BIOINFORMATION
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Volume 10(8)

Comparative Molecular Docking Studies with ABCC1 and Aquaporin 9 in the Arsenite Complex Efflux

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Received July 18, 2014; Revised August 03, 2014; Accepted August 04, 2014; Published August 30, 2014

Abstract:
Arsenic is the most toxic metalloid present in the natural environment in both organic and inorganic arsenic forms. Inorganic arsenic is often more hazardous than the organic form. Arsenite and arsenate compounds are the major inorganic forms which are toxic causing severe human health dysfunction including cancer. Excretion of arsenic from the system is found elusive. Therefore, it is of interest to screen channel proteins with the arsenic complex in the different combination of arsenic, GSH (glutathione) and arsenic, selenium using docking methods. The mode of arsenic removal. The complex structure revealed the mode of arsenic binding efficiency with the receptor aquaporine 9 and ABCC1 channel protein. This provides insights to understand the mechanism of arsenic efflux. These inferences find application in the design, identification and development of novel nutracetucal or any other formulation useful in the balance of arsenic efflux.

Keywords: Arsenic toxicity, Molecular Docking, Transport Channels.

Background:
The rapid industrialization and mainly geological sources cause inadvertent exposure of arsenic (As) to humans through water pollution **[1].** The non-anthropogenic sources of arsenic [both arsenate (AsV) and arsenite (AsIII)] are the major causes of their chronic exposure to 100 million people worldwide, in the form of drinking water with a very high unacceptable level **[2].** In present, mainly in the several regions of India and Bangladesh, a major population drinks groundwater with arsenic concentrations above than the World Health Organization (WHO) acceptable standard **[3, 4].** Apart from their presence in water bodies, in clinical medicine, arsenic is used as arsenic trioxide in tandem with all-trans-retinoic acid to treat acute promyelocytic leukemia, **[5, 6],** and in some drugs along with antimony to treat parasitic infections, african sleeping sickness, and leishmaniasis **[7].** The Multidrug resistance-associated protein1 is a protein, which is in humans is encoded by the ABCC1 gene. It is a polytopic transmembrane protein that belongs to the ABC Transporter family, and acts as an efflux pump. It transports conjugated organic anions and also co -transports certain unmodified xenobiotics e.g. vincristine with glutathione (GSH). It is equally important for the transports inorganic arsenic as a GSH conjugate and GSTP1-1 may have a synergistic role in this process **[8, 9].** ABCC1 mediates the cellular efflux of a variety of xenobiotics, typically as glucuronide sulfate, or glutathione conjugates. It may also play a role in protecting the seminiferous tubules from methoxychlor-induced damage **[10].**

The Aquaporins, major intrinsic protein are a family of water-selective membrane channels aquaporin super family and, responsible for transporting small uncharged molecules such as glycerol and urea whereas, water, plays a pivotal role in the uptake of AsIII **[11-13].** It is reported that, both the channels involved in the efflux of arsenic in presence of GSH, and it has also been reported that ABCC1 and Aquaporin are involved in the arsenic efflux **[14].** Evidence suggests that at least some of these substrates are co-exported with GSH across the plasma membrane. The purpose of the current study is to identify the arsenics associated complex in best fit structure and the mode by which they get transported with a greater affinity and that are substrates for ABCC1 and Aquaporin 9.

