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Research Article

Molecular Docking Assessment of Efficacy of Different Clinically Used Arsenic Chelator Drugs

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Arsenic contamination of ground water has become a global problem affecting specially, south-east Asian countries like Bangladesh and eastern parts of India. It also affects South America and some parts of the US. Different organs of the physiological system are affected due to contamination of inorganic arsenic in water. Animal studies with different chelators are not very conclusive as far as the multi/differential organ effect(s) of arsenic is concerned. Our docking study establishes the molecular rationale of blood test for early detection of arsenic toxicity; as arsenic has a high affinity to albumin, a plasma protein and actin, a structural protein of all cells including Red Blood Cells. This study also shows that there is a little possibility of male reproductive organs toxicity by different forms of inorganic arsenic; however, female reproductive system is very much susceptible to sodium-arsenite. Through comparative analysis regarding the chelating effectiveness among the available arsenic chelator drugs, meso-2,3 dimercaptosuccinic acid (DMSA) and in some cases lipoic acid is the most preferred choice of drug for removing of arsenic deposits. This computational method actually reinforces the clinical finding regarding DMSA as the most preferred drug in removal of arsenic deposits from majority of the human tissues.

1. Introduction

The source of arsenic poisoning comes through drinking water and has now become a major concern throughout the globe. It affects a large population of Bangladesh [1] and eastern part of India. Arsenic occurs in nature in both organic and inorganic forms; the latter being more toxic. Inorganic form combines mainly with oxygen and sulfur. In drinking water, it is mainly present as arsenious acid in trivalent [As(III)] state [2]. Arsenic trioxide (ATO) (As_2O_3) dissolves in water under conditions dependent on pH, presence of redox chemicals, reducing bacteria, and so on to produce this arsenious acid [3]. Arsenic rarely occurs in the zero valent metalloid state and mostly occurs in the trivalent state and occasionally in the pentavalent state. Pentavalent arsenic compounds tend to decompose into trivalent state when ingested. Within physiological system, pentavalent arsenic uses ADP and uncouple oxidative phosphorylation to change to trivalent arsenic [4]. So, the main toxicity of inorganic arsenic comes from trivalent arsenic. The toxicity of trivalent arsenic [As(III)] arises from the fact that it binds to free thiol (–SH) groups in proteins, especially vicinal thiol groups [5].

Clinical reports suggest that arsenic toxicity affects a variety of organs, having highest concentrations in tissue where proteins have *sulfhudryl* (thiol) groups like skin, nails, and hair. When larger doses of arsenic are ingested, the tissue distribution appears to change. The LD_{50} for oral administration to mice are as follows: 3 mg/kg for arsine; 14 mg/kg for arsenite [As(III)]; 20 mg/kg for arsenate [As(V)]; 700–1800 mg/kg for monomethylarsonic acid (MMA), 700–2600 mg/kg for dimethylarsinic acid (DMA); and >10000 mg/kg for arsenobetaine and arsenocholine showing that inorganic arsenic is much more toxic [6] and the ingested dose determines the duration of chelation therapy combined or alone like with BAL (British Anti Lewsite) or dimercaprol (DMC) [7]. Many studies have been performed on humans after fatal arsenic poisoning. Results of these studies showed widespread distribution of arsenic in all organs; the highest concentrations

is in the liver and kidneys, which had, respectively, 10- and 3-fold higher concentrations than in the other organs like brain, cerebellum, lung, heart, pancreas, spleen, muscle, and skin [8].

To understand arsenic removal from the physiological system and detoxification processes, experimental animal models are treated with acute intoxicating doses of inorganic arsenic and followed for hepatic and renal tissue distribution. These studies indicate the role of these organs in the detoxification and elimination of arsenic. These studies reveal that the liver is the site of inorganic arsenic methylation that helps in detoxification and elimination of arsenic [9]. Initial study showed that methyltransferases present in liver, transfers methyl group from S-adenosylmethionine to arsenite, thus forming monomethylarsonic acid (MMA) [10]. Further studies revealed that the high concentrations of arsenic are deposited in muscle and heart—the third and fourth highest arsenic concentrations after the liver and kidneys tissue. Therefore, the fatal rhabdomyolysis observed in animals treated with arsenic poisoning is followed by heart failure [8, 11–14]. Table 1 summarizes a list of affected organs by arsenic poisoning and the abundant protein(s) that are present in that organ. In case of ingestion of pentavalent arsenic [As(V)], toxicity occurs primarily by reduction to trivalent arsenic [As(III)].

