Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Virtual screening of natural anti-filarial compounds against glutathione-S-transferase of *Brugia malayi* and *Wuchereria bancrofti*

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Received April 3, 2018; **Accepted** October 21, 2018; **Published** October 30, 2018

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Doi: http://dx.doi.org/10.14715/cmb/2018.64.13.13

Abstract: Glutathione-S-transferase also referred as GST is one of the major detoxification enzymes in parasitic helminths. The crucial role played by GST in various chronic infections has been well reported. The dependence of nematodes on detoxification enzymes to maintain their survival within the host established the crucial role of GST in filariasis and other related diseases. Hence, this well-established role of GST in filariasis along with its greater nonhomology with its human counterpart makes it an important therapeutic drug target. Here in this study, we have tried to explore the inhibitory potential of some of the well-reported natural ant-filarial compounds against the GST from *Wuchereria bancrofti* (*W.bancrofti)* and *Brugia malayi* (*B.malayi).* In silico virtual screening, approach was used to screen the selected natural compounds against GST from *W.bancrofti* and *B.malayi.* On the basis of our results, here we are reporting some of the natural compounds which were found to be very effective against GSTs. Along with we have also revealed the characteristic of the active site of BmGST and WbGST and the role of important active site residues involve in the binding of natural compounds within the active site of GSTs. This information will oped doors for using natural compounds as anti-filarial therapy and will also be helpful for future drug discovery.

Key words: Virtual screening; *Brugia malayi; Wuchereria bancrofti;* Natural compounds.

Introduction

Infectious diseases have a threatening effect on human health and life. It's a well-known fact that a number of parasitic diseases are considered to be the diseases of poor or " neglected tropical diseases" some of the parasitic diseases are trypanosomiasis, leishmaniasis, schistosomiasis, lymphatic filariasis, and onchocerciasis. The parasites responsible of these diseases are real monsters and proves to be fatal they are responsible for high death rates in many endemic countries each year. The other dreadful part is that there is a lack of vaccines, as well as proper treatment, is out of the reach of poor people. So, it an alarming signal and inevitable for us to discover affordable medication and therapy against such threatening ailments. It can only be achieved by launching such pharmaceutical industries comprising the use of natural resources like plants etc. as plant kingdom is the immense treasure of medicinal compounds

Lymphatic filariasis (LF) is a debilitating and chronic disease majorly affecting the people in sub-tropical and tropical areas of Africa, Asia, and areas of the United States of America (1-3). LF is usually caused by the parasites *Wuchereria bancrofti* (*W. bancrofti*), *Brugia malayi (B. malayi)* and *Brugia timori (B. timori),* which are transmitted by *Anopheles, Culex* and other mosquitoes. Among all the filarial infections, LF is the most prevalent one (4-6). Commonly known as "elephantiasis," this disease has affected ~1.4 billion of the global population. The current estimate shows that 553 million people in India live in endemic areas and around 48 million having either circulating microfilariae (mf) or overt diseases like elephantiasis, lymphoedema, and hydrocele (6, 7). Filariasis is one of the most common causes of permanent disability among tropical diseases worldwide creating the highest disease burden in terms of disability-adjusted life years (DALYs) (8). Those affected also suffer psychosocial stigmatization and economic suffering as it can lead to job loss or "inability to work." The disease is, therefore, a major cause of poverty as it causes an economic burden for those affected, on their dependents, their communities and the country as a whole (1, 9).

Great efforts have been made since the last three decades, in order to control and tackle LF; however, to get rid of these diseases is still a hard nut to crack and actually a heinous human health issue. Albendazole is being used world wide for the global control of LF (10). Other recommended treatment of filariasis patients is the administration of albendazole combined with iver-

mectin or DEC (11, 12). However, all these treatments have a narrow approach and limited activity. Moreover anthelminthic resistance in another tough challenge against parasite control.

