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Research Article

IN SILICO SCREENING AND IDENTIFICATION OF POTENT ANTIPROTOZOAL DRUGS AGAINST AQUAPORIN PROTEIN OF *NOSEMA* SPECIES INFECTING SILKWORM AND HONEY BEE

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Abstract- The Microsporidian diseases are becoming major constraint for the production of silkworm and honeybees. The diseases are causing considerable yield loss to sericulture and apiculture industry. At present, no proper protozoan disease management strategies are available. Hence, there is a need for identification and screening of pathogen protein targeted drugs for the containment of diseases in rearing condition. In the present study, the different available drugs were screened against the active sites of aquaporins of *Nosema bombycis* and *Nosema cerenae* by using different *in silico* methods. Three dimensional structures of aquaporins of *N. bombycis* and *N. cerenae* were elucidated by using protein modelling tools. The active sites of the proteins were identified by using CASTp server. The docking between the drugs and active site of aquaporins was carried out by using Auto Dock Vina. The interaction between drugs and active site was visualized by using Chimera. Based on the results of the present study the existing antiprotozoan drugs *viz.,* paramomycin sulfate, pentamidine, quinapyramine and proguanil can be used as potent drugs that can block the active sites of aquaporin proteins of both *Nosema* species.

Keywords- *Nosema, Silkworm, Honeybee, Aquaporins, Antiprotozoan drugs*

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Introduction

Microsporidia is a group of unicellular, intracellular, eukaryotic parasite that invades a variety of pathogens from protists to mammals. The invasion of microsporidia can be lethal to the production of insects resulting in an enormous loss to the industries [1]. The *Nosema* is a genus of microsporidian parasites containing 81 species affecting diverse species of silkworms, honeybee, wasps, and beetles. The microsporidian diseases like pebrine and nosemosis cause a massive loss in the rearing of commercial insects [2]. *Nosema bombycis* is an obligate destructive parasite and a silkworm pathogen which is the first microsporidia species identified in 1857 [3] and it is found in host cells where it undergoes repeated asexual divisions followed by spore formation causes a deadly protozoan disease called Pebrine by vertical and horizontal transmission [4]. *Nosema ceranae* is a microsporidian, that mainly affects *Apis cerana*, the Asiatic honeybee in Taiwan (2004). In adult honey bees, it causes the disease nosemosis along with *Nosema apis*, [5]. *N. ceranae* lives as dormant long-lived spore, and which remains resistant to extreme temperature and dehydration [6,7]. Microsporidian spores are the resting stage that lives inside or outside their host. The spores are characterized by a presence of right and thick wall with polar filament which is coiled having a posterior vacuole and polaroplast. Commonly infection progress through 5 stages namely spore adhesion to host cells, spore activation, increases in the internal osmotic pressure of the spore, polar filament ejection and sporoplasm injection into the plasma membrane through the polar filament. Though the mechanism not fully explored for spore germination, a stimulus such as osmotic pressure, pH, radiation, ionic species, and the temperature was needed [8-10]. It has been theorized that no matter the mode of activation, microsporidia exhibit an equivalent response to the stimuli and increasing the intrasporal osmotic pressure [11-14]. This increase in osmotic pressure results in an influx of water into the spore accompanied by swelling of the polaroplasts and posterior vacuole prior to spore discharge [15,16]. It is this pressure which forces the eversion of the polar tube and expulsion of the sporoplasm. In hyperosmotic solutions, polar tube discharge is restrained or slowed down, so sporoplasm passage won't occur; therefore, it provides clear evidence for the osmotic pressure theory. Water flow across the spore wall, and cell wall could be a clear demand for osmotic theories of spore discharge. Using D2O, showed that the water inflow into spores occurs through the particular transmembrane pathway (aquaporin) which is sensitive to $HgCl_2$ [16]. Spores germinate by an increase in the intrasporal osmotic pressure, and it is associated with the proteins mainly aquaporins. Aquaporin Aquatic channel proteins play a major role in sporulation and spore germination process. It belongs to integral membrane protein (MIP) superfamily and transport water and solute. Aquaporins in species of *N bombycis* and *N cerenae* show a greater amino acid sequence homology greater than that of aquaporins from other microsporidian species. Putative aquaporins were isolated from the species of *N bomycis* [17] and expressed in *Xenopus laevis* oocyte [18]. After expression of *N bomycis* aquaporins in *Xenopus laevis* oocytes, it was observed that it could promote rapid penetration of water into oocytes. Antiprotozoal agents are a class of pharmaceuticals used in the treatment of protozoan infections such as microsporidiosis, malaria, amebiasis, giardiasis, cryptosporidiosis, leishmaniasis, babesiosis, trypanosomiasis, Chagas disease, and toxoplasmosis. The mechanism of action of antiprotozoals varies from drug to drug due to the large variation in the characteristics and function of the protozoans. In the present study, an attempt has been made to explore the *N bombycis* and *N cerenae* aquaporins and their potential inhibitors especially antiprotozoal, which may yield novel therapeutic agents for microsporidian infections.

