Snake Venom Research

A. Antivenom/Alternative Antivenom Studies

Research Projects

1. Identification of antigenic proteins from the venom of Malaysian venomous snakes using tandem mass spectrometry

Investigators: Dr. Syafiq Asnawi, Prof. Iekhsan Othman, Assoc. Prof. Rakesh Naidu

Project Brief:

Information from venom proteomic (venomic) studies may lead to the improvement of antivenom through the aspects of its design, manufacturing and control. Information on venom composition, variability and immunological cross-reactivity are of utmost importance to future research on effective antivenom. Additionally, antivenom proteomics (antivenomics) study between the cross reactivity of venom and antivenom from non-conventional pairings may also prove useful in a clinical scenario where certain antivenom supplies are lacking or clinical manifestations of snake envenomation (that are often times too similar even between different snake families) are the only available information for clinicians to make a provisional diagnosis. The identified protein families in common between the venom-antivenom interactions of a family of snakes typically have specific signs and symptoms that can guide a clinician to make an educated guess of the culprit species and thus antivenom selection.

Our initial study using king cobra (*Ophiphagus hannah*) crude venom and king cobra antivenom using the agarose gel assay has revealed several antigenic protein families that interacted with the antivenom such as L-amino acid oxidase and neurotoxins. These findings demonstrated that our approach using the agarose gel assay were successful in revealing important antigenic toxins that is responsible for the production of antivenom in the host organism. Therefore, we aim to identify the antigenic proteins via the venom-antivenom interaction assay from at least 6 major venomous snake species in Malaysia with medical importance e.g *Calloselasma rhodostoma* (Malayan pit viper), *Cryptelytrops purpuromaculatus* (Mangrove pit viper), *Tropidolaemus wagleri* (Temple pit viper), *Naja sumatrana* (Spitting cobra), and *Naja kaouthia* (Monocled cobra). The study will provide valuable information on the potent antigenic proteins from all snake venom and may contribute towards the development of antivenom with high efficacy.

2. Preliminary investigation on the potential anti-venom activity from Malaysian common herbs and medicinal plant on the venom of Malaysian venomous snakes

Investigators: *Dr. Syafiq Asnawi, Prof. Iekhsan Othman, Assoc. Prof. Rakesh Naidu, Dr. Md. Farooq Shaikh*

Project Brief:

Snake envenomation is a risk that every Malaysians and visitors to the country are facing. It is estimated that Malaysia has up to 650 snakebites per 100, 000 populations per year with a higher number of mortality and morbidity recorded from the northern states of peninsular Malaysia. The only treatment against snake envenomation is antivenom produced from horses, however, it is associated with many drawbacks including anaphylactic reactions. Extracts from some medicinal plants have been described to have potential antivenom activity but none has been investigated using common herbs/medicinal plant from Malaysian on our local venomous snakes. In this proposed study, we aimed to investigate the potential antivenom reaction from sources such as ginger (*Zingiber officinale*), and Light blue snakeweed (Pokok ekor ular, *Stachytarpheta jamaicensis*) on the venom of Malaysian common cobras (*Ophiophagus hannah, Naja kaouthia*). Potential antivenom activity will be investigated by using simple agarose gel approach. Proteins that interact with the extract will form a white precipitate on the gel and will be subjected for protein identification by LCMSMS. We hypothesized that the interaction between the venom protein and the extract may have potential antivenom activity. The outcome of this study will provide early evidence on the potential antivenom activity from Malaysia commonly found herb/medicinal plant

B. Snake Venom Proteomics (Venomics)

Research Projects

1. Comprehensive Proteomics Analysis of Malaysian Venomous Snakes Using Multiple Chromatography Techniques and Tande Mass Spectrometry (LC-MS/MS)

Investigators: Dr. Syafiq Asnawi, Prof. Iekhsan Othman, Assoc. Prof. Rakesh Naidu

Project Brief:

Snake venom is a highly complex mixture of proteins and polypeptides with a myriad of biological activities. It functions as an important tool for defense against predators, prey immobilization, and facilitation of prey digestion. There are two families of terrestrial venomous snake that cause the majority of envenoming cases in Malaysia: Elapidae and Viperidae. Information on the venom proteome is important for understanding and predicting the clinical consequences of envenomation and for formulating an effective antivenom that will target and neutralize venom components that are common between different species. Furthermore, snake venom has been associated with various therapeutic applications—as a thrombolytic agent in cardiovascular disorders, anti-cancer activities, anti-microbial activities, as an anti-viral agent and in antiparasitic, and antifungal activities. Advancement of proteomic technique through mass spectrometry could identify important components from the snake venom with potential therapeutic applications.

