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Seasonal variation in biological and biochemical properties of Russell's viper (*Daboia russelli siamensis*) venom

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Biological, biochemical and electrophoretic properties of venom of four captive Russell's vipers (*Daboia russelli siamensis)* milked monthly for 11-24 months were studied. In general, all biological activities such as lethal, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing remained fairly stable within the first 3 months in captivity. Variation in activity (except haemorrhage and necrosis) was observed in captivity. Marked reduction in activity; (4-5 times) of lethal and defibrinogenating in July (raining) and November through January (winter) and coagulant (35 - 42 times) in April/May (summer) was observed. However, 4-7 times reduction in capillary permeability increasing activity (CPI) was recorded in July through September (raining). It is noteworthy that marked decrease in capillary permeability increasing activity was observed in two out of four Russell's vipers from 3rd month onward following captivity. There was a variation in protein content, L-amino acid oxidase, esterolytic and phospholipase A_2 activities in between seasons. However, no variation in venom yield was observed. Electrophoretic studies of venoms showed quantitative and qualitative variation in protein bands.

INTRODUCTION

Russell's viper bite is endemic in Myanmar. Myanmar Pharmaceutical Factory is the sole manufacturer of antivenom in Myanmar. Live Russell's vipers are bought every year for venom extraction. Snakes were milked, venom pooled, desiccated and stored at 4˚C. Venom is used for raising antivenom and the latter is used for treating snake bite cases. Batch to batch variation of Russell's viper (*Daboia russelii siamensis)* venom used for raising antivenom has been reported [1]. Venoms used for raising anti-venom are pooled from different milkings. Because of variation in venom property, possibility of seasonal variation of venom is considered. Studies on toxicity and yield of venom following repeated milking of venom from single snake or groups of snakes and of varying ages have been reported [2-10]. However, there were few reports on study of seasonal variation in composition and toxicity of snake venoms [11-13]. In this communication, biological and biochemical properties of four Russell's vipers milked at monthly intervals for 11 to 24 months were studied.

MATERIALS AND METHODS

Snakes

The snakes measuring 84cm (A), 88cm (C), caught in November 1993, snake E (89cm) in May 1994 and snake B (99cm) in July 1994 were housed in separate wooden cages at the Myanmar Pharmaceutical snake farm, Yangon where the environmental temperature ranged from 20º-32ºC. These snakes originated in the Kokekogwa, Taungdwingyi district of Magway Division.

The snakes were fed on 1 to 2 mice per week with water *ad libitum*. During winter, straw was provided for insulation.

Venom

Individual snake was milked and venom was lyophilised at monthly intervals following capture up to the time of death. The total length of the snake and volume of venom yielded (dry weight) were also measured at each extraction. Primary milking of two wild caught Russell's vipers measuring 84 and 88 cm in length, caught in November 1993 and another two measuring 88 and 89 cm in total length, caught in May 1994 from the same locality were also available for the study.

Assays

Biological properties (lethal, coagulant, haemorrhagic, necrotic, defibrinogenating and capillary permeability increasing activities) of venom were determined according to the WHO recommended techniques [14]. Biochemical tests such as L-amino acid oxidase, phospholipase, esterolytic activities, protein concentration (1 mg/ml of dry venom) and SDS-PAGE electrophoresis were also determined by the methods previously described [8].

Statistics

Values expressed were means ± 1 SD. Statistical analysis of the samples was carried out by comparison of means by Analysis of Variance and Student's t test. Level of significance was taken at $p < 0.05$ in the former and $p < 0.001$ in the latter.

RESULTS

Biological activities of the venoms

Results of mean biological activities of two Russell's viper venoms are shown in Table 1. In general, the pattern of individual biological activity of the four venoms was comparable except in the capillary permeability increasing activity (CPI) which waned from 3rd month onward following captivity in 2/4 snakes. All activities remained fairly stable within the first

