Research Article

Chronic toxicity of the total aqueous extract of the stem bark of *Sacoglottis gabonensis* in Wistar rats

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Abstract

Sacoglottis gabonensis is a plant used in the traditional treatment of infectious diseases such as Buruli ulcer and leprosy in Côte d'Ivoire, which lasts an average of six months. The objective of this study was to verify the safety of the total aqueous extract of *Sacoglottis gabonensis* stem bark (TAESg) in *Wistar* rats over a six-month period. 100 rats were divided into six groups, namely A, B, C and D of 20 rats/group and E, F of 10 rats/group. Groups A and E received distilled water ; groups B and C received TAESg at doses of 3.5 and 17.5 mg/kg bw; groups D and F received 35 mg/kg bw orally at a rate of 1 mL/100 g bw. Groups E and F were used to assess the persistence, reversibility or appearance of delayed toxic effects. The animals were weighed at the beginning and then weekly during the experiment. The quantities of food consumed were measured daily. At the end of the experiment, venous blood taken from the retro-orbital sinus was collected in EDTA tubes to determine the chronic effect of TAESg on hematological parameters. This study showed that daily oral administration of TAESg for six months had no effect on clinical signs of toxicity. No significant variation in feed intake and weight gain was observed. The erythrocyte count and erythrocyte indices ; the leukocyte count and leukocyte indices did not change. However, only the MCHC increased very highly in groups C and D. As for the average platelet count, it increased significantly in group D. Ultimately, TAESg had no effect on clinical signs of toxicity or anthropometric parameters, and was nontoxic on hematological parameters for six months. It is also necessary to evaluate the chronic effect of this extract on clinical biochemistry in rats.

***Keywords*:** *Sacoglottis gabonensis*, safety, anthropometric, hematological parameters

Introduction

According to the WHO, 80% of populations in developing countries rely on medicinal plants for their primary healthcare (OMS, 2002). Medicinal plants are known and used to maintain and/or restore health as an alternative to Western medicine (Abayomi, 1996). Numerous ethnobotanical studies have shown that hundreds of plant species are used in traditional medicine in Africa for the treatment of various pathologies (Adjanohoun, 1990 ; Abayomi, 1996). Among these plant species, some are known to be toxic and can present a real danger or cause minor disorders if ingested (Tsakala et al., 2001). Indeed, any biologically active substance is likely, at low or high doses, associated with prolonged administrations, to produce undesirable or even harmful effects (Gilles, 2004 ; Berrezoug and Berradia, 2014). However, the empirical use of these medicinal plants exposes patients to risks of poisoning which can sometimes prove fatal (Fennell et al., 2004). In addition, studies have shown that 35% of cases of acute renal failure and liver damage are associated with the use of herbal medicines (Nortier et al., 1999 ; Peyrin-Biroulet et al., 2004). However, in Côte d'Ivoire, *Sacoglottis gabonensis* (*S. gabonensis*) is a plant used orally and cutaneously in the treatment of Buruli ulcer (Vangah et al., 2000). Buruli ulcer is a chronic, debilitating infection of the skin and soft tissues caused by

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Mycobacterium ulcerans (OMS, 2012). In addition, the aqueous extract of the stem bark of this plant had an inhibitory effect on the growth of different strains of *Mycobacterium ulcerans* (Koné et al., 2007). Furthermore, acute toxicity tests carried out in mice showed that TAESg has an LD_{50} greater than 5000 mg/kg bw. Subacute toxicity tests by oral and cutaneous routes (Koné et al., 2009), the subchronic oral toxicity test (Kouassi, 2018) showed that total aqueous extract of *Sacoglottis gabonensis* stem bark (TAESg) was non-toxic at the practitioner's therapeutic dose on the various biological parameters studied. Other studies carried out have shown that TAESg possessed healing activity and hemostatic potential (Nagalo et al., 2022a ; 2022b). The chronicity of the treatment, which lasts on average six months, leads to assessing the chronic oral toxicity of this extract in order to guarantee its safety over a long period of use and to recommend it as an alternative means in the management of UB in Côte d'Ivoire. To achieve this objective, it will be specifically a question of assessing the effects of TAESg on clinical signs of toxicity, on anthropometric and hematological parameters following repeated oral administration for six months.

Materials and Methods

Plant material

This material consists of the stem bark of *S. gabonensis* (Baille) Urban (Humiriaceae). This bark was harvested in March 2021 in Ingrakon in the Alépé region, a town located approximately 45 km from the district of Abidjan. A sample was identified according to the one preserved at the National Floristic Center (CNF) under number 1154 dated June 16, 1965.

