



Protective Effect of *Lactobacillus sporogenes* against Arsenic-Induced Hematological Alterations in Male Albino Wistar Rats

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Abstract

The aim of the present study was to evaluate the arsenic-induced hemotoxicity and to evaluate the protective effects of *Lactobacillus sporogenes* in male albino Wistar rats. A total of 36 adult male albino Wistar rats were procured and divided into 3 groups of 12 animals each. Group 1 rats served as control, group 2 rats were administered sodium arsenite (@5 mg/kg BW/day), groups 3 rats were supplemented with *L. sporogenes* (@15 million spores/kg BW/day) along with sodium arsenite administered along with sodium arsenite orally daily for 28 consecutive days. Weekly body weights, hematological profile, and erythrocyte morphology were assessed. Significant ($P < 0.05$) reduction in mean weekly body weights (g) was observed in group 2 than group 1; however, a significant ($P < 0.05$) increase in weekly body weights was observed in group 3 as compared to group 2. A significant ($P < 0.05$) decrease in erythrocyte-related parameters and platelet counts, and a significant ($P < 0.05$) leukocytosis, relative lymphopenia, absolute neutrophilia, and monocytosis were noticed among arsenic-treated rats when compared to the control group. Blood smear of arsenic-treated rats contains echinocytes, microcytes, and spherocytes when compared to control. Scanning electron microscopic examination of blood revealed altered erythrocyte morphology in arsenic-treated rats with poikilocytosis and blebbing of the erythrocyte membrane. Supplementation of *L. sporogenes* along with arsenic resulted in improvement of all the hematological parameters and reduction in morphological abnormalities in comparison to the toxic control group. It is concluded that supplementation of *L. sporogenes* can effectively alleviate the arsenic-induced hematological alterations.

Keywords Arsenic · Lactobacillus · Hemotoxicity · Wistar rats

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Introduction

Heavy metal contamination is a global problem affecting not only humans but also animals. Arsenic (As) is one of the most important naturally occurring environmental toxicant, which is ubiquitously present in the earth's crust; hence, it is a major concern worldwide and also in India. Southeast Asian countries viz. Bangladesh, India, Pakistan, China, Nepal, Vietnam, Burma, Thailand, and Cambodia, are the most affected. In India, 20 states and 4 Union Territories have so far been affected by arsenic contamination in groundwater [18]. Geologic and anthropogenic activities result in leaching out of arsenic from the earth's crust into water bodies, continuous drinking of which leads to chronic arsenic toxicity which is also known as arsenicosis [22]. In natural water bodies, arsenic is mostly found in two states trivalent (As^{3+} , arsenite) and pentavalent (As^{5+} arsenate), both forms are highly toxic inorganic species [6] while the trivalent form is more toxic when compared to pentavalent.

The formation of $O_2^{\bullet -}$ and H_2O_2 in response to arsenic exposure in different cell lines was summarized by Shi et al. (2004). Arsenic exposure leads to its accumulation in erythrocytes by binding to Cys-13 α of hemoglobin which results in retention of arsenic for a longer period in the body [12].

The World Health Organization (WHO) has defined probiotics as “live microorganisms, which when administered in adequate amounts confer a health benefit on the host” [21]. Probiotics are considered functional food. Although many bacterial genera like *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Propionic bacterium*, and *Streptococcus* have been used as probiotics, the most beneficial effects in man are *Lactobacilli* and *Bifidobacteria* [5]. Probiotics, which are capable of colonizing the intestinal tract, are reported to combat many diseases through modulating intestinal microorganisms [9, 15].

Materials and Methods

Experimental Animals

A total of 36 male albino rats (*Wistar* strain) weighing 180–220 g were procured from M/s Vyas Labs, Hyderabad. The rats were housed in solid bottom polypropylene cages at the laboratory animal house facility, College of Veterinary Science, Rajendranagar. Rats were maintained in a controlled environment (temperature 20–22 °C) throughout the course of the experiment. All the rats were provided with a standard pellet diet (M/s VRK Nutritional Solutions, Pune) and distilled water ad libitum throughout the experimental period and were allowed to acclimatize for 7 days. All the experimental animals were observed daily for clinical signs and mortality, if any, during the entire period of study. The experiment was carried out according to the guidelines and with prior approval of the Institutional Animal Ethics Committee (IAEC approval No.6/22/C.V.Sc., Hyd/IAEC I).

