PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES ON NAJA NIGRICOLLIS VENOM

THESIS

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By

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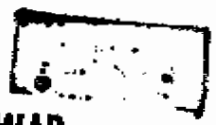

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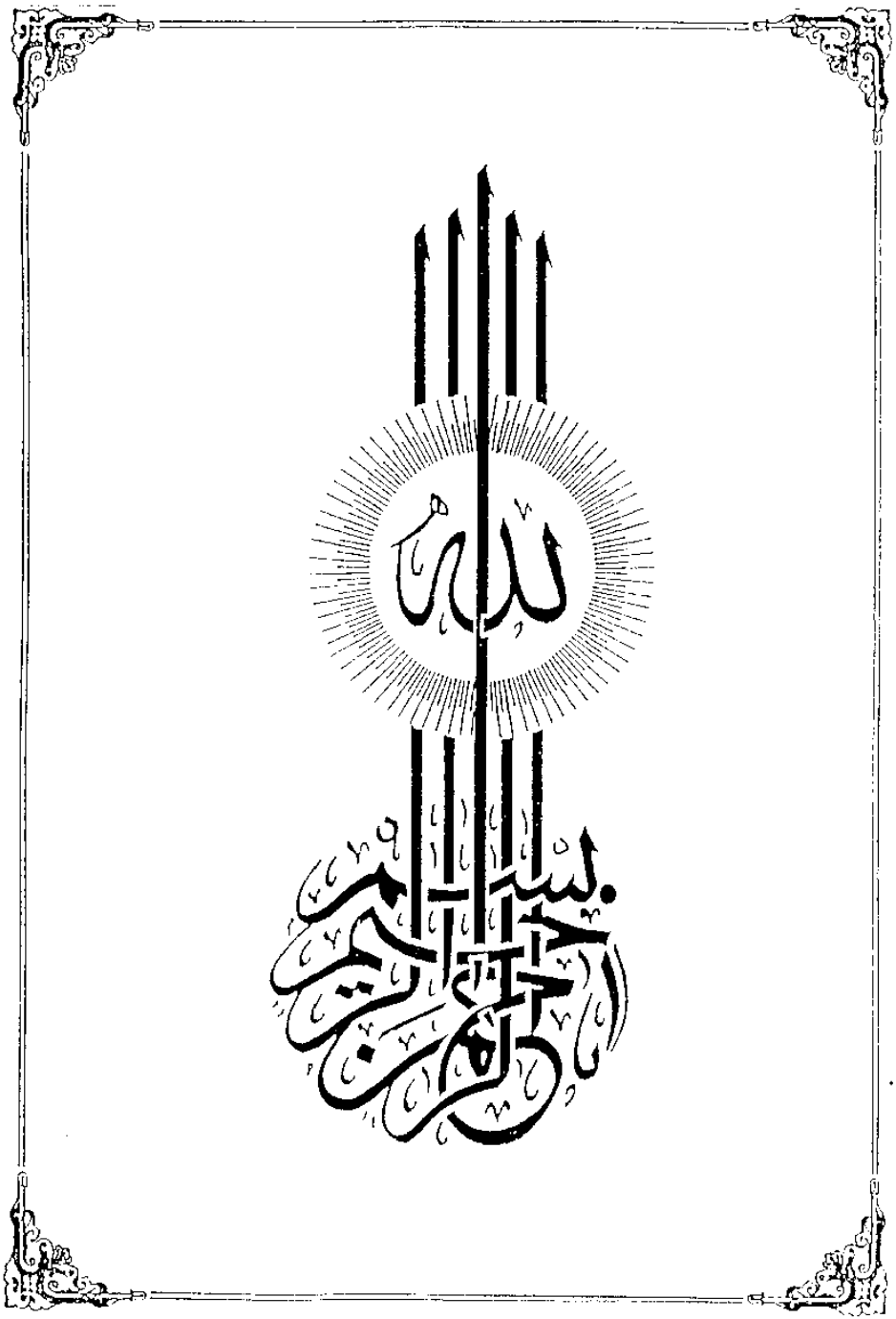

