CHAPTER 5

DISCUSSION

AND CONCLUSION
Snakebite is a neglected community health issue of India. The deaths due to snakebites remained controversial and the true scale monitoring of death and injuries of snakebites are not available. The deaths noticed by the authorities are underestimated, as WHO estimated that there are around 35,000-50,000 deaths due to snakebite in India. The variation in estimates is due to inappropriate reporting systems; conventional healers give treatment to many of the snakebite victims, and many of them lose their life before being admitted to the hospital and remain unnoticed. The records of snakebite deaths primarily based on victims admitted to the government hospitals. In emerging countries like India, the data reporting systems are improper, due to that the deaths outside hospitals remain unnoticed; this may be the main cause for the underestimation of the deaths due to snakebite in India. Snakes are scattered in the world except Arctic regions. In India, about 305 snake species observed among them 52 are venomous and the majority of the snakebites are due to four medically significant snake species called as India’s Big 4.

Deaths and serious injuries due to snakebites can conquered with early identification and treatment of the victims. The antivenom is the only effective remedy against snakebite, but equal consideration given to the possible adverse drug reactions due to the antivenom. In India to cure snakebite victims, generally, polyvalent ASVA is used, which has the venom-neutralizing activity for four medically significant snakes of India. Although the ASVA is polyvalent, the supportive treatment varies and plays an important role during snake envenomation treatment resulting in the saving of lives and serious injuries to the victims (Warrell D.A., 2012). Many authors reported that use of specific monovalent ASVA is ideal, as it has antibodies against particular venom, which efficiently neutralizes particular venom. The monovalent antiserum avoids the unnecessary burden of non-specific antibodies present in polyvalent antiserum to the victim resulting in the use of less antiserum. Monovalent ASVA treatment is cost saving than polyvalent antiserum as it avoids unnecessary administration of non-specific antibodies resulting in a saving of valuable ASVA, which has shortage world widely. In many regions of the world, polyvalent ASVA is preferred for treatment of snakebite victims as no standard methods is available to identify the snakebite envenomation and species prior to the administration of ASVA. For practical application of monovalent ASVA, there is a need to develop a trustworthy method.
to detect snake envenomation at the early stage. Although, after extensive research for development of a suitable method to identify the offending snake species, we are not able to develop a reliable, specific, rapid, and cost effective method for field application, which will provide a key tool for serving effective utilization of antivenom and snakebite management (Selvanayagam Z. E. et al., 2002).

Snakebite envenomation identification is a crucial part of snakebite treatment, as several bites do not lead to systemic envenomation mentioned by Sharma et al. (2004). In many snakebite cases, signs, and the symptom expressed by the victims are due to fear of snakes, several non-poisonous snakes lookalike to poisonous snakes to guard themselves against the predator. In India, snakebites identified mainly based on symptomatic approach, bitten part examination, signs, and the symptoms shown by victims, also using biochemical tests like urine examination and 20-min WBCT. For medical practitioners, it becomes difficult to identify the actual envenomed victims using available tests (Warrell, D.A., 2010, 2012; Alirol et al., 2010). The above-mentioned test does not give clear indications of the severity of systemic envenomation and offending snake species. Various serological tests to are available for identification of the systemic envenomation, but ELISA turns into a method of choice for the identification snakebite envenomation. The use of ELISA for venom detection was first reported by Theakston et al. (1977), and afterwards widely applied for snake species identification without conferring any cross-reaction (Steuten et al., 2007; Selvanayagam Z.E. and Gopalakrishnakone, 1999).

To understand better about the snakebites, it is important to know about the composition of snake venom. Many authors confirmed that the venom contains numerous proteins and it varies greatly from one species to other. The variation among venoms may be due to the change in season, habitat, and available prey. In this study, the SDS-PAGE of CV, KV, RV, and EV venoms (Fig.4.1) confirmed that venoms have specific protein patterns, and contains some proteins, which resembles each other. Similar molecular weight proteins or same antigenic parts of dissimilar proteins may add the degree of cross-reaction between the venoms. Many research articles illustrated that there is a significant cross-reaction between monovalent antiserum of two different species; these cross-reacting
antibodies can hinder the specificity of the test. (Selvanayagam Z.E. et al., 1999; Dong L.V. et al., 2003; Gao J. F. et al., 2013).

