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Amelioration of arsenic-induced toxic effects in mice by dietary supplementation of *Syzygium cumini* leaf extract

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ABSTRACT

Arsenic created a serious public health problem in Bangladesh due to its presence in groundwater and dissemination of the toxic effects to millions of people. The scarcity of the treatment options to manage this affected population has made the situation much worse. To find a promising treatment option, this study was undertaken to examine the ameliorating roles of *Syzygium cumini* leaf extract (SLE) against arsenic-induced toxic effects in mice. Swiss albino mice were divided into four groups where 'control' group received pure water + normal feed, 'arsenic (As)' group received sodium arsenite ($NaAsO_2$)-containing water (10 μg/g body weight/day) + normal feed, 'As+SLE' group received $NaAsO_2$-containing water + feed supplemented with SLE (50 μg/g body weight/day) and finally the 'SLE' group received pure water + feed supplemented with SLE. A gradual increase in body weight gain was observed in control mice; however, the body weight gain in As-exposed mice was decreased. This decrease in body weight gain was prevented in As+SLE group mice that received SLE supplemented feed. Arsenic showed a secondary effect by causing enlargement of spleen, kidney and liver of 'As' group mice and this enlargement of the organs was minimized with SLE supplementation. In addition, SLE abrogated arsenic-mediated elevation of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), uric acid and glucose. These results, therefore, suggest that SLE might have future therapeutic value for preventing or reducing arsenic-induced toxic effects.

Key Words: Sodium arsenite, Mice, *S. cumini* leaf extract, Serum parameters, Organ enlargement

INTRODUCTION

Arsenic, a poisonous heavy metal, is persistent in the environment and possesses potential for a wide range of deleterious health consequences. Millions of people in Bangladesh and many other countries are exposed to elevated levels of arsenic through drinking contaminated ground water.[1,2)] Arsenic exposure thereby created a serious public health concern worldwide. The effect of this heavy metal poisoning is apparent either in short time or after prolonged

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exposure depending on the dose and route of its entry, body defense mechanism, and nutritional status of an individual. Long-term exposure to arsenic causes a wide range of adverse effects on health, including weight loss, skin lesions, cancer, cardiovascular disease (CVD), diabetes, liver disorders, immunotoxicity etc.[3-7] Most of the arsenic compounds are known to be soluble in water to some extent, thereby easily transported through the blood to various organs of the body. Although some of the arsenic that enters the body is excreted, however, a significant portion is reported to be absorbed by various tissues/organs including hair, nails, liver, kidney, heart, lung and spleen causing adverse physiological effects.[8-10]

Among several hypotheses that have been proposed for understanding the mechanism of arsenic toxicity, the involvement of arsenic-induced oxidative stress is considered as the most prominent. Arsenic-induced oxidative stress results mainly from its ability to generate reactive oxygen species (ROS) and to interact with sulfhydryl groups of proteins/enzymes.[11-13] Various studies have demonstrated that this oxidative stress is capable of disrupting multiple cellular signaling pathways that may play prominent roles in arsenic-mediated disease manifestation.[14,15] Despite recognition of the global public health threat of arsenic toxicity; its effective, reliable and safe treatment still remained mostly unknown. Considering the existence of a correlation between arsenic toxicity and oxidative stress, researchers are looking forward to utilizing the antioxidant properties of different plant extracts to combat arsenic poisoning.

Recent studies have demonstrated potential roles of antioxidants in the prevention and/or management of arsenic toxicity.[16,17] Consequently, plant-based natural compounds and their active constituents with high antioxidant potential have received great attention because of their ability to counteract the toxic effects of arsenic.[18,19] Natural antioxidants present in tea extract have been shown to protect against arsenic-induced toxicities.[20,21] We have recently reported that arsenic-induced loss of mice body weight and enlargement of various organs were prevented by dietary supplementation of *Phyllanthus emblica* leaf extract.[9] Therefore, the search for antioxidants in fruit, vegetable and medicinal plants to ameliorate the toxic effects of heavy metals is drawing great attention around the globe.

Syzygium cumini Linn. (family *Myrtaceae*), a well-known fruit plant, is widely distributed in tropical and subtropical regions including Bangladesh. *S. cumini* has been greatly valued for possessing bioactive compounds such as flavonoids, glycosides, tannins, anthocyanins and ascorbic acid; all of which have excellent antioxidant properties.[22,23] In this context, multiple therapeutic applications of *S. cumini* have so far been described; among them is anti-diabetic, anti-inflammatory, anti-diarrheal, anticancer, and antimicrobial activities.[24-27] In the present investigation, extract of *S. cumini* leaves was evaluated for its preventive activity against arsenic-mediated adverse effects in experimental mice.

MATERIALS AND METHODS

Plant materials

The leaves of *S. cumini* were collected from orchards at Curzon Hall campus, University of Dhaka, Bangladesh. The plant was identified and authenticated and a voucher specimen (Accession no. 34742) of the plant was deposited in Bangladesh National Herbarium.

