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RESEARCH ARTICLE

ATTENUATION BY FRUIT EXTRACT OF (AMLA) *Emblica-officinalis*: ARSENIC ELEVATED CONCENTRATION OF SERUM MATRIX METALLOPROTEINASE-2 AND -9 WITH HIGH RISK OF CARDIOVASCULAR DISEASES IN MICE

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ABSTRACT

Exposure to arsenic in individuals has been found to be associated with immune related problems via oxidative stress and inflammation. In earlier studies, we have demonstrated that amla protects against arsenic induced oxidative stress and apoptosis in thymus and spleen of mice. In continuation to that the present study has therefore been focused to investigate the protective efficacy of amla in arsenic induced elevated concentrations of serum matrix metalloproteinase (mmp)-2 and -9 and their associations with high risk of cardiovascular diseases in mice. Arsenic treatment in mice, significantly increased mmp-2 (2.16 fold, $p<0.001$) and mmp-9 (1.45fold, $p<0.01$) level as compared to controls. No significant changes were found in control as well as amla alone group. While co-treatment with arsenic and amla significantly decreases mmp-2(0.65 fold, $p<0.01$) and mmp-9 (0.81fold, $p<0.05$) level as compared to arsenic alone group. The study results underscore the protective effects of amla in arsenic induced serum up regulation of mmp-2 and mmp-9 level as a high risk of cardiovascular disease, the fruit extract of amla balance the mmps level due to hypolipidemic and cardioprotective nature.

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INTRODUCTION

Arsenic is widely distributed in the environment due to its natural existence and anthropogenic activities. Due to the presence of high levels of inorganic arsenic in ground water in many parts of the world, human health related disorders are of major concern (Brinkel *et al*., 2009). High levels of arsenic has been reported in three districts Ballia, Varansi and Gazipur of Uttar Pradesh in the upper and middle Ganga plain, India (Ahamed *et al*., 2006). According to the World Health Organization (WHO) guidelines, the recommended value of arsenic is 10 µg/L (Chakraborti *et al*., 2010). More than 50% of the aquifers in the Ganga-Meghna-Brahamaputra plain of India and Bangladesh have arsenic level above the WHO recommended limit (Chakraborti *et al*., 2013). Several reports suggested that arsenic has entered the food chain including rice and vegetables (Zhu *et al*., 2008), presence of excessive amount of arsenic in foods and vegetables indicate that exposure to arsenic is unavoidable. Cardiovascular diseases (CVDs) and cancers are the major causes of chronic arsenic exposure-related morbidity and mortality. Matrix metalloproteinase-2 (MMP-2) and −9 (MMP-9) are deeply involved in the pathogenesis of CVDs and cancers.

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Studies reported chronic exposure to moderate and/or high levels of arsenic in drinking water may lead to the development of disease in humans, MMP-9 is the most prominently studied in the associated with a variety of lung diseases (Atkinson *et al*., 2003). The imbalance between MMP-9 and TIMP-1 is considered to contribute to the progression of airway remodeling in part due to changes in epithelial wound response (Wesley *et al*., 2007).In cancer pathology; MMP-2 and MMP-9 are mainly implicated in the formation of new blood vessels through angiogenesis. Among the MMPs, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been widely studied that degrade both gelatins and collagens of extracellular matrix (ECM) proteins. MMP-2 and MMP-9 activities are largely regulated by tissue inhibitor of metalloproteinase-1 (TIMP-1) (Murphy, Willenbrock, 1995). These MMPs have been implicated in cardiovascular through the degradation of ECM to be linked atherosclerotic plaque formation and plaque instability (Li *et al*., 1996). There is also increasing evidence that environmental factors/contaminants (especially heavy metals) contribute to the cardiovascular diseases including hypertension, atherosclerosis, blackfoot disease and dyslipidemia (Prozialeck *et al*., 2008). On the other side authors are reported that arsenic up-regulates the expression of various inflammatory molecules and inhibits key regulators of lipid homeostasis, these two are key components in the

