Venom Yield and Toxicities of Six Egyptian Snakes with a Description of a Procedure for Estimating the Amount of Venom Ejected by a Single Snake Bite

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Abstract:

The venom yield, body length and toxicity of six Egyptian poisonous snakes (*Naja haje*, *Naja nigricollis*, *Cerastes cerastes*, *Cerastes vipera*, *Cerastes cerastes cerastes* and *Echis carinatus*) were studied. The percentage of dry weights of these venoms were 17.1-38.2 % that of the wet weight. The LD_{50} (i.p.) of the six venoms were 0.495, 1.670, 0.950, 0.625, 1.350 and 0.920 mg/kg mice respectively. A procedure for estimation of the venom ejected by a single bite of *Naja haje* or *Cerastes cerastes* is described. There was a correlation between the snake body length and the amount of venom ejected by a single bite or that milked from each snake. The percentage of recovery of *Naja haje* and *Cerastes cerastes* venom, injected manually in rabbit muscles, was 68.1 and 97.5 %. From the recovery of the snake venom, the amount of the venom ejected by a single bite was estimated. This procedure may be helpful for determining the dose of the specific antivenin.

Key Words:

Snakes; Venom; Egyptian snakes; Venom yield; Venom toxicities.

Introduction:

Snake venoms are rich sources of toxic proteins with many medical importance and consequently, in treating snake bite victims. The dose of artivenom to be administered depends on the amount of these toxic proteins injected, in addition to the kinetics of venom distribution in the body (Hutton and Warrell, 1993; McNally *et al*., 1993; Warrell, 1995; Chippaux, 2006; 2007 and Narvencar, 2006). Several studies have been carried out on the venom ejected by the bite of poisonous snakes (Kochva *et al*., 1982; Tun-Pe and Khin-Aug-Cho, 1986; Chippaux *et al*., 1991; Westhoff *et al*., 2005 and Mirtschin *et al*., 2006). The amount of venom delivered per bite under experimental conditions not only varied with species of snakes (Morrison *et al*., 1982) but other factors also influenced the venom yield such as the age, the length and the diet of the snake (Fix, 1980; Furtado *et al*., 1991; Tun-Pe *et al*., 1995; Andrade and Abe, 1999; Mirtschin *et al*., 2002 and Peterson, 2006a,b). Moreover, the sympathetic nervous system is one of the factors that influenced the venom yield and production

(Yamanouye, *et al* 1997). In all previous studies, the injection of venom in the rubber diaphragm of a collecting flask, by a hand hold snake with digital massage of venom gland did not approximate the natural biting situation. Therefore, one of the important requirements to develop improved antivenins and its suitable dose is the precise information of the quantity of venom ejected by a single bite. The estimation of venom delivered by a single bite is recommended than successive biting because the second or third bite, delivered by the same snake, yield was less than the venom weight (27 %) of the first bite (Morrison *et al*., 1982; Brunda *et al*., 2006 and Peterson, 2006a,b). It was found that the first bite of all snakes in previous studies was strikingly more efficient than the successive bites (Morrison *et al*., 1982 and 1983 and Hayes, 1992). During the medical treatment of snake bite, excessive doses of antivenin are maintained for long periods, due to the inability to decide the amount of venom ejected by bite, and consequently, it was suspicious that the antivenin gave rise to serious medical problems (Christensen, 1981, Theakston, 1986; Gillissen *et al*., 1994; Peterson, 2006a,b and Gutiérrez, *el al*., 2007).

In the present paper, the biometrics, venom yields and toxicity of six Egyptian snakes were studied. Additionally, a method was described to measure the amount of venoms ejected by a single bite of Naja haje and Cerastes cerastes venoms.

Materials and Methods :

Snakes and venoms:

Poisonous snakes used were Naja haje, Naja nigricollis, Cerastes cerastes, Cerastes vipera, Cerastes cerastes cerastes and Echis carinatus collected from different areas of Egypt as shown in table (1).

