E:/Biomedica Vol.22 Jul. – Dec. 2006/Bio-11 (A)

# EFFECTS OF PROLONGED POISONING BY COBRA VENOM ON BLOOD COAGULATION, PLATELETS AND FIBRINOLYSIS

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The effects of Cobra Venom (CV) on blood coagulation, platelets and fibrinolytic system were studied in rabbits after injecting repeated doses of 0.05 MLD of the Venom. Thrombocytopenia was the earliest change to appear. It was followed by rise in serum fibrinogen degradation products and prolongation of prothrombin time, (PT) activated partial thromboplastin time (APTT) and thrombin time(TT) indicating a progressive consumption coagulapathy and activation of fibrinolysis. Red blood cell morphology was unchanged during first three weeks; whereas fragmentation appeared after fourth week and it increased in severity with further envenomations i.e., when chronic disseminated intravascular clotting was established.

The venomous reptiles of the Indo-Pakistan subcontinent are the Russell's Viper, Echis carinatous (family: Viperidae), Cobra and Kraits (family: Elapidae)<sup>1</sup>. Cobra Venom (CV) is rich in neurotoxins and it causes death due to respiratory paralysis. On the other hand, Russell's Viper Venom (RVV) is rich in haemorrhagins & cytotoxins, therefore, it produces coagulation defects predominantly.<sup>2</sup>

Reports on coagulation changes in victims of Cobra bite and experimental venom work are conflicting<sup>3</sup>. This is because in most of the experimental work high doses (2, 1 and 0.5 MLD) of venom were used and animals died within 24 hours<sup>4,5</sup>; whereas most of the patients receive smaller quantities of venom during bite. Martins et al<sup>6</sup> have also postulated that toxin persists at the site of bite and minute concentrations are released off and on resulting in prolonged defibrination. They have also noted thrombin like action and myotoxic activity of the venom. This type of chronic snake venom poisoning may also be observed amongst snake charmers and workers in research laboratories who do milking of snakes.

The purpose of the present study was to demonstrate the effects of repeated small doses (0.05 MLD) of CV on coagulation & fibrinolytic system.

## MATERIALS AND METHODS

*Snake Venom*: Lyophilized Cobra Venom was obtained from National Institute of Health, Islamabad. The venom was reconstituted in phosphate buffered saline (pH 7) in such a way that one ml of diluted fluid contained 0.6 mg of crude CV. A minimal lethal dose (MLD) for a rabbit weighing 1.5 kg was found to be 0.6 mg. The dilutions of venom were made with normal saline just before injection.

*Animals:* A total of 16 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:**

*Experimental Group:* Eight male animals were included in this group. Each of them received 10-18 doses of 1:20 dilution of CV at intervals of 3 days. Seven animals died after receiving 10-18 doses of the venom; whereas the remaining animals were sacrified at the end of the experiment, when it had received 18 doses of CV. Blood samples were taken for haematological determinations after 24 hr, 3 days and then at weekly intervals until the experiment was terminated at the end of the eighth week.

*Control Group:* Eight male animals were included in this group. Each animal received 1.0 ml of physiological saline through the intramuscular route at the time of envenomation of each experimental animal. Blood samples were drawn at the time they were obtained from the animals in the experimental group.

*Investigations:* Platelets were counted on Counter Electronic Counter. The peripheral smears were studied for red blood cell morphology after staining with Giemsa stain. Reticulocytes were stained with brilliant cresyl blue and counted by use of an oil immersion lens according to Dacie and Lewis<sup>7</sup>. Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen levels were determined according to Dacie and Lewis<sup>7</sup> to evaluate the coagulative activity of the venom.