Chelation is the process by which the metal is leached out of the body, for a long time the mainstay was BAL; then 2,3 Dimercapto propane-1-sulfonic acid (DMPS) and Dimercapto succinic acid (DMSA). The former drug was administered by intramuscular injection while the latter drugs can be easily administered through oral route. DMSA is also known as succimer; its various analogs like MonoisoamylDMSA (MiADMSA), MonomethylDMSA (MmDMSA), and MonocyclohexylDMSA (MchDMSA) were developed. A good chelating agent should be expected to have the following qualities:

(i) greater affinity for the metal,

(ii) same distribution as the metal,

(iii) low toxicity,

(iv) ability to compete with natural chelators,

(v) ability to penetrate cell membranes,

(vi) rapid elimination of the toxic metal,

(vii) high water solubility,

(viii) capacity to form nontoxic complexes [11].

In experimental models, tested chelators are DMSA, DMPS as its sodium salt, BAL, and Lipoic acid. The relative effectiveness of these dimercapto compounds in protecting mice from the lethal effects of an LD_{99} of sodium arsenite (SAN) ($NaAsO_2$) is DMSA > DMPS > BAL [15]. No direct comparison with alpha lipoic acid (α-LA) has been made. BAL, however, increases the arsenic content of the brain of rabbits injected with sodium arsenite. These results raise an issue regarding the appropriateness of BAL as the treatment for systemic arsenic poisoning. DMPS and DMSA have effectiveness as prophylactics for the prevention of the vesicant effect of Lewisite. The sodium arsenite inhibition of the pyruvate dehydrogenase (PDH) complex can be prevented and reversed *in vitro* or *in vivo* by these chelators and for this action, DMPS is most potent. Otherwise, DMSA is the most potent chelator of arsenic in clinical practice. Lipoic acid, also an arsenic chelator, is used for its excellent blood brain barrier permeability to leach out brain deposits of arsenic [16, 17]. Different chelator drugs that are in use in arsenic toxicity are evaluated through empirically based observations of animal experimentations. Here, we explore the computational/quantitative rationale of those clinically used chelator drugs at molecular level.

Table 1: Organs affected by arsenic and the most abundant protein(s) in that organ.

Affected organ by arsenic	Abundant protein of the organ
Skin [18]	Collagen
Heart muscle [19]	Lactate dehydrogenase (LDH)
Reproduction, carcinogenicity [20]	Estrogen receptor alpha (ER-α)
Liver [21]	Thioredoxin reductase (TDR)
Liver [22]	Glutathione reductase (GTH)
Energy metabolism in all cells [23]	Pyruvate dehydrogenase (PDH)
Cytoskeletal structure [24]	Tubulin
Cytoskeletal structure [25]	Actin
Blood (serum) [16, 26]	Albumin
Liver, kidney and brain [17, 21, 26]	Spermine and spermidine

2. Methods

Present study is aimed to make a computational assessment of the efficacy of different chelator drugs in different tissues involved with open source software on a Linux platform. Molecular docking of small molecule was done using Autodock3 which is free for academic user. For performing molecular docking procedures, we have chosen several proteins from the organs that are reported to be affected by arsenic toxicity (Table 1). The name and the collected source of structural detailing of the proteins in the PDB format are mentioned in Table 2. The chosen drug molecules and collected structures are mentioned in Table 3. The detailing steps of the molecular docking processes [27] are as follows.

(1) The PDB files of receptor (macromolecule) and ligand were obtained from the Protein Data Bank and the Drug bank or from any other source as mentioned in Tables 2 and 3.

(2) Preparing the receptor PDB files, as the PDB files often contained added waters, the files were read in the GUI of ADT, water selected as HOH* from a string and deleted after the warning.

