ABSTRACT

Lymphatic filariasis infects more than 128 million people worldwide in 80 endemic countries. It continues to be a formidable problem especially in Asian subcontinent and Africa. Around 20% of world population is at risk of infection and 44 million people are affected with various clinical forms of the disease. About 76 million people are carrying the parasites in their blood with no clinical symptoms. Within the last few years, the currently available antifilarial drugs, diethylcarbamazine and albendazole are principally microfilaricidal with little or negligible effect on adult filarial parasites. However, the treatment results into reappearance of microfilariae after a period of therapy. Therefore, there is an urgent need for the identification of new drug target and understanding of its role in pathogenesis.

Thioredoxin reductase in many parasites systems has been identified as a potential drug target. The discovery of selective inhibitors which are unique to TrxRs will aid in the development of potential drug/vaccine candidate. Hence, there is a great prospect to exploit filarial TrxR and its inhibitors as a potential chemotherapeutic drug target against lymphatic filariasis.

The major findings of the work described in this thesis have been enumerated below:

➢ Screening of thioredoxin reductase in filarial parasite S. cervi

- Significant amount of TrxR activity was detected in adult female, male, and microfilarial life stage of bovine filarial parasite, S. cervi. Highest activity was detected in the adult female crude extract followed by microfilariae and adult male. While ES did not show any TrxR activity.
- Presence of TrxR activity was evident in the sub-cellular fractions of adult female extract of cytosolic fractions having highest activity followed by mitrochondrial, nuclear fractions.
Abstract

➢ **Purification and biochemical characterization of thioredoxin reductase from**

  *S. cervi*

- *S. cervi* TrxR of approximately 50 KDa was purified to homogeneity (90% fold purification) by the use of ammonium sulphate precipitation, DEAE-Sephadex A50 column, and 2’5’ ADP agarose affinity chromatography.
- The purified ScTrxR showed pH and Temperature optima of 7.0 and 35°C respectively.
- Presence of FAD in purified ScTrxR was observed by measuring absorbance peak at 450 nm suggesting the association of FAD with the enzyme. It was further confirmed by the fluorescence emission spectra at 450 nm which was found to be similar to that of FAD.
- ScTrxR showed a significant antigenic cross reactivity with human filarial infected sera. IgG and subclasses IgG1 and IgG4 order of cross reactivity was observed to have a similar pattern to Mf > CH > EN.
- The Km values for DTNB was determined in our study which were found to be very similar to those obtained in *Hemonchus, Schistosoma, Fasciola hepatica* and Bovine erythrocytes.
- Lineweaver-Burk plot for ScTrxR using CDNB inhibitor showed noncompetitive type of inhibition whereas auranofin showed competitive type of inhibition.
- The selenium content was found to be 20 ng/mg of purified enzyme.
- ScTrxR gene amplification partial (~1.2 Kb) product showed 75% nucleotide sequence match with *BmTrxR* gene and predicted protein sequence matching showed 65% homology to *BmTrxR*. 
Thioredoxin reductase as a potential Chemotherapeutic target in *S. cervi*

- 1-Chloro 2, 4-dinitrobenzene (CDNB) and auranofin are two specific TrxR inhibitors which were found to be effective against the adult filarial parasites.
- It caused a concentration and time dependent decrease in the motility and viability of parasites leading to their death.
- Inhibition of *S. cervi* TrxR leads to the downregulation of the antioxidant system followed by generation of oxidative stress in these parasites. The increased ROS level induced lipid peroxidation and protein carbonyl formation and decrease the level of PTP, which might alter the mitochondrial membrane permeability leading to release of cytochrome c from the mitochondria. We have found decreased level of cytochrome c oxidase activity in mitochondrial fraction of treated parasites.
- High peak intensity of calpain was found to be up-regulated in case of Auranofin treated parasites. Increase in calpain activity can be directly related to release of calcium from the cytosol. Increase calcium level change the mitochondrial function and promote caspase-3 activity and initiating a apoptotic like event in parasite.
- CDNB and auranofin inhibitors significantly down regulated the level of ced-9, and up regulated ced-3, a homolog of mammalian caspase-3 suggesting initiation of intrinsic pathway of apoptosis.

Effect of thioredoxin reductase inhibitors on proteome of *S. cervi*

- The proteomic profile of CDNB (50 µM) and auranofin (20 µM) treated parasites showed marked alteration in different proteins spots in comparison to the control parasites.
- We observed a down regulation in the glycolytic enzymes such as enolase and PGK suggesting reduced ATP formation in the parasite in both CDNB and auranofin treated parasites.
Abstract

- Calcium regulated proteins like calpain, calreticulin and coiled-coil domain were found to be upregulated in auranofin treated parasite.
- An increased mitochondrial calpain activity might also help in mitochondrial dysfunction and further induction of apoptosis in parasites.
- This study suggests that TrxRs inhibitors disrupt the cellular homeostasis thereby generating oxidative stress followed by mitochondrial mediated apoptosis leading to the death of the parasites.

CONCLUSIONS

✓ *S. Cervi* possesses abundant redox system containing enzyme in all the life stages. TrxR/Trx is the prominent redox system present in adult female *S. cervi*.

✓ Biochemical characterization studies revealed that filarial TrxR differ from their host TrxR.

✓ *S. cervi* TrxR showed much lower Km and Vmax when compared to human thioredoxin reductase, a clear possibility to explore as a drug target.

✓ TrxR inhibition disrupts the cellular homeostasis thereby generating oxidative stress followed by mitochondrial mediated apoptosis in filarial parasites leading to the death of the parasites.

✓ TrxR is essential for survival of parasite within the host and could be taken as potential drug target for synthesis and screening of new antifilarial against the host.