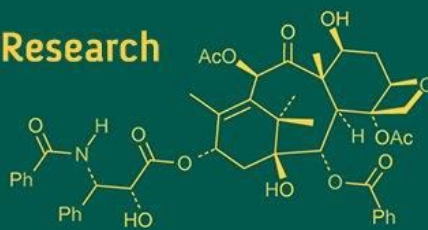


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Assessment of ameliorative potential of *Aegle marmelos* fruit extract against hematological and biochemical alterations in sub-acute arsenic toxicity in mice

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Abstract

The study was conducted to assess the changes in hematology and serum enzymatic profile of mice in sub-acute toxicity of sodium arsenite and the mitigating effect of supplementation of *Aegle marmelos* fruit extract during this toxicity. A total of 60 BALB/c mice were randomly divided into 5 groups (control-I, vehicle-II, and treatment groups III, IV and V). Group III was administered with sodium arsenite orally for 28 days, group IV was administered orally with *A. marmelos* fruit extract, and group V was administered sodium arsenite and *A. marmelos* fruit extract for 28 days. The clinical signs observed were decreased activity, anorexia, diarrhea and alopecia. Hemoglobin, packed cell volume, and total erythrocyte count were increased in arsenic treated group as compared to the control group, which could be because of dehydration due to diarrhea. Levels of serum alanine aminotransferase, aspartate transaminase, creatinine and urea of male and female mice increased in arsenic toxicity group. The co-administration of *A. marmelos* was shown to counteract the toxic effect of sodium arsenite. *A. marmelos* administration reduced damage to the liver and kidney, as it was noted that levels of serum enzymes in group V were less than those in group III and did not differ from control. It is concluded that administration of *A. marmelos* has reduced the toxic effects of sodium arsenite, particularly to the liver and kidney, which in turn helped to maintain the serum enzyme levels.

Keywords: Arsenic toxicity, anemia, bael fruit, hepatotoxicity, nephrotoxicity

Introduction

Water is the most essential nutrient for sustaining life. But the surface water has usually been contaminated with sewage and industrial waste disposal, rendering it impotable. Groundwater is also extensively used globally for drinking and domestic water needs. The contamination of groundwater by heavy metals like arsenic has been detected globally. The contamination of groundwater occurs mostly through natural processes, industrial activities, agricultural practices and veterinary drugs (Ratnaike, 2003; Zubair *et al.*, 2017) ^[1, 2]. An estimated 108 countries are affected by arsenic contamination with arsenic levels in drinking water above the World Health Organization (WHO) provisional guideline value of 10 µg/L (or 10 ppb) (WHO, 2022) ^[3]. The major countries affected are from Asia and Europe, followed by Africa, America and Australia. In Asia, Bangladesh, India, Pakistan, China, Nepal, Vietnam, Burma, Thailand and Cambodia, are the most affected (Mukherjee *et al.* 2021) ^[4]. In India, 20 states and 4 Union Territories have so far been affected by arsenic contamination in groundwater (Shaji *et al.*, 2021) ^[5]. Chronic arsenic exposure results in severe health issues like cancers of the skin, lung, kidney and bladder, coronary heart disease, bronchiectasis, hyperkeratosis, muscle and joint pains and various gastric problems, sensory and motor polyneuritis, hepatitis/chronic liver disease, chronic diarrhea, and aplastic anemia (Pal *et al.*, 2009; Shaji *et al.*, 2021) ^[5, 6]. Acute cases of Arsenic poisoning occur more commonly from accidental ingestion of insecticides or pesticides by the animals. The animals clinically develop nausea, vomiting, excessive salivation, abdominal pain, and profuse watery diarrhea (Ratnaike, 2003) ^[1]. Arsenic exposure leads to imbalance between reactive oxygen species and antioxidants in the cells resulting in oxidative injury to cell.

