

AD 648822

AD

**EDGEWOOD ARSENAL
TECHNICAL REPORT**

EATR 4074

**DOSE-RESPONSE RELATIONSHIP OF CRUDE COBRA
VENOM (Naja naja) IN THE DOG**

by

Henry P. Ciuchta, Ph.D.
James A. Vick, CPT., MSC
James H. Manthei

March 1967



**Medical Research Laboratory
Research Laboratories
EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010**

MAR 24 1967

ARCHIVE COPY

EDGEWOOD ARSENAL TECHNICAL REPORT

EATR 4074

DOSE-RESPONSE RELATIONSHIP OF CRUDE COBRA
VENOM (Naja naja) IN THE DOG

by

Henry P. Ciuchta, Ph. D.
James A. Vick, CPT., MSC
James H. Manthei

Experimental Medicine Department

March 1967

Distribution of this document is unlimited

Task 1L013001A91A02

Medical Research Laboratory
Research Laboratories
EDGEWOOD ARSENAL
EDGEWOOD ARSENAL, MARYLAND 21010

FOREWORD

The work described in this report was authorized under Task 1L013001A91A02, In-House Laboratory Independent Research and Development (U). This work was started in May 1965 and completed in February 1966.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Reproduction of this document in whole or in part is prohibited except with permission of the Commanding Officer, Edgewood Arsenal, ATTN: SMUEA-TSTI-T, Edgewood Arsenal, Maryland 21010; however, DDC is authorized to reproduce the document for US Government purposes.

DIGEST

This study was to determine the dose-response effects of crude cobra venom (Naja naja) in the anesthetized dog. It was found that the LD50 for crude cobra venom in the anesthetized dog is about 0.105 mg/kg, and the LD99 is 0.148 mg/kg.

CONTENTS

	<u>Page</u>
I. INTRODUCTION	7
II. MATERIALS AND METHODS	7
III. RESULTS	9
IV. DISCUSSION	9
V. CONCLUSIONS	11
LITERATURE CITED	13
DISTRIBUTION LIST	15
DD FORM 1473 (DOCUMENT CONTROL DATA - R&D)	23

BLANK PAGE

DOSE-RESPONSE RELATIONSHIP OF CRUDE COBRA VENOM (Naja naja) IN THE DOG

I. INTRODUCTION.

Poisonous snakes and their venoms have been the subject of many investigations. There still seems to be a relative lack of information, however, concerning the dose-response relationship of snake venoms in different species of animals. Studies have usually dealt with smaller animals, that is, mice, guinea pigs, and rabbits^{1,2} and then were primarily concerned with the evaluation of some therapeutic approach in relation to a specific lethal dose, for example, a LD50.

The purpose of this study was to determine mortality in dogs after the intravenous administration of increasing doses of crude cobra venom (Naja naja, King Cobra) and then to prepare a dose-response curve. Crude cobra venom was used because the physiologic changes it produces in the dog have been well defined.^{3,4,5} It is one of the most available, and it has possible therapeutic applications.^{6,7}

II. MATERIALS AND METHODS.

Forty-four adult mongrel dogs, weighing 8 to 10 kg and anesthetized with 30 mg/kg pentobarbital sodium* administered intravenously, were used. The number of animals given each dose and the doses are listed in the table.

The lyophilized crude cobra venom** was dissolved in distilled water to give a final concentration of 1 mg/ml. The venom was refrigerated at 5°C until its use, when it was allowed to return to room temperature. The venom was injected directly into the femoral vein.

* The influence of this anesthetic on the dose-response relationship of toxins, in dogs, is minimal, as shown by previous studies conducted by the authors.

** Ross Allen Reptile Institute, Miami, Florida.

Table. Survival and Mortality Data for Dogs Administered Crude Cobra Venom

No.	Survival time									
	Dose (mg/kg)									
	0.06	0.09	0.10	0.11	0.12	0.25	0.50			
1	Perm	210	210	190	105	52	25			
2	Perm	Perm	Perm	Perm	90	20	5			
3	Perm	Perm	Perm	120	120	40	3			
4	Perm	Perm	900	Perm	160	45	18			
5	-	Perm	Perm	210	150	33	27			
6	-	-	Perm	Perm	Perm	20	28			
7	-	-	Perm	-	150	-	-			
8	-	-	900	-	150	-	-			
9	-	-	Perm	-	-	-	-			
Average survival time	-	-	-	173	132	35	18			
Mortality rate	0/4	1/5	3/9	3/6	7/8	6/6	6/6			
Percent mortality	0	20.0	33.3	50.0	87.5	100	100			

NOTE: Perm - indicates permanent survival

Arterial blood pressure was monitored by a catheter inserted into the femoral artery and connected to a Statham pressure transducer and the E and M physiograph recorder. Respiratory rate, heart rate, and electrocardiogram (EKG) were monitored by placing two needle-tipped electrodes in either side of the chest wall and attaching them to the physiograph with appropriate preamplifiers.

