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Snake Venom: A deadly toxin with Therapeutic potential and immunization

[1]Gourab Biswas, [2]Anamika Saha*, [2]Sujayita Mazumder, [1]Arijit Seal, [3]Anwesha Naskar

[1] Department of pharmaceutical Technology, Brainware University, 398, Ramkrishnapur Road, near Jagadighata Market, Barasat, Kolkata, Pin-700125, West Bengal

[2]The Neotia University, School of pharmacy, Jhinger Pole, Sarisha, Dimond Harbour, Pin – 743368, West Bengal

[3]Nibedita pharmacy College,Bagmara, P. O-D/Parashpur, Murshidabad, Pin - 742305.

*Corresponding author:
Address: Ranaghat, Nadia, West Bengal
Email id: sahaanamika780@gmail.com

Contact Number:8597897360/ 7872064057

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ABSTRACT:

Venom is a biochemical mixture of proteins that have both positive and negative aspects. Snake venom is used in the production of a number of pharmaceutical formulations. It plays a vital role in medical field including a large number of drug formulation and also production of genetically engineered venom provide an effective result in a shorter period of time. Once snake bites a person, the person may die immediately due to blockage of nervous system or may take some time to envenom including renal dysfunction, neural blockage, toxicity and hemorrhagic diathesis. On the other hand, venom is considered as a blessing that treat many diseases like blood sugar control, cancer therapy and used in preparation of therapeutics for smallpox or wound healing. Venom is available in various forms at different price depending on the type of venom to be taken and availability of rare to rarer reptile to produce the venom. In fact, however in most of the cases, the venoms of fatal snakes are found to be clinically and diagnostically potential and active.

KEYWORDS: Venom, fangs, antibody, antidote, Immunosorbent Assay

INTRODUCTION :

Snake venom is basically a glandulous mucous that snakes secret to inactive or paralyze their pray and to absorb them before digestion. Venom acts as both, a life-threatening toxin and life-saving medication as well. Snakes also use venom as a defensive toxin to save themselves. Snake venom acts as versatile therapeutics including its action on nervous system, circulatory system, muscular system and cardiovascular system.Venom is sparkled by their teeth known as **fangs** from oral gland. Venom tastes as sugary water consisting of a number of chemical constituents along with small peptides, proteins, amino acid, lipid, carbohydrate, nucleic acid and other pharmacologically active molecules. Snake venom is found to be a blood coagulating agent in many cases.(1)

Venom is a fundamental biochemical complex made up of a mixture of enzymatic and non-enzymatic proteins. It has several applications that help advance medical science by enabling various scientific studies for the benefit of medical technology and society at large. Conversely, though, venom can also be used as bio-weapon to fight against and provide self-defense.(2)

Snakes are thus a jewel contribution of nature that needs to be preserved and taken care of properly.

Snake bite is very common issue in rural areas. Snakes can be categorized based on effective of their toxicity, route of administration of venom and their habitat.(3)

Systemic snake envenomation in humans results in the injected venom being absorbed and entering the systemic circulation, which can have a range of clinical effects depending on the particular snake and the venom components. The families Elapidae (cobras, kraits, mambas, Australian dangerous terrestrial snakes, coral snakes, and sea snakes) and Viperidae (true vipers and pit vipers) are responsible for most cases of serious human envenomation.(42) Venoms can induce a broad range of local and systemic effects due to the diversity of physiologically active proteins they contain. Tissue necrosis and localized discomfort and edema are among the local consequences. Numerous myotoxic and cytotoxic substances, including SVMP, cytotoxins, and non-catalytic PLA2 mycotoxins, can induce severe tissue necrosis and may necessitate debridement and amputation. The systemic effects coagulopathy, neurotoxicity, acute renal damage, and myotoxicity—have the potential to be fatal. The most common systemic envenoming disease in the world is probably venom-induced consumptive coagulopathy, which can be produced by a variety of vipers and Australasian elapids.Neurotoxicity, mainly following an elapids bite, is the paralysis of the facial, bulbar, respiratory, and limb muscles, which can result in respiratory failure and death.(4)

