

Effects of Cobra Venom on Blood Coagulation, Platelets & Fibrinolysis

ABDUR RAHMAN A H NAGI* ABDUL HAYEE**

ABSTRACT

The effects of Cobra Venom (CV) on blood coagulation, platelets and fibrinolytic enzyme system were studied after injecting 1 MLD, 0.5 MLD & 0.1 MLD CV intramuscularly in rabbits. Thrombocytopenia was the earliest change to appear. No prolongation of Prothrombin Time (PT), Activated partial thromboplastin Time (APTT) and Thrombin Time (TT) was seen in any of these animals. Fall in fibrinogen levels and raised levels of FDPs were noted indicating activation of fibrinolysis. RBC Morphology was unchanged in all these animals. Reticulocyte counts also remained unchanged. On histological examination lungs, liver and kidneys showed intravascular clotting and cellular damage.

Key words: Cobra venom, fibrinolysis, blood coagulation

INTRODUCTION

In Pakistan, important poisonous snakes are Cobra (Naja Naja), Russell's Viper (Vipera Russelli), Saw-Scaled Viper (Echis Carinatus) and Kraits¹. The deaths due to bites by these snakes amount to 10,000 to 12,000 annually in the subcontinent of India and Pakistan². Venom of these snakes contain many active components which act upon the blood constituents, Nervous System and other systems of body. Cobra Venom is characterized predominantly by its neurotoxic effect³. This neurotoxic effect is due to toxin, a protein in the Cobra Venom^{4,5}. The haematological abnormalities produced by Cobra bite are variable. In about 50% cases of Cobra bite moderate neutrophilic effect has been reported⁵. In a series of cases studied by Warrell et al⁵ platelet counts were normal and in only two cases the counts were significantly low. No direct action of CV on Coagulation have been observed^{5,7}. CV has got mild accelerating effect on the lysis of normal euglobulin clot, indicating a fibrinolytic action^{5,7,8,9}. However one of the Cobras i.e., Naja Nigricollis has got antifibrinolytic activity^{6,19}.

Another commonest serious effect of Cobra poisoning in man is tissue necrosis. Reid¹⁰ observed that local necrosis rather than neurotoxicity was the main clinical feature of poisoning by Cobra (Naja Naja). However spontaneous haemorrhage has also been reported in a few cases⁵. Hypofibrinogenemia and raised levels of FDP has been noted by Li et al²⁰.

The purpose of present study was to observe the effects of CV on Coagulation, fibrinolytic system and morphological changes in tissues using variable doses of venom (1 MLD, 0.5 MLD & 0.1 MLD).

MATERIALS AND METHODS

Snake Venom: Dried lyophilized venom of Cobra (Naja Naja) was obtained from National Institute of Health, Islamabad. The venom was reconstituted in Phosphate Buffered Saline (pH 7) in such a way that each ml of diluted fluid contained 0.6 mg of crude CV. Minimal lethal dose (MLD) for a rabbit weighing 1.5 kg was found to be 0.6 mg. Further dilutions of venom were made with normal saline just before venom injection.

Animals: A total of 32 local domestic rabbits were used as experimental animals. The average weight of the animals at the commencement of experiment was 1.5 kg.

Injection Schedule:

Group I: Eight male animals were included in this group. Each of these animals received 1.0 ml of the CV using intramuscular route. All these animals died six hours after envenomation. Blood samples were taken one hour and five hours after the envenomation.

Group II: Eight male animals were included in this group. Each of these animals received single dose of 1:2 dilution of CV i.e., 0.5MLD. All these animals died within 12-13 hours after the envenomation. Two blood samples were taken for haematological tests i.e., after 4 hours & 12 hours of envenomation.

Group III: Eight male animals were included in this group. Each of these animals received three doses of 1:10 dilution of CV at the interval of four days. All these animals died within 12 days of first envenomation. Three blood samples were taken for haematological tests after 24 hours, 3 days and 10 days of first envenomation.

Group IV: Eight male animals were included in this group. Each of these animals received 1.0 ml of Physiological Saline using intramuscular route at the time of envenomation of each experimental animal.

Department of Pathology & Pharmacy, University of Sargodha

*Department of Pathology, L M D C & ** King Edward Medical University, Lahore

Correspondence to Dr. Abdur Rehman

Blood samples were drawn with each experimental sample to serve as control for coagulation and other tests. Kidneys, hearts, lungs and liver of all experimental and control animals were subjected to tissue processing for histological studies. Both gross morphological changes and microscopic features were noted.