Table (1) : Localities of the venomous snakes used in the study

Species	Location
Naja haje	El-Fayum – Baniswif
Naja nigricollis	Assuit – Souhage – El-Oxor
Cerastes cerastes	El-Fayum–Banisuif – El-Esmaelia
Cerastes vipera	El-Behira
C. cerastes cerastes	Aswan – Abu Simple
Echis carinatus	El-Wahaat

After milking for the first time, snakes were maintained in large cages attempting to recreate seasonal variations with adjustable lighting and temperature. Each snake venom was extracted manually every 3 weeks in the Venom Research Lab of Zoology Department, Faculty of Science, Ain Shams University. The venom obtained from each snake, during six months (summer and winter seasons), was weighed after milking and after lyophilizing to determine the wet and dried weight. The length of each milked snake was measured. The lethality of each pooled venom was determined.

Experimental animals:

CD-1 mice of both sexes weighing 20-25 g were used to determine lethal toxicity of venoms. Albino adult rabbits (1.5-2 kg) were used to estimate the amount of venoms introduced in their muscles either by snake bite or manually injection.

Determination of lethal toxicity:

The lethal toxicity (LD50) of snake venoms or rabbit muscle homogenate was determined in CD-1 mice (20-25 g) injected intraperitoneally with several dilutions of each venom or muscle homogenate in total volume of 0.2 ml balanced physiological saline (BPS). The number of deaths for each injection was noted after 24 hr. The LD50 values were calculated according to the method described by Poll and Bieber (1981).

Extraction and determination of snake venom weight ejected by a single bite from rabbit muscle:

Spontaneous strike by snakes:

Pieces of fresh muscles weighing 20 g were removed from a killed rabbit and attached to a hook in the tip of a long staff. The muscle in the staff was then placed in the snake cages to receive a spontaneous strike by Naja haje or Cerastes cerastes with lengths ranging from 100-170 and 40-59 cm respectively. The number of trials for each snake was 20.

Injection of venoms manually:

Another experiment was carried out to estimate the recovery rate of Naja haje and Cerastes cerastes venoms injected manually in rabbit muscles. The doses amounting 113.14, 70.74 and 28.28 mg of Naja haje venom were injected in pieces of 20 g rabbit muscle. In another muscles pieces with the same weight, 19.52, 12.67, 9.77 mg doses of Cerastes cerastes venom were injected. Each venom dose was injected in three pieces of muscles. After the two set of experiments had carried out, the piece of muscle containing the venom was immediately minced with a pair of scissors, ground in 50 ml of BPS with a mortar and pestle and homogenized with a 'blender'. Total volume of the homogenate was 100 ml. The homogenate immediately filtered and the filtrate, was assayed for toxic effects.

The procedure for determining the quantity of venom ejected in rabbit muscle either by a single bite or injected manually was as follows:

$$\text{weight of venom (mg)} = \frac{\text{Total MLD ejected}}{LD_{50}}$$

$$\text{Where, total } MLD_{50} = \frac{\text{MLD/ ml muscle extract } \times 100 \text{ ml } \times 100}{\text{Average recovery of the venom}} \quad \text{......... (a)}$$

LD_{50} of the venom=mg / 20g mice .... (b)

The percentage of the venom ejected to the total available venom present in the venom glands was calculated from the formula :

$$\frac{a / b}{c} \times 100$$

The total weight of venom after milking was designated as (c)

Statistical analysis:

Data of body lengths of snakes (mean + S.E.), their venom yield extracted either manually or by spontaneous snake strikes (mean + S.E.) were analyzed using one-way analysis of variance to evaluate the correlation between them (Campbell, 1994).

Results:

Venom yields and toxicity of Egyptian poisonous snakes:

The venom yields of six Egyptian poisonous snakes obtained manually, snake lengths and their toxicity are shown in table (2).