Anthelmintic resistance, secondary effects and limited macrofilaricidal activities of the known anti-filarial drugs have paved the way to hunt new and alternative methods of treatment and of course medicinal plants prooves to be very useful for cutting corners or be used as a cheap source and affordable for poor people (13). Since prehistoric times, medicinal plants are considered to be therapeutic in traditional health care systems and are still considered as a major health care source (14). It is estimated, globally a large population (~60%) rely on traditional natural medicine for their primary health care needs (15).

Helminths exhibit few detoxification enzymes so the important cytochrome P-450 dependent detoxification reactions also lacks. By the time it was discovered that Glutathione-S-transferases are multifunctional enzymes their major function is to catalyze the conjugation of glutathione thiolate anion with a multitude of second substrates or non-covalent binding proteins for a range of hydrophobic ligands. The important aspect of this protein is that it serves as a major detoxification enzymes and serves an important function for the survival of the parasite.

When we discuss the defense system of filarial nematodes, GSTs (glutathione S transferase(s) are of utmost importance. These secreted enzymes provide effective resistance to the filarial parasites against immune effector mechanisms and their persistence in the mammalian host. They perform this role by inhibiting the oxidative burst of leukocytes and neutralization of secondary lipid products . GSTs are immense family of multifunctional dimeric enzymes providing defense against oxidative attack through a mechanism of conjugation of electrophiles to glutathione and reduction of lipid hydroperoxides. Now a days enormous research on GSTs are conducted as an effective drug target for the development of anti-schistosomal, antimalarial and antifilarial drugs because of their role in drug metabolism. Survival of the parasite depends upon the Parasitic GST, and the inhibition of parasitic GST very well supports in increasing the activity of currently accessible antifilarial medicines. Human filarial parasite GST is different from human GST in structure and sequences.

This perspective of GSTs can be easily proved by considering the ability of helminths to combat against the attack on the cell membrane by reactive oxygen species (releasing cytotoxic products) (16). The inhibition of GST(s) gradually becomes more fascinating, since these the appearance of these enzymes in drug resistance and their involvement in the biosynthesis of a various important arachidonic acid metabolites. In order to relate the efficiency of known natural anti-filarial compounds against GST of *B. malayi* and *W. bancrofti,* we have used in silico approach to study the interaction of these compounds against the chosen GSTs of particular compounds used in this study. Few compounds are found to be very effective against BmGST or WbGST or both of them. This generated result will not only help in the use of natural compounds as ant-filarial theraputic candidate but will also provide an insight towards the designing of new anti-filarial inhibitors for chemotherapy against filariasis.

Materials and Methods

Preparation of protein

The three dimensional structure of GST for *W.Bancrofti* (WbGST) was available with RCSB protein databank, and was retrieved in ".pdb" format (pdb id: 3T2U). All the heteroatoms and water molecules were removed from this structure. While the three dimensional structure of GST for *B.malayi* (BmGST) was unavailable with RCSB protein databank, so we computationally modelled its structure using its amino acid sequence. The 301 amino acid long sequence of BmGST was retrieved from uniprot database (accession number O02636). This sequence was subjected to Blastp search against protein databank to identify template structure (17). The structure Of *W.Bancrofti* Glutathione S-Transferase which shares 100% query coverage and 99% identity was taken as a template to model the structure of BmGST. MODELLER9v8 (18), an automated comparative protein modeling program was used to model the structure of BmGST. A total of five different models were generated and the best one was selected on the basis of their DOPE score.

Structure Validation

The overall stereochemical quality of the modeled structure of BmGST was validated using PROCHECK (19).

Ligand preparation

Literature survey was performed to collect all the natural compounds carrying anti-filarial properties. The 3D structure of all the selected natural compounds was extracted from from pubchem compounds database (20). All the selected structures were compiled together in a single library.