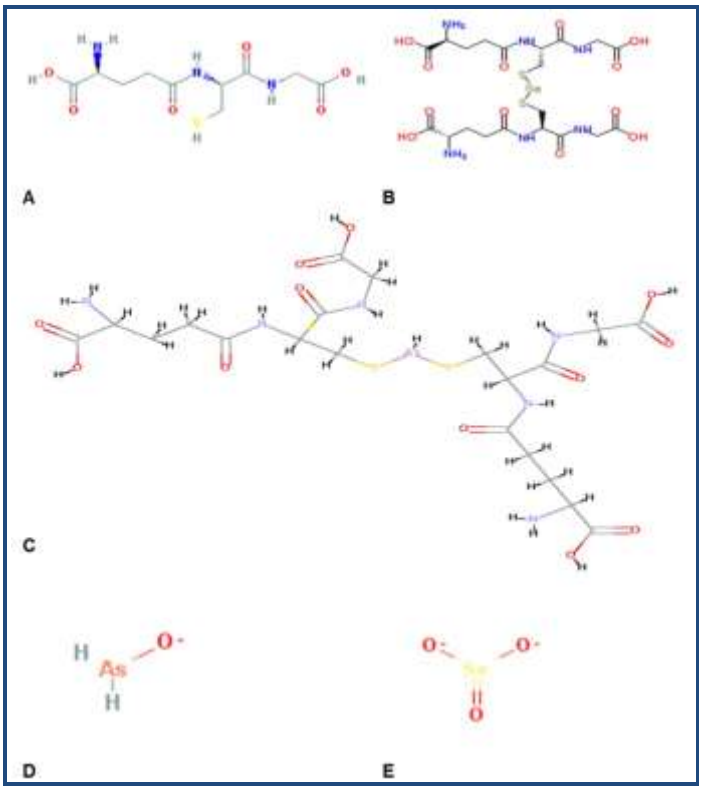

Figure 1: **A)** Chemical structure of Glutathione, Pubchem ID: CID 124886, ChemSpider ID: 111188; **B)** Chemical structure of Diglutathione selenide (GSH-Se-GSH), ChemSpider ID: 97171, Pubchem ID:CID 108069 **C)** Chemical structure of GSH-AsH-GSH; **D)**Arsinite, Pubchem ID:CID 5460562; **E)** Chemical structure of Selenite, Pubchem ID:CID 1090**.**

Methodology:

Preparation of Ligand structures

Ligand files of Glutathione, Diglutathione selenide/ Seleno-diglutathione (GSH-Se-GSH), Arsinite and Selenite were downloaded in .mol format **(Figure 1A - E)** from ChemSpider Chemical Database. These files cannot be directly used by Patch Dock tools **[15],** thus they were converted it into .pdb files using Discovery Studio Visualizer version 3.5. Discovery Studio is a software package of biological molecular design solutions for computational chemists and computational biologists. Discovery Studio makes it easier to analyze the properties of large and small particles. GSH-AsH-GSH (Figure-2A) was drawn and the SMILE ID generated by using ChemSketch Software, after that CORINA (http://www. molecular-networks.com/ online_demos/corina_ demo) was used for the conversion of SMILE ID (Simplified Molecular Input Line Entry System) to 3D structure in .pdb format. Further the ligand was submitted for the minimization using Chimera version 1.5.3 using with Genetic Algorithm Steps 2000 and 0.5 grid units Optimized **[16, 17].**

Preparation of protein structures

The structures of Proteins involved in this study Auqapurine-9 and ABCC1 both, were not available in the RCSB Protein Data Bank, so that we have generated both structures by Homology Modeling (Modeller9.10) and validated by the Ramachandran Plot (RAMPAGE). The Chimera was used for the energy minimization, removal of steric collision with the steepest descent steps 1000, steepest descent size 0.02 Å, Conjugated gradient steps 1000 and the conjugate gradient step size 0.02 Å for the conjugate gradient minimization **[17-19].**

Protein- Chemical Molecular Docking Studies

All the in silico protein-chemical docking analyses were performed by using of PatchDock. The Auqaporin-9 and ABCC-1 were docked with the Glutathione, GSH-AsH-GSH, GSH-Se-GSH, Arsinite and Selenite as shown in figure 2 and 3. Patch Dock online Server done the molecular docking on Geometry-based algorithm **[15, 20].** This sever was applied to recognize the binding scores and binding residues of Glutathione, GSH-AsH-GSH, GSH-Se-GSH, Arsinite and Selenite with the Auquaporin-9 and ABCC-1, that were treated as a rigid body. To yield good molecular shape complementary with high competency, this method engaged 3D transformations driven by local characteristic matching and spatial pattern detection techniques, such as the geometric hashing & poses clustering. After the fast transformational search, the best geometric fit obtained the highest scores, while the low scores exhibited poor matches. Clustering RMSD was chosen as 4.0Å. The result obtained through the email address provided and the docked complex structure was downloaded **[21, 22].**

