REVIEW OF LITERATURE
Arsenic as metalloid

Arsenic is a metalloid - a substance that is not actually a metal, but has some metallic properties. It is a naturally occurring element with atomic number 33 and atomic mass of 74.9 g mole\(^{-1}\). Arsenic can exist in many different chemical forms and also in combination with other elements both in inorganic and organic forms. The inorganic form is considered to exert more toxic effect on human health\(^{33}\). The organic form of arsenic may be produced by biological activity mostly in the surface waters, but are rarely quantitatively important. Most trace metals occur in solution as cations (eg Pb\(^+\), Cu\(^+\), Ni\(^{2+}\), Cd\(^{2+}\)) which generally become increasingly insoluble as the pH increases; but in case of arsenic, it can persist in solution at relatively high concentrations even at near neutral pH values\(^{34}\), which cause a severe hindrance in removing arsenic from water. Arsenic enters the atmosphere through inputs from wind erosion, volcanic emission, marine aerosols and pollution and is returned to the earth’s crust by wet and dry deposition. The most important anthropogenic inputs are from smelter operations and fossil fuel combustion. In several developing countries in Asia, the exposure of arsenic through drinking arsenic-enriched groundwater has serious adverse impact on human health. The situation is particularly alarming in Bangladesh, West Bengal (India) and other countries in South and South East Asia\(^{35}\). Arsenic is widely considered as one of the worst environmental health disasters, with an estimated 50 million people at risk of cancer and other arsenic-related diseases. More recently, the impact of arsenic on irrigation water and on crops and aquatic ecosystems have also become a matter of great concern\(^{36}\). Recent studies have revealed enhanced arsenic concentration in the areas with active volcanic chains such as in Costa Rica, Nicaragua and El Salvador where soil and groundwater resources often exhibit elevated arsenic concentrations above the regulatory standard of 10 \(\mu\)g/L \(^{37}\). Some countries have accepted lower safety standards in order to
increase water supplies; however these are likely to have higher costs in the long run. Growing pressure on governments and science to protect human health and the ecosystem from arsenic contamination has stimulated increased research using a wide range of approaches and techniques.

Figure R1: Exposure to arsenic-contaminated drinking water

**Arsenic in groundwater of West Bengal**

The source of arsenic in groundwater can be traced out by establishing the relations between the drainage patterns of the area. In this regard Geological Survey of India and Central Ground Water Board have done commendable job and come out with certain findings. The problem of groundwater pollution by arsenic is found in the interfluvial region of the Bhagirathi-Hugli and the Jalangi-Ichamati rivers lying mostly in the eastern part of the Bhagirathi-Hugli river of West Bengal\(^3\)\(^8\) The arsenic contamination in ground water beyond permissible limit of 0.05 mg/l has been found within the shallow aquifer (20-60 m below ground level). Apart from this area, other areas where higher incidence of arsenic has been reported are four blocks (adjacent to the river Ganga) in Malda district, Purbasthali block of Bardhaman district and Balagarh block of Hugli district.
Arsenic-endemic areas in the world

Before the year 2000, there were five major incidents of arsenic contamination in groundwater in Asian countries: Bangladesh, West Bengal, India, and sites in China. Between 2000 and 2005, arsenic-related groundwater problems have emerged in different Asian countries, including new sites in China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, DPR Korea, and Pakistan. There are reports of arsenic contamination from Kurdistan province of Western Iran and Vietnam, where several million people may have a considerable risk of chronic arsenic poisoning. An elevated arsenic level in the drinking water is the major cause of arsenicosis around the world. Ground water contamination with arsenic has been also reported from USA, Canada, Chile, Argentina, Vietnam, Hungary, Laos and Cambodia.\(^{39}\)
Health issues related to arsenic exposure

The chronic health effects of inorganic arsenic exposure from consumption of arsenic-contaminated water include skin lesions, skin cancer, internal malignancies, neurological effects, hypertension, peripheral vascular diseases, cardiovascular disease, respiratory diseases, and diabetes mellitus⁴⁰.

