JNEPHROL 0000; 00(00): 000-000

DOI: 10.5301/jn.5000129

In vivo assessment of bacteriotherapy on acetaminophen-induced uremic rats

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Abstract

Objectives: **Acetaminophen is a commonly used antipyretic agent which, at high doses, causes renal tubular damage and uremia. Bacteriotherapy affords a promising approach to mitigating uremic intoxication by ingestion of live microbes able to catabolize uremic solutes in the gut. The present study evaluates the nonpathogenic soil-borne urease-positive bacterium Sporosarcina pasteurii (Sp) as a potential urea-targeted component for such an "enteric dialysis" formulation.**

Methods: **Twenty-four albino male rats were randomly divided into 4 groups: The control group (group NC) received distilled water intraperitoneally for 7 days. The positive control group (group U) received 500 mg/kg acetaminophen intraperitoneally for 7 days. The tested group (group UP) was administered Sp at a dosage of 10^9 cells/day for 5 weeks, after receiving 500 mg/kg per day of acetaminophen intraperitoneally for 7 days. Vehicle control (group VC) received only Sp at a dosage of 10^9 cells/day for 5 weeks without acetaminophen treatment. Blood, kidney, liver and stool samples were collected after scarification, for biochemical (urea, creatinine, malondialdehyde, superoxide dismutase, catalase, glutamate oxaloacetate transaminase [GOT] and glutamate pyruvate transaminase [GPT] of blood, kidney and liver) tests. Limited fecal analysis was performed.**

Results: **Blood urea nitrogen (urea, creatinine) and toxicity indicators (GOT, GPT) were increased, and antioxidant enzymes were decreased in group U. Blood urea nitrogen and toxicity indicators were reduced, and antioxidant enzymes were increased significantly in the group UP ($p<0.05$) compared with group U. The number of Sp was increased in Sp-treated groups compared with groups NC and U.**

Conclusions: **The study demonstrated that the bacteria tested reduced blood urea nitrogen levels significantly.**

Key words: *Acetaminophen, Enteric dialysis, Sporosarcina pasteurii, Uremia*

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. In the United States the cost of treating patients with renal replacement therapy and renal injury will be US $28 billion by the year 2010, and in India 90% patients suffering from kidney disease are not able to afford the cost of uremia management (2). 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.

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). In the early stage of acetaminophen toxicity, formation of the reactive intermediate *N*-acetyl-p-benzoquinone imine (NAPQI) through cytochrome P450 occurs. At therapeutic doses, NAPQI is removed by conjugation with glutathione sulfhydryl (GSH). High doses of acetaminophen when in-

gested result in the reduction of cellular GSH and allow NAPQI to be attached to cellular proteins and begin lipid peroxidation, along with renal injury (3, 4).

"Enteric dialysis" is an alternative approach using live microbes for solute removal in uremia, based on the fact that concentration gradient make solutes disperse from plasma into the lumen when the concentration of uremic solute becomes greater in plasma than in the lumen, and major parts of uremic solutes become distributed throughout the intestine (5) by binding to large amounts of ingestible solute-specific sorbents within the gut. To mitigate uremic solutes, the use of live bacteria which degrade uremic toxins within the gut has been the preferred approach to date (6, 7). Spore-forming ureolytic gram-positive Bacillus, lactic acid bacteria and yeast were tested for bacteriotherapy of azotemia by some researchers, and it was demonstrated that feeding with Sporosarcina pasteurii and Lactobacillus sporogenes minimized blood urea levels, slowed the progression of end-stage renal disease and increased the life span of experimental rats (8, 9). Supplementation with probiotics for 16 weeks lowered blood urea nitrogen (BUN) levels in nephrectomized rats, slowed the progression of azotemia observed in the placebo group and prolonged the life of uremic rats (10). Sporosarcina pasteurii (formerly classified as Bacillus pasteurii) produce much more urease enzyme than Proteus vulgaris, and they do not introduce human or environmental toxicity or pathogenicity. S. pasteurii can be considered for management of uremic patients, due to its breaking down of BUN, as it uses urea as its sole nitrogen source and producing ammonia.

