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# ENZYMATIC ACTIVITIES OF SOME SNAKE VENOMS FROM FAMILIES ELAPIDAE AND VIPERIDAE

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**ABSTRACT**

Alkaline phosphomonoesterase, phosphodieslerase, L-amino acid oxidase, hyaluronidase, 5'-nucleotidase, arginine ester hydrolase, phospholipase $A_2$ and proteinase activities were determined in eight snake venoms, including three from sea snake, of families Elapidae and Viperidae from Pakistan. The species includes three sea snakes *Hydrophis cyanocinctus, Enhydrina schsitosa, Mic*r*ocephalophis gracifis gracilis* and two land snakes *Naja naja naja, Bungarus caeruleus* of family Elapidae while three land snakes *Vipera russelli russelli, Echis* carinatus and *Eristocophis macmahoni* of family Viperidae. The venoms of family Elapidae are characterized by low levels to traces of proteinase, L-amino add oxidase and arginine ester hydrolase activities with the exception of *Naja* naja *naja* and a moderate to high levels of phospholipase $A_2$ activities. The venoms of family Viperidae, on the other hand, are characterized by the presence of moderate to high levels of 5'-nucleotidase, proteinase, phosphodiesicrase and phosphomonocsterase activities.

## Introduction

The snake venoms arc known to be complex mixtures of organic compounds, mainly proteins, a number of which have enzymatic activities (Russell, 1984). The snake venoms are reported to contains atleast twenty five such enzymes including protcolytic enzymes, phospholipases, oxidases and the enzymes effecting circulatory system (Russell, 1983). The identification of those enzymes in past three decades not only lead to the better understanding of clinical manifestations produced by snake envenomation in human victims but also the production of drugs, antivenom and antidotes against the venom and its components.

In Pakistan, a number of medically important snake species are found in abundance and remains a health hazard for the community, mainly in villages and undeveloped areas. Although a number of studies have been carried out in Pakistan on some snake venoms to isolated active components (Shafgat et al.,' 1994 Siddigi et al., 1991) but no report is available on detailed enzymology of medically important snake species of Pakistan.

The present study was thus initiated to determine the enzyme activities of snake species from two important families Elapidae and Viperidae found in Pakistan. The members of family Viperidae are only terrestrial (land-oriented) while members of family Elapidae also includes sea snakes. The species selected were three sea snakes *Hydrophis cyanocincros, Enhydrtna schsitosa, Microcephalophis gracilis gracilis and* two land snakes *Naja naja naja, Bungams caendeus* of family Elapidae while three land snakes *Vipera russelli russelli, Echis carinatus* and *Eristocophis macntal:oni* of family Viperidae.

## Materials and Methods

*Materials:*

The sea snakes were captured near and around coastal areas of Karachi whereas other snakes were supplied by serpent charmers and identified by Zoological Survey department. Venom was collected manually by milking technique from live snakes and lyophilized. Enzyme substrates were products of Sigma Chemical Company (St. Louis, M.O.). Other chemicals were of analytical grade and products of Sigma Chemical Company (St. Louis, M.O.).

*Enzyme activities:*

Alkaline phosphomonoesterase, phosphodiesterase and Camino oxidase activities were determined by the methods described by Aird and da-Silva (1991) using p-nitrophenyl phosphate, bis-p-nitrophenylphosphate and L-leucine-p-nitroanilide, respectively, as substrates. Hyaluronidase, 5'-nucleotidase, phospholipase At, arginine ester hydrolase and proteinase activities were determined as described previously (Collins and Jones, 1972; deHass et al., 1968; Ellman et al., 1961; Mori et al., 1984; Sughihara et al., 1985; Tan and Ponnudurai, 1991) using human umbilical cord hyaluronic acid, 5'AMP, egg yolk phophatidylcholine, a-benzoyl-arginine ethyl ester and casein respectively as substrate. Hyaluronidase and proteinase activities are ex-pressed as units/mg while other enzyme activities are expressed either inp mole (phospholipase A2, arginine ester hydrolase) or nmole (alkaline phosphomonoesterase, phosphodiesterase, 5'nucleotidase, L-amino oxidase) of substrate transformed or product released per min per mg of venom (Tan and Ponnudurai, 1991). Each experiment was repeated atleast thrice and results are expressed as mean ± SE.