<table>
<thead>
<tr>
<th>System</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Skin</td>
<td>Skin lesions</td>
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<tr>
<td>Cardiovascular</td>
<td>Blackfoot disease</td>
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<tr>
<td>Nervous</td>
<td>Peripheral neuropathy, encephalopathy</td>
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<tr>
<td>Hepatic</td>
<td>Hepatomegaly, cirrhosis, altered heme metabolism</td>
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<tr>
<td>Hematological</td>
<td>Bone marrow depression</td>
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<tr>
<td>Endocrine</td>
<td>Diabetes</td>
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<td>Renal</td>
<td>Proximal tubule degeneration and cortical necrosis</td>
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Table 2: Common health issues in chronic arsenic exposure
Skin lesions are one of the most common features of chronic arsenic poisoning and these lesions are used as diagnostic criteria of endemic arsenic poisoning in Inner Mongolia, China\textsuperscript{41}. There are several reports that discuss the relative risk or prevalence rate of chronic health effects in arsenic-exposed populations, although few papers attempt to define dose–response relationships between arsenic exposure via the drinking water and chronic toxicity because of the difficulty in estimation of individual exposure. Epidemiological study by \textit{Das et al}\textsuperscript{12} demonstrates that chronic exposure of arsenic through drinking water in the population of West Bengal has caused increased liver dysfunction, autoimmune deregulation and cardiovascular risk. In clinical investigations it was found that inorganic arsenic induces immunotoxicity in humans which cause hypertrophy of immune organs and liver. It is also reported that arsenic intoxication causes vascular inflammation and subsequent atherosclerosis\textsuperscript{42}. Chronic exposure to arsenic has been reported to elicit multiple health consequences. Several inflammatory clinical conditions like hypertrophy of liver and spleen are observed in patients who ingested arsenic-contaminated drinking water over a long time\textsuperscript{43}. \textit{Santra et al}\textsuperscript{44} reported hepatic abnormalities among inhabitants living for a long term in arsenic-contaminated areas. The incidence of hepatomegaly was found to have a linear relationship proportional to increasing exposure to arsenic in drinking water\textsuperscript{45}. All these factors together contribute toward progression of liver cirrhosis and liver cancer.
Mechanism of Arsenic toxicity

![Arsenic poisoning](image)

**Figure R4: Toxic effects of arsenic**

Arsenate has the potency to replace phosphate groups from many biochemical reactions because they have similar structure and properties as that of the corresponding biomolecule\(^46\). Specific functional groups within enzymes, receptors or coenzymes, such as thiols or vicinal sulfhydryls, have a major role in metabolism. Trivalent arsenicals readily react with thiol-containing molecules such as GSH and cysteine and hinder its function\(^48, 47\). Binding of MMA and DMA to protein occurs to a greater extent in comparison with the pentavalent form\(^49\) of arsenic. The binding of trivalent arsenic to critical thiol groups may inhibit important biochemical events which could lead to toxicity. Methylated trivalent arsenicals such as MMA are potent inhibitors of GSH reductase\(^50\) and thioredoxin reductase\(^51\). Besides replacing functional groups from the metabolic enzymes the principle reason behind arsenic toxicity in involvement of oxidative stress.
**Arsenic-induced oxidative stress is one of the major mechanisms of arsenic toxicity:**

Oxidative stress is defined as a biochemical condition that is characterized by the imbalance between free radicals and the anti-oxidative defense mechanisms\(^{52}\). Reactive Oxygen Species (ROS) are products of cellular metabolism which plays a vital role in stimulating various signaling pathways both in plants and animals. Superoxide anion (O\(_2^–\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radical (OH) and organic peroxides are normal products of the biological reduction of molecular oxygen\(^{53}\). Arsenic, during its sequential reduction from trivalent to pentavalent state, interferes with the functional group of critical enzymes and disrupts the important biochemical reactions which thereby aid in ROS generation\(^{54}\). Prolonged and sustained ROS can cause a significant amount of damage to cell structure and function and may induce somatic mutation and neoplastic transformation\(^{55}\). ROS are involved in a wide spectrum of diseases including chronic inflammation, and in the initiation and progression of various kinds of cancers\(^{56}\).

