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Hemostatic potential of total aqueous extract of Sacoglottis gabonensis (Baille) Urban (Humiriaceae) stem bark in Wistar rats pretreated with warfarin

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Research

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ABSTRACT

Background: *Sacoglottis gabonensis* is a medicinal plant used in traditional treatment of Buruli ulcer and leprosy in Côte d'Ivoire. A study conducted on the healing potential of this herb on induced wounds showed good results. The objective of this study is to evaluate the hemostatic potential of the total aqueous extract of *Sacoglottis gabonensis* (TAESg) in rats after a pretreatment with warfarin.

Methods: Thus, 42 rats were evenly distributed into seven groups of six rats each. The rats in group 1 did not receive treatment whereas those in groups 2, 3, 4, 5, 6 and 7 pretreated with warfarin received by oral route, distilled water, 100 mg/kg body weight (bw) of Vitamin K1 and doses of 3.5; 17.5; 35 and 350 mg/kg bw of TAESg, respectively. Blood samples were collected from the rats' retro-orbital sinus before the experiment, after induction the blood hypocoagulation with warfarin and after the treatments with vitamin K or the extract in orderto determine hemostatic parameters such as the prothrombin time, the activated partial thromboplastin time and the International Normalized Ratio, the fibrinogen level, the calcium level and the thrombocyte level.

Results: The results showed disturbances in the hemostatic parameters of warfarin-induced hypocoagulability rats. The treatments with TAESg significantly normalized these parameters by reducing the prothrombin time, the activated partial thromboplastin time and the INR and by increasing fibrinogen levels. However, the levels of calcium and thrombocytes which were increased after the administration of warfarin did not experience any significant change after the treatments with TAESg or Vitamin K, depending on the group.

Conclusion: TAESg has some hemostatic properties similar to those of vitamin K.

Key words: hypocoagulation, hemostatic parameters, rats, *Sacoglottis gabonensis.*

1. INTRODUCTION

The occurrence of a vascular injury triggers a physiological defense mechanism that fights against the loss of blood from the vascular system. Thus, hemostasis is the entire physiological process intended to limit blood loss during a vascular breach through the formation of a thrombus [1,2]. A dysfunction in this process can induce a hemorrhagic or thrombotic pathology[3]. Among these pathologies, thrombopathies where the function of the platelet is altered,[4] thrombocytopenia, hemorrhage was found [5,6]. According to the World Health Organization projections, the complication of arterial or venous thrombotic diseases is a consequence of cardiovascular mortality. It is one of the main causes of death in the world with 35% in men and 40% in women [7]. The treatments used in the context of dysfunctions in hemostasis are varied. The therapy used is based on drugs that reduce the haemorrhagic phenomenon with platelet action such as catecholamines, vasopressin and relatives [8].

Despite the progress made by modern medicine, the management of all kinds of bleeding disorders constitutes a public health problem [9]. Moreover, in Africa, the inadequacy and inaccessibility of health centers and concerns about the harmful effects of modern medicines have caused a large part of the population of African countries to turn to traditional medicine [10]. Resorting to easily available and accessible natural resources would constitute a palliative solution to so-called modern medicines. About 80% of Africans use traditional medicine to meet their primary health and care needs. Thus, the WHO recommends countries to conduct scientific studies about the efficacy and safety of medicinal plants used empirically [11]. It is in this context that many works have been conducted by several researchers on the therapeutic virtues of medicinal plants. Among them there are plants that are used to stop bleeding (hemorrhages) [12,13]. In order to contribute to the management of haemorrhages by plants, *Sacoglottis gabonensis* drew our attention. Indeed, this plant is used orally and by cutaneous route in the treatment of Buruli ulcer in Côte d'Ivoire [14]. Phytochemical screening revealed the presence of several phytochemical compounds, namely terpenes, polyphenols, flavonoids, tannins, quinones and alkaloids [15]. A recent study conducted in our laboratory showed that the extract of this plant accelerates wound healing induced in rats [16].

This work aims to show hemostatic potentials of the total aqueous extract of the stem bark of *S.gabonensis* in rats pretreated with warfarin by evaluating prothrombin time (PT), activated thromboplastin time (APT), the International Normalized Ration (INR) and fibrinogen, calcium and thrombocyte levels.

