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A comparative study of biological and biochemical properties of white, pale yellow and yellow Russell's viper (*Daboia russelii siamensis)* venoms

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Biological and biochemical properties of white, pale yellow and yellow venoms of Russell's viper (*Daboia russelii siamensis*) of same length (81-90cm) from Nyaungdon, Ayeyawady Division were studied by standard techniques. The results showed that biological and biochemical properties of these venoms were comparable except that the white venom possessed slightly potent defibrinogenating activity and lacked L amino acid oxidase activity compared to the yellow. The pale yellow venom had 1/3 and the white ½ of the arginine esterase activity possessed by the yellow. SDS-PAGE of the venoms showed that the white venom had fewer bands than the pale yellow and yellow venoms. Such variation in biological properties of the coloured venoms may be attributed to age (length) rather than colour variation of the Russell's viper venom.

INTRODUCTION

While studying geographical variation of Russell's viper (*Daboia russelii siamensis*) venom, we came across coloured venoms namely white, pale yellow and yellow. It has been observed that white venom contains less L amino acid oxidase than yellow venom [1]. Master and Kornalik [2] found that there are antigenic differences between yellow and white venom and the latter has weak necrotic activity compared to the former. Variation in colour of young and adult Russell's viper (*D.r.siamensis*) venom has been reported [3]. In the present study, we compared biochemical, biological and SDS-PAGE properties of white, pale yellow and yellow venoms of Russell's viper (*D. r. siamensis*) from our collected pools.

MATERIALS AND METHODS

418 Russell's vipers measuring 61-120 cm collected from 15 localities of 5 divisions of Myanmar were milked individually, lyophilized, pooled according to their length, colour and locality and stored at +4ºC. Venoms were pooled according to their length into two groups (<90 cm) (young) and (>90 cm) (adult).

Biological,biochemical and SDS-PAGE properties were determined on ten (white 3, pale yellow 3 and yellow 4) young viper (81-90cm) venoms of Nyaungdon, Ayeyawady Division.

Biological and biochemical properties of venom were also carried out on pooled 14 young (white) and 14 adult (yellow) Russell's vipers venom collected from Danubyu, Ayeyawady Division.

Methods

Biological properties of the venoms such as lethality, coagulant, haemorrhagic, necrotic, capillary permeability increasing and defibrinogenating activities were studied according to the WHO recommended techniques [4]. Biochemical properties: esterase activity was measured using substrate

Table 1. Biological properties of Russell's viper venoms from Nyaungdon, Ayeyawady Division

Length /color	No of snake	$LD_{50\ iv}$ µg/mouse*	MHD µg/rat	MND µg/rat	MDD µg/mouse	MCD µg/ml	MCPID µg/ml	Phospholipase activity** µg/mg/min	L amino acid oxidase activity** µg/mg/min	Arginine esterase activity** µg/mg/min
81-90 cm White	3	4.77 ± 1.8	24.6	26.9	2.4	0.07	0.0065	800 ± 5	ND	35.5 ± 0.3
Pale yellow	3	4.68 ± 1.0	24.0	29.2	4.2	0.09	0.0003	880 ± 2	6.6 ± 0.3	22.2 ± 1.2
Yellow	4	5.53 ± 2.5	24.3	31.6	3.3	0.06	0.0007	960 ± 1	6.6 ± 0.3	75.7 ± 0.3

Data are means of duplicate determination
** Mean ± SD (n=4)
*95% confidence limit

Table 2. Biological properties of Russell's viper venoms from Danuphyu, Ayeyawady Division

Length /color	No. of snake	LD_{50iv} µg/mouse *	MHD µg/rat	MND µg/rat	MDD µg/mouse	MCD µg/ml	MCPID µg/ml	PLA_2 activity** µg/mg/min	L amino acid oxidase activity** µg/mg/min	Arginine esterase activity** µg/mg/min
71-90 white	14	4.42± 1.2	30.9	30.9	4.2	1.995	0.0004	1000± 5	ND	142± 1.5
91-120 yellow	14	6.38 ± 1.0	30.2	27.5	5.1	2.291	0.0018	1026 ± 3	13.6 ± 0.7	133± 0.6

Data are means of duplicate determination
** Mean ± SD (n=4)
*95% confidence limit

TAME [5], phospholipase activity using phosphatidyl choline [6], L-amino acid oxidase activity using L-leucine as substrate [7] and pyruvate formed was measured following development of colour with dinitro phenylhydrazine substrate. Enzyme activities were expressed in units/mg/min. SDS-PAGE of the venoms was carried out according to the method of Laemmli [8].

RESULTS

Lyophilised venom of 418 Russell's vipers from collected pool showed that 64% (131/204) of young venom were white and 85% (182/214) of adult were pale yellow and yellow. Eighty to ninety percent of young viper venom from Danubyu (Ayeyawady Division), Indaing, Hmawbi and Kungyankone (Yangon Division) were white.

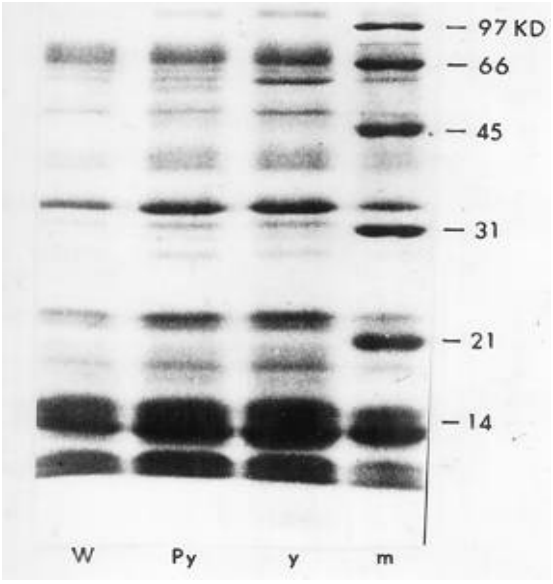

Fig. 1. SDS-PAGE patterns of white, pale yellow and yellow Russell's viper venoms of Nyaungdon (81-90cm). W=white, Py=pale yellow, Y= yellow venoms and m= molecular weight markers.

