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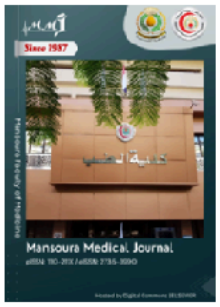

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INTRODUCTION

Since pre-historic times, man has always suffered from snake bite. Nowadays, there is still a high incidence of snake bite especially to the people living in infested areas, (Rsosenfeld, 1971). This is especially true among persons Who handle poisonous snakes routinely . Therefore, it is considered as an occupational hazard among professional amateur, herpetologists, biologists, museum reptile curators and religious faddists who use poisonous snakes as a part of thelr ceremonial regalia, (Chiristensen 1969 and Reid, 1972).

Snake venoms are complex organic substances containing a wide variety of pharmacologically active chemical components, so it is the most toxic poison, (Oehme et al.,1980).

Earlier studies on snake venom were primarly directed towards the explanation of their mode of action and detecting a proper line of treatment, (Russell, 1971). Recently, advanced knowledge of the physiological effects and biochemical properties of the venoms was obtained, (Russell, 1971; Sarangi et al., 1980 and Saini, 1985) which lead us to perform this work in an attempt towards a better and clear understanding of the histopathological and histochemical effects of snake venom on the different tissues of the experimental animals. Assessment of the results occurring after the use of antivenom in the treatment of

envenomation is done.

MATERIAL AND METHODS

Venom :

The venom used in this study was obtained from venom Research centre, Physiology Department, Faculty of Medicine, Ain Shams University. It was collected from adult snakes (Egyptian Cerastes Cerastes Viper) and dried in a dissicator and stored at 3 C^{o} in the dark, prior to use. For assay the dried venom was reconstituted in 0.9 % sodium chloride (Physiological saline solution) in a concentration of 1 mg/ 1 ml. on the day of experiment, (Sosa et al., 1979 and Ruiz et al., 1980).

Anti-Venom:

The type of the anti-venom used is in the form of polyvalent antivenom ampoule of 10 ml., Purchased from Sera and Vaccines institute, Agouza, Cairo, Egypt. It was stored in a refrigerator at + 4 C^{o} and used before the date of expiry given on the pack. Once the ampoule has been opened its contents were used immedietly.

Experimental Animals :

70 male and female mature guina pigs ranging from 400 800 gram in weight were used. They were divided into the following four major groups :

(I) Group I :

- This group is composed of 40 animals and were used for the comparative study of the effect of single different doses of Cerastes cerastes venom. They were divided into 4 subgroups, each composed of 10 animals.

1. Ist subgroup : The animals were given venom solution in a dose of 0.5 ug/gm body weight intraperitoneally.

2. 2nd subgroup : The animals were given venom solution in a dose of 1 ug/gm, body weight intraperitoneally. This dose is the maximum non lethal dose, (Rechnic et al., 1962).

3. 3rd subgroup : The animals were given venom solution in. a dose of 1.5 ug/gm, body

weight intraperitoneally. This dose is considered the minimum lethal dose , (Rechnic et al., 1962).

4. 4th subgroup : The animals were given venom solution in a dose of 2 ug/gm, body weight intraperitoneally.

(2) Group II :

This group is composed of 10 animals and they were used to study the effect of repeated small doses of Cerastes cerastes venom solution. Each dose contained 0.5 ug/gm body weight that was administered intraperitoneally as six injections every other day, (Rechnic et al., 1962).

(3) Group III :

This included 10 animals, they were given 1 ug/gm, body weight venom solution intraperitoneally, followed immediately by intraperitoneal injection of polyvalent antivenom. The dose of the polyvalent antivenom used in this study was calculated according to Rechnic et al., (1962), who reported that 1. ml of the antivenom was able to neutralize 1.25 mg of dried venom.

(4) Group IV :

This group consisted of 10 animals kept as control.

