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STUDIES ON ECHIS CARINATUS VENOMS AND ANTIVENOMS OF DIFFERENT COUNTRIES. *

M. Latifi and M. Tabatabai

Abstract*

Studies on the venom extracted from the *Echis carinatus* indicated that there are only slight differences in the yield and lethality of venoms of specimens in a single locality. Echis venoms of Iran, Pakistan and Eritrea were compared for their lethality (i. v. LD_{50} in mice), minimum coagulant dose (MCD-P), minimum defibrination dose (MDD), minimum haemorrhagic dose (MHD), minimum necrotizing dose (MND) and their rate of neutralization tests (i. v. mg/ml in mice). The antigenic components of the venoms were compared by gel diffusion tests using specific and polyspecific antivenoms prepared by immunization of horses. The crude antivenoms were purified and concentrated. The antivenom prepared against Echis carinatus (Eritrea) neutralized the venom of *Echis carinatus* (Iran) whereas that prepared against *Echis carinatus* (Iran) venom neutralized only partially the venom of *Echis carinatus*(Eritrea).

To obtain the best results from antivenom treatment, the commercial antivenom should be tested for potency by using the local reference venom.

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Introduction

The problem of how best to treat persons bitten by venomous snakes in tropical regions is high-lighted in the case of *Echis carinatus* accidents. It was found that an antivenom against *Echis carinatus* was effective in some areas of Nigeria but not in other areas (Warrell, D. A. et al, 1974). The same difficulty was faced in Pakistan (Latifi, M. personal observation, 1970), where the serum used in the treatment was from the same source as that employed in Nigeria and the snakes causing the bites where correctly identified.

An attempt has been made to compare the characteristics of *Echis carinatus* venoms of Iran, Eritrea and Pakistan, firstly to test the possibility of choosing a representative of Echis for the production of an antivenom having the same capacity to neutralize the Echis venoms of diverse localities and secondly, to improve the effectiveness of the polyspecific antivenoms available.

Materials and Methods

Two pooled *Echis carinatus* venoms, each from Iran and Eritrea, were selected for monospecific antivenom preparations. The collected venom from Iran was prepared at the Razi Institute, Tehran (Latifi, M. 1984): the Eritrean venom was from Ballardini, A., Keren-Eritrea; venom from Pakistan was provided by National Institute of Health of Pakistan.

Monospecific and polyspecific antivenoms against each venom were prepared by immunization of horses. The plasma was purified and concentrated by pepsin digestion and ammonium sulphate precipitation (Latifi, M. 1978). The polyspecific antivenom No. 63 was prepared at the Razi Institute against the venoms of *Naja naja oxiana, Echis carinatus* (Iran), *Pseudocerastes persicus, Vipera lebetina, Vipera latifii* and *Agkistrodon halys.* The polyspecific antivenom No. 125 was obtained from the Haffkine Institute of India and prepared against the venoms of *Naja naja, Echis carinatus, Vipera russelli* and *Bungarus caeruleus.*

The polyspecific antivenom No. 24 was obtained from the National Institute of Health of Pakistan and prepared against the venoms of *Naja naja* and *Vipera russelli* only but deemed effective against *Echis carinatus* (Pakistan) venom. Venom lethality was determined in 18-20 g mice by i.v. injection of 0.5 ml of various dilutions of venom in saline, and estimated by the Spearman-Karber method (Finney, D.J. 1964). The potencies of antivenoms were expressed as mg of venom neutralized by one ml of antivenom (Latifi, M. 1978). The antigenic components of venoms were determined and compared with each other by the immunodiffusion technique (Latfi, M. 1972). The minimum coagulant dose (MCD), minimum haemorrhagic dose (MHD), minimum necrotizing dose (MND) and minimum defibrination dose (MDD) for *Echis carinatus* venoms were determined (Theakston, R.D.G. and Reid, H.A. 1983).

Results and Discussion

Variations of the lethality of *Echis carinatus* venoms from the different localities were noted, with the toxicity of that from Iran markedly the highest and the venoms of Eritrea and Pakistan with almost the same lower values (Table 1.).

The results of gel diffusion tests indicated that there were slight antigenic differences between the venoms of Iran, Eritrea and Pakistan. A number of precipitin lines were obtained from the diffusion of selected venoms against their specific and non-specific antivenoms. With specific sera, lines were more numerous and sharper while with the non-psecific antivenoms, lines were less in number and with less sharpness, in the different ranges of the cross-reactions (Fig. 1). Some relationship was noted between the venoms of Eritrea and Iran and between Iran and Pakistan (Fig. 1). However the three venoms do differ from one another as shown by the varying potencies of the antivenoms against the venoms tested (Tables 1 & 2). It appears that the antivenom prepared against *Echis carinatus* (Eritrea) venom can partially neutralize the venom of

Echis carinatus (Iran) while the antivenom prepared against *Echis carinatus* (Iran) venom can not neutralize the venom of *Echis carinatus* (Eritrea) to the same degree. The same phenomena were observed in the case of Iranian *Echis* antivenom when tested against *Echis* venom of Pakistan and with *Echis* (Eritrea) antivenom against *Echis* (Pakistan) venom. Some relationships were also observed between the *Echis* venoms of Eritrea with Iran and of Iran with Pakistan when monospecific and polyspecific antivenoms were used in the neutralization tests (Tables 1 & 2).

With reference to the results obtained from comparative potencies, testing venoms against different antivenoms, it seems that sometimes the paraspecific activity enhances the potency value; the serum No. 24 which was not originally prepared against *Echis carinatus* (Pakistan) venom had a good capacity of neutralization.

