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ORIGINAL ARTICLE

Protective Role of Selected Probiotics on Experimentally Induced Kidney Failure in Rats

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ABSTRACT

Uremia, acute kidney injury (AKI), chronic kidney disease (CKD) and end-stage renal disease (ESRD) have become worldwide public health problems. There are many causes for acute renal failure which mainly includes acute tubular necrosis which occurs either due to ischemia or toxins. CKD or ESRD is a gradual decrease of kidney function in both kidneys over a period of time. These conditions increase patient morbidity and mortality risks and treatment option is only dialysis and kidney transplantation which is very much expensive and not free from side effects. So that the maximum people of our country cannot afford this therapy to treat kidney disorders. However, In absence of reliable and effective modern nephroprotective drugs and traditional medicines, different scientists in different part of the World is trying to employed for the treatment of the diseases by using complementary or alternative medicine of the disease treatment and /or prevention which are free from side effects and cost effective also. The present review work was revealed that the nonpathogenic bacteria, such as *Sporosarcina pasteurii* MTCC No. 1761 and chicken isolated strain *Lactobacillus ingluviei* ADK10 were tested for their probiotic characteristic. The nonpathogenic bacteria *S. pasteurii*, and *L. ingluviei* ADK10 were useful as potential urea-targeted component. Blood urea nitrogen and toxicity indicators were reduced, and antioxidant enzymes were increased significantly in the bacteria treated groups ($p<0.05$) compared with experimentally induced uremic groups. So, these probiotics were beneficial for prevention of kidney diseases.

INTRODUCTION

Currently chronic kidney disease (CKD) appears to be a foremost problem across the world, and it is ranked fourth among the key diseases in the United States, affecting over 20 million people and growing at 8% yearly (1). Worldwide, the number of patients with CKD is rising, and it is now being recognized as a major public health problem that may reach epidemic levels over the next decade. Uremia is a potentially lethal syndrome of kidney disease demanding instant treatment. The most often used treatment options for uremia include kidney transplantation and dialysis, which are very expensive and not free from side effects. At present, worldwide statistical data on the incidence and prevalence of kidney disease, the resulting mortality and the high cost of treatment demonstrate the requirement for an effective alternative.

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Acetaminophen remains one of the most effective, chemotherapeutic analgesic-antipyretic agents belonging to the para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs). The use of analgesics such as acetaminophen regularly over long durations of time can cause analgesic nephropathy, another cause of kidney disease. Acetaminophen overdose may result in potentially fatal hepatic and renal necrosis in humans and experimental animals (2). An acute over dose of acetaminophen (paracetamol, *N*-acetyl*p*- aminophenol) overdose may result into potentially fatal hepatic and renal necrosis in humans and experimental animals (3). At higher doses, acetaminophen produces a centrilobular hepatic necrosis that can be fatal. The initial step of its toxicity is the formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with glutathione sulfhydryl (GSH). High doses of acetaminophen result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury and oxidative stress (4). Urea is the main nitrogen along with toxic waste product of protein catabolism (5). The mechanisms involved in urea's toxicity are poorly understood (6), it is well established that urea contributes to the synthesis of other toxic moieties including guanidines and carbamylation products (7), or, at the very least, it appears to parallel the severity of

renal dysfunction. Moreover, excess urea induces generation of free radicals and oxidative stress, which have been implicated in cellular damage (8). Azotemia or uremia is a precondition to renal failure wherein nitrogenous waste products, chiefly urea, accumulate in the blood. In most cases of renal failure, there is usually an increased retention of waste metabolites (mainly urea and creatinine) in the blood and body tissues due to a substantial decrease in urinary excretion.