Naja haje:

The mean wet and dried venom yield of Naja haje after milking from adult snakes (mean length = 149.0 ± 4.6 cm) were 353.7 ± 4.6 and 110.8 ± 8.3 mg respectively. The range of wet Naja haje venom was 160 - 449 mg and the range of the dried venom was 34 - 200 mg. The wet or dried venom yield extracted manually correlated with the length of Naja haje snake ($r = 0.861$, $P < 0.01$ or $= 0.776$, $P < 0.001$; $n = 20$ for wet and dried venom respectively). The LD50 of Naja haje venom was 0.495 mg / kg.

Naja nigricollis :

The spitting cobra Naja nigricollis gave 408.1 ± 41.5 mg wet venom which decreased to 117.1 ± 15.0 mg dried venom per adult snake after lyophilizatian. The range of wet and dried venom obtained from adult snakes were 281 - 552 and 76 - 168 mg respectively. The venom yield either wet or dried correlated with the snake length (mean length = 125.7 ± 6.6 cm ; $n = 7$). The r values for wet and dried venoms were 0.789 ($P < 0.05$) and 0.760 ($P < 0.05$) respectively. The lethal dose of Naja nigricollis venom was 1.670 mg / kg.

Cerastes cerastes:

The venom yield of horned Egyptian sand viper, Cerastes cerastes, with the mean length of 52.9 ± 2.0 cm ($n = 20$) was 179.3 ± 13.4 mg in case of wet venom while that of dried venom was 42.3 ± 3.0 mg per adult viper. The ranges of wet and dried Cerastes cerastes venom were 86 - 295 and 23 - 75 mg respectively. There was correlation between the length of the viper and wet or dried venom yield ($r = 0.815$; $P < 0.001$ and $r = 0.628$; $P < 0.01$ respectively). The lethal dose of Cerastes cerastes was 0.95 mg / kg.

Cerastes vipera:

On milking Cerastes vipera, adult vipers produced 58.4 ± 5.6 and 22.3 ± 2.2 mg wet and dried venom per adult viper respectively. The minimum amounts of wet and dried Cerastes vipera venom were 17 and 9 mg while the maximum amounts were 94 and 43 mg respectively. The LD50 of this viper venom was 0.625 mg / kg. The correlation coefficient (r) between the length of the viper (52.9 + 2.5 cm; $n = 20$) and the wet venom yield was 0.657 ($P < 0.05$) and that in case of dried venom was 0.578 ($P < 0.05$).

Cerastes cerastes cerastes:

The wet and dried venoms of Cerastes cerastes cerastes were 145.5 ± 16.6 and 28.9 ± 2.8 mg per adult viper. The ranges of wet and dried venom extracted from ten adult vipers were 72 - 220 and 15 - 40 mg respectively. The venom yield correlated with the viper length either in case of wet ($r = 0.949$; $P < 0.001$) or dried ($r = 0.857$; $P < 0.001$) venoms. The LD50 of Cerastes cerastes cerastes venom was 1.350 mg / kg.

Echis carinatus:

Ten adult carpet vipers were used with the mean length of 46.9 + 1.7 cm. The average of wet and dried venoms were 83.6 ± 11.6 and 14.3 ± 2.8 mg with ranges of 30 - 131 and 5 - 22 mg respectively. There was a significant ($P < 0.001$) correlation between wet or dried venoms and the viper length ($r = 0.909$ for wet and $r = 0.908$ for dried venoms). The lethality of Echis carinatus was 0.922 mg / kg.

Recovery of lethal toxicity in muscle extracts:

The recovery of Naja haje and cerastes cerastes venoms was shown in table (3). Three experiments for each venom gave no significant variation in recovery of the lethal toxicity. The average recovery of Naja haje and Cerastes cerastes venoms were 68.1 and 97.5% respectively. The periods between the injection and extraction and also between the extraction and the toxicity assay were varied so that it become so hard to compare the recoveries of the toxic effects. The recovery was not affected by different intervals between the injection and the extraction and the toxicity determination, provided that the intervals between two successive procedures did not exceed 1 hr. The toxic effect of the venom was not affected by homogenization and the homogenate of non-injected rabbit muscle did not cause any appreciable skin reaction.

