Chapter 5

# Mechanisms of Arsenic-Induced Toxicity with Special Emphasis on Arsenic-Binding Proteins

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**Abstract**

The importance of different arsenic forms in public health is well recognized owing to its distinct physical characteristics and toxicity. Chronic arsenic exposure has left a trail of disastrous health consequences around the world. However, the mechanisms behind the toxicity and the consequential diseases occurring after acute or chronic exposure to arsenic are not well understood. The toxicity of trivalent arsenic primarily occurs due to its interaction with cysteine residues in proteins. Arsenic binding to protein may alter its conformation and interaction with other functional proteins leading to tissue damage. Therefore, there has been much emphasis on studies of arsenic-bound proteins, for the purpose of understanding the origins of toxicity and to explore therapeutics. This book chapter illustrates the molecular mechanisms of arsenic toxicity with a special emphasis on arsenic binding to proteins and its consequences in alteration of tissue homeostasis.

**Keywords:** arsenic, gap junction intercellular communication (GJIC), gap junction proteins, connexin 43, DJ-1, sulfhydryl groups

## 1. Introduction

Long-term exposure to arsenic has resulted in the largest mass poisoning of the human population, making more than 100 million people defenseless against cancer and other arsenic-related diseases [1, 2]. Epidemiological studies have revealed that arsenic exposure

spans a wide geographical area spread across continents, with contaminations originating from soil, water, air and even food. Arsenic pollution gets aggravated through natural processes like volcanic eruptions, weathering, and biological activity. Anthropogenic activities, such as ore smelting, mining, well drilling and combustion of fossil fuels, also accelerate infusion of arsenic into places of human habitation [3]. Owing to its toxic nature, arsenic is a threat not only to humans but also to other living species. **Figure 1** illustrates natural and anthropogenic sources of arsenic.

Many mechanisms of arsenic-induced carcinogenicity have been proposed like DNA repair inhibition, oxidative stress, epigenetic events, effect on signal transduction and genotoxic damage. Studies have been focused to understand the molecular mechanisms of arsenic-induced carcinogenesis with an emphasis on oxidative stress and related signal transduction pathways. One of the hallmarks of oxidative stress is generation of reactive oxygen species (ROS) which triggers the antioxidant pathways as a cellular defense response. Two of the major players of cellular defence response on arsenic exposure are nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and Parkinson's disease protein 7 (DJ-1), and their interplay results in activation and upregulation of several genes like glutathione-S-transferase A2 (GSTA2), NAD (P)H dehydrogenase quinone 1 (NQO1) and thioredoxin (Trx). There has been increasing evidence correlating arsenic exposure to reactive oxygen species (ROS) generation, DNA

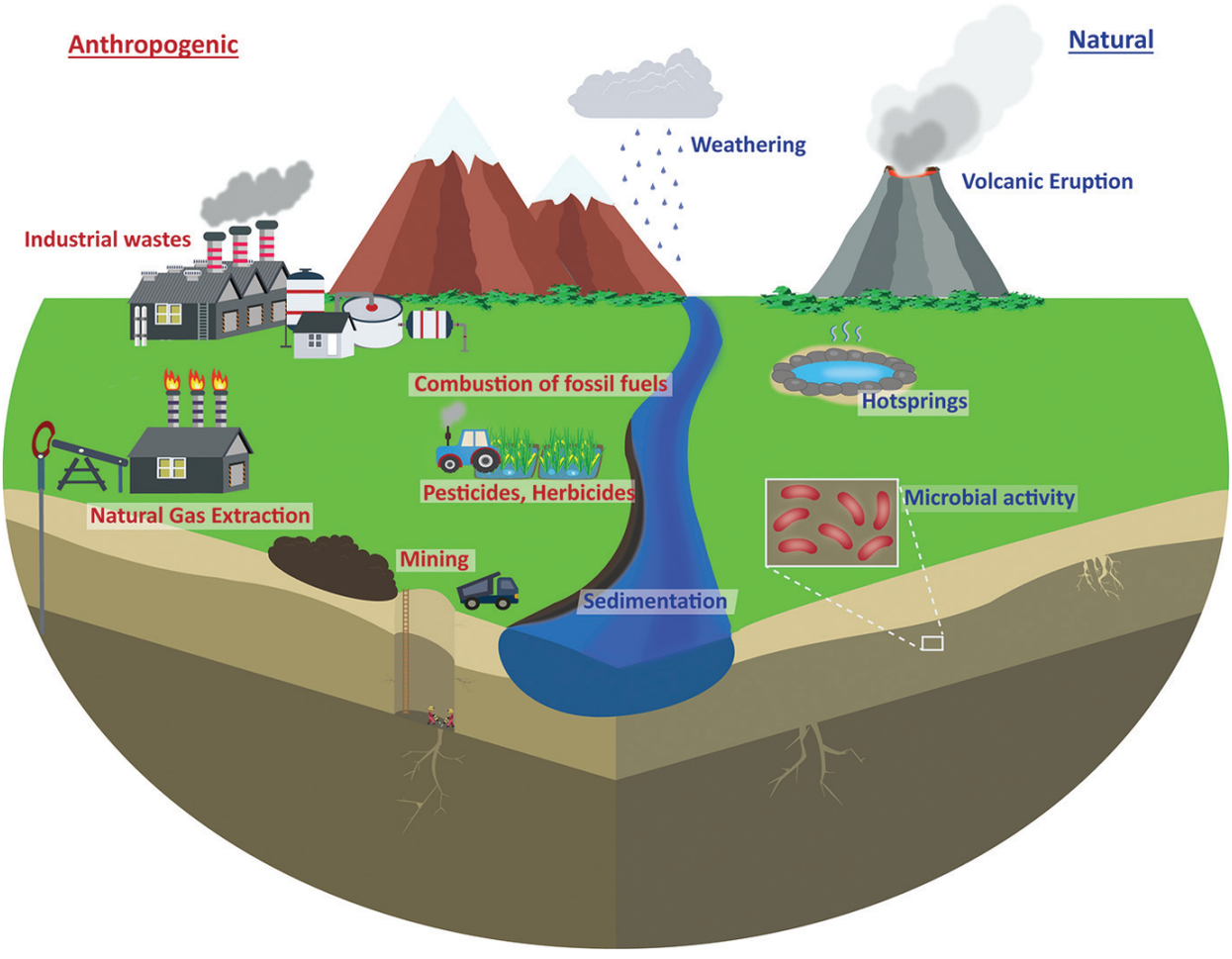


**Figure 1.** Mobilization of arsenic into environment.

damage and tumor promotion. Inorganic arsenic has been recognized as a potent human carcinogen. A number of epidemiological studies have found that human populations exposed to arsenic are prone to different types of cancers including that of the bladder, lung, skin, liver and kidney [4, 5]. Human body responds to arsenic ingestion through a set of concerted metabolic actions starting with methylation of the inorganic arsenic to monomethylarsonic ($MMA^V$) acid, which is then methylated again to dimethylarsinic acid ($DMA^V$) to permit its excretion through urine. However, this response may result in persistent methyl exhaustion in the event of chronic arsenic exposure leading to hypomethylation of DNA, which can alter the gene expression making the cells susceptible to carcinogenesis [6]. Interestingly, arsenic alone is considered to be a very weak mutagen; however, its synergistic association with genotoxic agents like ultraviolet radiation is reported to make it a potent mutagen [7]. Notwithstanding, the diverse mechanisms of arsenic toxicity need far greater elucidation, though the health hazards are well understood.

From the mechanistic standpoint, arsenic binding to cellular proteins can be a plausible mechanism of toxicity based on two hypotheses premised on functional disruption arising out of (a) sulfhydryl groups in proteins forming covalent bond with arsenite [8] and (b) the phosphate groups in proteins replaced by an arsenate. Arsenic binding to a specific protein could change the conformation and interaction with other functional proteins [9]. Therefore, many studies have been undertaken to examine the direct binding of arsenic to proteins, for the understanding mechanisms of arsenic toxicity and designing therapeutics against it.