Animal materials

The experiments were conducted on male and female albino rats of the *Wistar* strain *Rattus norvegicus* species. They were all obtained from the animal facility of the Laboratory of Physiology, Pharmacology, and Pharmacopoeia at Nangui Abrogoua University (UNA). They were six to eight weeks old, with body weights ranging from 84 to 101 g. They were housed in plastic cages with stainless steel lids and provided with feeding bottles. A layer of wood shavings was placed at the bottom of the cages to constitute bedding. The animals were kept at a temperature of 22 ± 2°C with a 12-hour photoperiod. The rats were fed daily with IVOGRAIN® pellets and tap water continuously from feeding bottles. The experimental protocol and animal handling procedures were conducted in accordance with good laboratory practices (OCDE, 1998).

Technical equipment

The entire apparatus consisted of a grinder (Retsch SM 100, Hann, Germany) to finely powder the *S. gabonensis* stem bark ; a Denver S-234 electronic balance (Belgium) for weighing ; a Selecta® oven (Spain) to dry the extracts ; and an Ovan MCG05E magnetic stirrer (Europe) to homogenize the extracts. Other equipment included a funnel, absorbent cotton, and Wattman No. 1 filter paper for filtering the decoction ; a 500 mL graduated cylinder to measure the water content ; and a gastric tube for force-feeding the animals. For the determination of hematological parameters, purple tubes containing EDTA were used for blood sampling. An automated counter analyzer (Sysmex XT 2000i, Japan) for the determination of hematological parameters ; Slides and coverslips, Olympus BX41 microscope (Olympus, Tokyo, Japan) and camera (Samsung Galaxy A50, Korea) for taking blood smears and observation.

Chemical substances and pharmacodynamics

The chemical substances and reagents consisted of diethyl ether (Cooper) for animal anesthesia ; 90% alcohol for disinfection ; and May Grünwald-Giemsa for fixation and staining of blood smears.

Preparation of the TAESg

The total aqueous extract was prepared according to the method described by Kouassi (2018). Four hundred grams (400 g) of *S. gabonensis* stem bark powder was dissolved in two liters (2 L) of distilled water and brought to a boil for 30 minutes. After cooling, the decoction was filtered, first through absorbent cotton and then through Wattman No. 1 paper. The filtrates were oven-dried at 50°C for 48 hours. A dry powder with an TAESg content was obtained. This powder, which was used to prepare different concentrations of TAESg was stored in the refrigerator at -5°C until the days of manipulation.

Chronic oral toxicity assessment

This study was conducted according to OCDE protocol 452 (OCDE, 2009). It consisted of daily oral administration of the test substance to the test groups and distilled water to a control group for a period of at least six months, or 12 months or more, at increasing doses. Since *S. gabonensis* is traditionally used for the treatment of Buruli ulcer over a six-month period, in this study, TAESg was administered daily for six months.

Composition of animal groups and administration of TAESg

Eighty (80) rats were evenly divided into four groups of 20 rats, each consisting of 10 male and 10 female rats. They were separated by sex in cages. Two other groups, called satellite groups, consisting of 10 rats, including five male and five female rats, were used to assess the reversibility, persistence, or delayed onset of toxic effects. All animals in these different groups received a volume of 1 mL/100 g of

body weight by gavage via a cannula once daily, starting at 8:00 a.m., for six months of experimentation. The different administrations were as follows :

- Group 1, the control group, received distilled water ;
- Groups 2, 3, and 4, the test groups, received the TAESg at doses of 3.5, 17.5, and 35 mg/kg bw, respectively.
- Groups 5 and 6, the satellite groups, received distilled water and the TAESg at a dose of 35 mg/kg bw, respectively.

At the end of the six-month experiment, at 8:00 a.m. the following day, the animals in satellite groups 5 and 6 received no further substance for 60 days to assess the persistence of toxic effects, their reversibility, or the delayed onset of toxic effects. These animals had access to water and food ad libitum.

Clinical signs

General clinical observations of the animals were performed twice daily, in the morning after gavage for 3 hours and in the evening from 5:00 p.m. to 7:00 p.m. throughout the study. These clinical observations included behavior, gait, fur, respiration, fecal characteristics, and any acts of cannibalism (OCDE, 1998).