Quantity of venom ejected by a single bite of Naja haje and Cerastes cerastes:

Each 20-g piece of rabbit muscle was subjected to an actual attack by the snake Naja haje and the viper Cerastes cerastes. The venom ejected was extracted from the muscle piece and the extract was assayed for lethality. The number of LD50 dose of Naja haje and Cerastes cerastes venoms was estimated in the muscle extract and was shown in tables 4 and 5. The mean volumes of muscle extracts after a single bite of Naja haje and Cerastes cerastes causing death were 0.0350 + 0.010 and 0.0744 + 0.011 ml respectively. The amounts of Naja haje and Cerastes cerastes venom ejected in pieces of 20 g muscles were 79.39 + 15.58 and 34.53 + 3.51 mg. There was a correlation between the body length of Naja haje and Cerastes cerastes and the amount of venom ejected by a single bite ($r = 0.648$; $P < 0.01$; $n = 20$ and 0.636; $P < 0.01$; $n = 20$ respectively).

Discussion:

The present work may establish a valuable procedure for determining the amount of Naja haje and Cerastes cerastes venoms ejected by a single bite of snakes. A known quantity of the venom was injected into pieces of fresh rabbit muscle with a syringe. The venom was extracted and the recovery of the toxic effect was determined. The toxic effect of snake venom was determined by either using lethal activity (Tu, 1982 and Warrell, 1995) or skin reactions of experimental animals (Morrison et al., 1982 and 1983; Furtodo et al., 1991 and Tun-Pe et al., 1995). However, the present study suggested that determination of lethal effect by using mice is more practical for most species of snakes. The amount of venom ejected by snakes has previously been studied using mice (Morrison et al., 1982; Hayes, 1992 and Gutiérrez, el al., 2007). The basic milking or venom extraction techniques were done manually by using rubber covered glass (Ditada et al., 1978) and electrical extraction by applying electricity to the maxillary region, thereby stimulating contraction of the muscles surrounding the venom gland (Glenn et al., 1972). In the present study, manual extraction was preferred as it is similar to the natural ejection of venom during biting. The amount of venom was not influenced by the interval between milking snakes and consequently regular milking did not adversely affect the average amount of venom collected. According to Kochva et al. (1982), cobra venom glands stimulated by milking apparently did not discharge all the venom stored.

The average percent of dried venoms with respect to the wet venoms collected from Naja haje, Naja nigricollis, Cerastes cerastes, Cerastes vipera, Cerastes cerastes cerastes and Echis carinatus were 31.3, 28.7, 23.6, 38.2, 19.9 and 17.1 %. This has obvious practical implications for antisera production against the venoms of these dangerous species. The recovery by this procedure was 68 % and 98 % in terms of lethal toxicity for Naja haje and Cerastes cerastes venoms respectively. These results encouraged us to recommend the use of this procedure in estimating the maximum and minimum amounts of venoms ejected by the actual bite of poisonous snakes that will help in bites treatments.

