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# EFFECT OF COBRA *NAJA HAJE* VENOM ON THE ADRENAL ACTIVITY IN RABBITS

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## ABSTRACT

Injection of rabbits with a sublethal dose (0.2mg/kg) of *Naja haje* venom induced an increase in serum cortisol, free cholesterol, glucose and potassium ions. A decrease in total cholesterol and sodium ions was observed while the progesterone level was not changed. These results suggest that the cobra venom has induced a stimulatory effect on the glucocorticoid activity paralleled with a depressed mineralocorticoid function.

## INTRODUCTION

The vertebrate adrenal cortx secretes a number of steroid hormones involved in the regulation of carbohydrate, fat and protein metabolism as well as the hydromineral balance (Ashmore, 1960; Bellamy *et al.*, 1968). Some studies suggest that the adrenal gland is one of the main endocrine organs affected by snake (Mohamed *et al.*, 1964; Kadyrov, 1978), scorpion (Bałozet, 1971) and bee venoms (vick *et al.*, 1972). However, most of the reports on snake venoms were directed toward investigating the histopathological effects on the gland and measuring the levels of the steroid metabolites. Mohamed *et al.* (1978) found that the venom of the Egyptian cobra; *Naja haje* induced changes in the adrenal gland of mice usually associated with increased adrenocortical activity such as depletion of cortical lipid and ascorbic acid.

The present study was initiated to investigate the effects of the *Naja haje* venom on the circulating levels of cortisol and two of its precursors, cholesterol and progesterone. In addition, serum glucose, sodium and potassium levels were determined.

## MATERIALS AND METHODS

*Naja haje* venom was obtained from the serpentarium of Venom Research Centre, Faculty of Medicine, Ain Shams University. Sixteen male rabbits weighing 1—1.5 kg were allocated eight to each of two treatment groups. The first group served as controls and injected with the saline vehicle. The second group was treated with a single intravenous injection of the cobra venom sub-LD 50 dose (0.2 mg/kg). All injections were into the marginal vein of the ear in a volume of 0.2 ml. Two hours after the treatment, the rabbits were sacrificed and blood samples were collected. Serum was separated and stored deep frozen at -20°C prior to assay. Cortisol and progesterone were measured by radioimmunoassay (RIA). $^{125}I$ - labelled cortisol and Amerlex cortisol antiserum were supplied by Amersham, U.K. Progesterone was assayed using progesterone antiserum (SH. P001), purchased from Steranti, U.K. and labelled hormone TRK 413 (1,2,6,7)-$^{3}H$- progesterone supplied by Amersham. The RIA protocols were as described by the manufacturers. Serum glucose was estimated using the glucose oxidase method (Trinder, 1969). Determination of serum total and free cholesterol was performed according to the method of Courchaine *et al.* (1959). Sodium and potassium were determined using Corning 455 flame photometer. All data were paired and statistically analysed by student's t-test.

## RESULTS

**Table 1**

Effect of *Naja haje* venom on serum cortisol, progesterone, total and free cholesterol, glucose, sodium and potassium ions of rabbit two hours after treatment.

| Measurement | Control | Treated |
|---|---|---|
| Cortisol ($\mu$g/100ml) | 4.5 ± 0.39 | 7.4 ± 0.93** |
| Progesterons (ng/ml) | 5.7 ± 0.78 | 6.5 ± 0.61 |
| Total cholesterol (mg/100) | 117.6 ± 11.30 | 84.2 ± 7.30* |
| Free cholesterol (mg/100ml) | 31.6 ± 6.00 | 52.7 ± 5.90* |
| Glucose (mg/100 ml) | 120.0 ± 15.20 | 260.4 ± 29.70*** |
| Sodium ions (m Eq/L) | 158.4 ± 6.40 | 135.8 ± 3.30** |
| Potassium ions (m Eq/L) | 6.1 ± 0.70 | 14.9 ± 1.60*** |

**Values are expressed as means ± SE., *P < 0.05, **P < 0.02, ***P < 0.001**

Table (1) shows that *Naja haje* venom induced a significant increase ($p<0.02$) in serum cortisol concentration whereas progesterone level was not statistically different from the control value. Serum total cholesterol of envenomated rabbits decreased significantly ($p<0.05$) while there was a significant ($p<0.05$) increase in free cholesterol. Serum glucose and potassiumion levels were increased by the venom treatment ($p<0.001$) while the sodium ion level was decreased ($p<0.02$).

## DISCUSSION

The increased serum cortisol reflects a stimulatory effect of the venom on the glucocorticoid activity. This result agrees with a previous report on the black snake venom where a sublethal dose increased the 17-ketosteroid secretion (Mohamed *et al.*, 1964). It is also in agreement with the work of Mohamed *et al.* (1978) who reported stimulatory effects of *Naja haje* venom on the adrenal gland as assessed by histological evidence. Bee venom was also found to increase cortisol secretion in monkeys (Vick *et al.*, 1972) and rabbits (Mohamed *et al.*, 1986).