The role of antioxidants is very successful in combating arsenic-related diseases (Patrick, 2003) [7]. *Aegle marmelos correa* (Bael fruit) belongs to the Rutaceae family. It is a tropical deciduous tree that grows throughout India, including the Sub-Himalayan forests of Bengal, Central and South India, and Burma (Singh and Chaurasiya, 2014) [8]. Bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, and terpenoids (Bhanot, 2018) [9]. So the present study was conducted to analyze the protective effect of *Aegle marmelos* fruit extract on hemato-biochemical changes induced by arsenic toxicity in mice.

Materials and Methods

To induce sub-acute toxicity in mice, Sodium Arsenite was dissolved in distilled water and was given daily for 28 days @ 3 mg/kg body weight by oral intubation (Singh *et al.*, 2014) [10].

To prepare the ethanolic extract of *A. marmelos* fruits, the unripe fruits of *A. marmelos* (Bael) were selected and procured from the local area and were identified by the Faculty of Pharmacy, Bhupal Nobles' University, Udaipur (Raj.), India. After washing and breaking off the shell, the pulp was scooped, and slurry was prepared and sieved through a 20 mesh sieve and spread onto flat trays for drying under shade. The dried flakes were grinded to powder. The powder obtained was stored in sterile polythene bags under refrigerated conditions until further use. The powder obtained was used for ethanolic extraction. For this 10 gm of *A. marmelos* fruit powder was mixed with 100 mL of ethanol and kept for 2 days at room temperature with intermittent shaking. This solution was then filtered and heated at 40 °C in water bath until the solvent was evaporated and a dry extract was obtained (Bagri and Kumar, 2024) [11]. The ethanol extract was stored in a refrigerator at 4 °C till further use.

Experimental animals

Sixty (60) clinically healthy BALB/c mice weighing 25-30 g between 2-3 weeks of age, of either sex, the males and females were housed separately and kept in polypropylene cages under standard conditions (Temperature 23±2 °C; R.H. 55±10% and light-12 hours light: 12 hours dark cycle). Standard mice pelletized feed and water was provided throughout the experimental period. The necessary approval from the Institutional Animal Ethical Committee (IAEC) was obtained. After acclimatization for 10 days, the total of 60 mice were randomly divided into 5 groups, viz. Groups I, II, III, IV, and V each contain 12 mice. Each Group was subdivided into 'm' (male) and 'f' (female) with 6 mice in each. Group I (6 male, 6 female) served as the control group, and Group II served as the vehicle. Group III (6 male, 6 female) served as the toxicity group (SA), which was administered with sodium arsenite @ 3 mg/Kg body weight (Singh *et al.* 2014) [10]. Group IV (6 male, 6 female) received ethanol extract of *A. marmelos* (AME) fruit @ 200 mg/body weight (Akhouri *et al.*, 2020) [12]. Group V (SA+AME) (6 male, 6 female) received sodium arsenite @ 3 mg/Kg body weight as well as ethanolic extract of *A. marmelos* fruit @ 200 mg/body weight by oral intubation for 28 days. Mice were observed individually after dosing, daily for a total of 28 days. Clinical signs and mortality were observed daily during the experimental period. The body weight of each mouse from all groups was recorded weekly.

Hematological parameters

On the 28th day of the study, blood was collected in ethylene diamine tetra acetic acid (EDTA) vials from the retro-orbital sinus of each mouse (under Isoflurane inhalation anesthesia) of all the groups and hematology was performed by using automatic analyzer (Mindray, RM-303-03) at the Department of Veterinary Physiology and Biochemistry. The parameters analyzed were Hemoglobin estimation (Hb), Total erythrocyte count (TEC), Packed cell volume (PCV), Total Leukocyte count (TLC), and Differential leukocyte count (DLC).

Biochemical parameters

For estimation of serum biochemical levels, blood samples were collected in sterile tubes without an anticoagulant. After centrifugation of blood at 3000 rpm for 15 min at 4 °C, and serum was separated and stored at -20 °C till further analysis. The serum alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine, urea, total protein, albumin and globulin levels were analyzed using the semi-automatic biochemistry analyzer (Biogen-BGS-246) using a liquid stable reagents kit (Aspen Laboratories Pvt. Ltd.).