Molecular docking

GOLD (Genetic Optimization for Ligand Docking) 5.0 (21) was used to was carried out the molecular docking of all the selected natural compounds against GST of both *B.Malayi* and *W.Bancrofti*. All the operations were performed using default parameters. The best ranked poses were further analysed using a consensus scoring function of X-Score to calculate the binding affinity (22).

Molecular dynamics simulation

The complex of top scoring natural compound with WbGST and BMGST were subjected for MD simulation. All the MD simulation calculations were performed on GROMACS using the GROMOS 96 force field (23). In this study, we selected the conformations with lowest binding energies as the starting conformation for MD simulations. The complex of BmGST-Strychnine and WbGST-Curcumin were selected in this study for performing MD simulation. The proteins were immersed within a cubic box solvated by simple point charge (spce) water molecules. The dimensions of the box was set to ensured that in both the protein, all the atoms should be at least 1.5 Å away from the wall of

the box with periodic boundary conditions. NaCl counter ions were added subsequently added to equilibrate the electro-neutrality condition. Finally, for the selected complex structures, molecular dynamics simulation was performed for a time period of 10-ns was carried out for. At every 2 ps, the trajectories were stored. The RMSD (root-mean-square deviations) and the root-mean-square fluctuations (RMSF) were calculated during this time period (10ns simulations).

Results and Discussion

Glutathione-S-transferase(s) (GST) has been widely reported as a therapeutic target in lymphatic filariasis (16, 24, 25). A large number of researches are being carried out toward designing of inhibitory molecule targeting GST (16, 26, 27). The use of natural compounds as a drug candidate has been an area of great research interest in drug design (27-29). Here we have utilized in silico virtual screening approach to search for the natural compound carrying inhibitory potential against GST of both *B. malayi* and *W.bancrofti*. It was found that the GST of both *B. malayi* and *W.bancrofti* shares great homology with each other (98% identity). This high level of identity is due to the fact that both belong to Onchocercidae family. While the structure of GST for W.bancrofti was available with RCSB protein databank but the structure for its B.malayi counterpart was unavailable. So we moved forward toward modelling the structure for GST (*B.malayi*) using computational modelling methods. Here we modelled the structure of BmGST using 3D structure of WbGST (pdb id: 3T2U), sharing 98% identity as a template. Later the modelled structure of BmGST was validated by Ramachandran Plot, which shows 94% amino acid residues in the most favoured region, while no residue was found in the disallowed region. All the selected compounds used in this study had been reported to be carrying anti-parasitic properties (11, 27, 30, 31). These compounds are reported to be carrying potential to interrupt the proliferation of parasites into their human host. Molecular docking provides an overview of the binding affinity of ligands against the selected target enzyme (32). Here all the selected natural compounds were docked against both BmGST and WbGST, and the topmost compounds were screened on the basis of their gold fitness score. In this study, curcumin, capsaicin, and piperine were found to be the most active compounds active against both BmGST and WbGST. For instance curcumin was found to bind with gold fitness score of 50.19 and 34.05 against WbGST and BmGST respectively (Table 1 and 2). Piperine was found to be equally effective against both the GSTs, where it shows the gold fitness score of 39.76 and 37.91 against WbGST and BmGST respectively (Table 1 and 2). Geraniol and Strychnine were found to show selective inhibition, where Geraniol was found to be very effective against WbGST, while not better binding affinity was found against BmGST. On another side Strychnine was showing higher binding affinity against BmGST, bit was not effectively binding within the active site of WbGST. Previous studies have reported the application of natural compounds for the treatment of several neglected parasitic diseases. The medicinal role of curcumin has been well established, the role of curcumin in successfully combating the parasitic infections has also been well reported (33, 34). In this study the interaction plot of the complex provides a binding insight into the binding of compounds within the active site of target protein. The binding analysis of the selected compounds in complex with their target protein provides the mode of binding and the reveal information of the important active site residues helpful in accommodating the natural compounds within the binding site of GSTs. It was revealed that the interaction of all the natural compounds within the binding site of both GSTs was mainly dominant by hydrophobic interactions, while there were just few amino acids found to be actively involved in making hydrogen bond formation with the natural compounds. For instance R95 and V153 were the only active site residues of WbGST which were involved in making hydrogen bond formation. In BmGST and WbGST, G12, L13, T102 and Y106 were found to be the common residues playing crucial roles in the orientation of compounds within the active site (Table 1 and 2) (Figure 1-4). The important role of these residues had been reported earlier. The com-