Table-1 *Physico-chemical properties of Aquaporin proteins*

Accession No.	Length	M.wt	PI	-R	+R	EC	II	AI	GRAVY
A0A1L4APG2 (*Nosema bombycis*)	249	26694.26	5.12	17	13	26150	33.73	113.29	0.788
C4VBN2 (*Nosema cerenae*)	249	26935.68	9.3	13	19	34045	24.24	106.99	0.653

Table-2 *Secondary structural characterization of Aquaporin proteins*

Accession No.	Alpha helix	Extended strand	Beta turn	Random coil
A0A1L4APG2	35.34%	26.10%	3.21%	35.34%
C4VBN2	32.13%	25.70%	4.82%	37.35%

Table-3 *Docked conformations of Aquaporin proteins with antiprotozoal drugs*

Protein	Ligands	Affinity (Kcal/mol)	Number of Hydrogen Bonds	Hydrogen bond Threshold (angstrom)	Number of Contacts
Nosema bombycis (Aquaporin)	Paramomycin sulfate	-6.7	4	3.5	74
	Pentamidine	-5.5	4	3.5	50
	Quinapyramine	-6.7	2	3.5	39
	Proguanil	-5.4	3	3.5	44
Nosema cerenae (Aquaporin)	Paramomycin sulfate	-6.7	9	3.5	75
	Pentamidine	-6.7	4	3.5	44
	Quinapyramine	-6.5	3	3.5	45
	Proguanil	-7.3	2	3.5	44

Table-4 *Information about the interacting residues to form hydrogen bond*

Protein Models	Drugs	Hydrogen bonds				
		Donor (D)	Acceptor (A)	Hydrogen (H)	D--A dist	DH--A dist
Nosema bombycis (Aquaporin)	Paramomycin sulphate	het O	PRO 156 O	het H	2.635	1.673
		het O	SER 159 OG	het H	3.04	2.09
		het N	GLU 163 O	het H	2.946	1.964
		het N	GLU 164 OE1	het H	2.999	2.046
	Pentamidine	het N	GLN 158 OE1	het H	3.068	2.394
		het N	GLN 158 OE1	het H	3.34	2.438
		het N	GLN 158 OE1	het H	3.121	2.36
		het N	SER 159 OG	het H	3.34	2.619
		het N	ALA 175 O	het H	2.807	2.228
	Quinapyramine	het N	GLY 153 O	het H	2.799	2.048
		het N	GLY 165 O	het H	3.445	2.629
	Proguanil	het N	GLN 158 O	het H	2.803	1.802
		het N	GLN 158 O	het H	3.402	2.544
		het N	GLY 165 O	het H	2.969	2.291
Nosema cerenae (Aquaporin)	Paramomycin sulphate	het N	SER 33 OG	het H	3.23	2.469
		het N	GLN 35 O	het H	3.052	2.265
		het N	THR 36 O	het H	3.009	2.174
		het N	THR 36 O	het H	3.267	2.432
		het N	THR 36 OG1	het H	2.905	2.048
		het O	GLN 35 O	het H	2.981	2.362
		het O	THR 36 O	het H	3.525	2.742
		het O	GLN 38 O	het H	2.841	2.23
		het O	ASN 115 OD1	het H	2.923	1.981
	Pentamidine	het N	SER 33 OG	het H	3.158	2.198
		het N	THR 36 OG1	het H	3.032	2.15
		het N	ILE 180 O	het H	3.056	2.179
		het N	GLU 176 OE1	het H	3.179	2.254
	Quinapyramine	het N	SER 33 O	het H	3.264	2.437
		het N	SER 33 O	het H	2.762	1.778
		het N	SER 33 OG	het H	2.998	2.265
	Proguanil	het N	PRO 70 O	het H	3.321	2.623
		het N	THR 73 O	het H	3.027	2.016