Anthropometric assessment

The animals were weighed at the beginning and then weekly during the experiment to determine their weight gain. Similarly, the quantities of water and food consumed were measured daily, as was the quantity of droppings excreted, to determine food consumption indices (IC) and weight gain (WG), according to the following formulas (Gasnier and Vachel, 1952) :

$$IC = \frac{quantity\ of\ food\ consumed\ during\ a\ period}{weight\ gain\ during\ the\ same\ period}$$

$$WG = mass\ of\ the\ animal\ at\ a\ period - initial\ mass$$

Hematological test

Blood samples were collected from the retro-orbital sinus of the rat eye using a sterile Pasteur pipette (Laroche and Rousselet, 1990). The blood sample was immediately collected in tubes containing EDTA. A complete blood count was performed immediately using an automated analyzer (Sysmex XT-2000 i, Japan) (Smith et al., 1986 ; Bellier and Cordonnier, 2010). At the end of the six-month experiment, a blood smear was taken from each sample. The slide was air-dried for five minutes. The smear was then fixed with May Grünewald alcoholic stain for three minutes. The slide was rinsed with tap water and stained with 10% diluted Giemsa stain for ten minutes. After rinsing with tap water, the smear is dried at room temperature of 25 ± 2 °C for ten minutes (Pouletty, 2010). Finally, the smears are observed under a 10 x 100 objective after immersing a drop of oil on the slide to look for any anomalies.

Statistical analysis

Data were analyzed using Graph Pad Prism 8.0.1 software (San Diego, CA, USA). Results were expressed as the mean followed by the standard error of the mean (M ± SEM). Statistical significance was determined using a 2-way ANOVA followed by Turkey's test. These tests provided the level of significance at $p < 0.05$. In the presentation of results, the symbols (*, **, ***, ****/ $^{\#}$, $^{\#\#}$, $^{\#\#\#}$, $^{\#\#\#\#}$) indicate significant decreases and increases compared to controls.

Results

Effect of TAESg on the general condition of animals

TAESg was administered to apparently healthy rats. However, after administering the different doses of extract, all rats were calm compared to control rats for approximately 10 minutes (Table 1). Changes in stool

Table 1. Observation of clinical signs in animals

Groups	Doses (mg/kg bw)	Tranquility Behavior							Normal Behavior						
		M_0	M_1	M_2	M_3	M_4	M_5	M_6	M_0	M_1	M_2	M_3	M_4	M_5	M_6
A	**0**	0	0	0	0	0	0	0	20	20	20	20	20	20	20
B	**3.5**	0	20	20	20	20	20	20	0	0	0	0	0	0	0
C	**17.5**	0	20	20	20	20	20	20	0	0	0	0	0	0	0
D	**35**	0	20	20	20	20	20	20	0	0	0	0	0	0	0
		Appearance of stools													
Group	**Doses**	**Loose stools**							**Normal stools**						
	(mg/kg bw)	M_0	M_1	M_2	M_3	M_4	M_5	M_6	M_0	M_1	M_2	M_3	M_4	M_5	M_6
A	**0**	0	0	0	0	0	0	0	20	20	20	20	20	20	20
B	**3.5**	0	2	1	2	1	3	1	0	18	19	18	19	17	19
C	**17.5**	0	3	2	4	2	2	1	0	17	18	16	18	18	19
D	**35**	0	6	2	4	4	2	3	0	14	18	16	16	18	17

n = 20: number of rats that received the substances during the six months; 0 = none; M_0 = start of the experiment; M_1 = first month; M_2 = second month; M_3 = third month; M_4 = fourth month; M_5 = fifth month; M_6 = sixth month

appearance, priapism, morbidity, and mortality were observed as clinical signs of toxicity during this study. Indeed, after administration of the different substances, changes in stool appearance in some rats at different doses were observed from the first month until the end of the experiment (Table 2). Stools had become soft and not liquid, unlike diarrhea. The fur of some rats was falling out due to their young age, revealing new fur. As for the state of the genital system, a non-ischemic priapism effect (Figure 1) was observed in male rats of the different doses of TAESg in the first month for the dose 35 mg/kg bw and from the second month for doses of 3.5 and 17.5 mg/kg bw. This effect lasted until the end of the experiment. Thus, after gavage, the rat enters a state of prolonged erection for a period of time. Regarding morbidity and mortality (Table 2), we observed one case of morbidity in the second month at the dose level of 3.5 mg/kg bw, two and four respectively for the doses of 17.5 and 35 mg/kg bw. Similarly, one case of mortality was observed in groups 1 and 2 respectively at the fourth and third month while three and four cases were observed respectively for groups 3 and 4, from the second month to the fourth month.

