'The Serpents' Grace

Chitra Nair*
BITS Pilani Goa Campus, Goa
Email: chitranair04@gmail.com

To science I bow, for in its magnanimity, I found my humility.

The summer of 2015 was an incredible time for me. With India's recent exit from the ICC World Cup, I had nothing much to do and yet a lot to look forward to. I had just bid farewell to my short-lived stint in the roaring corporate sector to pursue a career in scientific research. Fuelled by the confidence that's characteristic of theearly 20s, I was looking for what they call 'fulfilment'.Patience, not my strongest suit then, was fading. Desperation peaked and 'where to begin' continued to be my biggest dilemma. This is whenI saw an advertisement for a research fellow which caught my attention. 'Study of anticoagulants and platelet aggregation inhibitors from Indian cobra venoms' it said. I wasted no time in perusing the background of this research topic and two hours later, I had made up my mind.

That's where my journey began and this is my story.

About five centuries ago, when Paracelsus, a man with an exemplary knowledge across a myriad of disciplines, amalgamated his wisdom in chemistry and biology, it gave birth to a promising branch of science – Toxicology. Honoured as the 'Father of Toxicology', his famous quote, "the dose makes the poison" pioneered the search for therapeutics from toxins.

Out of all the toxins studied, what makes snake venom toxins so intriguing is not just the enigma associated with them but also the wealth of life-saving molecules present in them.

* Ms. Chitra Nair, Ph.D. Scholar from Bits Pilani Goa Campus, Goa, is pursuing her research on "Blood Coagulation and Platelet Aggregation Inhibiting Toxins of NajaNaja (Indian Spectacled Cobra) Venom." Her popular science story entitled "The Serpents' Grace" has been selected for AWSAR Award.

Snakes, especially cobras, have been a vital element of mythology and culture for several hundred years. While greatly feared, these ophidians are also hugely revered and even worshipped in some cultures. Despite their lethality, snake venoms are comprised of some biomolecules that can serve therapeutic purposes too. Snake venoms are categorised based on the area they target in their prey: Neurotoxins, affecting the nervous system; Cytotoxins, affecting the cells; and Hemotoxic, affecting blood cells. Their pathophysiology resulting in bleeding disorders makes snake venom a goldmine for anticoagulants and antiplatelet agents.

In the event of an injury, clotting of blood is vital to prevent excessive loss of blood. However, formation of an undesirable clot, called an embolus, impedes the smooth flow of blood through the blood vessel by obstructing its path. This embolus could travel through the blood vessels to and lodges itself in other parts of the body, mostly the leg or the arteries of the lungs, which can be fatal. Despite the availability of many synthetic anticoagulants like Heparin, Streptokinase, Urokinase, etc., the accompanied side effects press the need to look for alternative sources of these drugs. The goal is to find a drug, which can dissolve blood clots or prevent unnecessary clotting by drawing a perfect balance between haemostasisand haemorrhage. This can be achieved by exploring the mechanisms of blood coagulation and identifying the major arsenals that need to be targeted in manipulating and regulating haemostasis.

The process of blood coagulation is akin to a chain reaction and hence called 'The coagulation cascade' and is initiated when collagen,a protein found in skin, is exposed in the event of an injury. Following this, a battery of chemicals, called clotting factors, are recruited at the site to draw the attention of the surrounding platelets and direct them to the injury site to achieve the three phases of haemostasis:

Binding of platelets at the site of the injury and to each other,

Formation of Fibrin sheets, (a protein derived from the plasma protein Fibrinogen), which encapsulate all the bound and aggregated platelets into a haemostatic plug, and

Gradual dissolution of the clot which promotes wound healing.

Cobra venom mainly affects the nervous system, resulting in respiratory failure and eventually the death of the prey. This points to the fact that cobra venom mainly consists of neurotoxins but, in our laboratory, we have found that it possesses some biomolecules that show potential as an anticoagulant.