Against those pro-oxidative reactions, biological defense systems exist in cells which include both enzyme-based and non-enzyme-based systems\(^{57}\). The enzyme-based defense system includes copper-zinc superoxide (Cu-Zn SOD), glutathione peroxidase (GPx) and catalase (CAT) whereas the non-enzymatic defense system of the cell includes ascorbic acid (vitamin C), α-tocopherol, glutathione (GSH), β-carotene and vitamin A. Super oxide dismutase (SOD) converts superoxide anion (O\(_2^–\)), to hydrogen peroxide (H\(_2\)O\(_2\)). The generated H\(_2\)O\(_2\) is then converted to water and oxygen by glutathione peroxidase (GPx) and catalase (CAT) (maydani 2001). Since most of the oxidative methylation and biotransformation of arsenic occurs in liver\(^{58}\), the tendency of generation and accumulation of free radicals in hepatocytes is highest.
**Figure R5: Arsenic participation in generation of free radicals**

*The intricate relations between oxidative stress and inflammation:* Oxidative stress and inflammation may be referred to as two sides of the same coin. Studies to decipher the role of oxidants in inducing inflammation have been conducted earlier, but the question how they are interlinked remains largely unresolved. Most of the studies ended with a conclusive note that inflammation and oxidative stress are two parallel phenomena, which have a summated adverse effect on the physiological system. Inflammatory stimulus initiates ROS-mediated activation of signaling pathways. ROS acts both as a signaling molecule and a mediator of inflammation. For instance, superoxide can readily bind with reactive nitrogen species (RNS) which in turn induces nitrosative stress and adds to the pro-inflammatory burden of the cell.
In this way both inflammation and oxidative stress goes hand in hand to create an inflammatory milieu. Elaborate background studies provide evidence that strongly suggest that arsenic has a significant role in eliciting both oxidative stress and inflammation\(^59\).

**Metabolism of arsenic in liver**

In the liver, the metabolism of arsenic involves enzymatic and non-enzymatic methylation. The most frequently excreted metabolite (\(\geq 90\%\)) in the urine of mammals is dimethylarsinic acid (DMA\(\text{V}\))\(^60\). In humans inorganic arsenic is reduced non-enzymatically from pentoxide to trioxide, using glutathione (GSH) or GST. Reduction of arsenic pentoxide to arsenic trioxide increases its toxicity and bioavailability. Methylation occurs through methyltransferase enzymes. S-adenosylmethionine (SAM) may serve as methyl donor\(^61\). Resulting metabolites are monomethylarsonious acid (MMA\(\text{III}\)) and dimethylarsinous acid (DMA\(\text{III}\)). Methylation has been regarded as a detoxification process. While in fact reduction from +5 to +3 arsenic may be considered as bio-activation\(^62\). Arsenite inhibits members of the disulfide oxidoreductase family like glutathione reductase and thioredoxin reductase\(^62\). The remaining unbound arsenic (\(\leq 10\%\)) accumulates in cells, which over time may lead to skin, bladder, kidney, liver, lung, and prostate cancers\(^60\). Other forms of arsenic toxicity in humans have been observed in blood, bone marrow, cardiac, CNS, GI, gonadal, kidney, liver, pancreatic, and skin tissues.
Medicinal use of arsenic derivatives

Medicinal use of arsenic and its derivatives dates back more than 2400 years to ancient Greece and Rome. Arsenic was viewed as both a therapeutic agent and a poison\textsuperscript{64}. Hippocrates administered orpiment (As\textsubscript{2}S\textsubscript{3}) and realgar (As\textsubscript{2}S\textsubscript{2}) as an ulcer remedy; Dioscorides used orpiment as a depilatory. Arsenic has also been used to treat the plague, malaria, and cancer and to promote sweating. Physicians prescribed arsenic for both external and internal use throughout the 18th century. Arsenides and arsenic salts were key ingredients in antiseptics, antispasmodics, antiperiodics, caustics, cholagogues, hematinics, sedatives, and tonics\textsuperscript{65}. Approximately 60 different arsenic preparations have been developed and distributed during the lengthy history of this agent. More than 20 of these preparations were still in use at the end of the 19th century, including Aiken’s Tonic Pills, Andrew’s Tonic, and Arsenauro\textsuperscript{66}.