Biological and biochemical properties of white, pale yellow and yellow venoms of Russell's viper of the same length (81~90cm) from Nyaungdon (Table 1) were comparable except that the white venom possessed slightly potent defibrinogenating activity and lacked L amino acid oxidase activity compared to the yellow. The pale yellow venom had 1/3 and the white 1/2 of the arginine esterase activity possessed by the yellow venom. SDS-PAGE of the venoms showed the white venom had fewer bands than the pale yellow and the yellow venoms (Figure 1).

Comparative study of biological and biochemical properties of white (young) and yellow (adult) venoms of Danubyu (Table 2) shows that the former was more lethal and possessed more potent capilllary permeability increasing activity than the latter. L-amino acid oxidase activity was not detected in the former. Phospholipase and protease activities (argnine esterase) were comparable.

DISCUSSION

The study highlighted that 64% of young Russell's viper (*D.r.siamensis*) (<90cm) venom were white and 85% of the adult (>90cm) were pale yellow to yellow. However, 80~90% of the young viper venom from Danubyu, Indaing, Hmawbi and Kungyankone were white.

Study of young Russell's viper venom of same length (81~90cm from Nyaungdon, Ayeyawady Division) suggested that biological properties of white, pale yellow and yellow venoms were comparable except that the white venom possessed slightly more potent defibrinogenating activity. However, on comparing white venom of young Russell's viper (*D.r.siamensis*) venom of Danubyu with that of adult yellow venom, the former was more lethal and possessed potent capillary permeability increasing activity than the latter. It was reported that the white venom of Indian Russell's viper possesses weak necrotic activity compared to the yellow [2]. However, the other activities are comparable as observed in our Russell's viper (*D.r.siamensis*) venoms. It has also been documented that juvenile venom is more potent than that of adult [3] and such variation in biological properties of the coloured venoms may be attributed to age (length) rather than colour variation of the Russell's viper venom. Variation in protein bands of venom in SDS PAGE between young and adult [3] has been reported. A similar observation was made in our study.

Study on biochemical activity of the venom showed Indian Russell's viper (*Vipera russelli*) venom was found to have the same concentration of L amino acid oxidase and protease activities in both yellow and white venoms [2]. Our findings agree with observation made by Master and Kornalik [2] that proteolytic activity of *Vipera ammodytes* is comparable in both yellow and white venoms except the latter lacks L amino acid oxidase. In contrast, white venom of the Indian Russell's viper is found to have same concentration of L amino acid oxidase in both venoms. Age, species and geographical variation of venom composition [9-11] may account for the differences.

REFERENCES

1. Zeller, E.A. Enzyme of snake venoms and their biological significance. Advances in enzymology, Nord, F. F. (ed.) Interscience Publishers, New York, 1948; 459-495.
2. Master, R.W.P. and Kornalik, F. Biochemical differences in yellow and white venoms of *Vipera ammodytes* and Russell's viper. *Journal of Biological Chemistry* 1965; 240: 139-142.
3. Tun Pe, Nu Nu Lwin, Aye Aye Myint, Kyi May Htwe and Khin Aung Cho. Biochemical and biological properties of the venom from Russell's viper (*Daboia russelli siamensis*) of varying ages. *Toxicon* 1995; 33:817-821.
4. Theakston, R.D.G. and Reid, H.A. The development of simple standard assay procedures for characterization of snake venoms. *Bull. W.H.O*. 1983, 61:949-956.

5. Gutierrez, J.M., Avila, C., Camacho, 2. and Lomonte, B. Ontogenetic changes in the venom of the snake *Lachesis muta stenophy*. (Bushmaster) from Costa Rica. *Toxicon* 1990; 28: 419-416.

6. Magee, W.L.O. and Thompson, R.H.S. The estimation of phospholipase A activity in aqueous systems. *Biochemical Journal* 1960; 77: 526~534.

7. Dimitrov, G.D. and Kankonkar, R.C. Fractionation of *Vipera russelli* venom by gel filtration. I. *Toxicon* 1968; 5: 213-221.

8. Laemmli, U.S. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) 1970; 227:680-685.

9. Johnson, E.IE., Kardong KV and Ownby.E.L. Observations on white and yellow venoms from an individual southern pacific rattle snake (*Crotalus viridis helleri*) *Toxicon* 1987; 25:1169-1180.

10. Tun Pe, Nu Nu Lwin, Aye Aye Myint, Kyi May Htwe and Khin Aung Cho. Geographical variation of biological properties of Russell's viper venom: a preliminary report. *Myanmar Health Sciences Research Journal* 1993; 5: 107-112.

11. Jayanthi, G.P. and Gowda, T.V. Geographical variation in India in the composition and lethality potency of Russell's viper (*Vipera russelii*) venom. *Toxicon* 1988; 26: 257-264.

12. Aye Aye Myint, Tun Pe, Kyi May Htwe and Khin Aung Cho. Variation in biological properties of venom from different localities of Ayerwaddy division. *Myanmar Health Science Research Journal* 1993; 5: 121-124.