Research studies on snakebite have tested snake venom in blood, despite the fact that such tests are rarely feasible in a clinical setting. Numerous test techniques have been developed to identify snake venom and venom antigens (toxins) in biological materials. Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are two of these techniques. To identify the species of snake involved in envenomation and evaluate the degree to which antivenom binds to free venom, Theakston and Research have primarily utilized venom assays.First venom-specific ELISA developed in 1977 by Theakston and coworkers had good sensitivity and specificity, with the possible exception of some circumstances where cross-reactivity with closely related

snake species may occur. It has been observed that the limits of detection for a toxin or venom from snakes range from 0.1 to 20 mcg/L.[36]The stated coefficients of variability for intra- and inter-assays are 1 to 20% and 4 to 20%, respectively. ELISA is less time-consuming, more cost-effective, easier to use, and poses no health risks while handling radioisotopes than RIA does. Although the ELISA approach analyses skin swabs for venom rather than blood, it has been applied to build snake venom detection kits that act as diagnostic tools to help clinicians choose the proper antivenom.[5]

The mainstays of treatment for snake envenomation are supportive care and the delivery of certain antivenoms tailored to the species or kind of snake in question. Snake antivenoms are defined as antibodies or antibody fragments derived from the plasma of animals (often sheep and horses) inoculated with snake venom. Intravenous injections of antibodies (IgG immunoglobulin) or antibody fragments (F(ab')2 or Fab) bind and neutralize free venom in the patient's plasma to counteract or stop additional deleterious effects. However, there is also a risk of hypersensitivity reactions since animal-derived antivenoms include foreign proteins. Severe anaphylaxis may result from these reactions, which can also cause extremely hazardous cutaneous and multiorgan reactions. Improved dosage and antivenom use may increase the cost-effectiveness of antivenoms, which are nevertheless costly and frequently scarce in some areas.[6]

VENOM CLASSIFICATION:

Venoms can be classified on the basis of their site of action and toxifying mechanism.

Table 1. Classification based on mechanism of action[7]

Type of venom	Responsible for destruction/malfunctioning of:	Adverse effect on target cell or tissue	Examples
Hemotoxic	Circulatory system or blood	Platelet assembly formation,[34] Disorganized blood clot	Rattlesnakes, Russell’s viper, Copperheads
Cytotoxic	Cell functioning	Unnatural growth or death of healthy cells	Cobra, Elapids
Neurotoxic	Nervous system	Disturbs impulse transmission	Black mamba
Proteolytic	Essential protein	Hamper various metabolic activities	Red bellied black snakes

CHEMISTRY OF SNAKE VENOM:

Snakes use their venom to control their prey and enable them to live in their natural environment[32]It is well recognized that venom is a highly toxic mixture of many chemicals including lipids, proteins, peptides, nucleosides, carbohydrates, and amino acids.[44]

Snake venoms consist of various types of toxins. The pathophysiology of snakebite envenomation is determined by the ratio and mix of each toxin, as several toxins found in snake venom work in concert with one another. Different clinical manifestations are caused by the chemical makeup of venom from vipers and elapids. While venoming by vipers usually results in myotoxicity and hemotoxicity, venoming by elapids usually causes neurotoxic, cytotoxic, and cardiotoxic symptoms.[8]

Although there are many exceptions and significant diversity at the species level, secreted phospholipases A2 (PLA2s) and three-finger toxins (3FTxs) are frequently major constituents and play a dominant role in the action of the venom. Elapid venoms are primarily composed of peptides and proteins from seven families.For instance, there are noticeable variations in the makeup and function of these poisons in the venom of certain Australian snakes as well as snakes belonging to the Dendroaspis genus, or mambas.[37,40]The latter have a very low percentage (<6%) of 3FTx, while the former do not contain PLA2. It's interesting to note that these snakes all have quite strong venoms.[9]

An average of 6% of elapid venom is made up of additional toxins, such as L-amino acid oxidases (LAAOs), snake venom serine proteases (SVSPs), and snake venom metalloproteinases (SVMPs). A class of serine protease inhibitors with the Kunitz domain fold is known as the Kunitz-type peptides.[41,43]Their effectiveness and selectivity in inhibiting K+ channels are well recognized, and they typically comprise 5% of elapid venom. In mambas, kinitz-type peptides are highly frequent. The remaining identified protein families are found in smaller amounts.[10]

Nine protein families' worth of poisons are mostly found in viperid venoms. Once more, there are a great deal of outliers as well as significant variability within specific species and subspecies. The PLA2, SVMP, and SVSP toxins are predominant in the majority of species, accounting for approximately 70% of the total venom proteome. Despite having a considerable percentage of sequence identity with the PLA2s of elapids, many of which are neurotoxic, the majority of viperid PLA2s are myotoxic. Lesser quantities (4–7%) of other toxins include C-type lectins, LAAOs, natriuretic peptides, and C-type lectin-like proteins.[11]

Since each species' venom may contain hundreds of proteins, enzymes, and peptides, this analysis merely scratches the surface of snake venom complexity.