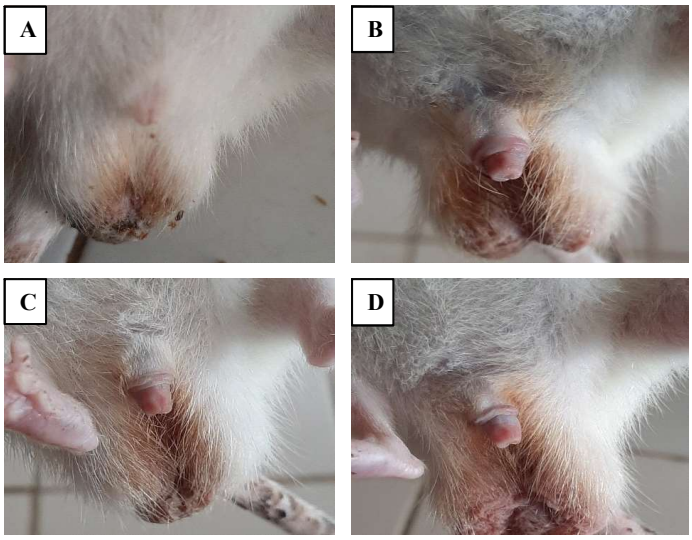

Figure 1. Effect of TAESg on the genital tract of male rats. *A: Normal genital tract in rats treated with distilled water; B: Priapism in rats treated with TAESg at a dose of 3.5 mg/kg bw; C: Priapism in rats treated with TAESg at a dose of 17.5 mg/kg bw; D: Priapism in rats treated with TAESg at a dose of 35 mg/kg bw.*

Effect of TAESg on anthropometric parameters

Effect of TAESg on food consumption indice (IC)

Throughout the experiment, a progressive increase in IC was observed in all animals (Figure 2). Rats receiving 3.5, 17.5, and 35 mg/kg bw of extract consumed almost the same amounts of food as controls during the first, second, fourth, fifth, and sixth months of the experiment. However, at the third month, a very highly significant decrease ($p< 0.0001$) in the food consumption indice was noted in animals force-fed with ETASg at doses of 17.5 and 35 mg/kg bw. This decrease was 67.46 ± 1.40 g and 66.29 ± 1.63 g respectively for doses of 17.5 and 35 mg/kg bw compared to the control which is 77.29 ± 0.95 g.

Effect of ETASg on Weight Gain (WG)

Before administration of the different substances, the rats' weights were 72.9 ± 3.88; 73.2 ± 3.55; 73.3 ± 4.82 and 72.9 ± 3.61 respectively for groups A, B, C and D. A change in

Table 2. Observation des signes cliniques des animaux (suite)

Condition of the genital system																
		Non-ischemic priapism							Normal							
Group	Doses (mg/kg bw)	M_0	M_1	M_2	M_3	M_4	M_5	M_6	M_0	M_1	M_2	M_3	M_4	M_5	M_6	
A	0	0	0	0	0	0	0	0	10	10	10	10	10	10	10	
B	3.5	0	0	10	6	5	7	8	10	10	0	4	5	3	2	
C	17.5	0	0	10	8	8	7	8	10	10	0	2	2	3	2	
D	35	0	10	10	10	7	8	10	10	0	0	0	3	2	0	
		Morbidity								Mortality							
Group	Doses (mg/kg bw)	M_0	M_1	M_2	M_3	M_4	M_5	M_6	Total (%)	M_0	M_1	M_2	M_3	M_4	M_5	M_6	Total (%)
A	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5
B	3.5	0	0	1	0	0	0	0	5	0	0	0	1	0	0	0	5
C	17.5	0	0	1	1	0	0	0	10	0	0	1	1	1	0	0	15
D	35	0	0	3	1	0	0	0	15	0	0	2	1	1	0	0	20

n = 20: number of rats that received the substances during the six months; 0 = none; M_0 = start of the experiment; M_1 = first month; M_2 = second month; M_3 = third month; M_4 = fourth month; M_5 = fifth month; M_6 = sixth month.

