General Introduction
Metals have been used by man since antiquity. Anthropogenic uses and exploitation of the reserves have led to global dispersion of metals in the environment. The most notable environmental metals that pose a threat to public health are lead, arsenic, chromium, cadmium and mercury. Among the other naturally occurring heavy metals, arsenic is classified as metalloid because arsenic has both metallic and non-metallic properties and has also been denoted as the most notorious one. In nature, arsenic occurs in three valance state (i.e., -3, +3, +5). The trivalent arsenic (As3+) and the pentavalent arsenic (As5+) are widely present in natural waters and are soluble over a wide range of pH. The physico-chemical property of arsenic increases the level of difficulty in its separation. The removal technology of arsenic and disposal of arsenic-bearing waste, presents a challenging task to the environmental engineers. Unlike organic waste, inorganic arsenic cannot be degraded biologically to harmless products. The chemistry of arsenic is quite complex, which also adds to the problem of its segregation from water. Arsenic can be stable in four oxidation states, the arsenic states are interchangeable and thus its removal is largely dependent on pH of the medium, oxidation state and redox potential.

Over the centuries, arsenic has been used for a variety of purposes. Arsenic was a constituent in cosmetics, and was used more extensively than at present in agriculture to protect crops from pests. Arsenic as copper acetoarsenite was used as a pigment in paints, the best known being “Paris green”. Arsenic present in Paris green used to react with hydrogen liberated from coal fires and from gas for lighting, to form arsine, a toxic gas. A fungus Scopulariopsis breviculis present in damp wallpaper also metabolized the arsenic in Paris green to arsine. Inhalation of arsine caused toxic consequences on humans.

Arsenic contamination in drinking water has a detrimental impact on human health which profoundly impairs the quality of life. To the inhabitants of arsenic-endemic areas, arsenic intake mainly occurs through contaminated drinking water. The mischievous physical
properties of arsenic have attracted the attention of scientific fraternity to delve into it and find a strategy to counter the problem. When dissolved in water, arsenic has no color, no odor and no taste. Moreover it exists in water at even near neutral pH; thus unknowing intake of arsenic-water produces a long term effect.

![Figure I.1: Route of exposure of arsenic in humans and animals](image)

Millions of people around the world are exposed to arsenic contaminated drinking water, of which, the worst situation is observed in the Bengal delta plain. The three mighty rivers that are flowing in this region have their origin in Himalayas and bring a lot of alluvial sediments and minerals with them to be deposited in this region. Over the period of time these deposits have grown in mass and made a significant contribution towards ground water contamination with hazardous elements.

Another significant reason behind increased arsenic contamination of drinking water is installation of deep tube wells. In 1961, outbreak of cholera in the adjoining regions of
Bengal and Bangladesh at regular intervals took a toll of over thousands of lives\textsuperscript{10}. With a view to provide safe drinking water, about 4.5 million shallow and deep tube wells were randomly installed around the region. This installation program mitigated the casualties caused due to cholera but left the population endangered with another severe problem of arsenic intoxication. By 1983, the first report of arsenic was published and it was found that all deep tube wells were tampering the bedrock and bringing up the arsenic to contaminate drinking water\textsuperscript{11}.

A current statistical survey in West Bengal and Bangladesh showed that 9 out of 18 districts of West Bengal are having elevated concentrations of arsenic and 42 districts in Southern Bangladesh are reported to have arsenic contamination\textsuperscript{12}. To date, there has been a paucity of conducted studies to develop suitable programs to limit arsenic contamination, and the problem of accessing safe drinking water still persists.

Arsenic damages biological systems through multiple mechanisms, one of them being generation of reactive oxygen species\textsuperscript{13} (ROS). Experimental studies have suggested that arsenic increases the production of reactive oxygen species such as hydrogen peroxide\textsuperscript{14}, hydroxyl radicals\textsuperscript{15}. The antioxidant response is the major defense mechanism in vertebrates.
used to neutralize ROS elicited by toxic exposure and maintain cellular redox homeostasis\textsuperscript{16}. The induction of oxidative stress by arsenic may in turn mediate abnormal gene expression and inflammatory responses\textsuperscript{17}.