Statistical analysis

The data obtained was subjected to statistical analysis by applying the analysis of variance (ANOVA) technique employing SPSS software. Statistical significance between means of various groups was tested using the Duncan comparison test at $p < 0.05$.

Results and Discussion

Sub-acute arsenic toxicity was experimentally induced in mice and alterations developed in erythrocyte and leukocyte related parameters of blood and serum enzymes related to liver and kidney function were studied. Further, the mitigating effect of administration of *Aegle marmelos* fruit extract on these hemato-biochemical parameters was assessed. Clinical signs observed were abnormal gait, dullness, anorexia, depression, hair loss and ill health. Reduced feed and water intake were observed after 21 days in arsenic treated group III. Hair fall was also observed in toxicity group III after 21 days of dosage. No change in feed and water intake was observed in groups I, II and IV. No mortality occurred during the experimental period in any of the groups. Similar clinical signs were observed by Sandhu & Brar (2008) [13] and Satapathy *et al.* (2021) [14]. The ameliorated group V (SA + AME) did not show loss of hair or change in food and water intake.

Weekly Body weight

The body weight (g) of mice (Table 1) on 0, 7th, 14th, and 21st day of the experiment did not show a significant effect. The body weight of male mice on 28th day showed significantly lower values in group III (SA) as compared to control group I (C) and group V (SA+ AM). The decreased body weight of group III male mice on the 28th day might be due to feed and water avoidance and impairment in the function of the digestive system due to arsenic toxicity. Similar observation was recorded by Bhattacharya & Halder (2012) [15], Barai *et al.* (2017) [16] and Satapathy *et al.* (2021) [14]. There was no significant difference between the control and the ameliorated group V.

Hematological Parameters

The mean values of hematological parameters (Hb, PCV, TEC, TLC and DLC) of different experimental groups are shown in Tables 2 & 3. The hemoglobin concentration showed a significant increase in the sodium arsenite-treated group (Group III) in both male and female mice compared with the control group. The increased Hb levels observed in arsenic-exposed animals may be associated with hemoconcentration due to dehydration as decreased water intake was noted or compensatory erythropoietic responses triggered by oxidative stress and tissue hypoxia caused by arsenic toxicity. Arsenic exposure has been reported to influence erythropoiesis and alter hematological parameters through oxidative damage to blood cells and interference with bone marrow function (Flora, 2011) ^[17]. Similar increases in hemoglobin concentration following arsenic exposure have been documented in experimental animals subjected to sub-chronic arsenic toxicity (Mazumder, 2008) ^[18].

The packed cell volume (PCV) values also showed a significant increase in the arsenic-treated group compared with the control group in both sexes. The elevated PCV values could be due to reduced plasma volume due to dehydration or hemoconcentration (Jain, 1993) ^[19]. The total erythrocyte count (TEC) in both male and female mice was significantly higher in the arsenic-treated group compared with the control group. The increased TEC value could be due to dehydration as diarrhea was also observed clinically or it could be due to a compensatory response to oxidative stress or hypoxic conditions induced by arsenic toxicity (Flora *et al.*, 2008) ^[20]. In the *A. marmelos* extract-treated group (Group IV), the values of Hb, PCV, and TEC were also higher than those of the control group. This may be attributed to the action of bioactive phytochemicals such as flavonoids, phenolics, present in *Aegle marmelos*, that are known to improve hematopoiesis and protect blood cells from oxidative damage (Sharma *et al.* 2011) ^[21]. The reduced levels of ROS will enhance erythrocyte stability and support normal hematological function. In the sodium arsenite plus *A. marmelos* extract-treated group (Group V), the values of Hb, PCV, and TEC remained higher than the control group but were lower than those of the arsenic toxicity group III. This suggests that *Aegle marmelos* extract may exert a modulatory effect on arsenic-induced hematological alterations, possibly by reducing oxidative stress through its phytochemicals. Similar protective effects of *A. marmelos* against heavy metal-induced hematological disturbances have been reported in earlier studies (Flora, 2011; Panda *et al.*, 2007) ^[17, 22].