In this study to raise antibodies against snake venom, immunization of rabbits carried out by using the detoxified venoms. The detoxified venoms in the immunization process minimized harmful side effects of the venom and allow to use a more amount of antigen per dose, which resulted in a rapid enhancement of ASVA. Although, the detoxified venom was utilized for the immunization process, generated antibodies have strong neutralizing activity towards the native venom samples (Fig.4.2). During immunization, relatively large molecular weight proteins are more immunogenic compared to lower ones. The CV observed less immunogenic than RV, SDS-PAGE profiles depict that CV contains lower weight proteins than RV (Fig.4.1).

In the results, ARV-Abs and ACV-Abs confirmed cross reactivity towards the heterospecific venoms (Fig. 4.6). The figure (Fig. 4.6) showed that ARV-Abs have strong cross reactivity against EV and ACV-Abs against KV; the reason behind this cross reactivity may be the snakes related to the same family have more similar proteins or common epitopes on different proteins resulting in a high degree of cross-reaction. In view of this, it becomes a necessity to obtain SSAb’s against particular venom. Antibodies purified from hyper-immune plasma by the process of protein-A affinity chromatographic purification. Protein-A selectively separates IgG’s from whole plasma, during purification all IgG’s were separated which contains the specific, nonspecific and cross reacting IgG’s may contributes the cross-reactivity among the venoms. In advance, to eliminate the non-specific and cross-reacting IgG’s, immuno-affinity chromatography was carried out using CNBr activated Sepharose 4B beads bounded to conspecific and heterospecific snake venoms separately. The specificity and efficiency of resulting antibodies (RV & CV SSAb’s) to conspecific snake venoms depicted in (Fig.4.7). In the present study, it was observed that obtained SSAb’s are sensitive and specifically detected the conspecific venom.
Today, there are many ELISA based assays available to detect snake venom from snakebite victims, but these assays require more time, therefore not useful for snake venom detection before administration of ASVA. In view of this, In Australia CSL developed SVDK kit to identify snake venoms from victims; it requires 30-40 min to demonstrate the results. The time required for SVDK is less but there is a scope to reduce time by use of advanced immunological methods. The methods like LFA are sensitive also specific and suitable for field application. The results of LFA obtained within least time, at present, LFA plays a vital role in the field diagnostics like identification of pregnancy, HIV, Malaria, Dengue, and more (Wong and Tse, 2008; Wild, 2001). Due to above facts, we here tried to apply LFA platform for selective and rapid detection of snake envenomation.

Generally, Most of the commercially available LFA platforms utilize two different MAbs for impregnation and conjugation for detection of single antigen. The application of MAbs for envenomation detection has restrictions as snake venom composed of many proteins, only some of them are characterized, and there is huge seasonal and geographical variation amongst the venoms of snakes belongs to same species. Application of MAbs may hamper the sensitivity as the circulating venom in the blood of victims is very low, in addition to the venom composed of numerous proteins, and their amount in the venom can vary. So it becomes incredibly difficult to develop sensitive and reliable LFA for detection of snakebite using MAbs. To encounter this problem, LFA was developed by using purified specific polyclonal antibodies. Use of SSAb’s amplified the sensitivity of the assay as it includes only venom specific antibodies. Beside this, the use of two different species antibodies for impregnation and conjugation enhanced the sensitivity, the reason may be different species reacted differently with the same venom and antibodies generated recognize antigen in a different way leads to increase in sensitivity of SEDIA.

The developed SEDIA to detect the RV & CV on a single device observed selective to detect RV and CV at respective test zones and sensitive to detect venom as low as 0.1 ng/ml (Fig 4.11). The device able give results between five to ten min. The conjugate cannot get capture in the test zones in the absence of venom. A control zone was impregnated with anti-horse antibodies and the conjugate in presence or absence venom giving red line at
control zone, representing the validity of the assay. The test becomes invalid in the absence of a red line at control zone (Fig. 4.9). The experimental envenomation performed in Swiss albino mice by introducing a specified quantity of venoms and blood was collected after particular duration to obtain plasma samples. The diluted plasma applied to SEDIA device to verify the functionality. The SEDIA distinctively detected RV, CV, and showed 100 % conformity between SEDIA and ELISA results (Fig. 4.12 and Table 4.2).

In conclusion, for the management and detection snakebite, the SEDIA has an immense potential to become a technique for field application, which will help in avoiding unnecessary utilization of valuable antivenom by the identification of only envenomed victims. Early detection and specific supportive treatment can imparted due to SEDIA, may result in the saving of lives of victims. This work illustrates a process prototype; in the Indian scenario, the application of SEDIA achieved by the inclusion of EV and KV venom detection on a single device with further advancements and case studies.