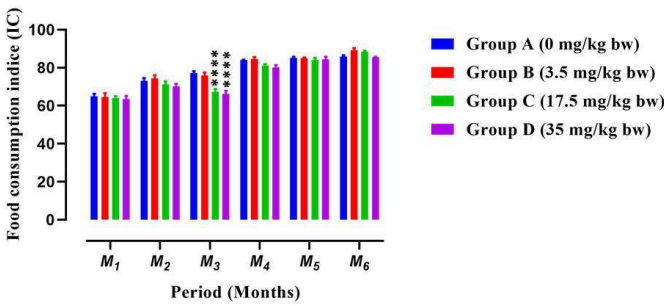

Figure 2. Effect of ETASg on food consumption indice in all rats. *Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group 1, control, and groups 2, 3 and 4 treated respectively at doses of 3.5, 17.5 and 35 mg/kg bw for six months of treatment; M_1: first month; M_2: second month; M_3: third month; M_4: fourth month; M_5: fifth month; M_6: sixth month.*

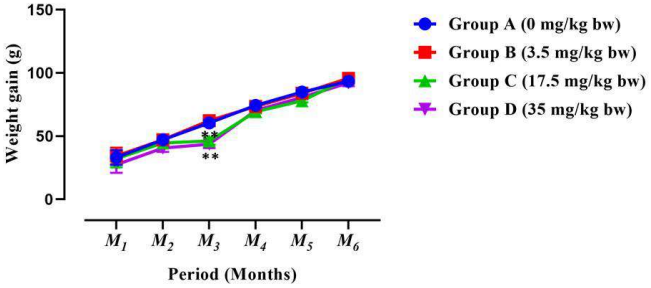

Figure 3. *Effect of TAESg on weight gain in all rats. Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. p< 0.05. Comparisons are made between group 1, control, and groups 2, 3 and 4 treated respectively at doses of 3.5, 17.5 and 35 mg/kg bw for six months of treatment; M_1: first month; M_2: second month; M_3: third month; M_4: fourth month; M_5: fifth month; M_6: sixth month.*

weight gain during the six months of treatment was observed in all animals in the different groups (Figure 3). Animals at doses of 3.5, 17.5 and 35 mg/kg bw of extract had a statistically identical weight gain (p> 0.05) to that of the controls during the first, second, fourth, fifth and sixth months of the experiment. However, at the third month, a very highly significant decrease (p< 0.0001) in body weight gain was noted in animals at the 17.5 and 35 mg/kg bw doses. This decrease was 46.15 ± 2.87 g and 43.69 ± 3.09 g respectively for the 17.5 and 35 mg/kg bw doses compared to the body mass of the controls which is 60.48 ± 2.30 g.

Reversible and delayed effect of ETASg administration on anthropometric parameters

At the sixth month, the last day of administration of TAESg, all anthropometric parameters (IC and WG) of animals treated with a dose of 35 mg/kg bw of extract were similar to those of animals receiving distilled water (Table 3). Two months after stopping the administration of the extract, the various anthropometric parameters changed in both control rats and rats receiving the 35 mg/kg bw dose. However, no significant variation (p< 0.05) in these parameters was observed.

Effect of TAESg on hematological parameters

Effect of TAESg on erythrocyte parameters in rats

Quantitatively, the results obtained after oral administration of the TAESg do not indicate any significant increase (p> 0.05) in erythrocyte counts in rats treated at doses of 3.5, 17.5, and 35 mg/kg bw compared to those in the control group (0 mg/kg bw) (Table 4). These values are 7.74 ± 0.20, 7.65 ± 0.17, 7.43 ± 0.16, and 7.59 ± 0.18.10^6/mm^3, respectively, for 0, 3.5, and 4.5 mg/kg bw. 17.5 and 35 mg/kg bw. The erythrocyte indices, namely hemoglobin, hematocrit, and MCV, also showed no significant variation (p> 0.05) in the treated groups compared to the control group (0 mg/kg bw). However, MCHC showed a very highly significant increase (p< 0.0001) in the groups treated at doses of 17.5 and 35 mg/kg bw compared to the control group. These values were 22.19 ± 0.20 Pg and 21.80 ± 0.40 Pg respectively in the group treated at doses of 17.5 and 35 mg/kg bw and 19.12 ± 0.37 Pg in the control group. Qualitatively, the TAESg had no significant influence on the morphology of erythrocyte cells. Indeed, these cells retained their size, color, and shape in rats treated at doses of 3.5; 17.5 and 35 mg/kg bw compared to those of the control group (0 mg/kg bw) (Figure 4).

Table 3. Effects on anthropometric parameters after discontinuation of TAESg administration

Groups	Doses (mg/kg bw)	Food consumption indice		Weight gain	
		Month 6	Month 8	Month 6	Month 8
E	**0**	81.07±1.06	124.8±2.58	94.44±0.95	166.8±8.24
F	**35**	82.63±0.82	131.3±1.09	91.92±1.53	159.9±5.76

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 10 rats/group. Comparisons are made between group 5, control, and group 6 treated at a dose of 35 mg/kg bw. Month 6: sixth month; Month 8: eighth month. p> 0.05.