Animals alive at the end of a 72-hr observation period were considered to be permanent survivors.

III. RESULTS.

Individual survival data, average survival time, and percentage of mortality at each dose level are shown in the table. All four dogs given 0.06 mg/kg venom survived, but all six animals given 0.25 mg/kg died within 52 min. The smaller dose produced a slight fall in blood pressure, which immediately returned to a normal level. Respiration increased slightly and became more shallow but returned to the preinjection level within 1 to 2 min; transient bradycardia occurred. Blood pressure of the animals that received 0.25 mg/kg of venom decreased markedly, but returned to a normal level in about 20 min and remained stable until it fell terminally. Respiration decreased until complete apnea was reached about 20 to 45 min after injection. Tachycardia occurred immediately after the administration of the venom and was followed by bradycardia and subsequent return to normal rate. About 20 to 45 min after injection, bradycardia occurred and heart rate decreased progressively until the animal died.

The intermediate doses (0.09 to 0.12 mg/kg) produced responses that were modifications of those just described.

The figure depicts the dose-response curve for crude cobra venom with 95% confidence limits. The LD50 for the venom is about 0.105 mg/kg, and the LD99 is approximately 0.148 mg/kg.

IV. DISCUSSION.

Progressively increasing doses of crude cobra venom produced corresponding mortality in anesthetized dogs. The injection of 0.06 mg/kg of venom caused relatively little change in blood pressure, respiratory rate, EKG, or heart rate, and all animals survived. The administration of 0.12 mg/kg venom killed 87.5% of the dogs, and 0.25 mg/kg venom was lethal to all animals. Because of the narrow margin between the least toxic and the most toxic doses, the dose-response curve has a steep slope.