Similarly, total leukocyte count (TLC) was markedly elevated in male mice of the arsenic toxicity group compared with the control group, indicating possible stimulation of immune or inflammatory responses. Leukocytosis may be due to protective reaction of the body against toxic stress and tissue injury induced by arsenic exposure. Increased leukocyte counts following heavy metal exposure have been reported previously and are considered indicative of inflammatory or immune responses to toxic insult (Weiss & Wardrop, 2010) ^[23]. The values of TLC in the sodium arsenite plus *A. marmelos* extract-treated group (Group V), were lower than those of the arsenic-treated group III, particularly in females, indicating that *Aegle marmelos* extract may reduce arsenic-induced inflammatory or immune responses. The normalization of TLC following

plant-based antioxidant treatment has been reported in several toxicological studies and is attributed to the anti-inflammatory and immunomodulatory properties of phytochemicals (Sharma *et al.*, 2011) ^[21].

Differential leukocyte counts, including lymphocytes, neutrophils, and other granulocytes, are important indicators of immune status and physiological responses to toxic agents. Alterations in leukocyte levels reflect presence of inflammation, immune modulation, or stress-related changes in blood induced by toxic substances such as arsenic. Researchers have reported that Arsenic exposure affects immune function by inducing oxidative stress, altering cytokine production, and modifying leukocyte distribution in blood (Flora, 2011; Rahman *et al.*, 2012) ^[17, 24].

The percentage of lymphocytes in both male and female mice of different experimental groups did not show statistically significant differences as compared to the control group. However, non-significantly increase was observed in the *A. marmelos* extract-treated male mice of group IV. It is well known that lymphocytes play a key role in adaptive immunity, and alteration in their levels is seen in stress, toxicity, and immune stimulation (Weiss & Wardrop, 2010) ^[23]. Arsenic exposure has been shown to modulate lymphocyte proliferation and function, sometimes resulting in immunosuppression or altered immune responses depending on the exposure level and duration (Flora *et al.*, 2008) ^[20]. The less variability in lymphocyte counts observed in the present study indicate that the present duration or dosage of arsenic exposure did not produce marked suppression of lymphoid cells.

The neutrophil percentage showed a slight increase in the arsenic-treated group (Group III) in both male and female mice compared with the control group, although the differences were not statistically significant. Neutrophils constitute the primary line of defense against infections and are often elevated during inflammatory or stress conditions. (Jain, 1993) ^[19]. The mild increase observed in neutrophil counts in arsenic-treated mice therefore reflect a response to oxidative stress or inflammatory processes induced by arsenic toxicity. In the *Aegle marmelos* extract-treated group (Group IV), neutrophil percentages were slightly reduced compared with the arsenic-treated group. This finding may suggest an anti-inflammatory or immunomodulatory effect of the plant extract. *Aegle marmelos* is known to contain several phytoconstituents such as flavonoids, alkaloids, and phenolic compounds that exhibit antioxidant and anti-inflammatory activities (Sharma *et al.*, 2011) ^[21]. These compounds may help reduce oxidative stress and inflammatory reactions, thereby stabilizing leukocyte distribution in the bloodstream.

The other granulocyte population (including eosinophils and basophils) showed relatively minor variations among the experimental groups. In male mice, the control group exhibited slightly higher values compared with the arsenic-treated group III. Granulocytes other than neutrophils are generally involved in allergic responses, parasitic infections, and inflammatory processes (Weiss & Wardrop, 2010) ^[23]. The decreased percentage of other granulocytes in group III could be due to altered leukocyte dynamics during toxicity, where neutrophils increased while other granulocytes decreased. In arsenic toxicity, inflammatory and stress responses may favor neutrophilia, which proportionally reduces the relative percentage of other granulocytes in differential leukocyte counts (Jain, 1993) ^[19].

