



ANTIVENOM PROPERTIES OF THE STEM BARK OF *SCHUMANNIOPHYTON MAGNIFICUM* ON *BITIS GABONICA* VENOM

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ABSTRACT

The aim of this study was to evaluate the antivenom properties of *Schumanniphyton magnificum*; a plant of the Rubiaceae family used in the treatment of snake bites in several rural localities of Cameroon. After harvesting and identification, hydro ethanolic solvent was used for the extraction of the plant by using 2.526 kg of powder of stem bark of the plant. *In vitro* activity tests were carried out to evaluate the effect of the plant on the activity of phospholipase A2 using egg yolk, and on hemolysis induced by *Bitis gabonica* venom on red blood cells of rats. *In vivo*, the effect of *Schumanniphyton magnificum* on the venom was assessed by evaluating the response on the fever induced by the venom. Hydro ethanolic extracts of the *Schumanniphyton magnificum* stem bark showed significant inhibition of phospholipase A2 with optimal activity. The plant also inhibited the hemolytic activity of viper venom on red blood cells. *Schumanniphyton magnificum* decreased the rectal temperature of animals. It appears from the present study that *Schumanniphyton magnificum* has antivenom properties against the bites of *Bitis gabonica* viper.

KEYWORDS: Antivenom, *Bitis gabonica*, *Schumanniphyton magnificum*, Venom.

INTRODUCTION

The morbidity and mortality associated with snakebites is a neglected health problem in tropical and subtropical countries [1]. Each year, there are 5,000,000 snake bites worldwide, resulting in up to 2,500,000 cases of poisoning; at least 125,000 deaths (100,000 in Asia, 20,000 in Africa and 5,000 in Latin America); and about more than 300,000 permanent amputations and disabilities. Most cases occur in Asia, Africa and Latin America [2]. In Africa, there are an estimated 1,000,000 annual snake bites, about half of which require treatment. It is the rural poor in emerging countries who are most often affected, and mainly those with few

medical resources. In Cameroon, epidemiological studies are still recent; but it is possible to estimate the importance of snake bites from the annual reports of the Pasteur Institute, which provide the antivenom sales figures for the country since 1960 [3]. Between 1960 and 1970, an average of 1,500 vials per million inhabitants were dispensed each year, 2,300 vials per year in the 1980s and about 30 doses per million inhabitants since the 2000s [3]. Lethality is proportional to the absence of antivenom treatment and mainly due to two major families dangerous for humans: *Elapidae* (cobras and mambas) and *Viperidae* (vipers). In 2016, 1673 snakebites were recorded [4] in Cameroon and the Far North and North regions account for 76% of the

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deaths that occurred. The treatment of a snake bite is a serotherapy that must be undertaken as soon as possible [5]. They are F (ab ') 2 fragments of polyvalent or monovalent purified equine immunoglobulins directed against the venom of the most frequently encountered species (*Bitis*, *Echis*, *Naja* and *Dendroaspis*). The management of venomous snake bites is a medical emergency whose serotherapy remains the only treatment that is justified and effective at the moment; this should be available and accessible to the most vulnerable populations. But the cost of treatment remains relatively high and its unavailability in rural areas where snake bites are more frequent leads people to resort to the use of medicinal plants [6]. The study was focused on the evaluation of the antivenom properties of *Schumanniphyton magnificum*; a plant of the *Rubiaceae* family used in the treatment of snake bites [7] in several localities of Cameroon.

MATERIALS AND METHODS

Plant material

Schumanniphyton magnificum stem barks were collected from different places at the Nyong and Soo district (Abang, Nkoumadjap and Nkolnguet) during January 2018. The specimen was identified at the National Herbarium of Cameroon. The stem bark was shade dried, pulverized, labelled and stored. The powdered stem bark obtained (2.526 kg) was extracted with ethanol-water mixture in proportions 70/30 by macerating for 72 hours [8]. The extract was then evaporated in rotary evaporator at 78 °C to afford a greenish-brown residue considered as the crude ethanol extract of *Schumanniphyton magnificum* stem bark.

Experimental animals

Thirty healthy adult Wistar rats weighing about 80-220g between 2 and 3 months of age were used for the study. All the animals for experimentation were kept in the animal house of Pharmacology and Toxicology laboratory of the Faculty of Medicine and Biomedical Sciences of the University of Yaounde 1 under controlled temperature. All the animals were acclimatized for one week before experimentation. They were fed with standard rat pellet diet and water.

