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RESEARCH ARTICLE

IN VIVO ELIMINATION OF ARSENIC THROUGH LACTOBACILLUS SPOROGENES.

Ranjit Kumar[1]*, Roushan Kumari[1], Arun Kumar[1], Vikash Kumar Ravi[2] and Md Ali[1]
1. Mahavir Cancer Institute & Research Centre, Phulwarisharif, Patna (Bihar), India.
2. Veer kunwar Singh University,Ara.

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Abstract

Groundwater arsenic contamination is a global phenomenon affecting more than 70 countries on six continents. Arsenic is one of the more ubiquitous minerals in the Earth's crust. It is also concentrated in hydrous iron oxides. Arsenic can be easily solubilized in ground waters depending on pH, redox conditions, temperature and solution composition. Therefore the study is designed to evaluate *in vivo* effect of *Lactobacillus* sporogenes on arsenic degradation in mice. Mice were administered arsenic for four weeks followed by 8 weeks administration of Lactobacillus. Arsenic causes increased level of urea and uric acid. While urea and uric acid level were restored effectively in *Lactobacillus* administered group. Glomerulus and Bowmens capsule were restored effectively in eight weeks *Lactobacillus* administered group. Cytoplasm of PCT and DCT were restored effectively after eight weeks of Lactobacillus administration. Therefore it is concluded that *Lactobacillus* causes *in vivo* elimination of arsenic from mice. It acts well against arsenic induced toxicity in mice and restores biochemical parameters effectively. It also maintains normal morphology of kidney tissues. *Lactobacillus* reduces nephrotoxicity caused by arsenic exposure effectively. In this way it acts effectively against arsenic induced toxicity. Therefore it is one of the most effective antidote against arsenic induced toxicity.

Introduction:-

Ground water arsenic contamination had already been reported in 20 countries out of which major incident were from Asia. Arsenic-related groundwater problems have emerged in different Asian countries, including new sites in China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan and Pakistan [1]. Groundwater arsenic contamination is a global phenomenon affecting more than 70 countries on six continents. The Ganga–Meghna–Brahmaputra plain is extensively populated. These populations intensively exploits groundwater resources, experiencing a myriad of environmental impacts associated with local, regional, and global issue[2]. Arsenic contamination of groundwater in the Bengal Basin has been called 'the largest mass poisoning in human history[3]. In south Asia, enrichment of arsenic in groundwater possesses a serious health threat not only limited to human being but also accumulate in plant and fish [4]. Arsenic contamination of ground water may occur in two ways: anthropogenic activities and aquifer naturally contain. Ground water contamination cross the permissible limit of WHO (0.01mg/L), excess arsenic has been detected in drinking water supply in many country about 200 million people under risk [5]. Arsenic is one of the more ubiquitous minerals in the Earth's crust. It is also concentrated in hydrous iron oxides. Arsenic can be easily

Corresponding Author:- Ranjit Kumar.
Address:- Mahavir Cancer Institute & Research Centre, Phulwarisharif, Patna (Bihar), India.

solubilized in ground waters depending on pH, redox conditions, temperature, and solution composition. Many geothermal waters contain high concentrations of arsenic. Natural arsenic in ground water at concentrations above the drinking water standard of 10μg/liter is not uncommon [6].

Probiotics strains are natural components of the human oral and intestinal microflora and are ubiquitous within the environment making the overall risk of infection by these species low. Hence many members of the *Lactobacillus* genus have obtained the generally recognized as safe status for use in food in the United States and European Food Safety Authority (EFSA).

Lactobacillus is the most commonly used as probiotics [7]. The use of probiotics in food products as well as a stand-alone therapy is very attractive, as they present an effective and noninvasive approach to providing health benefits, such as reducing inflammation.

Arsenicosis or chronic exposure leads to hypertension, cardiovascular diseases, developmental abnormalities, diabetes, hearing loss, hematological, neurological, reproductive problem, black foot diseases and cancer. This may affect a number of organs lungs, kidney, bladder, liver, testis, uterus and prostate gland [8].

Therefore the study is designed to evaluate In Vivo elimination of arsenic through *Lactobacillus sporogenes* in mice.

Materials and Methods:-

Arsenic:-

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

Microbes Used:-

Lactobacillus sporogenes was used as antidote.