C O N T E N T S

GENERAL INTRODUCTION

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There are six hundered known species of venomous snakes in the world; and of these more than fifty species occur in Africa. Snakes are characterised by the presence of lidless eyes, a movable lower jaw and absence of external ears. They posses a forked tongue by which they detect vibrations and taste varions odours. In the poisonous species the tongue is connected with the venom apparatus. The teeth are pointed and curved, and in addition to acting as feeding organs, may be modified in venomous snakes to form fangs. Snakes are limbless creatures of variable body length, from less than six inches to as long as 30 feet. The body skeleton is composed simply of a back-bone and ribs. Snakes are characterised by marked elasticity of the region of the neck. They move in horizental undulations and never in a straight line. The skin is covered by a layer of bony scales which is shed periodically; the so called sloughing. The number of rows, the shape of the scales and their arrangement from a basis for classification; some snakes are viviparous and produce living young and others are oviparous. Eggs are laid during the very early summer

and after an incubation period of eight weaks the eggs hatch giving little serpents which grows rapidly to reach twice their size by autumn. In winter snakes hibernate to reappear in May (The breeding season). Snakes feed at intervals of five to twelve days. They may feed on birds, frogs, fish, lizards or on warm-blooded animals and rodents and even on other snkaes. The victim may be killed by constriction or poisoning but is usually swallowed alive; after being first investigated by the tongue. Snakes do not chew their food but swallow it whole.

Snakes have a body temperature usually one degree lower than that of the air and their activity is influenced by heat and cold. They are most active at a temperature 21 - 32°C below 21°C their activity slows down, at 10°C they become nearly helpless, while at 5°C they are almost lifeless. At a temperature above 32°C snakes seek cooler shelter.

The venom apparatus :

This apparatus reaches its highest development in poisonous snakes. Other species in the animal kingdom that also have the capacity to elaborate poisons include frogs, poisonous fishes, spiders, scorpions and wasps.

The venom glands in snakes are actually speciolized salivary organs, consisting of a pair of glands lying above the roof of the mouth, which open by poisonous ducts into the poison fangs. These fangs are pierced by a canal which opens near the end and are enclosed by a pouch of mucous membrane, so venom inoculation is through the canalized or grooved teeth. When the snake bites, the digastric muscle opens the jaw, the ptyregoid bone is pushed foreward, the poison fangs are erected.

The poison glands are so situated that the opening of the jaws and erection of the fangs squeezes the poison out of them through the fangs into the prey. The venom not only immobilizes the prey but aids in the subsequent digestion of the animal tissues.

Snake bite :

Generally snake bite is at its highest incidence in the dry months of June, Jully and August and at its lowest level during the relatively cold months.

Bite by Elapidae as Naja nigricollis may or may not be followed by burning pain at the bitten site. It is usually associated with a noticeable soft swelling at the bitten site and any or all of the following symptoms

may be observed : nausea, vomiting lassitude, drowsiness, salivation, sweating, slurred speech, unstady gait and a fealing of thickness in the throat. There is marked ptosis as the voluntary eye movements and accomodation are abolished. The intercostal muscles become increasingly paralysed and the respiration becomes laboured and diaphragmatic. Involuntry urination and defecation and agonal convulsions may occur. Death due to respiratory failure may follow at any time from one minute to one how after the accident. Spitting snakes as Naja Nigricollis are capable of delivering a profuse amount of venom and if this strikes the eyes, an intensely painful inflammatory reaction is set up.

A side from the species of the snake, the following factors may contribute to the chance of death from snake bite :

1. The amount of the venom injected; and this in turn depends upon :- the content of the venom in the snake; number of bites; thickness of clothing at the site of the bite; thickness and degree of penentration of subcutaneous fat and the molecular weight of the venom, if molecular weight decrease, toxicity increases.

2. The amount of poisonous substances in the venom. These vary widely from snake to another even of the same species; and may depend upon : the size and age of the snake; time since its last bite, and physiological factors such as time of day, state of nutrition, parturition, mating... etc.

3. The physiological state of the victim particularly the age (children under five years are a poor risk); health, nutritional status and previous exposure to snake venoms. There is evidence of reduced susceptibility with repeated exposure.

4. Finally the speed with which the treatment is given, and the type of treatment greatly affect mortality.

NAJA NIGRICOILLS

Naja nigricollis also known as the spitting cobra or Black necked cobra. It belongs to the famely Elapida, it is a very dangerous snake not only in Egypt but also in Africa. In Egypt it has a wide geographical distribution from upper Egypt then south westerly to Anglo and on the west coast. It is also a common snake in India. It has a life span from 4-15 years.