(3) Preparation of the macromolecular files—Polar hydrogens were then added with no bond order. ADT was then used to add charges, and Kollman

charges added (by default; ADT adds Kollman charges for a peptide (determined by checking whether all of its component residue names appear in the standard set of 20 commonly occurring amino acids) and Gasteiger charges if not so). Finally, solvation parameters were added and the files were saved as molecule.pdbqs (where "q" and "s" represent charge and solvation, resp.).

(4) Preparation of the ligand file. Generally all hydrogens are added and nonpolar hydrogens are merged. Gasteiger were charges added unless the ligand is also a peptide, in which case the above procedure would be followed. ADT automatically takes care of solvation and checks for aromatic carbon atoms and hence lone pairs and nonpolar hydrogens merged. ADT then determined the best root (the best root is the atom in the ligand with the smallest or largest subtree; in case of a tie, if either atom is in a cycle, it is picked as the root, and if neither or both is in a cycle, the first to be found is picked). Next we defined rotatable bonds in the ligand, making all amide bonds nonrotatable, and set the number of active torsions to fewest atoms. The ligand file was then saved with a ligand.out.pdbq extension ("q" representing charge).

(5) Preparation of the grid and the grid parameter file. For the calculation of docking interaction energy, it is necessary to create 3D box (grid) in which the protein molecule is enclosed. The grid volume should be large enough to allow the ligand to rotate freely, even when the ligand is in its most fully extended conformation. The parameters required to create such a grid are stored in the grid parameter file, molecule.gpf.

(6) Now, autogrid3 was run to create a map for every atom type in the ligand and create the corresponding macromolecular file with the extension molecule.glg either from Run of the GUI or the command line autogrid3 -p molecule.gpf -l molecule.glg and when finished, it writes Successful Completion.

(7) Preparation of Docking Parameter File. The macromolecular pdbqs and ligand.out.pdbq files are read. The search methods of AutoDock include the Monte Carlo simulated annealing method, the genetic algorithm, local search, and the hybrid genetic algorithm with local search. The latter is also referred to as the Lamarckian genetic algorithm because offsprings are allowed to inherit the local search adaptations of their parents, and this was the chosen algorithm for the analysis. The docking job can similarly be run from Run of the GUI or from command line autodock3 -p molecule.dpf -l molecule.dlg, and

(8) When finished and Successful Completion is written, the PDB file and Analyze open docking log are done and choosing different color for receptor and ligand, the conformations can be played by energy. The dlg files can be opened in a terminal and each run's (the number of runs can be fixed by the user) final docked energy, Gibbs free energy, Inhibition Constant is written. Also, given in the file are the RMSD values. A conformation can be chosen and it's coordinates written to run the next set of docking and this should be done till Gibb's free energy is no longer significantly reduced.

(9) Diagrams are drawn with the protein and the ligand attached to it in its lowest energy conformer. Protein and ligand can be made of different width and color and the background white. The image can be saved in various formats of which we chose the tif format [28].

Table 2: Sources of PDB files of proteins in our study.

Protein	PDB ID/otherwise ID	Reference
Thioredoxin reductase	2CFY (edited)	[29]
Glutathione reductase	1GRT (edited, A34E + R37W mutant)	[29]
Collagen	1BKV (edited)	[29]
Actin	1J6Z (edited)	[29]
Estrogen receptor alpha (ER-Alpha)	1X7E (edited)	[29]
Pyruvate dehydrogenase	2BUB (edited)	[29]
Albumin	2BXI (edited)	[29]
Tubulin	2HXH (edited)	[29]
Keap1 (Keich-like ECH-associated protein-1)	3ADE (edited)	[29]
Kallikerin/KLK-7	3BSQ (edited)	[29]
Lactate dehydrogenase	3H3F (edited)	[29]
Spermine	HMDB, Acc no. HMDB01256	[30]
Spermidine	HMDB, Acc no. HMDB01257	[30]

Table 3: Sources of PDB files of ligands in our study.