Table 1. Binding affinity of compounds against WbGST.

Compounds	Goldfitness Score	X-score (Kcal/mol)	Residues involved	
			Hydrogen bond	Hydrophobic interaction
Curcumin	50.19	-6.63	R95, V153	G12, L13, P16, V94, R95, H98, T99, T102, Y106, E156, I160
Capsaicin	44.46	-6.22	R95	L13, P16, V94, R95, H98, T99, T102, K103, V153, E156
Piperine	39.76	-6.45		L13, P16, Q49, L50, P51, S63, R95, H98, V153
Geraniol	38.86	-6.44	R95	L13, P16, R95, H98, E156, I160

Table2. Binding affinity of compounds against BmGST.

Compounds	Goldfitness Score	X-score (Kcal/mol)	Residues involved	
			Hydrogen bond	Hydrophobic interaction
Strychnine	39.87	**-6.8**	G204	F8, I10, G12, L13, T102, G204
Piperine	37.91	**-6.23**	N203	F8, I10, G12, L13, W38, H98, T102, G204
Capsaicin	36.58	**-6.93**		F8, I10, G12, L13, T102, Y106, P201, G204, N205, G206
Curcumin	34.05	**-6.58**	H98	F8, I10, G12, L13, A35, T102, P201,

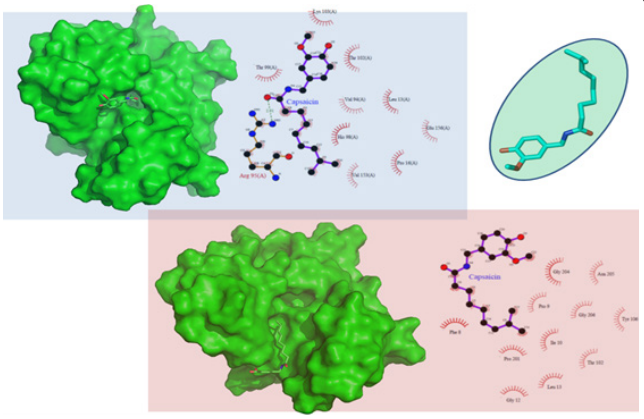

Figure 1. Binding pattern of Capsaicin within active site of BmGST and WbGST.

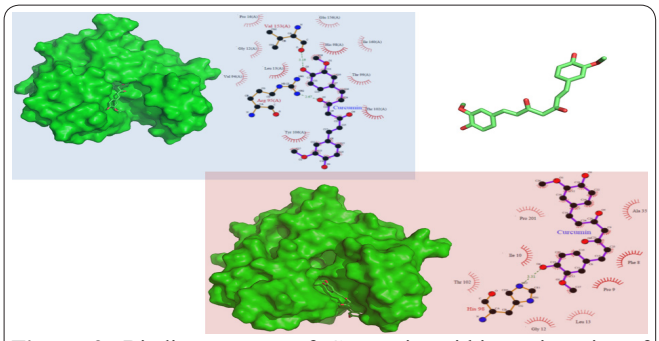

Figure 2. Binding pattern of Curcumin within active site of BmGST and WbGST.