Preparation of S. cumini leaf extracts (SLE)

SLE was obtained as described previously.[25] Briefly, the leaves were cleaned and air-dried at room temperature keeping them away from direct sunlight for 7–10 days followed by grinding to a coarse powder. Leaf powder (250.0 g) thus obtained was soaked in 1 L ethanol (95%) in a

flask and kept for extraction at room temperature for 1 week. The extract was then filtered using Whatman filter paper (no. 11) to collect the filtrate. The residue was again soaked in ethanol to get additional extractive. All the collected filtrates were then concentrated using vacuum rotary evaporator at reduced temperature and pressure. A gummy substance obtained thereby was subjected to drying at room temperature to prepare the powdered form. The powdered extract was weighed and stored at 4ºC for further work. From 250.0 g of dried leaf powder, 35.5 g (14.2%) of the extract was finally obtained. The extract was mixed with mice feed purchased from International Centre for Diarrheal Disease Research, Bangladesh (icddr,b).

Animal maintenance

Swiss albino mice (male, 6 weeks of age) were purchased from icddr,b. Mice were randomly selected and housed in plastic cages with wood-cob bedding (6 mice/cage). After one week of acclimation, mice were divided into four groups namely control, arsenic (As), As+SLE and SLE. 'Control' mice were supplied with miliQ water using feeding bottles and normal mice feed. The 'As group' mice were given normal feed and sodium arsenite ($NaAsO_2$) containing water (prepared in miliQ water, 10 µg/g body weight/day) while the 'As+SLE group' mice were provided with SLE (50 µg/g body weight/day) containing feed and As-containing water. The 'SLE group' was provided with SLE containing feed and miliQ water. These different groups of mice were maintained for 12 weeks. All these procedures and experiments using mice were undertaken following the ethical issues set by the Faculty of Biological Sciences, University of Dhaka, Bangladesh.

Measurement of the body and organ weight of mice

Each mouse of all groups was weighed in every two weeks using an analytical balance and recorded accordingly. After 12 weeks of maintenance, the mice were sacrificed by cervical dislocation and the abdomen was exposed surgically by ventral incision. The kidney, liver and spleen were removed carefully, cleaned of all fat and connective tissue and weighed. The average organ/body weight (mg/g) ratio was then calculated.

Blood collection and assay of various serum parameters

Surgical blade (size 11) was pinched sharply between the ear and eye of the mice. Blood came out as drops and collected in test tubes. Serum was then separated from the collected blood and kept at –80°C until the assays for various parameters were done. Serum glucose and uric acid levels were measured, and alkaline phosphatase (ALP), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were determined using commercially available assay kit following manufacturer's protocol (Human Diagnostic, Germany; DiaSys Diagnostic Systems, Turkey; and Biosystems S.A., Spain). All serum samples were analyzed in duplicate and then mean values were used.

Measurement of arsenic deposition in tissue samples of As-exposed mice

Levels of arsenic in the tissue samples of As-exposed mice were measured by the method described previously.[28] Briefly, liver and spleen samples were taken in a 15 ml polypropylene tube in the presence of 3 ml of nitric acid (61%). The tubes were capped properly and incubated at 80°C for 48 hrs, followed by cooling for 1 hr to room temperature. After cooling, 3 ml of hydrogen peroxide (30%) was added to each tube, followed by incubation at 80°C for 3 hrs. After suitable dilution of the digested materials with ultrapure water, levels of arsenic in the samples were determined by an inductively coupled plasma-mass spectrometer (ICP-MS; 7500cx, Agilent Technologies, Inc.) with a reaction cell for the absence of ArCl ion interference.

Statistical analysis

Statistical analyses for this study were performed using software of Statistical Packages for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL). Data were shown as mean ± SD. Data were analyzed by one-way ANOVA followed by Bonferroni multiple comparison tests. A value of $p<0.05$ was considered statistically significant.

RESULTS

Arsenic-mediated decrease in mice body weight was partially rescued by SLE

The initial average body weight of the 'control', 'As', 'As+SLE' and 'SLE' group of mice were 18.20±0.71, 18.06±0.80, 18.49±1.47 and 17.80±1.34 g, respectively. Each group of mice was supplied with their respective feed and drink as described in the materials and methods section. The weight of each group of mice was noted at every two-week interval, although the data of 0, 4, 8 and 12 weeks were plotted as shown in Fig. 1. After 12 weeks, the average body weight of the control, As, As+SLE and SLE group became 32.25±1.5, 19.81±1.3, 25.57±0.73 and 31.78±0.65 g, respectively. It was observed that the control mice gained weight gradually with time; however, the normal gain of the body weight in As-exposed mice was disrupted. Body weight gain in As-exposed mice was significantly reduced compared with control at 4, 8 and 12 weeks ($p<0.05$). Interestingly, SLE supplementation partially rescued the mice from impaired growth observed in the As-exposed group. The body weight of As+SLE group mice was significantly different ($p<0.05$) from the As group mice. This result indicated a potential role of SLE in mitigating arsenic-mediated toxic effects for growth retardation. The pattern of growth in control and SLE group mice were found to be similar indicating no apparent effects of SLE alone on mice growth.