initiation of atherosclerosis (Mazumder *et al*., 2011). The whole plant of *Emblica-officinalis* has high medicinal value; however fruits possess most of the pharmacological properties. The fruit extract of amla and its active constituents have long been used in Chinese and Indian traditional system of medicine (Singh *et al*., 2015). The most common and important pharmacological action of amla is antioxidant, anti-inflammatory and hypolipidemic activity. Nutritious diet may be able to inhibit and/or reverse the toxic mechanism of arsenic, whereas a deficient diet can increase the susceptibility to adverse effects of arsenic in drinking water. Some studies suggested that nutritious diet reduces the arsenic toxicity by increasing methylation of arsenic (Prozialeck *et al*., 2008). In our previous studies, we have suggested that arsenic induced enhanced oxidative stress linked with apoptosis in thymocytes of mice could be protected through simultaneous treatment with amla (Singh *et al*., 2013). Arsenic induced hepatic toxicity associated with impaired antioxidant status has also been protected following simultaneous treatment with arsenic and amla (Maurya *et al*., 2011). Further, we have also reported that arsenic induced immunotoxicity linked with inflammation has been significantly protected through simultaneous treatment with arsenic and amla that was due to anti-inflammatory, antioxidant and metal binding property of amla which could reduce the load of arsenic in spleen and thymus and help to decrease the generation of reactive oxygen and nitrogen species and also alterations in inflammatory biomarkers and lipid profile in blood of mice and imparts its protective effects (Singh *et al*., 2014a). In view of the previously published reports, the present study was designed to assess the effect of supplementation of amla extract on arsenic elevated the concentration of serum MMP-2 and MMP-9 in mice especially associated to the circulating biomarkers of CVDs, the current scenario we examine the mechanism of fruit extract of amla as an anti-inflammatory and anti-dislipidemic property of amla.

MATERIALS AND METHODS

Animals and Treatment

The study was approved by the institutional animal ethics committee of King George's Medical University, Lucknow (No. 121 IAH/Pharma-11), India, and all experiments were carried out in accordance with guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India. Male Balb/c mice (15 ± 2 g) were obtained from the animal breeding colony of CSIR-Indian Institute of Toxicology Research (Lucknow). Mice were housed in an air-conditioned room at 25± 2ºC with a 12 hours light/dark cycle under standard hygiene conditions. Mice had *ad libitum* access to a pellet diet and filtered water. The dose of fruit extract of Amla and arsenic is based on our previous studies (Singh *et al*., 2013; 2014; 2015) and for the study, the mice were randomly divided into four groups with 8 animals/group: and the dose of arsenic and amla were given orally with the help of canula after dissolving it in suitable solvent:

Group I - Mice treated with vehicle (2% gum acacia) for duration of treatment and served as control.

Group II - Mice treated with sodium arsenite (dissolved in distilled water at 3 mg/kg body weight, *per os* daily for 30 days).

Group III - Mice treated with fruit extract of *Emblica officinalis* (500 mg/kg body weight, suspended in 2% gum acacia, *per os* daily for 30 days).

Group IV - Mice co-treated daily with arsenic and fruit extract as in Groups II and III.

Blood collection

At the end of the experimental period (30 days), animals were sacrificed by cervical dislocation. Immediately after, heart puncher blood was quickly collected in 10 % EDTA tubes for the separation of serum for the assessment of different MMPs markers.

Assay of MMP-2 and MMP-9 level in mice serum

The concentrations of MMP-2 and MMP-9 in serum of mice exposed to arsenic and simultaneous treatment of arsenic and amla were quantified by using an enzyme-linked immunoassay kits (MMP-2, 9 kits purchased from Sigma Aldrich, USA). According to the manufacture's protocols. A micro-plate reader (Synergy HT of BIO-TEK International, USA) was used for the measurement of color development. All standards and samples were analyzed in duplicate. The absorbance was read at 530 nm in a micro plate reader.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by a Newman–Keuls test for multiple pair-wise comparisons among the groups. All values were expressed as mean (±SEM). P value < 0.05 was considered significant.

RESULTS

Effect of fruit extract of amla on the serum mmp-2 level in mice

Arsenic has been found to be associated with the increased concentration of serum mmp-2. Effect of arsenic and co-treatment of arsenic and amla on elevated concentration of mmp-2 has been presented in Figure-1.