Conventional chemotherapeutic options available to either treat or prevent the progress of uremia, are still restricted. In absence of reliable and effective modern antioxidative drugs for the disease treatment, concerted efforts are presently channeled toward exploring complementary or alternative chemotherapy in the disease treatment and/or anticipation. Among lactic acid bacteria, *Lactobacillus* has attracted a lot of attention for their potential probiotic effects in human health. *Lactobacillus* composed of 100 recognized species, particularly those belonging to beneficial and non-pathogenic genera. The origin of lactic acid bacteria may come from mammals and birds intestines or from naturally fermented foods. Lactic acid bacteria (LAB) along with *Lactobacillus* have some probiotic functions, such as antagonistic reaction against pathogenic bacteria, adjusting the balance of intestinal flora, reducing of blood cholesterol, inhibiting and reducing the risk of tumors and cancer, stimulating the immune system, stimulation of Vitamin C production and enhancement of digestion etc (9, 10). Oxidative damage has been found to play a major role in arthrosclerosis, cancer, arthritis and kidney disease. Generation of harmful free radicals and reactive oxygen species (ROS) may cause endogenous metabolic process. These free radicals are responsible for oxidative damage and finally to cell death. Some *Lactobacilli* have been shown to possess antioxidative activity, and are able to decrease the risk of accumulation of ROS during the ingestion of food.

The urease positive and antioxidative activity expressed by some Lactobacillus strains used as food components and lactic acid bacteria may have a substantial impact on kidney failure human welfare. In this perspective, some urease positive strains were collected. Even also lactic acid bacteria were isolated from different sources such as of chicken intestine, curd, cow milk, pickles, cheese, and sea fish were collected from local markets in Midnapore town, West Bengal, India.

This paper summarizes findings of the review, which includes a discussion on the following areas: preliminary screening of lactic acid bacteria, antioxidant activity of bacteria, selected bacteria acts as a probiotics and the protective role of such probiotics on experimentally induced kidney failure and oxidative stress.

MATERIALS AND METHODS

Collection of bacteria from MTCC (Microbial Type Culture Collection), Pune and selection of urease positive bacteria

Eight urease positive strains from MTCC i.e *Lactobacillus rhamnosus, L. casei, L. plantarum, L. fermentum, Enterococcus faecalis, Streptococcus thermophilus, Sporosarcina pasteurii* and *Escherichia coli were collected*. The potent and effective one was selected on the basis of urease positive, survival under the human GI tract conditions, antimicrobial activity and antioxidetive activity.

Collection of sample for Isolation, selection of urease positive lactic acid bacteria isolates

Chicken intestine, curd, cow milk, pickles, cheese, and sea fish were collected in sterile container from markets in three topical district of West Bengal (22º34'0''N, 88º22'0''E), India and brought these to the laboratory for microbial screening.

Primary screening of lactic acid bacteria

Samples with 0.85% NaCl (w/v) saline solution were plated onto de Man-Rogosa-Sharpe (MRS) agar [composition (w/v) 1.0% peptone, 0.8% meat extract, 0.4% yeast extract, 2.0% glucose, 0.5% sodium acetate trihydrate, 0.1% polysorbate 80 (also known as Tween 80), 0.2% dipotassium hydrogen phosphate, 0.2% triammonium citrate, 0.02% magnesium sulfate heptahydrate and 0.005% manganese sulphate tetrahydrate, pH 6.5], by dilution plating technique and incubated at 37°C under anaerobic condition for 24-48 h. After 2 days of incubation the bacterial colonies were primarily selected on the basis of phenotypic characteristics (colony characteristics, gram staining, cell shape, and catalase production), and isolates of lactic acid bacteria were collected (11).

Secondary screening of selected bacteria

All the bacteria collected from MTCC and isolated previously selected were subjected for secondary screening process. It is performed on the basis of urease positive, survival under the human GI tract conditions, antimicrobial activity and antioxidative activity. The potent bacteria among those selected were maintained routinely on slant at 4 ºC (12).

Urease positive: All the collected bacteria were tested for presence of urease enzyme using Nesler Reagent. In bacterial culture broth brown color precipitate indicated that the bacteria was urease positive (11).

Antimicrobial activity : Twenty-four hours culture of selected bacteria (log phase) were taken and turbidity was adjusted to 0.5 Mc Farland (corresponding to 10^8 CFU/mL) with phosphate buffer. Culture supernatants were obtained by centrifuging (6000 rpm for 30min at 4°C). One mL cell-free culture supernatants of selected bacteria were retained as untreated filtrate. To determine the organic acid function, 1 mL cell-free culture supernatant was adjusted to pH 6.5. In order to test the heat sensitivity, 1 mL cell-free culture supernatant was incubated at 100◦C for 15 min and treated with protease-K. The antimicrobial activity of all samples was tested using the agar-well assay against 24-hour old indicator organisms. A lawn of an indicator strain was made by spreading the cell suspension containing 10^7cfu/ml over the surface of MRS plates with a cotton swab. The plates were allowed to dry and a sterile cork borer of diameter 7.0 mm was used to cut uniform wells in the agar plates. Each well was filled with 70 µl of filter sterilized