Fibrinogen degradation products (FDPs) were estimated by the latex agglutination technique (Thrombo-Wellcotest of B. Wellcome), which employs antibody to human FDPs. As the sensitivity of the reagent was so adjusted that in the presence of FDPs at a concentration of 2  $\mu$ g per ml or higher the latex particles clumped together giving macroscopic agglutination. Therefore, double dilutions of serum were made with glycinesaline buffer and each dilution was tested. The agglutination pattern at the end of the test period indicated the presence of at least 2  $\mu$ g/ml FDPs in the respective dilution. We used the reagent for measuring FDPs in rabbit sera without introducing any conversion factor.

# RESULTS

During the first week, the platelet counts in the experimental animals mostly remained within the normal limits. The counts of the experimental groups were 280 to 370 x  $10^3/\mu$ ; whereas in control animals were 290 to 400 x  $10^3/\mu$ l. Then there was a gradual fall in the platelet count and it ranged between 140 to 200 x  $10^3/\mu$ l at the end of the 2<sup>nd</sup> week and 160 to 240 x  $10^{3}/\mu$ l at the end of the 3rd week. The maximum fall was observed at the end of the 4<sup>th</sup> week when the platelet counts were 100 to 110 x  $10^{3}/\mu$ l. Later there was a gradual rise in the platelet counts, which were 130 to 140 x  $10^{3}/\mu$ l at the end of the 5<sup>th</sup> week, 130 to 160 x  $10^{3}/\mu$ l at the end of the 6<sup>th</sup> week, 140 to 180 x  $10^{3}/\mu$ l at the end of the 7<sup>th</sup> week and 120 to 140 x  $10^{3}/\mu$ l at the end of the 8<sup>th</sup> week (Table-I). The



**Fig. 1:** Photomicrograph of red blood cells of rabbits receiving 0.05 MLD of Cobra venom. Giemsa stain × 450.

counts in control animals remained between 290 to  $400 \times 10^3/\mu$ l.

No abnormality in the red blood cell morphology was seen during the first three weeks of the experiment. At the end of the fourth week, a mild degree of crenation of red blood cells appeared. The morphological changes increased progressively and at the end of the 8<sup>th</sup> week, the blood smears showed a marked degree of crenation of red cells, fragmentation and anisocytosis (fig. 1). Red blood cell morphology remained normal in the control animals. No significant difference in the reticulocyte count was observed in the experimental and control animals (Table 1).

Prothrombin time was within the normal limits during the first week of experiment when it ranged from 9 to 10 sec in experimental animals (Table 1). It was significantly prolonged in the subsequent samples and maximum prolongation was seen at the end of the 8<sup>th</sup> week when it was found to be 18 sec (Table 1). In the control animals, it remained 9 to 10 sec.

Activated partial thromboplastin time remained within the normal limits during the first three weeks of the experiment when it was from 26 to 32 sec in the experimental group and 25 to 32 sec in the control animals. It was slightly prolonged in the samples taken at the end of the 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> weeks when it was found to be from 35 to 36, 30 to 35, 35 to 36 and 30 to 36sec respectively. Maximum prolongation was seen in the samples taken at the end of the 8th week when it was 40 sec (Table 1). Similarly TT was almost normal during the first three weeks of the experiment when it ranged from 8 to 10 sec in the experimental groups and 7 to 8 sec in the control animals. This was followed by a gradual prolongation of TT in the subsequent samples. Maximum prolongation was seen at the end of the 8<sup>th</sup> week when it was 15 sec (Table 1).

The fibrinogen level was from 360 to 410 mg/dl during the first three weeks in experimental animals. This was followed by a gradual fall in the fibrinogen contents and it ranged between 210 to 300 mg/dl in the subsequent samples (Table 1). It remained between 380 to 410 mg/dl in control animals throughout the experiment.

Serum FDPs were normal only in the first two samples. Then there was a gradual rise in the level of serum FDPs, and at the end of the  $3^{rd}$  week it ranged between 12 and 14 µg/ml (Table 1); whereas in control animals it was 2 to 5 µg/ml. In the subsequent samples serum FDPs were 30-40 µg/ml.