Although arsenic was found to be beneficial in many disease states and side effects or later repercussions of therapy were inconsistent, concerns among medical professionals about toxicities associated with arsenic use, especially long-term use, surfaced in later years. The IARC first evaluated the carcinogenicity of arsenic and arsenic compounds in 1973. It found
a “causal relationship between skin cancer and heavy exposure to inorganic arsenic in drugs, drinking water with a high arsenic content, or in the occupational environment.” However, experimental studies with arsenic in animals were considered inadequate, and the causative role of arsenic remained largely unclear.

**Arsenic use in cancer treatment**

Arsenic has been reported to have a therapeutic effect on acute pro-myelocytic leukemia (APL). But arsenic treatment showed several side effects like increased AST, ALT levels, high leukocyte count, hemorrhage of teeth nose and skin. Arsenic showed a preferential selectivity for malignant cells both clinically and in radioactive tracer studies. Much of the recent decline in the medical use of arsenic (other than limited use in parasitic infections) can be attributed to concerns about its toxicity and potential for carcinogenicity, particularly skin cancer. Environmentalists refer to arsenic as the number one carcinogen. In 1979, the International Agency for Research on Cancer introduced an overall classification system for carcinogens and placed arsenic and certain arsenic compounds in Group 1, agents that are carcinogenic to humans.

**Liver: The arsenic-target**

Among the various internal organs affected by chronic exposure to arsenic in humans, liver is one of the most important targets. Epidemiological studies have shown association between chronic arsenic exposure and liver disease including hepatomegaly, hepatoporal sclerosis, liver fibrosis and cirrhosis of liver. Abnormal liver functions as manifested by severe gastrointestinal problems and clinical elevations of liver enzymes in plasma including alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) also are associated with chronic arsenic exposure. Exposure of mice to arsenic in drinking water causes elevation of liver enzymes in plasma and capillarization of liver.
sinusoidal endothelium\textsuperscript{72}. In fact, liver is the major site of arsenic metabolism\textsuperscript{73} and hence arsenic exposure causes liver disease in exposed humans\textsuperscript{70}.

**Figure R7:** Progression of liver disease due to arsenic exposure

**Arsenic and the immune response**

**Figure R8:** Immune organs of the human body
Clinical investigation has demonstrated that inorganic arsenic induces an abnormal inflammatory response associated with hypertrophy of organs. Reports indicate that arsenic can have effects on certain aspects of the immune system which includes suppression of contact hypersensitivity responses\textsuperscript{74}, increased interleukin-1α (IL-1α) expression\textsuperscript{75}, loss of adhesion, impairment of function, and morphologic changes in human macrophages\textsuperscript{76, 77}. Sodium arsenite present in drinking water has been correlated with a suppression of human peripheral blood lymphocyte activation and responses\textsuperscript{78}. Stress of various types has been proposed as one of the mechanisms for arsenic-induced tissue damage and cell death\textsuperscript{79, 80}. Arsenic can potentially produce stress, either directly or indirectly through uncoupling of mitochondrial oxidative phosphorylation, and/or increased cellular production of H\textsubscript{2}O\textsubscript{2}\textsuperscript{80, 81}. However, the capability of arsenic to modulate stress-related gene expression in intact animals is unknown.

Arsenic has been identified as an immunomodulatory agent in certain experimental models and epidemiologic studies\textsuperscript{82}. It was recently reported that chronic low-dose arsenic exposure can profoundly alter the gene and protein expression of many regulators of the innate immune system in a mouse model of exposure\textsuperscript{83}. Arsenic trioxide has been shown to suppress the activity of human myeloid and mononuclear cells and is being developed as a treatment for myelodysplastic syndromes and certain leukemias\textsuperscript{84}. There have been few studies on the immunotoxicity of arsenic following inhalation exposures\textsuperscript{27}. Sporadic reports indicate that arsenic can have significant effects on certain aspects of the immune system in experimental systems, like, suppression of contact hypersensitivity responses\textsuperscript{74}, altered expression of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF)\textsuperscript{85}, loss of adhesion, impairment of function, and morphologic changes in human macrophages\textsuperscript{77}. 
The mechanisms of arsenic toxicity on the immune system are still not clear. In the present study we have analyzed the differential role of arsenic in modulating a pro versus anti-inflammatory effect.