2. MATERIAL AND METHODS

2.1. Plant

The plant material used was composed of the stem bark of *Sacoglottis gabonensis* (Baille) Urban (Humiriaceae). They were harvested in March 2021 in Ingrakon (Alépé, Côte d'Ivoire) a town located at about 45 km from Abidjan. A sample was identified in accordance with that kept at the National Floristic Center, under number 1154 of June 16, 1965.

2.2. Animal

The experiments were conducted on male and female albino *Wistar* rats (*Rattus norvegicus*). They were kept in the animal facility of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of Nangui Abrogoua University (UNA). They were 3 months old and their body weight varied between 109 to 120 g. They were housed in plastic cages with a stainless slid and provided with feeding bottles. A layer of wood shavings was placed at the bottom of the cages to form the bedding. The animals were subjected to a temperature of 22 ± 2°C with a light/dark cycle. The rats were fed daily with standard granules and tap water ad libitum. The experimental protocol and the animal handling procedures were conducted according to good laboratory practices [17].

2.3. Preparation of total aqueous extract of the plant

The method was as previously described by Kouassi [18]. The fresh harvested barks were crushed into small pieces and subsequently dried in the laboratory on the bench at a temperature of 25°C for four weeks. The dried barks were reduced into a fine powder using the Retsch SM 100 grinder. Four hundred grams (400 g) of the powdered were dissolved in two liters (2L) distilled water and boiled for 30 minutes. The decoction was filtered on absorbent cotton and Wattman filter paper N°1. The filtrates were dried in the oven at 50°C for 48 hours. A dry brown powder which is total aqueous extract of *Sacoglottis gabonensis* (TAESg) was stored in air-tight bottles until ready for use.

2.4. Evaluation of hemostatic potential

2.4.1. Induction of blood hypocoagulation in rats

The method used was that of Handan *et al.* with a slight modification in terms of dose and induction time [19]. Forty-two rats were distributed evenly into seven groups of six rats at the rate of three male rats and three female rats. To do this, except the rats in group 1, the rats were pretreated with 3 mg/kg body weight of warfarin (Coumadine®) dissolved in distilled water by oral route using a gastric tube, for two consecutive days.

2.4.2. Animal treatment

All animals in the different groups were given a volume of 1 mL/100 g body weight by oral route once a day for three days of experimentation. The different administrations were as follows:

- Group 1: control group; non-hypocoagulated rats were administered with distilled water.
- Group 2: positive control; hypocoagulated rats were administered with distilled water.
- Group 3: negative control; hypocoagulated rats were treated with 100 mg/ kg body weight of Vitamin-K.
- Group 4: hypocoagulated rats were treated with 3.5 mg/kg body weight of TAESg.
- Group 5: hypocoagulated rats were treated with 17.5 mg/kg body weight of TAESg.
- Group 6: hypocoagulated rats were treated with 35 mg/kg body weight of TAESg.
- Group 7: hypocoagulated rats were treated with 350 mg/kg body weight of TAESg.

2.4.3. Blood sampling

Blood samples were taken according to the method described by Waynforth [20]. Three blood samples were taken, before the induction of hypocoagulability, on the third day after the induction of hypocoagulability and at the end of the experiment. Thus, the blood taken immediately was collected into three different types of tubes. The first one contained the anticoagulant ethylene diamine tetra acetic acid (EDTA), the second one, sodium citrate and the last one, dry tubes.

2.4.4. Determination of hemostatic parameters

The coagulation potential was performed directly using a Sysmex machine from samples taken in tubes containing sodium citrate. The tubes were centrifuged at 3000 rpm for 10 min. the sera obtained were used to determine the prothrombin time (PT) [21]. The activated partial thromboplastin time and the fibrinogen level [22,23]. The method used for the determination of calcium was that of Clark *et al.* done in serum collected in dry tubes after a centrifugation at 3000 rpm for 5 min [24]. As for the dosage of thrombocytes, it was determined from blood samples taken in tubes containing EDTA for complete blood count [25,26].