In Group V (sodium arsenite plus *A. marmelos* extract-treated group), lymphocyte and neutrophil percentages were generally comparable to those observed in the control group. This suggests that the plant extract may have moderated the potential immunotoxic effects of arsenic exposure. The antioxidant properties of *Aegle marmelos* might have neutralized reactive oxygen species and reduced oxidative damage to immune cells (Panda *et al.*, 2007) [22]. These findings support previous reports suggesting that medicinal plants rich in phenolic compounds can help mitigate oxidative stress and maintain immune function during toxic exposure (Flora, 2011; Sharma *et al.*, 2011) [17, 21].

Biochemical Parameters

The Mean values of aspartate transaminase (AST), alanine aminotransferase (ALT), Creatinine and Urea of different experimental groups are shown in Table 4. The results showed a significant increase in AST values of both male and female mice of toxicity group III as compared to the control. Similar observations were made by Bhattacharya & Haldar (2012) [15] and Biswas *et al.* (2017) [25]. The AST values of ameliorated group V were significantly lower than group III and did not differ from the control. The ALT values of toxicity group III (male and female) were significantly higher than those of the control group. Moreover, the ALT values of group V (male and female) were significantly lower than group III and did not differ from the control. Therefore, present study demonstrated a significant elevation in AST and ALT activities in both male and female mice of the arsenic-treated toxicity group (Group III) compared with the control group. The levels of these enzymes indicate the hepatocellular health, and so their increased serum levels suggest that hepatocytes are damaged and the enzymes have leaked out due to membrane permeability alterations and cellular necrosis. Similar elevations in liver enzymes following arsenic exposure have been reported by Mazumder (2008) [18] indicating impaired liver function and hepatocellular degeneration. Research reports show that arsenic exposure damages hepatocytes through oxidative stress, mitochondrial damage, and lipid peroxidation, which lead to the leakage of intracellular AST and ALT into the bloodstream (Flora, 2011; Rahman *et al.*, 2012) [17, 24]. The reduced level of serum AST and ALT in group V (SA+ AM) suggests a protective role of the fruit extract against arsenic-induced hepatic damage. Furthermore, the enzyme levels in group V did not differ significantly from those of the control group I, indicating restoration of near-normal hepatic function in group V. The hepatoprotective activity of *Aegle marmelos* might be due to the neutralization of ROS generated during arsenic metabolism by the flavonoids, phenolics, coumarins, and tannins present in *Aegle marmelos* fruit extract (Panda *et al.*, 2007; Sharma *et al.*, 2011) [21, 22].

The serum creatinine levels in male mice of the group III (arsenic toxicity) were significantly higher than those of the control group I. As creatinine is a sensitive indicator of glomerular filtration rate, the elevated serum levels in group III indicates damage to renal tissues and reduced kidney function. Similar changes have been observed by other researchers (Gaim *et al.*, 2015) [26]. It is reported that arsenic induces tubular degeneration and inflammation in renal tissues (Flora *et al.*, 2008) [20]. The results further revealed that creatinine values of Group IV were significantly lower than those of Groups II, III, and V, indicating comparatively

better renal functional status in group IV (AM extract treated). However, female mice in the group V (SA + AM) exhibited significantly higher creatinine values compared with the control group, suggesting that although the *Aegle marmelos* treatment provided partial protection, complete normalization of renal function in females was not achieved. The serum urea levels were also significantly higher in both male and female mice of group III (SA) compared with the control group I. This indicates impaired renal function and decreased clearance of nitrogenous metabolic waste products. Earlier researchers have also reported elevated blood urea and creatinine levels in arsenic-induced nephrotoxicity in laboratory animals and human studies (Rahman *et al.*, 2012; Mazumder, 2008) [18, 24]. Furthermore, in the Group V (SA+AM), the urea values of male mice did not differ significantly from those of the control group I, indicating that *Aegle marmelos* supplementation has ability to restore renal function in male mice. However, urea levels in females of this group were lower than group III but still remained significantly higher than those of the control group I, suggesting relatively less protection in female mice. Such sex-related differences in the protective response may be associated with hormonal influences or differences in detoxification pathways and antioxidant enzyme activities (Flora *et al.*, 2008) [20].