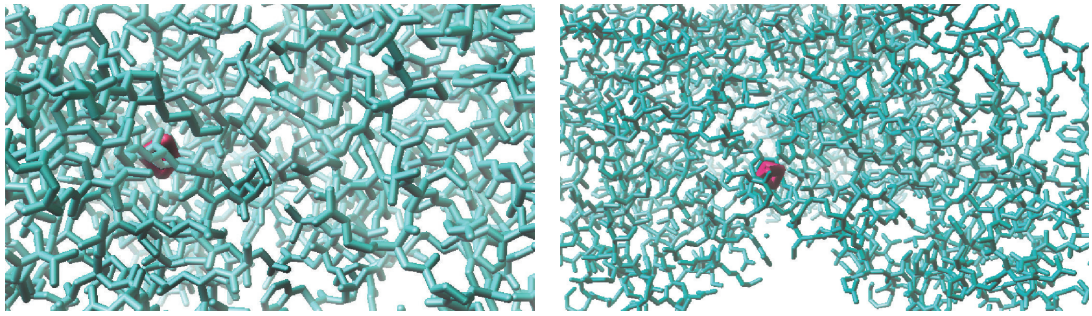
Ligand	Accession no.	Reference
Arsenic trioxide (ATO)	DB01169	[31]
Sodium arsenite (SAN)	CID443495	[32]
Lipoic acid (α-LA) (ALA)	DB00166	[31]
Dimercaprol (BAL)	DB06782	[31]
DMSA (Dimercapto succinic acid) or succimer	DB00566	[31]
DMPS (2,3 Dimercapto propane-1-sulfonic acid)	12405pdb	[33]

3. Results

3.1. Gibb's Free Energy and Binding Energy. The output results of AutoDock as mentioned in previous section are denoted by ΔG. AutoDock gives us the most important parameter in studying a closed system and it's minimum value of Gibbs Free energy or ΔG for every conformer of the ligand docked to the protein, also, calculating the equilibrium binding constant K in each case as these are related by the simple relation $\Delta G = -RT \ln K$.

Table 4: Minimum Gibbs' free energy (ΔG) in Kcal/mol.

Receptor/protein	Drug					
	ATO	SAN	ALA	DMC	DMPS	DMSA
TDR	−1.42	−1.81	−3.97	−2.99	−4.23	−4.93
Collagen	−0.91	−1.37	−4.09	−2.21	−3.14	−4.27
GTR	−1.48	−2.11	−4.61	−3.29	−4.87	−5.88
Actin	−1.66	−2.19 (Figure 2)	−5.33	−2.67	−4.94	−5.12
ER-Alpha	−1.61	−2.23 (Figure 2)	−6.01	−3.00	−4.28	−6.42
PDH	−1.35	−2.17	−4.92 (Figure 3)	−3.53	−4.85	−5.88
Albumin	−1.94 (Figure 1)	−1.99	−5.68 (Figure 3)	−3.02	−4.95	−5.85
Tubulin	−1.73	−2.08	−5.55	−3.06	−5.82 (Figure 4)	−5.90 (Figure 4)
Keap1	−1.84	−2.22	−7.03	−3.15	−5.12	−8.55
KLK−7	−1.91 (Figure 1)	−1.88	−6.37	−3.48	−6.17	−7.25
LDH	−1.48	−1.9	−6.67	−3.23	−4.32	−8.30
Spermine	−0.26	−0.36	−6.68	−0.89	−0.27	−8.95
Spermidine	−0.26	−0.34	−6.74	−0.98	−0.26	−9.92



(a) Albumin (b) KLK-7

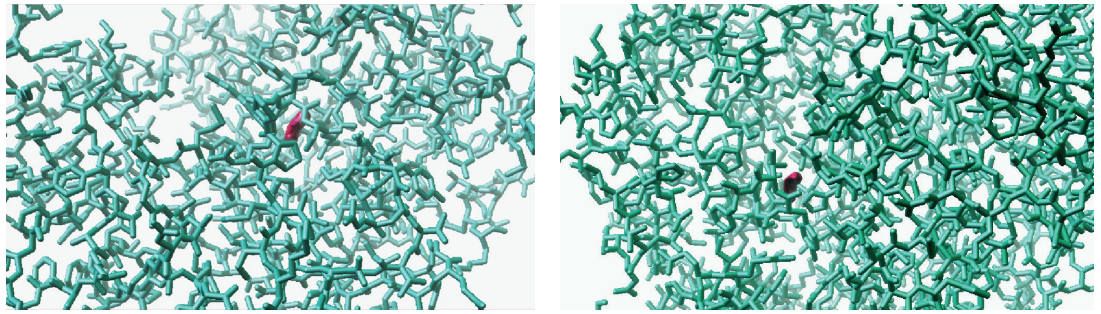
Figure 1: ATO binding with albumin (a) and KLK-7 (b).