Our present study aims to evaluate the nonpathogenic, soil-borne, alkalophilic, urea-degrading bacterium *S. pasteurii* (Sp) with its enhanced ability for urease production (phenotypic mutants of *S. pasteurii* MTCC 1761), as a remedy for uremia in acetaminophen-induced uremic rats. As this strain of Sp has not been considered for management of uremic patients nationally or internationally.

Material and methods

Selection of animals and care

The study was conducted on 24 healthy, adult, male albino Wister rats strain (Ghosh Animal, Animal Foods and Animal Cages Supplier, Kolkata, West Bengal, India) with a body weight of 100 ± 15 g. They were acclimatized to laboratory conditions for 2 weeks prior to experimentation. Animals were housed 6 per cage in a temperature-controlled room (22ºC ± 2ºC) with 12/12 hour dark/light cycle (8:00-20:00 light and 20:00-8:00 dark) at a humidity of 50% ± 10%. They were provided with standard food (pellet diet) and water ad libitum. The principles of laboratory animal care of the US National Institutes of Health were followed throughout the duration of the experiment (11), and our institute's ethics committee approved the experimental protocol.

Grouping of animals and experimental procedure

Animals were randomized and divided into 4 groups (NC, U, VC and UP) of 6 animals in each group. Group NC served as untreated control and was injected with distilled water 5 ml/kg body weight daily for 7days. Group VC rats were similarly treated as group NC. Groups UP and U animals were treated with 500 mg/kg body weight of the acetaminophen for 7 days intraperitoneally.

Formulation preparation

For the present study, food balls were arranged for a casein-based diet with Sp, sterile 10% honey and milk mixture (12). The formulation was stored in a –70°C freezer in aseptic conditions. For formulation, composition and dosage specifications, see the Table I.

Hematological study

After 5 weeks, animals were sacrificed by diethyl ether anesthesia. Blood samples were collected by hepatic artery puncher under diethyl ether anesthesia, using 21-gauge (21G) needles mounted on a 5-mL syringe (Hindustan Syringes and Medical Devices Ltd, Faridabad, India) into heparin-coated sample bottles for analysis of hematological parameters including red blood cell (RBC) count by hemocytometer and hemoglobin (Hb) by standard kit method (Merck, Japan).

Blood uremia profile

Biochemical estimation of blood urea

The collected blood was centrifuged, and the plasma fraction was separated. Plasma urea levels were measured by commercially available standard blood urea kit (Merck, Japan) with a semiautoanalyzer (Merck, Japan) following a standard protocol for photometric determination of urea according to the glutamate dehydrogenase method (13).

Biochemical estimation of blood creatinine

The collected blood was centrifuged, and the plasma fraction was separated. Plasma creatinine levels were measured

by commercially available standard creatinine kit (Merck, Japan) with a semiautoanalyzer (Merck, Japan) following a standard protocol for photometric determination of creatinine based on the Jaffé kinetic method without deproteinization (14).

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 (H_2O_2) 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 (15).

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 (16). 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%.

Estimation of lipid peroxidation from the levels of malondialdehyde

Kidneys and livers were homogenized separately at the tissue concentration of 50 mg/mL in 0.1 M of ice cold phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000*g* at 4ºC for 5 minutes individually, and the supernatants were used for the estimation of malondialdehyde (MDA) levels. The MDA in the sample was calculated by using the extinction coefficient of 1.56×10^5 M/cm and expressed in the unit nM/mg of tissue or nM/mL of plasma (17).

Toxicity study

Biochemical estimation of glutamate oxaloacetate transaminase and biochemical estimation of glutamate pyruvate transaminase

For the assessment of toxicity in serum, liver and kidney, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were measured according to the method of Goel (18).