Effect of TAESg on leukocyte parameters in rats

The results obtained after oral administration for six months of the TAESg show that the mean values of the leukocyte count in rats treated at doses of 3.5, 17.5 and 35 mg/kg bw did not undergo any significant variation ($p > 0.05$) compared to the control group (0 mg/kg bw) (Table 5). These values are 13.50 ± 0.73; 15.33 ± 0.72; 12.25 ± 0.90 and 12.41 ± 1.28.10^3/mm^3 respectively for doses of 0, 3.5, 4.5 and 5.5 mg/kg bw. 17.5 and 35 mg/kg bw. The same observation was made at the level of leukocyte indices, namely lymphocytes, monocytes and granulocytes, where no significant variation ($p > 0.05$) was recorded in all the rats treated at the different doses compared to those of the rats in the control group.

Effect of TAESg on platelet count in rats

The mean platelet count of rats treated with the TAESg increased non-significantly ($p > 0.05$) at doses of 3.5 and 17.5 mg/kg bw and significantly ($p < 0.05$) at 35 mg/kg bw compared to that of control rats (Table 6). The mean platelet count varied from 421.8 ± 61.24 × 10^3/mm^3 ; 432.3 ± 62.85 × 10^3/mm^3 and 461.9 ± 55.91 × 10^3/mm^3 in rats treated with doses of 3.5, 17.5 and 35 mg/kg bw and from 408.7 ± 29.26. 10^3/mm^3 at a dose of 0 mg/kg bw.

Table 4. Effects of TAESg on erythrocyte parameters in all rats

Erythrocyte parameters	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)
Erythrocytes (10^6/mm^3)	7.74 ± 0.20	7.65 ± 0.17	7.43 ± 0.16	7.59 ± 0.18
Hemoglobin (g/dL)	14.71 ± 0.19	14.44 ± 0.24	14.23 ± 0.49	14.95 ± 0.21
Hematocrits (%)	41.33 ± 0.79	40.44 ± 0.67	39.38 ± 7.03	41.81 ± 0.87
MCV (fL)	53.54 ± 0.87	52.98 ± 0.62	55.04 ± 1.41	55.21 ± 0.82
MCHC (Pg)	19.12 ± 0.37	18.90 ± 0.21	22.19 ± 0.20$^{\#\#\#\#}$	21.80 ± 0.40$^{\#\#\#\#}$

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group 1, control, and groups 2, 3 and 4 treated respectively at doses of 3.5, 17.5 and 35 mg/kg bw; $p<0.05$. MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Content.

Table 5. Effects of TAESg on leukocyte parameters in all rats

Leukocyte parameters	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)
Leukocytes (10^3/mm^3)	13.50 ± 0.73	15.33 ± 0.72	12.25 ± 0.90	12.41 ± 1.28
Lymphocytes (10^3/mm^3)	11.04 ± 0.73	12.00 ± 0.63	10.87 ± 0.70	10.31 ± 1.13
Monocytes (10^3/mm^3)	1.24 ± 0.08	1.36 ± 0.08	1.33 ± 0.12	1.26 ± 0.14
Granulocytes (10^3/mm^3)	1.01 ± 0.16	0.97 ± 0.15	1.05 ± 0.22	1.04 ± 0.11

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group 1, control, and groups 2, 3 and 4 treated respectively at doses of 3.5, 17.5 and 35 mg/kg bw.

Table 6. Effects of TAESg on thrombocyte counts in all rats

Thrombocyte parameter	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)
Thrombocytes (10^3/mm^3)	408.7 ± 29.26	421.8 ± 61.24	432.3 ± 62.85	461.9 ± 55.91$^{\#}$

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 20 rats/group. Comparisons are made between group 1, control, and groups 2, 3 and 4 treated respectively at doses of 3.5, 17.5 and 35 mg/kg bw.

Reversible and delayed effects of TAESg

Two months after discontinuation of TAESg administration, no delayed effects were observed on erythrocyte and leukocyte parameters in all rats (Table 7). However, the disturbances observed in MCHC and platelet levels in rats treated with TAESg at a dose of 35 mg/kg bw after six months of administration virtually disappeared two months after discontinuation.

