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Herbal treatment of scorpion envenomation: Plant extracts inhibited Opisthacanthus capensis venom phospholipase A_2 activity

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Abstract: The inhibitory effects of *Momordica charantia linn, Isoberlinia doka, Terminalia avicennioides, Tamarindus indica and Crotalaria retusa L* aqueous leaves extracts on *Opisthacanthus capensis* (Black creeping scorpion) venom phospholipase A_2 (PLA_2) activity was investigated. The enzyme from O. *capensis* venom had a pH and temperature optima of 5 and 60˚C respectively with an activation energy of 5.20 Kcal/mol. Different concentrations (4mg, 6mg and 8mg/ml) of *Isoberlinia doka* and *Momordica charantia* Linn inhibited the activity of O *capensis* venom PLA_2 in vitro displaying an uncompetitive inhibition pattern with a decrease in the computed index of efficiency (K_{cat}). Different concentrations (4mg, 6mg and 8mg/ml) of *Terminalia avicennioides, Tamarindus indica and Crotalaria retusa L* also inhibited O. *capensis* venom PLA_2 activity in vitro but the inhibition pattern was competitive inhibition with K_{cat} remaining unchanged. This study reveals that the use of these plants by herbalists in northern Nigeria in the treatment of scorpion bites could be justifiable.

Keywords: Opisthacanthus Capensis, Venom, Phospholipase A_2, Scorpion, Envenomation

1. Introduction

Scorpion venom envenomation in humans caused by scorpion sting is a serious public health problem worldwide. Mammalian cells elicit a strong inflammatory response as a result of scorpion venom envenomation which is characterized by increased levels of local and circulating leukocytes, and that in severe cases, can lead to pulmonary edema and death in Children [1].

Scorpions are predatory arthropod animals of the order scorpions within the class *Arachnida[2].Opisthacanthus capensis* belongs to the family *liochelidae* and it is among the scorpion species that is dangerous to humans. Its venom contains powerful neurotoxins and it is especially potent that it indirectly contributes to tissue damage [3]. The venom constituents include mucopolysaccharide, hyaluronidase, phospholipase, serotonin, histamine, enzyme inhibitors, and proteins namely neurotoxic peptides [4,5,6]. Phopholipase A_2 catalyzes the hydrolysis of glycerophopholipids at the Sn-2 position producing free fatty acids and lysophospholipids. One such reaction product, arachidonic acid is the precursor in the biosynthesis of prostaglandins which belong to the group of compounds called Eicosanoids. Prostaglandins are important in the inflammatory processes and are implicated in a numerous pathophysiological conditions. Due to the increased presence and activity of PLA2 in the venom of the scorpion, arachidonic acid is released from phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site [7]. Medicinal plants are the back bone of Traditional medicine. Traditionally, the use of plant preparation as sources of drug are based on the experience and superstitions passed from generation to generation, virtually by the word of mouth [8]. In northern parts of Nigeria, mostly in rural areas, health centers are inadequate and the victims of scorpion bite mostly depend on traditional healers and herbal antidotes as an alternative treatment. *Momordica charantia linn, Isoberlinia doka, Terminalia avicennioides, Tamarindus indica and Crotalaria retusa L* are plants that are used commonly by the herbalists in the

treatment of scorpion bite. *Momordica charantia* L. fruits, leaves, seeds and roots are considered as valuable traditional medicine. It is considered as anti-dotal, and anti-pyretic and tonic in many parts of the worlds [9]. *Isoberlinia doka* is a hardwood tree native to African tropical savannah and Guinean forest – savannah Mosaic dry forests where it can form single specie stands. This hardwood tree is quick to colonise clearings and abandoned land and grass gregariously, often establishing near pure stands [10]. *Isoberlinia doka* is used in carpentry and for making furniture, although the wood is somewhat difficult to work with hand – tools. It is also used as fuelwood. It is used for treating muscular – skeletal system disorders in traditional West American Medicine. An infusion of the leaves is used for treating jaundice. *Isoberlinia doka* is also used for the treatment of scorpion bite, treating of infectious diseases and scientific investigations have confirmed its antibacterial activity [11]. *Terminalia avicennioides* belongs to the combratacea family, and its commonly found in the savannah region of west Africa [12]. It is a yellowish brown tree with hard and durable wood. The root of the tree is used as chewing stick in some parts of Nigeria and have been claimed to cure dental caries and skin infection [13]: the root bark decorction is used in Ivory coast to treat severe jaundice [14]: In Senegal the plant is used to cure sores and ulcer [15]: In Nigeria it is used in the treatment of gastrointestinal disorders [16] as well as syphilis [17]. One of the most effect use of this plant is in the treatment of bloody sputum and cough by the Nupe tribe in the north central Nigeria [18]. *Tamarindus Indica* belongs to the family leguminosae and it's moderate to large in size, evergreen tree, up to 24 m in height and 7m in girth [19]. *Tamarindus indica* is used as a Traditional medicine in Nigeria, India, Sudan, Bangladesh, and most of the tropical countries. Contrary to pharmaceuticals, it is often freely and readily available [20,21]. Almost all parts of the tree find one use or the other in food, chemical, pharmaceutical, and textile industries, and as fodder, timber, and fuel [22,23]. *Crotalariaretusa* L is a genus of herbaceous plant and family fabaceae that is locally used as a traditional medical plant in many parts of Nigeria [9]. An herbaceous shrub with erect growth habit, the stem is ridged and velvety, leaves are abovate, having a rounded leaf tip and a wedge shaped leaf base (3.3 – 9.2cm long, 1 – 3.8com wide) [9]. *Crotalaria* species are used as food by the larvae of some Lepidoptera species including *Endoditasericcus, Etiellazinekenella*and *Utetheisaornatrix*. The toxic alkaloid produced by some members of this genus are known to be incorporated by utelleisialarve and used to secure their defense from predators [24].