Bitis gabonica viper venom

The venom of *Bitis gabonica* was obtained from the Mvog Beti Zoo and stored at 4 °C in a refrigerator before use.

Antivenom

The antivenom used in this study (INOSERP™ manufactured by Inosan Biopharma SA LAB, Batch No 5IT11003, Manufacturing Date: 10/2015, Expiry date: 10/2018) was obtained from the pharmacy of the Urgency Center of Yaounde.

Effect of the extract on hemolysis

This was assessed using the method described by Dougherty in 1976 and Surabhi and al in 2011. Blood was collected from healthy adult wistar rats in ethylene ditetramine (EDTA) tubes. The collected blood was centrifuged at 900 rpm and washed three times with saline solution; 1% rat's red blood cells (RRBC) was prepared. *Bitis gabonica* venom was dissolved in physiological saline solution 0.9% to make a final stock solution of 100 µg/mL. Then 800 µL mL of venom, 800 µL of phosphate buffer (pH 7.2) and 800 µL of 1% RRBC was put in different tubes. Different concentrations of *Schumanniphyton magnificum* extracts (100, 200, 400 and 800 µg/ml) were added. Tubes were then incubated for 30 minutes at 37 °C and centrifuged at 1000 rpm for 3 minutes. The absorbance of each tube was measured at 540 nm using spectrophotometer. The percent inhibition of hemolysis was calculated [9].

$$\% \text{ Hem} = \frac{A_{540} \text{ Ct} - A_{540} \text{ Tt}}{A_{540} \text{ Ct}} \times 100$$

Effect of the plant on phospholipase A2 activity

A 100 µL volume of the crude venom of *Bitis gabonica* viper was incubated with 100 µL of egg yolk at 37° C for 30 minutes. The mixture was immersed in boiling water during 5 minutes and titrated using sodium hydroxide 20mM and phenolphthalein as indicator. This procedure was repeated using *Schumanniphyton magnificum* extracts incubated with the venom. The volume of sodium hydroxide used to neutralize free fatty acids was recorded and the activity of phospholipase A2 calculated [10].

Effect of the plant on fever

This test was performed by the Al-Ghamdi method enunciated in 2001 by measuring the rectal temperature of the animals before and after venom inoculation [11]. The rectal temperature of the animals is taken beforehand with a clinical thermometer before injection of the viper venom and then at 15 and 60 minutes after subcutaneous

administration of the antivenomous serum or the extract of the plant at 200, 400 and 800 mg / μ L.

Statistical analysis

Results were presented as mean \pm standard deviation. Comparison between different groups was done using analyses of variance (ANOVA). The rate of significance was fixed at a probability value less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Table 1 shows the results of hemolysis test. The study of the effect of *Schumanniohyton magnificum* extract on venom-induced hemolysis on red blood cells of rats showed that the plant extract was able to neutralize this hemolysis from 19% to 30% for concentrations of 100 to 800 μ g / mL. Surabhi Pandey, Emmanuel Toppo and Preeti Chauhan who, working on a comparative study of *Calotropis gigantea* Linn and *Cassia fistula* Linn on the venom of certain snakes, showed that these plants inhibited venom-induced hemolysis [9].

Figure 1 shows the effect of *Schumanniohyton magnificum* extract on phospholipase A2 of viper venom. The results showed that from 200 to 1600 μ g / ml, the plant extract inhibited the activity of that enzyme from 100% to 48.8%. These observations are similar to those of Thushara and al which showed in their study entitled "In vivo and In vitro neutralizing potential of *Rauwolfia serpentina* plant extract against *Duboisia russelli* venom" that this plant reduced the activity of phospholipase A2 of viper venom up to 35 % [12].

Table 2 shows the rectal temperature variation of rats before and after envenomation. Rectal temperature decrease was observed while using the plant extract on wistar rats. This observation was obtained with the study of Rafael SF who also showed in 2009 that the aqueous extract of *Mikania glomerata* leaves brought the temperature back to normal in the rats treated with the plant after inoculation of the venom [13].

CONCLUSION

Works in this study have shown that this plant inhibits phospholipase A 2 activity, hemolysis and elevated temperature in young rats treated immediately with the plant after subcutaneous inoculation with *Bitis gabonica* venom.