The results of venom yield and LD50 of the six adult Egyptian snake venoms used in the present study demonstrate well-defined difference between the venoms yield and their LD50. An apparent correlation between the snake body length and amount of venom ejected was observed. This may help in approximating the amount of antivenom or any drug recommended in clinical management. This finding is in agreement with the other studies using other snake venoms (Fix, 1980; Glenn and Straight, 1982,1985; Furtado et al., 1991; Tun-Pe et al., 1995; Peterson, 2006a,b and Richardson et al., 2006). Therefore, the total available venom as well as the amount of the venom ejected by a single bite from the body length of Naja haje and Cerastes cerastes could be estimated. Percentages of dried venom (17 - 38%) of the six Egyptian snakes were in the general range recorded for other venomous snakes (Russell, 1980, Branch, 1981; Wit 1982 and Mirtschin et al., 2006). Branch (1981), studying the venom yield from three species of side-biting snakes of the genus Atractaspis, reported that the percentage of dry weight of those venoms was in the range of 22–27 % of the wet weight. Tun-Pe and Khin-Aung-Cho (1986), in a study to measure the amount of venom injected by strike of Vipera palaestinae, reported that the average amount of venom injected in the first bite was 45 % of the glands' content. The venom yield expressed per length of snake, showed that cobras or vipers produce venom in proportion to their size with a linear relationship. This is in agreement with other similar studies (De Lucca et al.,1974; Kochva, 1978 and Kochva et al., 1982).

table (2): Biometrics, venom yields and toxicity of venoms extracted manually from Egyptian snakes.

Species	Number of snakes	Body length (cm) (1)	Venom yield (mg)			Lethality (LD_{50}) mg/ kg mice	Correlation coefficient (r)	
			Wet (2)	Dry (3)	%*		(1) x (2)	(1) x (3)
Naja haje	20	149.0 ± 4.6 (120 – 185)	353.7 ± 4.6 (160 – 449)	110.8 ± 8.3 (34 – 200)	31.3	0.495	0.861	0.776
Naja nigricollis	7	125.7 ± 6.6 (100 – 150)	408.1 ± 41.5 (281 – 552)	117.1 ± 15.0 (76 – 168)	28.7	1.670	0.789	0.760
Cerastes cerastes	20	52.9 ± 2.5 (30 – 60)	179.3 ± 13.4 (86 – 295)	42.3 ± 3.0 (23 – 75)	23.6	0.950	0.815	0.628
Cerastes vipera	20	32.8 ± 0.9 (28 – 40)	58.4 ± 5.6 (17 – 94)	22.3 ± 2.2 (9 – 43)	38.2	0.625	0.657	0.578
C. cerastes cerastes	10	43.8 ± 1.3 (40 -40)	145.5 ± 16.6 (72 - 220)	28.9 ± 2.8 (15 – 40)	19.9	1.350	0.949	0.857
Echis carinatus	10	46.9 ± 1.7 (40 – 53)	83.6 ± 11.6 (30 - 131)	14.3 ± 2.8 (5 – 22)	17.1	0.922	0.909	0.908

Lethality (LD_{50}) results are represented as mg venom / kg mice, venom injected through i.p. route.
All other results are represented as mean ± S.E.M.
*% of dried venom compared with the wet venom.

Table (3): Lethal toxicity recovery of *Naja haje* and *Cerastes cerastes* venoms injected into fresh rabbit muscle.

Species	Experiment No.	mg venom #	Number of LD_{50} dose injected	Number of LD_{50} dose extracted	Recovery Rate (%)	Average recovery rate (%)
*Naja haje**	1	113.14	11428	7407	64.8	68.1
	2	70.74	7145	4761	66.6	
	3	28.28	2857	2083	72.9	
*Cerastes cerastes***	1	19.53	1028	1000	97.3	97.5
	2	12.67	667	652	97.8	
	3	9.77	514	500	97.3	

 # mg venom equivalent to the number of LD_{50} injected into 20 g rabbit muscle.

*** LD_{50} of *Naja haje* = 9.9 µ / 20 g mice.**

**** LD_{50} of *Cerastes cerastes* = 19 µ / 20 g mice.**

Data are expressed as a mean of three trials.

Table (4): Quantities of venom ejected spontaneously by a single bite of *Naja haje*.

