OnLine Journal of Biological Sciences 3 (9): 824-833, 2003
ISSN 1608-4217

Pharmacological Characterization of the Rat Paw Edema Induced by *Echis pyramidum* Venom

Abdulrahman Khazim Al Asmari
Research Center, Armed Forces Hospital, Riyadh, Saudi Arabia

Abstract: The present study was conducted to investigate the inflammatory response induced by ***Echis pyramidum*** venom (EpV) in the rat hind-paw by measuring paw edema. Non-heated EpV (3.125 - 50 μg paw^{-1}) caused a marked paw edema (plateau) accompanied by intense haemorrhage(not shown); whereas heated venom (97° C, 30 s; 3.125-50 μg paw^{-1}) produced a dose and time-dependent non-haemorrhagic edema. The response with heated EpV was maximal within 15 min, disappearing over 24 h. Heated EpV (25 μg paw^{-1}) was optimized to test the effect of various drugs on edema induced by the EpV. The results showed that dexamethasone, H_1 and H_3 receptor antagonist cyproheptadine and H_1 receptor antagonist chlorpheniramine markedly ($P<0.001$) reduced the EpV-induced rat paw edema, whereas the cyclooxygenase inhibitor diclofenac and the proteinase inhibitor (aprotinin) produced a moderately significant ($P<0.01$) inhibition of edema. The commercially available antivenom was found to be ineffective when administered intravenously, whereas local administration by coupling the EpV and antivenom had a highly significant ($P<0.001$) effect. We conclude that EpV induced edema; while the expected principal mediators of this inflammatory response were serotonin, histamine, cyclo-oxygenase, other prostaglandins (PGs) and cytokines. Finally, the antivenom given intravenously failed to reduce edema.

Key words: Rat paw edema, ***Echis pyramidum*** venom (EpV), inflammatory mediators, antagonist, antivenom

Introduction

The saw-scaled viper (genus ***Echis***) has an extensive geographical distribution from Senegal in the west, through the north of Africa, south as far Kenya, the Middle east, Turkmenistan, Uzbekistan as far as the Aral Sea the Indian subcontinent, including SriLanka (Gasperetti, 1988; Cherlin and Brokin, 1990; Warrell, 1995). Gasperetti (1988) stated that, through personal communication with Professor Reid, snakes of the genus ***Echis*** are the snakes most dangerous to man and according to their range from Nigeria to Srilanka, they are responsible for more snake bite fatalities than all other snakes together. ***Echis carinatus*** average about 35 cm in length. Their bites probably kill more people than those of any other genus of snakes and untreated cases had a mortality rate of about 7-15% (Warrell and Arnett, 1976; Warrell ***et al.***, 1977). There are three species of ***Echis*** distributed throughout the Arabian Peninsula, ***Echis pyramidum***, ***Echis carinatus sochureki*** and ***Echis coloratus***. These three species are responsible for most envenomation cases

each year. Gasperetti (1988) reported that in Arabia, the species ***Echis pyramidum*** was found from the south edge of the Harrat al Birk near the 18th Parallel (its existence North of this border, that had been reported by Corkill and Cochrane (1965) and Farag and Banaja (1980) was negated by the works of Gasperetti that had been going on since 1960; southwards to Yemen, eastward to Aden Hadramut, an isolated area near Dhofar and in the area of Hakimah near Jizan.

Clinical symptoms of ***Echis*** envenoming are characterized by highly complex pathophysiological features of local as well as systemic nature (Warrell, 1993). The local manifestations caused by ***Echis*** venoms include edema, pain, haemorrhage and necrosis (Rosenfeld, 1971; Kingston, 1981; Kamiguti ***et al.***, 1991; Warrell, 1993; 1995; Milani-Junior ***et al.***, 1997; Al-Jammaz ***et al.***, 1999; Jorge ***et al.***, 1999). The systemic complications are characterized by hypofibrinogenemia, thrombocytopenia and a decline in coagulation factor V and VIII:C (Weiss ***et al.***, 1973; Schaeffer ***et al.***, 1986) Other viper venoms led to the same situations (Lobo ***et al.***, 1994, 1998). Edema is a common feature of the cutaneous inflammatory response and is dependent on a synergism between the mediators of vascular permeability and blood flow (Williams and Morley, 1973; Williams and Peck, 1977; Williams, 1979; Brain and Williams, 1985). One of the important consequences of altered capillary permeability in local inflammation is the extravasation of leucocytes (Hamblin, 1994). The degree of accumulation of these cells at inflammatory sites in the skin is related to local blood flow (Issekutz and Movat, 1979; Issekutz, 1981; Buckley ***et al.***, 1991).