Exposure of arsenic to mice caused an elevated level of mmp-2 (2.16 fold, $p < 0.001$) in serum as compared to controls. Co-treatment with arsenic and amla decreased the mmp-2 level (0.65 fold, $p < 0.01$) in serum as compared to mice treated with arsenic alone suggested the antioxidant and hypo-lipidemic activity of amla. No significant effect on the level of mmp-2 was observed in mice treated with amla alone as compared to controls (Figure-1).

Defense of fruit extract of amla on the serum mmp-9 level in mice

Effect of arsenic and co-treatment of arsenic and amla on the serum level of mmp-9 in mice is presented in Figure- 2. Mice exposed to arsenic exhibited significant increase in mmp-9 level (1.45 fold, $p<0.01$) as compared to controls. Co-treatment with arsenic and amla decreased the serum level of mmp-9 as compared to arsenic treated group (0.81-fold, $p<0.05$). No significant effect on serum mmp-9 level was observed in mice treated with amla alone as compared to controls (Figure- 2).

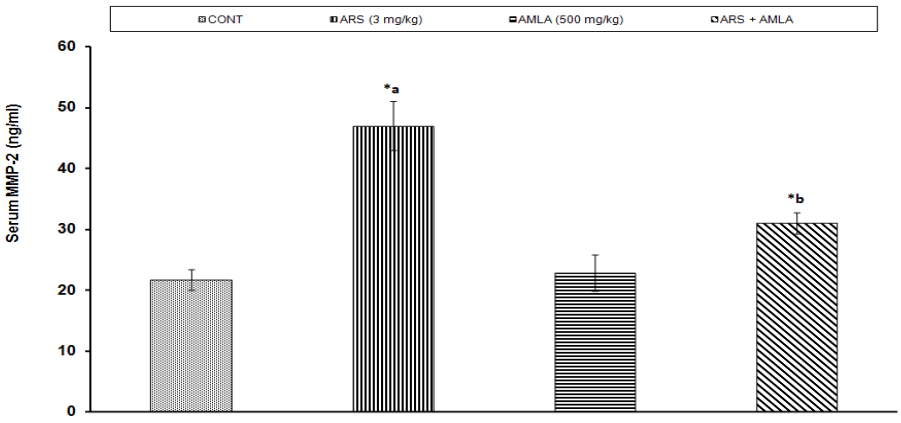

Figure- 1. Effect of serum MMP-2 (ng/ml) in mice exposed to arsenic, amla and their co-treatment in serum of mice

Values are mean ±SEM of five animals in each group
a-compared to control group, b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

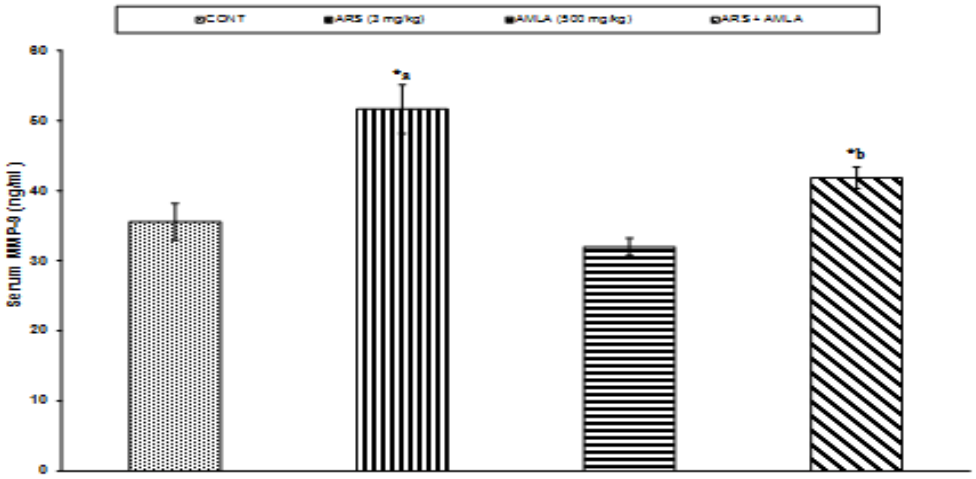

Figure- 2. Effect of serum MMP-9 (ng/ml) in mice exposed to arsenic, amla and their co-treatment in serum of mice

Values are mean ±SEM of five animals in each group
a-compared to control group, b-compared to arsenic treated group
*Significantly differs ($p < 0.05$)

