal in appearance, i.e. smooth and firm to the touch (Figure 1, A). This normal liver appearance was also observed in rats given 3.5, 17.5 and 35 mg/kg bwTAESg (Figure 1, B; C and D). Similarly, none of the rats in any of the treated groups showed blackish, flabby livers. Macroscopic abnormalities such as whitish nodules were not observed in rats from any of the treated groups. Furthermore, administration of TAESg resulted in no significant ($p > 0.05$) change in the mean relative liver mass of rats treated at doses of 3.5, 17.5 and 35 mg/kg bw compared with that of control rats (Table 5). These mean values were 3.73 ± 0.15, 3.54 ± 0.20 and 3.72 ± 0.16 % for 3.5, 17.5 and 35 mg/kg bw respectively, compared with 3.13 ± 0.07 % for the control.

Microscopically, the livers of rats treated with distilled water showed a normal structure (Figure 2, A). The centro-lobular vein is located in the center of each lobule. Hepatocytes form flattened laminae with large, clearly visible nuclei. Blood flows to the centrilobular vein via capillary sinusoids located between the hepatocytes. No hepatic lesions such as steatosis, blood congestion or necrosis were observed in the hepatic architecture of rats treated with 3.5, 17.5 and 35 mg/kg bwTAESg (Figure 2, B; C and D). Dilatation of capillary sinusoids was noted in 30 % of rats treated with TAESg at 35 mg/kg bw (Figure 2, D).

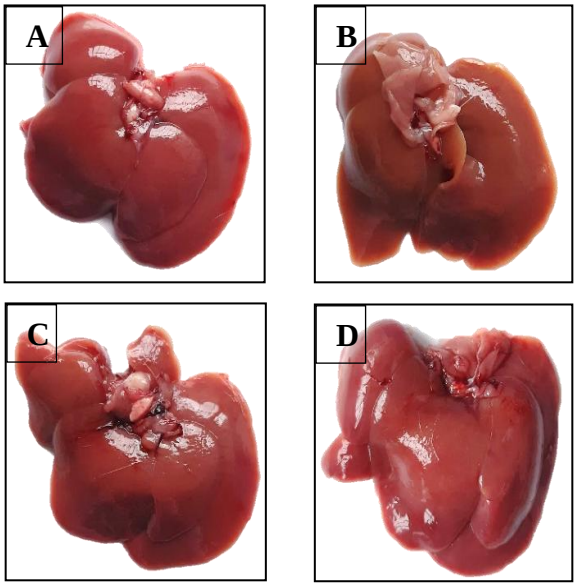

Fig 01: Macroscopic appearance of rat liver

A: liver of a control rat treated with distilled water; B: liver of a rat treated with TAESg at 3.5 mg/kg bw; C: liver of a rat treated with TAESg at 17.5 mg/kg bw; D: liver of a rat treated with TAESg at 35 mg/kg bw.

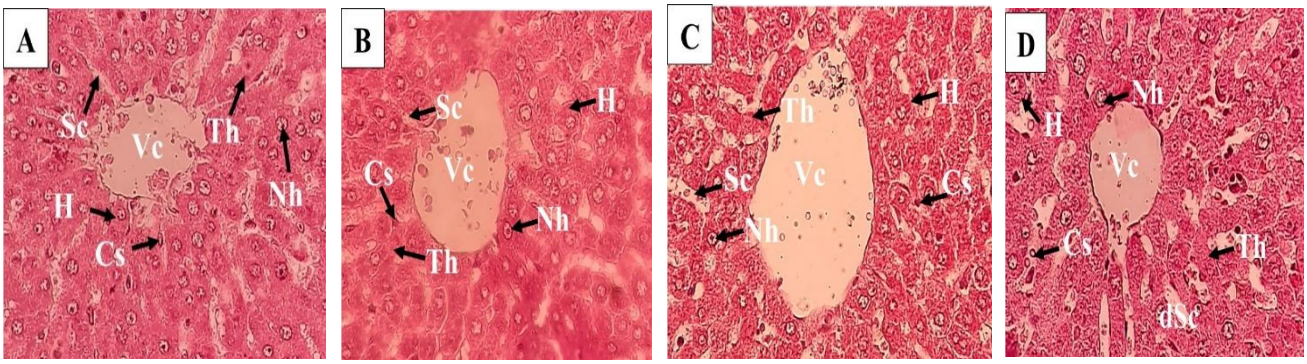

Fig 02. microphotographs of rat liver tissue

A: liver tissue from a control rat treated with distilled water (G X 100); B: liver tissue from a rat treated with TAESg at 3.5 mg/kg bw (G X 100); C: liver tissue from a rat treated with TAESg at 17.5 mg/kg bw (G X 100); D: liver tissue from a rat treated with TAESg at 35 mg/kg bw (G X 100); Hematoxylin-eosin staining; Vc: Centrilobular vein; H: Hepatocytes; Nh: Hepatocyte nucleus; Sc: Capillary sinusoid; Cs: Blood cells; Th: Hepatocyte trabeculae; dSc: Dilatation of capillary sinusoids.