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Effects of Cobra venom have been studied by many workers mainly in patients of Cobra bite<sup>10-12</sup> and rarely experimental models have been produced.<sup>17</sup>

In the present study, there was a progressive thrombocytopaenia in experimental animals and lowest counts were observed at the end of the 4<sup>th</sup> week. In the experimental models produced by Urizar etal<sup>17</sup>, thrombocytopaenia has been reported. Other workers<sup>16</sup> have also noted lowering of platelet counts in patients of Cobra bite.

The results of this experiment show that a picture of DIC develops after repeated injections of smaller doses of Cobra venom. This type of chronic snake venom poisoning may be observed in snake chambers and workers in research laboratories while milking the snakes. Some workers<sup>3</sup> have postulated that depot of toxin may persist at the site of bite and minute concentrations are released off and on. These events can cause total defibrination.

Most of the workers<sup>15-17</sup> did not study red blood cell morphology whereas in the present experiment schistocytosis / fragmentation of red blood cells was noted in rabbits receiving multiple doses of CV. However this change has been observed in other patients of Dic.<sup>8,9,14</sup>

Prolongation of PT, APTT and TT in these animals particularly in terminal stages and raised FDPs at later stages of experiment suggest that CV can lead to consumption coagulopathy and can activate fibrinolysis. Warrell et al<sup>16</sup> have reported that Cobra venom has no direct coagulant action. The coagulant action seems to be indirect i.e., release of thromboplastin like substance from necrotic tissue.<sup>13</sup> Many workers<sup>6,11,13</sup> have noted tissue necrosis due to myotoxic activity of the CV. It has also been observed that tissue destruction is directly related to the amount of Cobra venom<sup>10</sup>. As smaller doses were given in these animals hence slow and progressive tissue destruction lead to mild changes in coagulation profile.

More marked changes in coagulation profile were noted in a similar study by Rehman et al<sup>4</sup> where higher doses of venom were used leading to severe necrosis and early changes in coagulation. The FDP levels were significantly raised in terminal stages of present experiment. The raised FDP levels may be due to fibrinolysis after intravascular clotting as is commonly seen in DIC.<sup>8,9</sup> Some workers<sup>15</sup> have also reported a direct fibrinolytic action of the Cobra venom that may be another cause of increased FDP levels.

In **conclusion** the results of this experiment show that a picture of progressive consumption coagulopathy is produced after repeated injections

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	24 Hours	3 Days	1 <sup>st</sup> Week	2 <sup>nd</sup> Week	3 <sup>rd</sup> Week	4 <sup>th</sup> Week	5 <sup>th</sup> Week	6 <sup>th</sup> Week	7 <sup>th</sup> Week	8 <sup>th</sup> Week	Control
$\begin{array}{l} Platelet \ Count \\ \times 10^3 / mm^3 \end{array}$	320-370	290-350	185-280	140-200	160-210	100-110	130-140	130-160	140-180	120-140	190-400
RBC Morphology	NN	NN	NN	NN	NN	Frag. $\pm$	Frag. +	Frag. ++	Frag. ++	Frag. ++	NN
Reticulocyte Count %	1-2	0.5-1	0.5-1	1-2	1-2	2-3	1-2	1-3	1-2	1-3	2-3
PT Seconds	9-10	9-10	13-14	12-13	12-14	15-16	15-16	14-15	13-14	18	9-10
APTT Seconds	26-29	27-30	30-32	30-32	32-33	35-36	30-35	35-36	30-36	40	25-32
TT Seconds	8-9	7-8	9-10	9-10	9-10	13-14	13-14	12-14	12-14	15	7-8
Fibrinogen mg/dl			360-410				210-300				380-410
FDPs µg/ml	2-5	2-5	12-14	10-14	10-12	14-18	30	30	40	40	2-5

 Table 1:
 Changes in Coagulation Parameters, RBC Morphology and Reticulocyte Counts in Rabbits Receiving Repeated Doses of 0.05 MLD CV.

NN= Normochromic and Normocytic

Frag. = Fragmentation