4 hours after injection in 1st and 3rd groups and one day after the last injection in the 2nd group, the animals were sacrificed by cut throat, they were dissected and livers kidneys, spleens, lungs, hearts and brains were taken and each organ was cut in half. One half was fixed in 10% formaline and processed as paraffin sections and stained with haematoxylin and Eosin stain for hi~topathological study. The other half was cut into fresh frozen sections and processed for histochemical localization of alkaline phosphatase activity - (Gomori's method, Bancroft, 1975)- as a marker for transport through cellular plasma membrane.

RESULTS

1- Mortality rate :

The death rate was Increased with the increasing venom dose (table I) .

II - Histopathological findings :

(1) Liver :

Animals given 0.5 and 1 mg/gm body weight of the Venom showed granularity and vacuolization of the hepatocytes (Fig.I). Mild cellular infiltration composed of lymphocytes, plasma cells and macrophages was seen together with few scattered polymorphnuclear leucocytes in between the liver cells were present. There was also dilatation of the central veins with congestion of the sinusoids and hyperplasia of the Vonkupffer cells. Adminis-tration of 1.5 and 2 mg/gm body weight of the venom produced in addition to the previous changes fatty change Which was focally distributed in the former dose and diffuse in the latter one (Fig. 2) on the other hand. The proliferation of the bile ducts in the portal tracts with round cells infiltration around them (Fig. 3) were the stricking features in the group given repeated small doses.

(2) kidney :

In the four subgroups of animals given different single doses of the venom, the lesions in the kidney were mainly tubular. the glomeruli were almost normal. Tubular cells degenerative changes were present. These were in the form of cloudy swelling and vacuolization of the proximal convoluted tubules (Fig. 4) in the subgroups given 0.5 ug/gm and 1 ug/gm body weight the cloudy swelling extends to affect the Henle's loop with shedding of the epithelial lining cells in some areas in the animals administered 1.5 and 2 ug/gm body weight. Congested blood vessels that contained haemolysed blood were seen which was more marked and associated with interstitial haemorrhage in the larger doses. On the other hand, kidneys of guinea pigs received repeated. small doses, both glomeruli and tubules were affected. Atrophy of some glomeruli that appeared small in size and contracted in the center was

noticed, others showed hypertrophy and increase in size with proliferation of the parietal layer of Bowman's capsule. There was also cloudy swelling of proximal convoluted tubules with small foci of tubular necrosis, interstitial haemorrhages as well as small focal areas of fibrosis at the corticomedullary junction.

(3) Spleen :

Administration of single different doses of the venom produced hyperplasia of the lymphoid follicles with prominent germinal center (Fig.5) and proliferation of histiocytes in the blood sinusoids (sinus histiocytosis).

In chronic administration, in addition to the previous changes, congestion of the red pulp and interstitial haemorrhages were the prominent findings. Haemosiderin granules were present indicating haemolysis.

(4) Lung :

Oedema and congestion of few alveoli, with focal collapse of others were present. In addition, haemorrhages in the alveoli that contained haemolysed blood in animals injected with 1 or more ug/gm body weight of venom.

With repeatied administration of small doses of the venom, the lung showed hypertrophy of the lymphoid follicles with focal collapse of the alveoli and mild interstitial fibrosis . Interstitial haemorrhage was present in some areas. (Fig.6) .

(5) Heart :

Cloudy swelling of the cardiac muscle fibers was present after administration of 0.5 ug/gm body weight of the venom. Dilated and congested blood vessels and paravascular round cells infiltration in addition to the previous changes appeared after administration of 1 ug/gm body weight. These changes were seen in association to haemorrhages in

between muscle fibers after administration of larger doses of the venom (1.5 and 2 ug/gm body weight). (Fig.7).

Histopathological examination of the heart after repeated administration of small doses showed mild swelling of the muscle fibers with loss of muscle striations in focal areas -together with paravascular round cells infiltration (Fig.8)

(6) Brain:

No histopathological changes were seen in the brain apart from mild oedema indicated by the separated cellular component in either administration of single different doses or repeated small doses of the venom.

III- Histochemical findings :

Guinea pigs received single dose of 0.5 ug/gm body weight of cerastes cerastes venom showed slight increase in the alkaline phosphatase activity in all the organs except the brain which remained unchanged comparing to the control animals. The enzymatic activity was decreased with doses more than 0.5 ug/gm with the decreasing activity parallel to the increasing dose of the venom. The decreased activity in the liver started in the peripheral zone after administration of 1 ug/gm body weight of the venom. Then it becomes diffusely decreased when the dose increased, (Table 2 and Figs.g, 10, 11, 12, 13, 14 and 15).