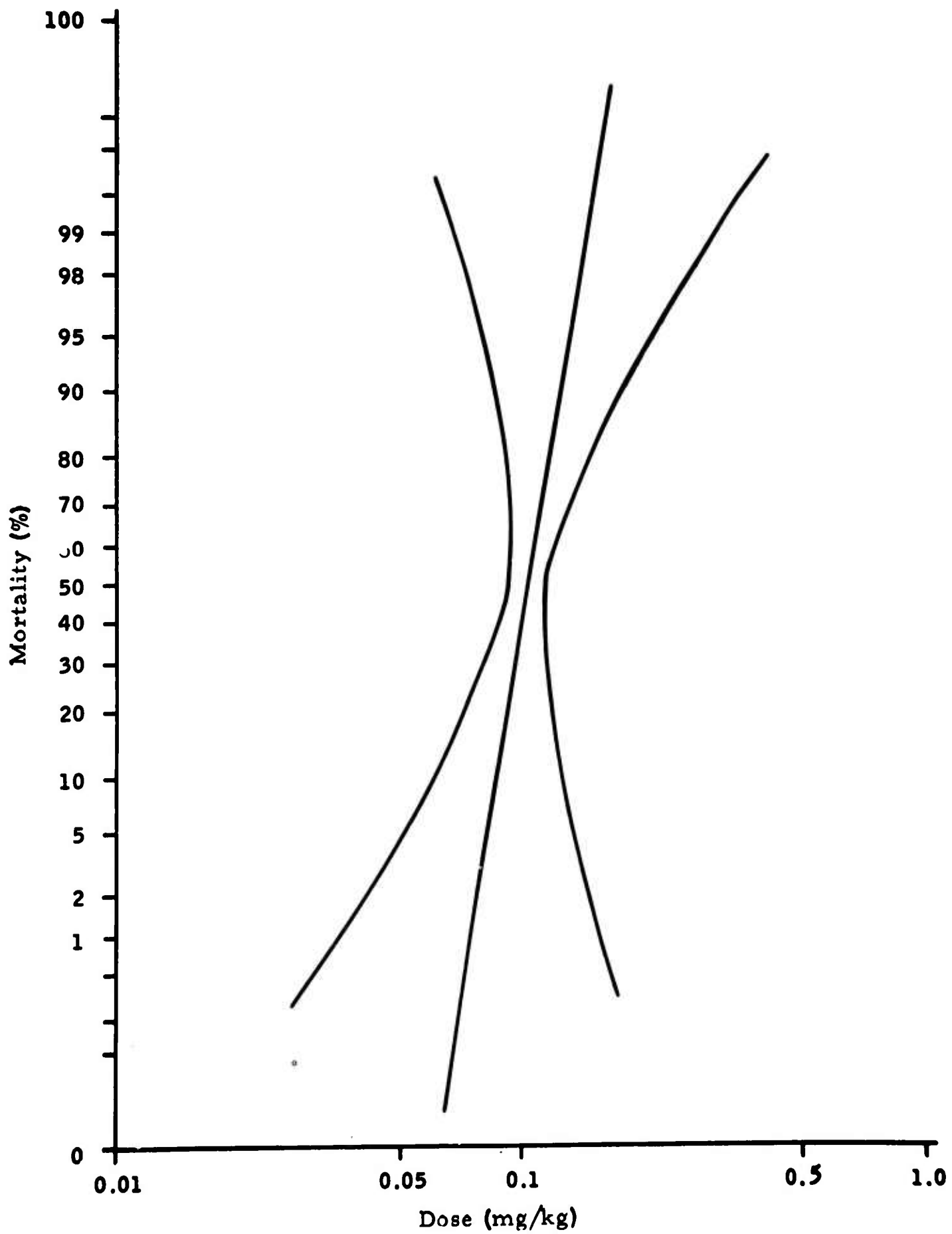


Figure. Crude Cobra Venom Dose-Response Curve With 95% Confidence Limits

V. CONCLUSIONS.

It was found that the LD50 for crude cobra venom in the anesthetized dog is about 0.105 mg/kg, and the LD99 is 0.148 mg/kg.

LITERATURE CITED

1. Minton, S. A. Some Properties of Pit Viper Venoms and Their Correlation With Phylogeny. ed. E. E. Buckley and N. Porges. Venoms. American Association for the Advancement of Sciences. Publ. No. 44, Washington, D. C., pp 145-151. 1956.
2. Kulkarmi, M. E., and Rao, S. S. Antigenic Composition of the Venoms of Poisonous Snakes of India. Ibid, pp 175-177.
3. Vick, J. A., Ciuchta, H. P., and Polley E. H. Effect of Snake Venom and Endotoxin on Cortical Electrical Activity. Nature 203, 1387-1388 (1964).
4. Vick, J. A., Ciuchta, H. P., and Polley, E. H. The Effect of Cobra Venom on the Respiratory Mechanism of the Dog. Arch. Inter. de Pharmacodyn. et de Therapie. 153, 424-429 (1965).
5. Meldrum, B. S. Actions of Whole and Fractionated Indian Cobra (*Naja naja*) Venom on Skeletal Muscle. Br. J. Pharmacology and Chemotherapy 25, 197-205 (1965).
6. Hills, R. G., and Firor W. M. The Use of More Potent Cobra Venom for Intractable Pain. The Am. Surgeon. 18, 875-879 (1952).
7. Braganca, B. M., and Badrinath, P. G. A Highly Selective Carcinolytic Agent Isolated From Cobra Venon. Nature 207, 534-535 (1965).

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R&D		
<small>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</small>		
1. ORIGINATING ACTIVITY (Corporate author) CO, Edgewood Arsenal ATTN: SMUEA-RME Edgewood Arsenal, Maryland 21010		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED
		2b. GROUP N/A
3. REPORT TITLE DOSE-RESPONSE RELATIONSHIP OF CRUDE COBRA VENOM (<u>Naja naja</u>) IN THE DOG		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) This work was started in May 1965 and was completed in February 1966.		
5. AUTHOR(S) (Last name, first name, initial) Ciuchta, Henry P. Vick, James A. Manthei, James H.		
6. REPORT DATE March 1967	7a. TOTAL NO. OF PAGES 23	7b. NO. OF REFS 7
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S) EATR 4074	
8c. PROJECT NO. Task no. 1L013001A91A02	8d. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) N/A	
10. AVAILABILITY/LIMITATION NOTICES Distribution of this document is unlimited.		
11. SUPPLEMENTARY NOTES In-house laboratory independent research-chemical	12. SPONSORING MILITARY ACTIVITY N/A	
13. ABSTRACT This study was to determine the dose-response effects of crude cobra venom (<u>Naja naja</u>) in the anesthetized dog. It was concluded that the LD50 is about 0.105 mg/kg, and the LD99 is 0.148 mg/kg.		
14. KEYWORDS Cobra venom Exotoxin shock Hypotension Respiratory failure Snake venoms Dose-response relationship Dog		