Results:

Generation and Validation of Protein Structures

We modeled Aquaporin-9 and ABCC1 Receptor protein structure by using Homology Modeling (MODELLER-9.10). The obtained DOPE percentage similarity of proteins was 42.12% for Aquaporin-9 and 89.98% for ABCC-1. After the protein modeling, we have validated our modeled structures by the use of RAMACHANDRA PLOT (RAMPAGE). The torsion angles of the 3D structure of Aquaporin-9 showed 93.9% amino acid residues in the favored regions, 5.5 % amino acid residues in the allowed region and 0.7% amino acid residues in the outlier region, whereas ABCC-1 showed 83.9% amino acid residues in the preferred regions, 10.9 % amino acid residues in the allowed region and 5.2 % amino acid residues in the outlier region.

Docking Study

To explore the binding and the efflux potential of Arsenite and other Arsenic based complexes alone and accompanied with Selenium and Selenium based complexes towards the Aquaporin-9 and ABCC-1 receptors, we have performed in silico protein-chemical docking analysis using Geometry Based Algorithm. The Auqaporin-9 and ABCC-1 were docked with the Glutathione, GSH-AsH-GSH, GSH-Se-GSH, Arsinite and Selenite **(Figure 2 & 3).** Patch Dock online docking Server used

in the molecular docking on Geometry-based algorithm **[1].** This server was applied to recognize the binding scores and binding residues **Table 1 (see supplementary material)** of GSH, GSH-AsH-GSH, GSH-Se-GSH, Arsinite and Selenite with the Auquaporin-9 and ABCC-1. The patchdock docking results is obtained through the email, where as Aquaporin-9 and ABCC1 showing different binding score against GSH-As-GSH, GSH-Se-GSH, Selenite & Arsenite. The binding affinity of aquaporine 9 is presented in the docking score which is 5852, for GSH-As-GSH, 6296, for GSH-Se-GSH, 1402 for Selenite and 798 for the Arsenite and similarly the docking score of ABCC-1 with GSH-As-GSH, GSH-Se-GSH, Selenite & Arsenite is 7248, 6678, 1500, 790 correspondingly when Arsinte and GSH-AsH-GSH interact with Selenite and GSH-Se-GSH docked with Aquaporin-9 and ABCC-1complexes, then the docking score increased from the earlier docking score when Aquaporin-9 and ABCC-1were untreated **Table 1.**

