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ISSN 6299 -0975

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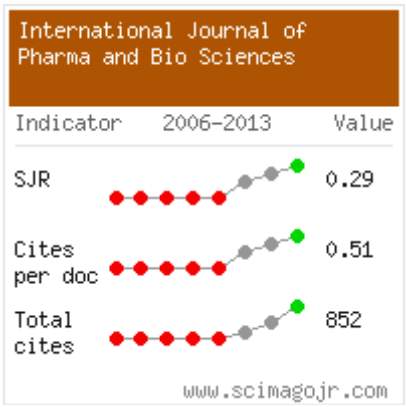

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Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ORAL TOXICITY STUDY OF THE VENOM OF *NAJA NAJA* IN *ALBINO RATS*

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ABSTRACT

The venom of *Naja naja* has been studied clinically as an analgesic. It has been recently determined that the venom of cobra is biologically active even when it is administered by mouth. As there are no published studies on the effects of orally administered cobra venom, a 72h toxicity study was conducted in the *Albino rats*. We have selected 1/50th LD_{50} dose (7µg/ kg body weight) in the present investigation and this dose was administered intragastrically. Group I (24h) received single dose, Group II (48h) received 2 doses with an interval of 24h and Group III (72h) received 3 doses daily with an interval of 24h. At the end of the experiment (*i.e.* 24h after administration of venom) the animals were sacrificed and the blood was examined. The alterations in the haeomogram after envenomation are of vital importance. The present study is aimed to investigate whether oral administration of venom damages gut lining and escapes into the blood stream and cause damage to the animals as seen in regular bites. The examination of various parameters demonstrates that cobra venom when administered orally at low doses is toxic to the animals. In the present study oral administration of snake venom severely altered all the parameters indicating that oral administration is also as toxic as compared to the injected venom. Snake venom is dangerous and should be treated with extreme caution and not ingested in any form.

KEY WORDS: **Venom, *Naja naja*, Intragastric administration, haemogram, serum electrolytes.**

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INTRODUCTION

In many countries snake envenomation is a major public health problem and recent studies indicate that there are more than 4 million envenomation cases throughout the globe annually, at least 20,000 cases if not more are fatal. South Asia is the world's most heavily affected region, due to its high population density, widespread agricultural activities, numerous venomous snake species and lack of functional snake bite control programs. In India alone, more than 2 million cases of snakebite are reported annually and it is estimated that 50,000 of these are fatal[1]. Four clinically important types of snake are found in India: cobras (*Naja naja and Naja kaouthia*), the common krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelii*), and saw scaled viper (*Echis carinatus*) [2]. Snake bite was included in the WHO's list of neglected tropical diseases, confirming the experience in many parts of this region that snakebite is a common occupational hazard of farmers, plantation workers and others, resulting in tens of thousands of deaths each year and many cases of chronic physical handicap. Much is now known about the species of venomous snakes responsible for these bites, the nature of their venoms and the clinical effects of envenoming in human patients. Despite increasing knowledge of 'snake venom' composition and mode of action, good understanding of clinical features of envenoming and sufficient production of antivenom by Indian manufacturers, snake bite management remains unsatisfactory in this region. Cobras (*Naja naja*) belong to the group of Elapidae and the venom of an elapidae contains postsynaptic neurotoxins that spread rapidly in its victim's bloodstream, causing respiratory failure and, eventually, death. Snake venom is complex mixtures containing predominantly proteins and polypeptides and small amount of organic compounds and minerals. Many of the proteins exhibit enzymatic activities, whereas the polypeptides include neurotoxins, cardiotoxins, myotoxins and cytotoxins. There are numerous publications on the clinical use of cobra venom by injection as an analgesic in addition to numerous laboratory studies. The venom of *Naja naja* is orally administered in countries like China for the treatment of pain, arthritis and cancer and orally administered cobra neurotoxins have been found to be effective in controlled trials . The present study was undertaken to establish the pathology of cobra venom administered intragastrically for a period of 72h.

MATERIALS AND METHODS

Lyophilized powder of *Naja naja* venom was obtained from Irula Snake Catchers, Industrial co-operative society, Vadanamelli, Tamilnadu. Healthy adult *Albino rats* of the same age (100 ± 10 days) and weight (150 ± 10 g) were procured from Indian Institute of Sciences, Bangalore. Rat feed was supplied by Sai Durga feeds and foods, Bangalore. All the animals were divided into four groups having six animals each. Animals of Group I received saline by intragastric administration (Control). Group II animals received a concentration of $1/50^{th}$ of LD_{50} intragastrically. Group III animals received 2 doses intragastrically with an interval of 24h and Group IV received 3 doses with an interval of 24h. The blood was collected 1 day after the administration of venom for further use of all the groups and stored in deep freezer at -80ºC for biochemical analysis.