Investigations & procedures: Platelets were counted on Sysmex Electronic Counter. Prothombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Thrombin Time (TT) were determined using Ortho Brain thromboplastin, Ortho Activated thrombofex reagent and Ortho fibrindex respectively. Fibrinogen levels were determined using Boerrhinger Fibrinogen kit. Methods were according to Dacie and Lewis¹¹. Fibrinogen degradation products were estimated using latex agglutination technique (Thrombowellcotest of B. Wellcome). Peripheral smears for red blood cell morphology were studied after staining with Giemsa stain. Reticulocytes were stained with Brilliant cresyl blue and counted under oil immersion lens. Methods were according to Dacie and Lewis¹¹.

RESULTS

Haematological Observations: Group-I animals receiving single dose of CV showed a mild reduction in platelet counts. The counts after 5 hours of envenomation were between 160-180 x 10³/mm³. whereas in control animals the counts were between 360-380 x 10³/mm³. There was no change in RBC morphology and reticulocyte counts also remained within normal limits. There was no significant change in PT, APTT and TT (Table-I). Fibrinogen was significantly reduced and fibrinogen level in experimental animals of this group fell to 180-200 mg/dl whereas in control animals it remained between 380-410 mg/dl. Fibrinogen degradation products were also increased upto 10ug/ml whereas in control animals FDPs were 2ug/ml (Table-I).

In animals of group-II, a mild degree of thrombocytopenia was observed and lowest counts were 180-200 x 10³/mm³, whereas in control animals counts were between 380-420 x 10³/mm³. The reticulocyte counts were within normal limits in both experimental and control animals. RBC Morphology was also normal. There was no significant change in PT, APTT and TT (Table-II). Fibrinogen in the experimental animals fell to 250-300 mg/dl whereas in control animals levels were between 380-410 mg/dl. There was a mild degree rise in FDPs level and it was upto 14ug/ml (Table-II).

In animals of Group-III, a moderate fall in the platelet counts was observed. The lowest counts were 180-200 x 10³/mm³ whereas in control animals

it remained between 380-420 x 10³/mm³. There was no change in RBC morphology and reticulocyte in any of these animals. There was also no change in PT, APTT and TT in any of these animals. The fibrinogen levels were between 280-320 mg/dl in all samples of experimental animals, whereas the control animals have level between 385-415 mg/dl. There was a progressive rise in serum and FDP levels. In animals of this group level went upto 14 ug/ml on 10th day of experiment whereas in control animals it remained between 2-5 ug/ml (Table III).

Table I: Group-I animals

	1 Hr	5 Hrs	Control
Platelet count x 10 ³ /mm ³	160-310	160-180	360-360
RBC Morphology	NN	NN	NN
Reticulocyte count%	0.5-2.0	0.8-1.0	0.5-2.0
PT Seconds	11-14	13-14	9-10
APTT Seconds	24-31	33-35	25-28
TT Seconds	10-11	12	7-8
Fibrinogen mg/dl	260-330	180-200	380-410

NN= Normochromic and Normocytic

Table II: Group II animals

	24 Hrs	3 days	10 days	Control
Platelet count x 10 ³ /mm ³	180-240	190-220	180-200	380-420
RBC Morphology	NN	NN	NN	NN
Reticulocyte Count %	1-2	0.5-1.0	1.0	0.5-2.0
PT Seconds	11-12	12-13	11-12	9-10
APTT Seconds	26-31	28-30	26	28-30
TT Seconds	8-10	10-11	10-11	8-9
Fibrinogen mg/dl	260-300	250-300	250-290	380-410
FDPs ug/ml	5	5-14	14	2-5

NN= Normochromic and Normocytic

Table III: Group II (CV 1:10 mild)

	24 Hrs	3 days	10 days	Control
Platelet count x 10 ³ /mm ³	NN	NN	NN	NN
RBC Morphology	1-2	0.5-1.0	1.0	0.5-2.0
Reticulocyte Count %	11-12	12-13	11-12	9-10
PT Seconds	26-31	28-30	26	28-30
APTT Seconds	8-10	10-11	10-11	8-9
TT Seconds	280-300	280-320	280-320	385-415
Fibrinogen mg/dl	5	5-14	14	2-5
FDPs ug/ml	NN	NN	NN	NN

NN= Normochromic and Normocytic

Morphological Changes: Morphological Changes were studied in lungs, liver, kidneys and Heart of the envenomated rabbits in all these groups. Changes were similar in all these experimental animals.