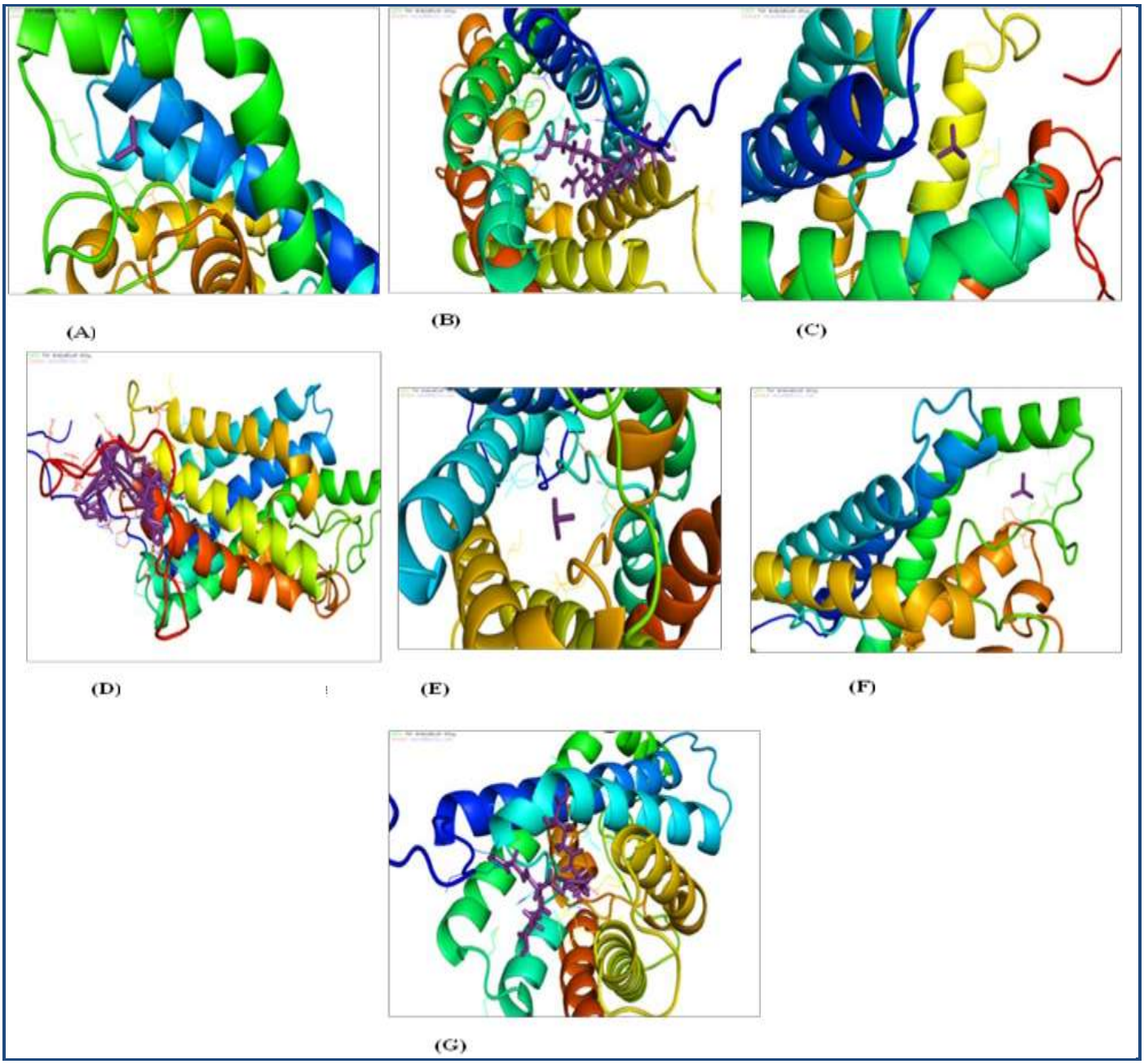

Figure 2: A-G molecular interactions of Aquaporin 9 and arsenite, selenite and GSH complex showing 3D graphics generated by PyMol Visualizer.

Discussion:

Here, we report the transportation of toxic compound through ion channels and in the face of multidrug receptor protein, which acts as multi specific anion transporter, glucuronides and sulfate conjugates of steroid hormones and bile salts **[23].** We explored the complex molecule such as arsenite, selenite and GSH complex proximity with the ABCC1 transporter protein, as we know the role of ABCC1 transportation involved MRP1-dependent transport system where of AsIII requires GSH and the glutathione transferase P1 (GSTP1) **[8].** Currently, it is unknown whether the formation of ArsenicIII(GS)2 under physiological conditions, and what proximity was best fitted.