Several investigators have studied the biochemical and pharmacological effects of the venoms collected from different species that belong to the genus ***Echis*** (Moav ***et al.***, 1963; Theakston ***et al.***, 1982; Theakston, 1983; Theakston and Reid, 1983; Al-Jammaz ***et al.***, 1999). However, to our knowledge, the venom inflammatory effect of EpV had not been studied so far, especially on members of the Arabian Peninsula. This investigation reflects dose-related and time-course inflammatory effects of EpV using rat's paw oedema model. The effects of various drugs including dexamethasone, cyproheptadine, chlorpheniramine, diclofenac , aprotinin and a commercial antivenom were also examined. The objective of this study was to examine the ability of EpV in inducing paw edema in the rats and the pharmacological mechanisms underlying this effect.

Materials and Methods

Reagents

Chlorpheniramine and diclofenac were obtained from Hikma, APM, Jordan. Dexamethasone and cyproheptadine from Merck and Co., Inc., Rahway, NJ, USA and aprotinin from Buyer AG, Germany.

Venom and antivenom

EpV was obtained from the Zoology department, King Saud University, Riyadh, Saudi Arabia. Snakes were collected from the wild, throughout the Kingdom by professional hunters and kept in the serpentarium facility at the previously mentioned site. Scientific classification, milking of specimens, lyophilization and storage of the venom was done by the specialized team of the Venomology Unit there.

The venom was dissolved in saline (final concentration 10 mg ml^{-1}) and immediately stored at -20°C until used. The antivenom used in this study was a national product, obtained from Al-Hayatt Company, Riyadh, Saudi Arabia. This antivenom was prepared in hyperimmunized horses using a mixture of ***Echis carinatus***, ***Echis coloratus***, ***Bitis arietans***, ***Cerastes cerastes***, ***Naja haje and Walterinnesia aegyptia*** venoms. the antivenom was dialized to remove the preservatives.

Measurement of rat paw edema

Male Wistar rats weighing 145-155 g (mean = 150 g) were used for all the experiments and were provided by the Armed Forces Hospital, Research Center (Animal House Services). All experiments were carried out according to the methods described by Faria ***et al.*** (2001). The animals were injected into the subplantar region of the right hind paw with 0.1 ml of either heated [30 s, 97°C; according to the method employed by Perales ***et al.*** (1992) for destruction of haemorrhage inducing factors] or non-heated EpV (3.125-75 μg paw^{-1}). The left paw was used as control and received the same volume (0.1 ml) of sterile saline. The edema was measured at 0.25, 0.5, 2, 4, 6 and 24 h using Plethysmometer (Model 7150, Ugo, Basile, Italy). The results were expressed as mean differences between the final and initial volumes of the injected paws.

Influence of various substances on EpV-induced edema

The group of rats (n = 6 each) were pretreated with different classes of drugs, as follows: (B) dexamethasone (4 mg kg^{-1}, s.c., 2 h before); © H_1 and H_3 receptor antagonist, cyproheptadine (8 mg kg^{-1}, i.p.,15 min before); (D) H_1 receptor antagonist, chlorpheniramine (16 mg kg^{-1}, i.p., 15 min before); (E) cyclooxygenase inhibitor, diclofenac (20 mg kg^{-1}, i.p., 30 min before); (G) a proteinase inhibitor, aprotinin (2000 KIU kg^{-1}, i.p., 30 min before). Following the appropriate time intervals, the animals received an intraplantar injection of EpV [25 μg paw^{-1} (0.17 mg kg^{-1})] and edema was measured (15 min later) as described before. (F) the commercial antivenom was either injected intravenously [1.3 mg kg^{-1} of total $F(ab)_2$] or locally together with venom during a 30 min incubation period at 37°C.

