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In silico Discovery of Potential Natural Inhibitors against Trypanothione Synthetase in *Canine leishmaniasis*

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ABSTRACT

Background: *Leishmania infantum,* one important intracellular parasite causes most potentially lethal diseases such as leishmaniasis *i.e.* Visceral leishmaniasis in dogs. Although certain chemical drugs such as pentostam, amphotericin B, miltefosine have been trailed against this disease, but all these drugs induced antibiotic resistance and toxicity in the host. Further, the trypanothione synthetase, a key enzyme of this parasite which catalyzes a reaction, maintaining thiol redox within the cell

Methods: The binding study was carried out with selected natural/synthetic phytocompounds/ drugs against the modeled trypanothione synthetase though molecular docking.

Result: The generated protein model with lowest discrete potential energy (DOPE) -19960.97 was found good in quality with z score of -5.19 and quality factor of 61.83% and it was found that the natural inhibitors glycyrrhetic acid (GRA) and Theaflavin (TFN) showed highest binding energies of -7.34 and -6.95 Kcal/mol. This study may be concluded that the natural inhibitors glycyrrhetic acid (GRA) and Theaflavin (TFN) would be potential regimen in treatment of canine leishmaniasis.

Key words: Homology modeling, *Leishmania infantum*, Molecular docking, Trypanothione synthetase.

INTRODUCTION

Leishmania infantum, one important vector-borne protozoan parasite, belong to the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) causes deadly disease *i.e.* visceral leishmaniasis in dogs which causes 1.3 million new cases and 20,000 deaths every year according to WHO 2015 (Singh *et al*., 2012). This protozoa is transmitted by species of sandfly belonging to the genus Phlebotomus and prevalent throughout the tropical and temperate regions including Africa, China, India, Nepal, Southern Europe, Russia and South America (Johan *et al.,* 2012). The infected dogs showed the clinical signs of fever, low RBC count, skin ulcer and an enlarged liver (Barrett and Croft, 2012). Further, the trypanothione synthase (TS), which catalyses glutathione to spermidine, a key intermediate in maintaining thiol redox within the cell and defending against harmful oxidative effects in such protozoa and can be utilized as drug target against these protozoa (Tetaud *et al*., 1998). This enzyme has important role in generation of free energy from ATP hydrolysis which conjugates glutathione and spermidine to form the intermediate of glutathionyl spermidine and the final product of trypanothione, helps in the survival of these protozoa (Oza *et al*., 2006). The lack of crystal structure of *Leishmania infantum* TS prompts to perform homology modelling of *Leishmania infantum* TS by utilizing the crystal structure of *Leishmania major* (Fyf *et al*., 2008). Although certain drugs such as pentavalent antimonials, Amphotericin B, Miltefsine, Paromomycin, Pentamidine and Sitamaquine have been used against this disease since long time, but all these drugs have limitations of increased drug resistance, cytotoxic side effects, cost and avaibility (Singh *et al*., 2012). So now this traditional paradigm has changed to search for natural herbal compounds which would be trailed against *leishmania infantum.* For this, different herbal as well as synthetic compounds were thought to be useful for treating heart diseases and an effect on the permeability of capillaries (Hooper *et al*., 2012), can be utilized as identifiable natural inhibitor against *Leishmania infantum.* So this prompts to undertake this current study with objective of identification of most potent herbal compound against trypanothione synthetase of *Leishmania infantum* through molecular docking experiment.

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MATERIALS AND METHODS

The study was conducted to discover natural inhibitors against trypanothione synthetase (TS) of *Leishmania infantum* at the Biochemistry Division, Indian Veterinary Research Institute, Bareilly, U.P. from December 2020 to July 2021.

Template selection

The lack of crystal structures of *Leishmania infantum* trypanothione synthetase (LiTS) caused to opt homology modeling to determine the structure of LiTS. The sequence of LiTS was retrieved from the NCBI protein database with accession number A0A3S7WXK4 and PSI-BLAST (Altschul *et al.,* 1997) was performed against Protein Data Bank. Trypanothione synthetase of Leishmania major (PDB ID: 2VPM) was considered for template for the reason that the resolution was 2.80 Å and the identity and similarity with Leishmania infantum were found 31% and 67% respectively.