In local medicine, the leaf is an excellent remedy for scorpion sting, ptyalism, diarrhea, scabies and impetigo. It's seed are used in ethano-medicine for the treatment of fever as vermifuge and as an antispasmodic uterus and intestinal agent [25].

Here, we are attempting to validate this claim by targeting, in vitro, opisthacanthus capensis venom phospholipase A_2 to see if one or more of these plant extracts can inhibit the enzyme activity.

2. Materials and Methods

2.1. Chemicals

All chemicals used in this study were of analytical grade. Tris (hydrxymethyl)-aminomethane, Hydrochloric acid, Sodium hydroxide and Phenophthalein were purchased from BDH Chemicals Ltd Poole England. Coomassie brilliant blue G250 and bovine serum albumin were from Fluka Biochemika, USA.

2.2. Animals

Black creeping scorpions were collected in Maiduguri, Nigeria and authenticated by Dr. A.M. Kokori of the department of Biological sciences, University of Maiduguri, Maiduguri Nigeria as *Opisthacantus capensis*.

2.2.1. Plant Material

The plants, *Momordica charantia linn, Isoberlinia do ka, Terminalia avicennioides, Tamarindus indica* and *C rotalaria retusa L* were collected also in Maiduguri, N igeria and authenticated by a plant taxonomist, Prof. S. S. Sanusi of the department of Biological sciences, Un iversity of Maiduguri, Maiduguri Nigeria.

2.2.2. Venom Extraction

The venom was extracted from the scorpion tail into a 50 mM tris-HCl buffer pH 8.

2.2.3. Phospholipase A_2 Assay

The phospholipase A_2 activity assay was carried out by a modified egg yolk coagulation method as described by Haberman and Neumann [26]. To one millilitre (1ml) of the homogenized egg yolk in 50mM tris-HCl buffer pH 8 was added 10µl of crude phospholipase A_2 and incubated for 10 mins. At the end of the incubation period, the mixture was immersed in boiling water for two minutes to stop the reaction. The liberated free fatty acids were then titrated against 20 mM NaOH using phenolphthalein as an indicator.

2.2.4. Effect of pH on Venom Phospholipase A_2

This was determined by assaying enzyme activity at varying pH ranging from pH 4-9.

2.2.5. Effect of Temperature on Venom Phospholipase A_2

This was done by incubating a mixture of enzyme and its substrate at varying temperature (30, 40, 50, 60, 70, and 80ºC) for 10 minutes and activity assayed.

2.3. Activation Energy

Activation energy (Ea) was estimated by preincubating the enzyme and its substrate at various temperature for 10 minutes before assaying for activity. Logarithm of initial velocity was plotted against reciprocal of the temperature in Kelvin (Arrhenius plot) and the slope was used to determine Ea.

2.4. Plant Extract Preparation

Fresh leaves of *Momordica charantia linn, Isoberlinia doka, Terminalia avicennioides and Crotalaria retusa L* were separately subjected to the following treatments. The leaves were treated according to the method of Joslyn [27] and dried in an oven for about six hours at 60ºC followed by sun drying for days. The dried leaves were ground into fine powder and the powder was sieved through a 0.25mm sieve (Endicott's test sieve Ltd., London, UK). One hundred grammes (100g) of fine powder was soaked in 300ml distilled water and left for 24 hrs (Cold extraction). The extract was filtered with whatmann's filter paper and the filtrate used in inhibition studies.

2.5. Initial Velocity Studies

This was carried out by incubating Phospholipase A_2 with varying concentrations of the substrate to obtain their corresponding V_o. Then, the data obtained was used for double reciprocal plot from which K_m and V_{max} were estimated.