Naja nigricollis is one of the dull scaled snakes. It may be blue black or greenish black in colour, but mainly blackish having three horizontal black lines beneath the hood giving the neck a characteristic appearance. It is about seven feet long (200-300 cm). It feeds on rodents. It attacks its victim in a very rapid method ejecting its venom in big amounts trying to injure the eyes of the victim. It can eject its venom to a distance of eight feet or more and so may reach the eyes of a standing person. The ejection of poison is an instantaneous operation, the jaws are slightly opened then closed quickly and during this time the poison leaves the fangs, without dribbling or spilling of the fluid. To eject the poison, the snake contracts the temporal muscles on the poison glands producing pressure and so forces the toxic fluid out to a considerable

distance. This occurs with such force, that its impact can be distinctly heard against ordinary glass, five feet away, with a sharp hiss heard instantaneously. Astonishing amounts of the poison are ejected, and the snake may repeat the performance half a dozen times. Such characters make the capture of Naja nigricollis an exciting though difficult operation. However this character facilitates collection of the venom. Thus a considerable amount of the venom can be collected by holding a glass screen in front of the snake. The spray of the venom deposited on the glass dries rapidly, than can be scraped off and collected.

Venom Composition :

Snake venoms are very complex mixtures of many different substances; and these are either protein or non-protein in nature.

Non-protein constituents : In 1919 Delezenne described the presence of zinc in Elapidae venoms. Kaye (1955) confirmed its presence and estimated its concentration in cobra venom to be 5 mg/g. Fischer and Neauman (1954) reported that both Naja haja and Naja nigricollis venoms contain high concentrations of zinc and sulfur. Magnesium was also found in cobra venom.

Long and Penny (1957) have repated that zinc was capable to inhibet certain venom enzymes e.g. phospholipase A., and phosphatases especially 5-nucleotides as stated by Kaye (1955); Michael et al. (1974) claimed that amounts of aluminium, copper and zinc about 10 to 100 times higher than would be expected, may be found in venoms, they also mentioned that high concentration of adenosine and adenosine phosphates are present in Elapidae venoms. Cholesterol free and protein bound lecithin are also present in cobra venom.

Protein constituents : About 90 % of the solid constituents of most snake venoms are proteins, some of which are enzymically active. The toxic activity of snake venoms is mostly associated with proteins. The proteins in Elapid venom are polypeptides or small proteins. By electrophoresis and by immunological fractionation 5-10 antigenic protein fractions have been described.

Non-enzymatic protein toxins are known to have a variety of pharmacological actions, and of these the most important are neurotoxin.

Elapid neurotoxin: According to Kellaway (1939) venoms contain neurotixins which exert various actions on the nervous tissue. The principle actions of Elapid neurotoxins are blocking of neuromuscular transmission, without

affecting nerve conduction or the ability of muscles to contract (curare-like). They are also responsible for respiratory inhibition by blocking impulses to respiratory muscles; while the respiratory center is not directly affected. Neurotoxins produce heart block.

Mohamed et al. (1971) separated neurotoxins from the Egyptian cobra. Subsequently, Mohamed et al. (1975) separated and identified two neurotoxins from the dialyzed venom of Egyptian cobra. One neurotoxin A in the dialysate and the other neurotoxin B which was fraction III of the dialysed venom.

Constituents with enzyme activity :

1- Phospholipases : These act on the phospholipids resulting in their hydrolysis. They are four types. A, B, C, D, and each one is characterised by a specific site of action on the phospholipids Mohamed et al.(1971, 1976) separated phospholipase A from the Egyptin cobra venom.

2- Proteinases : These are responsible for the proteolytic actions of the venoms. Mohamed et al. (1969) demonstrated the marked proteinase activity of Naja haje and Naja nigricollis venom. The anticoagulant effects of snake venoms may also be due to their proteolytic activities.

3- Choline-esterases : In 1938 Lyengar and his associates reported that the neurotoxic activity of cobra venom was due to its cholinesterase activity, splitting acetyl cholin and blocking transmission of nerve impulses.

Elapidae venoms are rich in cholinesterase (Meldrum 1965, Russel 1967, and Christense, 1968) Zeller 1977 showed that all species of the family Elapidae contain cholinesterase to a lesser or greater extent.

4- Phosphatidases : e.g. lecithinase, nucleases which take part in haemolysis. Lecithinase attacks capillary endothelium, acts on the heart and produces lung haemorrhages while nucleases disintegrate the myelin sheath, fragment nerve fibers and breakdown the nuclei of ganglion cells.

5- Phosphoesterases : These participate in haemolysis.

6- Hyaluronidase : This diffusing factor was stated by Boquet (1964) to be present in Elapidae venoms . Although not toxic by itself hyaluronidase enhances the effects of the true toxins by its spreading action (Zeller, 1948).

L-amino acid oxidase is also present in Elapidae venoms. Zeller and Maritz (1944). Toxic proteins include crotoxin, crotamine, neurotoxin, and cardiotoxin are found in Elapidae venoms.