

HEPATOPROTECTIVE EFFECT OF *LACTOBACILLUS SPOROGENES* AGAINST ARSENIC INDUCED TOXICITY IN *MUS MUSCULUS*Ranjit Kumar^{1*}, Pratibha Kumari², Shalini Kumari³, Arun Kumar¹, Md. Ali¹ and Ashok Ghosh¹¹Mahavir Cancer Institute and Research Centre, Phulwarisharif, Patna (Bihar), India.²Banasthali University, Rajasthan.³B.R.A. Bihar University, Muzaffarpur.***Corresponding Author: Dr. Ranjit Kumar**

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ABSTRACT

Arsenic found widely in the earth's crust. It is mostly found in organic, inorganic and gas form. In some areas of the world, high levels of arsenic are naturally present in drinking water which leads to high toxicological concern. Arsenic commonly causes arsenicosis, melanosis, renal dysfunction and prolonged exposure may cause cancer of skin, bladder and liver. Probiotics are reported to have antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis and immuno-modulatory effects. *Lactobacillus sporogenes* is common probiotics reside in intestine, it secretes a bacteriocin and coagulin, which is active against a broad spectrum of enteric microbes and causes detoxification. The present study is designed to evaluate hepatoprotective effect of *Lactobacillus sporogenes* against arsenic induced toxicity in *Mus musculus*. Mice were administered arsenic for four weeks followed by four weeks administration of Lactobacillus. Blood were collected for biochemical assay. Liver tissues were fixed for Light microscopic study. Arsenic causes many fold increase in SGPT and bilirubin level. Hepatic cells and kupffers cells were degenerated to greater extent with fragmented nuclei in arsenic exposed group. Sinusoid and central vein were dilated in arsenic exposed group. While lactobacillus causes marked restoration in biochemical and histological parameters of liver of mice. It is concluded from study that *Lactobacillus* causes effective restoration in SGPT and bilirubin level. Hepatic cells, kupffers cells, sinusoid and central vein were also restored effectively. It also maintains normal architecture of liver tissues. Lactobacillus reduces hepatotoxicity caused by arsenic effectively.

KEYWORDS: Probiotic, Melanosis, Hepatic cells, Bilirubin.**INTRODUCTION**

Arsenic found widely in the earth's crust, It is mostly found in organic, inorganic and gas form. In some areas of the world, high levels of arsenic are naturally present in drinking water leads to high toxicological concern. Ingestion of contaminated drinking water is the major routes for human exposure to arsenic. The contamination of groundwater from arsenic and its impact on health have formerly been recited from 23 regions in different parts of the world. The consequence of this particular problem is found to ascetic in Bangladesh^[1] ensued by west Bengal, India^[2] and China.^[3] In India, the major or highly affected area from arsenic contamination has been detected in the states of Bihar, Uttar Pradesh, Jharkhand, Assam, Chhattisgarh and Andhra Pradesh.^[4] The skin lesion induced from arsenic may be consociated with elevated imminent of skin, bladder and lung cancer,^[5] but the risk of cancer may be consequences even without skin lesion.^[6]

Lactobacillus sporogenes unique among probiotics in that it possesses a protecting, spore-like protein covering, which allows it to survive stomach acid, arrive at the small intestine, germinate, and grown.^[7,8] The therapeutic benefit is partly due to the ability of *L. sporogenes* to secrete a bacteriocin and coagulin, which is active against a broad spectrum of enteric microbes and causes detoxification.^[9] Most existing probiotics have been isolated from the human gut microbiota and plays an important role in human health, not only due to its participation in the digestion process, but also for the function it plays in the development of the gut immune system.^[10] Probiotics are reported to have antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis and immuno-modulatory effects.^[11] Probiotic microorganisms supports the balance of enteric microflora, which can be altered by antibiotics.^[12]

Thus the present study is designed to evaluate hepatoprotective effect of *Lactobacillus sporogenes* against arsenic induced toxicity of *Mus musculus*.

MATERIALS AND METHODS

Arsenic

In the present study Sodium Arsenate (Merk, Mumbai) was used for experiment.

Microbes Used

Lactobacillus sporogenes was used as antidote procured from Synzyme Pvt Ltd Uttarakhand.

Experimental model

Swiss albino mice (*Mus musculus*) weighing 30 ± 2 gm were selected as an experimental model in the present study. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Ethical approval was obtained from Institutional Animal Ethics Committee of the institute.