Experimental Design

A total of 36 male albino *Wistar* rats were randomly divided into 3 groups consisting of 12 animals in each group.

Group 1: Control

Group 2: Sodium arsenite @ 5 mg/kg BW/day orally for 28 days

Group 3: *Lactobacillus sporogenes* @50 million spores/kg BW/day + sodium arsenite @ 5 mg/kg BW/day orally for 28 days

The dose of sodium arsenite selected is one-eighth of the oral median lethal dose (LD50) values in rats [1]. The dose of probiotic is according to previous studies [17]. Sodium

arsenite and probiotic mixed in distilled water were administered daily to the rats through oral gavage.

Body Weights

Individual body weights of all the rats were recorded by using the electronic balance on day zero and subsequently on the 7th, 14th, 21st, and 28th day of the experiment to study the body weight variations.

Hematology

Two to three milliliters of blood was collected from retro-orbital plexus of rats, with the help of a capillary tube into an anticoagulant-coated vacutainers {K2-EDTA tube, 13 mm \times 75 mm, 4 mL (Rapid Diagnostics Pvt. Ltd., Delhi)} to carry out all hematological parameters. Prior to blood collection, the selected experimental rats were put to fast for 12 h. The blood samples were used for estimation of total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (DLC), and platelets count by using an automatic whole blood analyzer (BC-2800Vet).

Light Microscopy

A peripheral blood smear was prepared on grease-free glass slides and photographed using a light microscope using 1000 \times magnification after staining with the Leishman stain [20].

Scanning Electron Microscopy (SEM)

The blood was directly collected and fixed overnight in 2.5% glutaraldehyde (S.D fine chemicals, Mumbai) in 0.1 M phosphate buffer (pH 7.3 stored at 4 °C), washed in buffer, postfixed in 1% osmium tetroxide (Sigma Aldrich, USA) in 0.1 M phosphate buffer, and dehydrated with ascending grades of acetone (Qualigens Fine Chemicals, Mumbai). The dehydrated specimens were subjected to vacuum desiccation for 45 min and mounted on stubs over the double-sided carbon conductivity tape and uniform sputtering of gold was done with a sputter coater (JEOL-JFC-1600) for 180 s as per the standard protocol of Ruska Labs [11]. Later specimens were observed under a scanning electron microscope (JEOL; JSM-5600, Japan).

Statistical Analysis

Data obtained were subjected to statistical analysis by applying one-way ANOVA using a statistical package for social sciences (SPSS) version 20.0. Differences between means were tested by using Duncan's multiple comparison tests and the significance level was set at $P < 0.05$ [19].

Results

Body Weights (g)

Significantly ($P < 0.05$) lower mean values of weekly body weights (g) were recorded in group 2 than group 1 throughout the experiment. A significant ($P < 0.05$) increase in weekly body weights was observed in group 3 as compared to group 2 Table 1.

Hematology

A significant ($P < 0.05$) decrease in total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean

corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet counts, and a significant ($P < 0.05$) leukocytosis, relative lymphopenia, absolute neutrophilia, and monocytosis were noticed among arsenic-treated rats when compared with the control group on the 29th day of the experiment. Supplementation of *Lactobacillus sporogenes* along with arsenic resulted in improvement of all the hematological parameters in comparison with the toxic control group Table 2.

Light Microscopy

The blood smears of group 1 showed normal erythrocyte morphology Fig. 1 while the blood smear of arsenic-treated rats contains echinocytes, microcytes, dacrocytes, schizocytes, and spherocytes when compared to control Fig. 2. *Lactobacillus sporogenes* co-administration effectively reduced the morphological alterations in erythrocytes Fig. 3.

