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Pharmacognostic and Biochemical Properties of Certain Biomarkers in Snake Venom

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ABSTRACT

Snake venom is a special liquid which is produced by the poison gland of the poisonous snake. Snake venoms generally consist of a complex mixture of substances, each of which may exhibit one or more distinct toxic actions. Many of these proteins are harmless to humans but some are toxic. Snake venoms effects include, anti-blood coagulation, neurotoxicity, mycotoxicity, nephrotoxicity, cardiotoxicity and necrotoxicity. Snake venom is hemolytic and neuropathic-type venom. The hemolysis venom is more effective than the neuropathic-type venom and it will work almost immediately to the prey. According to traditional system of medicine, snake venom is widely used in various disorders in skin and blood. It performs good antitumor activity. This study reports on its traditional, chemical and pharmacological properties such as antioxidant, anticancer and analgesic activity.

Key words: Snake venom, antitumour, analgesic, coagulation, neurotoxicity, prothrombin, hemotoxic

INTRODUCTION

Snake venoms are generally produced by specialized glands which are related to salivary glands and are toxic to the prey (Kochva, 1987). Snake venoms contain a large number of biologically active proteins and peptides that are usually similar in structure but not identical to that of prey physiological systems. Snake venom is a mixture of different enzymes and having toxic and non-toxic activities, including pharmacological properties. The mechanism of toxin secretion is highly conserved and diversification of matured toxin sequences shows existence of multiple protein isoforms in the venom to adapt within prey environment (Fry, 2005). A duct to the fang base, where it is transported into the victim either by a groove in the fang or through a fang duct delivers the venom, once produced. The marine organisms (Cone snails) deliver their complex venom through a specialized radular tooth that serves as both a harpoon and disposable hypodermic needle (Joseph *et al*., 2010a). Cone snails are large group of recently evolved, widely distributed marine molluscs of the family Conidae. The evolution of conotoxin in the venom of predator snails may be influenced by selective pressures imposed by the nature of the prey, with peptides mixtures from piscivorous, molluscivorous and vermivorous snails exhibiting differences (Olivera, 1997; Ramasamy *et al*., 2011). The action of venom is the combined effect of all components present in the venom and the snakes escape the effect of own toxins due to specific

resistance mechanism and modulation of acetylcholine receptors. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted (Joseph *et al*., 2010b). Many of the most potent snake toxins have evolved highly specific targets, such as the neuromuscular junction or components of the haemostatic system. Phospholipase A_2 or phosphatide acylhydrolase 2, is hemolytic and myolytic present in snake venom which results in damage to cell membranes, endothelium, skeletal muscle, nerves and erythrocytes. It is an enzyme that catalyzes the hydrolysis of the acyl group attached to the 2-position of intracellular membrane phosphoglycerides (Shah *et al*., 2011). Snake venoms are widely used to develop anti-venom vaccines and medicine for rescuing snake venom poisoning patients. Presence of antibacterial molecules in the snake venom that would protect the snakes during feeding. Some of the first reports about antibacterial activity of snake venom was in 1948 and in 1968, involving Elapidae and Viperidae venoms (White, 2000; Glaser, 1948) . Enzymes and proteins are also very important bioactives in snake venom. Apart from enzymatic proteins, antimicrobial peptides have also been purified in recent studies. Cathelicidins isolated from *Bungarus fasciatus* and *Ophiophagus hannah* have reported potent antimicrobial activity against many strains of Gram negative bacteria (Wang *et al*., 2008; Chen *et al*., 2009; San *et al*., 2010). Russel Viper Venom (RVV) X and V enzymes and ecarin from Echis carinatus venom are proteins used for factors X and V and prothrombin determination in blood (Magalhaes *et al*., 1981; Rosing *et al*., 2001). Due to their characteristics, RVV enzymes have been used for the improvement of the detection of von Willebrand disease (Gold *et al*., 2002; Nahas *et al*., 1979) Venom molecules are good examples, such as in homeostasis, where they act as pro- and anticoagulant factors and also as inducers and inhibitors of platelet aggregation(Gornitskaia *et al*., 2003; Markland, 1997; Braud *et al*., 2000). Snake venom has been reported to include antioxidant, antibacterial, hypotensive, cancer suppressive, anticoagulant and analgesic activity. This study was aimed to present an overview of traditional, chemical and pharmacological investigations of bioactives present in snake venom.