Limited analysis of fecal microbiota

Limited analysis of fecal microbiota was performed for rats from all groups. Fresh fecal samples were obtained from individual animals at baseline (before treatment) and after 8 weeks of feeding. Samples were stored at –20°C for analy-

TABLE I
COMPOSITION OF MICROBIAL ADDITIVE FED TO DIFFERENT GROUPS

Serial no.	Groups	Microbial additive	No. of rats	CFU/dose per day
1	NC	No microbe(s)	6	-
2	U (acetaminophen)	No microbe(s)	6	-
3	UP (acetaminophen)	*Sporosarcina pasteurii* (Sp)	6	1×10^9 CFU
4	VC	*Sporosarcina pasteurii* (Sp)	6	1×10^9 CFU

CFU = colony-forming units; NC = control group: distilled water intraperitoneally for 7 days; U = positive control group: 500 mg/kg acetaminophen intraperitoneally for 7 days; UP = tested group: administered Sp at a dosage of 10^9 cells/day for 5 weeks; VC = vehicle control: received only Sp at a dosage of 10^9 cells/day for 5 weeks without acetaminophen treatment.

sis. Fecal mass was resuspended in physiological peptone saline at an approximate concentration of 0.02 g/mL. Serial dilutions in peptone saline were prepared, and 0.1-mL aliquots from dilutions of 10^{-5} and 10^{-7} were placed on Man-Rogosa-Sharpe agar (MRS agar; Highmedia, India) agar to obtain *Lactobacillus* sp. counts and on TSB agar plates (pH 9.0) to enumerate the population of Sp and other alkalophilic bacteria. Plates were incubated at 37°C for 48 hours (12).

Withdrawal study

Twelve Wister male rats were taken and divided randomly into 2 groups: group 1 was treated as controls, and group 2 was treated by Sp feeding at a dose of 10^9 bacterial cells for 7 days. Then after a regular interval (at 5, 10 and 15 days) stools of experimental animals were collected and cultured from dilutions of 10^{-5} on MRS agar (Highmedia, India) to obtain *Lactobacillus* sp. counts and on TSB agar plates (pH 9.0) to enumerate the population of Sp and other alkalophilic bacteria. Plates were incubated at 37°C for 48 hours (12).

Statistical analysis

Analysis of variance (ANOVA) followed by a multiple 2-tailed *t*-test with Bonferroni modification was used for statistical analysis of the collected data. Differences were considered significant when p was <0.05.

Results

Body weight

Body weight increased at the end of the experiment in groups U, NC, VC and UP compared with their initial body weight (Tab. II). In group U, the percentage of elevation in body growth was dramatically higher than in the other groups, due to the uremia-induced edema. After administration of the Sp in group UP, these indices returned toward the control levels.

Levels of hemoglobin and total RBC

Hb level and total RBC count were significantly decreased in group U animals (the acetaminophen-treated control group), compared with group NC. But in group UP, significantly higher levels of Hb and total RBC count were observed, compared with group U, and the values returned to those of the control group. In group VC, both levels of Hb and total RBCs were increased significantly after receiving Sp for 5 weeks (Tab. II).

Levels of blood urea and creatinine

Urea and creatinine levels were significantly increased in group U animals (the acetaminophen-treated control group), compared with group NC. But in group UP, significantly lower levels of urea and creatinine were observed, compared with group U, and the values returned to those of the control group (Tab. II).

Quantification of MDA

Quantities of MDA altered significantly in blood, liver and kidney, among all test groups (Tab. II).

Activity levels of CAT and SOD

After receiving acetaminophen (group U), the activity of this enzyme in blood and kidney were decreased significantly, compared with group NC. In group UP, CAT activity in blood, liver and kidney was elevated, compared with group U. There was a significant protection in CAT activity after treatment with Sp; administration of Sp to group UP resulted in a significant elevation in the activity of SOD in blood liver and kidney, compared with the animals in group U. The activity of this enzyme was decreased significantly in blood, liver and kidney in group U, in comparison with groups NC and VC (Tab. II). Treatment of Sp in the animals in group UP resulted in a significant restoration of SOD activity, compared with group U, and the values returned to the control levels. Activity of CAT and SOD was increased in the non-acetaminophen-treated control group (VC).

