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Effects of the ethanolic extract of P*arinari curatellifolia* on cat blood pressure and rabbit jejunum preparations

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Parinari curatellifolia has been used with great success in the treatment of snake bites by herbal medicine practitioners in Benue State, Nigeria. The effect of the ethanolic extract of Parinari curatellifolia was investigated on the cat blood pressure and rabbit jejunum to determine its possible relevance in the alleviation of the cardiotoxic and neurotoxic effects of snake envenomation. The statham guage cannulation method was used to investigate the effects of the extract on the cat blood pressure while the isolated tissue response method was used to test the effects of the extract on the spontaneous movement of the rabbit jejunum. At a concentration of 1 mg/ml, the extract produced a significant decrease of 9.0cm amplitude in cat blood pressure which is comparable to 8.7cm decrease produced by Acetylcholine (10 µg/ml). On the rabbit jejunum, the extract exhibited an antagonistic effect on the action of the Cobra venom. The extract was found to be potentially useful in the treatment of the species of snakes that causes increase blood pressure, tachycardia and neurotoxicity in their victims.

***Key words**; Parinari curatellifolia, envenomation, cardiotoxicity, neurotoxicity.*

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INTRODUCTION

Many plants are used in traditional medicine to treat snake bites and most of the rural populace in Africa relies on plants for the treatment of snake bites [1]. Indeed the local traditional medicine practitioner is the first port of call for victims of snake bites in most parts of Africa. Medicinal plants with anti venom properties effectively illustrate the cultural context of medicine and ethnicity [2, 3]. *Parinari curatellifolia* has been widely used by the Idoma ethnic group of Benue State, Nigeria to treat various snake bites with great success. The bark, leaves and the roots of the plant have been claimed to have anti-snake venom properties.

Snake venoms are very potent toxins of complex polypeptides having unique pharmacodynamic and pharmacokinetic properties [4]. There are two major classes of venoms produced by venomous snakes; the cobra and mambas produced neurotoxins and cardio toxins while the vipers and adders produced mainly cytotoxins and haemolysins [5]. The cardiovascular effects of snake venom are complex and it varies with the type of

snake. The crotalids and viperids release vasoactive amines such as histamine, serotonins and peptides such as bradykinins and prostaglandins. These contribute significantly to the circulatory shock that is the hallmark of envenomation by these snakes. Antivenoms effective in the treatment of bites by these snakes possessed vasodilatory and blood pressure reducing properties. Saw scaled viper and puff adders produced a significant fall in systemic blood pressure, this could be as a result of decrease in circulating fluid volume due to loss in splanchnic circulation [6]. Other snake venoms such as elapid snakes have a stimulant effect on the myocardium while cobra envenomation produced a persistent fall in systemic arterial blood pressure. Cardiotoxin has been found to have both stimulating and inhibitory effects on Na^+ - K^+ ATPase depending on the amount of venom injected [7]. The depressant effects of elapid snake venom are probably due to phospholipase A_2 (PLA_2) that liberates histamine, 5HT and prostaglandins from the mast cells and basophills. Another important pathophysiology of snake envenomation is the neuromuscular toxicity. Some snake venom effects are as a result of enzymatic activities while others are as a result of polypeptides having neurotoxic effects, these could lead to respiratory distress, convulsion and gastrointestinal paralysis [6,8]. Post synaptic neurotoxins could block acetylcholine to receptors at the neuromuscular junctions of the heart and gastrointestinal tract causing circulatory failure and gastrointestinal paralysis respectively. However, the greatest effects are generally exerted on respiratory muscles causing respiratory paralysis [8]. Presynaptic neurotoxins and postsynaptic neurotoxins blocked the pre and postjunctional transmission of acetylcholine through different mechanisms. The nerve impulse is either reduced or never reaches the nerve endings. Three groups of presynaptic neurotoxins are known; phospholipase A_2 (PLA_2), dendrotoxins and fasciculins. All elapids, hydrophids, crotalids and viperids share the same groups of PLA_2 toxins [8]. This work was aimed at investigating the effects of *Parinari curatellifolia* on cat blood pressure and smooth muscles of the gastrointestinal tract, since the two are indices of envenomation.