SLE supplementation blocked arsenic-induced enlargement of kidney, liver and spleen

We next examined whether any changes in physical appearance of internal organs such as kidney, liver and spleen occurred in arsenic-exposed mice or not. Organs such as spleen, kidney and liver were collected and the organ weight-to-body weight ratio was calculated as shown in Table 1. In control mice, mean ± SD organ-to-body weight ratios for spleen, kidney and liver was 3.6±0.32, 12.5±1.29 and 42.2±4.76, respectively. The organ-to-body weight ratios for spleen, kidney and liver of As-exposed mice, however, were significantly increased ($p<0.05$) compared to control and the values became 4.9±0.24, 19.1±3.39 and 54.2±2.97, respectively. It was evident from this result that the mice of As-group were associated with spleen, kidney and liver enlargement. Interestingly, SLE supplementation mostly blocked this increase in organ-to-body weight ratios as evident from the comparable organ-to-body weight ratios of both the control and As+SLE group mice (Table 1). These results indicated that SLE might have played an important role in reducing the arsenic-mediated toxic effects on those affected organs.

Arsenic-induced elevation of ALP, ALT and LDH was partially blocked by SLE

The liver is known as one of the most important organs in the body for its ability to metabolize nutrients, detoxify harmful substances and perform many other vital functions. The liver enlargement caused by arsenic poisoning in this study might have linkage with liver dysfunction. We, therefore, measured the levels of the enzymes e.g. ALP and ALT in serum as the elevated activity of these enzymes is known to have an association with liver as well as some other organ dysfunction. Compared to the levels of serum ALP and ALT of control mice (188.91±10.43 and 56.01±9.41 U/L, respectively), the levels of these enzymes in As-exposed mice were increased

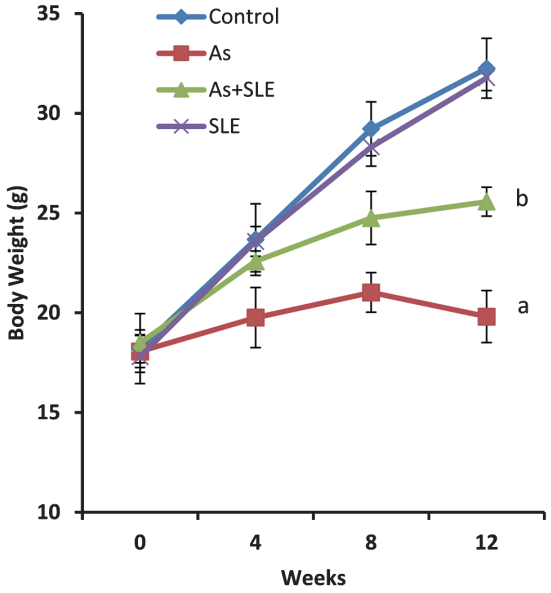

Fig 1 SLE partially rescues As-induced loss of body weight.
Body weight of each group mice was taken at every two weeks and continued up to 12 weeks from the starting date of the experiment (0 week). Data of 0, 4, 8 and 12 weeks were plotted. *X*- and *Y*-axis represented the duration (week) of diet and body weight (g), respectively. Data shown as mean ± SD (n=6 per group). [a]Significantly different (p<0.05) from the control group at 4, 8 and 12 weeks. [b]Significantly different (p<0.05) from the As group at 4, 8 and 12 weeks. The overall difference (heterogeneity) between four groups (Control, As, As+SLE and SLE) in all cases of 4, 8 and 12 weeks is statistically significant (p<0.01).

Table 1 Organ weight-to-body weight ratios of all four groups of mice after 12 week

Organ wt/body wt (mg/g)	Control	As	As + SLE	SLE
Spleen	3.6±0.32	4.9±0.24[a]	3.9±0.24[b]	3.7±0.43
Kidney	12.5±1.29	19.1±3.39[a]	14.5±1.28[b]	12.8±1.49
Liver	42.2±4.76	54.2±2.97[a]	46.1±3.37[b]	41.5±3.35

Values shown as mean ± SD (n=6 per group).
[a]Significantly different (p<0.05) from the control.
[b]Significantly different (p<0.05) from the As group.
The overall difference (heterogeneity) between four groups (Control, As, As+SLE and SLE) in all cases of spleen, kidney and liver is statistically significant (p<0.01).

(260.59±20.67 and 82.61±8.43 U/L, respectively) as shown in Fig. 2A. These increases in the enzyme activities were statistically significant (p<0.05). When the As-exposed mice were supplemented with SLE, the As-induced elevations of ALP and ALT activity were significantly blocked (p<0.05). In addition, we also measured the level of LDH in serum which might also be elevated due to damage of the heart, liver and kidney cells caused by arsenic exposure. Serum LDH level in As-exposed mice (694.25±39.21 U/L) was increased significantly compared to control (405.22±22.81 U/L) (Fig. 2B). This result indicated arsenic-mediated possible damage of heart and other tissues that might have caused elevation of serum LDH level. We again observed