Activity levels of GOT and GPT

The activity levels of GOT and GPT altered significantly in serum, liver and kidney among all test groups, as shown in Table III.

Result of limited fecal analysis

Sp revealed no effect on the population of *Lactobacilli* sp. in the rat intestine. There was no significant difference in *Lactobacilli* counts between the Sp-treated groups (group VC and UP) and group NC. The population of alkalophilic bacteria in the feces obtained from both VC and UP animals was higher compared with group NC, due to daily intake of Sp which is an alkalophilic bacteria and the taking of stool samples immediately after stopping treatment with Sp. Significant differences in the counts of alkalophilic bacteria were observed after 5 weeks of daily feeding with 10^9 colony

forming units (CFU) of Sp as compared with NC. Data are expressed as means ± SE (n=6) in Table IV.

Withdrawal study

Significant increases in the counts of alkalophilic bacteria were observed in group 2 after withdrawn of 5 days, 10 days and 15 days, after feeding with 10^9 CFU of Sp, in comparison with group 1 (Tab. V). We even observed that Sp count remained more or less the same 5, 10 and 15 days after withdrawal of bacterial feeding. There were no changes in *Lactobacillus* sp. counts in these 2 groups. Thus we can state that Sp might not be completely removed after stopping of Sp feeding, and it might be proliferating in the rats' intestines.

TABLE II
EFFECT OF ACETAMINOPHEN AND SP TREATMENT ON ACETAMINOPHEN-INDUCED OXIDATIVE STRESS AND UREMIA IN RATS

Parameters	Group NC: control	Group U: acetaminophen-receiving control	Group VC: vehicle control	Group UP: Sp treatment followed by acetaminophen
Body weight increase (g)	11.6 ± 3.77*	1.044 ± .002†	15.46 ± 4.05*	15.125 ± 1.99*
Hb (mg/dL)	10.863 ± 1.29*	8.861 ± 0.58†	14.629 ± 1.34‡	11.849 ± 0.59*
Total RBC%	7.03 ± 1.37*	4.537 ± .19†	11.465 ± 0.54‡	11.327 ± 1.36‡
Urea (mg/dL)	15.9 ± 2.4*	40 ± 2.17†	15.9 ± 1.83*	15 ± 1.77*
Creatinine (mg/dL)	0.288 ± 0.033*	0.696 ± 0.04†	0.31 ± 0.03*	0.452 ± 0.04‡
Blood malondialdehyde (nM/mL)	722.49 ± 189*	2,246.45 ± 217†	719.82 ± 130*	1,451.66 ± 643‡
Kidney malondialdehyde (nM/mg of tissue)	171.8 ± 48.1*	552.2 ± 52.5†	115.7 ± 43.4*	208.8 ± 64.3†
Liver malondialdehyde (nM/mg of tissue)	82.51 ± 8.51*	83.78 ± 9.49*	224.76 ± 31.98†	85.78 ± 3.99*
Blood superoxide dismutase (mmol/ml/min)	4.112 ± 0.44*	1.85 ± 0.2†	4.51 ± 0.61*	3.421 ± 0.55*
Kidney superoxide dismutase (mmol/mg/min)	2.473 ± 0.26*	0.97 ± 0.16†	3.44 ± 0.54*	2.382 ± 0.24*
Liver superoxide dismutase (mmol/mg/min)	6.78 ± 0.46*	1.95 ± 0.62†	5.64 ± 0.56*	4.16 ± 0.8*
Blood catalase (mmol/ml/min)	0.6497 ± 0.12*	0.12858 ± 0.022†	0.63376 ± 0.16*	0.36452 ± 0.010‡
Kidney catalase (mmol/mg/min)	0.5004 ± 0.014*	0.236 ± 0.05†	0.6883 ± 0.03*	0.298 ± 0.043†
Liver catalase (mmol/mg/min)	0.381 ± 0.016*	0.17 ± 0.08[b]	0.4437 ± 0.085*	0.375 ± 0.016*