Experimental model:-

Swiss albino mice (*Mus musculus*) weighing 30±2gm 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 6 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 at 6 mg/kg b.wt for 4 weeks was followed by the administration of *Lactobacillus sporogenes* for 8 weeks at 50 thousand spores/kg body weight. Animals were sacrificed on 4th week and 8th week of microbial administration.

Histopathological Studies:-

Mice were sacrificed from each group for histological analysis. The kidney 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 Hematoxylene-Eosin (H & E) stains and examined under light microscope.

Biochemical and Hormonal Assessment:-

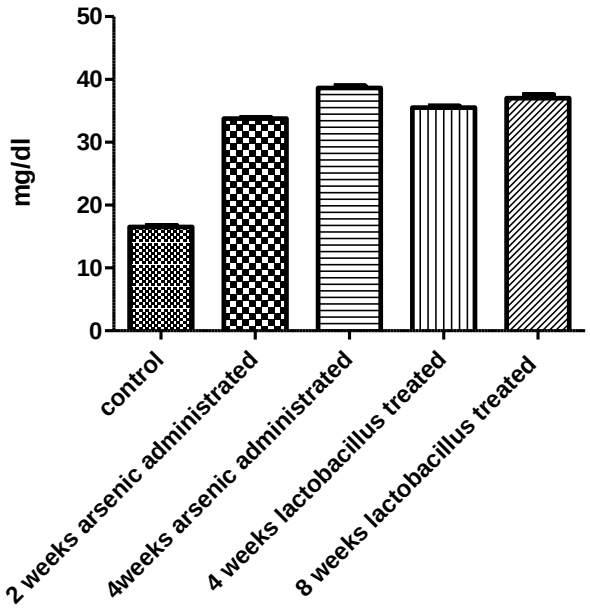
Blood were collected by orbital puncture and centrifuged to separate the serum to carry out biochemical analysis. Biochemical analysis were performed through serum by standard kit process (Coral crest) through U.V vis spectrophotometer.

Results:-

Biochemical assay:-

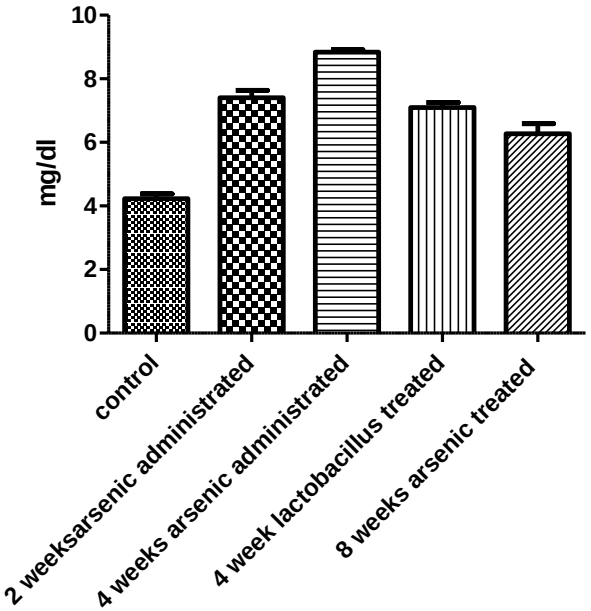
Kidney function test were analyzed in different study group. In control group urea level were 16.50 ± 0.28 mg/dl while after two weeks and four weeks arsenic administered group it become 33.78 ± 0.15 mg/dl and 38.65 ± 0.32 mg/dl. While in arsenic four weeks administered group followed by *Lactobacillus* four weeks and eight weeks administered group it was 35.50 ± 0.28 mg/dl and 37.00 ± 0.57 mg/dl respectively. (Graph: I).

Graph - I: Urea in serum of mice



In control group uric acid level were 4.233 ± 0.14 mg/dl while after two weeks and four weeks arsenic administered group it become 7.407 ± 0.22 mg/dl and 8.837 ± 0.076 mg/dl. While in arsenic four weeks administered group followed by *Lactobacillus* four weeks and eight weeks administered group it was 7.100 ± 0.15 mg/dl and 6.267 ± 0.31 mg/dl respectively. (Graph: II).