Table 05. Influence of TAESg on macroscopic liver appearance

Groups	Doses (mg/kg bw)	Sex	Color		Aspect		Whitish nodules		Body weight (g)	Liver mass (g)	Relative liver mass (%)
			Normal	Blackish	Smooth and firm to the touch	Soft consistency	Absence	Presence			
A	**0**	**M+F**	100 %	0 %	100 %	0 %	100 %	0 %	173.8 ± 5.14	5.43 ±0.16	3.13 ± 0.07
B	**3.5**	**M+F**	100 %	0 %	100 %	0 %	100 %	0 %	175.5 ± 4.11	6.48 ± 0.33	3.73 ± 0.15
C	**17.5**	**M+F**	100 %	0 %	100 %	0 %	100 %	0 %	176.3 ± 4.26	6.11 ± 0.32	3.54 ± 0.20
D	**35**	**M+F**	100 %	0 %	100 %	0 %	100 %	0 %	175.5 ± 3.41	6.44 ± 0.26	3.72 ± 0.16

Values are given as means followed by the standard error of the mean. n = 20 rats in each group and n= 10 rats/sex. Comparisons are made between control and TAESg-treated groups at doses of 3.5, 17.5 and 35 mg/kg bw. For the same period and for a given parameter: p> 0.05.

2.2 Effect of TAESg on kidneys

Control rats showedbrownkidneys of normal appearance (Figure 3, A). Rats treatedwithTAESg at doses of 3.5, 17.5 and 35 mg/kg bwshowedbrownkidneyswith normal appearance and no abnormalitiescomparedwith control rats (Figure 3, B; C and D). Similarly, none of the rats in the varioustreated groups showedblackishkidneyswith a soft consistency. Macroscopicabnormalitiessuch as nodules and atrophywere not observed in rats fromany of the treated groups. Furthermore, TAESgdid not cause anysignificant variation in the mean relative kidney mass of rats treatedwith 3.5 mg/kg bw (0.61 ± 0.03%), 17.5 mg/kg bw (0.60 ± 0.04%) and 35 mg/kg bw (0.63 ± 0.03%) respectively, comparedwiththat of control rats (0.53 ± 0.01%) (Table 6).

Sections takenfrom the kidneys of rats treatedwithdistilled water showedhistologically intact and normal kidneys (Figure 4, A). The glomerular capsule, glomerularspace or urinary pole, visceral and parietalleaflet, proximal and distal convoluted tubule wereclearly visible and distinct. The proximal convoluted tube has a brush border thatalmostfills the lumen, while the distal convoluted tube is simple and reveals a larger lumen. Chronic administration of TAESgdid not induceanylesions in the renal tissues of rats treated at doses of 3.5, 17.5 and 35 mg/kg bwrespectively, comparedwith the renal tissues of control rats (Figure 03, B; C and D).

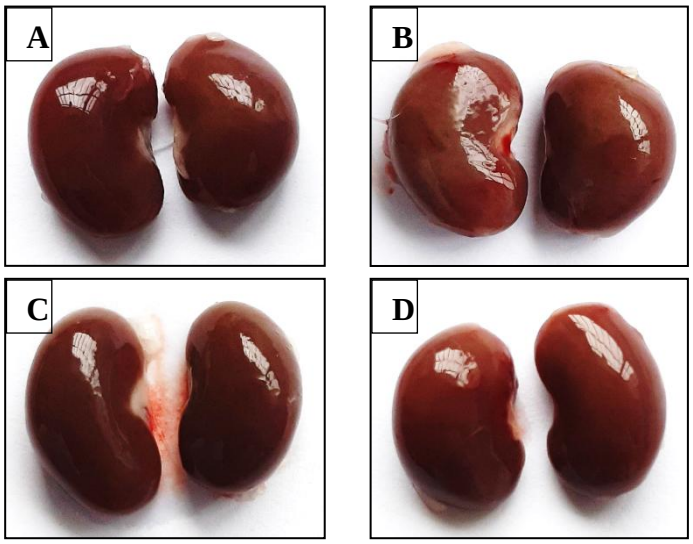

Figure 02. Macroscopicappearance of rat kidneys

A:kidneys of a control rat treatedwithdistilled water; B: kidneys of a rat treatedwithTAESg at 3.5 mg/kg bw; C: kidneys of a rat treatedwithTAESg at 17.5 mg/kg bw; D: kidneys of a rat treatedwithTAESg at 35 mg/kg bw.