Scanning Electron Microscopy (SEM)

Scanning electron microscopic examination (SEM) of the blood revealed normal blood cells morphology with the

Table 1 Effect of arsenic and probiotic on weekly body weights (g)

Group	1st week	2nd week	3rd week	4th week
Group 1	219.25 ± 1.77	232.75 ± 2.80	246.50 ± 2.88	267.67 ± 3.41
Group 2	190.75 ± 1.21*	207.17 ± 1.53*	222.50 ± 1.61*	235.50 ± 3.03*
Group 3	203.58 ± 1.74# [§]	220.12 ± 3.46# [§]	236.67 ± 2.04# [§]	256.33 ± 3.22# [§]

Values are mean + SE ($n = 6$); one-way ANOVA

Means with different superscripts in a row differ significantly at $P < 0.05$

*Control versus arsenic; #control versus probiotic; [§]arsenic versus probiotic

Table 2 Effect of *Lactobacillus sporogenes* supplementation on hematological profile of sodium arsenite treated male *Wistar* rats on the 29th day

Parameters	Control (group 1)	Arsenic-treated (group 2)	Probiotic supplemented (group 3)
TEC ($10^6/\mu\text{L}$)	8.67 ± 0.11	6.56 ± 0.10*	7.68 ± 0.12 [§]
Hb (g %)	16.47 ± 0.14	13.28 ± 0.19*	14.98 ± 0.11 [§]
PCV (%)	45.57 ± 0.28	43.15 ± 0.44*	45.67 ± 0.70 [§]
MCV (fL)	55.38 ± 0.25	49.98 ± 0.23*	51.92 ± 0.26 [§]
MCH (pg)	17.43 ± 0.10	16.05 ± 0.23*	17.15 ± 0.28 [§]
MCHC (g/dL)	31.87 ± 0.23	29.15 ± 0.25*	30.17 ± 0.29 [§]
TLC ($10^3/\mu\text{L}$)	8.63 ± 0.23	18.20 ± 0.13*	12.85 ± 0.25 [§]
Lymphocytes (%)	80.67 ± 1.09	71.00 ± 0.93*	77.83 ± 1.42 [§]
Neutrophils (%)	16.67 ± 0.33	24.67 ± 0.42*	20.00 ± 0.45 [§]
Monocytes (%)	1.83 ± 0.17	2.50 ± 0.22*	1.98 ± 0.02 [§]
Eosinophils (%)	1.75 ± 0.11	1.98 ± 0.04	1.83 ± 0.17
Basophils (%)	1.0 ± 0.23	1.0 ± 0.25	1.0 ± 0.21

Values are mean + SE ($n = 6$); one-way ANOVA

Means with different superscripts in a row differ significantly at $P < 0.05$

*Control versus arsenic; #control versus probiotic; [§]arsenic versus probiotic

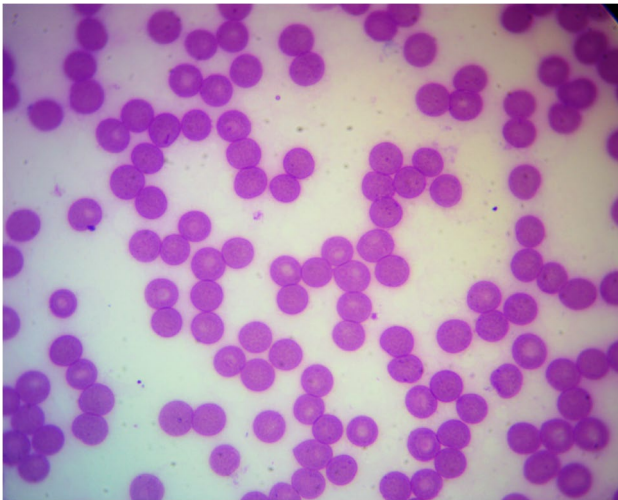


Fig. 1 Control—blood smear showing normal erythrocytes morphology (Leishman, 100X)

discoid appearance of erythrocytes in group 1 rats Fig. 4. However, cell morphology was altered in arsenic-treated rats with poikilocytosis characterized by the presence of macrocytes, microcytes, echinocytes, spherocytes, schizocytes, and blebbing of erythrocyte membrane Fig. 5. The size variation among erythrocytes was decreased along with less membrane defects in concomitant feeding of *L. sporogenes* along with arsenic Fig. 6.