MATERIALS AND METHODS

Collection and preparation of the plant extract

The bark of *Parinari curatellifolia* was collected and authenticated by Mr. O. Owa of the Agric Extension Services, Federal College of Forestry, Jos, Nigeria. The plant material was dried and powdered using mortar and pestle. 60 g of the powder was subjected to extraction process in a soxhlet extractor using ethanol as solvent. The extract was evaporated in a water bath at about 45°C until dried. The dried extract was preserved in the refrigerator until required for use.

Phytochemical analysis

The phytochemical analysis of the extract was done using the methods described by Trease and Evans [9].

Statham gauge cannulation of the cat artery

The statham gauge was calibrated using a mercury manometer. Various adjustments were made to exclude all the air in the plexi glass dome. The statham gauge was set up horizontally in a plane at the level of the heart (Phlebostatic level) thereby

assuming zero atmospheric pressure. The statham guage was connected to the recorder through a transducer. Pressure was applied through the 5 ml syringe filled with normal saline to deflect the pen of the recorder upwards.

Various adjustments of the stopcock were made through 45ºC to an intermediate position. The stopcock B was then opened to the atmosphere to achieve zero atmospheric pressure. Several adjustments of the balance and sensitivity controls were made to allow free transmission of the pressure through the transducer. The speed of 2.5mm/second was set on the recorder. After successful calibration of the statham gauge, the cat was then anaesthetized using urethane. The carotid artery of the cat was carefully exposed and cannulated, this was then connected to the stopcock B through a transducer to the recorder. The femoral vein of the cat was also exposed and cannulated and was used for drug administration. Heparin was infused periodically to the carotid artery to prevent clotting of the blood that may block the pressure from the heart.

After satisfactory calibration and cannulation, the following drugs were administered through the femoral vein and their responses were recorded.

Drugs	concentration	volume
Normal saline	0.9%	0.1ml
Acetylcholine (Ach)	10 µg/ml	0.1ml
Ach	1 mg/ml	0.1ml
Extract of *P. curatellifolia*	1 mg/ml	0.1ml
Adrenaline	10 µg/ml	0.1ml
Atropine	1 mg/ml	0.1ml

Effects of each agent was recorded and the vein was flushed with normal saline after each administration to return the pressure to normal and sufficient resting time was allowed before subsequent drug administration.

Increase in blood pressure was recorded as upwards deflection of the pen while decrease in blood pressure was recorded as downward depression of the pen.

Effects of the extract on rabbit jejunum

An adult male rabbit was stunned and exanquinated. The abdomen was quickly opened to expose the intestines. About 2 cm long of the jejunum was cut and placed in a beaker containing tyrode physiological solution. The tissue was mounted in a 560 ml organ bath containing physiological solution. The tissues was constantly aerated and kept warm at about 37^0C. Various agents including the extract and venom were administered into the organ bath and the effects were recorded as shown in table 1.

Ethical Consideration

Ethical clearance and approval for this study was obtained from the University of Jos Animal Ethics Committee. The ethical approval protocol number is PCL/ECC/008/2011. The study was carried out according to the guidelines on laboratory animal care outlined by the committee.

RESULTS

The phytochemical analysis showed the presence of cardiac glycosides, tannins, saponins, anthraquinones, carbohydrates, steroids and flavonoids.

TABLE 1 Effects of the ethanolic extract of *P. curatellifolia* on blood pressure of cat using statham guage cannulation

Drugs/Strength	Volume (ml)	Response (cm)
Normal saline	0.1	0.0 No response
Ach 10 µg/ml	0.1	8.7 Relaxation
Ach 1 mg/ml	0.1	10.4 Relaxation
P. curatellifolia 1 mg/ml	0.1	9.0 Relaxation
Adrenaline 10 µg/ml	0.1	7.0 Contraction
Atropine 1 mg/ml	0.1	7.5 Relaxation

Relaxation = Decrease blood pressure
Contraction = Increase blood pressure

The extract produced a significant decrease in the blood pressure of the cat compared to Acetylcholine and Atropine.