Lungs: Gross: Lungs were congested with Paticheal subpleural hemorrhages. Cut section showed odema. Histology: Microscopic examination showed RBC sludging in Arteries and Viens. These also showed Fibrin network deposition (fig-3). Early thrombus formation & odema was also seen. Some of the blood vessels showed endothelial proliferation.

Liver: Gross: Liver was congested in all the animals. Histology: Microscopic examination showed congestion with RBC sludging & sticking of RBCs to Endothelium of central veins & sinusoids. Platelet thrombi and Fibrin deposition was also seen. Hepatocytes were normal (fig-1).

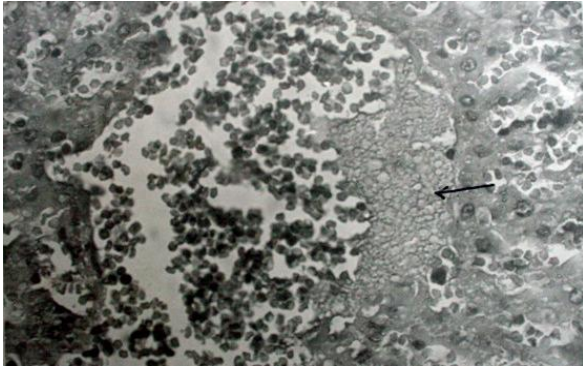


Fig. 1: Photomicrograph of a hepatic vessel showing RBC sludging in rabbits receiving ½ MLD of CV. Haematoxylin and Eosin x 450.

Kidney: Gross: Kidneys were having normal corticomedullary pattern. Histology: Glomerular capillaries were congested. Platelet thrombi were seen in major blood vessels (fig-2:4). There was slight increase in mesangial matrix. Interstitial blood vessels showed congestion and RBC sludging. Tubules showed necrosis with Hyaline casts.



Fig: 2 Photomicrograph of a renal proximal: Convoluted tubules showing epithelial cell degeneration in rabbits receiving 1 MLD of CV. Haematoxylin and Eosin x 450.

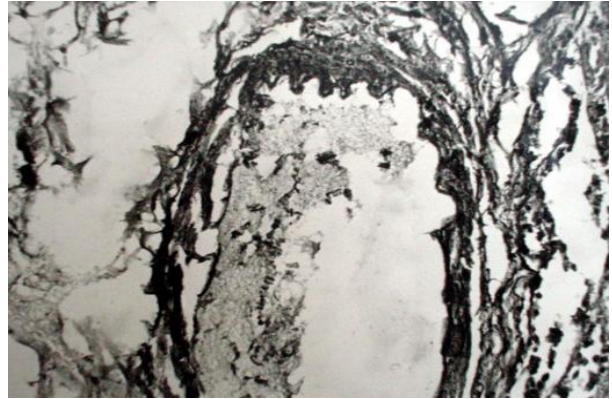


Fig: 3 Photomicrograph of a pulmonary blood vessel: Containing a fibrin and RBC thrombus in rabbits receiving 1 MLD of CV. Haematoxylin and Eosin x 450.

Heart: Gross: Chambers were filled with clotted blood. Histology: Pericardium, Myocardium and Endocardium were normal. Clotted blood was seen in chambers.

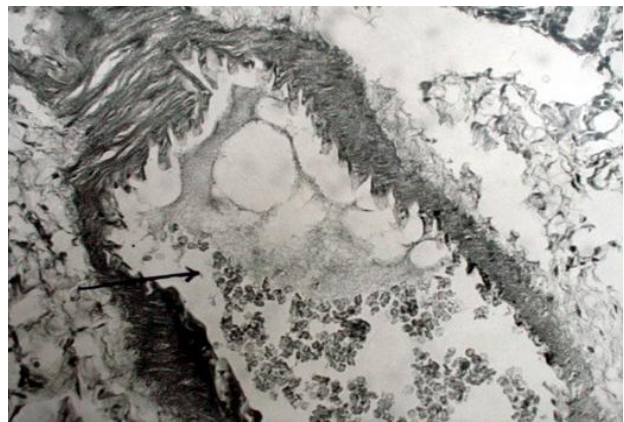


Fig: 4 Photomicrograph of a medium sized renal vessel: Containing Showing fibrin, platelets and RBCs forming thrombus in rabbits receiving ½ MLD of CV. Haematoxylin and Eosin x 450.