Table 1. Biological activities of Russell's viper venoms in three seasons

Biological activity	Code	Winter (Nov-Feb)	Summer (Mar-Jun)	Raining (July-Oct)
L aminoacid oxidase (ug/mg/min. venom)	A	164 ±3.57	168 ± 2.37	220.09 ± 3.04
	C	215 ±3.88	215.75 ± 5.36	234.5 ± 6.61
Proteolytic (ug/mg/ min. venom)	A	289.6 ±5.12	339.83 ±7.42	267 ± 9.44
	C	185.53 ±3.16	185.8 ±5.56	214.8 ± 11.66
Phospholipase A_2 (ug/mg/ min. venom)	A	1237.1 ±35.22	1179.3 ±25.64	1265.18 ± 9.38
	C	1258.7 ± 6.11	1135 ±8.06	1247.7 ± 16.2
Protein (1mg/ml)	A	821.7 ±18	850 ±25.2	823.6 ± 32.02
	C	805.5 ±15	845 ±15.66	815.2 ± 36.43
Venom yield dry (mg)	A	53.75 ±11	40 ±13.5	73.3 ± 30.5
	C	82.5 ±3.53	113.75 ±12.5	113.3 ± 25.6

Table 2. Comparison of biological properties of venoms of wild and captive Russell's vipers

Month	Status	Length (cm)	No.	LD_{50} µg / m	MHD µg / r	MND µg / r	MDD µg / m	MCD µg / ml	MCPID µg / ml
Nov	W	84 / 88	2	2.59 ±2.4	22.9	33.5	1.0	0.158	0.0024
	C	84	1	7.3 ±2.9	57	47.3	7.0	0.040	0.0018
May	W	88 / 89	2	8.1 ± 2.2	36.3	27.0	2.5	3.236	0.0017
	C	92	1	9.1 ± 2.3	84	50.0	6.0	0.263	0.0044

W = Wild C =Captive m = mouse r = rat

MHD = Minimum haemorrhagic dose
MND = Minimum necrotic dose
MDD = Minimum defibrinogenating dose
MCD = Minimum coagulant dose
MCPID = Minimum capillary permeability increasing dose
LD_{50} = Lethality

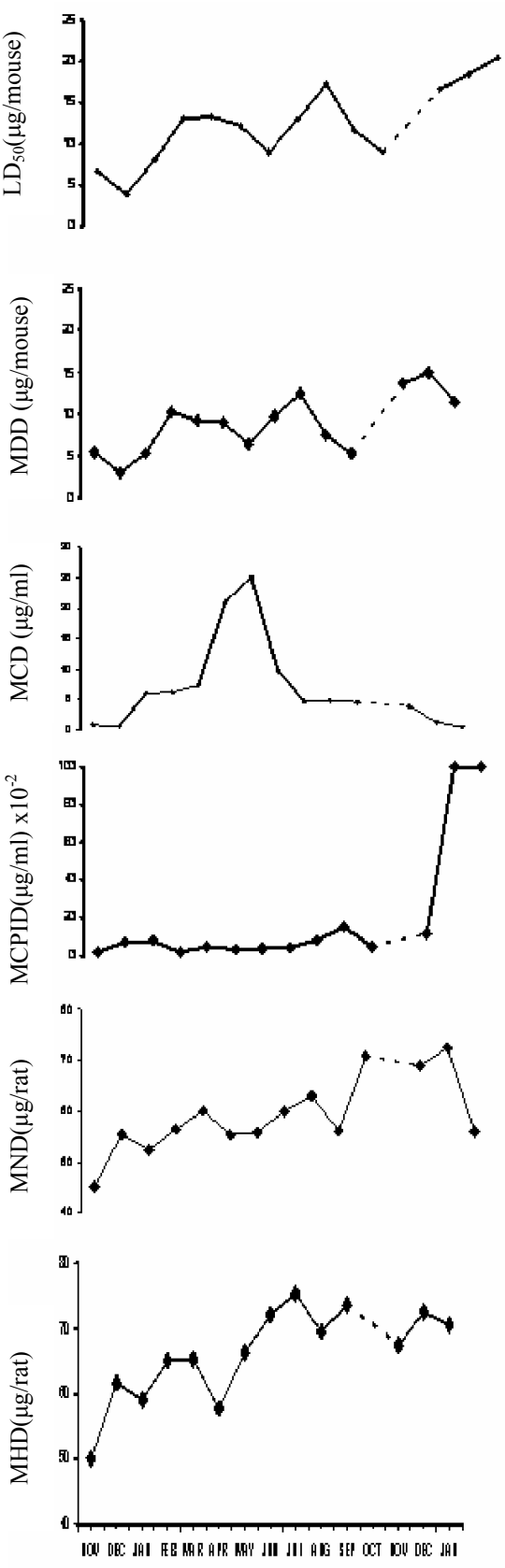

Fig. 1. Biological properties of Russell's viper venom* in captivity

*Each point represents a pooled venom of 4 snakes.