TABLE 2 Effects of the cobra venom and ethanolic extracts of *P. curatellifolia* on spontaneous movement of Rabbit Jejunum

Drugs/Strength	Volume (ml)	Response (cm)
Ach 1 x 10^{-5} g/ml	0.1	3.8 Contraction
	0.2	4.2 Contraction
	0.4	4.4 Contraction
	0.6	4.4 Contraction
	0.8	4.5 Contraction
P. curatellifolia 1 mg/ml	0.1	No Response
	0.2	No Response
	0.4	No Response
P. curatellifolia 5 mg/ml	0.1	No Response
	0.4	No Response
Cobra Venom 1 mg/ml	0.1	3.0 Contraction
	0.2	3.5 Contraction
Extract + venom incubate for one hour	0.1	No Response
	0.2	No Response
	0.4	No Response

Incubating the extract with the venom antagonizes the contractile effects of the venom on the rabbit jejunum. However the extract alone showed no response on the rabbit jejunum.

DISCUSSION

The cardiovascular effects of snake venom is complex and it varies with the type of snake, some of which are as a result of the release of vasoactive amines such as histamines; peptides such as bradykinins; enzymes such as phospholipase $A_{2;}$ and or blockade of neurotransmitters such as acetylcholine [5,6,7,10]. The cardiotoxins found in snake venom have been found to act specifically on the heart causing increased or decreased blood pressures in their victims [5,8]. In this study, the effects of the ethanolic extract of the bark of the *Parinari curatellifolia* produced a decrease (9cm in amplitude) on the cat blood pressure compared to 8.7cm in amplitude produced by acetylcholine (table 1). Acetylcholine produced its action of vasodilation, decrease rate and force of contraction as a result of increased permeability to potassium ion and parasympathetic stimulation of the cholinoceptors on the heart muscles [11]. One of the toxicities of cobra venom on the heart is blockade of Ach to its receptors. The extract could probably have acted via the cholinergic pathway since it increased cholinergic activities on the heart thus abolishing the cardiotoxicity of cobra venom on the heart (table 2). Atropine, one of the drugs used as a supportive therapy in victims of snake bites produced a decrease in blood pressure (7.5cm in amplitude) similar to the extract (table 1). Atropine decreases blood pressure by blocking the muscarinic (M_1) receptors on post ganglionic parasympathetic neurons thus relieving the synaptic effect of Ach [11]. On the other hand Adrenaline an adrenergic cholinoceptor increased (7.0 cm in amplitude) cat blood pressure. Investigation on the effects of the extract on the neuromuscular junction shows blockade of cobra venom on rabbit jejunum preparations (table 2), thus neurotoxicity being the hallmark fatality in some snake venom toxicities can be successfully abolished by the medicinal product from this plant. The extract alone produces no observable effects on the rabbit jejunum, while Acetylcholine produced a dose dependent contraction of the rabbit jejunum. Cobra venom was found to block or reduce the rhythmic contractile action of the jejunum, this is probably through the blockade of cholinergic activities. The blockade of the rhythmic contractility of the jejunum by the cobra venom was reversed by the extract as shown in table 2, which shows that the extract is effective against the snake venom.

CONCLUSION

The ethanolic extract of *P. curatellifolia* used in the treatment of snake bites produced a decrease in Cat blood pressure and antagonized the effect of cobra venom on the rabbit jejunum. The extract showed a demonstrable potential in the treatment of snake bites as claimed by the traditional medicine practitioners. Work is ongoing in our laboratory to elucidate the exact mechanism of anti-snake venom activities of *P. curatellifolia*.

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