## Results and Discussion

Results are summarized in Table I. It has been reported that enzymes are important constituents of elapid and viperid venoms (Tan, 1991) and are involved in many levels of venom action (Tan, 1991). In present study all the sea snake venoms examined exhibited low levels to traces of proteinase, phosphodiesterase, phosphomonoesterase, L-amino add oxidase, arginine ester hydrolase and hyaluronidase activities whereas a moderate 5'-nucleotidase and high phospholipase $A_2$ activities. The results are in good agreement with that reported for other sea snake species of *Lapernis hardwickii, Laticauda laticaudata* and *Laticauda cohtbrina* (Tan and Ponnudurai, 1991). The venoms of other elapid snakes, *N. naja naja* and *B. caenileus,* tested showed the usual characteristic of Elapidae species, that is low proteinase, alkaline phosphomonoesterase and phosphodiesterase and a moderate to high phospholipase $A_2$ activities (Tan and Ponnudurai, 1991).

The venom from species of family Viperidae showed moderate levels of proteinase, alkaline phosphomonoesterase, phosphodiesterase, arginine ester hydrolase, L-amino acid oxidase, hyaluronidase and a high nucleotidase and $PLA_2$ activities. Previously biological and lethal properties of venoms from many Viperidae species have been investigated (Tan and Ponnudurai, 1992) and support our findings. It has been reported that snakes from family Viperidae venoms exhibited moderate to high alkaline phosphomonoesterase and arginine ester hydrolase activities and were devoid of acetylcholinesterase activity (Tan and Ponnudurai, 1992). The presence of moderate to low levels of proteianse is reported to be a common characteristic among snake species of both families Viperidae and Elapidae (Russell, 1983) whereas absence of both L- amino acid oxidase and arginine ester hydrolase is the most common characteristic of sea snake species (Tan and Ponnudurai, 1991).

Venoms of many elapid snakes were reported to produce paralysis and respiratory failure in animals. These effects have been attributed to the presence of both enzymatic and non-enzymatic neurotoxins (Lee, 1972; Tan, 1991). In addition certain phospholipase $A_2$ enzymes, besides being a potent neurotoxin, also exhibited direct hemolytic, anticoagulant and myonecrotic activities (Rosenberg, 1986). Arginine ester hydrolase, on the other hand, were reported to be thrombin-like enzymes (Tan, 1991) and hyaluronidase *are* spreading factor for venom/toxins. Other enzymes of snake venoms of both elapid and viperid origin including L-amino oxidase, phosphodiestrase and 5'-nucleotidase were reported to contribute to the toxic action of snake venom (Zeller, 1977).

In conclusion, detailed enzymological study have been carried out in eight medically important snake species from families Elapidae and Viperidae. A number of enzyme activities, known to contribute in toxicology of these venoms, have been identified. The research is in progress to study the biological activities of these venom and to partially isolated the enzyme for specific characterization.