supernatant obtained from culture grown in MRS medium. All the assays were carried out in triplicate. After incubation at 37°C for 24 hrs, the diameter (mm) of the inhibition zone around the well was measured. The indicator organisms used were *Escherichia coli*, *Bacillus subtilis, Staphylococcus aureus*, *Shigella dysentery, Pseudomonas aeruginosa*, *Klebsiella pneumonia.* All the cultures were maintained in the refrigerator at 4°C (12).

Total antioxidative activity of bacteria: The measurement of antioxidative activity of isolates were performed by the thiobarbituric acid (TBA) method via the measurement of lipid peroxidation (13) based on the monitoring of inhibition of linoleic acid and rat plasma peroxidation by cell free extracts of bacteria. Linoleic acid was chosen as the unsaturated fatty acid (14). Linoleic acid (20 ml) emulsion was made up of 0.1 mL linoleic acid, 0.2 mL Tween 20, and 19.7 mL deionized water. Phosphate buffer (15 ml) solution (0.02 M, pH 7.4), 1 mL linoleic acid or rat plasma emulsion, 0.2 mL FeSO4 (0.01%), 0.2 mL H_2O_2 (0.56 mM), and 0.4 mL cell free extracts of bacteria were mixed and incubated at 37 °C. Cell free extract of bacteria was replaced by deionized water in the control samples. After 12 h of incubation, 2 ml reaction solution was mixed with 0.2 mL trichloroacetic acid (TCA; 4%), 2 mL TBA (0.8%), and 0.2 mL of butylated hydroxytoluene (BHT; 0.4%). This mixture were incubated at 100°C for 30 min and allowed to cool. Chloroform (2 ml) was then added for extraction. The extract was obtained and then absorbance was measured at 532 nm. The percentage of inhibition of linoleic acid peroxidation and rat plasma peroxidation were defined as follows: (1-A532 (experimental sample)/A532 (control) X 100%.

Animal study

Selection of animals and care : Healthy, adult, male albino Wistar strain rats (36) weighing 100±15 g (supplied by Ghosh animal, Animal Foods and Animal Cages Supplier, Kolkata-54) were used. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed six per cage in a temperature-controlled room (22 ± 2 ºC) with 12-12 h dark-light cycle at 50 ± 10 % Relative Humidity. They were provided with standard food (pellet diet) and water *ad libitum*. The principle of laboratory animal care of National Institute of Health USA guideline was followed throughout the duration of experiment (15) the Institute Ethics committee approved the experimental protocol.

Grouping of animals and experimental procedure: Animals were randomized and divided into three groups (NC, U, VC1, VC2, UP1 and UP2) of six animals each. Group NC served as untreated control and was injected with distilled water 5 mL/kg body weight daily for 14 days. Groups UP1, UP2 and U animals were treated with 550 mg/kg body weight of the acetaminophen by intraperitoneal injection for 14 days respectively for inducing uremia. VC1 -Vehicle control of *L. ingluviei* ADK10 and the animals were injected with distilled water 5 mL/kg body weight daily for 14 days VC2- Vehicle control *S. pasteurii* and the animals were injected with distilled water 5 mL/kg body weight daily for 14 days (16).

Formulation Preparation : Food balls were arranged by the casein-based diet with both the bacteria, sterile 10% honey and milk mixture. The formulation was stored in a 4 °C freezer in aseptic conditions. While no microbial additives were given to rats of NC and U groups, UP1 and VC1 groups were administered with 1 × 10^9 cfu of *L. Ingluviei* ADK10 and UP2 and VC2 groups were administered with 1 × 10^9 cfu of *S. pasteurii* for 21 days. After 21 days all experimental animals from all groups were sacrificed and blood, liver and kidney were collected (12).