BENIFITS OF VENOM:

As already discussed, a number of pharmaceuticals and other essential products are derived from snake venom. Many toxins from snake venom are investigated, researched and formulated into drugs for the treatment of conditions such as cancer, hypertension and thrombosis. Snake venom significantly lowers the blood pressure in human victims and experimental animals.[12]

- **Drugs from snake-venom**

Captopril:

Captopril was the first and most well-known medicine based on a snake venom component to be created successfully, coming out in 1975. John Vane, a Nobel Prize laureate, made the discovery, and Squibb, a major pharmaceutical company, later made it available for purchase. The medication mimics the effects of a peptide that increases bradykinin. Extracted from the venom of the Brazilian arrowhead viper Bothrops jararaca, it works by inhibiting the angiotensin converting enzyme, which is responsible for converting angiotensin I into angiotensin II. This makes it an effective treatment for hypertension and cardiovascular disease. The drug was awarded FDA approval in 1981 and is used to treat high blood pressure, diabetic renal disease, and heart failure following myocardial infarction. The drug has been developed across multiple generations.(12)

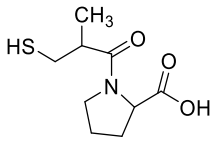

Figure 1. Chemical structure of Captopril

Aggrastat (Tirofiban):

Since the introduction of captopril, snake venoms have grown to be a significant source of bioactive chemicals that can be used to create new medications. Aggrastat (Tirofiban), an antiplatelet medicine based on snake venom disintegrins, is sold.(40)Originally developed by Merck, agrastat is currently sold by Medicure Pharma in the US and Correvio International outside of US. Aggrastat was created to reduce the incidence of thrombotic cardiovascular events, including as heart attacks. This antiplatelet drug belongs to the class of inhibitors of platelet glycoprotein (GP) IIb/IIIa. Its invention was based on the RGD sequence (Arg-Gly-Asp) motif found in snake venom disintegrins isolated from the venom of Echis carinatus. Originally derived from the venom of viperids, which commonly possess the integrin-binding RGD motif, disintegrins are a class of low molecular weight, cysteine-rich proteins. On May 14, 1998, the FDA authorized the medication, which is used to treat heart attack victims.(13)

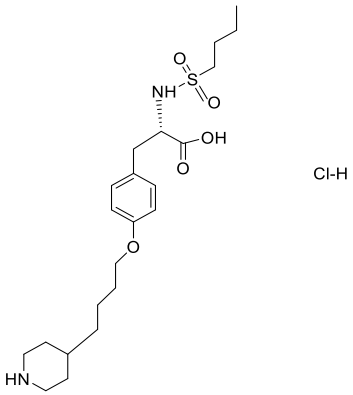

***Figure 2. Chemical structure of* Aggrastat (Tirofiban)**

Integrilin (Eptifibatide):[30]

Integrili was created by Millennium Pharmaceuticals, which is now a division of Merck and Takeda Pharmaceuticals, and was co-marketed by Schering-Plough. Acute coronary syndrome patients, especially those having percutaneous coronary intervention, receive an injection of integrilin to lower their risk of developing another heart attack or passing away.Integrilin is a peptide that resembles the glycoprotein (GP) IIb/IIIa inhibitor barbourin, which is found in the venom of the Southeastern pygmy rattlesnake (Sistrurus miliarus barbouri). The role of the GP IIb/IIIa integrin in mediating platelet aggregation is crucial. As opposed to Aggrastat, Intergrilin was made using the disintegrin's KGD sequence (Lys-Gly-Asp) from the venom of the Southeastern pigmy rattlesnake. In 1998, the FDA approved the medication integrilin, which is used to treat acute coronary syndrome.[14]