Therefore, the results clearly indicate that arsenic exposure induces significant hepatic and renal dysfunction in mice, as evidenced by elevated serum AST, ALT, creatinine, and urea levels. Similar observation was recorded by Bhattacharya & Haldar (2012) [15], Amer *et al.* (2016) [27]. Treatment with *Aegle marmelos* appears to mitigate these toxic effects, possibly through its phytochemicals that possess antioxidant properties. However, the degree of protection appears to vary between sexes and among different biochemical parameters.

The mean levels of total protein, albumin, and globulin of different experimental groups are represented in Table 5. The total protein levels in both male and female mice did not show statistically significant differences among the experimental groups when compared with the control group. However, a slight reduction was observed in the arsenic-treated group (Group III) in males. Flora (2011) reported that arsenic interferes with protein metabolism by inducing oxidative stress and altering hepatic synthetic activity. Moreover, in group IV (AM) comparatively higher total protein levels were observed which may be attributed to the nutritive and antioxidant properties of the plant extract, which may enhance protein synthesis. Group III (arsenic toxicity) revealed reduced level serum albumin levels as compared to the control group I in both sexes, although the reduction was not statistically significant. This might be due to the hepatocellular damage that has impaired albumin synthesis (Guyton & Hall, 2016) [28]. Previous studies have also reported decreased albumin concentrations in animals exposed to arsenic (Flora *et al.*, 2008) [20]. In contrast, the group IV (AM) exhibited comparatively higher albumin levels than the control and arsenic-treated groups, particularly in males. This suggests that the plant extract might have enhanced hepatic protein synthesis. In the Group V (sodium arsenite along with *Aegle marmelos* extract), albumin levels were lower than those of the control group I in males but relatively closer to normal in females. This suggests that although the *Aegle marmelos* extract provides some protective effect against arsenic toxicity, but the extent

of recovery may vary depending on the severity of toxicity and sexes. Significant differences were not observed in the globulin concentrations among different experimental groups in both male and female mice. Overall, the results suggest that arsenic exposure may disrupt protein

metabolism and liver function, and supplementation with *Aegle marmelos* fruit extract may help maintain normal serum protein levels through its hepatoprotective and antioxidant properties.

Table 1: Mean body weight (g) of mice (male and female) of all the experimental groups.

Groups	0 day		7 days		14 days		21 days		28 days	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
I (control)	38.54 ^a ±1.75	33.77 ^a ±2.37	38.09 ^a ±2.23	33.37 ^a ±2.76	38.84 ^a ±2.11	31.51 ^a ±3.33	39.16 ^a ±2.03	32.74 ^a ±3.59	40.35 ^b ±2.16	33.01 ^a ±3.63
II (Vehicle)	39.24 ^a ±1.44	33.14 ^a ±1.89	39.59 ^a ±1.57	32.55 ^a ±1.82	40.17 ^a ±1.40	31.47 ^a ±2.49	40.35 ^a ±1.62	31.22 ^a ±1.90	39.23 ^{ab} ±2.68	32.93 ^a ±1.93
III (SA)	39.31 ^a ±1.59	30.91 ^a ±2.45	39.34 ^a ±1.34	30.51 ^a ±2.87	39.79 ^a ±1.15	31.30 ^a ±2.62	39.89 ^a ±0.99	31.99 ^a ±2.52	34.00 ^a ±1.78	32.62 ^a ±2.35
IV (AME)	39.11 ^a ±1.03	31.99 ^a ±2.64	38.31 ^a ±1.66	31.00 ^a ±3.22	37.92 ^a ±1.91	31.27 ^a ±3.09	38.64 ^a ±1.41	31.24 ^a ±2.76	38.41 ^{ab} ±0.97	31.97 ^a ±2.73
V (SA + AME)	40.45 ^a ±1.13	30.49 ^a ±2.67	37.92 ^a ±0.85	29.28 ^a ±2.56	38.96 ^a ±0.85	30.07 ^a ±0.59	36.61 ^a ±1.28	29.37 ^a ±1.78	39.81 ^b ±0.91	30.61 ^a ±2.16

All values are represented Mean ± SE; n = 12 (6 male and 6 female) in each group; values bearing different superscript in the same column differ significantly at $p < 0.05$.