2.6. Inhibition Studies

Michaelis constant (K_m) and V_{max} were determined in the presence and absence of varying concentrations of each of the four (4) plant extracts (*Momordica charantia linn, Isoberlinia doka, Terminalia avicennioides and Crotalaria retusa L*).

2.7. Estimation of Total Protein

Total protein was estimated according to Bradford Method [28].

2.8. Statistical Analysis

Results are mean ± standard deviation for triplicate determination. Students' t-test was used to compare paired means and a difference was considered statistically significant at $P<0.05$.

3. Results

Figure 1 describes the effect of different pH on O. *capensis* venom phospholipase A_2 activity. Highest phospholipase A_2 was at pH 5.

The effect of different temperature on O. *Capensis* venom phospholipase A_2 is as depicted in figure 2. Highest phospholipase A_2 activity was recorded at 60°C. An activation energy of 5.20

Kcal/mol was calculated from the slope of the plot of $\log V_o$ vs reciprocal of absolute temperature (K) (Figure 3).

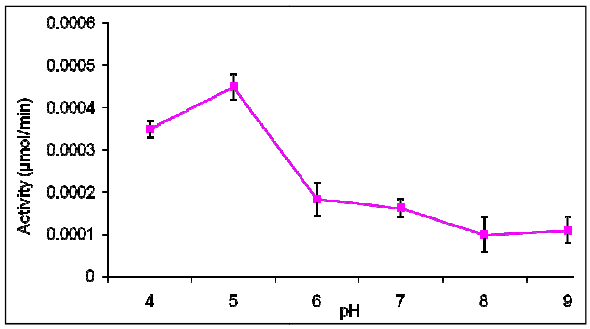

Figure 1. *Effects of pH on O. Capensis Venom Phospholipase A_2 activity*

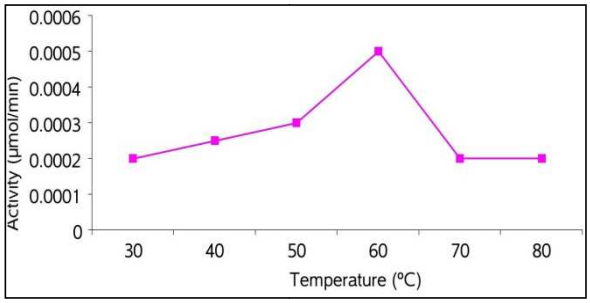

Figure 2. *Effects of Temperature on O. Capensis Venom Phospholipase A_2 Activity*

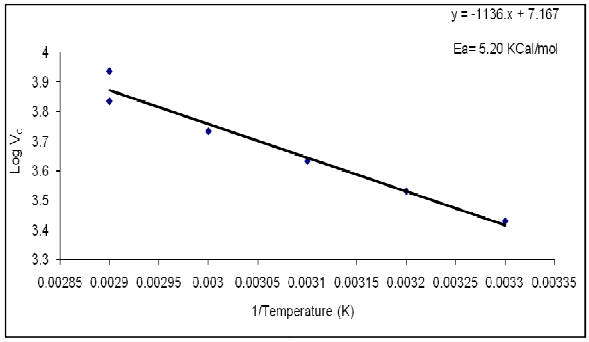

Figure 3. *Arrhenius Plot for O. Capensis Venom Phospholipase A_2*

Table 1 shows the Effects of different concentrations (4mg, 6mg and 8mg/ml) of *Isoberlinia doka (A)* and *Momordica charantia*(B) aqueous extracts on O. *capensis* Venom PLA_2 activity. Michaelis constant (K_m), V_{max+} and K_{cat} of the enzyme in the presence of different concentrations of I. *doka* and M. *charantia* decreased compared to the control.

Table 1. *Effects of different concentrations of Isoberlinia doka (A) and Momordica charantia(B) extracts on O. capensis Venom PLA_2 activity*

Kinetic parameters	Control (0%)	4mg/ml	6mg/ml	8mg/ml
Km (mg/ml) A	130.56±33.68^a	88.295±69.18^b	72.30±64.06^c	64.29±77.261^d
B	130.56±33.68^a	82.76±42.50^b	130.55±33.67^a	123.01±34.36^c
Vmax (A)	0.00074±0.015^a	0.00039±0.012^b	0.00092±0.0133^c	0.00062±0.0413^d
(µmol/min) (B)	0.00085±0.15^a	0.00063±0.19^b	0.00069±0.26^c	0.00080±0.37^d
K_{cat} (A)	0.000014±0.66^a	0.0000072±0.71^b	0.000016±0.24^c	0.0000113±0.75^d
(Min^{-1}) (B)	0.000016±0.66^a	0.000012±0.28^b	0.000013±0.45^c	0.000014±0.55^d

Values are mean±SD for triplicate determination. Values with different superscripts in the same horizontal row are significantly ($P<0.05$) different

Table 2 shows the result of different concentrations (4mg, 6mg and 8mg/ml) of aqueous extracts of *Tamarindus indica* (A), *Crotalaria retusa L* (B) and *Terminalia avicennioides* (C) on O. *capensis* venom PLA_2 activity. Michaelis constant (K_m) for the enzyme increased in the presence of the different concentrations of the plant extracts (A, B and C) while V_{max} and K_{cat} remained unchanged.