Discussion

In the present study, arsenic exposure has caused hematological alterations. A significant decrease in RBC count observed in the present study could be the result of the

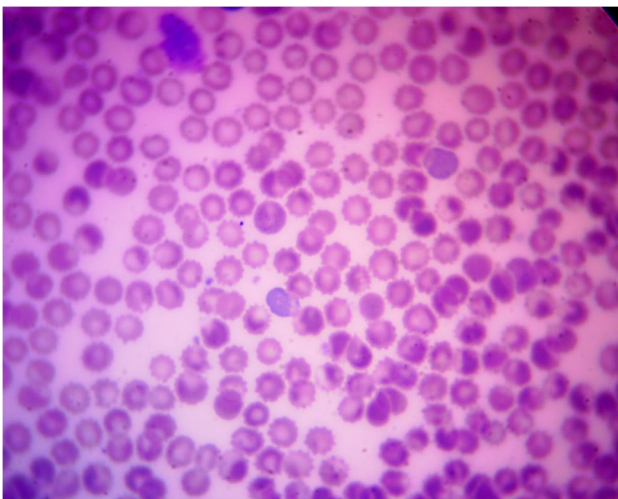


Fig. 2 Arsenic—blood smear showing poikilocytosis with echinocytes, microcytes, macrocytes, and spherocytes (Leishman, 100X)

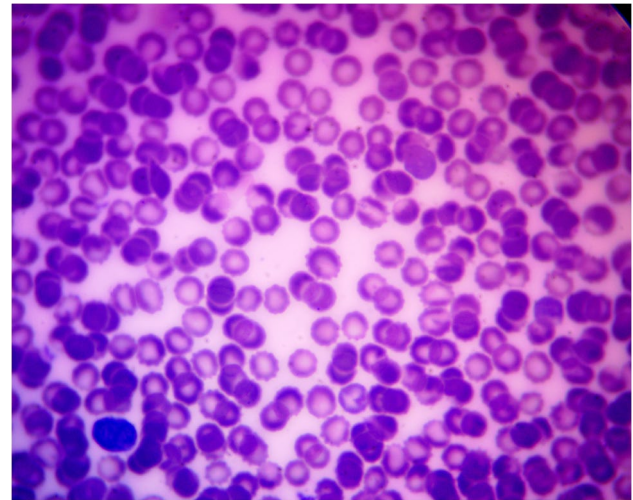


Fig. 3 Arsenic—blood smear showing near normal morphology of erythrocytes with few numbers of echinocytes (Leishman, 100X)

cytotoxic effect of arsenite in relation to the generation of free radicals that could have influenced the red cell membrane integrity via lipid peroxidation and caused anemia [10]. The anemia or deterioration of Hb levels was due to interference of metabolism and suppression of bone marrow as a residue of toxicant [16]. A significant decrease in PCV orchestrated by sodium arsenite in this study may be due to the inhibition of essential hematopoietic enzymes (Ex: ALAD) and destruction of RBCs by sodium arsenite [4]. A significant ($P < 0.05$) increase in TLC, noticed in group 2 rats, is indicative of leukocytosis which attributes to enhanced immune function to protect the animals from sodium arsenite-induced toxicity [14]. Increased oxidative stress and inflammatory cytokines production might have

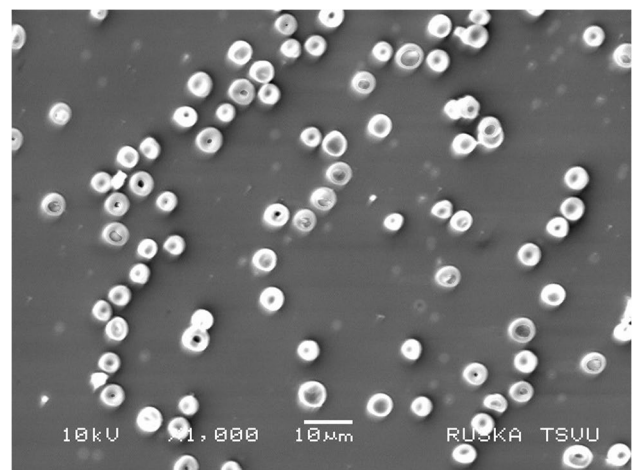


Fig. 4 Control—SEM of the blood showing the normal discoid appearance of erythrocyte (1000X)