2.5. Statistical analysis

Data were analyzed using Graph Pad Prism 8.0.1 software (San Diego, CA, USA). The results obtained were expressed as the mean followed by the standard error on the mean (M ± SEM). Statistical significance was determined using one-way analysis of variance (ANOVA1), followed by Turkey's test. For the presentation of the results, the signs (*, **, ***, **** / #, ##, ###, ####) indicated significant decreases while the letters (a, b, c and d / e, f, g and h) expressed significant increases compared to controls.

Table 1. Observation of the general condition of the animals

	Skin rash			Loss of motor skills			Diarrhoea			Heamaturia / Bleeding orifices			Salivation / vomiting			Aggressiveness		
	Be-fInd	Af-tInd	drgt rtm	Be-fInd	Af-tInd	drgt rtm	Be-fInd	Af-tInd	drgt rtm	Be-fInd	Af-tInd	drgt rtm	Be-fInd	Af-tInd	drgt rtm	Be-fInd	Af-tInd	drgt rtm
Grp 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grp 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
Grp 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
Grp 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
Grp 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
Grp 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6
Grp 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	6

n = 6: number of rats/groups; Group 1: untreated normal control;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively); Grp: Group; BefInd: before induction; Aft Ind: after induction; drgtrtm: during treatment.

3. RESULTS

3.1. Effect of the administration of warfarin on the general condition of animals

Prior to pretreatment of the rats with warfarin, all rats of the different group were doing well. After pre-treatment of the rats with warfarin and during the different treatments, no rat from group 1, 2, 3, 4, 5, 6 and 7 showed rash, loss of motricity, diarrhea, hematuria, orifice bleeding, salivation, vomiting (Table 1). However, except the control group 1, aggression was noted in the rats'behavior after warfarin administration and during their treatment. This aggressiveness was observed in both male and female rats of the different group.

3.2. Evolution of prothrombin time

The results of the effects of TAESg were presented in table 2. There was no significant difference between the prothrombin times of rats from different group at the beginning of the experiment. Similarly, for the same Group, the values obtained in male and female subjects showed no significant difference. After the administration of warfarin to the rats, a very highly significant increase in the prothrombin time was observed compared to the normal control group. These increases ranged between 49.10 ± 3.60; 47.62 ± 3.18; 48.25±2.73; 50.67 ± 2.96; 47.27 ± 3.12 and 46.98 ± 2.39 s respectively for group 2, 3, 4, 5, 6 and 7 compared to the normal control group whose time was 11.91 ± 0.30 s. After the treatment, the prothrombin times of the rats with Vitamin K and TAESg were significantly decreased compared to the values of the normal control group. The rats of the group treated with doses of 3.5; 17.5; 35 and 350 mg/kg bw of TAESg showed a decrease in prothrombin time in a dose -dependent manner compared to the values of the rats of group 3 treated with dose 100 mg/kg bw of vitamin K.

Table 2. Evolution of the prothrombin time

	Sex	Before induction	After induction	Aftertreatment (3 days)
Group 1	**M+F**	12.15 ± 0.09 s	11.91 ± 0.30 s	12.81 ± 0.07 s
Group 2	**M+F**	13.25 ± 0.31 s	49.10 ± 3.60^{d} s	31.68 ± 0.35^{d} s
Group 3	**M+F**	12.57 ± 0.18 s	47.62 ± 3.18^{d} s	14.21 ± 0.13$^{a\ ####}$ s
Group 4	**M+F**	12.79 ± 0.20 s	48.25 ± 2.73^{d} s	23.13 ± 0,48$^{d\ ####}$ s
Group 5	**M+F**	12.89 ± 0.14 s	50.67 ± 2.96^{d} s	20.33 ± 0.29$^{d\ ####}$ s
Group 6	**M+F**	12.70 ± 0.06 s	47.27 ± 3.12^{d}	17.50 ± 0.20$^{d\ ####}$ s
Group 7	**M+F**	12.65 ± 0.26 s	46.98 ± 2.39^{d} s	14.40 ± 0.32$^{b\ ####}$ s

The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2, 3, 4, 5, 6, and 7 on the one hand and between group 2 and group 3, 4, 5, 6, and 7 on the other. p< 0.05, n = 6 (3M, 3F): number of rats/ groups; a: significant, b highly significant, ## and c: very highly significant, #### and d: very highly significant; Group 1: normal control untreated;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively); M: male; F: female; s: second.

