ChemClass Journal, 2004 (19-23)

The anti-venom potential of the stem bark of *Boswellia dalzielli* on saw-scaled viper venom

E. L. Genwa* and H. Yero[1]
Chemistry Department, Boys' Secondary School
P.M.B. 01001, Gindiri, Plateau State.
[1]*Dept of Chemistry, College of Education, Gindiri, Plateau State*.

ABSTRACT

Boswellia dalzielli known as 'Ararabi' in Hausa belongs to the family Burceraceae. It is an aromatic tree with a height of up to 13 m and has a pale brown bark. It is widespread in tropical Africa in savanna woodland, often near inselbergs and rocky areas. Saw-scaled viper *(Echis carinatus)* is 40cm - 60cm long and is a widely distributed species of snake especially in Africa and Asia. Its venom is hematoxic i.e. the venom is injurious to blood vessels. Studies on the methanol, acetone, chloroform and water extracts of the stem bark obtained by the cold extraction method revealed that the methanol extract ED_{50} of 0.58 ml has the highest efficacy. This was determined by injecting a group of rats with a viper venom of LD_{50} whose LD_{50} was predetermined to be 0.076 mg. The study shows that *Boswellia dalzielli* stem bark could be used for viper antivenom.

* Author for correspondence

INTRODUCTION

The plant *Boswellia dalzielli* (frankincense tree) called 'ararabi' in Hausa belongs to the family Burceraceae. It is an aromatic tree with a height of up to 13m with a tall brown bark. Leaves pubescent; alternate, deciduous, leaflets 3-8 pairs, lamellate, margin senate. Flowers are white, fragrant, appearing before the leaves, small regular generally unisexual sepals ovate [3-5]. Petals ovate [3-5], joined; ovary superior. Fruit a drupe, Corriaceous surrounded by a scarious wing. *Baswellia dalziellA* is widespread in Africa.

The plant is said to possess some medicinal properties. Gum resin of the plant and fruits decoction of *Naulea latifolia* (African peach) is used for indigestion. Its fresh bark is emetic that is, it induces vomiting. The bark decoction as wash is used in the control of fever. The bark decoction is again used for correcting gastro-intestinal pains. The burnt gum resin used as insecticide to drive away flies and mosquitoes. It is also used as wash against rheumatism. Its gum is used as ingredient in the cure of many venereal diseases. The root and bark can be applied as antidote to arrow poison.

Only about one-third of the 2,500 snake species are poisonous. The presence of venom in snakes is a relatively recent evolutionary development. Venom refers to chemical substances that can cause severe injury to humans and other animals injected in rather small amounts or quantity. Venom is an extremely complex protein compound produced by cells lining several small tubes inside the gland. Each tube is connected to other tubes and they discharge their venom into a main duct that leads to the base of the fang.

Viper belongs to the family Viperidae and class reptalia. The snakes of this family are rather stout bodied and terrestrial. The saw-scaled viper ***(****Echis carinatus)* is 40- 60cm long and is a widely distributed species of snakes especially in Africa and Asia. This small viper is one of the most aggressive and feared venomous snakes in the world. *Echis carinatus* is sand-brown to dark brown and has light strips on its back. There are three distinctive behaviour patterns of this snake. Tail shaking, side winding movements and a rapid digging into the sand. In addition to the above mentioned behaviour patterns, it is able to produce rather loud rasping noise by rubbing together loops of its body. It eats insects in addition to the more usual feed of mice and small birds. The head is placed over the middle of the coiled body and remains nearly motionless while the rest of the body executes an undulating movement from front to back. One usually sees *Echis carinatus* on this threat posture, coiled up and with head ready to bite. The strongly hematoxic venom (which affects the blood vessels and heart muscles is estimated to be five times as potent as cobra

venom. It has fangs 5mm long. These relatively long fangs enable the viper to bite deep into the tissue and cause the victim to suffer severe necrosis. Viper bites are accompanied by prominent local irritation and symptoms of severe blood poisoning with burning pain, inflamed swellings pronounced discolouration, sudden drop in blood pressure, internal bleeding, and degeneration of the tissues and the formation of an abscess. Death ensues because the heart stops functioning. Some vipers whose venom also contains neurotoxin as well as hemotoxic substances are especially dangerous. Attempts to find snake antivenoms were being made in antiquity, but truly effective venom antidotes were not developed until the end of the 19th century with the work of Cesaire Phisalix and Gabriel Bertrand who produced Asp viper antivenom at the same time that Albert Calmelte developed his cobra antivenom. Asp viper antivenom became available from 1896 on.

EXPERIMENTAL

Sample collection

A sharp machete was used to remove the stem bark of *Boswellia daIzielli*. It was collected at Dutsen Bali, a mountain North East of the Federal Polytechnic Bauchi and was taken to the laboratory for identification at the Industrial Chemistry Department of Abubakar Tafawa Balewa University (ATBU), Bauchi and Biological Sciences Department of the same University. The sample was air-dried for four weeks and was pounded with the aid of pestle and mortar and then sieved.