COMPOSITION OF SNAKE VENOM

Fresh snake venom is a slightly fishy smell of the egg-like viscous liquid which color is always yellow, green or even colorless. Fresh snake venom is neutral or weak acid and it will become alkaline when it is placed for a long time. Water content 50 to 75%. When exposed to air, fresh venom is easy to produce foam. The composition of the venom is complex. It differs from species to species. The main ingredient is the toxic protein. Almost all venoms are composed of approximately 90% proteins. It contains more than 20 kinds of enzymes and toxins and all sorts of smaller molecules. Some toxins have multiple effects . Snake venoms also contain several peptides. They may vary from presenting neurotoxic (Mion *et al*., 2002; Francischetti *et al*., 1997; Harvey, 2001) cardiotoxic (Tsetlin and Hucho, 2004; Satora *et al*., 2003) or even an inhibitory platelet profile (Fry and Wuster, 2004; Russell, 1980; Rucavado *et al*., 1995; Ducancel, 2002; Morris *et al*., 1995). In addition, it also contains a number of amino acids, carbohydrates, lipids, nucleosides, biological amines and metal ions (Heise *et al*., 1995; Russell, 1980). The most important components are the substances with a cytotoxic effect, the neurotoxins and the coagulants.

CLASSIFICATION OF SNAKE VENOM

Two general types of toxins are known, neurotoxins and hemotoxins. Neurotoxic venom attacks the victim's central nervous system and usually result in heart failure and breathing difficulties. Cobras, mambas, sea snakes, kraits and coral snakes are examples of snakes that contain mainly

neurotoxic venom. After the bite, local symptoms were not obvious, less bleeding, swelling and slight fever. However, within a few hours after injury, the rapid systemic symptoms, patients with anxiety excitement, groaning with pain, difficulty swallowing, difficulty breathing, convulsions, respiratory muscle paralysis and the death will appear. In addition, some scientists are now studying this neurotoxin can be used to treat virus such as the rabies virus. Hemotoxic venom attacks the circulatory system and muscle tissue causing excessive scarring, gangrene, permanent disuse of motor skills and sometimes leads to amputation of the affected area. It can cause rapid swelling of the bite wound, bleeding and pain. The skin will become purplish, black and necrotic. After 6-8 h, it could be spread to the head, neck, limbs and lower back. If the bite wound has not treated effectively within 4 h at last death will occur due to heart failure or shock. The Viperidae family such as rattlesnakes, copperheads and cottomouths are good examples of snakes that employ mostly hemotoxic venom. Some snakes contain venom that contains combinations of both neurotoxins and hemotoxins. Snake venom proteins and polypeptides are classified into superfamilies of enzymes and non-enzymatic proteins. The members of each superfamily show similarity in their primary, secondary and tertiary structures. Among non-enzymatic proteins, superfamilies of three-finger toxins, serine proteinase inhibitors, C-type lectin-related proteins, atrial natriuretic peptides and nerve growth factors have already been well characterized (Zhong *et al*., 2006; Li *et al*., 2005; Ferreira *et al*., 1970). L-Amino acid oxidase, phospholipase A_2, metalloprotease and ribonuclease A are some examples of superfamilies of enzymes in this family (Takasaki *et al*., 1988; Gong *et al*., 1998; Wei *et al*., 2006; Wang *et al*., 2004; Wu *et al*., 2001). Based on the structure, activity and components, crude venom are also classified into cardiotoxin, neurotoxin, cytotoxin and myotoxin (Guinea *et al*., 1983; Barbosa *et al*., 2005).

TRADITIONAL USES OF SNAKE AND SNAKE VENOM

Among the earliest recorded use of snakes in Chinese medicine was the application of sloughed snake skin, described in the Shen Nong Ben Cao Jing (ca. 100 A.D.) It was originally applied in the treatment of superficial diseases, including skin eruptions, eye infections or opacities, sore throat and hemorrhoids. The use of snake gallbladder is first recorded in Ming Yi Bie Lu (Transactions of Famous Physicians; compiled by Tao Hongjing and written around 520 A.D.) which was an update of the Shen Nong herbal with double the number of ingredients. In addition to the gallbladder, the skin (fanpi) and the meat of a pit viper (Agkistrodon halys; fanshe) were used to treat skin diseases, pain and intestinal hemorrhage. There are at least three features of snakes that capture the attention of traditional healers: they have an incredible flexibility and speed, they shed their skin and certain snakes are extremely poisonous when they bite. The flexibility of snakes has suggested that they might be helpful in the treatment of stiffness, for example, arthritis. Two types of snakes, agkistrodon and zaocys, are currently used in several traditional and patent prescriptions for arthritis and they are sometimes soaked in alcohol to make an extract for stiff joints. The speed with which some snakes move indicated to traditional observers that as medicines their substance can move quickly around the body. Snakes are said to treat "wind" syndromes which tend to move around quickly. However, people are also cautioned not to consume snake wine when exposed to potentially pathologic wind, as the rapid movement of the snake medicine may aid the initial penetration of wind. Snakes which shed their skin has suggested that they have a regenerative quality for treating chronic skin problems. As a result, snake skin and whole snake are used in the treatment of skin diseases. This application is similar to the use of sloughed cicada skin for treating skin ailments. Acne, carbuncles, itching skin and psoriasis are