Homology modelling and structure validation

Homology modeling was performed for the LiTS protein sequence upon Modeller v9.21 (Sali and Blundell, 1993) using Trypanothione synthetase of *Leishmania major* as template. Ten 3D homology models were generated and the validation for the best model was done using GA341 (Melo *et al*., 2002) and DOPE (Shen and Sali, 2006) scores. Further the structures of best model were analysed by SAVES validation package (Eisenberg *et al*., 1997) and RAMPAGE server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php). ProSA program was also used to analyse the quality of the best model along with the template (Wiederstein and Sippl, 2007). ProQ analysis, ERRAT and Verify 3D analysis was done to check the quality and stability of the best model. Finally, the Model-template superimposition was performed using Pymol v2.1 molecular visualization program (De Lano, 2002).

Secondary structure prediction

The secondary structure of LiTS protein was predicted from its complete amino acid sequence (Accession: A0A3S7WXK4) using PSIPRED 4.0 algorithm (Buchan and Jones, 2019).

Selection and retrieval of ligand molecules

In this study, six natural compounds and four synthetic compounds and with antiprotozoal, anti-inflammatatory and antileishmania activities and one FDA approved antiprotozoal drug were selected for docking studies.

Molecular docking

In this study, molecular docking between above selected ligands and the drug target *Leishmania infantum* trypanothione synthetase (LiTS) was performed under AutoDock 4.2 (http://autodock.scripps.edu/) platform using Auto-Dock Tools 4 (Rizvi *et al*., 2013). For this, prior to docking, the macromolecule and the ligands were prepared with addition of Kollman charges and polar hydrogen atoms using ADT tool. The grid map was assigned around the drug binding cavity of the target protein with size of 126, 126, 126 in x, y, z direction with grid spacing of 0.375Å. In this, docking was performed with lamarckian genetic algorithm (LGA) of 10 runs (25000000 energy evaluation steps for each run), keeping the LiTS protein rigid and ligand molecules flexible with in drug binding pocket. Binding interactions between atoms and molecules and visualization were carried out using UCSF Chimera v1.14 and PyMol molecular graphics tool (www.pymol.org).

RESULTS AND DISCUSSION

In the current scenario, the canine visceral Leishmaniosis that causes approximately 20,000 deaths, annual incidence of 300,000 new cases and 1 billion of people at risk of infection drives the major concern around the glove for control of this disease (Hailu *et al*., 2016). Although various chemotherapeutics have been trailed against this disease, but all compounds cause serious side effects, such as renal, pancreatic and hepatic toxicity, teratogenicity, cardiac as well as gastrointestinal problems (Copeland and Aronson, 2015). Further, a single FDI approved drug miltefosine which has been used since 2014 against this disease but it has been facing lot of difficulties in its implementation due to problems of affordability and accessibility (Sunyoto *et al*., 2018). It has also been reported that the trypanothione synthetase has vital role in the survival and growth of protozoa as this enzyme mediates the final step of tryptophan synthesis. Moreover, it was reported that humans do not have tryptophan synthase, so this enzyme was explored as a potential drug target against *leishmania infantum* (Chaudhary and Roos, 2005). Although the binding interaction studies, targeting different enzymes of *leishmania infantum* such as dihydrofolate reductase, thymidylate synthase (Vadloori *et al*., 2018) Glutamyl cysteine Synthetase (Agnihotri *et al*., 2017), Inositol phosphoryl ceramide synthase (Norcliffe *et al*., 2018) with natural inhibitors have been reported in previous finding, but here authors focused on the discovery of potential natural compounds against thetryptophan synthase enzyme. As the crystal structure of *leishmania infantum* trypanothione synthetase (LiTs) was not avaible, the primary sequence analysis resulted a conserved domain *i.e* CHAP domain present between 109th to 257th amino acid which mediates the utilization of catalytic cysteine residue in a nucleophilic-attack mechanism during tryptophan synthesis (Bateman *et al*., 2003). To its support, the avaible crystal structure of *leishmania major* was retrieved and utilized for generation of protein models.

The model structure of *Leishmania infantum* trypanothione synthetase (LiTS) was generated using trypanothione synthetase of *Leishmania major* (PDB ID: 2VPM chain: A) as template. Out of ten models, Model-9 with lowest DOPE and GA341 score of -19960.97 and 0.02942 was considered for best model shown in Table 1 and performed for further structural validation studies. Further the structural superimposition of LdTS model with template 2VPM-A chain both before and after energy minimization (Fig 1) revealed a Root Mean Square Deviation (RMSD) score of 0.112 Å. The RAMPAGE analysis of this model showed (88.5%, 7.1%, 4.3%) amino acid residues in the favored, allowed in the outlier region (Fig 2A). The ProSA-web

Table 1: Ten LiTs protein models generated by homology modeling.