Extraction

Twenty grams of the powdered bark of *Boswellia dalzielli* was weighed and transferred into a 250ml conical flask. The sample was extracted in the cold using successive solvents according to their increasing polarity. In the first extraction, 100ml of hexane was added to the powder and the mixture was stirred and covered. It was allowed to stand for three days and filtered using Whatman No. I filter paper. A deep brown filtrate (extract) was left in the open for atmospheric evaporation of the solvent. The procedure was repeated with 100 ml each of acetone, methanol and chloroform. The acetone, methanol and chloroform extracts were brown, yellow and bright yellow respectively.

Milking of the snakes for the venom

Four snakes *(Echis carinatus)* were milked to obtain the venom used for the research work. The milking was done by holding the snakes at the head region with a pair of forceps and the fangs were brought in contact with a sterilised container. As the fangs came into contact with the container, the snakes began to release the venom into the container.

Determination of the lethal dose (LD_{50}) of the saw-scaled viper venom (Speamia and Karber method)

The venom was dissolved in normal saline at 2.33mg of venom per 10ml of solution. Two ml of the stock solution was used for further serial dilution factor of 0.25ml.

Venom administration

The experiment was designed so as to cover the span between 0 and 100 % mortality. Different concentrations of the 0.25ml were injected intra-muscularly into the white rats of both sexes, weighing 18g. Each group of thee rats injected with same dose was placed in a separate cage and kept at room temperature for observations and deaths recorded during a period of 48 hours.

Determination of effective dose (ED_{50}) of Boswellia dalzielli in methanol and Acetone extracts

Methanol and acetone extracts were used to define the 50% effective dose. The titration scheme comprise of four different volumes of extracts injected in three rats which had previously been injected with a constant volume of venom, with a concentration 0.076mg. Each group was placed in a separate cage and kept at 25ºC. Observation of deaths was followed and recorded during a period of 48 hours.

RESULTS AND DISCUSSION

Table 1 shows the results of the effect of the saw-scale viper venom following its administration to determine the LD_{50}. The results show that the venom of the viper is quite toxinomous and at higher concentration, all the rats died. There was the swelling of the body, weakness of the body, and the three rats died after 30- 40 minutes. Decreasing the concentration to 0.194mg also resulted in the death of all the three rats but survived a long period of time. The minimum concentration of venom in which 50% of the rats will

Table 1: Results of LD_{50} of viper venom

Experiment	Concentration of venom (mg)	Mortality	Observation
1	0.25	3	The body was swollen followed by weakness and death after 30 – 40 minutes
2	0.194	3	Bleeding at point of injection, weakness of the body followed by death after 1¼ hours
3	0.076	1	Bleeding at point of injection. Death occurs after 3 hours. Survivors were looking dull
4	0.039	0	No death recorded, all 3 survivors looked weak and very dull

Table 2:Results of ED_{50} of acetone extracts with a constant concentration of 0.076 mg viper venom

Experiment	Volume of extracts (ml)	Mortality	Observation
Control	1.00	0	All survived and were active
1	0.25	3	Bleeding at point of injection, general fear followed by immobility. Death occurred after 1 hour
2	0.50	2	Bleeding at point of injection. Weakness of the body. Death occurred after 1 hour. Survivor as immobile
3	0.75	2	Bleeding at point of injection, death occurred after 2 hours. Survivors were visibly weak.
4	1.00	0	Swollen bodies. Survivors were weak at first but strong afterwards.

Table 3: Results of ED_{50} of methanol extract with a constant concentration of 0.076 mg viper venom

Experiment	Volume of extracts (ml)	Mortality	Observation
Control	1.00	0	All survived and were active
1	0.25	3	Severe bleeding at point of injection, swollen bodies and dea occurred after 1 hour 15 minutes.
2	0.50	2	Bleeding at point of injection. Slight swelling of bodies. Dea occurred after 2 hours. Survivor was weak.
3	0.75	0	Swelling at point of injection. They were weak at first but becan active afterwards.

not survive is 0.076 mg; hence it is the LD_{50} of the viper venom.

Table 2 presents results of the effective dose ED_{50} of acetone extract of stem bark of *Boswellia dalzielli* with constant concentration of 0.076mg viper venom. Various volumes of extracts were administered. The result of 1ml of extract served without venom into thee rats intra-muscularly shows no death. This suggests that the extract has no adverse effects on the rats. However, administering 0.25ml of the acetone extract to rats previous injected with 0.076mg of venom resulted in bleeding, weakness of the body and subsequently all the rats died after 1 hour with subsequent increases in volume of the extract from 0.75ml with constant concentration of venom led to the death of two rats with only one survivor. The effective dose ED_{50} where 50 % of rats survived is 0.60ml (Appendix 2), which implies that at higher volume of extract, most of the rats envenomed by 0.076mg survived while at lower volumes, most of rats died.