examples of conditions that may respond to snake skin. Snake skin is also considered useful in reducing clouding (nebula) of the cornea, the "skin" of the eyes. Poisonous animals often cause paralysis when they bite and this is due to the presence of neurotoxins. They are then used medically by oral administration (which greatly reduces the toxicity) for the treatment of convulsions (by inhibiting intense muscle contractions). Also, some forms of paralysis are "tonic" in nature, that is, due to overcontraction of muscles and in such cases the nerve toxins can overcome paralysis. Agkistrodon (but not zaocys) is a poisonous snake used for epilepsy and paralysis. Scorpions and millipedes (scolopendra) are used similarly. Anti-convulsive activity is also ascribed to snake skin and cicada skin. In the *Ben Cao Gang Mu* it was said that "Agkistrodon penetrates the bone to expel the pathogenic wind and alleviate convulsion and is the essential material for wind arthralgia, convulsion, scabies and malignant scabies-because it travels everywhere, outward to the skin and inward to the viscera." It was noted in Illustrated Materia Medica that "Agkistrodon has a quicker effect in treating wind syndrome than that of other snakes." Several records in Chinese medical books indicate that snake slough is useful for malignant sores, such as mammary abscess and tumor, boils, carbuncles and furuncles. The slough is usually roasted and then used both internally and topically. Snake bile has long been valued as a tonic, characterized as such by its sweet aftertaste. It is used to make a special health drink at snake restaurants (which are today still found in southern China, Hong Kong and Taiwan). The bile of a snake to be eaten is mixed with some rice wine and consumed before the meal as an invigorating beverage and appetite stimulant. In the treatment of diseases, snake bile is used for whooping cough, rheumatic pain, high fever, infantile convulsion, hemiplegia, hemorrhoids, gum bleeding and skin infections. The antitussive action of bile from *Hydrophis cyanocinctus* (a sea snake) is one-ninth that of codeine when assayed in mice (adult human codeine dosage for treating cough is 20-30 mg). Snake bile is collected in spring and summer when the content of solids is highest. Snake gallbladder is sometimes combined with pinellia or citrus to produce an antitussive and phlegm-resolving powder for treatment of acute bronchitis. Snakes are also used in the treatment of cancer. The small agkistrodon is a common ingredient in modern treatments, especially for leukemia. A combination of *Agkistrodon halys* and *Natrix trigrina* (water snake), in the form of powder (3-5 g per day), it is used as an adjunct to herbal decoctions and drug to treat hepatoma. Snake venom is also sometimes used as medicine; recent research has shown that snake venom may have value in treating cardiovascular diseases and blood pressure. Anticoagulant properties have been identified and are especially prevalent in the vipers.

PHARMACOLOGICAL PROPERTIES

Anticoagulant activity: The crude venom of *Pseudechis australis* shows a dose-dependent anticoagulant action on human blood *in vitro* using computerized thromboelastography. Clot progress parameters (K and α) were affected at low dose levels and had no effect on onset of coagulation parameters (SP, R). At high dose there was a total anticoagulant effect. These results generally shows the anti coagulant effects of venom (Dambisya *et al*., 1995).

Anti-invasive activity: The human glioblastoma cell line (T98G) was treated with contortrostatin or Colloidal Gold-TNF-alpha (CG-TNF-alpha) alone or in combination. Vitronectin and fibronectin-dependent adhesion of untreated and treated glioma cells were studied. Contortrostatin significantly decreased cell adhesion to vitronectin and fibronectin. Contortrostatin binds to T98G integrins in an RGD-dependent manner, whereas protein kinase C (PKC) appears to be involved

in CG-TNF-alpha actions, leading to inhibition of cell invasion. The efficiency of contortrostatin in inhibiting cell invasion was enhanced by combination with CG-TNF-alpha. The combined use of contortrostatin and CG-TNF-alpha may have potential for malignant glioma therapy by effectively inhibiting glioma cell invasion (Schmitmeier *et al.*, 2000).