**Signaling pathways in arsenic toxicity**

Mammalian cells cope with oxidative insult by adapting anti-oxidant defense signaling mechanisms to neutralize ROS\(^{16}\). This adaptive antioxidant response is mediated by activation of NF-E2 related factor (Nrf2) through the antioxidant response element (ARE). This would indicate that such compounds that can elicit Nrf2-dependent adaptive responses may confer protection against arsenic intoxication. Polyphenolic compounds like curcumin, resveratrol, epigallocatechin gallate, ellagic acid etc., are reported to have anti-oxidant properties and have received considerable attention in this regard. Demerdash et al.\(^{86}\) have reported that curcumin can mitigate arsenic intoxication in rats. The most attractive chemopreventive compounds are those that potentially induce the Nrf2- dependent defensive response without eliciting toxic effects, that is, those that tip the balance toward the Nrf2-dependent beneficial response. Nrf2 activators identified so far include phenolic antioxidants (caffeic acid, epigallocatechin-3-gallate, butylated hydroxyanisole etc.)\(^{87}\). Up-regulation of the Nrf2-dependent defense response has proved to be beneficial in reducing arsenic-induced toxicity in a urinary bladder cell culture model\(^{88}\). Pre-treatment with chemicals that activate Nrf2 has been reported to enhance cellular resistance to arsenic-induced cell death\(^{89}\).
Nuclear factor-κB is a known cellular stress marker. In quiescent cells, the NF-κB dimers are sequestered in the cytoplasm by a family of inhibitors, called IκBs (Inhibitor of κB) the IκB proteins mask the nuclear localization signals of NF-κB proteins and keep them sequestered in an inactive state in the cytoplasm. Activation of NF-κB is initiated by signal-induced degradation of IκB proteins. This occurs primarily via activation of a kinase called the IκB kinase (IKK). There appears to be a significant role of NF-κB in arsenic induced cellular stress and apoptosis sup. A direct role of the NF-κB pathway in arsenic-induced apoptosis was reported in a study in which transient over-expression of NFκB–p65 in L540Cy Hodgkin/Reed-Sternberg cells protected them from arsenic-induced apoptosis.  

Figure R9: Oxidative stress management
A promising strategy in reversing damages caused by oxidative stress may be the use of antioxidant therapy. Diet is known to play important role in developing as well as ameliorating chronic diseases. Diets rich in fruits, vegetables, and pulses which are rich in polyphenols and possess greater antioxidant properties also reduce the risk of development of chronic inflammatory diseases like diabetes, coronary heart disease, obesity, hypertension etc.

**Antioxidants: Potential players in reversing arsenic toxicity**

Antioxidants are substances that interact with free radicals and neutralize them from causing cell damages. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Vegetables and fruits are rich sources of antioxidants. There is good evidence that intake of a diet with lots of vegetables and fruits are healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors. The body makes some of the antioxidants to neutralize the free radicals. These antioxidants are
called endogenous antioxidants\textsuperscript{94}. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements\textsuperscript{95}. Examples of dietary antioxidants includes beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol).

**Classification of antioxidants**

Kinetically antioxidants can be classified into six categories as below\textsuperscript{96}:

1. **Antioxidants that break chains by reacting with peroxylradicals having weak O-H or N-H bonds**: phenol, napthol, hydroquinone, aromatic amines and aminophenols
2. **Antioxidants that break chains by reacting with alkylradicals**: quinones, nitrones, iminoquinones
3. **Hydro peroxide decomposing antioxidants**: sulphide, phosphide, thiophosphate.
4. **Metal deactivating antioxidants**: diamines, hydroxyl acids and bifunctional compounds.
5. **Cyclic chain termination by antioxidants**: aromatic amines, nitroxyl radical, variable valence metal compounds.
6. **Synergism of action of several antioxidants**: phenol sulphide in which phenolic group reacts with peroxyl radical and sulphide group with hydro peroxide.