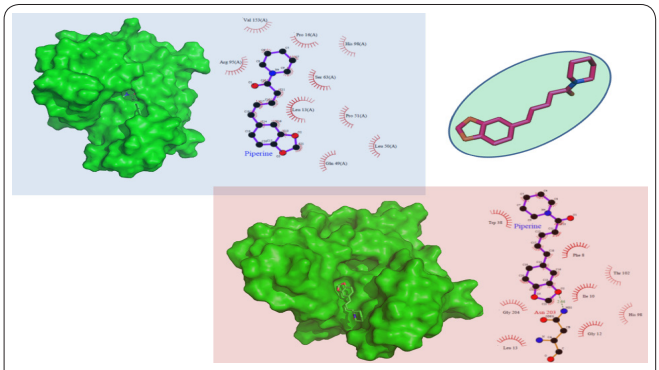

Figure 3. Binding pattern of Piperine within active site of BmGST and WbGST.

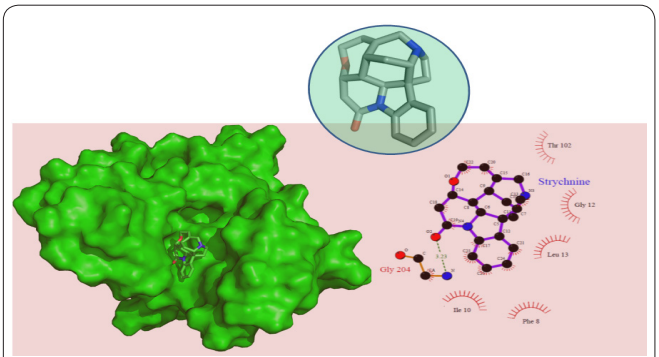

Figure 4. Binding pattern of Strychnine within active site of BmGST and WbGST.

plex of top scoring compound against both the GSTs i.e curcumin-WbGST and Strychnine-BmGST were further subjected to MD simulation for 10ns. The MD simulation was performed to gain a better insight into the binding of these compounds within the active site of their respective proteins. The calculation of RSMD, a crucial parameter for analysing the equilibration of MD

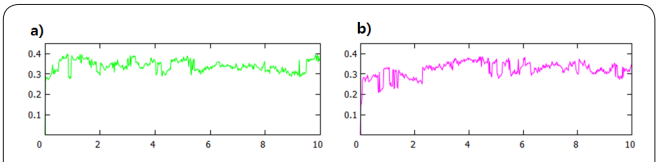

Figure 5. Root mean square deviation of the backbone atoms of docked complexes at 300K. (a) BmGST- Strychnine complex is shown in green color (b) WbGST-Curcumin complex is shown in pink color

trajectories for backbone atoms of WbGST-curcumin and BmGST-Strychnine complexes was performed. The measurements of the backbone RMSD is helpful in providing a better insight into the conformational stability of both the selected complexes. Figure 5 shows the RMSD value of WbGST-curcumin and BmGST-Strychnine complexes. Until 6 ns, an increase in the RMSD value of the WbGST-curcumin complex structure was noticed (~0.39 nm). Later the RMSD value of this complex dropped down to 0.3 and was found to fluctuate less during the rest of the time period. In case of BmGST-Strychnine the RMSD fluctuation was highest at 5.5 ns where the RMSD fluctuated upto 0.39 nm, later the continuous drop in the RMSD was noticed till 9ns, which tends to fluctuate after 9ns too. No major fluctuation in the RMSDs of both the complex was noticed. Based on the RMSD results, we conclude that the stability of the WBGST-Curcumin complex was higher than the BmGST- Strychnine complex.

This study provides an in silico overview of the use of selected natural compounds as inhibitor against BmGST and WbGST. Here we have listed some of the important natural compounds which could be used as a future therapeutic drug candidate against filarial GSTs towards preventing various parasitic infections. The docking study also reveals the role of crucial amino acid residues within the active site region of the targets and the mode of their interaction in accommodating the natural compounds within the binding cavity. These outcomes will be highly useful for better anti-filarial therapeutic design.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2018R1C1B5047470).

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