Graph - II: Uric acid in serum of mice



Histological study:-

Kidney tissues were studied in detailed in different groups. In control group of mice glomerulus, bowmens capsule, proximal convoluted tubule and distal convoluted tubule were normal with well defined cytoplasm and nuclear material (Figure: 1).

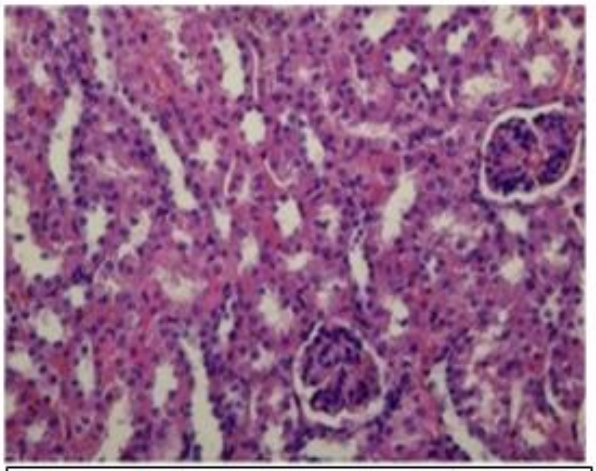


Figure – 1: showing kidney of control mice with normal glomerulus, bowmens capsule, proximal convoluted tubule and distal convoluted tubule. Well defined cytoplasm and nuclear material were observed.

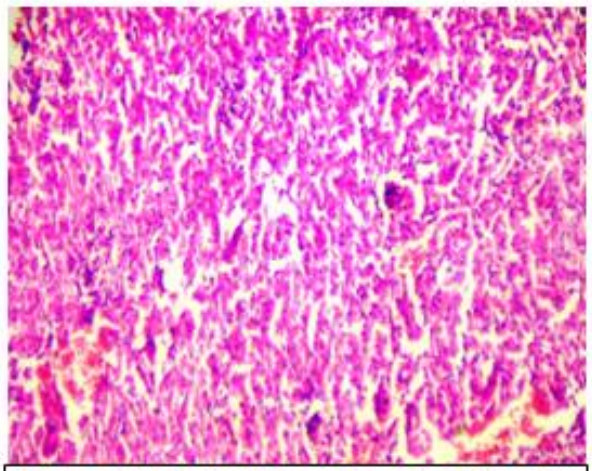


Figure – 2: showing kidney of arsenic eight weeks administerd group with many vacuolated spaces. Clustered nuclei were also observed in glomerulus. Degenerated cytoplasm were observed in tubules of PCT and DCT. Bowmens capsule were also degenerated.

In arsenic eight weeks administered group many vacuolated spaces were observed. Clustered nuclei were also observed. Degenerated cytoplasm was observed in tubules of PCT and DCT. Bowmens capsule was observed in degenerated condition with clustered nuclei in glomerulus (Figure: 2). In arsenic eight weeks followed by *Lactobacillus* four weeks administered group show restoration in glomerulus and bowmens capsule. PCT and DCT were also restored in structure to greater extent (Figure: 3). In arsenic eight weeks followed by *Lactobacillus* eight weeks administered group show restoration in cytoplasm of PCT and DCT. Least vacuolization were observed. Well defined bowmens capsule and glomerulus were observed (Figure: 4).

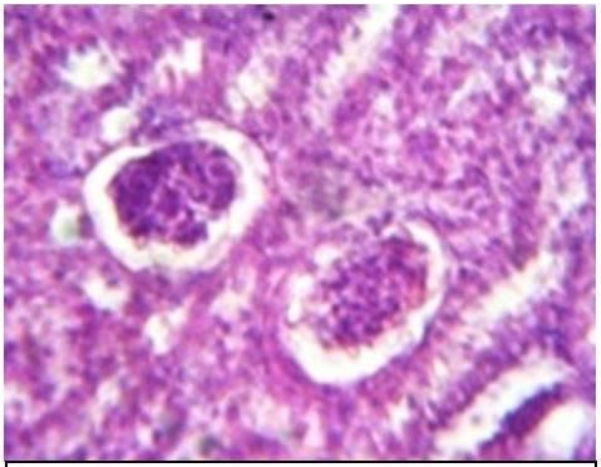


Figure – 3: showing kidney of arsenic eight weeks followed by lactobacillus four weeks administered group with restoration in glomerulus and bowmens capsule. PCT and DCT were also restored in structure to greater extent. Least vacuolated spaces were observed.

