ABSTRACT

EXPLORATION OF THERAPEUTIC EFFICACY OF POMEGRANATE IN REDUCING ARSENIC INDUCED OXIDATIVE STRESS, INFLAMMATION AND CELL DEATH

Pathological complications arising due to arsenic poisoning have become an alarming global plight. Countries like India, Argentina, and Bangladesh are under constant threat of the health adversities caused by the high content of inorganic arsenic present in the drinking water available in these areas. With no imminent solution for prevention or cure against the systemic damage resulting from arsenic consumption, the onus lies on the scientific community to unravel the mechanism of arsenic toxicity and put forward a widely accessible prevention option to help combat this social menace. Thus, in this study we have used both in vitro and in vivo models of arsenic exposure to simulate real time situations in order to delineate molecular changes associated with arsenic-mediated hepatic damage. We have also done a comparative analysis of popular polyphenols and have shown that pomegranate fruit extract (PFE) is most efficient in rescuing arsenic-induced hepatotoxicity.

To replicate environmental scenarios of chronic arsenic contamination, we administered doses of arsenic considered safe by WHO (0.01 mg/L) and five-fold and ten-fold higher concentrations to male Swiss albino mice for 30 days through drinking water. To understand why the host immune system fails to salvage arsenic-induced liver damage, we examined the effect of arsenic on thymus and observed progressive thymic dysfunction leading to organ injury. We found that arsenic modulates the IL-6/Stat-3/Foxp3 axis in thymocytes in a dose-dependent manner to fabricate an immune-suppressive milieu.

In addition we also provided PFE supplementation to another set of animals along with the mentioned arsenic doses. We analyzed activities of an array of enzymes like AST, ALT LDH etc both in serum and liver to ascertain that arsenic causes systemic damage and liver dysfunction. We also observed that PFE could mitigate such changes. Arsenic exposure
resulted in increased oxidative stress which caused activation and nuclear accumulation of p53 and caspases necessary for apoptotic cell death. To understand the molecular intricacies of arsenic-induced hepatic cell death, we studied the regulation miR-34a, a micro RNA widely reported to be closely associated with apoptosis. Data obtained by measuring the relative expression of miR-34a by qPCR and by silencing p53 reflected a direct co-relation between p53 activation and miR-34a. Our study also establishes that PFE treatment can reverse arsenic-induced damage by restoring changes made at molecular levels. Thus, PFE efficiently reverses hepatotoxicity by reducing oxidative stress and rescuing the hepatocytes from cell death.

In the present study, we have also compared the antioxidant potential of extensively reported dietary polyphenols viz., pomegranate fruit extract (PFE), green tea (GT) and resveratrol (Res). Comparison of the ALP, AST, LDH and ALT activities, oxidative stress signatures and expression profiles of redox-sensitive transcription factors upon co-treatment with the three dietary supplements revealed that PFE offered maximal protection against arsenic induced damage. Thereafter, came resveratrol and green tea ranked third in reducing hepatotoxic markers.

To conclude, our study shows that arsenic renders host immune system dysfunctional and causes progressive hepatic damage. Arsenic-mediated oxidative stress alters miR-34a levels resulting in activation of p53 and consecutive hepatic cell death. PFE intake confers extensive protection against such damages by modulating changes at molecular levels. We also prove that among PFE, GT and Res, PFE emerges as the most promising candidate for chemoprevention against arsenic related damage.

**Key words:** Arsenic, immune-suppression, hepatotoxicity, polyphenols, oxidative stress, apoptosis.

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