METHODOLOGY

Chronic Toxicity Study: Selected pathogen-free mice were sorted and sodium arsenate was administered at 5 mg/kg body weight dose for 4 weeks by Gavage method. Sacrifices were done at the end of 2nd week and 4th week of Sodium arsenate administration in each group.

Bioremediation: Sodium arsenate administration 5 mg/kg b.wt for 4 weeks was followed by the administration of *Lactobacillus sporogenes* for 4 weeks at dose of 15 million spores/kg body weight. Animals were sacrificed on 2nd week and 4th week of microbial administration.

Histological Studies: Mice were sacrificed from each group for histological analysis. The liver tissue were dissected out and washed three times in isotonic saline (0.85 w/v %), fixed in 10% neutral formalin solution and was processed. Slides were stained with Hematoxyline-Eosin (H & E) stains and examined under light microscope.

Biochemical and Hormonal Assessment: Blood were collected by orbital puncture and centrifuged to separate the serum to carry out biochemical analysis. Biochemical analysis were performed through serum by standard kit process (Coral crest) through U.V vis spectrophotometer.

RESULTS

Biochemical assay

In control group S.G.P.T. level were 19 ± 2.01 U/ml while after two weeks and four weeks of arsenic administration it was 98.33 ± 8.32 U/ml and 213 ± 17.0 U/ml. While in arsenic four weeks administered group followed by *Lactobacillus* two weeks and four weeks administration it was 49.33 ± 5.68 U/ml and 24.67 ± 6.02 U/ml respectively. (Graph: I).

In control group bilirubin level were 0.30 ± 0.11 mg/dl while after two weeks and four weeks of arsenic administration it was 3.86 ± 0.75 mg/dl and 6.03 ± 1.05

mg/dl. While in arsenic four weeks administered group followed by *Lactobacillus* two weeks and four weeks administration it was 2.50 ± 0.45 mg/dl and 0.80 ± 0.12 mg/dl respectively. (Graph: II).

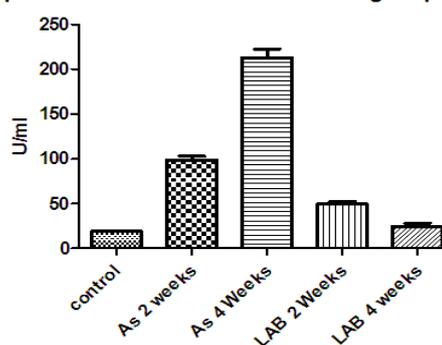
Histological study

In control group of mice well organised hepatic cells were observed. Central vein and hepatic vein was prominent. Both cytoplasmic and nuclear materials of hepatic cells were well organised (Figure: 1).

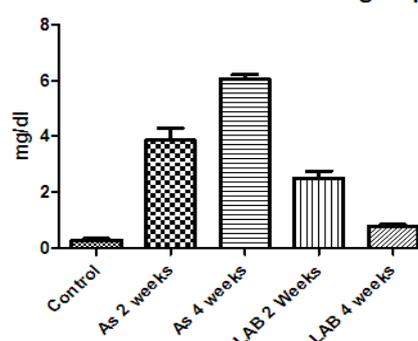
In arsenic two weeks administered group degenerated cytoplasmic material of hepatic cell were observed. Fragmented nuclear material was observed with many vacuolated spaces. Degeneration was also observed in kupffers cells. (Figure: 2). In arsenic four weeks administered group degenerated cytoplasmic material of hepatic cell were observed. Fragmented multi lobed nuclear material with elongated and clustered nuclei was observed. Degeneration was also observed in chromatin material of kupffers cells. (Figure: 3).

In arsenic four weeks followed by *Lactobacillus* two weeks administered group effective restoration in nuclear material of hepatic cell were observed. Chromatin material were well organised. Kupffer cell were restored effectively. Least vacuolated spaces were observed. (Figure: 4). In arsenic four weeks followed by *Lactobacillus* four weeks administered group restoration in nuclear material and cytoplasmic material of hepatic cell was observed to greater extent. Least vacuolization were observed in hepatic cells. Central vein and hepatic vein were also restored effectively. (Figure: 5).