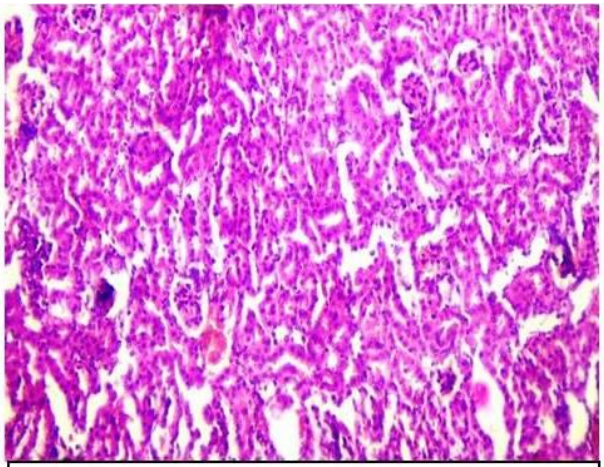


Figure – 4: showing kidney of arsenic eight weeks followed by lactobacillus eight weeks administered group with restoration in cytoplasmic material of PCT and DCT. Least vacuolization were osereved. Well defined bowmens capsule and glomerulus were observed.

Discussion:-

Exposure to arsenic imposes a big health issue worldwide[9]. Arsenical keratosis appears as a diffuse thickening involving palm and soles alone or in combination with nodules in severe cases crack may be seen in the soul[10]. Oxidative DNA damage, acquired tolerance to apoptosis, enhanced cell proliferation, altered DNA methylation, genomic instability and aberrant estrogen signaling have been reported to be involved in liver toxicity caused by arsenic[11]. Arsenic exposure also causes spontaneous abortion, still births, reduced birth weight, and infant mortality[12]. Kidney leads to oxidative phospholipids degradation, arsenic induce peroxidation in kidney induces oxidative damage leading to functional deterioration [13]. In our study we observed elevated level of urea and uric acid in arsenic exposed group. Glomerulus and Bowmans capsules were also degenerated to greater extent. Proximal convoluted tubule and distal convoluted tubule were degenerated to greater extent.

Since the cell wall of gram positive bacteria has higher metal binding activity[14]. The uptake of arsenic by bacteria via glycerol and phosphate transport system, once arsenic has entered the cell, other defense mechanisms are activated. These mechanisms are based on energy dependent efflux of arsenite from cell. *Lactobacillus* has also mar and ars operon system[15] which encode efflux transpoter which have ability to export metals out of their cell reduce damage to organism by lowering the concentration. The mechanisms by which Probiotics exert their effects are largely unknown, but may involve modifying gut pH, antagonizing pathogens through production of antimicrobial compounds, stimulating immunomodulatory cells, and producing lactase [16]. Cell wall of lactic acid bacteria is seems to be contain a large number of negatively charged functional groups, mainly carboxyl and phosphoryl. Electrophoratic studies have established that the net surface charge of lactic acid bacteria is negative at neutral pH [17, 18, 19, 20]. In present study, we observed restoration in urea and uric acid. Since arsenic has negatively charged species and this is problematic for bacterial binding interaction therefore overcome surface negative charge by methylatating 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[21]. In the present study restoration in nuclear and cytoplasmic material of Bowmans capsule and glomerulus were observed. Effective restoration was also observed in tubular system of kidney.

Escherchia coli uptake arsenic by non-specific phosphate transpoter pit, some other bacteria has more specific phosphate transporter pit which transport arsenic less specific[22, 23]. Probably water is absorbed in large intestine and this *Lactobacillus* also resides there. Arsenic is absorbed through walls of *Lactobacillus* and eliminated with them. In this way it is eliminated from mice and reduced arsenic induced nephrotoxicity were observed in them.

Conclusion:-

Therefore from the above study it is concluded that *Lactobacillus* causes *in vivo* elimination of arsenic from mice. It acts well against arsenic toxicity in mice and restores biochemical parameters effectively. It also maintains normal architecture of kidney tissues. Lactobacillus reduces nephrotoxicity caused by arsenic exposure effectively. In this way it will be the most effective for in vivo elimination of arsenic induced toxicity in mice.

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