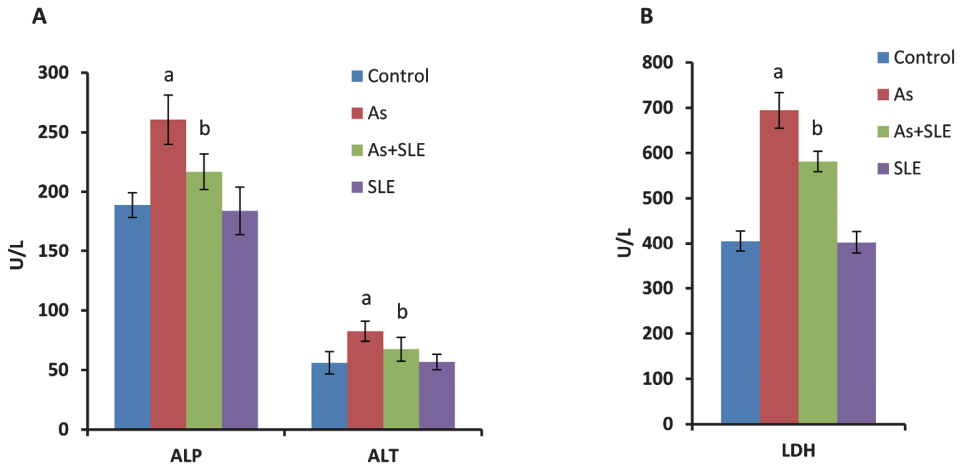

Fig. 2 SLE partially rescues arsenic-mediated elevation of serum enzymes.
Blood samples were collected after 12 weeks of diet for determining the levels of the serum enzymes ALP, ALT (A) and LDH (B). Data shown as mean±SD (n=6 per group). [a]Significantly different (p<0.05) from the control group. [b]Significantly different (p<0.05) from As group. The overall difference (heterogeneity) between four groups (Control, As, As+SLE and SLE) in all cases of ALP, ALT and LDH is statistically significant (p<0.01).

that this serum elevation of LDH was partially blocked when SLE was supplemented.

Arsenic deposition was higher in liver compared to spleen of As-exposed mice

We next determined the level of arsenic deposition in the liver and spleen by ICP-MS. Arsenic was deposited in a quite higher amount in the liver (6.01±1.80 mg/Kg body weight) than in the spleen (1.68±0.20 mg/Kg body weight) of the As-exposed mice (Fig. 3). A significant difference (p<0.05) of arsenic deposition in both liver and spleen was observed in As-exposed mice group compared to the control. We also tested whether SLE supplementation decreases arsenic deposition in those organs. Although SLE partially reduced the level of arsenic deposition in those organs (liver 3.76±1.01 and spleen 1.24±0.19 mg/Kg body weight), however, this reduction was not statistically significant (p>0.05). These results argued that the arsenic-mediated toxic effects could mostly be reduced by SLE, but not arsenic deposition in various organs.

Arsenic-induced elevation of uric acid and glucose was partially blocked by SLE

Arsenic-induced elevation of LDH and uric acid is reported to be associated with an increased risk of CVD.[29,30] As the elevation of LDH is already observed in this study, we next examined whether arsenic exposure is also associated with increased uric acid level. As shown in Fig. 4, serum uric acid in the As-exposed group (4.15±0.23 mg/dl) was increased compared to the control (3.44±0.29 mg/dl). Although SLE supplementation partially blocked the elevation of this enzyme, however, the difference was not statistically significant (p>0.05) when the As-exposed group was compared to the As+SLE group. Arsenic is known to induce diabetes mellitus,[6,31,32] while diabetes mellitus has been shown to be associated with uric acid level.[33-35] We, therefore, tested whether or not As-exposed mice accompany increased serum glucose level. We observed that arsenic exposure significantly (p<0.05) increased the serum glucose level (181.33±11.71 mg/dl) compared with control (110.66±9.01 mg/dl). SLE supplementation prevented partially, although not significantly (p>0.05), the As-induced increase in glucose level.

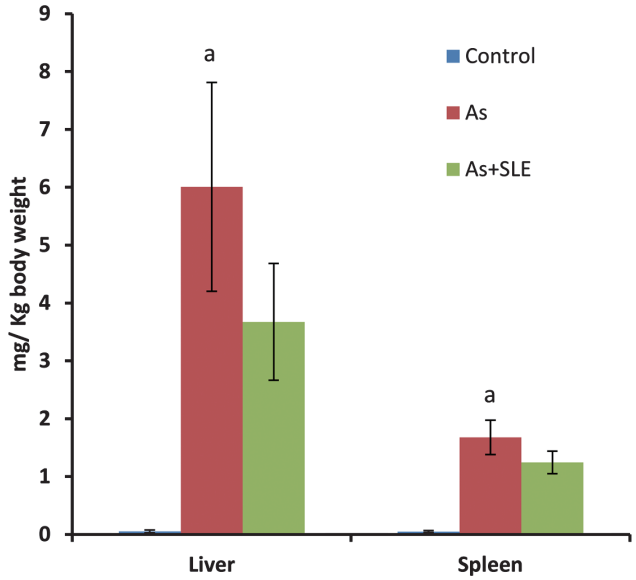

Fig. 3 Deposition of arsenic (mg/Kg body weight) in the liver and spleen of As-exposed mice. Data shown as mean±SD (n=3 per group). [a]Significantly different (p<0.05) from the control. The overall difference (heterogeneity) between three groups (Control, As and As+SLE) in both cases of liver and spleen is statistically significant (p<0.01).