Models	molpdf	DOPE Score	GA341 score
target.B99990001.pdb	2399.02197	-19669.74609	0.00918
target.B99990002.pdb	2497.18872	-19616.37305	0.02927
target.B99990003.pdb	2407.97656	-19712.56836	0.01068
target.B99990004.pdb	2505.65552	-19605.44531	0.01573
target.B99990005.pdb	2389.01807	-19724.93945	0.00991
target.B99990006.pdb	2393.02734	-19516.81445	0.01424
target.B99990007.pdb	2580.00928	-19826.70313	0.00999
target.B99990008.pdb	2574.49268	-19556.78711	0.02213
target.B99990009.pdb	2628.64624	-19960.97266	0.02192
target.B99990010.pdb	2297.84937	-19729.81836	0.01175

Molpdf= molecular pdf, DOPE Score= Discrete optimized protein energy score.

analysis revealed a Z score of -5.19 (Fig 2B). The ProQ analysis resulted Levitt-Gerstein (LG) and Max sub score of 2.86 and 0.73. Further the ERRAT programme showed the overall quality value for LiTS model of *Leishmania infantum* was 61.83% (Fig 2C). In addition, Verify3D plot of the modelled protein (Fig 2D) showed PASS and the 3D environment profile resulted 81.93% of the residues have averaged 3D-1D score~*0.2. The structural validation study suggested our model is best in terms of quality and stability.

Due to lack of crystal structure, the secondary structure of *Leishmania infantum* trypanothione synthetase (LiTS) protein was predicted from its primary sequence using PSIPRED web server which showed 1 long, 3 medium, 2 short helical regions and 3 medium, 11 short β-sheets within the structure of LiTS protein (Fig 3).

Few drugs such as pentavalent antimonial derivatives, sodium stibogluconate, paromomycin, pentamidine, miltefosine and amphotericin-B showed promising effect against this protozoa, but all these drugs showed potent toxic with serious side effects and also exhibit drug resistance (Oh *et al*., 2014). These drugs also cause severe adverse reactions such as reversible peripheral neuropathy; pancreatitis, nephrotoxicity cardiotoxicity, pancytopenia, myalgia and bone pain (Croft *et al*., 2006).

This study has been able to find the binding efficiency of selected phytochemicals (10-hydroxycamptothecin, Theaflavin, Hecogenin acetate, glycyrrhizic acid, convallatoxin, tubocurarine, cafestol, mundulone, pomiferin, catechin) against tryptophan synthase of the protozoa. The separate binding interaction study between selected natural inhibitors and antiprotozoal drug with LiTS protein was done to assay the better therapeutic agent against *L. infantum* based upon their free binding energy and the inhibition constant of each binding complex which was reported in Table 2.

The result showed that among all selected inhibitor, Glycyrrhetic acid (-7.34 kcal/mol; KI: 4.18 uM), Theaflavin (-6.95 kcal/mol; KI: 8.04 uM) showed best binding efficiency with LiTS protein. The binding interaction between LiTS with glycyrrhetic acid and Theaflavin are

Table 2: Docking scores of eleven ligands against *Leishmania infantum* trypanothione synthetase (LiTS) protein are reported.

Ligands (Phytochemicals/ drugs)	Docking energy scores (kcal/mol)	Intermolecular energy (kcal/mol)	Inhibition constant (KI)
10-hydroxycamptothecin	-5.94	-6.83	44.55 uM
Theaflavin	-6.95	-10.23	8.04 uM
Hecogenin acetate	-6.55	-6.84	15.93 uM
Glycyrrhetic acid	-7.34	-8.23	4.18 uM
Convallatoxin	-3.43	-6.12	3.04 mM
Tubocurarine	-4.97	-6.16	227.62 uM
Cafestol	-5.99	-6.89	40.55 uM
Mundulone	-5.98	-6.88	41.29 uM
Pomiferin	-6.77	-8.56	10.95 uM
Catechin	-6.32	-6.62	23.38 uM
Miltefosine	-6.6	-8.69	14.46 uM

SN= Serial number, pM= pico molar, nM= nano molar, uM= micro molar.

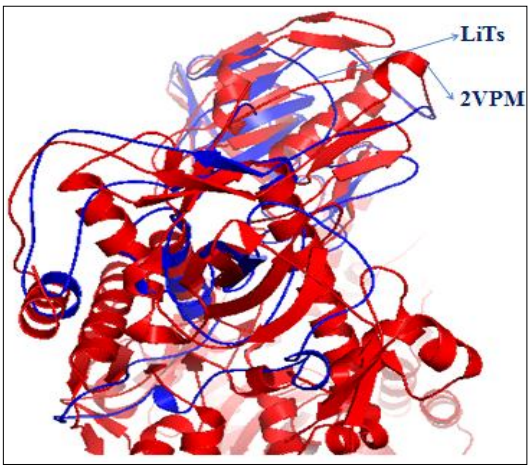


Fig 1: The structural super imposition between identified drug target trypanothione synthetase (blue color) of *L. infantum* and template (PDB ID: 2VPM, Chain A) (red color) of *L. major* represented.