Graph-1: S.G.P.T. Level in different group of mice



Graph-2: Bilirubin Level in different group of mice



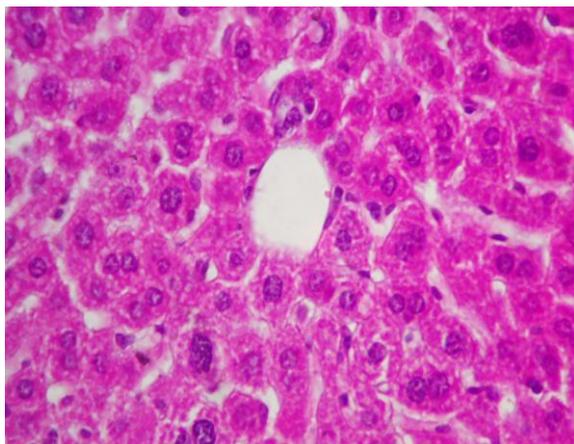


Figure 1: Shows liver of control mice with well organised hepatic cells. Central vein is prominent. Both cytoplasmic and nuclear material of hepatic cells were well organised.

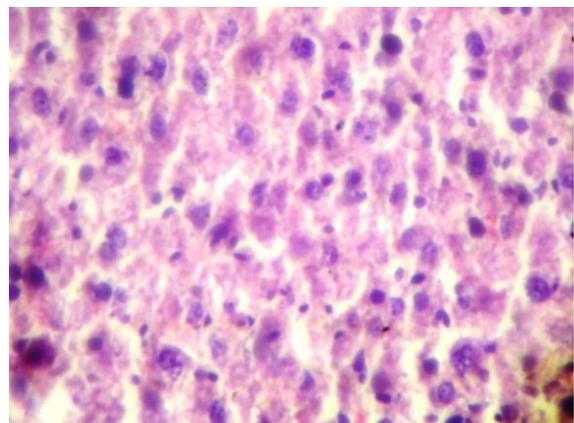


Figure 2: Shows liver of 2 weeks Arsenic administered mice with degenerated cytoplasmic material of hepatic cell. Fragmented nuclear material was also observed. Many vacuolated spaces were observed. degeneration were also observed in kupffers cells.

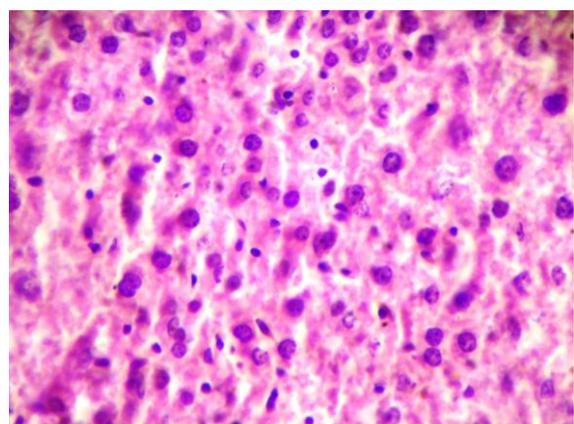


Figure 3: Shows liver of 4 weeks Arsenic administered mice with fragmented nuclear material of hepatic cells. Many vacuolated spaces were observed in hepatic cells. Degenerated nuclear material of kupffer cells were also observed.

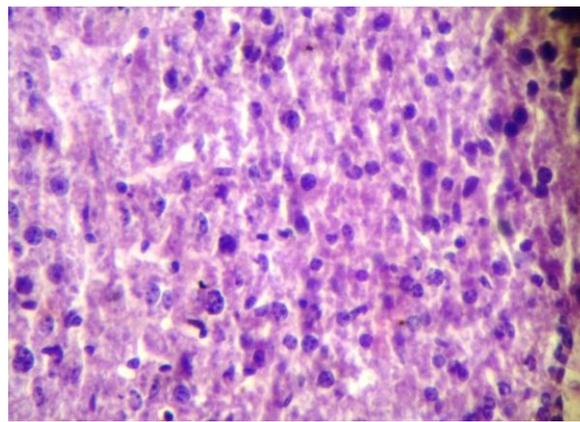


Figure 4: Shows liver of 4 weeks Arsenic administered mice followed by 2 weeks administration of *Lactobacillus sporogenes* with effective restoration in nuclear material of hepatic cell. Chromatin material were well organised. Kupffer cell were restored effectively. Least vacuolated spaces were observed.