All proteins with functionally important and conserved cysteine (Cys) residues, whose sulfhydryl groups are reactive nucleophiles or form disulfide bonds, are potential targets of functional disruption during chronic arsenic exposure. One such protein with conserved cysteines is the gap junction protein, connexin 43 (Cx43), belonging to the connexin family, and is the most commonly expressed member in different cell types. Our recent study showed that direct arsenic binding to this protein causes alteration in trafficking and the absence of gap junctional plaques on cell surface, resulting in propensity for cell proliferation. Given the hazardous nature of arsenic, the qualitative and quantitative analysis of arsenic is a much needed requirement. The conventional methods like neutron activation analysis and X-ray analysis, atomic absorption spectrometry (HG-AAS) and stripping voltammetry are very costly as well as complex. So, the quest for easy and cost-effective method continues till date. One such method gaining reputation in the field relies on optical sensors which have been discussed in this chapter. This chapter summarizes numerous traits of arsenic toxicity and emphasizes the interaction of arsenic with proteins to evaluate the chemical, biological, and physiological consequences.

## 2. Biogeochemical cycle: transformation and mobilization of arsenic in nature

Arsenic is commonly mobilized into the environment due to both natural and anthropogenic processes. The natural processes include geological (weathering of rocks and volcanic eruptions) and biological (microbial activity) events (**Figure 1**). Ancient or recent volcanic activities

results in the inclusion of arsenic in the environment [10]. The earth's atmosphere also has a significant presence of arsenic species owing to wind erosion processes, sea spray, hot springs, volcanic emissions, forest fires and volatilization (in cold climates). Human activities like pharmaceutical manufacturing, glassmaking industry, wood processing, chemical weapons, burning of arsenic-rich fossil fuels and electronics industry also contribute to the addition of arsenic compounds into the environment [11]. Industrial by-products and wastes, ore smelting, mineral mining and well drilling can also mobilize and intensify arsenic into the environment.

Microbial metabolisms like arsenate reduction, arsenite oxidation and methylation processes are also a determining factor of the occurrence of the various arsenic oxidation states in the environment. Reduction of arsenate to arsenite by arsenate reductase enzymes is a common feature in the microbial world, while incidences of oxidation of arsenite to arsenate have also been reported in contaminated environments. These reactions also contribute to the protective and/or energy metabolisms of the bacteria from various arsenic-induced stress conditions (**Figure 2**) [12, 13].

## 3. Cellular mechanisms of arsenic toxicity

The levels of ROS play a key role in normal cell signaling, and its alteration can result in aberrant expression of genes that are activated by redox mechanisms. Notably, genes associated with redox mechanisms include those regulating cellular proliferation, differentiation and apoptosis. The consequences of ROS production can further lead to DNA damage which typically involves the conversion of 2-deoxyguanine to 8-hydroxyl-2′-deoxyguanosine (8-OHdG),

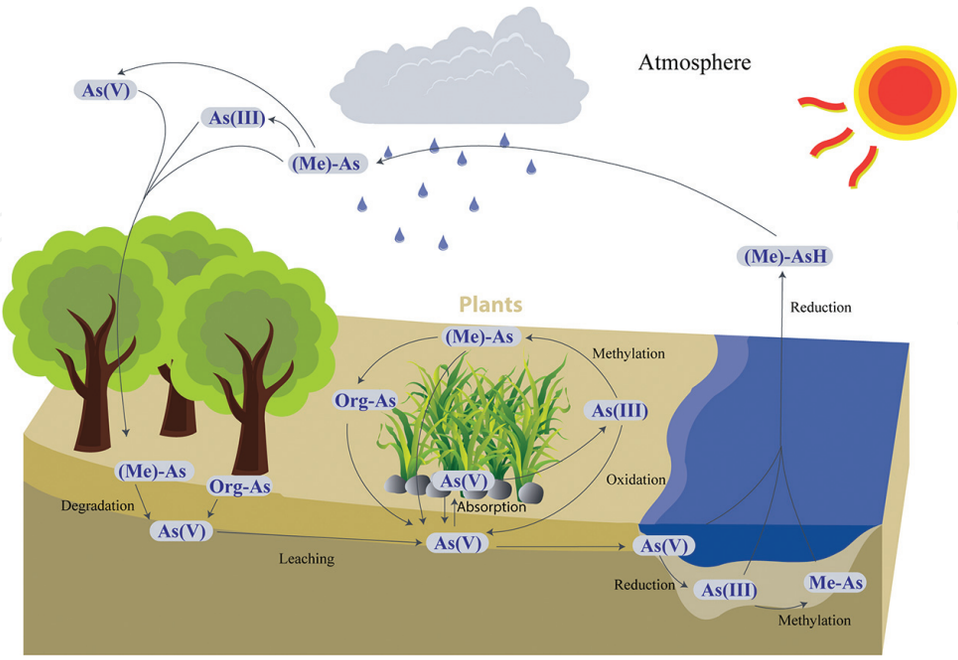


**Figure 2.** Biogeochemical cycle of arsenic.

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which is considered as a marker indicating oxidative stress of DNA. Arsenic was capable of inducing specific DNA lesions consistent with oxidative damage like 8-OHdG generation. Moreover, 8-OHdG has also been detected in the skin of patients with arsenic-related Bowen's disease and in the liver of rats exposed to dimethylarsinic acid ($DMA^V$). These results indicate that ROS generation is a major pathway for arsenic-mediated genotoxicity in mammalian cells [14, 15].

Glutathione and other aminothiols such as cysteine and cysteamine comprise the non-protein sulfhydryls (NPSHs) in a cell and have significant free radical scavenging abilities. Therefore, depletion of intracellular glutathione levels is known to have an effect on arsenic mutagenesis. Studies have shown that pretreatment of cells with an inhibitor of glutathione biosynthesis (buthionine sulfoximine) reduces NPSH levels in the cell, resulting in enhancement of both the cytotoxicity and mutagenicity of arsenic. In contrast, glutathione and cysteine pretreatments are capable of protecting mammalian cells against the toxic effects of arsenite [16].

In a similar way, various antioxidants also have a significant effect on arsenic-induced genotoxicity. The balance between the rate of generation of free radicals and the rate of their removal by various antioxidant enzymes dictates the deleterious effect of oxidative stress. Enzymes like superoxide dismutase (SOD) and catalase are capable of partially suppressing both the toxicity and the mutagenic potential of sodium arsenite. These enzymes catalyze the dismutation of superoxide anions and prevent the formation of hydroxyl radicals by removal of hydrogen peroxide, respectively. Therefore, catalase and SOD are capable of reducing the mutagenic potential of arsenic. This is also consistent with other reports which reveal the ability of sodium arsenite to induce heme oxygenase, an oxidative stress protein, and peroxidase in various human cell lines. Moreover, the arsenite-induced occurrence of sister chromatid exchanges is reduced by SOD in cultured human lymphocytes [16].

In mammalian liver, the methylation of arsenic to MMA and DMA occurs at a high level by an incompletely characterized methyltransferase (**Figure 3**) using S-adenosylmethionine (SAM) as a methyl donor. SAM is a global methyl donor, required for DNA methylations, and its depletion can lead to hypomethylation of DNA resulting in alteration of gene expression like c-Myc, c-Met, cyclin D1 and induction of carcinogenesis [17, 18].

DNA methylation is an epigenetic modification that plays an important role in controlling the expression of various genes. Methylation generally occurs at cytosine residues located in symmetrical CpG nucleotide sequences, and its alteration, both in the global and regional levels, has been associated with oncogenesis. Methylation of CpG islands in the promoter region suppresses gene expression, as 5-methylcytosine interferes with the binding of transcription factors or other DNA-binding proteins causing reduced transcription. On the other hand, promoter hypomethylation causes overexpression of associated genes. Therefore, aberrant DNA methylation could be an underlying epigenetic mechanism causing altered gene expression that contributes towards the formation of cancers. This has been studied well in hepatocytes where chronic arsenic exposure induces hepatic DNA hypomethylation, which can potentially lead to aberrant gene expression and oncogenic growth in the liver, therefore suggesting a plausible mechanism of hepatocarcinogenesis (major cellular effects of arsenic are summarized in **Figure 4**) [18].