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Table 1: Inhibition of hemolysis

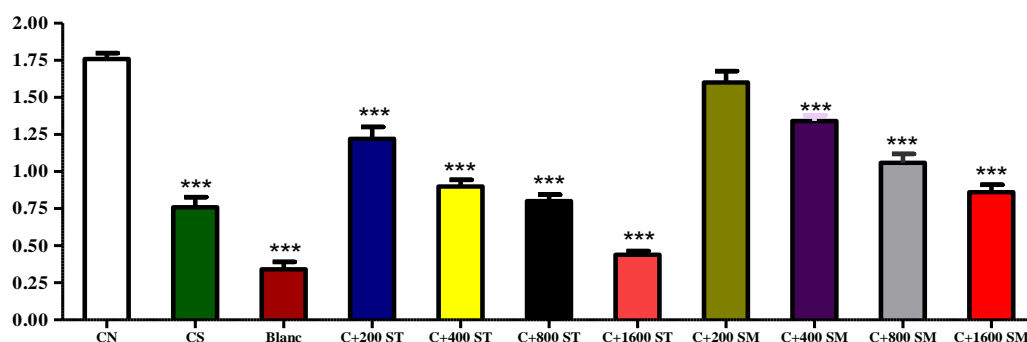
Groups	% Hem	Inhibition of hemolysis
RBC/water	100	0.00 ± 0.00
C+ 100 ST	85	0.15 ± 0.01
C+ 100 SM	81	0.19 ± 0.06 **
C+ 200 ST	83	0.17 ± 0.04
C+ 200 SM	79	0.21 ± 0.01 **
C+ 400 ST	85	0.15 ± 0.04
C+ 400 SM	76	0.24 ± 0.01 **
C+ 800 ST	71	0.29 ± 0.05
C+ 800 SM	70	0.30 ± 0.01 **

RBC = Red blood cells

SM = *Schumanniphyton magnificum*

ST = Serotherapy (INOSERPTM)

** Indicates a significant reduction in hemolysis by the plant



*** Significant inhibition of phospholipase A2 activity of

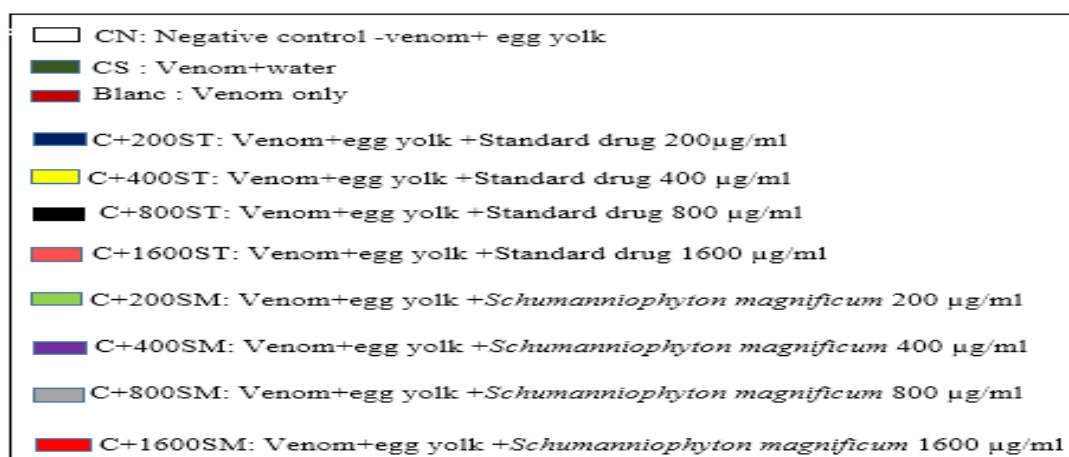


Figure 1: Effect of *Schumanniphyton magnificum* extract on Phospholipase A2 activity.

Table 2: Rectal temperature variation of rats before and after envenomation.

	T° avant injection du		
	venin/SAV/SM	T° 15 min	T° 60 min
Groupe sain	37.3 ± 0.27	37.0 ± 0.77	37.0 ± 0.25
Venom	37.0 ± 0.44	37.5 ± 0.43	36.1 ± 0.78
Venom +SAV	37.0 ± 0.23	37.4 ± 0.43	37.0 ± 0.36
Venom +SM 200	37.1 ± 0.90	38.9 ± 0.77	37.5 ± 0.37
Venom +SM 400	36.9 ± 0.56	37.6 ± 0.94	37.0 ± 1.24
Venom +SM 800	36.6 ± 0.37	37.5 ± 0.44	36.6 ± 1.37