The various test of morbidity effects of *Echis carinatus* venoms of different sources, the minimum coagulant dose (MCD-P), minimum defibrination dose (MDD) and the minimum necrotizing dose (MND) showed slight variations (Table 3).

In conclusion, from the findings that *Echis carinatus* venoms from different localities differ so markedly, the best results to be obtained from antivenom treatment will come from an antivenom made from venom of the local population of snakes or at least a commercial antivenom should be controlled for potency by testing with the local reference or approved venom.

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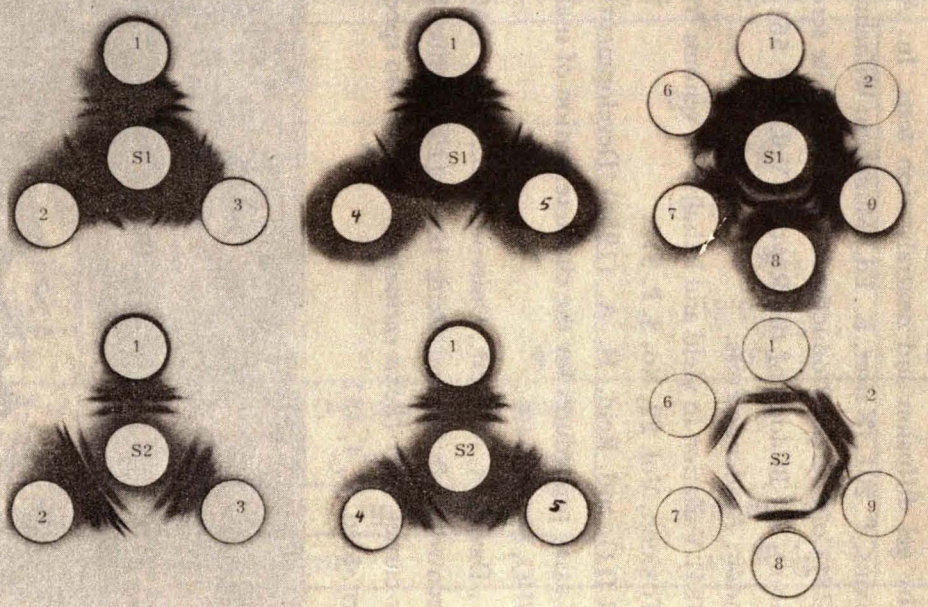
FIG. 1. Comparative precipitation bands formed in agar by Echis carinatus venoms against different antivenines.



Antivenines : S1 = Monovalent Echis No. 87, Iran. S2 = Monovalent Echis No. 9, Africa

Venoms : 1=Echis, Iran. 2=Echis, Africa. 3=Echis, W. Pakistan. 4=Echis, pooled male, Iran. 5=Echis, pooled female, Iran. 6=Echis, male, right fang, Iran. 7=Echis, male, left fang, Iran. 8=Echis, female, left fang, Iran. 9=Echis, female, right fang, Iran.

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(مارجعفری)Echis Carinatus

Table 1. Variation in Potency of *Echis Carinatus* Venoms and Antivenoms.

Sources of venom	LD_{50} (i.v.) (μg/mouse)	Antivenom neutralized (mg/ml)				
		Monospecific		Polyspecific		
		No. 8 7 (Iran)	No. 9 (Eritrea)	No. 63 (Iran)	No. 125 (India)	No.24 (Pakistan)
Echis carinatus (Iran)	4.6 (3.6–5.8)	2.8	0.8	2.6	1.4	0.6
Echis carinatus (Eritrea)	16.7 (13.7–21.6)	0.4	2.8	1.8	0.8	0.4
Echis carinatus (Pakistan)	15.6 (12.5–18.2)	1.4	0.6	2.0	0.8	2.6

The LD_{50} for each venom was calculated by the Spearman-Karber method (Finney, 1964).

The range of all values is given in parentheses.

Table 2. Comparative Potencies Testing of Snake Venoms Against Two Polyspecific Antivenoms

Venoms	LD_{50} (i.v.) (μ g/mouse)	Polyspecific antivenom neutralized (mg/ml)	
		No. 125 (India)	No. 24 (Pakistan)
Naja naja (India)	10.5	1.0	1.4
Naja naja (Pakistan)	5.1	0.6	1.2
Naja naja (Iran)	8.8	0.2	0.4
Bungarus fasciatus (India)	14.0	0.4	0.2
Echis carinatus (Pakistan)	15.6	0.8	2.6
Echis carinatus (Iran)	7.6	1.8	0.6
Echis carinatus (Eritrea)	18.1	0.8	0.4
Vipera russelli (Pakistan)	2.3	0.4	1.0
Vipera russelli (India)	2.2	1.4	1.0

Potency was determined intravenously in mice (16-18 g).

Table 3. Lethal, coagulant, Haemorrhagic, Defibrination and Necrotizing Effects of Echis Carinatus venoms of Different sources.

Sources	LD_{50} (i.v.) (μg/mouse)	MDD (μg/mouse)	MCD-P (μg/ml)	MND (μg/rat)	MHD (μg/rat)
Echis carinatus (Iran)	4.6 (3.6–5.8)	1.7	2.0	39.0	13.2
Echis carinatus (Eritrea)	16.7 (13.7–21.6)		12.0	21.6	12.0
Echis carinatus (Pakistan)	15.6 (12.5–18.2)		19.2	66.0	15.6

The LD_{50} for each venom was calculated by the Spearman-Karber method (Finney, 1964).
The range of all values is given in parentheses.