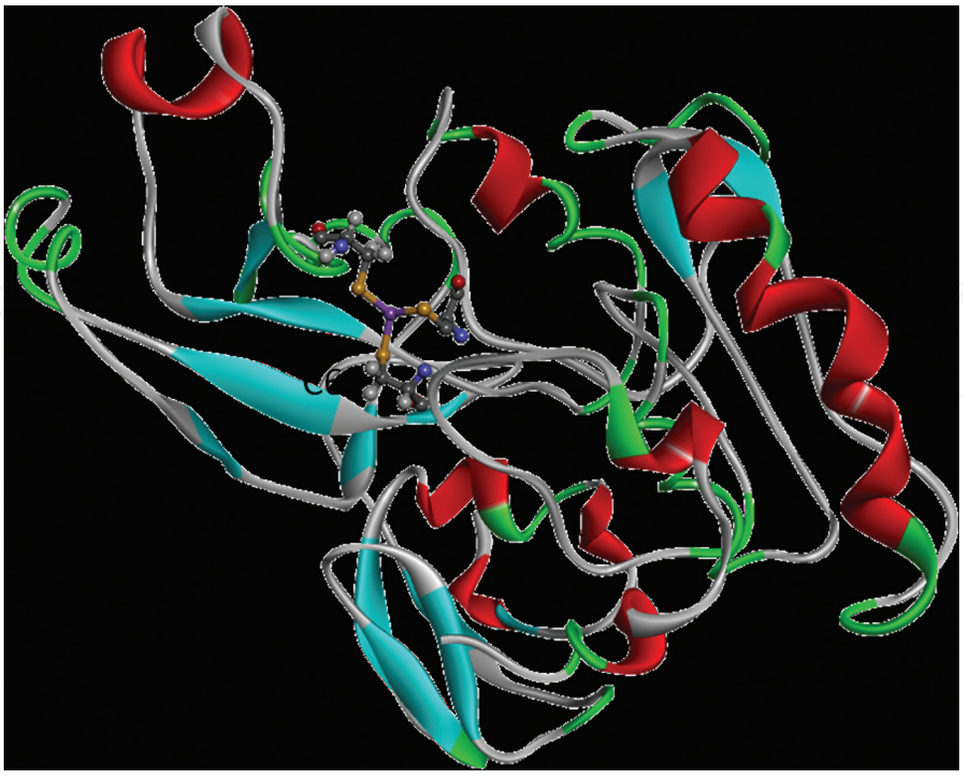


**Figure 3.** A homology model for arsenite methyltransferase from humans (AS3MT_HUMAN) showing arsenic bound to Cys residues. PDB ID: 5EVJ with 42% sequence identity spanning residues 38–327 was used to build the model. The coordinates were downloaded from https://swissmodel.expasy.org/repository/uniprot//Q9HBK9 and refined to introduce the arsenic atom.

Estrogens are considered to be liver carcinogens in rodents and are suspected to cause carcinogenesis in humans [19]. Evidence suggests that they cause hepatocellular proliferation and aberrant mitogenesis through ER-mediated mechanisms in addition to the likelihood that they confer epigenetic modifications. Hypomethylation of estrogen receptor-$\alpha$ (ER-$\alpha$) promoter region caused by arsenic exposure and ER-$\alpha$ overexpression have been found to trigger associated formation of proliferative lesions and hepatocellular carcinogenesis. Therefore, chronic arsenic exposure causes overexpression of ER-$\alpha$ creating hypersensitivity of hepatic cells to endogenous steroids. As evidenced by microarray analysis, various cell cycle-regulating genes like cyclin D1, cyclin D2 and cyclin D3 were overexpressed on exposure to arsenic. Liver cells that acquired malignant properties upon arsenic treatment also showed cyclin D1 overexpression. In addition, this overexpression had a direct effect on the observed malignant transformation, as selective cyclin D1 overexpression in the liver was sufficient enough to initiate hepatocellular carcinogenesis. Cyclin D1 can, therefore, be considered as a hepatic oncogene. Cyclin D1 is also known to be upregulated transcriptionally by various growth factors which potentially include estrogens. In estrogen-responsive tissues like the liver and uterus, proliferative lesions and co-overexpression of ER-$\alpha$ and cyclin D1 after chronic arsenic exposure are reported. Cyclin D1 activation by arsenic may be a secondary effect to ER-$\alpha$ overexpression as cyclin D1 is potentially an ER-$\alpha$-linked gene. Therefore, we can expect that aberrant expression of cyclin D1 along with that of other oncogenes leads to carcinogenic

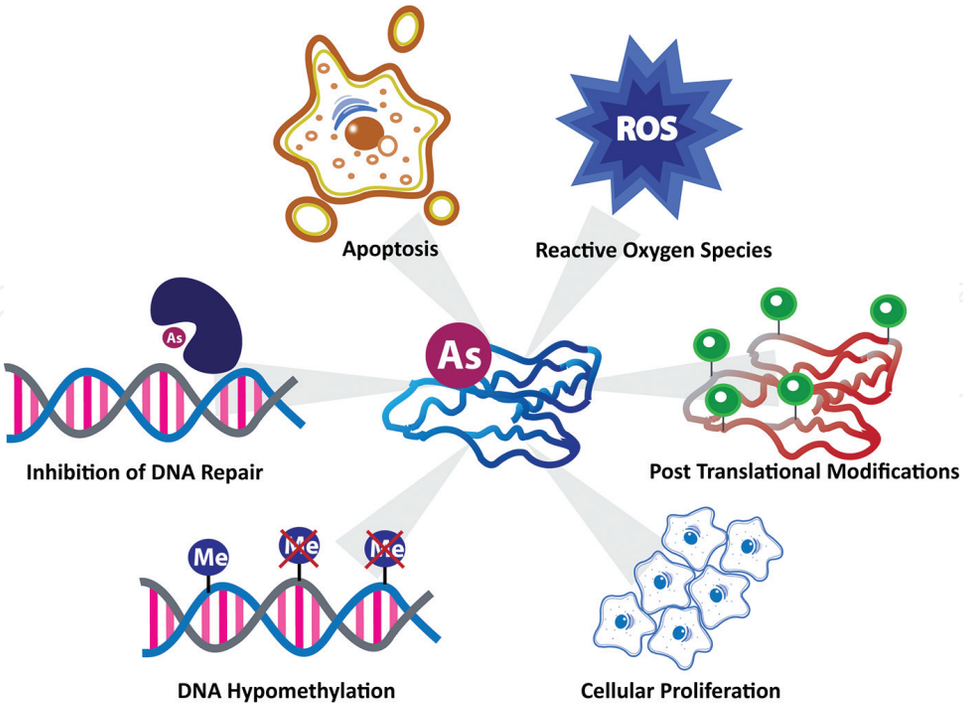



**Figure 4.** Cellular effects of arsenic toxicity.

transformation. Altogether, cyclin D1 overexpression was seen upon arsenic exposure in multiple in vitro and in vivo model systems of arsenic carcinogenesis, which includes skin and bladder cancers in rodents. Thus, under conditions of arsenic-induced carcinogenesis, overexpression of cyclin D1 is observed consistently [18–20].

In mouse lung tissue, reduced expression of proteins associated with cellular migration was observed when exposed to low dose of arsenic. On lung tissue of mice fed low-dose arsenic, changes in extracellular matrix (ECM) protein expression and a large increase in matrix metalloproteinase (MMP)-9 expression were revealed [21]. MMPs are responsible for ECM degradation among other proteolyses. MMP-9 is the most prominently studied MMP in the lung and has been associated with a variety of lung diseases [22]. An increase in the ratio of MMP-9 to tissue inhibitor of matrix metalloproteinase (TIMP)-1 was observed under low-level arsenic exposure [23]. This imbalance between MMP-9 and TIMP-1 can cause changes in epithelial wound response, thereby contributing to the progression of airway remodeling. Altered wound response is partly due to increased secretion and activity, upon increasing concentration of arsenic. Therefore, arsenic ingestion may alter wound response and, specifically, MMP-9/TIMP-1 ratios in the lung. To conclude, arsenic is capable of causing or exacerbating lung diseases by directly affecting signaling pathways involved in cell migration and remodeling of the airway [24].