This project will employ multiple chromatography techniques such as gel filtration, reverse-phase liquid chromatography, and ion exchange chromatography to fractionate complex crude snake venom. Sample processing will then be carried out using in-gel and in-solution digestion technique prior to tandem mass spectrometry analysis. Protein identification will be carried out using advanced and latest version of PEAKS Studio software (Peaks X).

C. Neurodegenerative Diseases/Alzheimers'

Research Projects

1. Investigation of the anticonvulsive effect of the venom from Malaysian venomous snakes (Malaysian pit vipers and Malaysian cobras)

Investigators: Dr. Syafiq Asnawi, Dr. Md. Farooq Shaikh, Prof. Iekhsan Othman

Project Brief:

Epilepsy belongs to a group of neurological disorders characterized by the tendency of recurrent seizures and interruption of normal brain function. Common treatment options for epilepsy include lamotrigine (LTG) and vigabatrin (VGB). However, several side effects have been associated with the use of these drugs ranging from headache and dizziness to liver failure and weight loss. There are limited information on the pathogenesis of seizures, however, several studies have shown that pro-inflammatory mediators released by the brain and peripheral immune cells may have a key role. In addition, oxidative stress has also been associated with the induction of epilepsy as evidenced by the increased rate of oxidative metabolism, low antioxidant defence and elevated concentration of polyunsaturated fatty acids in the brain. Therefore, these conditions may trigger epilepsy by exposing the brain to damage induced by free radicals.

Mega-biodiversity of Malaysia's natural resources from its myriads of flora and fauna species may hold answers for novel anticonvulsant agents and naturally derived drug candidates or templates. Animal venoms have been demonstrated with potential anticonvulsant activities in various in-vivo models. Non protein toxin isolated from king cobra (*Ophiophagus hannah*) provided significant protection against drug induced convulsions in male albino mice (Saha et.al, 2006). Furthermore, phospholipase A2, a protein commonly found in the venom of viper/pit viper and cobra species was demonstrated to have strong neuroprotection activity by reducing neuronal cell death (Armugam et.al, 2009). Anticonvulsant properties was also demonstrated from the venom of various insects such as wasps and spider. Denatured venom of a social wasp (*Polybia ignobilis*) was anticonvulsant against acute seizures induced by the intracerebroventricular injection of bicuculline, picrotoxin and kainic acid (Cunha et.al, 2005). Similar effect was observed from the *Agelenopsis* spider venom in which it reduced the behavioural convulsions in rats in a dose dependent manner (Jackson and Parks, 1990). These findings raised questions whether similar anticonvulsant properties could be identified from the venom of the two of the most common pit viper species in Malaysia; *Calloselasma rhodostoma* (Malayan pit viper) and *Cryptelytrops purpureomaculatus* (Mangrove pit viper). We have performed the proteomic characterization of the crude venom and have confirmed the presence of proteins such as phospholipase A2. To the best of our knowledge, this is the first study to investigate the anticonvulsant properties of Malaysian pit viper venom using a zebrafish model.

D. Anticancer

Research Projects

1. Cytotoxic activities of cytotoxin from Malaysian cobra venom in human colon cancer cells

Investigators: Assoc. Prof. Rakesh Naidu, Dr. Syafiq Asnawi, Prof. Iekhsan Othman

Project Brief:

Snake venom contains myriad of bioactive molecules and toxins with potential therapeutic application including anti-cancer. Cytotoxins are polypeptides unique within the cobra venom with various biological activities including cellular mitochondrial dysfunction. Cytotoxic activity of cytotoxins isolated from *Naja oxiana*, *Naja haje*, and *Naja kaouthia*, were demonstrated on human lung adenocarcinoma and promyelocytic leukaemia cells. We have successfully purified a cytotoxin from the venom of Malaysian *Naja kaouthia* and would like to investigate the cytotoxic activity on the protein on human colon cancer cells. We hypothesized that the purified cytotoxin would demonstrate a dose dependent cytotoxic activity on the colon cancer cells.