2.3 Effect of TAESg on the heart

Rats in the control group showed normal-looking, light-red hearts (Figure 5, A). Rats treated with TAESg at doses of 3.5, 17.5 and 35 mg/kg bw showed light-red, normal-looking hearts with no abnormalities compared with control rats (Figure 5, B; C and D). Similarly, none of the rats in the various treated groups showed blackish, hard hearts. Macroscopic abnormalities such as hypertrophy and atrophy were not observed in rats from any of the treated groups. Furthermore, TAESg did not cause any significant difference in the mean relative heart mass of rats treated with 3.5 mg/kg bw (0.37 ± 0.02 %), 17.5 mg/kg bw (0.37 ± 0.02 %) and 35 mg/kg bw (0.39 ± 0.03 %) respectively, compared with that of rats from the control group (0.35 ± 0.01 %) (Table 7).

Heart tissue from rats treated with distilled water showed normal anatomical structure (Figure 6, A). The cardiac muscle bundles were well connected, with interspersed bifurcations at their ends. Administration of TAESg did not induce any lesions in the cardiac tissues of rats treated at doses of 3.5, 17.5 and 35 mg/kg bw, compared with the cardiac tissues of control rats (Figure 6, B; C and D).

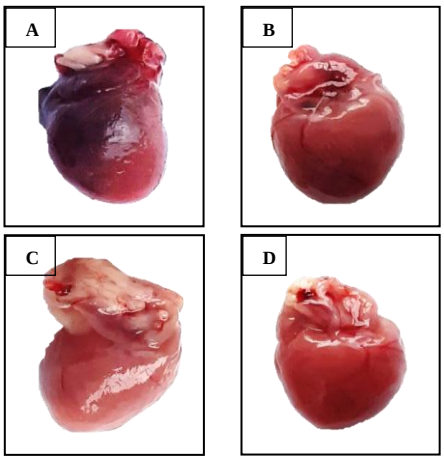

Fig 03. Macroscopic appearance of rat hearts

A: heart of a control rat treated with distilled water; B: heart of a rat treated with TAESg at 3.5 mg/kg bw; C: heart of a rat treated with TAESg at 17.5 mg/kg bw; D: heart of a rat treated with TAESg at 35 mg/kg bw.

Table 06: Influence of TAESg on macroscopicappearance of kidneys

Groups	Doses (mg/kg bw)	Sex	Color		Aspect		Abnormalities		Body weight (g)	kidneys mass (g)	Relative kidneys mass (%)
			Normal	Blackish	Smooth and firm to the touch	Soft consistency	Nodules	Atrophy			
A	**0**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	173.8 ± 5.14	0.94 ± 0.03	0.53 ± 0.01
B	**3.5**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	175.5 ± 4.11	1.07 ± 0.06	0.61 ± 0.03
C	**17.5**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	176.3 ± 4.26	1.04 ± 0.06	0.60 ± 0.04
D	**35**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	175.5 ± 3.41	1.19 ± 0.07	0.63 ± 0.03

Values are given as meansfollowed by the standard error of the mean. n = 20 rats in eachgroup and n= 10 rats/sex. Comparisons are made between control and TAESg-treatedgroups at doses of 3.5, 17.5 and 35 mg/kg bw. For the sameperiod and for a given-parameter: p> 0.05.