Studies have revealed that both c-Jun NH2-terminal kinases (JNKs) and extracellular signal-regulated protein kinases (Erks) are activated by arsenite, with their activation varying temporally and depending on the dosage. Various results also indicate that Erk activation but not JNK activation is required for arsenite-induced cell transformation. Expression of the dominant-negative mutant JNK1 blocked induction of apoptosis by arsenite or arsenate compared with

vector-transfected JB6 cells, indicating the role of activation of JNKs in arsenic-induced apoptosis. Studies have found that both arsenite and arsenate can cause transactivation of activation protein-1 (AP-1). Since increased activation of AP-1 by arsenite could be inhibited by either treating cells with MAP kinase Erk kinase (MEK)1 inhibitor or overexpression of dominant-negative protein kinase C $\alpha$ (PKC$\alpha$), this induction appears to occur through activation of mitogen-activated protein (MAP) kinases and PKC. Moreover, in AP-1-luciferase reporter transgenic mice, transactivation of AP-1 was caused by both arsenite and arsenate. Recent data also indicates that PKC, upstream from the MAP kinases, may be involved in mediating arsenite-induced signal transduction. Activation of PKC requires it to be translocated from the cytosol to the membrane, and this phenomenon is observed within 15 minutes when cells are treated with arsenite. Moreover arsenite-induced AP-1 activity, phosphorylation of Erks, JNKs and p38 kinase were blocked once PKC activation was inhibited. These results suggest that PKC plays a critical role in arsenite-induced activation of MAP kinases [25, 26].

Nuclear factor kappa B (NF-κB) is a rapidly induced stress-responsive transcription factor that may play an important role in arsenic-induced signal transduction, cell transformation and apoptosis [27]. Reports suggest that in arsenic-induced oxidative stress, $H_2O_2$ and superoxide are the predominant reactive species in endothelia cells and may be the mediators for the activation of the NF-κB pathway. It was also shown that arsenic could induce activation of NF-κB in different cell culture models. Expression of a dominant-negative inhibitory kappa-B-$\alpha$ blocked arsenic-induced activation of NF-κB and apoptosis [26].

## 4. Arsenic binding to proteins

The trivalent arsenite has a tendency to bind to sulfhydryl groups. The cysteine residues are a direct target of arsenite in proteins and peptides [28]. The chemical reaction involved in arsenic binding to cysteines has been well recognized. Some of the chemicals like arsine halides used in warfare during the First World War owe their toxicity to their ability of binding to protein dithiols. To defy the toxic effects of such warfare agents, the British government approved the use of β-chlorovinyldichloroarsine (dithioglycerol) which has the ability to form stable complexes with arsenic. The competitive binding of arsenic to dithioglycerol rescues cellular proteins from binding to arsenic [29, 30].

Arsenic affinity for proteins can result in conformational changes in the protein and loss of protein–protein and protein-DNA interactions. *Escherichia coli* consists of a repressor protein ArsR in which each subunit within its $\alpha$-helix contains two cysteine residues. The unraveling of this $\alpha$-helix is required in order to accommodate trivalent arsenite for binding to the protein. The unraveling of the helix causes the conformational change in the protein that dissociates ArsR from DNA resulting in induction of gene expression. Arsenite binds to three cysteine (Cys32, Cys34 and Cys37) residues in ArsR, where Cys32 and Cys37 are present in the $\alpha$-helix of the DNA-binding sites (**Figure 5**). The Cys residues in the protein are located in such a way that arsenite is unable to bind unless the protein unwinds for a conformational change [31].

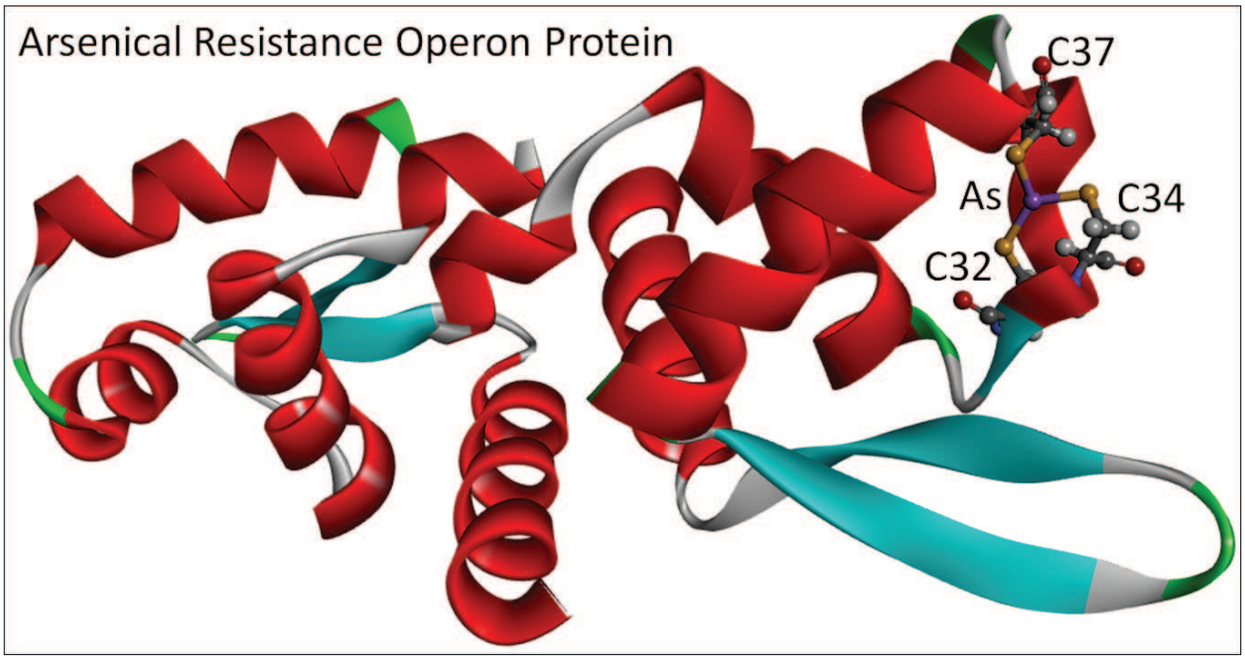



**Figure 5.** A homology model for arsenical resistance operon repressor protein from *E. coli* (ARSR_ECOLI) showing arsenic bound to Cys residues. PDB ID: 1SMT with 40% sequence identity spanning residues 8–90 was used to build the model. The coordinates were downloaded from https://swissmodel.expasy.org/repository/uniprot//P37309 and refined to introduce the arsenic atom.

### 4.1. Binding sites of arsenic in proteins

Cysteine and histidine residues are thought to be the most frequent targets of metals like zinc, copper and iron resulting in such metals binding to peptides and proteins [32, 33]. However, the binding of arsenic to histidine is not well understood and is yet to be established. There is no change in nuclear magnetic resonance (NMR) spectra once arsenic was added to a buffered solution of histidine signifying the absence of interaction between histidine and arsenic [34]. Many studies have also used site-directed mutagenesis to replace cysteine residues with serine residues on the reason that interaction between arsenic and serine is very weak. Arsenic is however known to bind to zinc finger protein in C3H1 motif and not in C2H2 motif, releases zinc, and thus decreases the capacity of the protein to bind to DNA. Selenocysteine—a cysteine analogue—also has the ability to bind to arsenic species. This amino acid is present in selenoproteins and has a lower pKa which increases nucleophilicity. The amino acid residues in the vicinity of cysteine (or selenocysteine) act as proton donors [35–37].

Studies with some enzymes reveal that serine residues can be potential targets of arsenic species, thereby inhibiting their function. It was found that in serine hydrolases and the arsenic moieties interacting with hydroxyl containing serine, pentavalent forms of arsenic rather than trivalent forms were prevalent. The complex between the serine residue and the pentavalent arsenic consists of a tripartite oxyanion hole in the proximity of the active site [38].