Table 7. Effects of TAESg on hematological parameters in all rats after cessation of administration

Hematological parameters	Group E (0 mg/kg bw)		Group F (35 mg/kg bw)	
	Month 6	Month 8	Month 6	Month 8
Erythrocytes (10^6/mm^3)	7.74 ± 0.20	7.42 ± 0.24	7.59 ± 0.18	7.49 ± 0.27
Hemoglobin (g/dL)	14.71 ± 0.19	14.55 ± 0.24	14.95 ± 0.21	14.72 ± 0.22
Hematocrits (%)	41.33 ± 0.79	40.09 ± 1.20	41.81 ± 0.87	41.22 ± 0.74
MCV (fL)	53.54 ± 0.87	54.25 ± 1.07	55.21 ± 0.82	55.26 ± 0.99
MCHC (Pg)	19.12 ± 0.37	19.73 ± 0.48	22.19 ± 0.20$^{\#\#\#\#}$	19.85 ± 0.63
Leukocytes (10^3/mm^3)	13.50 ± 0.73	12.81 ± 0.91	12.41 ± 1.28	13.25 ± 0.97
Lymphocytes (10^3/mm^3)	11.04 ± 0.73	10.59 ± 0.90	10.31 ± 1.13	11.07 ± 0.71
Monocytes (10^3/mm^3)	1.24 ± 0.08	1.18 ± 0.10	1.26 ± 0.14	1.27 ± 0.19
Granulocytes (10^3/mm^3)	1.01 ± 0.16	0.94 ± 0.15	1.04 ± 0.11	0.91 ± 0.07
Thrombocytes (10^3/mm^3)	408.7 ± 29.26	403.8 ± 33.70	461.9 ± 55.91$^{\#}$	421.8 ± 33.15

Values are expressed as means followed by the standard error of the mean (M ± SEM); n = 10 rats/group. Comparisons are made between groups 5, control, and 6 treated at a dose of 35 mg/kg bw; $p < 0.05$. MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Content.

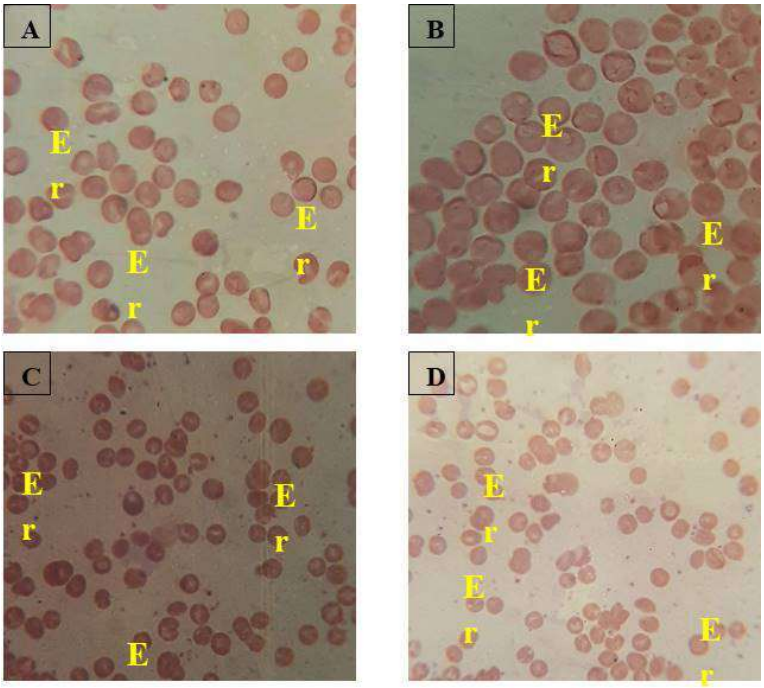

Figure 4. Effects of TAESg on erythrocytes in rats. *A: Blood smear from a rat in the control group; B, C and D: Blood smear from a rat treated with the TAESg at the respective doses of 3.5, 17.5 and 35 mg/kg bw; Er: Erythrocytes; May-Grünwald Giemsa staining; Magnification x100.*