Although our findings are consistent with the fact that some venoms are capable of inducing adrenocortical hormone release, the exact mechanism of this effect is not completely defined. The *Naja haje* venom, like most animal venoms, contains a large number of different constituents which makes it difficult to conclude which constituent acts upon what level of the hypothalamic-hypophyseal- adrenal axis to stimulate cortisol production. It was suggested that epinephrine, released from the adrenal medulla by snake and bee venoms potentiates the ACTH release from the adenohypophysis (Edery *et al.*, 1972; Mohamed *et al.*, 1978). It is probable that epinephrine may act directly on the adenohypophysis to stimulate endogenous ACTH release since this structure lies outside the blood-brain barrier (Wislocki and King, 1966). The possibility also exists that the venom could affect the adrenal cortex through histamine. This pharmacologically active autacoid is liberated with snake envenomation and is known by its ability to stimulate the adrenal activity (Schayer, 1967; Habermann, 1972; Rothschild and Rothschild, 1979). This assumption is supported by the fact that alpha-bungarotoxin and the related snake neurotoxins bind with high affinity to receptors in the central nervous system and the adrenal gland (Seto *et al.*, 1977; Schechter *et al.*, 1978).

In the adrenal gland, progesterone is mainly regarded as a precursor for the biosynthesis of gluco- and mineralocorticolids. However, the adrenal cortex secretes into the general circulation a significant amount of progesterone in a rate regulated by ACTH (Fajer *et al.*, 1971). Our results show that progesterone level was not changed after *Naja haje* venom administration. This may be explained by a small differential lag between its biosynthesis and utilization to form cortisol. It is also probable that the venom-induced endogenous ACTH has a pattern of selective effect favouring to increase cortisol secretion without accumulation of progesterone.

The decrease in serum total cholesterol and the increase in the free cholesterol are not consistent with previous results on scorpion venum where El-Asmar *et al.* (1979) found that a single dose of scorpion venom increased both total and free cholesterol in rats. The present results can be explained by increased adrenal uptake of circulating cholesterol due to stimulated cortisol production under the effect of released endogenous ACTH. Borkowski *et al.* (1967) reported that 80% of hydrocortisone produced in man was derived from plasma cholesterol under ACTH stimulation. Gwynne and Hess (1978) and Gwynne *et al.* (1979) showed that ACTH activates specific membrane receptors in adrenocortical cells to increase cholesterol uptake. The rise in free cholesterol may have resulted from stimulated hydrolysis of serum cholesteryl esters. It was reported that plasma cholesteryl esters are first hydrolyzed then penetrate the adrenal cells as free cholesterol (Shyamala *et al.*, 1966; Dexter *et al.*, 1970). It would seem reasonable to suggest that in the present study, the cholesteryl esters were hydrolyzed, in part, to free cholesterol and utilized by the adrenal cells which offors an explanation for the decrease in serum total cholesterol.

The hyperglycemic effect of *Naja haje* venom is similar to that reported by Mohamed *et al.* (1980) for some Egyptian and allied African snake venoms. This effect may be due to increased glycogen break down by the released epinephrine or the direct diabetogenic effects of the venom (Mohamed *et al.*, 1978). Cobra venom was found to exhibit contra-insulin effects resulting in decreased peripheral glucose utilization and subsequent high blood glucose level (Ambe and Crane, 1959; Tu and Passey, 1965). Although excessive cortisol secretion is known to produce hyperglycemia (Cooley and Janssens, 1977), it is unlikely that the hyperglycemia observed in the present study after two hours resulted from the increased cortisol and its gluconeogenic action. Cortisol is a slow hormone acting via a mRNA (O'Malley *et al.*, 1972) and two hours would probably not be long enough for cortisol to raise the blood glucose.

There was also a decrease in serum sodium level and an increase in potassium. These results agree with those reported with scorpion venom (Ismail *et al.*, 1978; Tash *et al.*, 1982). These findings reflect suppressed mineralocorticoid activity. and may be explained by a depressed activity of renin-angiotensin system, the main regulator of aldosterone secretion, under the effect of the venom. Huang (1984) reported an inhibitory effect of phospholipase A on renin and this enzyme was detected in the cobra venom by Sarker and Davis (1968) and Lee (1971). Another possible reason could be that the high levels of circulating cortisol affected the mineralocorticoid activity. Kenyon *et al.* (1984) reported that high levels of circulating glucocorticoids antagonize the effects of mineralocorticoids and inhibit the antinatriuretic and kaliuretic effects of aldosterone. This assumption needs assessment by direct measurement of aldosterone and renin levels.

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# تأثـــير سم الكوبرا ناجـا هـــاج على نشاط الغـــدّة الكظـريـــة في الأرنـــب

أحمـــد رفعـــت عـــزت و عـلي عبـد العـــال

يتناول هذا البحث تأثير سم ثعبان الكوبرا عند مستوى الجرعة تحت المميته ( ٢ر٠ ملجم/كجم ) على نشاط الغدة الكظرية للأرنب . وأوضحت النتائج أن إستخدام هذه الجرعة من سم الكوبرا له تأثيرات مختلفة ، فهي تؤدي إلى زيادة مستوى هرمون الكورتيزول ، والكوليسترول الحر والجلوكوز ، وأيونات البوتاسيوم في مصل الأرنب . كما تؤدي أيضـــا إلى خفض مستويات المصل من الكوليسترول الكلي وأيونات الصوديوم . وأشارت النتائج إلى أن هذه الجرعة ليس لها تأثير ملحوظ على مستوى هرمون البروجيسترون .

وأظهرت النتائج أن التأثير الحافز لسم الكوبرا على إنتاج الكورتيكودات السكرية يكون مصحوباً بتأثير مثبط على إنتاج الكورتيكودات المعدنية . وقد نوقشت هذه النتائج لتحديد الأسباب المحتملة لهذه العلاقات .