## 4.2. Arsenic binding to specific proteins

### *4.2.1. Arsenic binding to hemoglobin*

Arsenic species are cleared from the blood immediately in humans, but the time of clearance of arsenic from animal species varies noticeably. The retention of arsenic in rat blood is longer when compared with other species. Arsenic has been found to bind to transferrin in hemodialysis patients [39]. Hemoglobin in red blood cells (RBCs) were predicted to be the sites of arsenic accumulation, because hemoglobin constitutes 97% of dry weight of RBCs [40]. The affinity of hemoglobin in rat liver is much higher in rats as compared with humans. The rat and human hemoglobins are tetramers, each consisting of two $\alpha$-chains and two $\beta$-chains. The difference lies in the number of the cysteine residues with rat hemoglobin consisting of three cysteines (Cys111, Cys104 and Cys13) in $\alpha$-chain, while two cysteines (Cys125 and Cys93) in $\beta$-chain. On the other hand, human hemoglobin has only one cysteine in $\alpha$-chain and two cysteines in $\beta$-chain [3].

### *4.2.2. Arsenic binding to glutathione*

The metabolism of arsenic in the cells involves the reduction of pentavalent arsenic to trivalent arsenic. This reaction consists of a redox cycle involving a bio-thiol (glutathione) with the production of a *tris*-glutathionyl-arsenite species. The multiple methylations of arsenite by *S*-adenosylmethionine to the generation of trimethylarsine (hemolytic toxin) also involve glutathione. Glutathione presence in the intermediate conjugate forms of methylated arsenic species helps these molecules to be removed from the cells by the multidrug-resistant proteins (having ATP-binding cassette). Dimethylarsinic acid (carcinogenic end-metabolite) also reacts with glutathione having a high cytolethal effect on cells. Moreover, various enzymes and regulatory elements can contribute to the arsenic biotransformation by contributing individual or multiple cysteine thiol groups in vicinity in proteins, for example, thiol groups required for catalytic activity [41].

### *4.2.3. Arsenic binding to metallothioneins*

Metallothioneins are expressed by various organisms including bacteria, fungi, plants and vertebrates. They belong to a protein family of ubiquitous nature characterized by low molecular weight, high metal and cysteine content. They are capable of binding essential metal ions (zinc, copper) and toxic heavy metals (arsenic, cadmium).

Studies have revealed that bioaccumulation of arsenic in seaweed species *Fucus vesiculosus* is achieved through the binding of arsenite to the cysteine-rich metallothioneins. Moreover, arsenic is also known to bind to mammalian metallothioneins in rabbit and human species. It is present abundantly in the kidneys and liver of mammals. Further studies on human metallothioneins were consistent with the hypothesis that arsenite has a binding preference for three vicinal thiol groups, with $\alpha$ and $\beta$ domain of human metallothionein containing 11 and 9 cysteines, respectively. All the 9 cysteines were involved in binding to three arsenite molecules in $\beta$ domain, while in the case of $\alpha$ domain, only 9 out of 11 cysteine residues were involved in binding to three arsenites. This leaves two cysteine residues protonated with no fourth arsenite engaged in binding [42, 43].

*4.2.4. Arsenic binding to ArsD As(III) metallochaperone*

Arsenic being the most common toxic element in the environment has resulted in the evolution of arsenic detoxifying mechanisms in nearly all organisms. In archaea and bacteria, trivalent metalloids like arsenite are pumped out of the cell by ArsAB ATPases encoded by various *ars* operons. Three conserved cysteine residues (Cys12, Cys13 and Cys18) are required for the chaperone activity of ArsD. ArsD also helps to increase the arsenite affinity of Ars A enabling the detoxification of arsenite, even at low concentrations. In the case of ArsA, there are two cysteines (Cys113 and Cys422) in the high affinity metalloid-binding site along with the third cysteine that participates in activation of ATP hydrolysis. In the absence of arsenite, a low basal rate of ATPase activity is shown by ArsA [44].

*4.2.5. Arsenic binding to other proteins*

Trivalent arsenic species are also known to bind to other proteins like actin, tubulin, estrogen receptor and glucocorticoid receptors. Arsenite can bind to Kelch-like ECH-associated protein 1 (KEAP 1). This is a major antioxidant-sensing protein which acts at low $K_d$ values. One of the most common motifs present in many proteins consists of two cysteine residues separated by two amino acids (CXXC). The presence of cysteine residues increases with increasing complexity of the organisms, making humans vulnerable to arsenic toxicity because of the high affinity of arsenic for cysteine residues.

One such important cell surface protein consisting of highly conserved cysteine residues is connexin 43 (Cx43)—a widely expressed gap junctional protein important for cell death, proliferation and differentiation [45]. Cx43 has nine Cys residues, six of which are in the extracellular domain and three in the intracellular domain. Six connexin monomers form a hemichannel called connexin. In the plasma membrane, one connexin can dock to another connexin in the plasma membrane of an adjacent cell resulting in the formation of complete gap junction channel. A hemichannel formed by single type of connexin isoforms is called homomeric hemichannel or consists of multiple types of isoforms called heteromeric hemichannel. Two identical homomeric or heteromeric hemichannels dock to form a homotypic channel, and two different homomeric or heteromeric hemichannels dock to form a heterotypic channel (**Figure 6**). Recent in silico studies (Hussain et al., manuscript communicated) in combination with cellular and biochemical analysis revealed insights into the binding modes of arsenite to conserved Cys groups in Cx43. In Cx43, $As^{+3}$ can be bound to three cysteines in the intracellular domain in a monovalent fashion as they are free cys, while it can bind the extracellular domain cysteines in either monovalent, divalent or trivalent fashion depending on the state and location of the protein in the cell. Arsenite ion ($As^{+3}$) can attack the free sulfhydryl group until all the valencies of the $As^{+3}$ are satisfied by covalent bonding to the sulfur from the cys residues. This profoundly affects the Cx43 primary, secondary, tertiary and the quaternary structure. This study is the first of its kind which shows that arsenic can directly bind to Cx43 via its highly conserved cysteine residues causing misfolding of Cx43, which leads to alteration of transportation, localization and oligomerization of Cx43. Further experiments revealed that Cx43 was colocalizing with ER marker (calnexin), revealing the inability of Cx43 to be transported beyond endoplasmic reticulum/endoplasmic reticulum

