ABSTRACT

Snake venom is a rich concoction of pharmacologically active proteins and polypeptides that evolved as an aid of "offence and defence" for snakes, facilitating immobilization and digestion of prey as well as defence against threats. Snake venom proteins can disrupt the central and peripheral nervous systems, the blood coagulation cascade, the cardiovascular and neuromuscular systems, and the general homeostasis state, thus making envenomation a medical emergency and a potentially fatal multi-organ disease. A common target of snake venom proteins is the haemostatic system of the prey/victim, which is symptomized by haemotoxic conditions post envenomation. These proteins disrupt haemostasis by targeting various proteins and factors affecting the blood coagulation cascade as well as the platelet aggregation pathway. For instance, the venom of *Daboia russelii* is haemotoxic in nature, and symptoms observed post its envenomation include persistent and profuse bleeding accompanied by consumptive coagulopathy and thrombocytopenia. Over the years, numerous studies have been undertaken to understand the haemotoxic nature of *Daboia russelii* venom and a number of anticoagulant and procoagulant toxins have been identified. However, their exact mechanisms of action remain unclear and their ability to modulate platelet hyperactivity remains largely unexplored. Understanding the molecular mechanisms of such proteins and their manipulation can help in designing better therapeutic interventions against cardiovascular diseases as antiplatelet drugs or antithrombotics. Moreover, *Daboia russelii* is one of the deadliest snakes across the Indian sub-continent. Therefore, multiple studies assessing the neutralization potency and immunological cross-reactivity of commercially available antivenom against *Daboia russelii* venom have been performed. These assessments have pointed towards various limitations owing to variations in venom composition. As such, an inclusive venomics approach to study the venomous snakes belonging to different geographical locations becomes crucial for designing effective antidotes. *Daboia russelii*, being a category I medically important snake across most of the Indian sub-continent, numerous research endeavours have been undertaken to study and compare its venom composition. However, more venom profiling of this species from lesser explored regions needs to be done for formulation of better alternatives to the current antivenom therapy for efficient treatment against *Daboia russelii* bites.

In this study, we attempted to study *Daboia russelii* venom from Tanore, Rajshahi, Bangladesh in order to gain in-depth understanding of the venom composition and understand its haemotoxic nature. A detailed exploration of the venom proteome by mass spectrometric analysis revealed the presence of 37 different proteins belonging to 11 different snake venom protein families in the venom. Phospholipase A_2 was found to be the most abundant protein family contributing to 26% of the proteome closely followed by snake C-type lectin, snake venom serine protease, snake venom metalloprotease and cysteine-rich secretory protein with relative abundances of 17%, 15%, 9%, and 9% respectively. Other snake venom protein families detected in the proteome with relatively lower abundances were disintegrin, kuntiz-type serine protease, vascular endothelial growth factor, L-amino acid oxidase and vascular nerve growth factor. Proteomic analysis also revealed the presence of two major phospholipase A_2 toxins, Daboiatoxin (both A and B subunits) and Daboxin P in the same proteome. Biochemical studies revealed that the crude venom exhibited procoagulant activity, phospholipase A_2 activity, indirect haemolytic activity and inhibition of collagen induced platelet aggregation. The proteome profile as well as the results of the biochemical studies correlates with the pathophysiological implications of *Daboia russelii* envenomation.

Subsequently, the study was aimed towards identifying an anti-platelet protein in the venom. The crude venom was fractionated using reverse-phase HPLC and each fraction was tested for their inhibitory effect on collagen-mediated platelet aggregation. The fraction most efficient in inhibiting collagen induced platelet aggregation was found to be fraction number 9 (P9). It could also inhibit platelet aggregation induced by ristocetin and thrombin. Biochemical analysis revealed that P9 exhibited mild anticoagulant and phospholipase A_2 activity. SDS-PAGE profile P9 under reducing condition displayed a single band at 15 kDa, and two bands at 15 and 27 kDa under the non-reducing condition suggesting that P9 is a mixture of more than one protein. This was confirmed by LC-MS/MS analysis which identified P9 as a mixture of Dabocetin and Daboxin P. Dabocetin was already being reported as an inhibitor of ristocetin-mediated platelet aggregation. Therefore, we investigated the anti-platelet potential of Daboxin P in the subsequent chapter.

Daboxin P was purified from the venom of *Daboia russellii* according to the protocol of Sharma et.al., 2016 by a two-step chromatographic approach using gel filtration chromatography followed by RP-HPLC. Subsequently, Daboxin P was studied for its effect on platelet aggregation induced by various agonists. It was observed that thrombin-induced platelet aggregation was inhibited maximum whereas inhibition of collagen-induced platelet aggregation was found to be 50%. Dose dependent studies revealed that it could completely inhibit thrombin-induced platelet aggregation at a concentration of 200nM and Anti-Aggregation 50 (AD_{50}) dose was determined to be 55.166 nM. Daboxin P also dose dependently reduced the thrombin mediated calcium influx. The inhibitory activity was not affected by alkylation of active site of Daboxin P indicating that the anti-platelet activity is independent of catalytic activity. In-silico interaction studies revealed that Daboxin P binds to thrombin and blocks its interaction with its receptor on the platelet surface. Quenching of thrombin's emission spectrum by Daboxin P and electrophoretic profiles of pull-down assay further verifies the binding between Daboxin P and thrombin. Thus, the present study demonstrates that Daboxin P is a natural inhibitor of thrombin with antiplatelet potential which manifests this activity by binding to thrombin.