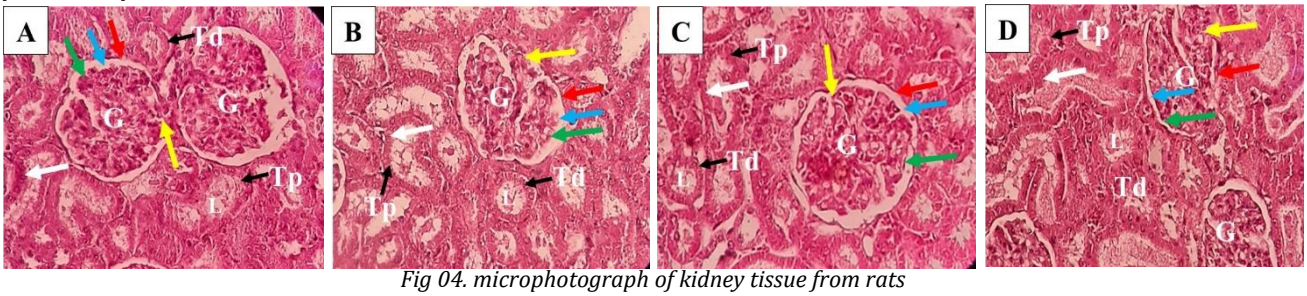

Fig 04. microphotograph of kidney tissue from rats

A: kidney tissue from a control rat treatedwithdistilled water (G X 100); B: kidney tissue from a rat treatedwithTAESg at a dose of 3.5 mg/kg bw (G X 100); C: kidney tissue from a rat treatedwithTAESg at a dose of 17.5 mg/kg bw (G X 100); D: kidney tissue from a rat treatedwithTAESg at a dose of 35 mg/kg bw (G X 100); Haematoxylin-eosinstaining; G: glomerulus; Tp: proximal convoluted

tubule; Td: distal convoluted tubule; L: tubular lumen; yellowarrow: vascular pole; redarrow: parietalleaflet; bluearrow: urinaryspace or urinary pole; green arrow: visceralleaflet; white arrow: normal brush border.

Table 07. Influence of TAESg on the macroscopic appearance of the heart

Groups	Doses (mg/kg bw)	Sex	Color		Aspect		Abnormalities		Body weight (g)	heart mass (g)	Relative heart mass (%)
			Normal	Blackish	Smooth	Hard consistency	Hypertrophy	Atrophy			
A	**0**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	173.8 ± 5.14	0.62 ± 0.02	0.35 ± 0.01
B	**3.5**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	175.5 ± 4.11	0.66 ± 0.03	0.37 ± 0.02
C	**17.5**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	176.3 ± 4.26	0.68 ± 0.02	0.39 ± 0.01
D	**35**	**M+F**	100 %	0 %	100 %	0 %	0 %	0 %	175.5 ± 3.41	0.68 ± 0.03	0.39 ± 0.03

Values are given as means followed by the standard error of the mean. n = 20 rats in each group and n= 10 rats/sex. Comparisons are made between control and TAESg-treated groups at doses of 3.5, 17.5 and 35 mg/kg p. For the same period and for a given parameter: p> 0.05.

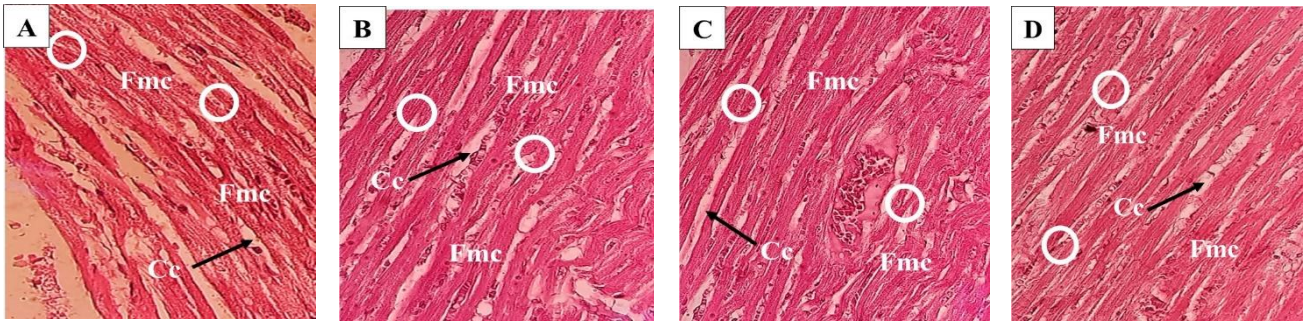

Fig 05. Microphotographs of rat heart tissue

A: heart tissue of a control rat treated with distilled water (G X 100); B: heart tissue of a rat treated with TAESg at a dose of 3.5 mg/kg bw (G X 100); C: heart tissue of a rat treated with TAESg at a dose of 17.5 mg/kg bw (G X 100); D: heart tissue of a rat treated with TAESg at a dose of 35 mg/kg bw (G X 100); Hematoxylin-eosin staining; circles: intercalated discs; Fmc: cardiac muscle bundle; Cc: cardiac cavity.

