SHORT ABSTRACT

Dihydroorotase, the third enzyme of the de novo pyrimidine biosynthesis pathway of *Leishmania donovani*, was cloned, expressed and biochemically characterized in both directions of enzymatic reactions. The data revealed a preference for biologically significant forward direction. The forward direction enzymatic reaction shows acidic pH optimum in contrast to slightly alkaline pH optimum of reverse reaction. Further, the enzyme shows both tetrameric and dimeric structures in equilibrium. Biotin sulfone and kaempferol are identified as potential inhibitors of the enzyme using computational docking and subsequently validated on recombinant dihydroorotase. Dihydroorotase inhibitor could not elicit parasitic death as the same was prevented by a parallel salvage pathway which was demonstrated by qRT PCR and DNA degradation. Overall these inhibitors have led us to a better understanding of pyrimidine metabolism in the parasite as a whole. The localization patterns of variants of asparaginase a key enzyme of the aspartate metabolic pathway which provides precursors to the de novo pyrimidine pathway were found out to be cytosolic in the *L. donovani* parasite. An understanding of the pyrimidine and its related aspartate metabolic pathway will aid in the development of therapeutics for the treatment of visceral leishmaniasis.