The most thermodynamically stable conformer in a bunch of conformers (say 20 or 50 runs) as that will have the minimum value of Gibb's free energy as per the two laws of thermodynamics. Thus, docking a ligand onto a protein we can predict it's most stable conformer and if there are a variety of ligands which ligand binds the best as in this case. Ligand docking (natural and drug) method was used to establish the rationale of long term toxicity of different adjuvant drugs of breast cancer [34].

Table 4 shows the minimum docked energy of different ligands (drugs) to the different protein molecules. Table 4 shows that arsenic trioxide (ATO) most strongly binds with the albumin protein and after that KLK-7, a tissue protein that is expressed on bacterial infection (Figure 1). It is to be noted here that experimental finding of arsenic toxicity and efficacies of different arsenic chelators are also revealed with sodium arsenite [15]. Hence, we make a comparative assessment between binding affinity of sodium arsenite (SAN) and ATO with the same proteins. Table 4 reveals that binding affinity of SAN is a little bit higher than ATO for most of the studied proteins (except KLK-7) due to more ionization potential. Among the studied proteins, SAN binds most strongly with ER-alpha and moderately with actin, a structural protein that is present in cells (Figure 2).

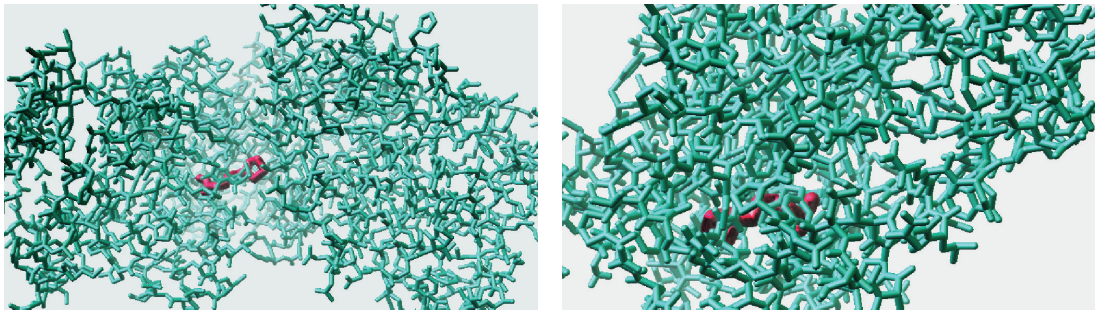
Docking study reveals further that DMSA and ALA (alpha-lipoic acid) binding to albumin are more than arsenic (Figure 3) (Table 4). Hence, they may be capable of removing arsenic from blood. In this connection, ALA could also become potent chelator of removing arsenic from tissue, as it binds strongly with actin, a structural protein of the cell. Of the available chelators, DMSA is the most potent and for chelation of arsenic from tubulin, a combination of DMPS and DMSA, would be effective (Figure 4). Arsenic chelation potentiality of DMSA is also helpful in protecting PDH, an important enzyme system of mitochondrial TCA cycle and the next most effective chelator for this enzyme system is the ALA (Figure 3) (Table 4).

ER-alpha is mostly affected by SAN as binding energy to this receptor protein is much more compared to other proteins studied. Our docking study also collaborate the experimental finding regarding the efficacy of different chelators in the order of DMSA > DMPS > DMC in protecting against SAN as these chelators have more affinity to ER-alpha [15].