Discussion

The study of chronic toxicity of the TAESg by oral administration revealed clinical signs of tranquility, changes in stool appearance, loss of dorsal fur, priapism, morbidity, and mortality at doses of 3.5, 17.5, and 35 mg/kg bw. These various signs of toxicity observed during this study are thought to be due to the nature and chemical composition of the extract, such as flavonoids, bergenin, alkaloids, sterols, and tannins, which appear to act on the central nervous system, the digestive system, and the reproductive system (Viau and Tardif, 2003). Indeed, the effects of a toxic substance on the central nervous system are manifested by temporary tranquility, depression, loss of appetite or drowsiness (Zayed et al., 2003). The change in the appearance of stools would be due to the nature of the extract which would act as a laxative, which leads to an acceleration of transit time and thus an increase in the frequency of stools (Ramkumar and Rao, 2005 ; Ford and Suares, 2011). Non-ischemic priapism, persistent non-painful erection observed after administration of the extract (gavage) is linked to an excessive influx of arterial blood within the corpora cavernosa of the penis (Kuefer et al., 2005) and would be due to the presence of certain phytochemical compounds such as sterols and flavonoids (Ondele et al., 2015). Also, after a phytochemical screening on *Caesalpinia benthamiana* (Caesalpiniaceae), a plant used in the treatment of erectile dysfunction, Bekro et al., (2007) revealed the presence of several phytochemical compounds including sterols and flavonoids which would be at the origin of the correction of these disorders. However, Koné et al., (2009) highlighted these phytochemical compounds and according to Bouquet, (1969), the decoction of the bark is used to treat diseases of the genital organs, which would justify non-ischemic priapism in rats. Mortality in the controls was due to cannibalism. The observed morbidity that later led to the death of the rats would be due to toxic manifestations *in vivo* (Viau and Tardif, 2003). Our results are contrary to those of Lyoussi et al. (2018), who, after chronic administration of the aqueous extract of *Calycotome villosa* seeds (Fabaceae) in rodents, did not observe clinical signs of toxicity.

Anthropometry is the only universally applicable and non-invasive method for assessing and evaluating nutritional and health risks (OMS, 1995). Administration of TAESg for six months showed no change in food consoumption indice and weight gain in animals treated with doses of 3.5, 17.5, and 35 mg/kg bw. A transient decrease in weight gain in animals treated with doses of 17.5 and 35 mg/kg bw was observed at the third month. This decrease in weight gain was associated with low feed intake. The decrease in weight gain is linked to the animal's nutritional status (Bertrand et al., 2003; Yaguiyan-Colliard, 2013). Indeed, any variation in the quantities of food consumed has an impact on weight gain, justifying the decrease in weight gain in the third month of the experiment (Besson et al., 2005). Our results are similar to those of Kouassi et al., (2018). These authors showed that subchronic administration of TAESg led to a decrease in food and water consumption and weight gain in rats at doses of 35 and 350 mg/kg bw in the third month. Two months after stopping TAESg administration, no clinical signs were observed in the general condition of the animals and anthropometric parameters.

At the hematological level, TAESg did not cause any variation in the rate of erythrocytes, hemoglobin, hematocrit, MCV, but caused an increase in MCHC at doses of 17.5 and 35 mg/kg bw after six months of treatment. This would mean that TAESg does not affect hematological parameters (Muthuraman and Singh, 2012). The increase in MCHC in rats at doses of 17.5 and 35 mg/kg bw could be due to an idiosyncratic effect (Narjoz et al., 2010). Our results are similar to those of Kouassi et al., (2018). These authors showed that subchronic administration of TAESg in rats at doses of 3.5, 35, and 350 mg/kg bw did not result in changes in erythrocyte parameters. However, our results are contrary to those of Osseni et al., (2017) who reported the formation of macrocytic erythrocytes on blood smears after subchronic administration of a total aqueous extract of *Carissa edulis* (Apocynaceae) leaves in rats at doses of 7.81, 31.25, and 125 mg/kg bw. At the leukocyte lineage level, TAESg did not modify their rate. As for thrombocytes, an increase in their average rate was observed at 35 mg/kg bw. The observed increase in the number of platelets suggests that the extract would act by promoting coagulation which could prevent hemorrhages (Li et al., 1999). Our results are in agreement with those of Kouassi et al. (2018) who obtained no variation in leukocyte parameters, but on the other hand an increase in thrombocytes after subchronic administration of TAESg in rats at doses of 3.5; 35 and 350 mg/kg bw. Two months after stopping TAESg administration, no variation was observed in erythrocyte and leukocyte parameters. The normalization of MCHC and thrombocyte levels shows that these changes were transient.

Conclusion

A study of the chronic toxicity of the total aqueous extract of S. gabonensis stem bark showed that administration of this extract resulted in the manifestation of certain signs such as non-ischemic priapism and facilitated intestinal transit. Furthermore, ETASg did not influence anthropometric, hematological, and biochemical parameters at the doses studied. However, Further Studies Of This Extract On Biochemical Parameters In Rats Are Needed.

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