Statistical analysis

Data were presented as the mean ± SEM, analyzed by analysis of variance (ANOVA) and followed by a Bonferroni test (SPSS Program). A P-value of less than 0.05 was considered to indicate significance.

Results

Effect of EpV on rat paw edema

Subplantar injection of non-heated EpV (25 μg paw^{-1}) caused intense haemorrhage and marked paw edema compared to saline-injected paws (n = 6; $P<0.001$).

Subplantar injection of heated EpV venom (3.125 - 50 μg paw^{-1}) produced a dose and time dependent non-haemorrhagic edema (Fig.1A). The maximal response was observed 15 min after venom injection, decreasing gradually over 24 h (Fig.1B). For further experiments, heated venom was routinely used at the dose of 25 μg paw^{-1}.

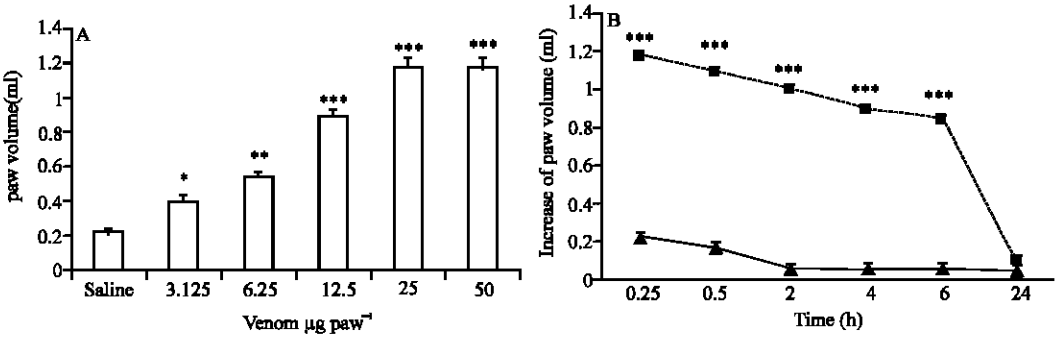

Fig.1: Rat paw edema induced by heated *Echis carinatus pyramidum* venom. Panel A shows that the intensity edema with venom doses varying from 3.125 μg to 50 μg paw^{-1}. Panel B shows the time course edema (15 min to 24 h) using 25 μg/paw of venom, (|) compared to saline (?). Venom was heated at 97°C for 30s. The control group received saline 0.1ml in the same experimental conditions. Each column represents mean + S.E.M. of five rats. Weak significance was *P<0.05, moderate significance was **P< 0.01 and high significance was ***P<. 001 compared to saline

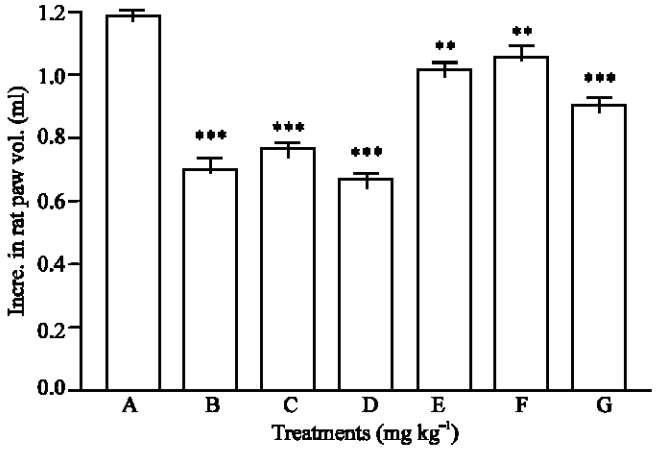

Fig. 2: The effect of (B) dexamethasone (1 mg kg^{-1}, s.c., 2h before), © cyproheptadine (8 mg kg^{-1}, i.p., 30 min before), (D) chlorpheniramine (16 mg kg^{-1}, i.p., 15 min before), (E) diclofenac (20 mg kg^{-1}, i.p., 30 min before) and (G) aprotinin (2000 KIU kg^{-1}, i.p., 30 min before) on the rat paw edema induced by heated EpV [25 μg paw^{-1} (0.17 mg kg^{-1})]. (F) Effect of coupling and incubating (for 30 min) the antivenom (1.3 mg kg^{-1}) with EpV [25 μg paw^{-1} (0.17 mg kg^{-1})]. (A) The positive control group received heated EpV [25 μg paw^{-1} (0.17 mg kg^{-1})] alone in the same experimental conditions. Each column represents mean ± S.E.M. of five rats. Moderate significance was (** P < 0.01) and marked (high) significance was (*** P < 0.001)