DISCUSSION

Effects of CV have been studied both in patients of Cobra bite⁵ and in experimental models^{7,12}. In the present study, animals of group-I & II (receiving 1 MLD & ½ MLD CV) showed moderate degree of thrombocytopenia. Low platelets counts have been reported by Warrell et al⁹ in a series of fourteen patients of Cobra bite. Urizar et al¹² observed mild degree of thrombocytopenia while studying effects of CV factor on Rats. However the thrombocytopenia produced by RVV in similar doses by Rehman et al¹³ was more marked as compared to present study, with CV. The reticulocyte counts remained within normal limits in our experiment. This parameter has not been included previously in patients of Cobra bite by Warrell et al⁵. There was no change in RBC

Morphology in animals receiving (1MLD, ½ MLD & 1/10 MLD CV). This could be due to short survival time after envenomation. A similar observation has been made Rehman et al¹³ in animals receiving 1 MD RVV. However they have reported Schistocytosis in animals receiving 0.1 MLD of RVV. These parameters were not included by Warrell et al⁵ and Urizar et al¹².

There was no significant change in PT, APTT & TT in animals of group-I, group-II & group-III. Warrell et al⁵ performed whole blood clotting time in his patients of Cobra bite to evaluate its effect on coagulation which he found was normal. However significant prolongation of these parameters have been reported by Khin and Meman et al^{17,18} in their experimental models using RVV and by Reid et al^{10,19} in patients of pit viper bite. The fall in fibrinogen level in animals of group I, II & III of this experiment was mild as compared to control group. Warrell et al⁵ have found that fibrinogen level, were normal in his patients of Cobra bite. However, in human studies fall in fibrinogen level has been reported after viper bites^{5,7}. A mild rise in FDP level was noted in animals of group I & II whereas the levels were upto 14 ug/ml in animals of group III at terminal stages. Mitrakul⁷ demonstrated that CV (Naja Naja) has got accelerating effect on the lysis of normal euglobulin clot. Some of the workers^{8,9} in their studies of crude Cobra Venoms reported them to be fibrinolytic. Warrell et al⁵ have noted levels of FDPs ranging from 5-40 ug/ml in patients of Cobra bites. These two changes have also been reported by Li et al¹⁹ in patients of Cobra bite.

Acute snake Venom poisoning is a common clinical observation (Analogous to 1 MLD, ½ MLD & 0.1 MLD group of present study). Prolonged defibrination after snake bites have been reported even after a single bite²⁴.

The result of this experiment show that Cobra Venom has an overall coagulant action in rabbits receiving variable doses of Venom^{22,23}. Mitrakul⁷ demonstrated that it has got no direct coagulant action on normal plasma. However tissue necrosis have been reported as a commonest serious effect of Cobra Venom in patients of Cobra bite⁵. Reid¹⁰ also emphasized that tissue necrosis rather than neurotoxicity is the main clinical feature of poisoning by Cobra Venom. The necrosed tissue may release thromboplastic substances that initiate intravascular clotting. Arnold⁴ has suggested the same mechanism of activation of coagulation cascade after pit viper bite that also produces massive tissue necrosis. However the coagulation action of CV is not so marked as compared to RVV in a similar study^{13,14}.

The raised FDPs in all experimental groups indicated a fibrinolytic activity of CV. It may be a

plasminogen plasmin system activation in response to intravascular clotting or a direct fibrinolytic activity as demonstrated by Mitrakul⁷. Mackay et al⁸ and Didisheim et al⁹ have also reported CV to be fibrinolytic.

Both Haematological & histological findings in this study demonstrate that Cobra Venom in doses of 1 MLD, ½ MLD & 1/10 MLD causes disseminated intravascular coagulation.

Urizar et al¹² has also described congestion of glomeruli, necrosis of tubular epithelium and fibrinoid thrombi in blood vessels of the kidneys of rats after injection of Cobra Venom factor. These findings were similar to the present study.

Chugh et al²⁰ have described congestion with fibrin thrombi in most of the blood vessels of the lungs, kidney & liver. Shastry et al²¹ have also reported focal mesangial proliferation in kidneys of patients of snake bite.

CONCLUSIONS

1. Cobra Venom showed a coagulant action in rabbits receiving variable doses of venom.
2. CV also activated fibrinolytic enzyme system.
3. Prominent changes were in platelets count and fibrinogen indicating that these are most sensitive parameters for Acute DIC.
4. Morphological changes in kidneys, liver & lungs were those of intravascular clotting with a few direct toxic effects on renal tubules and glomeruli.