3.3. Evolution of activated partial thromboplastin time

The administration of warfarin induced a significant increase of the activated partial thromboplastin time compared to that of group 1, the normal control. The activated partial thromboplastin time of control group was 17.99 ± 0.33 s whereas those of groups 2; 3; 4; 5; 6 and 7 reached 48.76 ± 3.52; 51.73 ± 5.10; 50.45 ± 4.28; 49.90 ± 3.74; 50.43 ± 4.46 and 49.89 ± 3.99 s, respectively. After the different treatments with vitamin K and TAESg, the activated partial thromboplastin time in all rats decreased significantly ($p < 0.001$) (Table 3)

3.4. Evolution of the International Normalized Ratio (INR)

Table 4 shows the effects of TAESg on the evolution of the International Normalized Ratio in rats pretreated with warfarin. The results highlighted a significant increase of the INR in the rats pretreated with warfarin compared to those of the control group. In fact, the levels were increased four times and reached 4.37 ± 0.46; 4.47 ± 0.32; 4.83 ± 0.25; 4.44 ± 0.36; 4.84 ± 0.22 and 4.81 ± 0.29 respectively for group 2, 3, 4, 5, 6 and 7.After treatment, the INR values of rats from group 3 treated with dose 100 mg/kg bw of vitamin K and those of groups 4, 5, 6 and 7 treated with TAESg at doses of 3.5; 17.5; 35 and 350 mg/kg bw were significantly reduced compared to the rats pretreated with warfarin without any treatment.

Table 3. Evolution of the activated partial thromboplastin time

	Sex	Before induction	After induction	Aftertreatment (3 days)
Group 1	**M+F**	17.95 ± 0.17 s	17.99 ± 0.33 s	18.84 ± 0.61 s
Group 2	**M+F**	17.89 ± 0.34 s	48.76 ± 3.52^{d} s	38.01 ± 1.94^{d} s
Group 3	**M+F**	17.87 ± 0.23 s	51.73 ± 5.10^{d} s	19.97 ± 0.35$^{####}$ s
Group 4	**M+F**	17.86 ± 0.38 s	50.45 ± 4.28^{d} s	25.04 ± 1.09$^{a\ ####}$ s
Group 5	**M+F**	17.77 ± 0.39 s	49.90 ± 3.74^{d} s	22.37 ± 0.28$^{a\ ####}$s
Group 6	**M+F**	18.30 ± 0.27 s	50.43 ± 4.46^{d} s	21.25 ± 0.29$^{####}$ s
Group 7	**M+F**	17.95 ± 0.37 s	49.89 ± 3.99^{d} s	20.84 ± 0.31$^{####}$s

The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2, 3, 4, 5, 6, and 7 on the one hand and between group 2 and groups 3, 4, 5, 6, and 7 on the other. p< 0.05, n = 6 (3M, 3F): number of rats/ groups; a: significant, b highly significant, ## and c: very highly significant, #### and d: very highly significant; Group 1: normal control untreated; Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively); M: male; F: female; s: second.

Table 4. Evolution of the International Normalized Ratio (INR)

	Sex	Before induction	After induction	Aftertreatment (3 days)
Group 1	**M+F**	1.06 ± 0.01	1.07 ± 0.01	1.07 ± 0.01
Group 2	**M+F**	1.07 ± 0.02	4.37 ± 0.46^{d}	3.78 ± 0.07^{d}
Group 3	**M+F**	1.06 ± 0.02	4.47 ± 0.32^{d}	1.07 ± 0.02$^{\#\#\#\#}$
Group 4	**M+F**	1.08 ± 0.03	4.83 ± 0.25^{d}	1.34 ± 0.02$^{\#\#\#\#}$
Group 5	**M+F**	1.07 ± 0.01	4.44 ± 0.36^{d}	1.20 ± 0.02$^{\#\#\#\#}$
Group 6	**M+F**	1.07 ± 0.02	4.84 ± 0.22^{d}	1.15 ± 0.01$^{\#\#\#\#}$
Group 7	**M+F**	1.09 ± 0.03	4.81 ± 0.29^{d}	1.08 ± 0.02$^{\#\#\#\#}$

The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2, 3, 4, 5, 6, and 7 on the one hand and between group 2 and groups 3, 4, 5, 6, and 7 on the other. p< 0.05, n = 6 (3M, 3F): number of rats/ group; a: significant, b highly significant, ## and c: very highly significant, #### and d: very highly significant; Group 1: normal control untreated;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively); M: male; F: female.