Pharmacological modulation of heated EpV on rat paw induced edema

The highest significant ($P<0.01$) inhibition of edema induced by the EpV was observed when employing dexamethasone, cyproheptadine and chlorpheniramine (Fig. 2). Whereas the treatment of the animals with the cyclooxygenase inhibitor diclofenac (20 mg kg^{-1}, i.p, 30 min before) and the proteinase inhibitor aprotinin (2000 KIU kg^{-1}, i.p., 30 min before) gave a moderately significant ($P<0.05$) effect.

Local injection of the coupled EpV and antivenom highly ($P<0.01$) reduced the EpV-induced paw edema, while intravenously administered (1.3 mg kg^{-1}) commercial antivenom failed to neutralise the venom-induced edema.

Discussion

Previous studies done on this species (***E. pyramidum***) were focussed on serum and tissue (***in vitro***) profile of experimental animals. Snake venom components, especially those of vipers, either activate, inhibit or liberate enzymes by cellular organelles destruction (Moustafa ***et al.***, 1974; Marsh ***et al.***, 1997; Abdel-Nabi ***et al.***, 1997). The different toxic effects exhibited by venoms of vipers were due to their contents of proteolytic and lipolytic enzymes (Tan and Ponnudurai, 1990). Common antecedent envenoming signs were hypoglycaemia (Abu-Sinna ***et al.***, 1993), general metabolic disturbances (Mahmoud, 1983), muscular dystrophy (Mohamed and Khaled, 1966), nephrotoxicity (Ickowitz ***et al.***, 1966) and induction of cytotoxicity (Bertke and Atkins, 1961).

This study shows that subplantar injection of either non-heated or heated EpV could cause a significant paw edema in the rat (Lobo ***et al.***, 2000). Viper envenoming produced edema and altered vascular permeability in the mouse hind paw (Trebien and Calixto, 1989; Lobo ***et al.***, 2000). Following these responses was an abundant leukocyte infiltration and haemorrhage ensued due to high doses of venom (Lobo ***et al.***, 2000). Since haemorrhage appeared soon after venom injection and interfered with the development of inflammatory edema, (Mandelbaun ***et al.***, 1975; Assakura ***et al.***, 1986; Faria ***et al.***, 2001). We decided to heat the venom in order to destroy the heat-labile proteolytic enzymes, the haemorrhagic factors, aiming to study the inflammatory components of this venom.

Increased vascular permeability and increased blood flow play an important role in edema formation (Williams and Peck, 1977; Williams, 1979; Brain and Williams, 1985). The capacity of exogenously applied vasodilators such as calcitonin gene-related peptide (CGRP), prostaglandin E_2 and prostacyclin to potentiate inflammatory edema in response to different inflammatory mediators is well known (Williams, 1983). Inverse comparisons based on the use of edema inhibitors showed that our tested drugs could reverse the edema induced by EpV. Our results showed that EpV alone induced rat paw edema which might indicate a direct or indirect relationship between local blood flow and the intensity of edema, as compared to Faria ***et al.*** (2001), Lobo ***et al.*** (2000) and Antunes ***et al.*** (1992).

In an attempt to further understand the pharmacological mechanisms involved in EpV-induced rat paw edema, different medications were used. These findings showed that treatment of the animals with cyproheptadine ($H_1$and H_3 receptor antagonist) and chlorpheniramine (histamine H_1 receptor antagonist) gave a highly effective edema reduction indicating the role of ***in vivo*** mast cell degranulation (Faria ***et al.***, 2001; Carneiro ***et al.***, 2002).