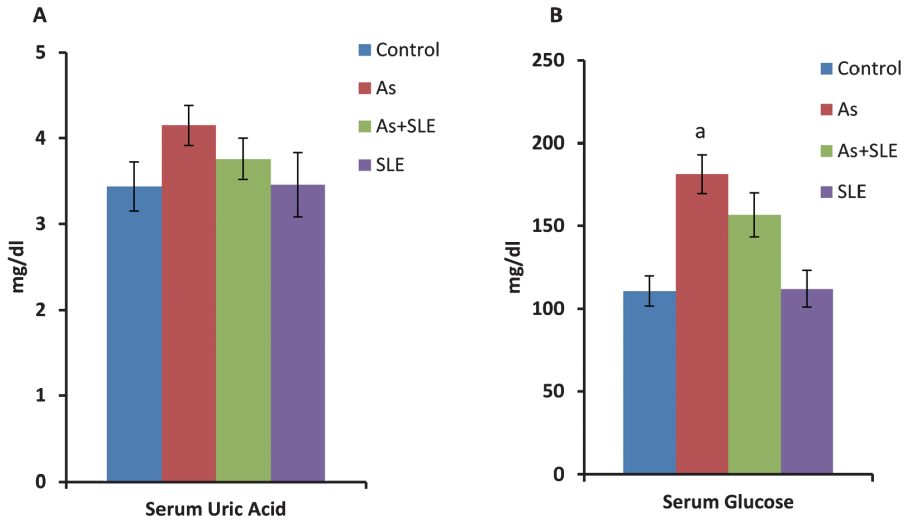

Fig. 4 SLE partially rescues arsenic-mediated elevation of serum uric acid and glucose Blood samples were collected after 12 weeks of diet for determining the levels of the serum uric acid and glucose. Data shown as mean±SD (n=3 per group). [a]Significantly different (p<0.05) from the control. The overall difference (heterogeneity) between four groups (Control, As, As+SLE and SLE) in case of serum glucose is statistically significant (p<0.01); however, in case of serum uric acid is found on the borderline of statistical significance (p=0.053).

DISCUSSION

Elevated intake of arsenic has been shown to interfere with a number of organ and body functions through perturbation of various biochemical and physiological activities.[36-38] The mechanism by which arsenic mediates toxic effects is not yet understood completely, however, generation of ROS by arsenic is thought to play a vital role in the process.[39] The present study evaluated the effect of SLE on arsenic-induced toxic effects in mice. The obtained results demonstrated that SLE significantly protected the experimental animals from the toxic effects. The gain of body weight usually serves as a useful indicator of animal growth which may be disrupted by exposing the animals to toxic substances. The reduction in body weight in As-exposed mice might be due to the toxic effects of arsenic which hampered several metabolic processes associated with retardation of growth and development.[9,19,40,41] However, the animals exposed to arsenic together with SLE supplementation were partially rescued from weight loss compared to arsenic alone exposure group indicating an effective ameliorating effect of SLE against arsenic-induced toxicity. Furthermore, the ineffectiveness of SLE alone to impose any visible change in growth suggests the nontoxic nature of SLE itself in the animals. The mechanism by which SLE prevented arsenic-mediated weight loss is still unclear; however, the high antioxidant content of SLE probably helped to scavenge free radicals generated by arsenic.[42,43] This view is supported by earlier reports that demonstrated prevention of arsenic-mediated weight loss by turmeric and *Phyllanthus emblica* leaf extract, each of which is known to have ROS scavenging activity.[9,19]

Though almost all organs are being affected by arsenic exposure, the liver, kidney and spleen are thought to be most susceptible to the toxic effects.[44-46] In our study, a significant increase in liver, kidney and spleen weight was observed in As-exposed mice demonstrated by increased organ/body weight ratio (Table 1). Our results comply with previous reports that showed an association between arsenic exposure and hepatomegaly, splenomegaly or kidney enlargement.[7,9,44] Co-treatment with SLE along with arsenic could significantly reinstate the organ weights to near-normal status, which is an indicative of the therapeutic potential of SLE against arsenic toxicity.

As a potential sulfhydryl-reactive compound, arsenic binds thiol groups of proteins/enzymes in the liver and undergoes biotransformation, thereby interfering with the integrity of hepatic plasma membrane leading to leakage of AST and ALT in serum.[47] ALT and ALP are usually measured to indicate damage of hepatic cells and problem with bones/gallbladder/kidney. ALT is known to be primarily localized inside liver cells; however, ALP is present in a wide variety of tissues including liver, bones, intestines, kidneys, and other organs. The levels of ALP and ALT are increased to some extent in most cases of liver injury or inflammation. In this study, we have shown that serum levels of ALP and ALT have been increased significantly in As-exposed mice compared to unexposed animals. Elevated levels of these enzymes indicated liver dysfunction in exposed animals as demonstrated in earlier reports.[5,16,48] In addition to an increase in serum ALP and ALT, we have also observed a considerable elevation in serum LDH, which is also in accordance with the observations, reported previously.[19,29,49] The increase in LDH levels might be due to the damage of liver, heart and kidney cells caused by arsenic exposure. Elevation of all these serum enzymes, in our study, was significantly prevented when SLE was co-administered with arsenic.