3 months in captivity. Variation in biological activities was observed during captivity except haemorrhage and necrosis. Mark reduction in lethal and defibrinogenating (4-5 times) in July (raining) and November through January (winter) and coagulant activity (35-42 times) in April-May (summer) was observed. About 4-7 times reduction in CPI activity was recorded in July through September (raining) and more than 50 folds reduction in the activity after a year in captivity was observed (Figure 1).

The pattern of biological activities of the venom collected from the wild caught Russell's vipers in November and May also showed a similar pattern of variation as in the venom collected over the same month of the year in captivity. However, the venoms from the wild caught snakes were far more potent than the latter except in coagulant activity (Table 2).

SDS PAGE electrophoresis

SDS-PAGE electrophoresis of monthly collected venoms of snake A is shown in Figure 2. Qualitative and quantitative differences in protein bands were observed among venoms. SDS–PAGE electrophoresis of monthly collected venoms of snakes B and C also showed similar qualitative and quantitative differences in protein bands (Figure not shown). The venoms collected from the wild in November 1993 and 1994 showed similar protein bands (Figures not shown).

Snake's length

While in captivity snake A grew from 84 cm to 106 cm (22 cm) in 24 months, snake E grew from 89 cm to 100 cm (11 cm) in 18 months, snake C grew from 88 cm to 108 cm (20 cm) and snake B, from 99 cm to 103 cm (4 cm) in 11 months. In general, growth in length of the snakes was observed in the first 4 to 6 months in two snakes (up to one year in one) following captivity and then it flattens out.

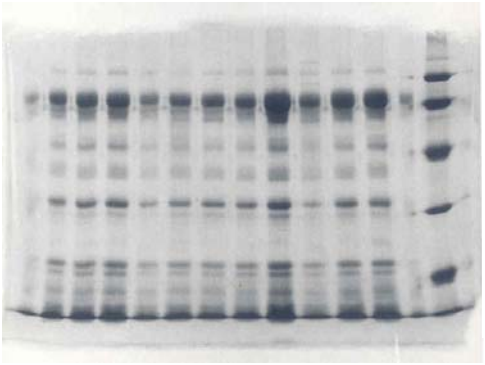

Fig. 2. SDS–PAGE electrophoroesis of venoms of Russell's viper A in captivity.

Lane 1-11 represent monthly collected venom samples of snake A
Lane 12 represent molecular weight marker

Venom yield

Mean volume of venom yield per milking of snake ranged from 0.2 ml to 0.7 ml. The average venom yield of the snakes in winter was 0.35 ml (n=10), summer 0.39 ml (n=14) and raining seasons 0.42 ml (n=11). No significant difference in venom yield of Russell's viper (*D.r.siamensis*) per milking in captivity was observed. However, seasonal variation in venom dry weight was observed in venoms A and C; (winter C 82.5±3.53 mg and summer C 113.75±12.5 mg ($P<0.01$), winter A 53.75 ±11 mg and summer A 40 ±13.5 mg ($P<0.01$), winter A 53.75 ±11 mg and raining A 73.3± 30.5 mg ($P<0.01$) and winter C 82.5± 3.53 and raining C 113.3± 25.6 mg ($P<0.01$). Venom yield was maximal in summer and raining in venom C and raining in venom A.

Biochemical activities of venom

Biochemical properties of venom were studied in two of four snakes (A and C). Seasonal variation in L-amino acid oxidase, esterolytic and PLA_2 activities of the venoms was observed. In venom A, a significant difference in L amino acid oxidase activity in between winter (164±3.57 ug/mg /min venom) and raining (220.09±3.04 ug/mg/min venom)($P<0.003$),PLA_2 between winter (1237.1±35.22ug/mg/min venom)and summer (1179.3 ± 25.64 ug/mg/min venom) ($P<0.2$), summer (1179.3±25.64 ug/mg/min venom) and rainy (1265.18±9.38 ug/mg/min venom) ($P<0.2$) was observed. The venom A has maximal L amino acid oxidase (220.09± 3.04 U/mg/min venom) and PLA_2 (1265.18± 9.38 U/mg/min venom) in raining and esterolytic activities (339.83 ±7.42U/mg/min venom) in summer.