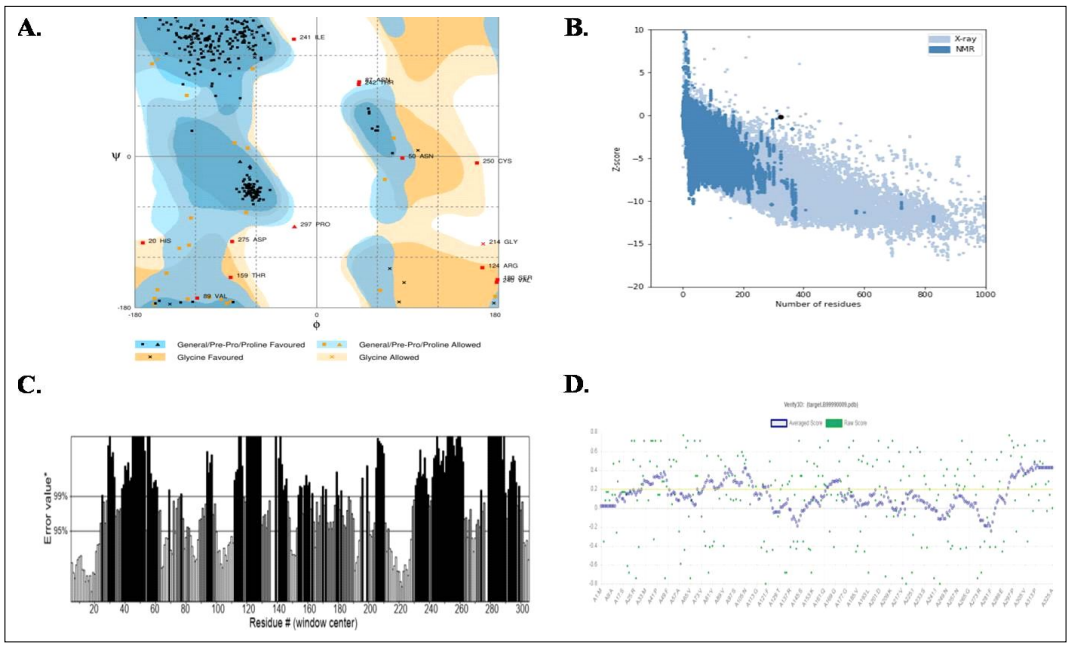


Fig 2: Ramachandran plot by RAMPAGE presented in (A), z plot which describes the overall quality of model evaluated and deciphered (B), Quality verification plot of the energy minimized model of the LiTs performed using ERRAT shown in (C), Verify 3D plot (D).

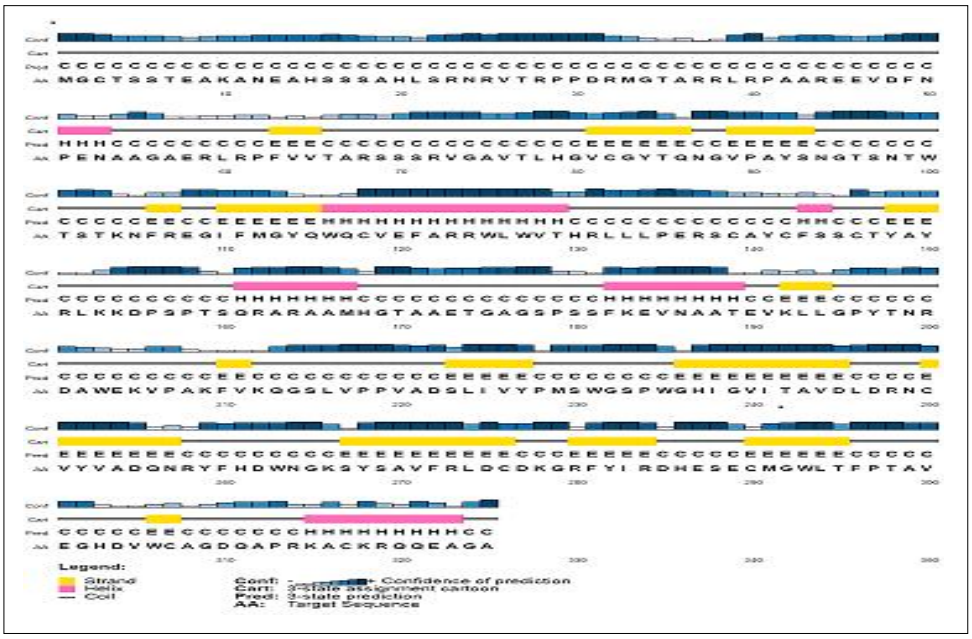


Fig 3: Predicted secondary structural elements for full length trypanothione synthetase of *Leishmania infantum*. Helix: Pink cylinder; Sheet: Yellow cylinder.