Arachidonic acid provides a number of inflammatory mediators, via the action of cyclooxygenase or lipoxygenase (Faria ***et al.***, 2001). The use of cyclooxygenase inhibitor (diclofenac) proved to be the least effective against the rat paw edema induced by EpV. The treatment of the animals with dexamethasone also caused a highly significant reduction in venom induced paw edema. This was expected as corticosteroids were known to indirectly inhibit the phospholipase A_2 action (Flower, 1989). Furthermore, corticosteroids also directly acted on leucocytes and other cell types inhibiting the release of cytokines and other inflammatory mediators (Angeli ***et al.***, 1999). Gutierrez ***et al.*** (1986) and Lomonte ***et al.*** (1993) had a similar report on the kinetics and cell composition of the inflammatory infiltrate observed in the foot pad of the rat. Aprotinin also significantly reduced the induced edema. It is an inhibitor of many proteases such as kallikrein whose products are bradykinin and kallidin (Erdos, 1963; Goth, 1978). These kinins are potent vasodilators that also increase the capillary permeability and are easily produced in tissues after injury; thus being cardinal agents in edema formation (Johnson and Erdos, 1973; Goth, 1978). The primary difference between our findings and others as those of Faria ***et al.*** (2001) was that EpV induced edema without potentiators. Secondly, the cyclooxygenase inhibitors (diclofenac) reduced the edema induced by EpV, which indicate that diclofenac metabolite might be involved in mast cell activation by this venom. Furthermore, H_1 and H_3 receptor antagonists reduced significantly rat paw oedema. Contrast results were obtained by Lobo ***et al.*** (2000), Trebiens (1989) and Perales ***et al.*** (1992).

Finally, we attempted to examine the ability of polyspecific antivenom to neutralize the edematogenic activity of this venom. Although local effects of antivenoms such as myonecrosis and haemorrhage (of other venoms) were largely studied (Bjarnason and Fox, 1994; Gutierrez and Lomonte, 1995), none of them were done on the edematogenic effect of EpV. When a mixture of venom and antivenom was administered in the paws, a high inhibitory effect was observed, whereas the separate antecedent intravenous administration of antivenom failed to reverse venom-induced edema. This situation could raise the query about employing viper antivenoms in treating victims. Benbassat and Shalev (1993) had investigated this by reviewing reported data on the effect of viper venoms ***in vitro***, laboratory animals and humans; and reexamined alternative treatment methods in order to assess the efficacy of using antivenoms. Tilbury ***et al.*** (1987) reporting on ***Echis coloratus***, had speculated and reviewed this situation.

In regards to edema, it could be suggested that antibodies raised against edematogenic component(s) of EpV were in very low amounts (highly diluted) in the total antiserum. This finding is in agreement with those of Faria ***et al.*** (2001). Further studies attempting to purify the edematogenic component(s) present in this venom are necessary to elucidate this aspect.

Acknowledgments

The author would like to thank Riyadh Military Hospital for supporting this work and his cordial thanks to Nasreddin M. Abdo for his help in this study. Author is deeply grateful to Mr Abbas M. for his assistance in the animal work.

References

Abdel-Nabi, I., R. Awadalla and I. El-Shamy, 1997. Biochemical effects of intraperitoneal injection of rats with the venom of the snake ***Echis carinatus***. Egyptian J. Zool., 29: 195-205.

Abu-Sinna, G., A.S. Al-Zahaby, A. Abd El-Aal, A. Abd El-Basit and N.A. Soliman, 1993. The effect of the viper ***Cerastes cerastes cerastes*** venom and venom fractions on carbohydrate metabolism. Toxicon, 31: 791-801.

Al-Jammaz, I., M.K. Al-Sadoon and A. Faheem, 1999. Effect of LD_{50} Dose of ***Echis coloratus*** venom on serum and tissue metabolites and some enzymes of male albino rats. J. King Saud Univ. Sci., pp: 61-68.

Angeli, A., R.G. Masera, M.L. Sartori, N. Fortunati, S. Racca, A. Dovio, A. Staurenghi and R. Frairia, 1999. Modulation by cytokines of glucocorticoid action. Ann. N. Y. Acad. Sci., 876: 210-220.