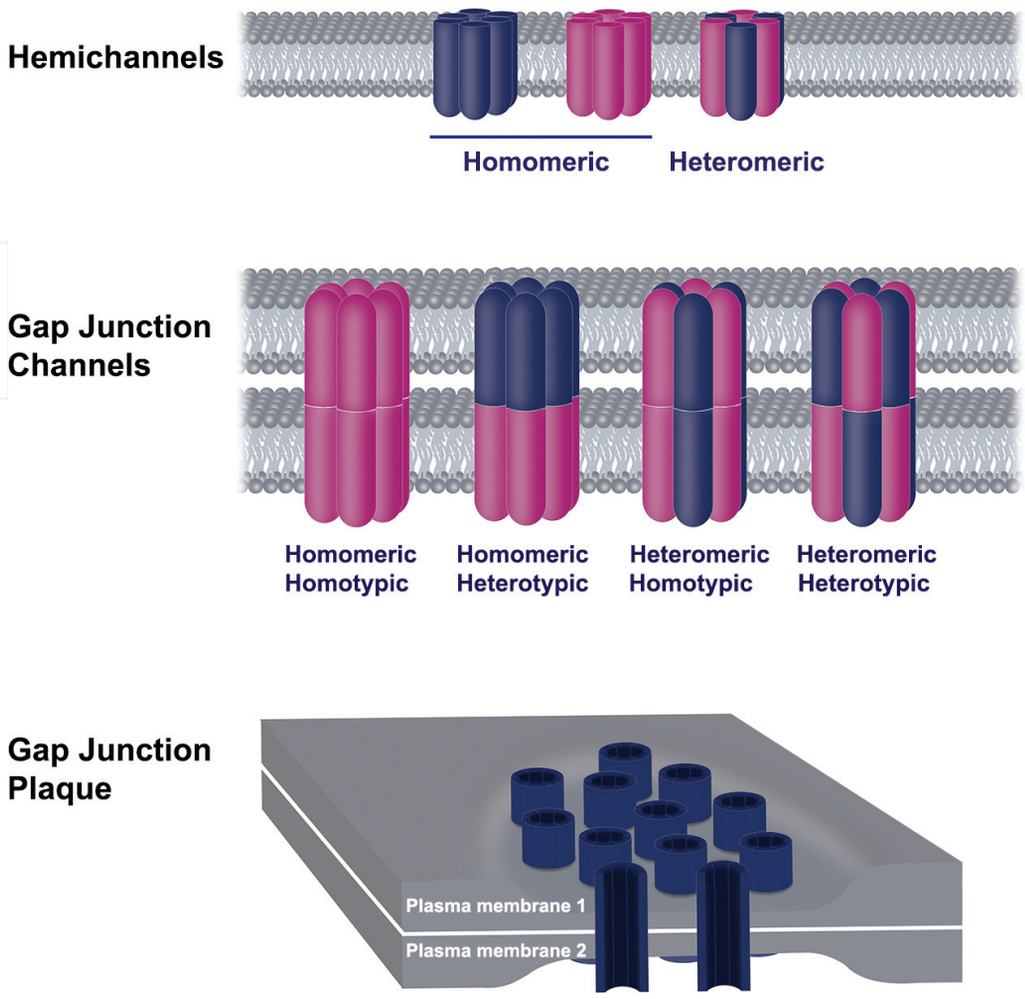



**Figure 6.** Hierarchy of structures involved in the formation of gap junction intercellular communication (GJIC).

Golgi intermediate compartment (ER/ERGIC) (Hussain et al., manuscript communicated). This loss of Cx43 composed of functional gap junctions on the cell surface has deleterious effect on cellular homeostasis (**Figure 7**).

Arsenic is considered a group 1 carcinogen by the International Agency for Research on Cancer (IARC) and causes cancers of the lung, liver and skin [46]. Gap junction intercellular communication has been found disrupted in many tumors and malignancies. Gap junctions are considered tumor suppressors, and the persistent downregulation of gap junction proteins makes cells susceptible to cancer [47]. Decreased or diminished expression and/or function of Cxs has been observed in most tumor cell lines and in solid tissue tumors, including melanomas. Our study revealed that arsenic causes disruption of gap junction intercellular communication both in vivo and in vitro. Arsenic is considered a weak mutagen; therefore, recent trends in the field have focused on deciphering the role of non-mutagenic pathways like cell–cell communication in arsenic-induced cancer. Our study revealed that arsenic induces disruption of gap junctions which are considered as tumor suppressors, thereby putting forward new non-mutagenic pathways which may be altered during the course of arsenic-induced carcinogenesis.

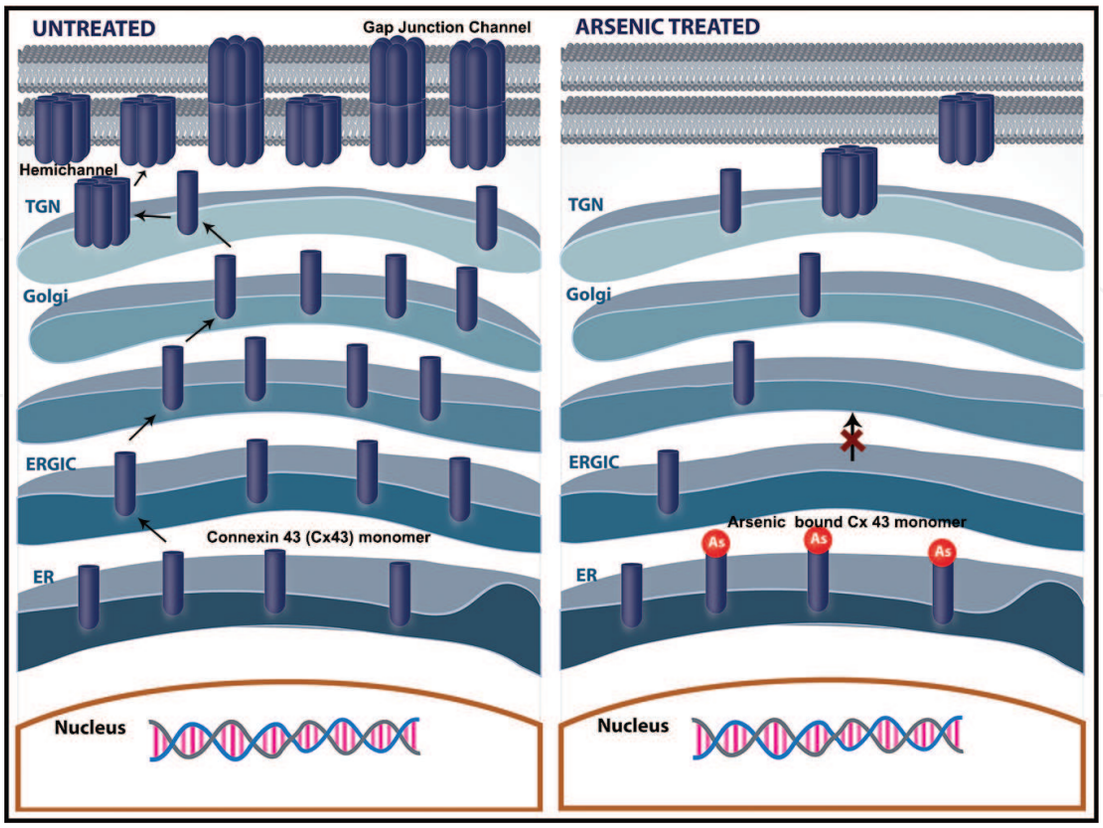



**Figure 7.** Arsenic binding causes alteration in trafficking of connexin 43 to the cell membrane.

Another such important cellular factor involved in cellular stress is DJ-1. DJ-1 is a 20KDa, homodimeric protein containing a nucleophilic elbow forming the active site of the protein. There are three important and conserved cysteine residues (Cys46, Cys53 and Cys106) in the DJ-1 protein, of which Cys53 and Cys106 are exposed. Cys106 has been found to be a prominent player in the nucleophilic groove that binds to divalent ions like zinc (II), copper (II) [48] and mercury [49] in vitro. Interaction with metal ions might be a possible mechanism of DJ-1-mediated cellular protection against metal-induced toxicity. Arsenic in the form of arsenite (As (III)) has been found to interact with three thiol group of cysteine residues [3]. Therefore, there is a possibility that arsenic binds to the nucleophilic groove in the homodimer of DJ-1. Oxidation state of the Cys106 is one of the determining factors behind the activity of the protein. Cysteine has the propensity to bind to three oxygen atoms resulting in the formation of the three forms—SOH, $SO_2H$ and $SO_3H$. The presence of the SOH and $SO_2H$ form activates the protein causing its translocation into the nucleus. Upon activation, DJ-1 regulates the activity of several transcription factors like nuclear factor erythroid 2-related factor 2 (Nrf2), polypyrimidine tract-binding protein-associated splicing factor (PSF) and sterol regulatory element-binding protein (SREBP), signal transducer and activator of transcription 1 (STAT1) and Ras-responsive element-binding protein (RREB1). DJ-1 has been found to inhibit phosphatase and tensin homolog (PTEN), an inhibitor of the AKT (protein kinase B) signaling pathway, resulting in enhanced cell proliferation. DJ-1 also functions in the sequestration of the death domain-associated protein (DAXX) in the nucleus. DAXX is required in the cytoplasm for

providing the second activation signal to the phosphorylated apoptosis signal-regulating kinase 1 (ASK1) protein, which then triggers the apoptotic pathway. As a result, unavailability of DAXX in the cytoplasm hinders the initiation of the apoptotic pathway. Under condition of excess oxidative stress, the $SO_3H$ form prevails which inactivates the protein and re-translocates back to the cytoplasm. As a result, the entire antioxidant response regulated by the activated DJ-1 protein is inhibited. Moreover, DAXX protein also becomes free, which then translocates into the cytoplasm and provides the required second activation signal to the phosphorylated ASK1 protein.