Anti tumour activity: Cerastes Cerastes Venom (CCV) from the Egyptian desert, at a concentration of 7μg mL^{-1} kills *in vitro* a significant number of mammary tumor virus-induced cells (≈55%) from mouse within a period of 48h. CCV (1μg/mouse), administered once per week directly into growing tumors for a period of 4 weeks, was found to reduce tumor load by 54% and as a consequence the CCV-treated mice lived for more than 35 days longer than untreated mice. Histological and ultrastructural examination of the cells and tumors, conclude that necrosis is most likely the underlying mechanism by which CCV inhibited the growth of tumor cells both *in vitro* and *in vivo* (El-Refael and Sarkar, 2009). The diluted snake venom (*Hydrophis spiralis)* exhibited a significant antitumor activity against EAC (Ehrlich Ascites Carcinoma cells) and detrimental toxicity on the liver of the treated animals (Karthikeyan *et al.*, 2007).

Antimicrobial activity: A panel of eight PLA_2 myotoxins purified from crotalid snake venoms, including both Lys 49 and Asp 49-type isoforms were found to express bactericidal activity, indicating that this may be a common action of the group IIA PLA_2 protein family. A series of 10 synthetic peptide variants, based on the original C-terminal sequence 115-129 of myotoxin II and its triple Tyr→Trp substituted peptide p115-W3, were characterized. *In vitro* assays for bactericidal, cytolytic and anti-endotoxic activities of these peptides suggest a general correlation between the number of tryptophan substitutions introduced and microbicidal potency, both against Gram-negative (*Salmonella typhimurium*) and Gram-positive (*Staphylococcus aureus*) bacteria (Santamaria *et al.*, 2005). Crude venoms from Viperidae species demonstrated significant inhibition zones between 6.6-12.5 mm. *Calloselasma rhodostoma* showed the largest inhibition zones between of 10.2-12.5 mm (San *et al.*, 2010).

Analgesic activity: Crotamine, a neurotoxic protein has been purified from *Crotalus durissus terrificus* venom by gel filtration on Sephadex G-75. When injected (i.p. or s.c.) in adult male Swiss mice (20-25 g), it induced a time-dose dependent analgesic effect which was inhibited by naloxone, thus suggesting an opioid action mechanism. Extremely low dose (133.4 μg kg^{-1}, i.p., about 0.4% of a LD_{50}) is more potent (30-fold) than morphine (w/w) as an analgesic (Mancin *et al.*, 1998).

Hemostatic activity: Venom proteins of the Viperidae snake family exert often with a narrow specificity, activating, inactivating or other converting effects on different components of the hemostatic and fibrinolytic systems. Purified snake venom proteins have become valuable tools in basic research and in hemostaseology. "Procoagulant" as well as "anticoagulant" venom components have been identified in invitro tests. Smaller doses of procoagulant venom components applied to large organisms as in the case of snake-bite accidents in humans may cause a consumption coagulopathy with localized or generalized bleeding. Highly purified, specific fibrinogen coagulant venom proteinases are used in human medicine to produce therapeutic defibrinogenation (Meier and Stocker, 1991).

Hypotensive activity: Vascular endothelial growth factor (VEGF165) exhibits multiple effects via the activation of two distinct endothelial receptor tyrosine kinases: Flt-1 (fms-like tyrosine

kinase-1) and KDR (kinase insert domain-containing receptor). KDR shows strong ligand-dependent tyrosine phosphorylation in comparison with Flt-1 and mainly mediates the mitogenic, angiogenic and permeability-enhancing effects of VEGF165. They also induced strong hypotension on rat arterial blood pressure compared with VEGF165 *in vivo* (Yamazaki *et al*., 2003).

Anti-Thrombotic activity: Snake venom act selectively on different blood coagulation factors, blood cells or tissues. Venom proteins affect platelet function in particular by binding and blocking or clustering and activating receptors or by cleaving receptors. They may also activate protease-activated receptors or modulate ADP release or thromboxane A_2 formation. L-amino acid oxidases activate platelets by producing H_2O_2. Many of these purified components are valuable tools in providing new information about receptor function and signaling (Clemetson *et al*., 2007).

CONCLUSION

The present study shows the pharmacological and traditional properties of various bioactive compounds present in the venom. Snake venom is a good antibiotic. It is also a good medicine in traditional system, recent research has shown that snake venom may have value in treating cardiovascular diseases, reducing blood pressure and also have anticoagulant properties. Snake venoms also contain several peptides. snake venom peptides have the potential for practical and therapeutic use. However, enzymes and proteins are also very important as some of them are described as laboratory diagnosis reagents. However, more Clinical and Pathological studies should be conducted to investigate the active principles present in snake venom.

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