Blood uremia profile

Biochemical estimation of blood urea: The blood will be centrifuged and plasma fraction will be separated. Urea level of plasma will be measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyser (Merck, Microlab-300 Japan) as per the standard protocol for phtotometric determination of urea according to the Urease GLDH method (kinetic UV test). First 10µl of urea standard (50 mg/ 100ml) will be mixed with 1000µl monoreagent (Composed of Tris pH7.8 - 120mmol/l, 2-Oxoglutarate-7mmol/l,ADP-0.6mmol/ l,rease-6ku/l,Glutamate dehydrogenase-1ku/l and NADH-0.25 mmol/l) to incubate for approx. 60 sec. at 25ºC and absorbance will be read at 37ºC for standardization, then 10µl sample is used above procedure and automatic calculated reading has taken (Standard kit method (17).

Biochemical estimation of blood creatinine: The blood will be centrifuged and plasma fraction will be separated. Creatinine level of plasma measured by commercially available standard Blood Urea Kit (Merck, Japan) and by Semiautoanalyser (Merck, Microlab-300. Japan) as per standard protocol for phtotometric determination of creatinine based on Jaffe kinetic method without deproteinization. First 100µl of creatinine standard (1 mg/ 100ml) will be mixed with 1000µl monoreagent (Buffer:NaOH-313mmol/l + 12.5 mmol/l and Picric acid-8.73mmol/l) to incubate for approx. 0-5 min at 25ºC and absorbance will be read at 37ºC for standardization then 100µl sample will be used above procedure and calculated reading will be taken standard kit method (17).

Biochemical estimation of Blood Uric Acid Levels : Enzymatic determination of uric acid occurred. End product of the reaction is quinoneimine and measured colorimetrically at 546 nm (18) using the diagnostic reagent kit manufactured by Merck Japan.

Antioxidant enzymes

Biochemical assay of catalase activity: Catalase (CAT) activity was measured biochemically. For the evaluation of CAT activity in blood, liver and kidney samples were homogenized separately in 0.05 M Tris-HCl buffer (Merck, India) solution (pH 7.0) at a tissue concentration of 50 mg/mL. These homogenates were centrifuged separately at 10,000*g* at 4ºC for 10 minutes. In a

spectrophotometric cuvette, 0.5 mL of hydrogen peroxide (H2O2) and 2.5 mL of distilled water were mixed, and a reading of absorbance was noted at 240 nm. Tissue supernatants and plasma were added at a volume of 40 µL separately, and the subsequent and 12 following readings were noted at 30-second intervals (19).

Biochemical assay of superoxide dismutase: Kidneys were homogenized in ice cold 100 mM Tris-cacodylate buffer (LOBA Chem, India) to give a tissue concentration of 50 mg/mL, which as centrifuged at 10,000*g* for 20 minutes at 4ºC. The superoxide dismutase (SOD) activity of these supernatants was estimated by measuring the percentage of inhibition of the pyrogallol (HIMEDIA, India) autooxidation by SOD (20). The buffer was 50 mM Tris (pH 8.2) containing 50 mM cacodylic acid (pH 8.2), 1 mM ethylenediaminetetraacetic acid (EDTA) (HIMEDIA, India) and 10 mM HCl. In a spectrophotometric cuvette, 2 mL of buffer, 100 µL of 2 mM pyrogallol and 10 µL of supernatant were added, and the absorbance was noted in a spectrophotometer at 420 nm for 3 minutes. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyrogallol by 50%.

Statistical analysis

All the values are expressed as mean ± SE (n = 6). Significant differences between the groups were determined with SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) for Windows using one-way analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). A difference was considered significant at the P < 0.05 level (21).

RESULTS AND DISCUSSION

Urease positivity and antioxidative property of microbes can able to utilize various uremic solutes including urea, uric acid, creatinine, and most of the other potentially harmful biochemical. By designing and formulating a regimen of probiotics targeting these substances in the bowel, can improve the patient's overall health. In this context, the present study conducted to search for potent bacteria from MTCC and samples of natural habitat of three topical district of West Bengal, India. Eight bacteria were collected from MTCC, India (Table 1) (11). A pool of 21 lactic acid bacteria (ADK1-ADK21) was isolated from different environmental samples (Table 2) by serial plating technique on MRS agar. Cell and colony character of all isolates were determined and tabulated (Table 3). Among the table two isolates ADK3 and ADK15 were catalase positive and proved that they were not a lactic acid bacteria (LAB) isolate. Growth pattern and survival under the simulated human gastrointestinal (GI) tract conditions and urease activity of Bacteria isolates of rest of the bacteria are represented in Table 4 (21).