REFERENCES

1. Zafar and Hafiz, Amtul: Serological identification of snake Venoms in envenomated Animals materials. Pakistan J Med. Re; 1975: 14; 1-2.
2. Minton, S.A.JR. Beware of Nonpoisonous snakes. Clinical Toxicology. 1979: 15(3)259-2653
3. Russell, Findlay E., Carison Richard W., Wainschel, Jack and Osborne, Arthur H: Snake Venom poisoning in the United States. J.A.M.M. 1975: 223; No. 4, 341-344.
4. Arnold, Robert E: Controversies and Hazards in the Treatment of Pit Viper Bites, Southern Medical Journal, 1979: 72; No. 8, 902-910.
5. Warrell D.A., Greenwood, B.M., Davidson, N. Med., Omerod L.D. Prentice R.M.: Necrosis, Haemorrhage and complement Depletion Following Bites by the Spitting Cobra (Naja Nigrocollis). Quarterly J. Ed. 1976: 177, 1-22.
6. Warrell, D.A. and Omerod L.D. Snake Venom Ophthalmia and Blindness Caused by the spitting Cobra (Naja Nigrocollis) in Nigeria Am. J. Trop. Med. Hygn. 1976: 25, No.3, 525-529.
7. Mitrakul Chulee: Effects of Five Thai Snake Venoms on Coagulation, Fibrinolysis and Platelet aggregation. Southeast Asian J. Trop. Med. Pub. Hlth., 1979: 10, No. 2, 266-275.

8. Mackay, H., Ferguson, J.C. and Mc Nicol, G.P. Effects of three Cobra Venoms on blood coagulation, platelets aggregation and Fibrinolysis. *J Clin. Path.* 1969: 22; 304.
9. Didisheim P, Lawis J.H: Fibrinolytic Coagulant activities of certain snake Venoms and Proteases. *Proc.Soc.Exp.Eiol;-Med-(NY)* 1959: 93; 10. (Cited by Mitrakul)
10. Reid, H.A: Snake bits in the Tropics. *British Medical Journal*, 3:359-362, 1968.
11. Dacie, J.V. Lewis, S.M.*Practical Haematology* 9th Ed., 2001.
12. Urizar RE, Dodds W.J, Roth, Marta, Rohloff, Jmie, Z, Micheal and Largent, J.A. Disseminated Intravascular Coagulation indaced by Liquoid in the Rat: Modification of the generalized Shwartz man reaction by Anerod and Cobra Venom factor. *Lab. Investigation.* 1979: 40; 645-654
13. Rehman A, Hayee A, Nagi A.H. Effects of Russell's Viper Venom on blood coagulation, platelets and fibrinolysis. *J.P.M.A.* 1983: 33, 81-86
14. Rehman A, Hayee A, Nagi A.H. Effects of Prolonged poisoning by Russell's Viper Venom on blood coagulation, platelets and fibrinolysis. *Japanese Journal of Med. Sc. And Biol.* 1984:37, 1-7.
15. Bick, R.L: Disseminated intravascular Coagulation and Related Syndromes: Etiology, Pathophysiology, Diagnosis and Management: *Am J Harm.* 1978: 5; 265-282.
16. Matsuda, Tamotsu: DIC (Disseminated I/V Coagulation) in the Aged. *ACTA HAEM. JAP.* 1978: 41; 1094-1101
17. Khin, M.A., K in , M.A.MA and Zin, Thaout: Effects of Russell's Viper Venom on blood coagulation, platelets and fibrinolytic Enzyme System.*Japan. J Med. Sci. Bol.,* 1977: 30;101-103
18. Memon S G, Khan F, Jagarey NA: Morphology Coagulation studies in Guinea Pigs Following Injection of Viper Russell Venom. *JPMA.* 1980: 63-68.
19. Q.B.Li, G.W. Hunag, K. Kinjoh, M. Nakamura, T. Kosugi. *Toxicon* 2001 Jul; 39: 943-8.
20. Chugh K.S, Aikat B.K, Sharma B.K, Dash S.C, Mathew M.T, Das K.C. Acute renal failure following snake bite. *Am. J. Trop. Med. Hyg.* 1975: 24; No 4, 692-697,
21. Shastry J.C.M., Date A, Carman R.H, Johny K.V. Renal failure following snake bite. *Am J Trop Med. Hyg.* 1977: 26; No 5, 1032-1038
22. Vinyl Kumar, Abbas AK. Pathological basis of disease: DIC. 2004: 655-658
23. Messmore HLJr, Wehrmacher W.H. DIC: A Primar for Primary Care Physicians: *Postgrad Med* 2002: 11(3)
24. Dong-Zong Hung, Ming Yi Liau, Shoei-Yn Lin Shiau. *Toxicon: Venom Detection in Patients of Cobra Bite:* 2003: 41: 409-15