Materials and methods

Preparation of ligand dataset

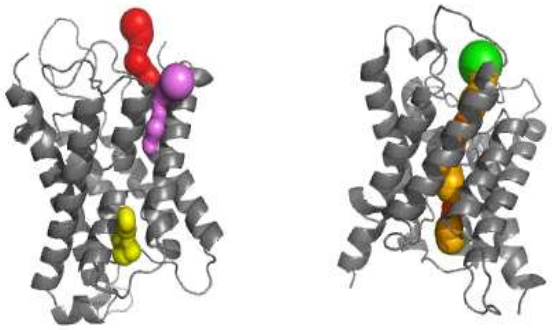
The chemical structure of the antiprotozoal drug was collected from published literature with their biological activity data. In this study, 2D structures of the antiprotozoal drug were retrieved from the PubChem database using the PubChem Structure search tool [19]. The 2D structures gathered were drawn and verified using marvin sketch, and the files were saved in MDL MOL format and converted to PDB format by OpenBabel software [20].

Functional characterization of Aquaporin

The aquaporin protein sequences of *Nosema bombycis* (Entry id: A0A1L4APG2) and *Nosema cerenae* (Entry id: C4VBN2) retrieved from the UniProt Knowledgebase (UniProtKB) [21]. The physico-chemical properties of the protein were computed by Expasy Protparam tool [22], and secondary structures were predicted using the Self Optimised Prediction from Multiple Alignment (SOPMA) server [23]. The aquaporin was characterized for the transmembrane regions, and these regions were predicted using the Transmembrane Hidden Markov Model (TMHMM) Program [24].

Preparation of protein structures

The structural information of *Nosema* aquaporins was not available in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RSCB PDB). The three-dimensional structure of aquaporins was generated using homology modelling. The structures were modelled using the most possible template matching more than e value using the Modeller software [25]. The best model was selected and analyzed by DOPE assessment score [26]. The modelled protein structures of *N bombycis* and *N cerenae* aquaporins was validated for evaluating model reliability using the web server RAMPAGE (mordred.bioc.cam.ac.uk/~rapper/rampage.php) and PDBsum (www.ebi.ac.uk/pdbsum).

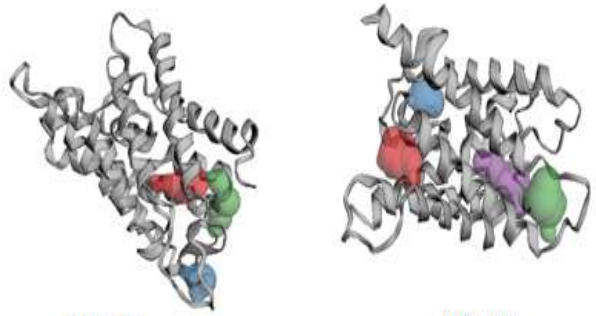


A. *Nosema bombycis* B. *Nosema cerenae*

Fig-1 Structural analysis of channels of aquaporin proteins

Identification of Active site of aquaporin protein

Active sites are the regions of the protein capable of binding to the ligand and create the biological response. The active sites of *N bombycis* and *N cerenae* aquaporin proteins were ascertained using using Computed Atlas of Surface Topography of proteins- CASTp web server (http://sts.bioe.uic.edu/) [27]. It provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins, which are prominent regions of proteins that are often associated with binding events.



