Chapter 8

8.1. Conclusions

Filariasis is parasitic disease affecting the people residing in the tropical and subtropical countries. It is a disabilitating disease causing elephantiasis and hydrocele. The infection in human caused by *B. malayi*, *W. bancrofti* and *B. timori*. In the present study the adult parasite were isolated from their respective host. *B. malayi* was isolated from human filariid infected experimental rodent model and *S. cervi* were isolated from bovine filarial infected cattles i.e. Indian water buffalos. The adult parasites were brought to the laboratory in normal saline. The mf was collected by dissecting the female adult filarial parasite. Both *B. malayi* and *S. cervi* mf were collected separately and were kept at -20° C until used.

The adult filarial parasites were used to prepare the somatic adult protein from both *S. cervi* and *B. malayi*. These adult proteins were evaluated using SDS-PAGE. The *B. malayi* adult protein yielded 25-30 protein bands in the resolving gel, while *S. cervi* adult protein showed 35-40 protein bands.

These adult filarial parasites antigens were also characterized for the presence of superoxide dismutase (SOD) in both *B. malayi* and *S. cervi*. Here the SOD activity was also determined in the microfilarial stage of the parasite and it was found that microfilarial stage have minimum activity.

The adult filarial protein prepared from *B. malayi* and *S. cervi* adult parasites were evaluated for their immunological properties. The cross reactivity of *B. malayi* and *S.
Cervi antigens were tested against human patients sera along with normal human sera living in non-endemic region using ELISA. It was found in the study that the antibody titer was 1:3200 therefore this titer was taken for further analysis in the present study studies. When different class of antibodies was used then ELISA result give the prevalence in the order of IgG > IgM > IgG4 antibodies in all antigenic preparation from a microfilaremic chronic infected patients. In this study symptomatic patients sera were using ELISA and it was found that reactivity of microfilarimic +ive patients were having highest titer value and the titer value was found in the order of microfilaraemic > endemic normal> chronic > acute whereas no reactivity was seen when non-endemic normal subjects sera was used.

The IgG reactivity in human patients sera were observed using western blotting. The chronic patients sera showed highest reactivity in ELISA recognized the immunogenic band at 89.26 kDa in CHMF -ive, 87.01 kDa in ACMF -ive and 75.54 kDa in mf +ive This indicated that all the human paitients sera showed +ive higher IgG reactivity and it was found that ~45% chronic patients sera were IgG +iv .While the EN sera gave a negative results.

In addition IFN gamma downregulation and IL4 upregulation showed the shifting of the immune response towards the th2 type therefore describing the magnificent tools used by parasite to create suitable conditions in host for a long term survival and uninterrupted transmission.

The adult filarial parasite of B. malayi and S. cervi were used to isolate the DNA using the manufactures protocol. The isolated genomic DNA of B. malayi and S. cervi to
were run on agarose gel electrophoresis and DNA bands visualized under UV at 365nm in transilumanator.

The DNA sequences from filarial DNA gene bank and ESTs were screened using *insilico* method. The DNA sequences from filarial DNA Genbank and EST’s were screened for homologous sequences for primer designing and alignments of homologous sequences using CLUSTAL W & X multiple alignment program. During analysis it was found that mitochondrial genome of *B. malayi* and *W. bancrofti* shares 89% homology with each other along with this the query coverage was found to be 99%. The sequences from filarial gene bank were also evaluated for designing primer for *B. malayi* and *S. cervi*. Primers targeting the different structural region of human filarial parasites *B. malayi* were designed ESTs and BLASTn program was used for the homology search. The PCR was performed using these primers of *B. malayi* and *S. cervi*. It was found that Hha1 gene of *B. malayi* was amplified using the Hha1 gene specific primers this was confirmed from the amplified fragment size of 322 bp for *B. malayi*. Thus it can be concluded that the Hha1 primer can be used to detect the *B. malayi* DNA specifically in the patient samples.

The primers targeting the different structural region of bovine filarial parasite, *S. cervi* were designed using DNA gene bank (*insilico* approach) and homology of the sequences were tested using BLAST. The cytochrome-c- subunit 1 (Cox1) gene was identified in *S.cervi* DNA as it showed the conserved region and therefore, can be used as an effective diagnostic marker using this primer. The *S. cervi* DNA was amplified using thermocycler and it was found that a fragment of 680 bp was amplified. This Cox1 primer and Hha1 primers together could be used as a novel target for differentiating the
filarial parasite from human and bovine filarial parasite. Thus will be useful tool for
diagnosis of filariasis.

Similarly, another gene glutathione-S-transferase (GST) was also evaluated as a
potential drug target using insilico approach. The different anti-filarial compound such as
albendazole, DEC and BHA were docked with the filarial GST sequences and were
ranked according to the gold fitness score. The top ranked poses were further analyzed
using a consensus scoring function of X-Score to calculate the binding affinity. This
study revealed amino acid residues crucial to the interaction of GST from both the filarial
parasite with albendazole, diethylcarbamazine and BHA. The binding modes exhibited by
various docked compounds illustrated the importance of specific residues within the
active site region of the targets. The roles of some important amino acids have also been
revealed, that were found to be playing an important role in the positioning of inhibitor
within the active site. Thus based on the above outcomes it is conclude of that these
inhibitors present within the active sites can behave as lead molecules to be a promising
anti-filarial compounds against the targets selected for our study.