Table 5. Evolution of fibrinogen levels

	Sex	Before induction	After induction	Aftertreatment (3 days)
Group 1	**M+F**	2.01 ± 0.05**g/L**	2.00 ± 0.02**g/L**	1.99 ± 0.02**g/L**
Group 2	**M+F**	1.98 ± 0.03**g/L**	0.92 ± 0.02******g/L**	0.94 ± 0.03******g/L**
Group 3	**M+F**	1.99 ± 0.01**g/L**	0.95 ± 0.02******g/L**	1.94 ± 0.04^{d}**g/L**
Group 4	**M+F**	2.00 ± 0.01**g/L**	0.97 ± 0.01******g/L**	1.46 ± 0.02^{d}**g/L**
Group 5	**M+F**	2.05 ± 0.05**g/L**	0.91 ± 0.02******g/L**	1.60 ± 0.04^{d}**g/L**
Group 6	**M+F**	2.00 ± 0.01 **g/L**	0.94 ± 0.02******g/L**	1.76 ± 0.01^{d}**g/L**
Group 7	**M+F**	1.99 ± 0.04**g/L**	0.97 ± 0.01******g/L**	1.84 ± 0.02^{d}**g/L**

*The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2, 3, 4, 5, 6, and 7 on the one hand and between group 2 and groups 3, 4, 5, 6, and 7 on the other. p< 0.05, n = 6 (3M, 3F): number of rats/ groups; **** and d: very highly significant; Group 1: normal control untreated;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively).; M: male; F: female.*

3.5. Evolution of the fibrinogen level

The results of the effects of TAESg were presented in table 5. The administration of warfarin to the rats induced a significant drop in the level of fibrinogen compared to the control group. These decreases were ranged between 0.92 ± 0.02; 0.95 ± 0.02; 0.97 ± 0.01; 0.91 ± 0.02; 0.94 ± 0.02 and 0.97 ± 0.01 g/L respectively for group 2, 3, 4, 5, 6 and 7 meanwhile the fibrinogen level was 2.00 ± 0.02g/L in the control group. The treatment of the rats with dose 100 mg/kg bw of vitamin K or TAESg with doses 3.5; 17.5; 35 and 350 mg/kg bw led to a significant increase (p< 0.0001) in this parameter compared to the rats pretreated with warfarin with no other treatment.

3.6. Evolution of the calcium level

Prior to warfarin pre-administration to rats, there was no significant difference of calcium levels between rats from different group. The rate was ranged from 11.38 ± 0.61; 11.53 ± 0.48; 12.10 ± 0.70; 11.58 ± 0.48; 12.06 ± 0.45; 11.81 ± 0.50 and 11.47 ± 0.56 mmol/L (Table 6). After the pretreatment of the rats with warfarin, a significant increase in calcium level was observed compared to the normal control rats. This increase was 32.11 ± 0.27; 32.27 ± 0.35; 32.89 ± 0.22; 32.78 ± 0.30; 32.20 ± 0.22 and 32.85 ± 0.36 mmol/L respectively for groups 2, 3, 4, 5, 6 and 7 compared to the control group whose calcium level was 11.65 ± 0.43 mmol/L. The treatment of the rats with TAESg or vitamin K induced a significant increase (p< 0.0001) in calcium levels compared to those which were not treated