Table 3 shows the results of methanol extracts at 0.076 mg venom for *Boswellia dalzielli*– we observed that when 1 ml of plant extract was served without venom into the three rats intramuscularly no death recorded. This suggests that the extract has no apparent toxic effect on the rats. However, on administering 0.25 ml of the extracts to rats previously injected with 0.076 mg of venom resulted in bleeding, weakness of body, swelling of body and death occurred after 1 hour 15 minutes with subsequent increase in volume of the extract to 0.5 ml led to the death of two rats with one survivor feeling weak. At higher concentration of 0.75 ml, no death was recorded with less mobility of rats at first. The effective dose (ED_{50}) where 50% of rats survived (Appendix 3) was 0.58 ml which implied that at higher volumes of extracts, most

of the rats envenomed by 0.076 mg survived but at lower volumes, most of the rats died.

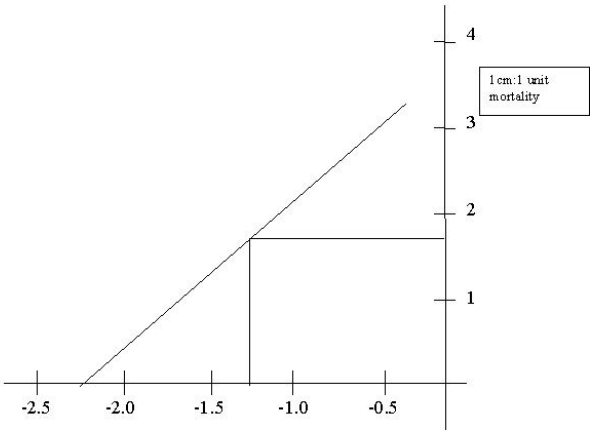
CONCLUSION

The study suggests that *Boswellia dalzielli* can be a good antidote for the management of viper bite. The methanol extract with ED_{50} equal to 0.5 8ml has a higher efficacy than the acetone extract with ED_{50} equal to 0.65 ml.

Appendix 1

Calculation of the lethal dose (LD_{50}) of saw-scaled viper using Speamia and Karber method

The log_{10} of the concentration of viper venom was



plotted against the mortality of the rats and the concentration at which 50% of the rats died was recorded as obtained from the graph.

Experiment	Venom Concentration	Log_{10} venom conc.	Mortality
1	0.250	-0.6020	3
2	0.194	-0.7120	3
3	0.076	-1.1190	1
4	0.039	-1.4090	0

2cm = 1 unit
Log_{10} conc. of venom

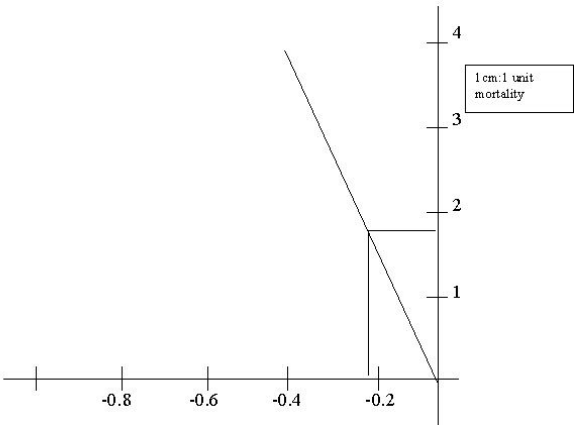
From the graph, the concentration at which 50% of the rats died is –2.0
Antilog = 0.01 mg
Therefore, LD_{50} = 0.01 mg

Graph 1: Log_{10} of viper venom

Appendix 2

Calculation of effective dose ED_{50} of acetone extract of *Boswellia dalzielli*

The log_{10} of volume of extracts was plotted against the mortality of the rats and volume of the extracts at which 50% death was obtained as shown in the graph.



Experiment	Volume of extracts (ml)	Log_{10} of extracts	Mortality
1	0.25	-0.6020	3
2	0.50	-0.3010	2
3	0.75	-0.1249	2
4	1.00	0.0000	0

2cm = 1 unit
Log_{10} volume of extract

From the graph, the volume at which 50% of the rats survived is –0.19.
Antilog = 0.646
Therefore, ED_{50} = 0.65 ml

Graph 2: ED_{50} Acetone extract – *Boswellia dalzielli*

Appendix 3

Calculation of effective dose ED_{50} of methanol extract of B*oswellia dalzielli*

The log_{10} of the volume of extract was plotted against the mortality of the rats and volume of extracts at which 50% death was recorded was obtained as shown in the graph.

Experiment	Volume of extract (ml)	Log_{10} volume of extract	Mortality
1	0.25	-0.6020	3
2	0.50	-0.3010	2
3	0.75	-0.1249	0
4	1.0	-0.0000	0