A close relationship has been noted between ROS generation and the inflammatory process\textsuperscript{18}. Although the specific events by which oxidants contribute to inflammation are not entirely elucidated, potential mechanisms include the activation of cyclooxygenage-2 (COX-2) and the transcription factor nuclear factor-kappa B (NF-κB) by pro-oxidants, thereby resulting in the initiation of expression of genes controlling several aspects of the inflammatory, immune and acute phase responses\textsuperscript{19}.

Despite years of scientific research, we are far away of providing an effective therapy against arsenic-related maladies. In recent years, worldwide attention is focused on the potential of dietary antioxidants in reducing free radical-induced cellular impairment during different stress types\textsuperscript{20}. In this context, we decided to examine the prospective of pomegranate fruit extract against arsenic contamination. From an earlier investigation done in our laboratory, it was established that pomegranate polyphenols are of huge health benefits and possess antioxidant properties which are capable of protecting hepatocytes from methotrexate (MTX) induced hepatotoxicity\textsuperscript{21}. MTX is an anti-folate drug that is extensively used in the treatment of cancer, autoimmune and inflammatory diseases\textsuperscript{22}. The use of this drug was highly restricted due to associated hepatotoxicity. Considering the findings of the mentioned study, we were interested to investigate the potency of pomegranate in ameliorating arsenic-induced hepatotoxicity.

From literature study it was apparent that oxidative stress lies at the heart of initiating arsenic-induced health consequences\textsuperscript{23}. Based on this information we hypothesized that supplementation of a diet rich in antioxidants that has the capability to scavenge free radicals could probably rescue cells from arsenic-toxicity.
Pomegranate can be categorized as a functional fruit and is cultivated over a wide region of Iran, India, Mediterranean countries, and drier parts of Southeast Asia, Malaysia and even in the United States. The principal components of pomegranate are flavonoids, ellagitannins, phenolics and pro-anthocyanidin compounds. Anthocyanins are the most important group of flavonoids present in pomegranate. Flavonoids are typically phenolic compounds and therefore act as potent metal chelators and free radical scavengers. The phenolic content of pomegranate varies depending on the region of cultivation, ripening time and season.

In the present study we have used a standardized extract of pomegranate fruit grown in California, POMx; to maintain experimental consistency. POMx includes the extracts of both skin and the fruit. HPLC analysis of the extract reports that it has 37-40% punicalagin and 3-4% free ellagic acid. Moreover in a comparative estimation of indigenous pomegranate fruit and POMx in our lab, it was found that POMx has a more potent action in scavenging nitric oxide and superoxide radical (Table 1). 5 ml of POMx extract contained 1000 mg of total phenolics. Data obtained by comparing the efficacy of indigenous pomegranate and POMx for an array of oxidative stress reducing parameters is presented in the table below.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>POMx</th>
<th>Indigenous PFE</th>
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<tbody>
<tr>
<td>1. Total polyphenolic content (Ellagic acid equivalents) (mg/ml)</td>
<td>4.89±3.7</td>
<td>2.41±2.4</td>
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<td>2. DPPH radical scavenging power (% with respect to control)</td>
<td>55±1.36</td>
<td>50±3.04</td>
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<td>3. Nitric oxide radical scavenging activity (% with respect to control)</td>
<td>49±2.66</td>
<td>44.31±1.57</td>
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<td>4. Superoxide radical scavenging activity (% with respect to control)</td>
<td>57.12±1.43</td>
<td>45.06±2.59</td>
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<td>5. H₂O₂ scavenging activity (% with respect to control)</td>
<td>53.79±3.04</td>
<td>47.09±1.94</td>
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<tr>
<td>6. Hydroxyl radical scavenging activity (% with respect to control)</td>
<td>59.07±1.94</td>
<td>51.7±2.19</td>
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Table 1: Comparison of the efficacy of POMx with indigenous pomegranate

Adverse health effects of arsenic largely depend on the dose of arsenic. Lack of exposure response relationship at low doses of arsenic prompted us to undertake this study. In this study, we selected a range of arsenic doses to have a full toxic account of the pollutant. The lowest dose selected in this study (0.01 mg/L) is the permissible dose recommend by WHO. The other two doses were the fivefold (0.05 mg/L) and tenfold (0.1 mg/L) of the recommended dose. Our study for the first time reports the pathogenesis of liver diseases at environmentally relevant doses.