In the secondary screening profile of the primary selected bacteria, only six bacteria were urease positive. Among that urease positive bacteria five bacteria show good survival condition in the simulated human GI tract conditions. In case of other secondary screening profiles such as antibacterial activity and antioxidant property two bacteria fulfill all the screening criteria i.e. *Sporosarcina pasteurii* collected from MTCC and LAB isolate-ADK10 (Table 5 and Table 6) (12). In previous study dietary supplements with *S. pasteurii* enhanced survival of $5/6^{th}$ nephrectomized rats while slowing the progress of renal injury induced by reduced nephron mass (22).

Table-1: Freeze-dried bacterial cultures collected from MTCC along-with MTCC number

MTCC. No.	Bacteria
903	*Lactobacillus fermentum*
439	*Enterococcus faecalis*
1423	*Lactobacillus casei*
4462	*Lactobacillus plantarum*
1408	*Lactobacillus Rhamnosus*
1938	*Streptococcus thermophilus*
723	*Escherichia coli*
1761	*Sporosarcina pasteurii*

Table-2: Place and type of environmental samples and respective lactic acid bacteria isoletes.

Place and district	Number and type nature of sample	Isolates
Hamalpara, Midnapore Town, Paschim Medinipore	Small Dairy- milk, cow dung	2 (ADK1,2) 3(ADK3,4,5)
Keranitola, Midnapore Town, Paschim Medinipore	Sweet shop-Curd Indian cottage cheese	2(ADK6,7) 2(ADK8,9)
Gate Bazar, Midnapore Town, Paschim Medinipore	Meet shop- chicken Intestine Local fisherman- fish intestine	3(ADK10,11,12) 2(ADK13,14)
Panskura, Purba Medinipore	Processed Food factory-Tomato puree	2 (ADK15,16)
Mohonpore, Nadia	Haringhata Dairy-milk curd	2 (ADK17,18) 3 (ADK19,20,21)
Total	**9**	**21**

The study of some scientists show that the manipulation of the gut microbiota by *Lactobacillus. ingluviei* results in weight gain in birds and mice. *L. ingluviei* were associated with an astonishing weight gain effect in ducks, chicks and mice but have never been studied in humans (23). Other study also show that some species of *L. ingluviei* made characterized by antagonistic properties toward bacterial pathogens as well as resistance to low pH and bile salts. This strain can potentially restore the balance of intestinal microflora in geese and can be considered for use as prophylactic agent or as an alternative to antibiotic therapy for infections by *Salmonella enteritidis, E. coli*, *S. aureus*, *C. perfringens*, *P. multocida*, or *R. anatipestifer* (24). Next-generation

human probiotic species should contain *Lactobacillus* spp. those are not associated with weight gain in humans. Finally, selection of the two potent urease positive bacteria could take part in the future management of the major health problems in the 21st century, kidney disease.

Acute large doses or chronic use of acetaminophen is commonly associated with nephrotoxicity and hepatotoxicity in humans as well as in animals (25). Thus, acetaminophen-induced nephrotoxicties are well established experimental models of drug-induced renal injury (26). Acetaminophen nephrotoxicity occurs due to its highly reactive metabolite N-acetyl-paraamino-benzoquinoneimine, which arylates proteins in the proximal tubule, initiating cell death of renal tubular cells.32 Drug-induced nephrotoxicities are often accompanied with marked elevations in BUN, serum creatinine and acute tubular necrosis and depletion of antioxidant enzyme (27).

Table-3: Comparative cell and colony character of all lactic acid bacteria isolates and collected strains and primary screening profile