Cellular and tissue accumulation of arsenic is thought to be a major concern because of its persistent damaging potential. Arsenic is known to accumulate in various organs including liver, kidneys, heart, lungs, muscles and spleen when ingested by human and animals.[8,9,50] Significantly high levels of arsenic accumulation were also observed in this study within the tested organs of the As-exposed group (Fig. 3). Although SLE was firmly able to prevent decrease in body weight gain and enlargement of organ weights in As-exposed mice (Fig. 1 and Table 1), its ability to

reduce arsenic deposition in organs, however, was not significant.

Evidence is accumulating in favor of an association between arsenic exposure and induction of diabetes in human and animal.[6,31,32] Our study demonstrates a significant difference in the level of blood glucose between control and As-treated mice (Fig. 4) supporting the above notion. The increase in glucose level might account for the cytotoxic effect of arsenic on the pancreatic β-cells.[51] In addition to increase in glucose level, the treated mice also accompany an increase in uric acid level. Our results act in accordance with the previous reports demonstrating a role of uric acid in the manifestation of diabetes[33-35] however, the relationship between uric acid and hyperglycemia is not always consistent. Many studies showed a positive correlation between the levels of serum uric acid and glucose, while others suggested an inverse relationship.[52,53] Although SLE significantly blocked arsenic-induced elevations of serum ALP, ALT and LDH activity, however, it could not reduce serum uric acid and glucose levels significantly.

In conclusion, the present study demonstrated a considerable effect of SLE against an arsenic-induced reduction in body weight gain, enlargement of organs and increase in various serum parameters. One of the major mechanisms behind arsenic toxicity has been attributed to oxidative stress. In connection with this view, SLE might show protection against arsenic-induced toxicity through its ability to counteract oxidative stress. The presence of a number of potential antioxidants in the test extracts[22,23,25,54] probably contributed to overall protection against the deleterious effects. Despite the great potential of SLE to ameliorate arsenic-induced adverse effects, the exact role of the SLE ingredients in the process of amelioration is still not understood clearly. Therefore, additional research about physiological, cellular and molecular mechanisms of the ingredients present in the extract is needed. This may lead us to develop a SLE-based therapeutic drug in future for the intervention of the complications due to arsenic exposure in human.

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CONFLICT OF INTEREST

All authors declare to have no actual or potential conflicts of interest.

REFERENCES

1) Chowdhury UK, Rahman MM, Mondal BK, Paul K, Lodh D, Biswas BK *et al.* Groundwater arsenic contamination and human suffering in West Bengal, India and Bangladesh. *Environ Sci*, 2010; 8: 393–415.
2) McLellan F. Arsenic contamination affects millions in Bangladesh. *Lancet*, 2002; 359: 1127.
3) Banerjee M, Sarkar J, Das JK, Mukherjee A, Sarkar AK, Mondal L *et al.* Polymorphism in the ERCC2 codon 751 is associated with arsenicinduced premalignant hyperkeratosis and significant chromosome aberrations. *Carcinogenesis*, 2007; 28: 672–676.
4) States JC, Srivastava S, Chen Y, Barchowsky A. Arsenic and Cardiovascular disease. *Toxicol Sci*, 2009; 107: 312–323.
5) Guha Mazumder DN. Effect of chronic intake of arsenic-contaminated water on liver. *Toxicol Appl Pharmacol*, 2005; 206: 169–175.
6) Tseng CH. The potential biological mechanisms of arsenic induced diabetes mellitus. *Toxicol Appl Pharmacol*, 2004; 197: 67–83.