Antunes, E., R.A. Marangoni, S.D. Brain and G. De Nucci, 1992. ***Phoneutria nigriventer*** (armed spider) venom induces increased vascular permeability in rat and rabbit skin ***in vivo***. Toxicon, 30: 1011-1016.

Assakura, M.T., A.P. Reichl and F.R. Mandelbaum, 1986. Comparison of immunological biochemical and biophysical properties of three hemorrhagic factors isolated from the venom of ***Bothrops jararaca*** (jararaca). Toxicon, 24: 943-946.

Benbassat, J. and O. Shalev, 1993. Envenomation by ***Echis coloratus*** (Mid-East saw- scaled viper): a review of the literature and indication for treatment. Isr. J. Med. Sci., 29: 239-250.

Bertke, E. M. and J.H. Atkins, 1961. Effect of ***Centroroides sculpturatus*** venom upon rat tissues: a histopathological study. Toxicon, 2: 205-218.

Bjarnason, J.B. and J.W. Fox, 1994. Hemorrhagic metalloproteinases from snake venoms. Pharmacol. Ther., 62: 325-372.

Brain, S.D. and T.J. Williams, 1985. Inflammatory mechanism of inflamed-tissue factor. Agents Actions, 3: 348-356.

Buckley, T.L., S.D. Brain, P.D. Collins and T.J. William, 1991. Inflammation edema induced by interactions between interleukin-1 and neuropeptide calcitonin gene-related peptide. J. Immunol., 146: 3424-3430.

Carneiro, A.S., O.G. Ribeiro, M. De Franco, W.H.K. Cabrera, F. Vorraro, M. Siqueira, O.M. Ibanez and N. Starobinas, 2002. Local inflammatory reaction induced by ***Bothrops jararaca*** venom differs in mice selected for acute inflammatory response. Toxicon, 40: 1571-1579.

Corkill, N.L. and J.A. Cochrane, 1965. The snakes of the Arabian peninsula. J. Bombay Nat. Hist. Soc., 62: 475-506.

Cherlin, V.A. and L.J. Brokin, 1990. Taxonomic revision of the snake genus Echis (Viperidae). II An analysis of the history of study and synonymy. USSR Acad. Sci. Proc. Zool. Inst. Leningrad, 207: 175-192.

Erdos, E.G., 1963. Structure and function of biologically active peptides: bradykinin, kallidin and congeners. Ann. N.Y. Acad. Sci., 104: 1.

Farag, A.A. and A.A. Banaja, 1980. Amphibians and reptiles from the western region of Saudi Arabia. Bull. Fac. Sci., King A. Aziz Univ., 4: 5-29.

Faria, L., E. de Antunes, C. Bon and A. Lobo de, 2001. Pharmacological characterization of the rat paw edema induced by ***Bothrops lanceolatus*** (Fer de Lance) venom. Toxicon, 39: 825-830.

Flower. R.J., 1989. Glucocorticoids and the inhibition of phospholipase A_2. In: Schlelmer, R.P., Clamor, H.N., Oronsky, A.L. (Eds.). Anti-inflammatory steroid action. Basic and Clinic Aspects. Acad. Press, New York, pp: 48-66.

Gasperetti, J., 1988. Snakes of Arabia. Fauna of Saudi Arabia, 9: 169-450.

Goth, A., 1978. Medical pharmacology. The C.V. Mosby Company, St. Louis, Missouri, pp: 766.

Gutierrez, J.M. and B. Lomonte, 1995. Phospholipase A_2 myotoxins from ***Bothrops*** snake venoms. Toxicon, 33: 1405-1424.

Gutierrez, J.M., F. Chaves and L. Cerdas, 1986. Inflammatory infiltrate in skeletal muscle injected with ***Bothrops asper*** venom. Rev. Biol. Trop., 34: 209-219.

Hamblin, A.S., 1994. Cytokines. In: Dale, M., Foreman, J.C., Fan Tai-Ping, D. (Eds.), Textbook of Immunopharmacology, 3rd ed. Blackwell Scientific Publications, London, pp: 179-192.

Issekutz, A.C., 1981. Effect of vasoactive agents on polymorphonuclear leukocyte emigration in vivo. Lab. Invest., 45: 234-240.