Data are expressed as means ± SE (n=6).
Hb = hemoglobin; RBC = red blood cells.
*p<0.05, vs. other column, (mean value of groups is not significantly different for these p values) by ANOVA followed by multiple 2-tailed *t*-test.
†p<0.05, vs. other column, (mean value of groups is significantly different for these p values) by ANOVA followed by multiple 2-tailed *t*-test.
‡p<0.05, vs. other column, (mean value of groups is significantly different from * and † for these p values) by ANOVA followed by multiple 2-tailed *t*-test.

TABLE III
EFFECT OF ACETAMINOPHEN AND SP TREATMENT ON ACETAMINOPHEN-INDUCED OXIDATIVE STRESS AND UREMIA IN RATS

Groups	SGOT (U/L)	SGPT (U/L)	GPT kidney (U/L)	GOT kidney (U/L)	GPT liver (U/L)	GOT liver (U/L)
Group NC: control	17.35 ± 0.83	26.85 ± 0.67	24.2 ± 1.0975	29.1 ± 0.8636	21.725 ± 0.99	25.35 ± 1.6
Group VC: vehicle control	12.67 ± 0.437	15.75 ± 0.0934	12.1 ± 0.47676	14.75 ± 0.008	18.175 ± 0.080	14.55 ± 0.36
Group U: acetaminophen-receiving control	62.1 ± 3.1	71.1 ± 0.4358	83.43 ± 0.717	72.9 ± 2.567	112.27 ± 8.76	92 ± 10.643
Group UP: Sp treatment followed by acetaminophen	19.25 ± 0.869	20.975 ± 0.199	28.825 ± 0.474	23.875 ± 0.98	20.7 ± 2.9936	25.05 ± 0.84

Data are expressed as means ± SE (n=6).
GOT = glutamate oxaloacetate transaminase; GPT = glutamate pyruvate transaminase; SGOT = serum glutamate oxaloacetate transaminase; SGPT = serum glutamate pyruvate transaminase; Sp = *Sporosarcina pasteurii*.

TABLE IV
RESULTS OF LIMITED FECAL ANALYSIS

Groups	Colony no. on MRS at 10^{-5} dilution (CFU) (immediately after stopping treatment)	Colony no. on TSB at 10^{-5} dilution (CFU) (immediately after stopping treatment)
Group NC: control	64 ± 6.1	30 ± 5.33
Group VC: vehicle control	77 ± 8.23	224 ± 23.98
Group U: acetaminophen-receiving control	72 ± 3.01	39 ± 0.96
Group UP: Sp treatment followed by acetaminophen	69 ± 4.09	203 ± 19.06

Data are expressed as means ± SE (n=6).
CFU = colony-forming unit; MRS = Man-Rogosa-Sharpe agar; TSB = Tryptic Soy Broth.

TABLE V
RESULTS OF LIMITED FECAL ANALYSIS OF WITHDRAWAL STUDY

Groups	5 days after stopping Sp treatment		10 days after stopping Sp treatment		15 days after stopping Sp treatment	
	Colony no. on MRS at 10^{-5} dilution (CFU)	Colony no. on TSB at 10^{-5} dilution (CFU)	Colony no. on MRS at 10^{-5} dilution (CFU)	Colony no. on TSB at 10^{-5} dilution (CFU)	Colony no. on MRS at 10^{-5} dilution (CFU)	Colony no. on TSB at 10^{-5} dilution (CFU)
Group 1	55 ± 6.9	65 ± 5.89	58 ± 5.5	49 ± 9.8	56 ± 4.1	52 ± 8.1
Group 2	47 ± 3.8	218 ± 20.4	61 ± 3.45	211 ± 16.2	59 ± 7.6	201 ± 13.3

Data are expressed as means ± SE (n=6).
CFU = colony-forming unit; group 1 = control; group 2 = Sp-administered group; MRS = Man-Rogosa-Sharpe agar; Sp = *Sporosarcina pasteurii*; TSB = Tryptic Soy Broth.