shown in surface and ribbon structure presented in Fig 4 A, B, C and D respectively.

In this study it was showed that among natural inhibitor, glycyrrhetic acid and Theaflavin showed highest binding affinity against LiTs which is a good agreement with the findings of Venkatesan *et al*. (2011) and It has been reported that Glycyrrhetic acid which is a pentacyclic triterpenoid aglycone, a product derived from the plant *Glycyrrhiza glabra* showed tremendous antiparasitic activity by activation through nitric oxide (NO) upregulation, proinflammatory cytokine expression and NF-κB activation through p38 kinase (Gupta *et al*., 2015). Similarly theaflavin is a class of natural flavonoids derived from the dried leaves of the plant *Camellia sinensis* (tea) and related plants with potent antioxidant properties. The antiprozoal activity of theaflavin might be due to inhibition of 1-deoxy-D-xylulose

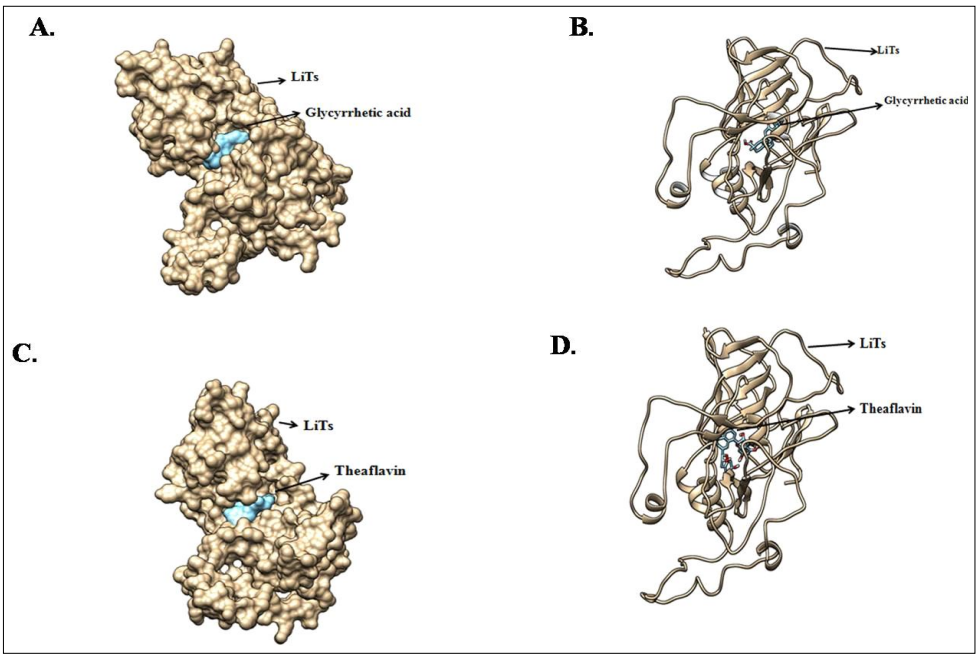


Fig 4: Binding interaction between *Leishmania infantum* trypanothione synthetase with glycyrrhetic acid showing by surface (A), ribbon (B) and with Theaflavin showing by surface (C), ribbon (D).

5-phosphate reductoisomerase, the key enzyme of the MEP terpenoid biosynthetic pathway previously reported by Hui *et al*. (2016).

CONCLUSION

This study concludes that the enzyme trypanothione synthase plays a major role in the tryptophan biosynthesis for the survival of *L. infantum*. Out of all the compounds in this study, Glycyrrhizic acid and Theaflavin which showed higher binding affinity, have been identified likely to be potential inhibitors of LiTs. This docking study hypothesized that Glycyrrhizic acid and Theaflavin could act as potent inhibitors of LiTs enzyme and would provide cost effective natural therapeutics against canine *leishmaniasis* in nearest future.

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Conflict of interest

All authors declare thet they have no conflict of interest.

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