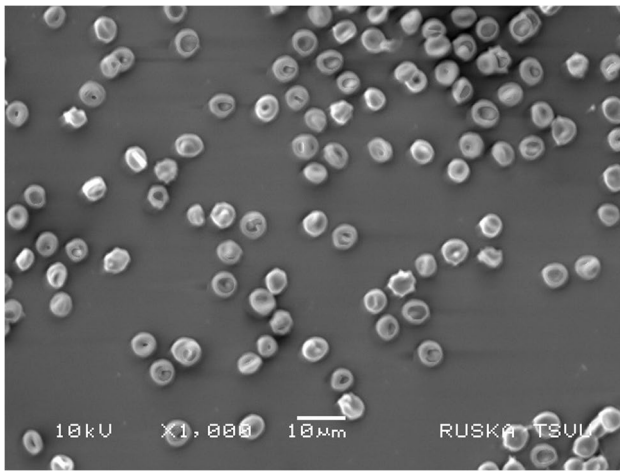


Fig. 5 Arsenic—SEM of the blood showing poikilocytosis with echinocytes, microcytes, and spherocytes. Blebbing of RBC membrane can also be seen (1000X)

facilitated the activation of polymorphs and mononuclear leukocytes which might also be the cause for significant ($P < 0.05$) relative lymphopenia, absolute neutrophilia, and monocytosis in group 2 rats compared to control. The neutrophilic leukocytosis in group 2 rats might be partly due to stress or partly due to the inflammatory changes noticed in the various organs. Supplementation of *L. sporogenes* significantly improved all the parameters suggesting the protective action against arsenic toxicity. This finding is in agreement with the recent report of the hemoprotective effect of *Lactobacillus reuteri* P16 against heavy metal toxicity [8]. The partial restoration of hematological parameters due to supplementation of *L. sporogenes* to arsenicated rats might be due to chelating and antioxidant ability. Probiotics have been shown to reduce the absorption of heavy metals in

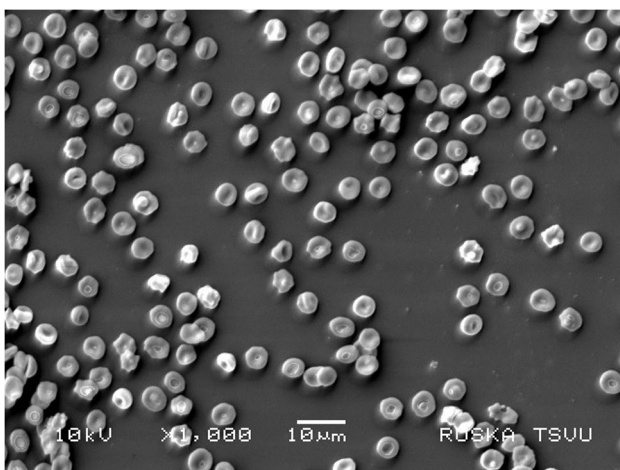


Fig. 6 Arsenic—SEM of the blood showing near normal discoid appearance of erythrocytes with few numbers of echinocytes (1000X)

the intestinal tract via the enhancement of intestinal heavy metal sequestration, detoxification of heavy metals in the gut, changing the expression of metal transporter proteins, and maintaining the gut barrier function [3].

Light microscopic and SEM studies have shown that arsenic exposure leads to the altered discoid appearance of erythrocytes and an increase in poikilocytic response characterized by hypochromic echinocytes, microcytes, macrocytes, and spherocytes associated with membrane blebbing, which are in agreement with observations of previous reports [2, 7]. These changes in erythrocytes can be attributed to the arsenic-induced increase in oxidative stress and decrease in antioxidant inputs which in turn are responsible for membrane damage, methemoglobin formation, osmotic fragility, and destruction of cells [13]. However, *L. sporogenes* supplementation has restored the morphology of erythrocytes to near normal suggesting the ameliorative effect of probiotic against arsenic-induced morphological alterations in erythrocytes which could be by protecting from free radical-induced RBC cell membrane damage.

Conclusion

Finally, it could be concluded that supplementation of *L. sporogenes* can ameliorate the arsenic-induced hemotoxicity. Furthermore, detailed molecular studies are needed to propose a scientific reason for the above observations.

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Declarations

Conflict of Interest The authors declare no competing interests.

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