Issekutz, A.C. and H.Z. Movat, 1979. The effect of vasodilator prostaglandins on polymorphonuclear leukocyte infiltration and vascular injury. Am. J. Pathol., 107: 300-309.

Johnson, A.R. and E.G. Erdos, 1973. Release of histamine from mast cells by vasoactive peptides. Proc. Soc. Exp. Biol. Med., 142: 1252.

Jorge, M.T., L.A. Ribeiro and J.L. O'Connell, 1999. Prognostic factors for amputation in the case of envenoming by snakes of the ***Bothrops*** genus (Viperidae) Ann. Trop. Med. Parasitol., 93: 401-408.

Kamiguti, A.S., J.L. Cardoso, R.D. Theakston, I.S. Sano-Martins, R.A. Hutton, P. Rugman, D.A. Warrell and C.A. Hay, 1991. Coagulopathy and haemorrhage in human victims of ***Bothrops jararaca*** envenoming in Brasil. Toxicon, 9: 961-972.

Kingston, M.E., 1981. Management of snake bite in Saudi Arabia. King Faisal Specialist Hospital Med. J., 1: 87-94.

Lobo de Araujo, A., F. Radvanyi and C. Bon, 1994. Acidic phospholipase A_2 from ***Bothrops lanceolatus*** venom: purification and molecular, enzymatic and pharmacological properties. Toxicon, 32: 1069-1081.

Lobo de Araujo, A., J.L. Donato and C. Bon, 1998. Purification from ***Bothrops lanceolatus*** (Fer de lance) venom of a fibrino(geno)lytic enzyme with esterolytic activity. Toxicon, 36: 745-758.

Lobo de Araujo, A., A. Olivia de Souza, M., Alice da Cruz-Hofling, C.A. Flores and C., Bon, 2000. ***Bothrops lanceolatus*** (Fer de lance) venom induces oedema formation and increases vascular permeability in the mouse hind paw. Toxicon, 38: 209-221.

Lomonte, B., A. Tarkowski and L.A. Hanson, 1993. Host response to ***Bothrops asper*** snake venom. Analysis of oedema formation, inflammatory cells and cytokine release in a mouse model. Inflammation, 17: 93-105.

Mahmoud, I., 1983. Biochemical and physiological studies on the action of viper venom ***Cerastes vipera*** on mice ***Mus musculus***. In: Masters Thesis (submitted) Fac. Sci. Cairo University, Egypt, pp: 139.

Mandelbaun, F.R., A.P. Reichl and M.T. Assakura, 1975. Some physical and biochemical characteristics of HF_2, one of the hemorrhagic factors in the venom ***Bothrops jararaca***. In: Ohsaka, A., K. Hayashi and Y Sawai, (Eds.). International Symposium on Animal, Plant and Microbial Toxins, 4, Tokyo, 1974. Proceedings of the Fourth International Symposium on Animal, Plant and Microbial Toxins, New York, Plenum Press, pp: 111-121.

Marsh, N., D. Gattullo, P. Pagliaro and G. Losano, 1997. The gaboon viper, ***Bitis gabonica***: haemorrhagic, metablic, cardiovascular and clinical effects of the venom. Lif. Sci., 61: 763-769.

Milani-Junior, R., M.T. Jorge, F.P. de Campos, F.P. Martins, A. Bousso, J.L. Cardoso, L.A. Ribeiro, H.W. Fan, F.O. Franca, I.S. Sano-Martins, D. Cardoso, C. Ide Fernandez, J.L. Fernandes, V.L. Aldrede, M.P. Sandoval, G. Puerto, R.D. Theakston and D.A. Warrell, 1997. Snake bites by jararacucu (***Bothrops jararacussu***): clinicopathological studies of 29 proven cases in Sao Paulo State, Brasil. QJM, 90: 323-334.

Moav, B., Ch. Moroz and A. de Vries, 1963. Activation of the fibrinolytic system of the guinea pig following inoculation of ***Echis colorata*** venom. Toxicon, 1: 109-112.

Mohamed, A.H. and L.Z. Khaled, 1966. Effect of the venom of ***Cerastes cerastes*** on nerve tissue and skeletal muscle. Toxicon, 3: 223-224.