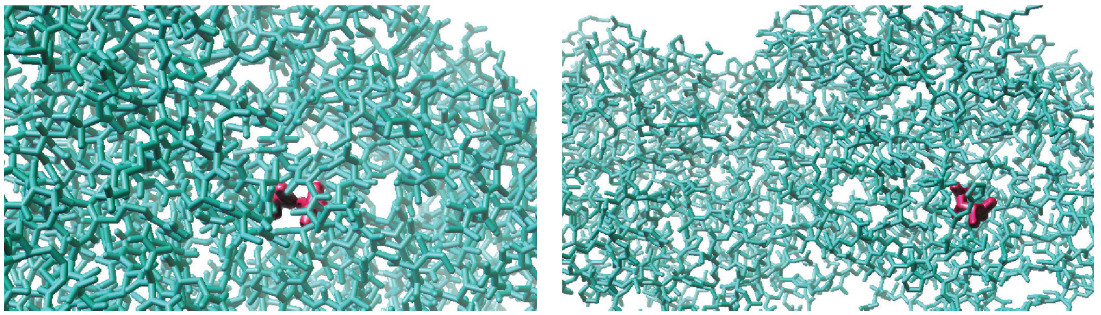
(a) Actin (b) ER-Alpha

Figure 2: SAN binding with actin (a) and ER-alpha (b).



(a) Albumin (b) PDH

Figure 3: α-LA binding with albumin (a) and PDH (b).



(a) DMPS (b) DMSA

Figure 4: DMPS (a) and DMSA (b) binding with the tubulin.

4. Discussion

The concern of this paper is to find out suitable arsenic chelator for arsenic toxicity due to repeated drinking of contaminated ground water. Inorganic form of arsenic is the major cause of arsenic poisoning through drinking of ground water. In drinking water arsenious acid in trivalent [As(III)] state is the major source for arsenic toxicity. Arsenious acid is produced from arsenic trioxide under conditions dependent on pH, presence of redox chemicals, and reducing bacteria [3]. Hence, arsenic toxicity may occur primarily due to arsenic trioxide (ATO) and inorganic arsenic produces more toxicity compared to organic form of arsenic [6]. However, understanding of toxic effect of arsenic and determination of the efficacies of different arsenic chelators in different experimental animal model are carried out with sodium arsenite (SAN), a component of pesticide [15, 32]. Hence, binding affinity of both ATO and SAN is carried out for different proteins.

Arsenic trioxide has poor affinity towards spermine and spermidine and highest towards albumin. Arsenic toxicity by repeated drinking of contaminated ground water is often detected by a low serum albumin/globulin ratio [35]. Arsenic tends to lower serum album and cause albuminuria. Hence, any chelating agent with higher affinity for the protein can wash out arsenic from that protein. Our study indicates that DMSA followed by ALA would be the most potent drug for removing arsenic bound to the albumin as the binding energy of these drugs are more than the binding energy of arsenic.

Arsenic binds with thioredoxin reductase, a thiol enzyme present in liver cytosol and binding of arsenic to this protein can be removed away most effectively by DMPS, or succimer (DMSA). However, for collagen, succimer is the best. Bound arsenic to the liver enzyme glutathione reductase, a detoxifying enzyme can be washed by dimercaprol, DMPS or DMSA. It is noteworthy that ALA can also work in all the above-mentioned cases. Actin is involved in cytoskeletal structure of all cells and for this protein, the potent chelators would be DMPS, succimer, and ALA (Lipoic acid) added to remove brain deposits. An exact similar case arises for tubulin and pyruvate dehydrogenase. In all these cases, dimercaprol works as a less potent chelator. Arsenic chelating efficiency from these proteins is in the order of DMSA (succimer) > DMPS ≫ dimercaprol (DMC) (BAL). Moreover, the efficacy of these chelators also collaborate the experimental finding with SAN as these chelators has also more affinity to ER-alpha [15].