DISCUSSION

Studies have been reported the efficacy of herbal extracts and synthetic agents also reduced the arsenic burden (Singh *et al*., 2013; 2014a; 2014b). Amla is widely accepted as an immune enhancer and have multiple pharmacological and immunomodulatory properties due to the presence of various phenolics and its derivatives (Yadav *et al*., 2017; Srivastava *et al*., 2014).Arsenic induced depletion of anti-oxidant defense enzymes including superoxide dismutase, catalase, and glutathione peroxidase associated with apoptosis, immunotoxicity and hepatic damage have been recently reported by us (Sreeramulu, Raghunath *et al*., 2009., Singh *et al*., 2015). Few studies have evaluated the effect of arsenic exposure on abnormal lipid profiles such as a large amount of total cholesterol (TC), especially low-density lipoprotein (LDL), and TG, and lower levels of high-density lipoprotein (HDL) are associated with a higher risk of CVD. MMP-9 is located at the shoulder region of plaques and areas of foam cell accumulation and plays an important role in several stages of atherosclerosis (Meuwese *et al*., 2007). MMP-9 expression was time-dependently increased in mice chronically exposed to 50–250 µg/L arsenic in drinking water. Myeloperoxidase (MPO) has been implicated as a catalyst for LDL oxidation (Hansson *et al*., 2011) and contributes to the overall ROS burden in the physiological milieu. Positive associations of circulating levels of MMP-9 and MPO with CVD have been reported (States *et al*., 2009). Studies are reported that Chronic arsenic exposure is well established as carcinogenic but interest in the non-cancer disease endpoints include cardiovascular disease (States *et al*., 2011) and immune dysfunction (Banerjee *et al*.,2008) in the arsenic exposed population of West Bengal, may influence inflammatory responses in the vascular cells. Increased serum concentrations of these cytokines and chemokines (IL6, IL8 and MCP-1) may act as early biomarkers of increased cardiovascular risk in the arsenic exposed subjects. Further, the pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 are also secrets due to the arsenic toxicity and generates inflammatory responses (Fainboim *et al*., 2007). These pro-inflammatory mediators are involved in the various biological and cellular comebacks including tumor progression, growth factor, transcription factor and activation of pro apoptotic proteins (Barbaro *et al*., 2014). MMPs act as inflammatory mediators (Ferroni *et al*., 2003). The major

cellular sources of MMPs are macrophages and neutrophils. MMPs degrade which proliferation and apoptosis of cells in vessel wall which are involved in the inflammatory process (Horstmann *et al*., 2006). Growing evidence suggests that MMP-2 and MMP-9 are deeply implicated in CVDs and cancers through vascular remodeling and the formation of angiogenesis and arteriogenesis (*Siasos et al*., 2012). Tissue remodeling is one of the several proposed mechanisms of toxicity, arsenic toxicity induce remodeling via methylation of genes occurs in early development, in wound repair, and in respiratory and cardiovascular disease. MMP-9 is also involved in the degradation of extracellular matrix proteins and is associated with carcinogenesis, (Chen *et al*., 2013) and cardiovascular disease. (Zheng *et al*., 2016) MMP-9 is present in low concentrations in serum in healthy adults, but increases within hours of disease onset (Chung *et al*., 2009). MMPs take part in the early stage of atherosclerotic process by enhancing migration and proliferation of smooth muscle cells as well as other inflammatory cells; whereas in advanced stage of atherosclerosis. In the present study simultaneously treatment with arsenic and amla significantly inhibited the serum MMP-2, MMP-9 as compared to those treated with arsenic alone. And also reduced arsenic burden in target tissues by metal chelating properties which may play a crucial role in its cardioprotective effect.

Conclusion

Chronic arsenic exposure causes a wide variety of diseases. Serum MMP-2 and MMP-9 concentrations showed associations with the several circulating markers of cardiovascular disease. Thus, the results of this study suggest that arsenic exposure-related elevation of serum MMP-2 and MMP-9 concentrations may be implicated in arsenic-induced CVDs .Simultaneously treatment with arsenic and amla also reduced mmp,s level in mice serum. The active constituents of amla have a hypo-lipidemic and cardio-protective in nature.

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