A. *Nosema bombycis* B. *Nosema cerenae*

Fig-2 The Modelled structure of aquaporin proteins and its binding sites

Docking studies

The Antiprotozoal drugs such as albendazole, aralen, atovaquone, benlate, carbendazole, eflornithine, furazolidone, lariam, metronidazole, nifursemizone, nitazoxanide, ornidazole, Paromomycin sulphate, Pentamidine, Plaquenil, Primaquine, Proguanil, Pyrimethamine, Quinapyramine, Tinidazole were used as ligands. The validated protein structure and ligands are prepared, and *in silico* protein-ligand docking analyses were performed by using of Autodock tool (ADT version 1.5.6) [28]. The protein and ligand structures were cleared by removing the explicit water molecule. Non-polar hydrogen was merged, and gasteiger charges were computed for protein and ligand. Grid box was generated to cover all the active site residues of the aquaporin proteins. The molecular docking study of antiprotozoal drugs with the modelled structure of aquaporins was done using Autovina tool [29]. The docking studies were visualized and analyzed under Chimera software [30].

Results and Discussion

The Aquaporin proteins of *Nosema bombycis* and *Nosema cerenae* was retrieved from the UniProt Knowledgebase (UniProtKB), and the physico-chemical properties of the protein were computed by Expasy Protparam tool is presented in [Table-1]. The aquaporins of *N bombycis* was characterized of seven transmembrane helices and these regions were 12-31, 41-60, 65-87, 91-113, 134-156, 180-202, 223-245 respectively. The Aquaporin of *N cerenae* was characterized of six transmembrane helices and these regions were 12-34, 39-61, 82-104, 132-154, 182-204, 224-246 respectively. The secondary structural feature of aquaporin protein infers that the random coil and alpha helix predominates and covers more than 65% followed by extended strand (>25%) and beta turn (>3%) and it was shown in [Table-2]. The Aquaporin belongs to the major intrinsic protein's family (PF00230, MIP). This family includes large members that form transmembrane channels and helps to transport water, small molecules, ions, etc. Aquaporins contain two tandem repeats that comprise three membrane-spanning domains and a pore-forming loop with two signature motif NPA (Asn-Pro-Ala) and NPG (Asn-Pro-Gly). The functional annotation of the aquaporin proteins shows the transmembrane activity, comprises of membrane (GO:0016020), integral component of membrane (GO:0016021), transmembrane transport (GO:0055085) and channel activity (GO:0015267). The aquaporins protein structure of *N bombycis* and *N cerenae* were modeled with the template of 4NEF_A, 4OJ2_X, 2ZZ9_A, 2D57_A that has more than 30% identity using Modeller program were shown in Figure. The modelled three-dimensional structure was validated using the RAMPAGE tool which exhibited the allowed and disallowed region and found to be reliable models. The percentage of residue