Bacterial strain	Cell character		Colony character		Catalase activity
	Shape	Gram Staining	Shape	Color	
Lactic acid bacteria isolates					
ADK1	Rod	Positive	Round	White	Negative
ADK2	Oval	Positive	Round	White	Negative
ADK3	Oval	Positive	Round	Gray	Positive
ADK4	Rod	Positive	Round	Milky white	Negative
ADK5	Coccus	Positive	Round	Grayish white	Negative
ADK6	Coccus	Positive	Round	White	Negative
ADK7	Rod	Positive	Round	White	Negative
ADK8	Rod	Positive	Round	Transparent	Negative
ADK9	Coccus	Positive	Round	White	Negative
ADK10	Plumped rod	Positive	Round	Grayish white	Negative
ADK11	Rod	Positive	Edge are Not equal	White	Negative
ADK12	Oval	Positive	Round	Gray	Negative
ADK13	Oval	Positive	Round	Milky white	Negative
ADK14	Coccus	Positive	Round	Grayish white	Negative
ADK15	Coccus	Positive	Round	White	Positive
ADK16	Rod	Positive	Round	Gray	Negative
ADK17	Plumped rod	Positive	Round	Milky white	Negative
ADK18	Oval	Positive	Round	Grayish white	Negative
ADK19	Oval	Positive	Irregular shape	Grayish white	Negative
ADK20	Rod	Negative	Round	White	Negative
ADK21	Coccus	Positive	Round	White	Negative
Strain from MTCC					
Lactobacillus fermentum	Plumped rod	Positive	Round	Gray	Negative
Enterococcus faecalis	Coccus	Positive	Round	Milky white	Negative
Lactobacillus casei	Oval	Positive	Round	Grayish white	Negative
Lactobacillus plantarum	Rod	Positive	Round	White	Negative
Lactobacillus Rhamnosus	Plumped rod	Positive	Round	Milky white	Negative
Streptococcus thermophilus	Coccus	Positive	Round	Grayish white	Negative
Escherichia coli	Rod	Positive	Round	White	Positive
Sporosarcina pasteurii	Cocci in chain form	Positive	Round	White	Negative

Table-4: Secondary screening profile of the primary selected bacteria survival under the simulated human GI tract conditions and urease activity of Bacteria strains In all cases data are expressed as Mean±SE (n=3). ANOVA followed by multiple two tail t-test. Different superscripts (a, b) differ from each other significantly ($p < 0.05$).

Strains	Urease test by Nesler reagent	0.3mg/ml Pepsin at pH 2 (log CFU/ml)		At pH 8.0 containing pancreatin (0.1 mg/ml, Sigma) and 0.3% (w/v) Oxgall (Sigma) (log CFU/ml)	
		0h	4h	0h	4h
Sporosarcina pasteurii	Positive	9.34±0.32	8.26±0.13	9.94±0.12	8.44±0.32
Lactobacillus rhamnosus	Negative				
L. plantarum	Negative				
L. fermentum	Negative				
Lactobacillus casi	Positive	9.77±0.52	8.74±0.43	9.74±0.32	8.63±0.16
Enterococcus faecalis	Negative				
Streptococcus thermophilus	Positive	9.77±0.31	4.64±0.23	9.22±0.11	2.64±0.56
Escherichia coli	Negative				
ADK1	Negative				
ADK2	Negative				
ADK4	Negative				
ADK5	Negative				
ADK6	Negative				
ADK7	Negative				
ADK8	Negative				
ADK9	Negative				
ADK10	Positive	9.51±0.12	8.89±0.24	9.84±0.16	8.34±0.56
ADK11	Negative				
ADK12	Negative				
ADK13	Negative				
ADK14	Positive	9.74±0.22	8.64±0.32	9.74±0.26	7.84±0.12
ADK16	Negative				
ADK17	Negative				
ADK18	Positive	9.29±0.51	8.24±0.21	9.14±0.13	7.87±0.17
ADK19	Negative				
ADK21	Negative				

Table-5: Secondary screening profile of the primary selected bacteria-Antibacterial activity

Indicator strains	Inhibition zone (mm)				
	Sporosarcina pasteurii	*Lactobacillus casi*	ADK10	ADK14	ADK18
E. coli (ATCC 25922)	3	2	11	4	5
Shigella dysenteriae (MTCC 1457)	6	-	12	9	9
Klebisella pneumoniae (ATCC 15380),	7	-	9	9	10
Staphylococcus aureus (ATCC 6538)	6	-	10	10	11
Pseudomonas aeruginosa (ATCC 15442).	6	-	13	13	12
Bacillus subtilis (ATCC6633)	5	2	7	13	10
Vibrio cholera (Hospital isolate)	4	3	10	6	5