Some of the popularly known natural products which contains huge amount of antioxidants as well as great therapeutic values include Pomegranate, Green tea, Curcumin, moringa,
theaflavin, resveratrol, amla, honey and many more. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidant. Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

![Figure R11: Protective role of polyphenols against several diseases](image)

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidant. Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

**Bioavailability of Polyphenols**

Bioavailability is the proportion of the nutrient that is digested, absorbed and metabolized through normal pathways. Bioavailability of each and every polyphenol differs however...
there is no relation between the quantity of polyphenols in food and their bioavailability in human body. Generally, aglycones can be absorbed from the small intestine; however most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in native form\(^{100}\). Before absorption, these compounds must be hydrolyzed by intestinal enzymes or by colonic microflora\(^{101}\). During the course of the absorption, polyphenols undergo extensive modification; in fact they are conjugated in the intestinal cells and later in the liver by methylation, sulfation and/or glucuronidation\(^{102}\). Importantly it is the chemical structure of polyphenols and not its concentration that determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma\(^{103}\).

Polyphenols influence the metabolism of pro-carcinogens by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens. They may also facilitate their excretion by increasing the expression of phase II conjugating enzymes. This induction of phase II enzymes may have its origin in the toxicity of polyphenols. Polyphenols can form potentially toxic quinones in the body that are, themselves, substrates of these enzymes. The intake of polyphenols could then activate these enzymes for their own detoxification and, thus, induce a general boosting of our defenses against toxic xenobiotics\(^{24}\).

**Pomegranate: A functional food**

Pomegranate belongs to the family Punicaceae. It is native from the area of Iran to the Himalayas in northern India, and has been cultivated and naturalized over the entire Mediterranean region since ancient times\(^{104}\). Actually, pomegranate is widely cultivated throughout Iran, India, Mediterranean countries, the drier parts of Southeast Asia,
Malaysia, the East Indies, and tropical Africa and, to some extent, in the United States (drier parts of California and Arizona), China, Japan, and Russia\textsuperscript{105}. Pomegranate is rich in polyphenols, major among them being anthocyanins and hydrolysable tannins. Pomegranate is considered as a functional food because the compounds present in different parts of pomegranate can play important role in ameliorating several diseases\textsuperscript{25}. The anthocyanins and hydrolysable tannins present in pomegranate can act as effective anti-oxidative\textsuperscript{106}, anti-tumor \textsuperscript{92} and anti-hepatotoxic\textsuperscript{107, 21} agents and can also improve cardiovascular disease conditions\textsuperscript{108}. They are also reported to have anti-microbial\textsuperscript{109} anti-inflammatory\textsuperscript{59} and anti-diabetic\textsuperscript{110} activities. The determination of the antioxidant capacity of pomegranate components and their derivatives is being given greater importance by researchers and those involved in the agro-food industry for use as natural additives to replace synthetic antioxidants. The action mechanism set in motion by the antioxidant activity of these compounds is still not clearly understood, although it is a known fact that antioxidant mechanisms involved in biological matrixes are quite complex and several different factors may play a role\textsuperscript{106}. Since the phenolic content of Pomegranate differs from its degree of ripening and change in cultivar there is a high possibility of difference in phenolic content among pomegranate fruits. Therefore we selected POMx for our experimental studies. A comparative study done in our laboratory between pomegranate fruit extract and POMx showed that it provides a consistent protection against free radical generation and has a similar reducing power as that of pomegranate.

**Reported investigations with pomegranate fruit extract**

POMx is a popular beverage produced from pomegranate fruit grown in California by Paramount Farms. Hong et al\textsuperscript{111} reported that polyphenols present in pomegranate juice possessed the capacity of arresting proliferation and induction of apoptosis in human androgen-dependent and -independent prostate cancer cells. Shukla et al. \textsuperscript{112} reported that
supplementation of POMx reduce joint inflammation and suppress the damaging effect of IL-β and TNF-α in arthritis and other disease associated with abnormal production of cytokines. Rasheed et al. 26 in a similar kind of study reported the therapeutic use of POMx derived compounds for the treatment of inflammatory disease by suppressing mast cells/basophil activation. A study by Rettig et al 113 proposes that POMx administration prevent the emergence of androgen-independent prostate cancer growth through a nuclear factor-kappa-B dependent mechanism. Patel et al114 did an interesting study to investigate if there is any adverse effect of consuming a standardized pomegranate beverage in sub-chronic doses. It was observed that 600 mg/kg/body weight did not show any observable changes. Khan et al.115 examined the potential role of oral consumption of pomegranate fruit extract on cell growth, progression, angiogenesis and signaling pathways in lung cancer cells. Their investigation showed that PFE significantly inhibited lung tumorigenesis and reported the chemopreventive action of pomegranate. The anti-proliferative role of pomegranate against human prostate cancer cell was also reported by Malik et al. 116. Another study by Malik et al. 117 suggest that PFE can modulate cyclin dependent kinase machinery which resulted in inhibition of cell growth followed by apoptosis. In all these studies a crucial role of oxidative stress and transcription factors are observed which indicates the putative role of natural polyphenols in cell proliferation, differentiation and cell death. Evidence of pomegranate as an anti-inflammatory and chemopreventive agent provided us an interesting aspect to investigate the anti-hepatotoxic effect of pomegranate and establishes its therapeutic efficacy against arsenic intoxication.
Apoptosis (programmed cell death) is a normal phenomenon during the process of development. Apoptosis helps in maintaining homeostatic mechanism among the cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. Agents that have the ability to modulate the life or death of a cell are highly appreciated for their immense therapeutic potential.