lying in the most favoured, additionally allowed, generously allowed and disallowed regions were 86.9%, 10.3%, 1.4% and 1.4% for *N bombycis* and 87.2%, 9.6%, 2.1% and 1.1% for *N cerenae*. Earlier, it was considered that the activation simply increases the permeability of the spore coat to water. However, data suggest while the spore coat functions as a molecular sieve, it is freely permeable to water. Another theory involved the creation of a proton gradient by the alkaline environment surrounding the spore [31]. The proton gradient drives a proton-cation exchange mechanism consisting of a carboxylic acid ionophore. As protons in the sporoplasm are depleted, the increase in alkalinity triggers the same mechanisms in the membrane of organelles, particularly the polaroplast and posterior vacuole. Water flows into the spore, due to the generalized osmotic imbalance, increasing the intrasporal pressure. Hence, modeled structures were uploaded to the CASTp server to predict the pores, tunnels and the active site of the models were shown in [Fig-1]. The possible binding sites of aquaporin protein were identified and found the residues that were considered as the most favorable binding site of protein for docking [Fig-2]. The germination process could be inhibited by the application of docked antiprotozoal drug showing the high degree of interaction. The efforts were made to obtain the entire orientation of the antiprotozoal drugs inside the binding pockets of the aquaporin protein. The Antiprotozoal drugs such as paramomycin sulfate, pentamidine, quinapyramine, and proguanil were shown the better binding and interaction with the aquaporin proteins. Paromomycin sulphate is an antiprotozoal drug and Pentamidine directly interact with the pathogen by binding to AT-rich regions of duplex DNA and the minor groove of DNA, thereby interfering with DNA replication [32]. Proguanil acts as a Dihydrofolate Reductase Inhibitor thereby by inhibiting parasitic dihydrofolate reductase enzyme whereas the mechanism of action of quinapyramine is still unclear. These drugs might directly interact with aquaporin protein by altering the structure of a protein or interact with the key cellular metabolism of parasites during the germination process [33,34]. Docking of the antiprotozoal drug with aquaporin protein was performed using the autodock and autovina program. *Nosema bombycis* aquaporin protein were docked with paramomycin sulfate, pentamidine, quinapyramine and proguanil and their docking energy were -6.7, -5.5, -6.7 and -5.4 respectively [Fig-3]. The interacting residues of four antiprotozoal drug with *N bombycis* aquaporin were Gly153, Pro156, Gln158, Ser159, Glu163, Glu164, Gly165 and Ala175. *Nosema cerenae* aquaporin protein was docked with paramomycin sulfate, pentamidine, quinapyramine and proguanil and their docking energy was -6.7, -5.5, -6.7 and -5.4 respectively [Fig-4].