Table 2. *Effects of different concentrations of Tamarindus indica (A), Crotalaria retusa L.(B) and Terminalia avicennioides (C) aqueous extracts on O. capensis venom PLA_2 activity*

Kinetic Parameter	Control(0%)	4mg/ml	6mg/ml	8mg/ml
K_m (mg/ml) A	130.56±33.68[a]	162.7±89.35[b]	99.75±22.20[c]	380.95±206.19[d]
B	130.56 ± 33.68[a]	392.54 ± 527.32[b]	145.38 ± 90.63[c]	203.70+115.85[d]
C	130.56 ± 33.68[a]	144.33±91.90[b]	138.9±96.2[c]	147.22±24.02[d]
V_{max}(µmol/min) A	0.00085±0.15[a]	0.0009.33±0.40[a]	0.00085±0.33[a]	0.00089±0.90[a]
B	0.00085 ± 0.17[a]	0.00086 ± 0.15[a]	0.00080 ± 0.19[a]	0.00086 ± 0.27[a]
C	0.00085 ± 0.17[a]	0.00096±0.38[a]	0.0009±0.36[a]	0.00085±0.58[a]
K_{cat}(min^{-1}) A	0.000016±0.66[a]	0.000017±0.71[a]	0.000016±0.58[a]	0.000016±0.16[a]
B	0.000016 ± 0.66[a]	0.000016 ± 0.60[a]	0.000015 ± 0.35[a]	0.000016 ± 0.50[a]
C	*0.000016 ± 0.66[a]*	*0.000018±0.72[a]*	*0.000017±0.67[a]*	*0.000016±0.12[a]*

Values are mean±SD for triplicate determination. Values with different superscripts in the same horizontal row are significantly (P<0.05) different. + The example for this table. The example for this table. The example for this table. The example for this table. The example for this table. The example for this table..

4. Discussion

The optimum pH of 5 displayed by the scorpion venom PLA_2 indicates that the enzyme is active at acidic pH. Enzymes in general are active only over a limited pH range and most have a particular pH at which their catalytic activity is optimal.

The optimum temperature of 60°C displayed by the scorpion venom PLA_2 indicates that the enzyme is active even at higher temperature. Lower molecular weight single polypeptide enzyme proteins with some disulphide bonds are likely to be heat-stable than large polymeric enzymes [29].

The mechanism of inhibition of *Isoberlinia doka* (A) and *Momordica charantia* (B) aqueous extracts (Table 1) on O. *capensis* venom PLA_2 reveals an uncompetitive pattern of inhibition. This indicates that the constituents of the extracts bind to the enzyme-substrate complex leading to the formation of a dead end complex and the inhibition cannot be reversed by increasing the substrate concentration. Both K_m and V_{max} were altered. There was also a decrease in the computed physiological index of efficiency O. *capensis* venom PLA_2. This indicates that there was a reduction in the number of molecules of phospholipids hydrolysed to products by the enzyme.

The decrease in the K_m of PLA_2 in the presence of various extracts of A, B, and C (Table 2) without any effect on the V_{max} is indicative of competitive inhibition. This suggests that the various extracts of A. B and C may contain an analogue that compete with the substrate for the active site of the enzyme. In this case, the computed physiological index of efficiency (K_{cat}) of O. *capensis* venom PLA_2 in the presence and absence of inhibitor remained unchanged.

This implies that the plant extracts have the potential to reduce the ability of PLA_2 to cause haemolysis of red blood cells, anticoagulant action and cardiotoxicity [30,31].

Similar result was reported by Abubakar *et al*.,[31] where snake venom PLA_2 activity was reduced significantly by extracts of *Indigofera pulchra, Aristolochia albida and Guiera senegalense*. Sailakshmi *et al.,*[32] also reported a dose dependent inhibition of snake venom PLA_2 activity by *Tamarindus indica seed extract*.

5. Conclusion

The aqueous leaves extracts of *Momordica charantia linn, Isoberlinia doka, Terminalia avicennioides, Tamarindus indica and Crotalaria retusa L* have exhibited some beneficial effects on O. *capensis* venom toxicity by inhibiting PLA_2 activity. Hence, the use of these plant extracts by herbalists for the treatment of scorpion bite may have some scientific basis.

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