Acetaminophen exposure significantly increased plasma level of blood urea nitrogen (Fig. 1 A), creatinine (Fig. 1 B), and uric acid level (Fig. 1 C), However, *S. pasteurii* and *L. ingluviei* ADK10 treatment on groups reduced the plasma level of blood urea nitrogen (BUN), creatinine, uric acid, compared to acetaminophen-exposed animals (group U) (21, 12). In renal disfunction, blood urea levels are raised because the rate of blood urea production becomes higher than the rate of renal clearance (28). Higher urea and creatinine levels in the blood are taken as an indicator of nephrotoxicity (29). Elevation of urea and creatinine levels in serum is taken

as an index of nephrotoxicity (30). Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown. Thus serum or blood urea concentration is often considered a more reliable renal function analyst than plasma creatinine. In the present study, acetaminophen-induced nephrotoxicity was characterized by marked elevations in the circulating levels of BUN and blood creatinine in control (group U) rats. In this study, acetaminophen induced nephrotoxicity showed a significant ($P < 0.05$) increase in the plasma urea, uric acid and creatinine concentrations in the Group U (acetaminophen induced) rats when compared to the normal group (Group NC) (Fig 1). Moreover, oral administration of *L. ingluviei* ADK10 and *S. pasteurii*, significantly ($P < 0.05$) decreased plasma urea and creatinine in group UP1 and UP2 when compared to the Group U (12, 21). The urease positive bacteria reduce the blood uremic toxins through enteric dialysis (22).

Oxidative stress is early events related to radicals generated during the hepatic metabolism of acetaminophen. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity (31). During kidney injury, superoxide radicals are generated at the site of damage, which modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radicals, which damage kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism (32). The present study also demonstrated that acute acetaminophen overdose resulted in a decrease in SOD and CAT activity levels, when compared with normal control rats (Table 7). This was due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When rats were treated with both the bacteria, SOD and CAT activity increased significantly, when compared with the uremic group ($p<0.05$). Both the bacteria improve the level of antioxidative enzymes. However, acetaminophen was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of reactive oxygen species appears as an early event which precedes cell damage in acetaminophen hepatotoxicity (33). Acetaminophen-induced nephrotoxicity was shown by biochemical measurements and histopathological changes, which coincide with the observations of other investigators (34).

Current evidence suggests that intracellular glutathione plays an essential role in detoxification of acetaminophen and prevention of acetaminophen-induced toxicity in liver and kidney (35). The generation of the reactive oxygen species appears as an early event which precedes intracellular glutathione depletion and cell damage in acetaminophen hepatotoxicity (33).

The present review study was revealed that *L. ingluviei* ADK10 and *S. pasteurii* supplementation has both beneficial probiotic characteristic and curative role against acetaminophen-induced nephrotoxicity, probably through its antioxidant and through enteric dialysis by producing urease. *L. ingluviei* ADK10 and *S. pasteurii* might, therefore, be considered as a potential safe therapeutic agent against renal injury caused by acetaminophen overdose.

CONCLUSION

We therefore conclude that *S. pasteurii,* and *L. ingluviei* ADK10 is safe for probiotic consumption in humans. *L. ingluviei* ADK10 and *S. pasteurii* possess a number of interesting important properties that constitute the requirement for their use as high potential probiotics with health-promoting properties (according to ICMR-DBT Guidelines for evaluation of probiotics in food, 2011). *L. ingluviei* ADK10 and *S. pasteurii* supplementation has both beneficial probiotic characteristic and curative role against acetaminophen-induced nephrotoxicity, probably through its antioxidant and through enteric dialysis by producing urease. *L. ingluviei* ADK10 and *S. pasteurii* might, therefore, be considered as a potential safe therapeutic agent against renal injury caused by acetaminophen overdose. Both the bacteria supplementation in a dose of 10^9 cfu /kg body weight has curative role against acetaminophen-induced nephrotoxicity, probably through its high urease enzyme production property as well as antioxidant property previously examined In case of group treated with *S. pasteurii* at a dose of 1×10^9 cfu and duration of 3 weeks show significant reduction of uremic profiles. In case of group treated with *L. ingluviei* ADK10 at a dose of 1×10^9 cfu and duration of 2 weeks show significant reduction of uremic profiles. Therefore, this alternative harmful bacteriotherapy management for kidney diseases are innovative as well as cost effective preventive, protective and promotive not only free from stressful but also delay the frequency of dialysis and increase the survible period after kidney transplant. Ultimately community people will be benefited.

CONFLICT OF INTEREST

I declare that I have no conflict of interest.

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