Discussion

Clinical biochemistry or pathological chemistry is based on the qualitative and quantitative study of molecules contained in the body's fluids, such as blood, urine and cerebrospinal fluid [25]. It focuses on the search for molecules involved in a pathology or the pathophysiological origin of a disease. It plays an important role in the identification, screening, diagnosis, monitoring and treatment of pathologies [26]. Thus, chronic administration of TAESg did not induce variations in ALAT, ASAT, PAL and LDH levels in all rats. Under normal conditions, these enzymes are found in liver, heart, muscle and kidney cells. When these organs are damaged, these enzymes enter the bloodstream [27]. Non-variation in their levels reflects the proper functioning of these organs [28]. TAESg would therefore have no adverse effects on liver parameters when administered chronically. These results are similar to those of Kouassi [15], who after subchronic administration of TAESg observed no variation in these enzymes at doses of 3.5 and 35 mg/kg bw. Regarding the effect of TAESg on serum metabolites, no significant variations in urea, creatinine, glucose, total cholesterol, HDL-cholesterol, triglycerides and total protein levels were observed in all rats at the doses tested. The role of the kidney is to purify the blood and eliminate metabolic end products such as urea, protons and creatinine [4]. The liver's role is to participate in metabolism, the storage of nutrients provided by digestion and the detoxification of harmful substances such as xenobiotics [1-3]. These results suggest that TAESg does not interfere with the renal mechanisms responsible for regulating renal functions, on the one hand, and the mechanism for regulating hepatic functions, on the other. Our results are similar to those of Lyoussi*et al.*[29] who found no significant variation in serum urea and creatinine levels after oral subchronicadministration of the aqueous extract of *Calycotomevillosa* seeds in rats at doses of 300 and 600 mg/kg bw. Also, our

results are in concordance with those obtained by Balogun and Ashafa [30], who after subchronic administration of aqueous extract of *Dicomaanomala* (Asteraceae) roots at doses 125, 250 and 500 mg/kg bw, observed no changes in carbohydrate, lipid and protein metabolism. As for serum electrolytes, no variation in serum potassium, chlorine or magnesium levels was observed in all rats. However, an increase in calcium and sodium levels was observed in rats treated with 35 mg/kg b.w. Electrolytes are maintained in a constant state of dynamic equilibrium in the body. Any variation in their level is essential for the detection of any hydroelectric disorder, and an abnormal electrolyte concentration is a sign of renal failure [31]. Thus, the increase in calcium and sodium levels could be explained by a defect in renal resorption due to excessive dietary intake of the salts in which Ivograin® granules are rich (10.73 g/kg for calcium and 0.15% for sodium). Our results are contrary to those of Kouassi [15], who observed no significant variation in calcium and sodium levels after subchronic oral administration of TAESg at doses of 3.5; 35 and 350 mg/kg bw. Two months after discontinuation of TAESg, calcium and sodium levels returned to normal, indicating that the effect of TAESg is transient.

From an anatomical-histological standpoint, TAESg did not alter the macroscopic appearance of the liver, kidneys and heart at doses of 3.5, 35 and 350 mg/kg bw. There were no significant changes in the relative mass of these organs. Our results are similar to those of Debelo*et al.*[32] for the liver, kidneys and heart. Indeed, these authors showed that aqueous extract of *Thymus schimperi* (Lamiaceae) leaves at doses of 200 and 600 mg/kg bw administered subchronically had no effect on the texture, color and morphology of these organs. Furthermore, histology of the liver, kidneys and heart showed no tissue damage after six months' administration. However, the dilatation of capillary sinusoids observed in rats at a dose of 35 mg/kg bw is thought to be due either to destruction of the sinusoidal wall, or to dissociation of the perisinusoidal matrix, thus atrophying the neighbouring hepatocyte trabeculae [33]. Our results at this level are similar to those of Asyura*et al.*[34], who also noted sinusoid dilatation after subchronic administration of the ethanolic extract of *Clinacanthus nutans* leaves on *Spraygue Dawley* rats at doses of 125 and 250 mg/kg b.w. These results confirm those of Kouassi [33], who highlighted the presence of sinusoid dilatation in rats subjected to the dose of 35 mg/kg b.w. for three months.

Conclusion

Chronic administration of TAESg has no influence on serum markers at the doses studied. Moreover, the anatomo-histological study showed that TAESg administration did not alter the tissue integrity of the vital organs, i.e. liver, kidneys and heart. This translate into a bio-tolerance of TAESg by the rat organism at the various doses of 3.5 mg/kg bw, the therapeutic dose proposed by the traditratician, and at doses of 17.5 and 35 mg/kg bw, TAESg is non-toxic.

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Conflict of Interest

The authors declare no conflict of interest.

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Nil

Informed Consent

We agree

Ethical Statement

None

Author Contribution

All Authors are contributed equally

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