Haemogram

RBC count was made with a Neubauer crystalline counting chamber as described by Samuel[3]. The hemoglobin concentration was estimated by the Acid - hematin method by Samuel[3]. PCV was estimated by Micro hematocrit method[3]. Mean Corpuscular volume and Mean Corpuscular Haemoglobin Concentration were estimated by Samuel[3]. Platelet count, WBC count and differential count were also estimated by Samuel[3].

Serum constituents

Serum creatinine levels were estimated by the method of Jaffe's reaction[4]. Serum Triglycerides were estimated by Samuel [3].

Liver function tests

Alkaline phosphatase activity was estimated by micro haematocrit method[6].Gamma glutamyl transferase levels were estimated by Samuel [3].

Serum Electrolytes

Serum sodium levels were estimated by the Trinder[7]. Serum potassium and calcium levels were estimated by the Kramer and Tisdall[8]. Serum phosphorous levels were estimated by the Gomorri and Bab[9]. Serum amylase activity levels were estimated by the Samuel[3]. Lactate dehydrogenase activity levels were estimated by Wootton[6].

Statistical Treatment

The data were subjected to statistical treatment. One way analysis of variance (ANOVA), and S-N-K tests were performed using SPSS (ver. 21) in the personal computer and $p < 0.01$ was considered as statistically significant.

RESULTS

The haematological alterations under envenomation of *Naja Naja* venom is presented in the Table 1. Oral administration of *Naja naja* venom produced statistically significant decrease in RBC, HB, PCV, MCV, MCH, MCHC and Platelet count (PC). On the other hand WBC showed increased level in 24 h, 48 h and 72 h. The red cell indicators like mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) are dependent on the RBC count, HB concentration and PCV values. MCV, MCH and MCHC and PC showed significant decrease after 24 h and 48 h and 72 h of intoxication in *Albino rats*. Serum creatinine levels increased in envenomed rats, whereas serum cholesterol and triglycerides showed a decrement when compared to the controls (Table 2). The liver function test was also carried out in control and experimental animals. Alkaline phosphatase and Gamma glutamyl transferase activities of envenomed rats were significantly decreased in all the time intervals studied in the present investigation (Table 2). Serum electrolytes in the blood were investigated after envenomation of snake *Naja naja* venom. The result indicates that an increase in the sodium, potassium, calcium and serum amylase in all the time intervals, however serum phosphorous and lactate dehydrogenase activity was decreased after snake envenomation (Table 3).

Table 1
Haemogram of orally administered Naja naja venom in Albino rats

Parameters	Control	24 h	48 h	72 h	F ratio
RBC (Cu. Mm)	9.186 0.762	5.428[a] 0.232 (-40.91)	5.578[a] 0.309 (-39.28)	4.679 0.371 (-49.06)	90.445*
Hb (g./dl)	16.866 1.138	13.709 1.396 (-18.71)	11.899 0.652 (-29.45)	9.474 0.855 (-43.83)	36.050*
PCV (%)	45.304 3.905	38.388[b] 1.967 (-15.27)	38.659[b] 3.371 (-14.68)	32.343 3.906 (-28.61)	15.151*
MCV (fl)	71.403 5.862	68.903 5.752 (-3.51)	66.518 4.654 (-6.84)	61.709 8.431 (13.57)	1.240[ns]
MCH (pg)	23.140 1.543	21.809 0.527 (-5.75)	18.764 1.014 (-18.91)	17.735 1.553 (-23.36)	18.337*
MCHC (%)	37.909 1.979	31.393[c] 0.732 (-16.75)	30.677[c] 1.086 (-18.65)	27.229 1.670 (-27.29)	41.218*
Platelet count (Lakhs/ Cu. Mm)	3.170 0.103	2.938 0.059 (-7.32)	2.222 0.083 (-29.90)	1.845 0.040 (-41.80)	285.560*
WBC	11117.149 95.826	12409.966 88.133 (11.63)	13455.792 71.972 (21.04)	14496.794 202.723 (30.40)	774.774*
Differential count					
Neutrophils	19.549 0.772	17.915 0.941 (-8.36)	16.149 1.200 (-17.39)	14.813 1.197 (-24.23)	22.598*
Lymphocytes	474.791 19.397	739.525 34.319 (55.76)	1213.848 80.036 (155.66)	1367.120 94.574 (187.94)	239.78*
Monocytes	1.952 0.089	2.991 0.086 (53.28)	5.308 0.827 (171.93)	8.962 0.292 (359.12)	294.727*
Eosinophils	3.792 0.323	5.984 0.366 (57.81)	8.219 0.723 (116.75)	12.011 0.297 (216.75)	336.85*
Basophils	0.933 0.020	1.220 0.117 (30.76)	1.512 0.125 (62.06)	1.985 0.104 (112.75)	111.461*