For estrogen receptors, most potent chelator would be DMSA (succimer) and for deposits in the brain, DMSA in combination with lipoic acid are used for the treatment especially for the cases of chronic arsenic poisoning. For the bacterial infected cells that secrete a serine protease Kallikerin/KLK-7, DMSA is a very potent chelator. DMSA is also effective for the removal of arsenic bound to Keap1 protein, a signalling protein. For lactate dehydrogenase which occurs in muscle, heart, and brain, DMSA is a very potent chelator compared to other chelators and Lipoic acid (ALA) is also the next best chelator so that it can be added to remove brain deposits of arsenic as it can cross the BBB (blood brain barrier) [36]. Reports suggest that other chelators when mixed with lipoic acid can pass the blood brain barrier easily and chelate brain deposits of arsenic due to the excellent lipophilic nature of lipoic acid [11].

Combination therapy was also suggested where two structurally different chelating agents are incorporated one is lipophilic and another is lipophobic simultaneously to chelate out both intracellular and extracellular metal deposits. Different antioxidants N-Acetyl cysteine, taurine, vitamin C, vitamin E, Zn, and Se enhance the effectiveness of different heavy metals chelation including arsenic by reducing the drug dose, preventing pro-oxidative damage, carcinogenic signalling is cut off and thus prevents tissue damage [11].

Arsenic binds to ER-α, the binding affinity of SAN is more than ATO. It might also get incorporated into spermine and spermidine, two peptides produced from the genital organs with high mitotic index and rapid turnover [37]. However, our study shows these peptides are less susceptible to arsenic toxicity (Table 4). Hence, male reproductive organ is less susceptible by both SAN and ATO; however, the female reproductive organ is more susceptible to SAN. Spermine and spermidine are interconvertible and occur in various organs like liver, kidneys, and cerebral regions of the brain. Dimercaprol and DMPS are both very poor chelators. Though trivalent arsenic has little affinity for spermine and spermidine; however, theoretically, DMSA is an extremely potent chelator as lipoic acid, and a combination of these would leach out arsenic very well from these two peptides. EDTA would be a poor chelator for arsenic, the logic can be found looking at the periodic table as it best chelates d and f block elements.

Arsenic, in the form of sodium arsenite is one of the most extensively studied metals that induce ROS generation and results in oxidative stress. Experimental results show that superoxide radical ion and H_2O_2 are produced after exposure to arsenite in various cellular systems [38]. Arsenic is known not only to produce ROS but also, nitric oxide ($NO^{\bullet}$) [39], dimethylarsinic peroxyl radicals $(CH_3)_2AsOO^{\bullet}$, and also the dimethylarsinic radical $(CH_3)_2As^{\bullet}$ [40]. Oxidative DNA lesions induced by arsenic were observed both *in vivo* and *in vitro* [41]. The molecular cause may be due to reduced activation of glutathione reductase (GTR). Our docking study indicates that SAN is more potent in binding with GTR than ATO. Similar observation is also made with lactate dehydrogenase and pyruvate dehydrogenase—both enzyme system are involved with energy production.

Our computational prediction of chelators of a heavy metal poisoning, especially useful when practical or experimental data is scanty or it is not known that which proteins would be affected by a specific toxin. Comparative analysis of interaction between a toxin with its known protein and to its off-targets (unknown protein) may indicate a weak interaction of toxin to its off-targets. Though the interactions are weak, however, the docking algorithm and computer simulation may provide the molecular rationale of long-term toxicity [34]. Our study further indicates that different forms of inorganic arsenic strongly bind with albumin (plasma protein) and/or actin (structural component of all cells including red blood cells). Hence, blood analysis could be the first hand indicator of arsenic toxicity and reinforces the earlier clinical investigation of blood for detection of arsenic toxicity [16, 26]. So, we suggest that albumin and actin (blood test) testing should be done for the detection of early arsenic poisoning. Present study also reinforces the clinical finding regarding the effectiveness of DMSA or succimer as the most potent arsenic chelator. And if any sort of arsenic toxicity is found, DMSA alone or in combination of lipoic acid is the first hand choice of treatment.

Clinical medicine can come to way after a considerable number of experimentations with animal model; however, with the objections raised by different animal ethical committee across the globe restrict the use of animal for biological experimentation [42–44]. It is to be noted here that our previous study [34] and the present study indicate a close alignment with the clinical findings. Therefore, it can be proposed that docking could be an alternative method of animal uses in the assessment of toxicity of an agent and/or pharmacological evaluation of a drug.

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