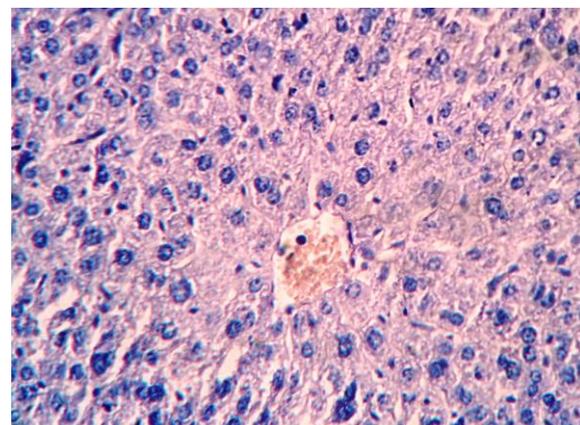


Figure 5: Shows liver of 4 weeks Arsenic administered mice followed by 4 weeks administration of *Lactobacillus sporogenes* with restoration in nuclear material of hepatic cells. Cytoplasmic material of hepatic cell was restored effectively. Least vacuolization were observed in hepatic cells. Central vein were also restored to greater extent.

DISCUSSION

The largest source of arsenic and other metals is usually food, of which the main dietary forms are seafood, rice, mushrooms and poultry.^[13] Arsenic disrupts the normal levels of metabolic enzymes in the body which are potent biomarkers of carcinogenesis.^[14] Chronic exposure to arsenic is associated with cancer of the skin and internal organs and with several non-malignant adverse health effects, such as weakness, edema, conjunctival congestion, diabetes mellitus, hypertension, and respiratory conditions.^[15,16] In India, though cases of arsenic toxicity including liver fibrosis due to drinking of arsenic contaminated water were reported from Chandigarh in early 1978,^[17] occurrence of large number of cases of arsenic induced skin lesions were reported

from Kolkata, West Bengal in 1984.^[18] Arsenicosis, melenosis and cancer cases were reported from Tilak rai ka hatta of Buxar district, Bihar.^[19]

Oxidative DNA damage, acquired tolerance to apoptosis, enhanced cell proliferation, altered DNA methylation, genomic instability. Aberrant estrogen signaling have been reported to be involved in liver toxicity caused by arsenic.^[20] Arsenic exposure also causes spontaneous abortion, still births, reduced birth weight, and infant mortality.^[21] The significance of oxidative stress is that trivalent arsenic inhibits the formation of glutathione, which protects cells against oxidative damage.^[22] Though pentavalent arsenic correspond to inorganic phosphate and replaces phosphate in glycolytic and cellular respiration pathways.^[23] In our study we observed increased level of SGPT and bilirubin in arsenic exposed group. Hepatic cells and sinusoid were also degenerated to greater extent in arsenic exposed group of mice with dilated central vein and sinusoid. Which indicates arsenic causes severe hepatotoxicity in mice.

Increasing evidence suggests that the *Bacillus* species could have an endo-symbiotic relationship with the host, being able to temporarily survive and proliferate within the gastro intestinal tract.^[24] The adhesion of the bacteria to intestinal epithelium represents the first step in the colonization process and adhesion characteristics are important when selecting probiotics.^[25] In our study we observed that *Lactobacillus* administration causes marked restoration in liver function test. Both SGPT and bilirubin were restored effectively.

Since arsenic has negatively charged species and this is problematic for bacterial binding interaction therefore overcome surface negative charge by methylating a selection of lactobacilli in order to neutralize the negative surface charge to foster more attraction between positively charged amino group on the cell wall and negative charged metals. Peptidoglycan layer and surface proteins, such as S-layer proteins, are known to contain positively charged groups. *Lactobacillus acidophilus* strains and *Lactobacillus crispatus* DSM20584 are known to produce S-layer proteins, which may explain their activity against arsenic.^[26] Hepatic cells and kupffers cells were restored in *Lactobacillus* administered group. While both nuclear and cytoplasmic material of central vein and hepatic vein were restored to greater extent in *Lactobacillus* administered group.

CONCLUSION

Therefore from the above study it is concluded that *Lactobacillus* causes effective restoration in hepatic cells on both biochemical and histological parameters of mice. It restores SGPT and bilirubin up to normal level. Hepatic cells, kupffers cells, sinusoid and central vein were also restored effectively. It also maintains normal architecture of liver tissues. *Lactobacillus* reduces hepatotoxicity caused by arsenic exposure effectively.

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