Table 2: Mean values of hematological parameters of different experimental groups.

Groups	Hb (g/dL)		Packed Cell Volume (%)		Total Erythrocyte Count ($\times 10^6/\mu\text{L}$)		Total Leukocyte Count ($\times 10^3/\mu\text{L}$)	
	Male	Female	Male	Female	Male	Female	Male	Female
I (control)	7.95 ^a ±0.30	9.35 ^a ±0.79	29.13 ^a ±1.54	34.95 ^a ±2.56	4.97 ^a ±0.29	5.45 ^a ±0.27	3.75 ^a ±0.44	4.00 ^a ±1.09
II (Vehicle)	9.73 ^{ab} ±1.02	10.86 ^{ab} ±1.08	35.08 ^{ab} ±3.81	41.50 ^{ab} ±3.64	5.29 ^a ±0.52	6.06 ^a ±0.56	4.36 ^{ab} ±0.79	3.98 ^a ±0.73
III (SA)	11.90 ^b ±0.97	13.20 ^b ±0.86	45.75 ^c ±3.45	49.10 ^b ±3.79	7.17 ^b ±0.43	7.51 ^b ±0.43	7.76 ^b ±1.03	7.60 ^a ±3.13
IV (AM)	12.28 ^b ±0.90	12.53 ^b ±0.45	43.11 ^{bc} ±3.55	48.00 ^b ±2.88	7.48 ^b ±0.48	7.18 ^b ±0.25	7.28 ^{ab} ±1.19	5.63 ^a ±0.67
V (SA + AM)	10.86 ^b ±0.69	12.88 ^b ±0.61	41.30 ^{bc} ±2.52	48.80 ^b ±2.13	6.55 ^b ±0.33	7.39 ^b ±0.28	7.03 ^{ab} ±1.90	3.30 ^a ±0.77

All values are represented Mean ± SE; n = 12 (6 male and 6 female) in each group; values bearing different superscript in the same column differ significantly at $p < 0.05$.

Table 3: Mean values of differential leucocyte count (%) of mice of different experimental groups.

Groups	Lymphocytes		Neutrophils		Other Granulocyte	
	Male	Female	Male	Female	Male	Female
I (control)	35.55 ^a ±2.65	54.72 ^a ±8.74	57.93 ^a ±2.35	41.78 ^a ±8.48	6.51 ^b ±0.49	3.50 ^a ±0.96
II (Vehicle)	37.05 ^a ±8.56	50.82 ^a ±7.99	56.28 ^a ±8.85	45.20 ^a ±7.60	6.67 ^b ±0.18	3.98 ^a ±0.92
III (SA)	36.18 ^a ±7.52	47.16 ^a ±5.45	59.31 ^a ±6.84	49.81 ^a ±4.48	4.50 ^a ±0.81	3.03 ^a ±0.80
IV (AM)	46.03 ^a ±5.00	54.52 ^a ±5.12	49.98 ^a ±4.47	38.64 ^a ±3.54	4.45 ^a ±0.89	6.84 ^a ±2.12
V (SA + AM)	31.78 ^a ±4.62	55.21 ^a ±4.53	62.56 ^a ±4.66	40.63 ^a ±3.65	5.65 ^{ab} ±0.54	4.16 ^a ±0.94

All values are represented Mean ± SE; n = 12 (6 male and 6 female) in each group; values bearing different superscript in the same column differ significantly at $p < 0.05$.

Table 4: Mean values of biochemical parameters of mice of different experimental groups.