For the arsenic efflux. In the current study, we explore the transport activities for AsIII(GS)2 and free AsIII in the presence of GSH . Binding simulation reveled Superficially, arsenic complex interaction with the channel protein these interaction showing higher arsenic efflux related most fitted structure, these results suggest that AsIII(GS)2 formation is spontaneous; however, high levels of plasma membrane- associated ABCC1 exist in the cell vesicles, and although further experimentation is required for the validation of ABCC1 related protein transportation efficiency. Here we found the arsenite-GSH complex, bind efficiently and showing best proximity.and it was compared with the other complex structure of selenite-GSH and selenium arsenic and GSH and found they showed close proximity with the ABCC1 transporter.

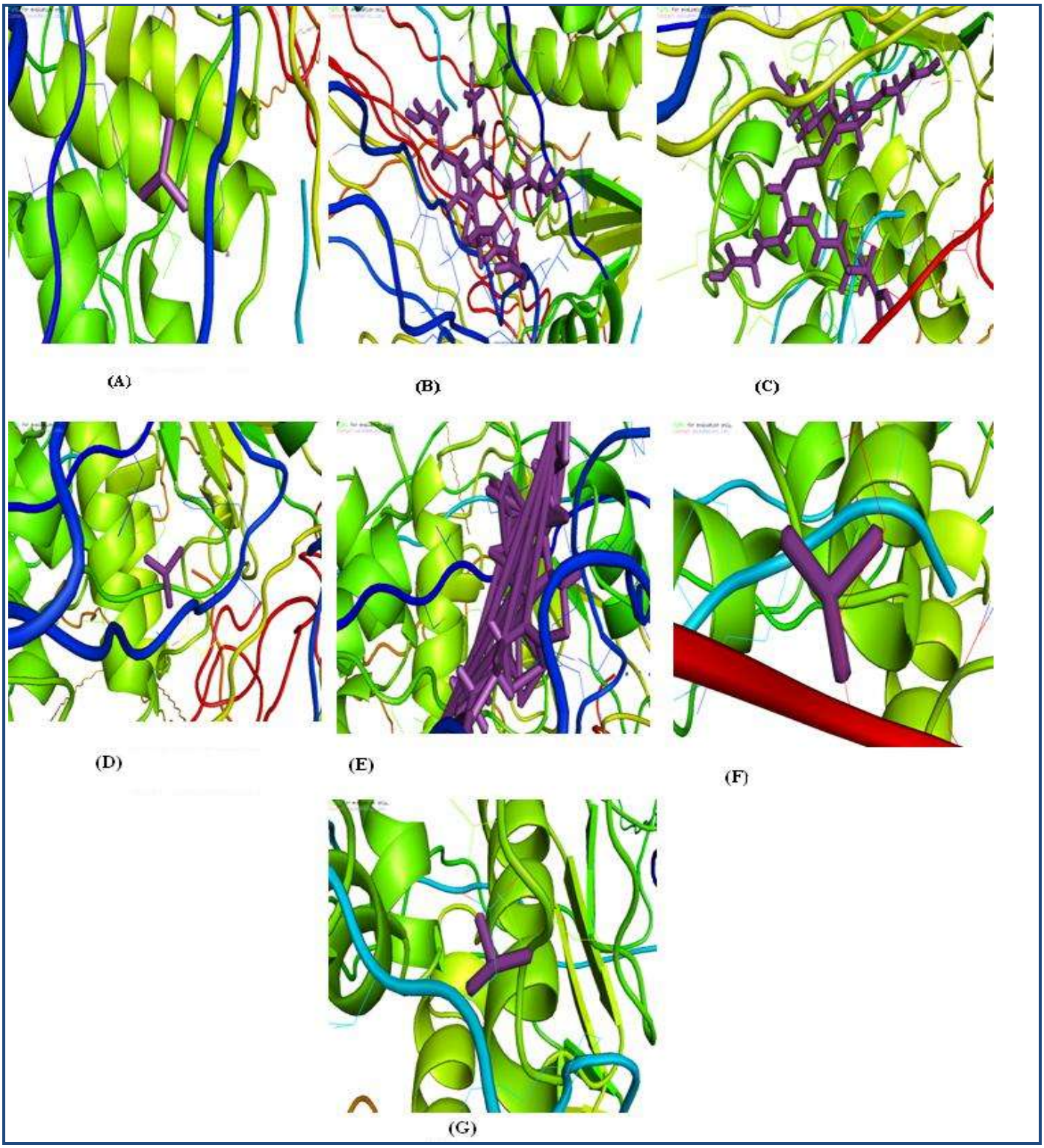

Figure 3: A-G Molecularinteractions of ABCC1 and Arsinite, Selenite and GSH complex showing Molecular Docking Simulation done by PatchDock, 3D structure Generated by Pymol Visualizer.

In the other protein transport channel aquaporin9, an integral membrane protein acts similarly for the arsenic efflux. Both protein complexes exhibited special binding with these complexes and the arsinite complexes. It is suggested that, if Selenite or Selenite complex and Arsenite or Arsinite complexes act together, then binding efficiency showed preferential over both the proteins. After the pre-exposure of Selenite or Selenite complexes with both protein structures it enhanced the binding affinity of Arsenite or Arsinite complexes was observed, which is related to the high efflux activity of Arsenic from the cell and it reduced the arsenic accumulation and arsenic detoxification.

ABCC1 and aquaporin 9 was found to deliberate cellular protection against arsenic in the presence of GSH- and it also reduced the arsenic cellular accumulation. Direct transport studies using this complex membrane vesicles revealed that Arsenic was a substrate for ABCC1 and aquaporin 9, but only in the presence of GSH or as As (GS)2. In the other hand transportation of Arsenic was not supported by selenium alone, selenium lacking a free thiol group, suggests that it also needed GSH for making conjugations. For the transport of As (GS) 2 was then characterized extensively.