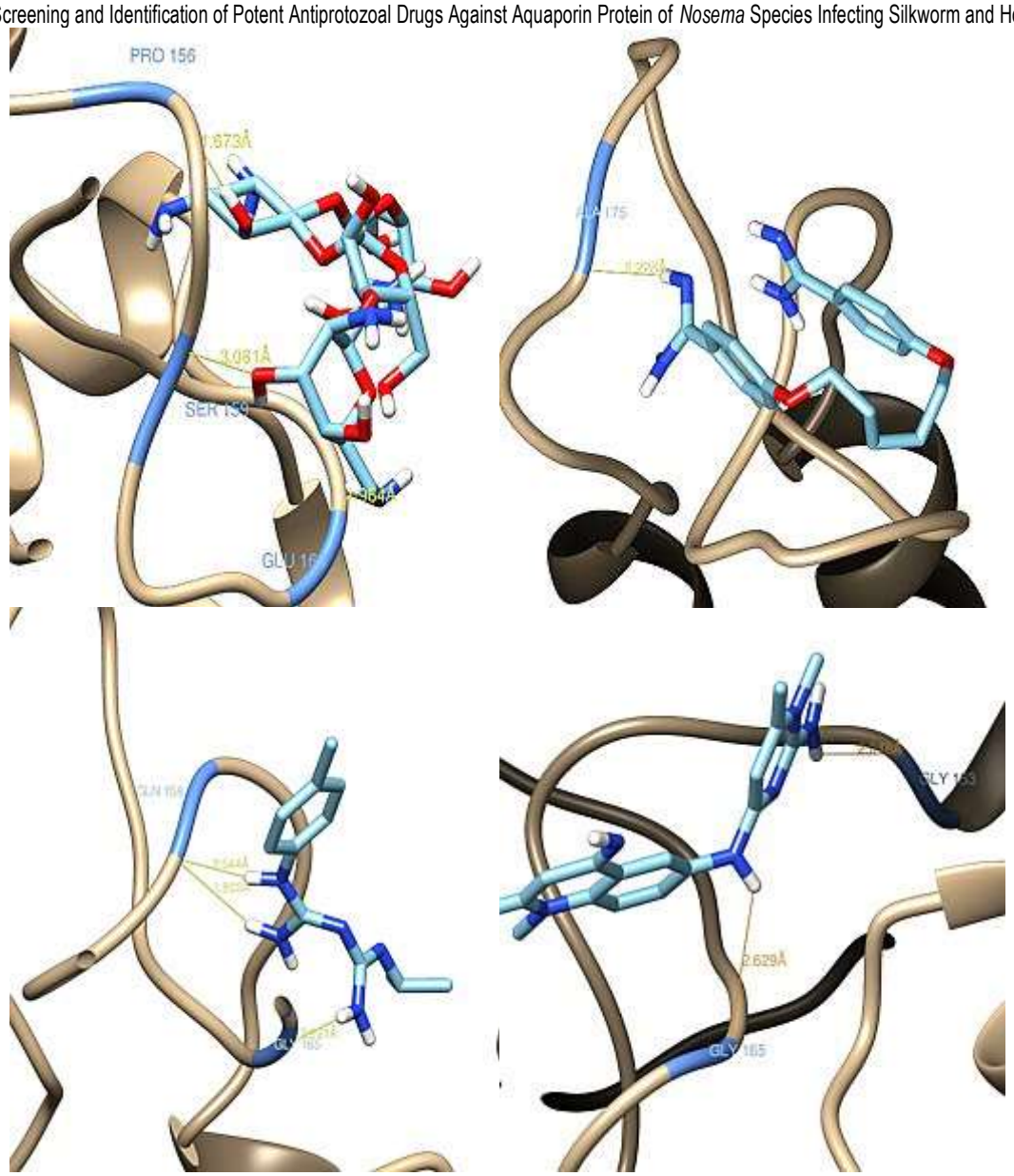


Fig-3 Interaction of compounds (A) Paramomycin sulphate, (B) Pentamidine, (C)Proguanil and (D) Quinapyramine with *N bombycis* aquaporin protein

The interacting residues of four antiprotozoal drug with *N cerenae* aquaporin were Ser 33, Gln35, Gln38, Thr36, Gln35, Pro70, Thr73, Asn115, Glu176 and Ile180 [Table-4].

Conclusion

The results of the present study revealed that, aquaporin proteins of *Nosema* species can be targeted for inhibition of microsporidian diseases in silkworm and honey bees. Screening of different drugs resulted in identification of potential inhibitors such as paramomycin sulfate, pentamidine, quinapyramine and proguanil which can block the water channel of *N bombycis* and *N cerenae* aquaporin protein.

Application of research: The findings of this study will help to design and develop novel inhibitor/s along with their analogues which binds to the aquaporin protein of microsporidia which ultimately results in controlling the diseases and better productivity of economic importance insects.

Research Category: *In silico* analysis

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Species name: *Nosema bombycis*, *Nosema cerenae*