Groups	Aspartate Transaminase (U/L)		Alanine aminotransferase (U/L)		Creatinine (mg/dL)		Urea (mg/dL)	
	Male	Female	Male	Female	Male	Female	Male	Female
I (control)	6.75 ^a ±0.83	7.85 ^a ±1.48	5.52 ^a ±0.36	26.92 ^a ±3.27	0.51 ^{ab} ±0.08	0.54 ^{ab} ±0.04	44.51 ^c ±2.87	26.80 ^a ±3.51
II (Vehicle)	27.26 ^a ±4.76	19.48 ^a ±2.87	26.80 ^a ±3.95	26.79 ^a ±4.59	0.56 ^b ±0.06	0.67 ^{abc} ±0.03	17.61 ^a ±1.77	30.06 ^{ab} ±3.57
III (SA)	83.49 ^b ±21.25	67.53 ^b ±17.47	72.01 ^b ±18.27	41.57 ^b ±4.44	0.75 ^c ±0.06	1.01 ^{bc} ±0.16	66.91 ^d ±8.18	39.42 ^{bc} ±3.01
IV (AM)	30.44 ^a ±4.66	22.41 ^a ±3.10	5.6 ^a ±0.55	22.19 ^a ±1.34	0.35 ^a ±0.02	0.38 ^a ±0.03	25.11 ^{ab} ±3.48	32.08 ^{ab} ±3.55
V (SA + AM)	38.74 ^a ±9.01	34.04 ^a ±8.62	22.76 ^a ±2.21	29.75 ^{ab} ±6.11	0.63 ^{bc} ±0.05	1.04 ^c ±0.29	36.59 ^{bc} ±3.78	42.79 ^c ±1.99

All values are represented Mean ± SE; n = 12 (6 male and 6 female) in each group; values bearing different superscript in the same column differ significantly at $p < 0.05$.

Table 5: Mean values of total protein, albumin, and globulin levels in mice of different experimental groups.

Groups	Total Protein (g/dL)		Albumin (g/dL)		Globulin (g/dL)	
	Male	Female	Male	Female	Male	Female
I (control)	6.79 ^a ±0.59	6.49 ^a ±0.53	3.60 ^b ±0.60	3.76 ^a ±0.44	3.18 ^a ±0.79	2.73 ^a ±0.70
II (Vehicle)	5.92 ^a ±0.52	6.33 ^a ±0.36	3.26 ^{ab} ±0.34	3.33 ^a ±0.40	2.66 ^a ±0.48	2.99 ^a ±0.63
III (SA)	6.05 ^a ±0.3	6.87 ^a ±1.09	3.24 ^{ab} ±0.21	3.20 ^a ±0.15	2.81 ^a ±0.49	3.66 ^a ±1.00
IV (AM)	7.22 ^a ±0.78	8.25 ^a ±1.35	4.22 ^b ±0.51	4.07 ^a ±0.50	3.00 ^a ±0.39	4.17 ^a ±1.34
V (SA + A M)	5.70 ^a ±0.69	6.84 ^a ±1.81	2.24 ^a ±0.28	3.54 ^a ±0.88	3.46 ^a ±0.67	3.29 ^a ±0.96

All values are represented Mean ± SE; n = 12 (6 male and 6 female) in each group; values bearing different superscript in the same column differ significantly at $p < 0.05$.

Conclusion

Overall, the present study demonstrated that sodium arsenite induces significant alterations in hematological parameters and serum levels of enzymes related to liver and kidney

function in mice. Further, the administration of *Aegle marmelos* fruit extract showed a protective effect against arsenic-induced toxicity, as evidenced by the improvement in several biochemical and hematological parameters. The

ameliorative action of the plant extract may be attributed to its antioxidant phytoconstituents, which help reduce oxidative stress to cells. Therefore, *Aegle marmelos* fruit possesses potential protective properties against arsenic-induced toxicity and further studies focusing on molecular mechanisms, dose optimization, and long-term exposure trials can be done to better understand its therapeutic potential and possible application as a natural protective agent against arsenic toxicity.

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Conflict of interest

The authors have no conflict of interests regarding the present research work.

Ethical approval

Approval was obtained from the Institutional Animal Ethics Committee (IAEC/RES/01/01/2021) before the initiation of the experiment on mice.

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