**Table I**

**Enzymatic activities of snake species of families elapidae and viperidae from Pakistan**

| | PRO | PDE | PME | LAAO | AEH | $PLA_2$ | NUC | HYA |
|---|---|---|---|---|---|---|---|---|
| **ELAPIDAE** | | | | | | | | |
| *H. CYAN* | 0.74 | 0.16 | 1.12 | ** | ** | 460 | 55 | 6.87 |
| | ± 0.020 | ± 0.016 | ± 0.024 | | | ± 9.12 | ± 2.38 | ± 0.43 |
| *E. SCHI* | 0.53 | 0.17 | 0.91 | ** | ** | 402 | 83 | 21.00 |
| | ± 0.020 | ± 0.010 | ± 0.021 | | | ± 8.54 | ± 1.70 | ± 1.30 |
| *M. GG* | 0.81 | 0.20 | 1.21 | ** | 1.08 | 330 | 50 | 11.00 |
| | ± 0.015 | ± 0.012 | ± 0.042 | | ± 0.05 | ± 13.0 | ± 1.84 | ± 0.91 |
| *N. NAJA* | 2.05 | 2.90 | 1.95 | 117 | 30.5 | 402 | 1100 | 45.00 |
| | ± 0.060 | ± 2.050 | ± 0.210 | ± 8.53 | ± 1.25 | ± 8.53 | ± 38.94 | ± 1.30 |
| *B. CAER* | 0.88 | 2.20 | 2.32 | 210 | 1.0 | 297 | 73 | 109.00 |
| | ± 0.040 | ± 0.210 | ± 0.260 | ± 14.7 | ± 0.08 | ± 8.54 | ± 2.21 | ± 4.11 |
| **VIPERIDAE** | | | | | | | | |
| *V. RR* | 0.58 | 33.0 | 5.50 | 105 | 3.3 | 318 | 1070 | 68.00 |
| | ± 0.003 | ± 2.08 | ± 0.640 | ± 6.45 | ± 0.17 | ± 8.53 | ± 50.70 | ± 1.08 |
| *E. CAR* | 2.77 | 9.75 | 7.12 | 46 | 70.7 | 105 | 2070 | 38.25 |
| | ± 0.080 | ± 0.850 | ± 0.310 | ± 1.38 | ± 1.25 | ± 6.45 | ± 50.66 | ± 0.85 |
| *E. MACH* | 3.25 | 8.87 | 8.65 | 285 | 2.8 | 320 | 1320 | 94.00 |
| | ± 0.320 | ± 0.420 | ± 0.210 | ± 13.2 | ± 0.11 | ± 8.53 | ± 50.00 | ± 2.94 |

- Results are expressed as mean ± S.E.
- PRO = proteinase, PDE = phosphodiesterase, PME = alkaline phosphomonoesretase, PLA = phospholipase A, NUC = 5'-nucleotidase, HYA = hyaluronidase, LAAO = L-amino oxidase, AEH = arginine ester hydrolase.
- Units of activity are given in the text.

** = Traces

*H. CYN = H. Cyanocinctus, E. SCHI = E. schistosa, M. GG = M. gracilis gracilis, N. NAJA = N. naja naja, B. CAER = B. caeruleus, V. RR = V. russelli russelli, E. CAR = E. carinatus, E. MACH = E. macmahoni*

## References

Aird S.D. and daSilva N.J. Jr. (1991). *Comp. Biochem. Physiol.* **99**: 287.

Collins J.P. and Jones J.G. (1972). Studies on the active-site of IRC-50 arvin, the purified coagulant enzyme from *Agkistrodon rhodostoma. Acta. Biophys. Biochem.* **56**: 91.

deHass G.H., Postema N.M., Niewenhuizen W. and Van Deenen L.L.M. (1968). *Biochem.* biophys. *Acta.* **159**: 103.

Ellman G.L., Courtney K.D., Andres V. Jr. and Featherstone R.M. (1961). *Biochem. Pharmacol.* **7**: 88.

Lee C.Y. (1972). *Annual Review of Pharmacology.* **12**: 265.

Mori N., Nikai T. and Sugihara H. (1984). *Toxicon* **22**: 451.

Rosenberg P. (1986). The relationship between enzymatic activity and pharmacological properties of phospholipases in natural poisons (Harris J.B. ed.) pp.129-184. Clarendon Press, Oxford.

Russell F.E. (1983). Snake venom poisoning. Scholium Intl. New York.

Russell F.E. (1984). Snake venoms. In: Structure, Development and Evolution of Reptiles (Ferguson M.WJ. ed.). Academic Press, London.

Shafqat J., Siddiqi A.R. Zaidi Z.H. and Jornvall H. (1991). *FEES* **284**: 70.

Siddiqi A.R., Shafqat J., Zaidi Z.H. and Jornvall H. (1991). *FEES* **278**: 14.

Sugihara IT., Mori N., Nikai T., Kishida M. and Akagi M. (1985). *Comp. Biochem. Physiol.* **82**: 29.

Tan N: H. (1991). *Trop. BioMed.* **8**: 91.

Tan N: H. and Ponnudurai G. (1991). *Comp. Biochem. Physiol.* **99**: 351.

Tan, N: H. and Ponnudurai G. (1992). *Intl. J. Biochem.* **24**: 331.

Zeller E.A. (1977). *Experientia* **30**: 143.