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

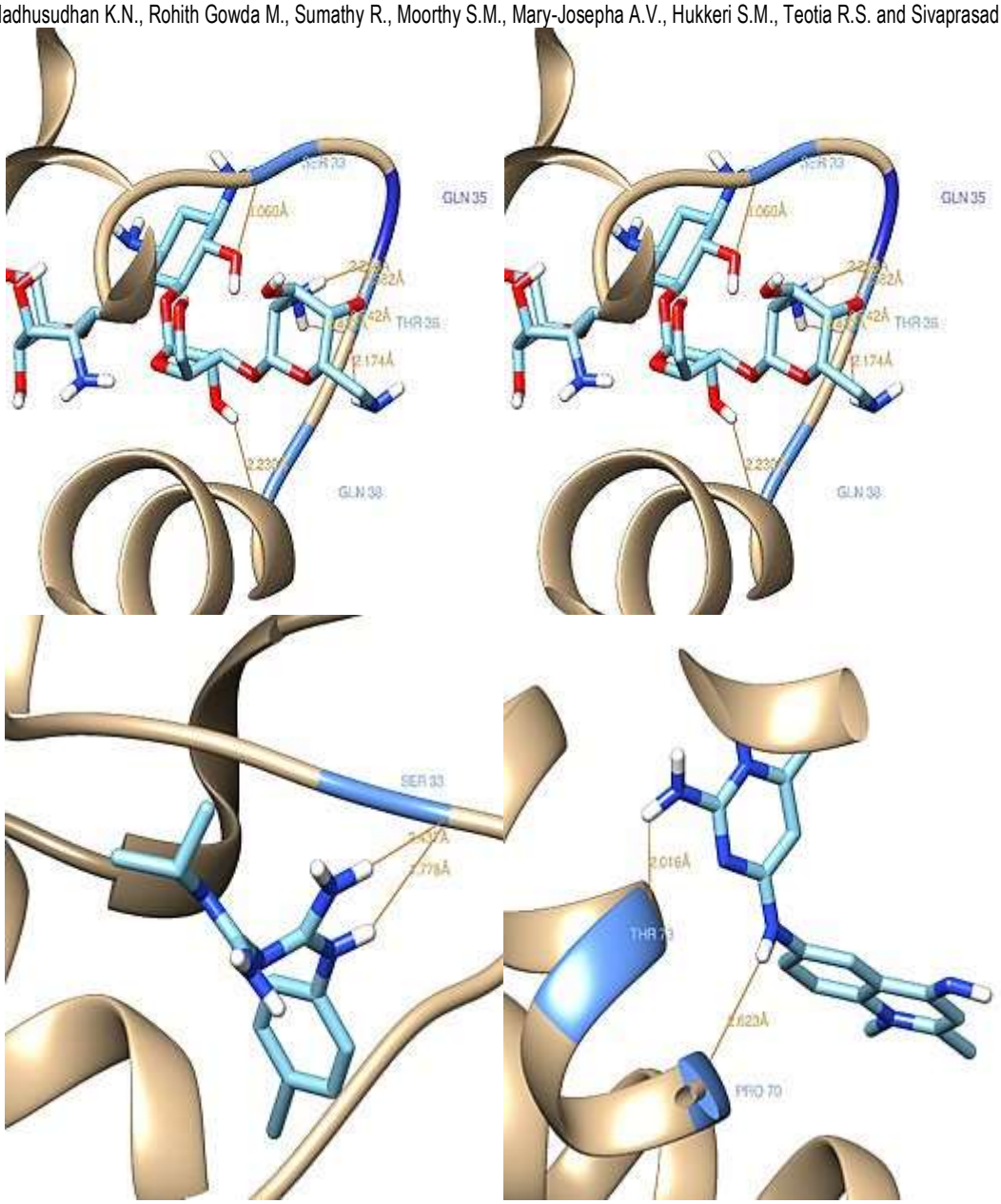

Fig-4 Interaction of compounds (A) Paramomycin sulphate, (B) Pentamidine, (C)Proguanil and (D) Quinapyramine with *N cerenae* aquaporin protein

References

[1] Pan G., Bao J., Ma Z., Song Y., Han B., Ran M., Li C., Zhou Z. (2018) *Dev Comp Immunol* 83,104-113.

[2] Berman H.M., Battistuz T., Bhat T.N., Bluhm W.F., Bourne, P.E., Burkhardt K., Feng Z., Gilliland G.L., Iype L., Jain S., Fagan P., Marvin J., Padilla .Ravichandran V., Schneider B., Thanki N, Weissig H, Westbrook JD, Zardecki C (2002) *Acta Crystallogr D Biol Crystallogr* 58(Pt 6 No 1), 899-907.

[3] Nageli C. (1857) *Bot. Ztg*, 15,760-761.

[4] Dunn A.M., Terry R.S., Smith J.E. (2001) *Adv Parasitol* 48,57-100.

[5] Cornman R.S., Chen Y.P., Schatz M.C., Street C., Zhao Y., Desany B., Egholm M., Hutchison S., Pettis J.S., Lipkin W.I., Evans J.D. (2009) *PLoS Pathog* 5(6),e1000466.