Administration of 1 ug/mg body weight of snake venom followed immediately with antivenom markedly decreases the previously described changes. The prominent features were mild degenerative changes of the parenchymatous organs.

DISCUSSION

The venom of Cerastes cerastes snakes used in the present work consists o f a mixture of toxic proteins and enzymes as well as other

pharmacologically active substances which have degenerating and necrotising properties, (Chugh et al., 1975; Sarangi et al., 1980).

The findings of the present study provides an evidence of the damaging action of the snake venom on various tissues of guinea pigs.

Administration of single doses of 0.5 and 1 ug/gm, body weight produced dilatation and congestion of the liver sinusoids together with vonkupffer cells hyperplasia. Degenerative changes of hepatocytes were present which were severer with larger doses of the venom. The congestion and, the dilatation of hepatic blood vessels may be attributed to the histamine content of the venom as well as the histamine released in the tissues of the animals. (Chen et al., 1984 and Tilminsany et al., 1986). The release of histamine in the tissues may be due to venom producing anaphylactic hypersensitivity reaction, (Jones & Mrcs,1985). Such reaction can be confirmed by the fact that snake venom is 90 % protein by the weight, that act as allergen, (Jones & Mrcs, 1985 and Schmutz &. Stahel, 1985). The reported vonkupffer cells hyperplasia may be due to the fact that these cells engulfed erythrocytes in various stages of haemolysis, (Rosenfeld, 1971). On the other hand, hepatocytes degenerative changes present in this study are suggested to be a direct toxic effect of the venom -that increased with the increasing doses. However, the haemorrhages demonstrated by Taube and Essex, (1937) and the coagulative necrosis reported by Okonogi et al. (1961) were not seen in the present study.

The kidneys of the animals exposed to single different doses of Cerastes cerastes venom, exhibited degenerative chanses of the tubular epithelium, while the glomeruli were almost normal, The blood vessels appeared congested and contained haemolysed blood. These lesions were more severe and associated with interstitial haemorrhage in the larger doses. These renal changes were also reported by several workers, (Gitter et al., 1962; Rechnic et al.,

1962; Mohamed et al., 1975 and Saini, 1985). Mohamed et al. (1975) stated that the tubular damage was prominent after snake venom as they are the sites of water reabsorption which results in concentration of the excreted venom. Intravascular haemolysis observed in this study may be attributed to the action of "phospholipase A" content of the venom, (Chugh et al., 1975 and Labib et al., 1979). This finding is consistent with that of Sarangi et al., (1980), Date and Shastry, (1981) and Amaral et al. (1985) and is in direct contrast with the finding of Myinb - Lwin et al. (1985) who reported that no haemolysis occurred with snake bite

In the group of animals administered small doses of venom repeatedly, glomerular affection was a stricking feature. The hypertrophied Bowmans capsular epithelium present in the present study are suggestive of tubulization of the parietal layer of Bowman's capsule as a part of the tubular regenerative process,(Saini, 1985). On the other hand the tubular system showed minimal lesion. This finding is in contradistinction with that is reported by Mohamed et al. (1974) who found that the main effects in repeatedly injected animals were demonstrated in the tubular system and not the glomeruli. Hamburger et al., (1968) found that foreign proteins injected in repeated doses produce experimental glomerulonephritis with localization of Antigen antibody complexes. Since the chief component of the venom seems to be protein, (Oehme et al., 1980) therefore, it can act as an antigen with production of allergic reactionss, (Rosenfeld, 1971). Therefore, the glomerular changes present in our study may be explained by the deposition of antigen antibody complexes or to a direct nephrotoxic effect or both.

Histopathological examination of the spleens and lungs after administration of single different and repeated doses of Cerastes cerastes venom showed evidence of toxicity. The hyperplasia of the lymphoid follicles in these organs may be attributed to an immunological response to the antigenic stimulation by the venom.