Snake Length (cm)	Quantity of venom ejected spontaneously by a single bite of *Naja haje*#		
	ml extract causing death	Number of extracted LD_{50} dose	mg venom
140	0.025	40000	58.15
150	0.025	40000	58.15
160	0.020	5000	72.69
140	0.030	3333	48.44
140	0.030	3333	48.46
130	0.030	3333	48.46
150	0.008	13000	193.82
140	0.030	3333	48.46
150	0.030	3333	48.44
130	0.030	3333	48.46
160	0.005	20000	290.75
150	0.010	10000	145.37
120	0.030	3333	48.44
100	0.095	1333	15.30
170	0.010	10000	145.37
110	0.200	5000	7.27
100	0.030	3333	29.07
160	0.010	10000	145.37
100	0.030	3333	48.44
120	0.030	3333	48.44
136.00 ± 4.83*	0.035 ± 0.0096*	9383.15 ± 2550.04*	79.87 ± 15.58*

*** Mean ± S.E.M.**

 # Correlation coefficient (r) between the snake length and the amount of venom ejected by a single bite is 0.648.

Table (5): Quantities of venom ejected spontaneously by a single bite of *Cerastes cerastes*.

Snake Length (cm)	Quantity of venom ejected spontaneously by a single bite of *Cerastes cerastes*#		
	ml extract causing death	Number of extracted LD_{50} dose	mg venom
50	0.0850	1176.50	22.92
50	0.0500	2000.00	38.97
50	0.0750	1176.50	22.92
50	0.1870	5347.60	22.92
50	0.0375	2666.67	51.97
50	0.0750	1176.50	22.92
50	0.0235	2857.14	55.68
50	0.0750	1176.50	22.92
50	0.0750	1176.50	22.92
50	0.1500	666.67	13.00
50	0.0375	2666.67	51.97
50	0.0235	2857.14	55.68
50	0.0200	5000.00	51.97
50	0.1870	5347.60	55.68
50	0.0235	2857.14	55.68
50	0.0375	2666.67	51.97
50	0.1000	1000.00	19.49
50	0.0750	1176.50	22.92
50	0.0750	1176.50	22.92
50	0.0750	1176.50	22.92
136.00 ± 4.83*	0.0744 ± 0.011*	2267.27 ± 331.37*	34.53 ± 3.51*

*** Mean ± S.E.M.**

 # Correlation coefficient (r) between the snake length and the amount of venom ejected by a single bite is 0.636.

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كمية السم المنتج وسميته لستة أنواع من الثعابين المصرية مع وصف طريقة لتقدير كمية السم المقذوف من لسعة ثعبان واحدة

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الملخص:

تتضمن هذه الدراسة تقدير إنتاج السم و طول جسم الثعبان و سميته لستة ثعابين مصرية سامة هي : الكوبرا المصرية والبخاخ والحية القرعاء والحية المقرنة والحية المقرنة بدون قرون و الحية الغريبة السمراء، تراوح نسبة الوزن الجاف لهذه السموم بين ١٧,١ - ٣٨,٢ ٪ بالنسبة لوزن السم السائل. الجرعة التي تسبب وفاة ٥٠ ٪ من الحيوانات لهذه السموم هي بالترتيب كالتالي ٠,٤٩٥ و ١,٦٧٠ و ٠,٩٥٠ و ٠,٦٢٥ و١,٣٥٠و ٠,٩٢٠ مليجرام لكل كيلو جرام وزن فأر، وأيضاً تحتوى هذه الدراسة على شرح لطريقة تقدير كمية السم التي تدخل الجسم عن طريق عضة واحدة للكبرى المصرية والحية المقرنة . و لقد اتضح من الدراسة أن هناك ارتباط مهم بين طول الثعبان وكمية السم التي تدخل في الجسم أو يتم استحلابها منه. أما نسبة وجود سم الكبرى المصرية والحية المقرنة بعد حقنها في عضلات الأرنب هي ٦٨,١٪ و ٩٧,٥ ٪ ويمكن من نسبة بقاء السم في العضلات حساب الكمية الحقيقية التي يقذفها الثعبان عند العض والتالي يمكن حساب كمية المصل اللازمة لعلاج حالات التسمم بهذه الثعابين.