Protein content of the venom (1 mg/ml=850±25 ug/ml) showed variation between winter (821.7 ±18 ug and summer (850±25.2 ug)($P<0.01$) in venom A and 805.5 ±15 ug and 845 ±15.66 ug respectively ($P<0.01$) in venom C.

DISCUSSION

Variation in yield of venom has been reported [2, 4, 9]. However, no significant variation in venom yield of Russell's viper (*Daboia russelii siamensis*) per milking was observed during captivity as snakes were kept in well-cared and feeding readily. Individual seasonal variation in venom yield was observed. Snake C yielded more venom in summer and rainy season compared to winter. Although the venom yield increased with increases in temperature, the maximum yield being obtained in the hottest summer months [15], it was unlikely that the difference seen in the two venoms was attributed to a change in environmental temperature since both were kept in the same environment. Because of small sample size, individual variation in yield could not be excluded. No seasonal variation in venom yield was observed in one C*rotalus atrox* milked monthly for 19 months [10]. It was reported that neither venom yield nor toxicity decreased following repeated venom extraction (16 times) over a period of two years in a group of water moccasins (*Agkistrodon piscivorus*) [3].

Maximum yield of *Naja naja oxiana* was observed in autumn and winter, that of *Vipera lebetina* and *Agkistrodon halys* in summer. However, no significant seasonal variation in venom yield was observed in venoms of *Pseudocerastes persicus*, *Vipera*

latifii, *Vipera xanthina* ssp. and *Echis carinatus* from Iran [9]. Marked variations in venom yield on successive milking for 2-7 months were observed in Australian elapids. Average secondary yield for all species was lower than the average primary yield with exception among the individual snakes [16].

Individual variation in growth in length was unlikely to be due to captive condition since all were kept in the same environment, probably genetic factor could not be excluded.

A significant decrease in PLA_2 activity in summer compared to winter or rainy season was observed in both venoms. There were no significant differences in PLA_2 activity between winter and raining and L amino acid oxidase activity between winter and summer of venom C. Protein content of the venoms showed variation between winter and summer ($P<0.01$). Variation in the composition of the venom from a single specimen of *Pseudonaja texilis* (common brown snake) over a year [12] and seasonal variation (winter and summer) in composition of venom of V*ipera ammodytes* based on comparison of different specimens of snake [11] have been observed. Intraspecific variation in venom colour (L amino acid oxidase), enzyme activity and lethality of V*ipera ammodytes* has been reported [17].

Biological properties of the venom remained fairly stable throughout captivity except marked decrease in coagulant activity in summer and capillary permeability increasing activity in a year end of captivity. It was noteworthy that CPI activity waned in 2/4 snakes from 3rd month onwards of captivity. However, toxicity of Notechis venom was approximately the same between primary and secondary yield provided the snakes were not diseased or starved [16]. Toxicity of the venom of a *Crotalus atrox* milked for 19 months tended to decrease during captivity and no seasonal variation in toxicity was observed [10]. Variation in biological properties of venom according to age in *Crotalus atrox* venom [5, 7] and Russell's viper (*D. r . siamensis*) [8] has been reported. Negligible seasonal intraspecific variability especially in coagulant activity of venom of carpet viper (*Echis carinatus*) has been observed [18].

SDS – PAGE electrophoresis of the venom B in captivity and venoms collected from the wild in November 1993 and 1994 (Figures not shown) showed similar results. Unlike venoms of *Vipera ammodytes* [11], there was no definite loss of protein band attributable to seasonal variation as in habu (*Trimeresurus flavoviridis*) venom [19] and in venom of *Bitis* sp. [20,21] following frequent milking or in snakes milked over a period of 20 months [13].

It has been reported that toxicity and the yield of venom depend not only on the age, size, forced feeding, fasted conditions of milking, stress, period of daylight, temperature and captive care [6] but also on the season and geographical origin of the snake [9].These seasonal variations in composition and biological properties of venom should be taken into consideration when collecting venoms for raising antivenoms.

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