[6] Aufauvre J., Biron D.G., Vidau C., Fontbonne R., Roudel M., Diogon M., Viguès B., Belzunces L.P., Delbac F., Blot N. (2012) *Sci Rep* 2,326.

[7] Doublet V., Labarussias M., de Miranda J.R., Moritz R.F., Paxton R.J. (2015) *Environ Microbiol* 17(4),969-83.

[8] Undeen A.H. (1990) *J Theor Biol* 142(2), 223–35.

[9] Undeen A.H., Frixione E. (1990) *J Protozool* 37(6), 561–67.

[10] Undeen A.H., Vander Meer R.K. (1990) *J Protozool* 37(3), 194–99.

[11] Kudo R. (1918) *J Parasitol* 4(4),141-47.

[12] Oshima K. (1937) *Parasitology* 29(2),220-24.

[13] Lom J., Vavra J. (1963) *Acta Protozool* 1, 81-92.

[14] Frixione E., Ruiz L., Santillán M., de Vargas L.V., Tejero J.M., Undeen A. H. (1992) *Cell motility and the cytoskeleton* 22(1),38-50.

[15] Weidner E., Byrd W. (1982) *J Cell Biol* 93(3),970-75.

[16] Frixione E., Ruiz L., Cerbon J., Undeen A.H. (1997) *J Eukaryot Microbiol*. 44(2),109-16.

[17] Chen G., Wang W., Chen H., Dai W., Peng X., Li X., Tang X., Xu L., Shen Z. (2017) *PloS one* 12(7),e0181703.

[18] Chen G., Zhang Z., Shang R., Qi J., Zhang Y., Tang S., Shen Z. (2018) *Parasitol Res* 117(11),3473-79.

[19] Kim S., Thiessen P.A., Bolton E.E., Chen J., Fu G., Gindulyte A., Han L., He J., He S., Shoemaker B.A., Wang J., Yu B., Zhang J., Bryant S.H. (2016) *Nucleic Acids Res* 44(D1),D1202-13.

[20] O'Boyle N.M., Banck M., James C.A., Morley C., Vandermeersch T., Hutchison G.R. (2011) *J Cheminform* 3,33.

[21] Pundir S., Martin M.J., O'Donovan C. (2017) *Methods Mol. Biol* 1558,41-55

[22] Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., et al. (In) John M. Walker (ed) (2005) *The Proteomics Protocols Handbook, Humana Press* 571-607.

[23] Geourjon C., Deléage G. (1995) *Comput Appl Biosci* 11, 681-84.

[24] Krogh A., Larsson B., von Heijne G., Sonnhammer E.L. (2001) *J Mol Biol* 305(3), 567-80.
[25] Webb B., Sali A. (2016) *Curr Protoc Bioinformatics*. 54, 5.6.1-5.6.37.
[26] Shen M.Y., Sali A. (2006) *Protein Sci*. 15(11),2507-24.
[27] Tian W., Chen C., Lei X., Zhao J., & Liang J. (2018*) Nucleic Acids Res* 46(W1),W363-W367.
[28] Morris G.M., Huey R., Lindstrom W., Sanner M.F., Belew R.K., Goodsell D.S., Olson A.J. (2009) *J Comput Chem* 30(16), 2785-91.
[29] Trott O., Olson A.J. (2010) *J Comput Chem*. 31(2),455-61.
[30] Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C., Ferrin T.E. (2004) *J Comput Chem*. 25(13),1605-12.
[31] Dall D.J. (1983) *J Theor Biol* 105(4),647-59.
[32] Fox K.R., Sansom C.E., Stevens M.F. (1990) *FEBS Lett*. 266(1-2),150-54.
[33] Desportes I., Le Charpentier Y., Galian A., Bernard F., Cochand-Priollet B., Lavergne A., Ravisse P., Modigliani R. (1985) *J Protozool*., 32(2),250-54.
[34] Weber R., Bryan R.T., Schwartz D.A., Owen R.L. (1994) *Clin Microbiol Rev* 7(4),426-61.