7) Das N, Paul S, Chatterjee D, Banerjee N, Majumder NS, Sarma N *et al.* Arsenic exposure through drinking water increases the risk of liver and cardiovascular diseases in the population of West Bengal, India. *BMC Public Health*, 2012; 12: 639. doi: 10.1186/1471-2458-12-639.
8) Benramdane L, Accominotti M, Fanton L, Malicier D, Vallon JJ. Arsenic speciation in human organs following fatal arsenic trioxide poisoning—a case report. *Clin Cheb*, 1999; 45: 301–306.
9) Sayed S, Ahsan N, Kato M, Ohgami N, Rashid A, Akhand AA. Protective effects of *phyllanthus emblica* leaf extract on sodium arsenite-mediated adverse effects in mice. *Nagoya J. Med. Sci*. 2015; 77, 145–153.
10) Rahman M, Al Mamun A, Karim MR, Islam K, Al Amin H, Hossain S *et al.* Associations of total arsenic in drinking water, hair and nails with serum vascular endothelial growth factor in arsenic-endemic individuals in Bangladesh. *Chemosphere*, 2015; 120: 336–342.
11) Pi J, Yamauchi H, Kumagai Y, Sun G, Yoshida T, Aikawa H *et al.* Evidence for induction of oxidative stress caused by chronic exposures of Chinese residents to arsenic contained in drinking water. *Environ. Health Perspect*, 2002; 110: 331–336.
12) Hei TK, Liu SX, Waldren C. Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species. *Proc Natl Acad Sci USA*, 1998; 95: 8103–8107.
13) Ruiz-Ramos R, Lopez-Carrillo L, Rios-Perez AD, De Vizcaya-Ruíz A, Cebrian ME. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. *Mutat Res*, 2009; 674: 109–115.
14) Druwe IL, Vaillancourt RR. Influence of arsenate and arsenite on signal transduction pathways: an update. *Arch Toxicol*, 2010; 84: 585–596.
15) Sumi D, Shinkai Y, Kumagai Y. Signal transduction pathways and transcription factors triggered by arsenic trioxide in leukemia cells. *Toxicol Appl Pharmacol*, 2010; 244: 385–392.
16) Zhang Z, Gao L, Cheng Y, Jiang J, Chen Y, Jiang H *et al.* Resveratrol, a natural antioxidant, has a protective effect on liver injury induced by inorganic arsenic exposure. *Biomed Res Int*, 2014; 2014: 617202. doi: 10.1155/2014/617202.
17) Yu H, Liu S, Li M, Wu B. Influence of diet, vitamin, tea, trace elements and exogenous antioxidants on arsenic metabolism and toxicity. *Environ Geochem Health*, 2016; 38: 339–351.
18) Singh MK, Yadav SS, Gupta V, Khattri S. Immunomodulatory role of *Emblica officinalis* in arsenic induced oxidative damage and apoptosis in thymocytes of mice. *BMC Complement Altern Med*, 2013; 13: 193. doi: 10.1186/1472-6882-13-193.
19) Karim MR, Khaque A, Islam K, Ali N, Salam KA, Saud ZA *et al.* Protective effects of the dietary supplementation of turmeric (*Curcuma longa* L.) on sodium arsenite-induced biochemical perturbation in mice. *Bangladesh Med Res Counc Bull*, 2010; 36: 82–88.
20) Aktar M, Islam NN, Sumit AF, Ahsan N, Hossain S, Ahmed M *et al.* Tea Extract Prevents Arsenic-mediated DNA Damage and Death of Murine Thymocytes *in Vitro*. *Dhaka Univ. J. Pharm. Sci*, 2015; 14: 79–85.
21) Messarah M, Saoudi M, Boumendjel A, Kadeche L, Boulakoud MS, El Feki A. Green tea extract alleviates arsenic-induced biochemical toxicity and lipid peroxidation in rats. *Toxicol Ind Health*, 2013; 29: 349–359.
22) Eshwarappa RS, Iyer RS, Subbaramaiah SR, Richard SA, Dhananjaya BL. Antioxidant activity of Syzygium cumini leaf gall extracts. *Bioimpacts*, 2014; 4: 101–107.
23) Mohamed AA, Ali SI, El-Baz FK. Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. *PLoS One*, 2013, 8: e60269. doi: 10.1371/journal.pone.0060269.
24) Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Mohan Kumar R, Aravindan P *et al.* Anti-inflammatory activity of *Syzygium cumini* seed. *Afr J Biotechnol*. 2008; 7: 941e943.
25) Ahsan N, Paul N, Shamma F, Islam N and Akhand AA. Leaf extract of *Syzygium cumini* shows anti-*vibrio activity* involving DNA damage. *Dhaka Univ J Pharm Sci*, 2012; 11: 25–28.
26) Goyal PK, Verma P, Sharma P, Parmar J, Agarwal A. Evaluation of anti-cancer and anti-oxidative potential of *Syzygium Cumini* against benzo[a]pyrene (BaP) induced gastric carcinogenesis in mice. *Asian Pac J Cancer Prev*, 2010; 11: 753–758.
27) Ayyanar M, Subash-Babu P, Ignacimuthu S. *Syzygium cumini* (L.) Skeels., a novel therapeutic agent for diabetes: folk medicinal and pharmacological evidences. *Complement Ther Med*, 2013; 21: 232–243.
28) Kato M, Kumasaka MY, Ohnuma S, Furuta A, Kato Y, Shekhar HU *et al.* Comparison of barium and arsenic concentrations in well drinking water and in human body samples and a novel remediation system for these elements in well drinking water. *PLoS One*, 2013; 8: e66681.
29) Liao YT, Chen CJ, Li WF, Hsu LI, Tsai LY, Huang YL *et al.* Elevated lactate dehydrogenase activity and increased cardiovascular mortality in the arsenic-endemic areas of southwestern Taiwan. *Toxicol Appl Pharmacol*, 2012; 262: 232–237.
30) Huda N, Hossain S, Rahman M, Karim MR, Islam K, Mamun AA *et al.* Elevated levels of plasma uric

acid and its relation to hypertension in arsenic-endemic human individuals in Bangladesh. *Toxicol Appl Pharmacol*, 2014; 281: 11–18.