### 4.3. Therapeutic applications of arsenic binding to proteins

Arsenous acid [$As(OH)_3$] formed by dissolving of arsenic trioxide ($As_2O_3$) was found to be an effective and safe treatment for acute promyelocytic leukemia (APL) in the 1970s. The United States Food and Drug Administration approved the use of $As_2O_3$ as a treatment for APL in September 2000 [50]. $As_2O_3$ treatment was shown to have a dual effect on APL cells, with low arsenic concentration (0.25–0.50 mM) favoring APL cell differentiation and high concentrations (1–2 mM) inducing apoptosis (programmed cell death). Direct arsenic binding to cysteine residues present in zinc fingers of promyelocytic leukemia fusion protein (PML-RARa) was found to be a mechanism underlying APL remission [51]. Arsenic binding induces a conformational change in the structure of the protein (PML-RARa), facilitating its oligomerization. This oligomerization enhances ubiquitylation and SUMOylation, resulting in its degradation [52]. Given the constant requirement for DNA and protein synthesis, thioredoxin (Trx) and thioredoxin reductase (TrxR) are observed to be overexpressed in various tumors. Moreover, in vivo data suggests that TrxR is necessary for the growth of tumor cells, making them plausible targets for anticancer therapies [53].

Arsenic has been proposed to induce cell death through thioredoxin reductase (TrxR) inhibition, with both N-terminal dithiols and C-terminal selenothiol interacting with arsenic compound [54]. Sensitivity of cells to arsenic can be attributed to high expression of membrane transporter aquaglyceroporin which allows arsenite uptake, along with a low, basal level of cellular glutathione. Multiple factors such as liver damage, cardiac toxicity and peripheral neuropathies caused by toxicity at higher dosage of $As_2O_3$, along with bioavailability of arsenic compounds, limit the widespread use of $As_2O_3$ against solid tumors [50].

## 5. Arsenic sensing

Considering the hazardous facts of arsenic, it is very important to detect arsenic both qualitatively and quantitatively. Many conventional methods like hydride generation atomic absorption spectrometry (HG-AAS), neutron activation analysis and X-ray analysis and stripping voltammetry are available to determine arsenic. Though these methods are available, they are not very cost-effective and are very complex [55–59]. To determine arsenic, easy and cost-effective methods are yet to be explored. In recent times various heavy metals and toxic anions are detected selectively and sensitively by using optical detection techniques (fluorescence and UV–Vis), which implement a viable and simple approach towards

the detection process. Except optical (fluorescence and UV–Vis) detection methods, other available methods need complex experimental setup; hence, they are far from 'on-field' application purpose. Simplicity, low-cost and 'on-field' application possibilities make optical sensing technique versatile.

Optical sensors can be of different types depending upon the material used for sensing. The first one is nanomaterial-based assays for the detection of the arsenic in different mediums. Though the detection of arsenic is tough, but researchers are able to draw an outline about the ligands which can bind arsenic, and these ligands can be used as a binding unit in a sensing material which leads to either color change or change in emission spectrum. As arsenic is very much labile towards thiol group, a bunch of thiolated ligands are reported for arsenic binding. These ligands are dithiothreitol (DTT), reduced glutathione (GSH) and cysteine, and **Figure 8** describes the chemical structure of these three ligands. Arsenic can bind with GSH and cysteine by forming As-O bond also, if no free –SH available. Except thiolated ligands, there are some ligands like humic acid [60] and N-(dithiocarboxy)-N-methyl-D-glucamine [61] which can also bind As(III) by forming As-O bond. Keeping this information in mind, gold nanoparticle-based sensors were reported for As(III) detection. The surface of the gold nanoparticles can be modified by the thiolated ligands, which after binding with As(III) showed a drastic color change to indicate the presence of the toxicant in the aqueous medium [62]. Aptamer-conjugated nanoparticles are also very effective composites which can detect arsenic in aqueous medium [63, 64] by changing the color. In all these types of detection assays, aggregation of the nanoparticles is the predominant factor to show the color change. Though these kinds of materials are responsive towards arsenic, but sensitivity is one of the issues which prevent these from field effectiveness.

Both selectivity and sensitivity are important for effective detection of arsenic. Small molecules are developed to detect different forms of arsenic in aqueous medium having good selectivity over other toxicants as well as good sensitivity. Baglan M et al. have reported a cysteine-fused tetraphenylethene, which can bind with $As^{3+}$, and showed aggregation-induced emission as a signal [65]. Here, also the thiol group of cysteine acts as the dominating factor for $As^{3+}$ binding and leading to the close proximity arrangement of the tetraphenylethene. More toxic $As^{3+}$ can be distinguished over less toxic $As^{5+}$ using this system, and the detection limit tends to 0.5 ppb, which is lower than the limit according to the World Health Organization (WHO) [66]. Keeping besides the thiol systems, Somentah et al. have designed a simple Schiff base system which can identify the most toxic $AsO_3^{3-}$ fluorometrically. 'Off–on' system in fluorescence is always most exciting and effective for the detection of pollutants. In this work they have designed a molecule which is initially not showing any fluorescence emission, but after selective addition of $AsO_3^{3-}$ fluorescence, signal is turned on due to intermolecular H-bonding leading to chelation-enhanced fluorescence (CHEF) [67]. Development of arsenic sensor is evolving year

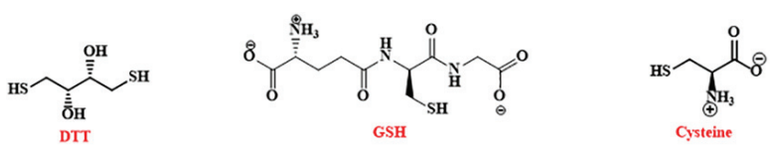
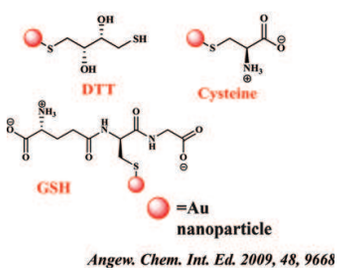
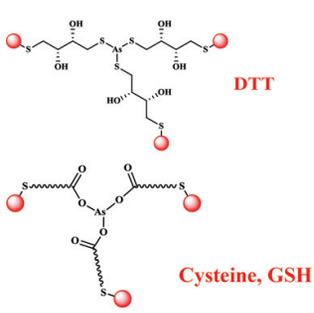
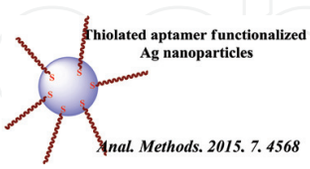
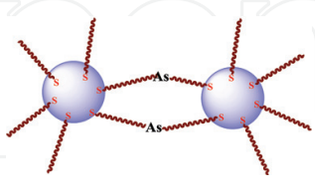
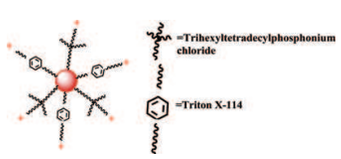
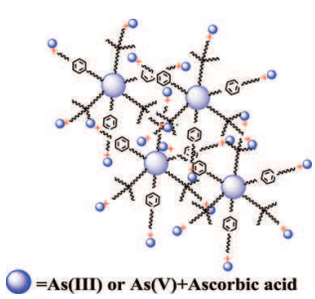



**Figure 8.** Chemical structures of thiolated ligands (DTT, GSH and cysteine).

after year due to the need of arsenic detection. A modified coumarin derivative was documented as an $As^{3+}$ sensor having a detection limit of 0.53 nM. Though the system has excellent sensitivity, but the main drawback is its incapability of detecting $As^{3+}$ in aqueous media. So, the sensing system which can work effectively in aqueous media for the detection of arsenic having fluorescence property is in tremendous search till date. In search of a suitable aqueous medium arsenic sensor, an inorganic co-crystal has been reported having a unique luminescent response to detect As(III), having a detection limit of 49 pM. But these types of systems are not that much useful for real-life application [68]. **Table 1** is prepared where available optical sensors are summarized.