All the values are Mean ± SD of 6 individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. Significance level * P < 0.01

Table 2
Alterations in the biochemical parameters of orally administered Naja naja venom in Albino rats

Parameters	Control	24 h	48 h	72 h	F ratio
Serum Creatinine (mg/dl)	0.891 0.015	0.985 0.016 (10.55)	1.228 0.106 (37.82)	1.632 0.108 (83.16)	112.094*
Serum Cholesterol (mg/dl)	61.688[a] 2.311	59.350[a] 4.595 (-3.79)	53.201 6.801 (-13.76)	47.327 4.479 (-23.28)	10.322*
Serum Triglycerides (g./dl)	64.713[b] 3.758	63.157[b] 4.071 (-2.40)	56.802 6.888 (-12.22)	48.627 5.705 (-24.86)	11.265*
Serum Alkaline Phosphatase (IU/l.)	906.173 52.953	1045.510 98.685 (15.38)	1151.051 72.527 (27.02)	1510.850 37.811 (66.73)	60.417*
Gamma glutamyl transferase (IU/l.)	3.952 0.500	6.038 0.227 (52.78)	7.805 0.572 (97.50)	9.881 0.545 (150.02)	163.599*
Serum LDH (IU/l.)	1772.573[c] 127.401	1721.847[c] 90.380 (-2.86)	1377.174 78.273 (-22.31)	1040.791 94.367 (-41.28)	67.708*
Serum Amylase (IU/l.)	178.858 13.934	306.817 14.454 (71.54)	512.053 43.005 (186.29)	639.832 40.413 (257.73)	259.314*

All the values are Mean ± SD of 6 individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. Significance level *P < 0.01

Table 3
Alterations in the serum electrolytes of orally administered Naja naja venom in Albino rats

Parameters	Control	24 h	48 h	72 h	F ratio
Sodium	145.785 12.849	146.346 5.813 (0.38)	147.387 10.658 (1.01)	145.402 17.733 (-0.26)	0.028[ns]
Potassium	4.872 0.279	5.888 0.226 (20.85)	6.814 0.421 (39.86)	7.475 0.491 (53.43)	52.264*
Calcium	5.945 0.607	8.461 0.242 (42.32)	8.931[a] 0.533 (50.23)	8.941[a] 0.318 (50.40)	59.988*
Phosphorous	19.391 0.726	22.482 0.925 (15.94)	14.362 0.964 (-25.94)	10.054 0.873 (-48.15)	222.456*

All the values are Mean ± SD of 6 individual observations. Values in the parenthesis indicate % change over control. Mean values with the same superscript do not significantly differ among themselves through S-N-K test. Significance level *P < 0.01

DISCUSSION

The present study was taken up to evaluate the biochemical changes in the *Albino rats* administered with *Naja naja* venom intragastrically. Generally, whatever may be the type of venom, it has to enter the bloodstream to have an effect. If a person swallows venom, provided he has no lesions in his gastrointestinal tract, the proteins will be broken down into harmless amino acids and absorbed, like the products of all protein digestion. On the other hand, high concentrations of venom given to animals orally can have lethal consequences because dangerous doses can get into the circulation through minor injuries to the mucous membrane of the digestive tract. When venom is given orally, death is the result of the venom itself and not a by-product of its digestion, administering antiserum saves the animal. The venom contains enzymes that damage tissues and let the poison in, and though toxic proteins certainly are deadlier when injected, some do pass through the gut wall. This factor is more important in infants, but even adults can die of swallowing small doses of very poisonous