Conclusion:

In the present study, it was apparent that the best transport was facilitated by the ABCC1 transport channel as aquaporin is showing less proximity that rivaled less removal of the arsenite from the cell. It also reports reduced arsenic clearance and increased toxicity in aquaglyceroporin-9-null mice aquaporin nul mice **[1]** and thereby aquaporin provides partial protection to animal from arsenic toxicity as compared to ABCC1. These results suggest that aquaporin 9 and ABCC1 are involved in controlling arsenic accumulation in cells, which then contribute to differential sensitivity to As (III) cytotoxicity between the cells. Mechanistic study was also needed to understand the structural integrity and molecular simulation in the chemical transportation. Therefore more in silico and biological interpretation was needed for the further investigation.

Acknowledgement:

The authors are grateful to The Vice-Chancellor Prof. S.W. Akhtar, Integral University, Lucknow, Uttar Pradesh, India for his support and providing necessary facility.

Conflict of interest statement:

We declare that authors have no conflict of interest.

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Edited by P Kangueane

Citation: **Poojan *et al.*** Bioinformation 10(8): 474-479 (2014)

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Supplementary material:

Table 1: List of Binding Score and interactive Residues of GSH, Arsenite, Arsenite related Complexes, Selenite and Selenite related complexes

S.No	Receptor	Ligand	Binding Score
1	Aquaporin9	GSH-As-GSH	5852
2	Aquaporin9	GSH-Se-GSH	6296
3	Aquaporin9	Selenite	1402
4	Aquaporin9	Arsenite	798
5	Aquaporin9-GSH-Se-GSH	GSH-as-GSH	5916
6	Aquaporin9-Selenite	Arsenite	778
7	Aquaporin9- GSH-Se-GSH	Arsenite	816
8	ABCC1	GSH-As-GSH	6778
9	ABCC1	GSH-Se-GSH	7248
10	ABCC1	Selenite	1500
11	ABCC1	Arsenite	770
12	ABCC1-GSH-Se-GSH	GSH-as-GSH	6988
13	ABCC1-Selenite	Arsenite	792
14	ABCC1- GSH-Se-GSH	Arsenite	816