Arsenic is associated with multitude of health effects. Besides liver disease, arsenic is also reported as an immunotoxic agent. In the present study we were particularly interested to find the impact of arsenic on host immune response at environmentally relevant doses. Innate immune response forms the front line of host defense mechanism and is the prerequisite for
potentiating adaptive response. Dampening of innate immune system disrupts immune homeostasis. In this study we have explored how arsenic exposure alters immune regulation and establishes a situation of immune incompetence.

The thymus is the primary site of T-cell lymphopoiesis during fetal life and early childhood. This period of thymic development is a critical window for developing proper immune function\textsuperscript{28}. The fetal thymus starts to produce T cells prior to mid gestation and this function is almost fully developed at birth\textsuperscript{29}. Human data, however, are scarce. Ahmed et al\textsuperscript{29} report that maternal exposure to arsenic through drinking water during pregnancy is associated with reduced thymic size in infancy. This may have serious consequences as a small thymic size at birth has been associated with increased risk of infection and mortality in infancy\textsuperscript{30}. An arsenic-related increase in infant morbidity has been observed, especially in patients of lower respiratory tract infection and diarrhea\textsuperscript{31}. The few previous epidemiological studies indicating adverse effects of arsenic exposure on the immune system, in particular immunosuppression, involved adults and children 6–10 years of age\textsuperscript{32}. In the present study we evaluated the relation between environmental dose responses of arsenic to immune function.

There are copious reports providing evidence that intake of polyphenols can protect against oxidative stress-induced damage to cellular constituents. For the execution of our study, we selected Swiss albino mice for developing the model of arsenic toxicity. The mice were exposed to arsenic through drinking water since oral route is the predominant way of arsenic exposure to humans. In parallel groups we co-administered pomegranate fruit extract along with arsenic exposure. We hypothesized that the polyphenolic content of pomegranate would possibly increase the antioxidant capacity of the host, which would increase the capability of eliminating/neutralizing arsenic-toxicity by the endogenous detoxification system. In this study we have examined only the efficacy of pomegranate against hepatotoxicity as liver is
most widely reported to be effected due to arsenic intake. The main motto behind evaluating immune aspects in this study is to evaluate the response of immune system after arsenic exposure and its role in progression of organ injury.

At the end of this study we did a comparative study between three dietary polyphenols (pomegranate, green tea, resveratrol) to determine their individual potency against reversal of arsenic induced hepatotoxicity. This study provides us with a close insight of the molecular event underlying arsenic toxicity and how dietary supplements impart protection against arsenic-induced hepatotoxicity.

The study was divided into three phases. The first phase of study confirms arsenic-induced toxicity both in liver and thymus. Subsequent to this finding, we analyzed the core molecular mechanisms behind how arsenic causes immune incompetence in the host and debars the repair mechanism. In the second phase we analyzed the anti-apoptotic, anti-hepatotoxic and anti-inflammatory role of pomegranate against arsenic induced hepatotoxicity. Positive outcomes of the selected polyphenols compelled us to screen two more dietary antioxidants in the third phase that might act as an antidote against hepatotoxicity.

The novelty of this work lies in the fact that we have evaluated the stress responsive pathways and cell death pathways closely to have a close view of the molecular happenings after arsenic exposure. We provided an array of phytochemicals that can effectively reverse arsenic-induced hepatotoxicity. Our data definitely forms a primary step towards more vigorous investigation of finding alternative ways of reducing arsenic ill effects.