31) Sung TC, Huang JW, Guo HR. Association between arsenic exposure and diabetes: a meta-analysis. *Biomed Res Int*, 2015; 2015: 368087. doi: 10.1155/2015/368087.
32) Liu S, Guo X, Wu B, Yu H, Zhang X, Li M. Arsenic induces diabetic effects through beta-cell dysfunction and increased gluconeogenesis in mice. *Sci Rep*, 2014; 4: 6894. doi: 10.1038/srep06894.
33) Johnson RJ, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M *et al.* Sugar, uric acid, and the etiology of diabetes and obesity. *Diabetes*, 2013; 62: 3307–3315.
34) Wei F, Chang B, Yang X, Wang Y, Chen L, Li WD. Serum uric acid levels were dynamically coupled with hemoglobin A1c in the development of type 2 diabetes. *Sci Rep*, 2016; 6: 28549. doi: 10.1038/srep28549.
35) Jia Z, Zhang X, Kang S, Wu Y. Serum uric acid levels and incidence of impaired fasting glucose and type 2 diabetes mellitus: a meta-analysis of cohort studies. *Diabetes Res Clin Pract*, 2013; 101: 88–96.
36) Anwar-Mohamed A, Abdelhamid G, Amara IEA, El-Kadi AOS. Differential modulation of aryl hydrocarbon receptor regulated enzymes by arsenite in the kidney, lung, and heart of C57BL/6 mice. *Arch Toxicol*, 2012; 86: 897–910.
37) Kapaj S, Peterson H, Liber K, Bhattacharya P. Human health effects from chronic arsenic poisoning—a review. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 2006; 41: 2399–2428.
38) Khan MMH, Aklimunnessa K, Ahsan N, Kabir M, Mori M. 2006. Case-control study of arsenicosis in some arsenic contaminated villages of Bangladesh. *Sapporo Med J*, 2006; 75: 51–61.
39) Shi H, Shi X, Liu KJ. Oxidative mechanism of arsenic toxicity and carcinogenesis. *Mol Cell Biochem*, 2004; 255: 67–78.
40) Golub MS, Macintosh MS, Baumrind N. Developmental and reproductive toxicity of inorganic arsenic: Animal studies and human concerns. *J Toxicol Environ Health*, 1998; 1: 199–241.
41) Kozul-Horvath CD, Zandbergen F, Jackson BP, Enelow RI, Hamilton JW. Effects of low-dose drinking water arsenic on mouse fetal and postnatal growth and development. *PLoS One*, 2012; 7: e38249. doi: 10.1371/journal.pone.0038249.
42) Ruan ZP, Zhang LL, Lin YM. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. *Molecules*, 2008; 13: 2545–2556.
43) Eshwarappa RS, Iyer RS, Subbaramaiah SR, Richard SA, Dhananjaya BL. Antioxidant activity of *Syzygium cumini* leaf gall extracts. *Bioimpact*, 2014; 4: 101–107.
44) Singh MK, Yadav SS, Yadav RS, Singh US, Shukla Y, Pant KK *et al.* Efficacy of crude extract of *Emblica officinalis* (amla) in arsenic-induced oxidative damage and apoptosis in splenocytes of mice. *Toxicol Int*, 2014; 21: 8–17.
45) Liu J, Waalkes MP. Liver is a target of arsenic carcinogenesis. *Toxicol Sci*, 2008; 105: 24–32.
46) Noman AS, Dilruba S, Mohanto NC, Rahman L, Khatun Z, Riad W *et al.* Arsenic-induced histological alterations in various organs of mice. *J Cytol Histol*, 2015; 6: 323. doi: 10.4172/2157-7099.1000323.
47) Watanabe T and Hirano S. Metabolism of arsenic and its toxicological relevance. *Arch Toxicol*, 2013; 87: 969–979.
48) Das N, Paul S, Chatterjee D, Banerjee N, Majumder NS, Sarma N *et al.* Arsenic exposure through drinking water increases the risk of liver and cardiovascular diseases in the population of West Bengal, India. *BMC Public Health*, 2012; 12: 639. doi: 10.1186/1471-2458-12-639.
49) Saad SY, Alkharfy KM, Arafah MM. Cardiotoxic effects of arsenic trioxide/imatinib mesilate combination in rats. *J Pharm Pharmacol*, 2006; 58: 567–573.
50) Dua TK, Dewanjee S, Gangopadhyay M, Khanra R, Zia-Ul-Haq M, De Feo V. Ameliorative effect of water spinach, *Ipomea aquatica* (Convolvulaceae), against experimentally induced arsenic toxicity. *J Transl Med*, 2015; 13: 81. doi: 10.1186/s12967-015-0430-3.
51) Yang B, Fu J, Zheng H, Xue P, Yarborough K, Woods CG *et al.* Deficiency in the nuclear factor E2-related factor 2 renders pancreatic β-cells vulnerable to arsenic-induced cell damage. *Toxicol Appl Pharmacol*, 2012; 264: 315–23. doi: 10.1016/j.taap.2012.09.012.
52) Bandaru P, Shankar A. Association between serum uric acid levels and diabetes mellitus. *Int J Endocrinol*, 2011; 2011: 604715. doi: 10.1155/2011/604715.
53) Nan H, Dong Y, Gao W, Tuomilehto J, Qiao Q. Diabetes associated with a low serum uric acid level in a general Chinese population. *Diabetes Res Clin Pract*, 2007; 76: 68–74.
54) Paul N, Akhand AA, Babu SU, Islam N, Ahsan N. *Syzygium cumini* leaf extract showed vibriocidal activity on selected diarrhea causing bacteria. *J Adv Lab Res Biol*, 2011; 2: 127–132.