A wide variety of stimuli and conditions, both physiological and pathological, can trigger apoptosis. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through a p53-dependent pathway. Apoptosis involves the activation of a group of cysteine proteases called “caspases” and a complex cascade of events that link the initiating stimuli to the final demise of the cell. Generally two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway are activated for execution of apoptotic pathway. However, there is now evidence that the two pathways are linked and that molecules in one pathway can influence the other. The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions whereas the intrinsic signaling pathways that initiate apoptosis involve a diverse array of non-receptor-mediated stimuli that produce
intracellular signals that act directly on targets within the cell and are mitochondrial-initiated events.

The control and regulation of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins\textsuperscript{120}. The tumor suppressor protein \textit{p53} has a critical role in regulation of the Bcl-2 family of proteins; however the exact mechanisms have not yet been completely elucidated\textsuperscript{121}. The Bcl-2 family of proteins governs mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic. Till date, a total of 25 genes have been identified in the Bcl-2 family. Some of the anti-apoptotic proteins include Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. These proteins have special significance since they can determine if the cell commits to apoptosis or aborts the process. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome \textit{c} release from the mitochondria via alteration of mitochondrial membrane permeability.

![Image of intrinsic and extrinsic pathways of apoptosis](image)

\textbf{Figure R13: Intrinsic and extrinsic pathways of apoptosis}
The extrinsic and intrinsic pathways both end at the point of the execution phase, considered the final pathway of apoptosis. It is the activation of the execution caspases that begins this phase of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, caspase-6, and caspase-7 function as effector or “executioner” caspases, cleaving various substrates including cytokeratins, PARP, the plasma membrane cytoskeletal protein alpha fodrin, the nuclear protein NuMA and others, that ultimately cause the morphological and biochemical changes seen in apoptotic cells\textsuperscript{122}. Apoptosis has to be tightly regulated since too little or too much cell death may lead to pathology, including developmental defects, autoimmune diseases, neurodegeneration, or cancer. Tumor cells can acquire resistance to apoptosis by the expression of anti-apoptotic proteins such as Bcl-2 or by the down-regulation or mutation of pro-apoptotic proteins such as Bax. The expression of both Bcl-2 and Bax is regulated by the p53 tumor suppressor gene\textsuperscript{123}. Certain forms of human B cell lymphoma have overexpression of Bcl-2, and this is one of the first and strongest lines of evidence that failure of cell death contributes to cancer\textsuperscript{124}.

Advances in understanding of the control of apoptosis at the molecular level have extended its potential oncologic significance far beyond the mere provision of a mechanistic explanation for tumor cell deletion. In particular, the discovery that apoptosis can be regulated by the products of certain proto-oncogenes and the p53 tumor suppressor has opened up exciting avenues for future research. A variety of anti-cancer drugs have been shown to induce extensive apoptosis in rapidly proliferating normal cell populations, lymphoid tissues, and tumors. Enhanced apoptosis is responsible for many of the adverse effects of chemotherapy and for tumor regression\textsuperscript{125}. 
Effect of apoptosis on micro RNAs