Table 6. Evolution of calcium levels

	Sex	Before induction	After induction	Aftertreatment (3 days)
Group 1	**M+F**	11.38 ± 0.61**mmol/L**	11.65 ± 0.43**mmol/L**	12.05 ± 0.35**mmol/L**
Group 2	**M+F**	11.53 ± 0.48**mmol/L**	32.11 ± 0.27^{d}**mmol/L**	32.77 ± 0.26^{d}**mmol/L**
Group 3	**M+F**	12.10 ± 0.70**mmol/L**	32.27 ± 0.35^{d}**mmol/L**	32.93 ± 0.11^{d}**mmol/L**
Group 4	**M+F**	11.58 ± 0.48**mmol/L**	32.89 ± 0.22^{d}**mmol/L**	33.56 ± 0.57^{d}**mmol/L**
Group 5	**M+F**	12.06 ± 0.45**mmol/L**	32.78 ± 0.30^{d}**mmol/L**	33.62 ± 0.66^{d}**mmol/L**
Group 6	**M+F**	11.81 ± 0.50**mmol/L**	32.20 ± 0.22^{d}**mmol/L**	33.20 ± 0.71^{d}**mmol/L**
Group 7	**M+F**	11.47 ± 0.56**mmol/L**	32.85 ± 0.36^{d}**mmol/L**	33.68 ± 0.76^{d}**mmol/L**

The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2. 3. 4. 5. 6.and 7 on the one hand and between group 2 and groups 3. 4. 5. 6. and 7 on the other. p< 0.05. n = 6 (3M. 3F): number of rats/ groups; d: very highly significant; Group 1: normal control untreated;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4. 5. 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw. respectively).; M: male; F: female

Table 7. Evolution of thrombocyte levels

	Sex	Before induction	After induction	After treatment (3 days)
Group 1	**M+F**	501.7 ± 22.09×**10^{3}/mm^{3}**	507.5 ± 21.82×**10^{3}/mm^{3}**	574.5 ± 53.15×**10^{3}/mm^{3}**
Group 2	**M+F**	459.2 ± 19.52×**10^{3}/mm^{3}**	1167 ± 136.3×**10^{3}/mm^{3} ^{d}**	1062 ± 28.50×**10^{3}/mm^{3} ^{d}**
Group 3	**M+F**	507.7 ± 15.85×**10^{3}/mm^{3}**	1158 ± 78.10×**10^{3}/mm^{3} ^{d}**	1090 ± 68.70×**10^{3}/mm^{3} ^{d}**
Group 4	**M+F**	476.3 ± 16.82×**10^{3}/mm^{3}**	1155 ± 158.8×**10^{3}/mm^{3} ^{d}**	1049 ± 46.93×**10^{3}/mm^{3} ^{d}**
Group 5	**M+F**	498.2 ± 22.97×**10^{3}/mm^{3}**	1197 ± 95.93×**10^{3}/mm^{3} ^{d}**	1116 ± 73.11×**10^{3}/mm^{3} ^{d}**
Group 6	**M+F**	516.0 ± 22.12×**10^{3}/mm^{3}**	1225 ± 134.4×**10^{3}/mm^{3} ^{d}**	1110 ± 110.2×**10^{3}/mm^{3} ^{d}**
Group 7	**M+F**	484.7 ± 17.42×**10^{3}/mm^{3}**	1221 ± 139.1×**10^{3}/mm^{3} ^{d}**	1088 ± 114.5×**10^{3}/mm^{3} ^{d}**

The values are presented as Mean followed by Standard Error on the Mean (M±ESM). The comparison is between group 1 and group 2. 3. 4. 5. 6.and 7 on the one hand and between group 2 and groups 3. 4. 5. 6. and 7 on the other. p< 0.05. n = 6 (3M. 3F): number of rats/ groups; d: very highly significant; Group 1: normal control untreated;Group 2: positive control (hypocoagulated rats treated with distilled water); Group 3: negative control (Hypocoagulated rats treated with vitamin k at 100 mg/kg bw); Group 4, 5, 6 and 7: test groups (hypocoagulated rats treated with TAESg at 3.5; 17.5; 35 and 350 mg/kg bw, respectively); M: male; F: female.