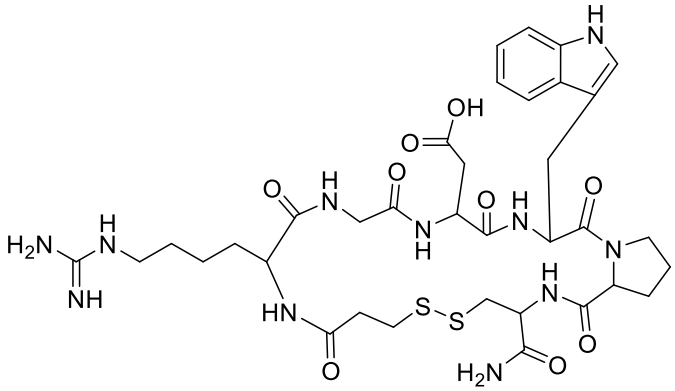

Figure 3. Chemical structure of Eptifibatide

Defibrase/Reptilase:

Although Defibrase/Reptilase (Batroxobin) has received approval for usage in other nations, it has not received clinical approval in the US. A thrombin-like serine protease enzyme called batroxobin was found in the venom of the Bothrops atrox and Bothrops moojeni subspecies of snakes.[16]

Batroxobin:

Batroxobin converts fibrinogen into fibrin with great force by releasing fibrinopeptide A from fibrinogen. Outside of the US (mostly in China), bravosis is used to treat a range of conditions, including as myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis, and postoperative bleeding. The Brazilian snake Bothrops atrox produces hemocoagulase in a similar way. [17]It has been used in plastic surgery, abdominal surgery, and human vitrectomy.

Exanta:

Exanta (Ximelagatran) is an anticoagulant thrombin inhibitor that is extracted from cobra venom and has been used as a thrombin inhibitor as well as a blood thinner.(15)

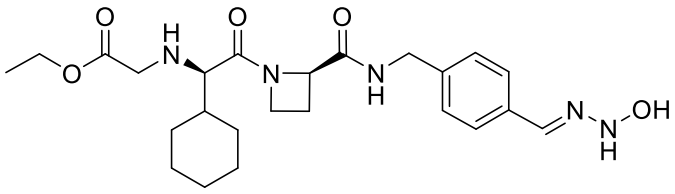

Figure 4. Chemical structure of Ximelagatran

Bivalirudin:

Patients receiving percutaneous coronary intervention for acute myocardial infarction are managed and treated with bivalirudin. This medication belongs to the class of direct thrombin inhibitors. When used with aspirin, bivalirudin reduces the blood's capacity to clot and helps shield blood arteries from dangerous clot formation. Patients undergoing specific heart and blood vessel operations, such coronary angioplasty, are administered this medication. (18)

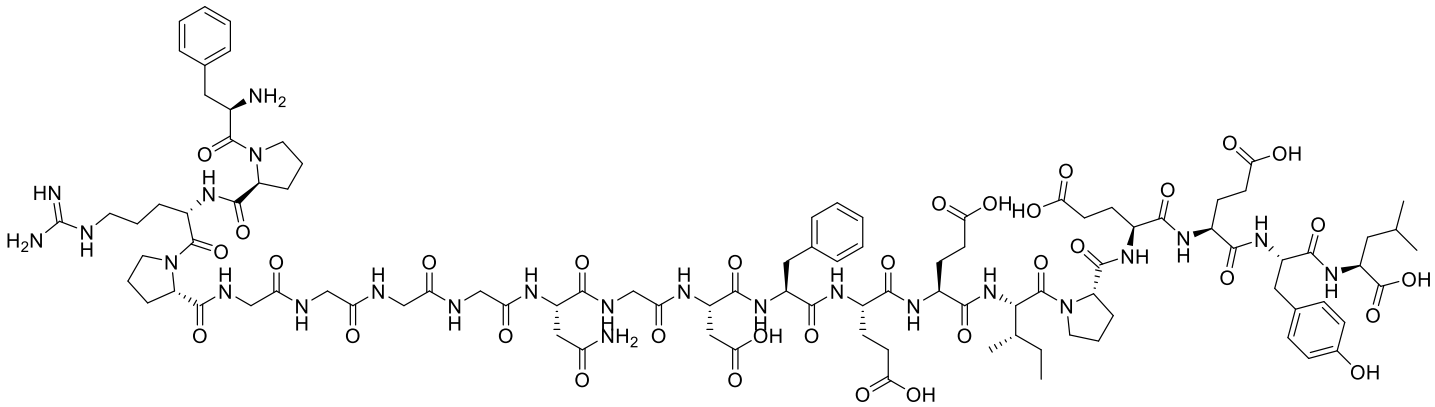

Figure 5. Chemical structure of Bivalirudin

Enalapril:

Enalapril is a drug that is used to treat congestive heart failure and hypertension. It is an inhibitor of the angiotensin-converting enzyme. This activity describes enalapril's actions, side effects, and indications. Enalapril is a useful medication for treating hypertension and other conditions.(20)

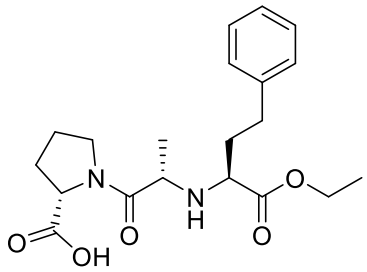

Figure 6. Chemical structure of Enalapril

Tirofiban: One antiplatelet medication is tirofiban. It works by stopping specific blood cells from adhering to one another, hence decreasing the likelihood of a dangerous clot forming. This medication should only be administered by your physician or under their close supervision. This is available in the form of solution.[19]

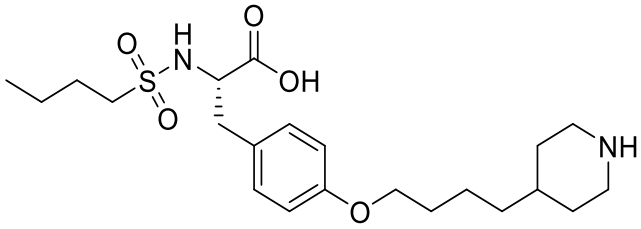

Figure 7. Chemical structure of Tirofiban

Ziconotide: A more recent intrathecal analgesic drug for treating chronic pain is ziconotate. The FDA has approved it for the treatment of severe chronic pain in patients who are not responding well to intrathecal morphine or systemic analgesics.

- **Beauty Products preparation:**[21]

  This deadly toxin is well known for its skincare treatment and different cosmetics therapy. Venom acts as an anti-ageing agent by preventing wrinkles on skin by relaxing the facial muscle. Snake venom facial treatment is a vividly used skincare worldwide.

  Venom is not only effective against pre-existing wrinkles but also prevent formation of new wrinkles. A number of marketed serumsare available that helps in expression of smooth appearance of skin.

- **Therapeutic application:**[22,23,28]

  Pharmaceutical preparations like ointment and medicated oil are widely available nowadays. It is used in the treatment of allergic reaction.

  1. **Fibrinogenolytic and fibrinolytic activity**:

     Snake venom enzymes remove fibrinogen from the circulation without converting it to fibrin. Venoms with anticoagulant properties are extensively studied for possible medical applications.[38]The drug Aggrastat (tirobifan) was developed from a compound in the venom of the saw-scaled viper (Echiscarinatus), and issued as an antiplatelet drug (glycoprotein IIb/IIIa inhibitors).

2. **Anti-Cancer activity:**[30]

   Venom is known to be used as an important component for preparation of many anti-cancer drugs due to its cytotoxic effect on malignant cells by controlling abnormal cell proliferation.

3. **Cardiotonic:**[24,25]

   Some venoms have been found to have blood thinning properties that can prevent platelet aggregation to control clotting. One of the most commonly used ACE inhibitors, Captopril, whose active ingredient was derived from snake venom.

4. **Anti-rheumatic activity:**[35]

   Rheumatoid arthritis is a chronic auto-immune disorder in which it attacks its own immune system erroneously assaults own body's tissue mistakenly. Venom is reported to reduce pain and inflammation of joints helping in curingRA. It contains a proteinases effect, that helps in preventing inflammation and immune complex causing arthritis by increasing the production of non-inflammatory cytokines.