My research is aimed at finding drug leads from *Najanaja* (Indian spectacled cobra) venom that can be used to developanticoagulants and antiplatelet agents by being potent at lower dosages with minimal side effects. My work is designed to achieve four milestones:

Finding an anticoagulant/antiplatelet protein from the Indian spectacled cobra venom and purifying it,

Characterisation of the protein(s) to ascertain their activity and potential,

Determining the effect of these proteins on human cells under laboratory conditions, and

Determining the mechanism by which the protein(s) exert their physical effect.

The venom was received in a dry, powdered form, devoid of all the moisture contentso that its biological activity is preserved. This was separated into its constituent proteins by ion exchange

chromatography that works one simple principle, opposites attract. The proteins present in the venom were separated on the basis of the charge they possess. The separated proteins, called fractions were then collected and subjected to a multitude of biochemical tests. The purity of these fractions is examined by Polyacrylamide gel electrophoresis (PAGE), which involves passing the protein fractions through a gel, under the influence of an electric field, which allows for separation into its constituent proteins on the basis of their mass and charge. The protein fractions were assessed for their fibrinogenolytic activity and the result was viewed using PAGE. Fibrinogen, a protein found in the plasma of blood, exhibits three bands on PAGE. Three of the protein fractions, – let's call them X, Y and Z – obtained from chromatography showed fibrinogenolytic activity. This was manifested by the digestion of one or more of Fibrinogen's bands, thus rendering it unavailable for cleavage into Fibrin sheets. The next step was to determine if these proteins had any effect on platelet aggregation that is induced by chemicals like ADP and collagen, also called agonists. For this, human blood was treated with X, Y and Z, followed by treatment with the agonists. On the surface of platelets certain protein molecules called receptors are present that facilitate the binding between them to form a clump of platelets. Proteins with antiplatelet activity cap these receptors, thus preventing the platelets from aggregating. The degree of platelet aggregation was measured by an electrical phenomenon called impedance. The effect of X, Y and Z on platelet aggregation was determined in comparison with the two standards: blood treated only with the agonist which showed maximum platelet aggregation and consequently, the highest impedance and untreated blood which showed the least impedance. Interestingly, only X and Y were found to effectively inhibit platelet aggregation and this activity was seen to increase with an increase in dosage. But, Z did not show any significant effect on platelet aggregation. This observation suggested that X, Y and Z target different components of the coagulation cascade.

While these assays were intended to prevent the formation of a clot, studying the effect of these protein fractions on preformed clots demanded equal attention. This facet of the challenge was studied by treating induced clots with X, Y and Z. The proteins that have fibrinolytic activity dissolve the clots by degrading the fibrin sheets which hold the clot together. The standards of comparison employed here were Fibrin treated with saline (which failed to dissolve the clot even after 48 hours) and fibrin treated with a known anticoagulant like Urokinase (which dissolved the clot in a few minutes).While proteins X and Y were found to dissolve the clot in 120 minutes, Z achieved this feat in 90 minutes, thus validating their immense anticoagulant potential.

It was also observed that when brought in contact with untreated blood, X, Y and Z were all found to significantly prolong its clotting time to 22 minutes, a process that would normally take 5 to 8 minutes.

While these protein fractions show promising potential as anticoagulant and antiplatelet drug leads under simulated conditions, one has to bear in mind the challenges that are likely to accompany their introduction into living cells. For this purpose, the effect of these proteins on cultured cells will be studied. The degree of invasiveness of the fractions, their cytotoxicity as well as the minimum dosage at which they harm the cells will be investigated.

The final leg of my research will involve cumulating all the results generated from the laboratory experiments to erect a framework based on which the correlation between the physical structure of these proteins and their biological activities,and the mechanisms by which these proteins exert their activities can be comprehended.

Although the work towards the completion of my PhD will end with the fulfilment of the aforementioned objectives, the journey of research is a never-ending one. Everyday, mankind faces a new challenge that prompts the curiosity of a hundred zealous minds. They say 'curiosity killed the cat' but I think, curiosity is the cornerstone of science. It is, in fact, the only path that helps us unravel the mysteries of this big wild world that we call home.