3.7. Evolution of the platelet count

The rats of the different groups pretreated with warfarin experienced a significant increase ($p < 0.0001$) in level of thrombocytes compared to that of the control group. Indeed, the thrombocyte levels of the rats of these group were 1167 ± 136×10^3/mm^3, 1158 ± 78.10×10^3/mm^3, 1155 ± 158.8×10^3/mm^3, 1197 ± 95.93×10^3/mm^3, 1225 ± 134.4×10^3/ mm^3 and 1221 ± 139.1×10^3/mm^3 respectively for groups 2, 3, 4, 5, 6 and 7 whereas that of the control was 507.5 ± 21.82×10^3/mm^3. After the different treatments with vitamin K and with TAESg, the level of thrombocytes did not undergo any significant variation compared to that of the positive control rats (Table 7).

4. DISCUSSION

The current study was undertaken to verify the hemostatic effect of the total aqueous extract of the stem bark *Sacoglottis gabonensis* (TAESg) on warfarin-pretreated rats. After the pretreatment of rats with warfarin, no clinical signs such as rash, loss of motility, diarrhea, hematuria, bleeding from the orifices, salivation, vomiting were observed, but they were aggressive. The aggressiveness observed in the rats pretreated with warfarin could be explained by the nature of the substance which seems to act on the central nervous system involved in many biological functions such as emotions and mood. These results are contrary to those of Handan *et al.* who reported bleeding, hematuria after pretreatment of rabbits for

four consecutive days with warfarin at a dose of 2mg/kg bw [19].

With regard to prothrombin time, activated partial thromboplastin time, increases in their levels were observed after rats 'pretreatment with warfarin inducing an increase in the INR. A lengthening of prothrombin time is a sign of a deficit of one or more factors, namely coagulation factors I, VII, V, X, II which are vitamin K dependent, i.e., their activations depend on the synthesis of vitamin K in the liver [27]. Prolongation of the APT indicates a pathology affecting factors XI, IX, VII and XIII or a treatment aimed at reducing the risk of thrombosis [28]. After treatment of the rats with vitamin K and TAESg, prothrombin time, TCA and INR decreased. Indeed, the role of vitamin K is very specific. Vitamin K is used to prevent the risk of bleeding due to vitamin K deficiency or to overcome an overdose of anti-vitamin K and intervene in the synthesis in the liver of the factors necessary for coagulation, including prothrombin, which could be responsible for the decrease in prothrombin time, TCA and INR [29].

The decrease in prothrombin time and INR observed in rats treated with TAESg could be justified by the probable presence of certain phytochemical compounds in the extract such as tannins and flavonoids which hemostatic properties have been demonstrated. Indeed, according to Nabil *et al.* tannins have a hemostatic activity that precipitate proteins to stop any bleeding, which would be the cause of the restoration of prothrombin time, TCA [30].

However, previous work done by Koné *et al.* showed that the total aqueous extract of the stem bark of *Sacoglottis gabonensis* contains catechic tannins and many other active phytoconstituents such as sterols, flavonoids, polyphenols, alkaloids and saponins [15]. In terms of fibrinogen levels, the results showed a drop in their level after pretreatment with warfarin. The drop in fibrinogen levels could be explained by the effect of warfarin on the liver. It modifies the level or prevent its synthesis in the liver [31].

The level of fibrinogen increased in rats treated with vitamin K and TAESg. Indeed, vitamin K is a powerful hemostatic that intervenes in the synthesis of factors necessary for the coagulation of which the factor I or functional fibrinogen, initiator of the cascade of coagulation of the extrinsic way. The increase in fibrinogen levels in rats treated with TAESg means that TAESg contains compounds capable of stimulating the synthesis of certain coagulation factors such as vitamin K, in the liver [32].

As for the levels of calcium and thrombocytes, the results showed increases after the pretreatment with warfarin. The increase in calcium and thrombocyte levels after warfarin pretreatment and their persistence after treatment with TAESg and vitamin K could be explained by the fact that thrombocytes are primarily responsible for coagulation by promoting platelet aggregation [33]. Hypercalcaemia could be explained by a defect in renal resorption due to the excessive dietary intake of salts in which Ivograin® granules are rich.

CONCLUSION

Total aqueous extract of the stem bark of *Sacoglottis gabonensis* has hemostatic properties. The extract reduced prothrombin time, activated partial thromboplastin time, INR and increased fibrinogen levels.

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