proteins, such as ricin. Gut enzymes break down some of the most dangerous venom too slowly to give much protection, and many types of venom resist or inhibit some of our digestive proteases. Bites by the Indian cobra (*Naja naja*) are common in India because of its close association with humans. Although no significant coagulopathy has been reported, *Naja naja* venom can form blood clots in vitro by activating prothrombin as demonstrated by thrombin-specific chromogenic substrate. Scanning electron microscopy demonstrates that the clots formed by venom lack the thin fibrin strands of normal blood clots formed by thromboplastin. The present study shows that oral administration of the venom alters RBC profile and has serious effects on blood clotting. Junaid Mohamood Alam [10] worked on different snake venoms namely *Physalia*, *H.spiralis*, *H. cyanocinctus* and *H. lapermoides.* Their studies reveal an increase in the serum LDH, gamma glutamyl transferase, alkaline phosphatase and amylase. In the present investigation, oral administration of the venom of *Naja naja* elevated levels of the serum enzymes such as LDH, γ- glutamyl transferase, alkaline phosphatase and amylase. Oral administration of the venom might have damaged the gut lining and considerable amount of toxin escaped into the circulation. This is probably responsible for the increase in serum constituents studied in the present investigation. This clearly indicates necrosis or cellular damage in liver, kidney, brain and heart induced by the snake *Naja naja* venom. The elevated levels of serum enzymes of snake *Naja naja* venom confirms the severe morphological alterations in liver, kidney, brain, heart. Shiomi[11] reported raised blood creatinine and blood urea levels in victims of sea snake bites with severe renal damage. Oral administration of the venom showed a decrease in the levels of triglycerides, cholesterol, and elevated levels of creatinine which indicates the possibility of hepatitis, glomerulonephritis and renal failure. Junaid *et al.*[10] recorded similar results in envenomation by venomous marine animals. Blood creatinine level in majority of the viper bite cases was found to be increased after 6h whereas in cobra bite cases this increase in creatinine was comparatively less which ultimately results in renal failure. Hence dialysis is often preferred. Pradeep kumar[12] studied the blood samples of viper and cobra bitten patients and analysed the blood sample from each case taken as soon as bite happened. They collected the blood samples at different intervals and studied clotting time, blood urea, serum creatinine, serum sodium, serum potassium, serum calcium. They reported that all the parameters they studied in both cobra and viper envonmated patients showed a significant increase except calcium. Ibrahim[13] studied the physiological effects of snake *Echis coloratus* crude venom in rat at different time intervals. They reported a decrease in serum cholesterol and an increase in serum triglycerides and alkaline phosphatase. The present results suggest that the snake venom escaped into the circulation might have mobilized lipids from adipose tissues and other tissues and the lipolytic enzymes which are present in the venom could have split tissue lipids with the liberation of free fatty acids.The disturbance in protein synthesis is due to cellular damage in hepatocytes together with haemorrhages in vital organs[13]. Moreover haemorrhages in vital organs together with increased vascular permeability, renal damage would further aggravate the accompanying hypoprotenimia and hypoalbuminae [13]. The severe hepatocellular injuries, necrosis of hepatocytes and kidney have been suggested to be the result of the significant rise in alkaline phosphatase levels after the envenomation of the snake *Naja naja* venom. The increased levels of serum alkaline phosphatase in *Naja naja* envenomation might be attributed to the destruction of the liver cells[14]. The alteration in the activity of LDH indicates the damage to the liver, kidney, brain and heart. Oral administration of the cobra venom also produces similar effects as those that are seen during biting. The present results indicate that oral administration of venom might damage the gut lining which results in the escape of dangerous quantities of venom into circulation. Al-Jammaz[15] reported a decrease in LDH level in the liver and kidney of envenomed rats which results in the elevated LDH enzyme level in the brain and heart might suggest the prevalence of anaerobic conditions in such vital organs as a result of enhancing the metabolic cycle to restore energy loss. Several studies were undertaken to determine the effect of the venom of some members of the viper family on

metabolism and important blood parameters of animals[16]. Though the venom of most viper snakes induces hyperglycemia in experimental animals[16], other venoms were reported to induce hypoglycemia[13]. Variations in serum physiological parameters can be used as biomarkers for monitoring the functions of vital organs of envenomed victims. The reduction in total serum cholesterol, triglycerides, LDH and the rise in total serum creatinine in envenomed rats are in accordance with observations of other investigators in this field[16]. The observed effects upon those parameters might suggest that the snake venom could have escaped into circulation and disturbed protein synthesis in hepatocytes in vital organs leading to protein loss. Similar observations were reported following various viper envenomation of rats[14]. In the present study oral administration of *Naja naja* venom resulted in the decrease in RBC, Hb, PCV, MCV, MCH, MCHC, platelet count, serum creatinine and increase in WBC. In differential count also, a gradual decrease in neutrophils and an increase in lymphocytes and monocytes, eosinophils and basophils was observed. The serum cholesterol and triglycerides showed a significant increase while serum alkaline phosphatase, gamma glutamyl transferase, and electrolytes showed an increment. The change in haemogram indicates that the snake *Naja naja* venom alters the biochemical pathways and causes cellular damage. In the present study oral administration of snake venom severely altered all the parameters indicating that oral administration is also as toxic as compared to the injected venom. Snake venom is dangerous and should be treated with extreme caution and not ingested in any form.

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