Moustafa, F.A., Y.Y. Ahmed and M.F. El-Asmer, 1974. Effect of scorpion ***Buthus minax*** L. Koch venom on succinic dehydrogenase and cholinesterase activity of mouse tissue. Toxicon, 12: 237-240.

Perales, J., C.Z. Amorim, S.L.G. Rocha, G.B. Domont and H. Moussatche, 1992. Neutralization of the edematogenic activity of ***Bothrops jararaca*** venom on the mouse paw by an antibothropic fraction isolated from opossum (***Didelphis marsupialis***) serum. Agents Act., 37: 250-259.

Rosenfeld, G., 1971. Symptomatology, pathology and treatment of snake bites in South America. In: Bucherl, W., Buckley, E.E. (Eds.). Venomous animals and their venoms, vol II. Acad. Press, New York, pp: 345-403.

Schaeffer, R.C., C. Briston, S.M. Chilton and R.W. Carlson, 1986. Disseminated intravascular coagulation following ***Echis carinatus*** venom in dogs: effects of a synthetic thrombin inhibitor. J. Lab. Clin. Med., 107: 488-97.

Tan, N. H. and G. Ponnudurai, 1990. A comparative study of the biological properties of venoms from snakes of the genus ***Vipera*** (true adders). Comp. Biochem. Physiol., 96: 683-688.

Theakston, R.D.G., 1983. The application of immunoassay techniques including enzyme-linked immunoassay (ELISA) to snake venom research. Toxicon, 21: 341-352.

Theakston, R.D.G. and H.A. Reid, 1983. Development of simple standard assay procedures for the characterization of snake venoms. Bull. W.H.O., 61: 885-895.

Theakston, R.D.G., H.A. Reid and D. Iddon, 1982. Standardization tests for estimation of defibrinating, coagulant, haemorrhagic and necrotizing effects of snake venom. Toxicon, 20: 363.

Tilbury, C.R., M.M. Madkour, D. Saltissi and M. Suleiman, 1987. Acute renal failure following the bite of Burton's carpet viper ***Echis*** coloratus Gunther in Saudi Arabia: case report and review. Saudi Med. J., 8: 87-95.

Trebien, H.A. and J.B. Calixto, 1989. Pharmacological evaluation of rat paw edema induced by ***Bothrops jararaca*** venom. Agents Act., 26: 292-300.

Warrell, D.A., 1993. Venomous bites and stings in Saudi Arabia. Saudi Med. J., 14: 196-202.

Warrell, D.A., 1995. Clinical toxicology of snake bite in Africa and the Middle East/Arabian Peninsula. In: Meier, J., White, J. (Eds.), Handbook of Clinical Toxicology of Animal Venoms and Poisons, Boca Raton, Florida, CRC Press, pp: 433-492.

Warrell, D.A. and C. Arnett, 1976. The importance of bites by the saw-scaled or carpet viper (***Echis carinatus***). Epidemiological studies in Nigeria and a review of the world literature. Acta Tropica (Basel), 33: 307-341,

Warrell, D.A., Davidson, N.McD. and Greenwood, B.M. (1977). Poisoning by bites of the saw-scaled or carpet viper (***Echis carinatus***) in Nigeria. ***Q. J. Med.*** 46(181), 33-62.

Weiss, H.J., L.L. Phillips, W.S. Hopewell, G. Phillips, N.P. Christy and R.F. Nitti, (1973). Heparin therapy in a patient bitten by a saw scaled viper (***Echis carinatus***), a snake whose venom activates prothrombin. Am. J. Med., 54: 653-662.

Williams, T.J., 1979. Prostaglandin E_2, prostaglandin I_2 and the vascular changes in inflammation. Br. J. Pharmacol., 65: 517-524.

Williams, T.J., 1983. Interactions between prostaglandins, leukotrienes and other mediators of inflammation. Br. Med. Bull., 39: 239-242.

Williams, T.J. and J. Morley, 1973. Prostaglandins as potentiators of increased vascular permeability in inflammation. Nature, 246: 215-217.

Williams, T.J. and M.J. Peck, 1977. Role of prostaglandin- mediated vasodilatation in inflammation. Nature, 270: 530-532.