The p53 protein is a sequence-specific transcription factor. Various cellular stresses, such as DNA damage, hypoxia, and nutrient deprivation, can activate p53 function via effects on p53 stability and nuclear localization and its interactions with other proteins, resulting ultimately in p53-stimulated transcription of perhaps a hundred or more genes. Based on the analysis of the results of a published genome-wide chromatin immunoprecipitation (ChIP) study from\textsuperscript{126}, it was noted that p53 binding region lies within 30 kb of the precursor transcription units for both miRNA34a and miRNA34bc. The transcriptional activity of p53 can be readily induced in cells by exposure to genotoxic stress, such as the chemotherapeutic agent adriamycin. This interesting finding has prompted us to find the relation between arsenic exposure apoptosis and miR-34a levels which may evolve as a key biomarker against toxicity. MicroRNAs are an abundant class of endogenous non-coding RNAs that are widely expressed in mammalian cells and are important in post-translational gene regulation, including cellular proliferation, differentiation and apoptosis\textsuperscript{127}. Functional studies have reported that the expression pattern of these microRNAs change in various human diseases including cancer\textsuperscript{128}. miRNAs are initially parts of primary transcripts (pri-miRNA) that are transcribed to 60-100 nucleotide long hairpin precursor RNA (pre-miRNA) by DROSHA, RNAase enzyme. This pre-miRNA is transported to the cytoplasm by a transporting factor known as exportin 5. In the cytoplasm the pri-miRNA is further excised by a DICER and unwound by a putative helicase to produce miRNA of size \~18-24 nucleotides. Mature miRNAs become part of RNA-induced silencing complex (RISC) that facilitates miRNA-induced gene silencing and thereby modulate post transcriptional regulation through base pairing between the 3’ untranslated region of the target mRNA. Binding of miRNA can either induce or reduce the translational rate or mRNA degradation\textsuperscript{129,130}. 
Though the biological functioning of miRNAs are not fully explained, it has been shown that the tissue levels of several miRNA correlate well with the pathological development of different cancers\textsuperscript{131}. Each miRNA might have multiple target genes and several miRNAs often regulate the same gene.

![Figure R14: MiR-34a functionality](image)

**Green Tea (GT) and Resveratrol (Res): Rich antioxidants**

![Figure R15: Green tea leaves and resveratrol](image)

Epidemiologic and preclinical evidence suggests that certain types of polyphenolic phytochemicals possess cancer chemopreventive properties and thus might be useful for clinical chemoprevention trials\textsuperscript{132, 133}. Among these phytochemicals, green tea catechins have
been shown to inhibit carcinogenesis or the growth of chemically induced cancers in various tissues, including the colorectum\textsuperscript{134}. Tea is a popular beverage worldwide because of its characteristic aroma, flavor and health benefits. Of the total commercial tea production worldwide about 80\% is consumed in the form of black tea and 20\% in the form of green tea\textsuperscript{135}. The characteristic aroma and health benefits of green tea are associated with the presence of epicatechin derivatives which has huge antioxidative property. Green tea is manufactured by steaming fresh tea leaves and drying them at elevated temperatures with care to avoid oxidation and polymerization of polyphenolic compounds\textsuperscript{135}. Animal studies have suggested that tea consumption suppress formation and growth of human cancer\textsuperscript{135}. Among epicatechin derivatives, EGCG has been most extensively examined because of its relative abundance and strong cancer preventive properties. Similar to green tea, resveratrol is a naturally occurring polyphenols phytoalexin that is present in grapes and several other common foodstuffs\textsuperscript{136}. Resveratrol demonstrates a broad range of potentially desirable biological activities including cancer prevention. In addition to its cancer prevention resveratrol confers significant protection against cancer induction in the skin, mammary gland, prostrate liver and colon\textsuperscript{137}. Resveratrol is the principal compound present in red wine. Concentration of resveratrol in wine varies from 0.2 and 5.8 mg/L depending upon the grape variety while white wine contains 0.68 mg/L of resveratrol\textsuperscript{138}. Red wine is extracted with skin intact grapes whereas white wine is fermented after removal of skin. Red wine has six times more trans-resveratrol than white wines while white wines have high concentrations of cis-resveratrol\textsuperscript{139}. Phytochemicals might be considered as ideal molecules due to their relatively low toxicity and capacity to target multiple signaling pathways which collectively influence cell survival and growth. In this study we have assessed the potential protective role of three popular dietary polyphenols against arsenic-induced toxicity.