A few small molecule sensors have been explored over the years, but the 'on-field' application is quite tough for small molecule sensors due to their low molecular weight and water solubility. To overcome such issues, polymeric sensing assays are developed as they have high molecular weight, tunable solubility by introducing hydrophilic functionality, high signal amplification and high sensitivity due to the number of more repeating units. In the field of materials science research, polymer-based substances have high priority. For sensing of arsenic, polymer-based sensing assay is very rare, with a few number of reports existing. A pyridylmethyl-appended 2-aminothiophenol with 2,6-diformyl-4-methylphenol was developed, which can detect arsenate (As(v)) selectively in aqueous medium. But the interesting fact

| Types of sensors | Compounds or materials | Mode of sensing with optical changes |
|---|---|---|
| 1.Nanoparticle-based | 1.   |   |
| | 2.   |   |
| | 3.   |   |

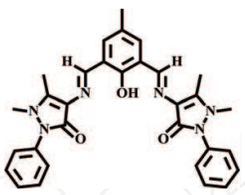
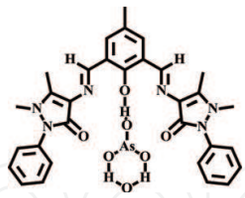
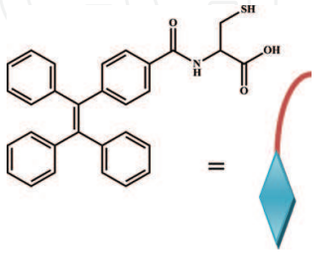
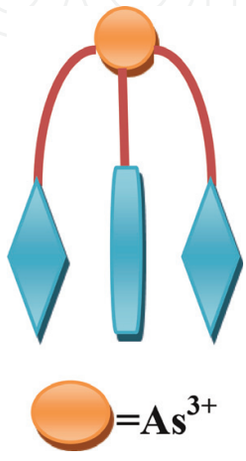
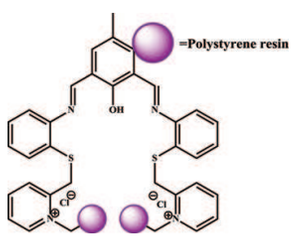
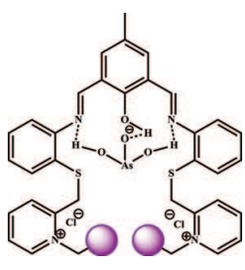
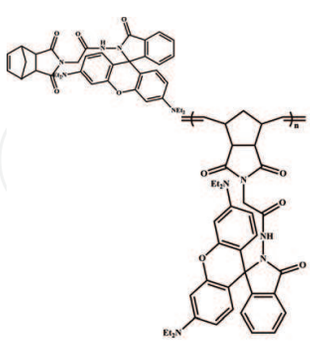
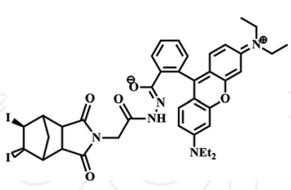
| Types of sensors | Compounds or materials | Mode of sensing with optical changes |
|---|---|---|
| 2. Small molecule-based | 1.  |  |
| | 2.  |   |
| 3.Polymer-based | 1.  |  |
| | 2.  J. Mater. Chem. A, 2013, 1, 8398 |  |

**Table 1.** Some available optical arsenic sensors and their mode of detections.

is that after attachment of the small molecule with polystyrene resin, the new material consists both sensing and removal property of As(V) which is very beneficial for the treatment of As(V) in drinking water practically [69]. All mentioned sensory assays are responding due to

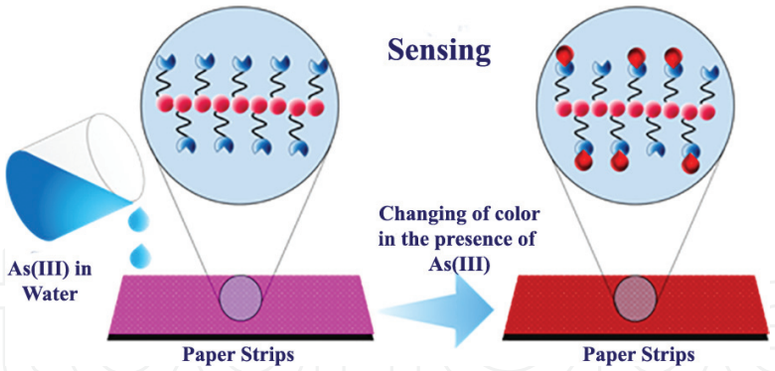



**Figure 9.** Cartoon representation to demonstrate change in color of PNor-Rh-coated paper strip in the absence and presence of As(III).

interaction of host and guest. But one of the best indirect As(III) sensors is reported in recent time. Sourav et al. reported one norbornene-derived rhodamine B, which is capable of detecting As(III) in aqueous medium up to 200 nM concentration [70]. Here, the main dominating factor is the oxidation of As(III) to As(V) in the presence of potassium iodate and concentrated HCl. During this oxidation procedure, iodine is liberated which coordinates with sensing molecule Nor-Rh, which leads to the colorimetric as well as fluorescence change. The effectiveness of this work is that the polymeric material of Nor-Rh can be used to make paper strip which will help to detect As(III) in real environmental samples. A cartoon representation is given in **Figure 9** to demonstrate the color change of polymer-coated paper strip with and without As(III).

In summary, though few reports are available for efficient detection of arsenic in aqueous medium with high sensitivity, research community continuously tries to develop sensory assay for 'on-field' application, with a tremendous impact in detection of arsenic in environmental samples with ease and real-life application.

## 6. Conclusion

Arsenic, having a high reactivity with cellular contents, can have diverse and deleterious effects on the cells. One of the important players of arsenic-induced toxicity is the generation of ROS, which can lead to DNA damage and lipid peroxidation. Another important effect is the arsenic exposure that causes the depletion of methyl groups in cellular milieu. Hypomethylation of promoter regions can lead to overexpression of genes which play a key role in cell proliferation, differentiation and apoptosis. As mentioned earlier, DNA hypomethylation upregulates receptors like ER-$\alpha$ making the cells more sensitive towards endogenous steroids. Arsenic is reported to activate PKC which activates MAPK pathway leading to the activation of various transcription factors like AP-1. AP-1 is considered as a crucial player in regulation of cell proliferation, differentiation and apoptosis. Arsenic effects extracellular matrix through upregulation of MMPs resulting in degradation of extracellular matrix having consequences in cellular migration, angiogenesis, proliferation and apoptosis. The biological effects of arsenic are so diverse that multiple

mechanisms have been proposed for the toxicity of the arsenic. The mechanisms involved in the arsenic-induced carcinogenesis are also diverse and complicated. DJ-1 is a multifunctional protein that is activated upon cellular stress response. Most of the studies on DJ-1 protein are related to oxidative stress, although implication of its activity in ER stress response has been shown. The interaction of arsenic with sulfhydryl groups in proteins is considered one of the principal mechanisms which triggers the cellular responses. The binding of trivalent arsenicals to thiols in intracellular and cell surface proteins often results in aberrations of normal cellular processes including alteration of cell–cell communication. Cell–cell communication mediated by connexins, especially Cx43, the most commonly expressed connexin in different cell types, is also disrupted by arsenic binding to its highly conserved cysteine residues. In general, the effect of direct binding of arsenic species to enzyme activity cannot be ruled out in toxicity-related investigations, where other factors like the reactive oxygen species are often implicated. New methodologies are needed to analyze the health effects of arsenic and how people cope with the socioeconomic consequences of the disease. Arsenic toxicity being a global phenomenon constitutes a major public health issue, and therefore an intense research is warranted.

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## Conflict of interest

The authors declare no conflict of interest pertaining to the contents of this chapter.

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