TREATMENT TO ENVRNOM:[26,33]

As envenoming is a major issue worldwide, it is very necessary to develop a therapy, process or any pharmaceutical formulation for the venom to be neutralized when injected in a healthy animal. Multiple formulations are available in market to treat envenoming. CroFab is the most commonly used drug. Though these drugs are capable to prevent damage due to envenoming but are not able to reverse the damage back that had already done by venom. [18]

- **First aid:**

  Whenever a snake bites, immediately enclose or immobilize the affected portion to prevent spreading of venom to the rest of the body. Wash out the affected area in domestic way using soap and water.

  Use a fresh dressing that is dry to cover the bite.

- **Antibody and vaccination:**[27]

Proteins called antibodies protects our body against foreign substances or antigens. Antibodies, which are generated by the immune system, attach themselves to these foreign chemicals and drive them out of body. Antibody is a Y-shaped structure, can also be referred to as immunoglobulin.

Following vaccination, some immune cells called B lymphocytes, which are in charge of defending against illness—identify the antigens in the shot. The B cells respond as body is invaded by the infectious agent. They proliferate to create a vast army of identical cells that are capable of reacting to the vaccine's antigens. [37] Following that, the cloned cells develop into one of two kinds of cells, either memory B cells or plasma cells.

- **Anti-venom production by immunization:**[29]

First, a huge, hyperimmunized animal is given a minimum dosage of venom that has been expertly extracted from a snake, taking care to avoid harming the host animal. When a foreign particle or antigen enters into the body, it starts creating antibody against the given venom to fight. Periodically dose of injected venom is increased as the animal become tolerant and immunized to it. Greater the dose injected; more will be the number of antibodies produced.[19]

Once, a considerable number of antibodies are produced, these are to be cultivated and cultured by sampling blood containing required antibodies that are separated out from blood undergoing different purification process. [39]From desired antibody, IgG is isolated, from which again F(ab)'2 is purified out. This fragment IgG is the core component for antidote of venom.

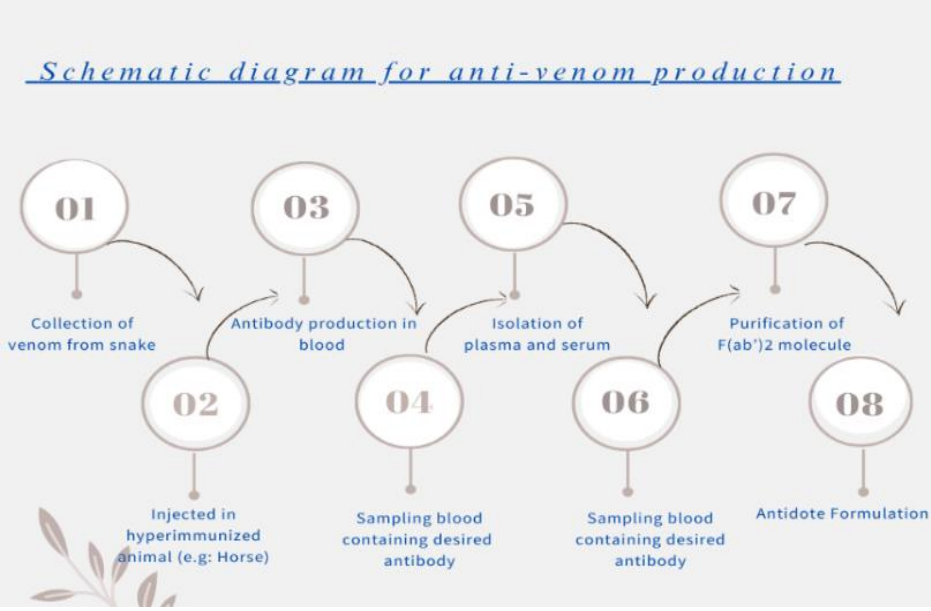

Figure 8. Schematic diagram for anti – venom production

CONCLUSION:[31,34]

For a very long time, snakes were thought to be some of the most dangerous and destructive creatures on Earth. Fortunately, snake-derived anti-venom formulations have had a major impact on the pharmaceutical sector.Since snake venom can be utilized to treat venom, create genetically modified anti-venom, and develop novel medications, studying snake venom is one of the most difficult tasks nowadays. Because venom is expensive to purchase, researchers are working to find more affordable ways to use it for a range of medical conditions. They are also attempting to lower the cost and increase the accessibility of venom for everybody. I'm praying for a pleasant and satisfactory conclusion soon.[22]

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Conflict of interest:

No conflict

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