Discussion

Acetaminophen overdose is often linked to many metabolic disorders. including those of blood electrolytes such as Na+ and K+, and urea and creatinine derangements. Higher concentrations of serum urea and creatinine are considered in the investigation of drug- and toxin-induced uremia in animals and human (19). The fundamental function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotics, makes the hematopoietic system unique as a target organ. Erythrocytes, leukocytes and platelets are produced at a turnover rate of about 1-3 million per second in a healthy human adult, and these values can be distorted in certain physiological or pathological states during hemolytic anemia or suppressive inflammation (20). Certain drugs including alkylating cytotoxic agents can also affect blood cell development rates and the normal range of hematological parameters. In the present study, receiving acetaminophen intraperitoneally for 7 days significantly decreased Hb levels and also lowered total RBC counts. The decrease in Hb is supposed to be due to destruction of RBCs and lower total RBC count due to hemolytic anemia or suppressive inflammation. After administration of Sp for 5 weeks, Hb levels become significantly higher with improved RBC counts, compare with the acetaminophen-induced uremic group U. However, this study shows that Sp could reverse the hematotoxic effect of acetaminophen, with ensuing improvement of hematopoiesis. BUN is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. In renal disfunction, blood urea levels are raised because the rate of blood urea production becomes higher than the rate of renal clearance (21). Higher urea and creatinine levels in the blood are taken as an indicator of nephrotoxicity (20). Elevation of urea and creatinine levels in serum is taken as an index of nephrotoxicity (22). 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, administration of a nephrotoxic dose of acetaminophen to rats resulted in progression of oxidative stress harmful to hepatic and renal tissues. In this study, acetaminophen-induced nephrotoxicity showed a significant ($p<0.05$) increase in the plasma urea and creatinine concentrations in group U (acetaminophen-induced uremic) rats when compared with the normal group (group NC). Moreover, oral administration of Sp significantly ($p<0.05$) decreased plasma urea and creatinine in group UP, when compared with group U. The intestinal mucosal surface functions as a semipermeable membrane. Driven by the concentration gradient, solutes with higher concentration in circulating blood diffuse from plasma into the intestine, and a large portion of uremic solutes are differentially distributed within the bowel. Thus urea-degrading bacteria *S. pasteurii*, when ingested, catabolize uremic solutes in the gut. Oxidative stress and lipid peroxidation are 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 (23). Previous studies have clearly demonstrated that acute acetaminophen overdose increases the lipid peroxidation and suppresses the antioxidant defence mechanisms in renal tissues (24). However, in acetaminophen-treated animals, MDA levels were significantly increased, when compared with normal control rats. On administration of Sp, the levels of MDA decreased significantly when compared with acetaminophen-induced uremic rats. 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 (25). 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. This was due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When rats were treated with Sp, SOD and CAT activity increased significantly, when compared with the uremic group ($p<0.05$). Sp improves 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 (26). Acetaminophen-induced nephrotoxicity was shown by biochemical measurements and histopathological changes, which coincide with the observations of other investigators (27). After Sp treatment, serum GPT, serum GOT, kidney GOT and kidney GPT, liver GOT and liver GPT levels in acetaminophen-induced uremic rats were decreased compared with the untreated uremic group, which may be due to toxic compound removal by the bacteria. Significant increases in the counts of alkalophilic bacteria in the stool of groups fed with Sp (in Tab. IV) immediately after 5 weeks of daily feeding with 10^9 CFU of Sp, as compared with group NC, may have been due to

the intake of alkalophilic Sp daily. Results of the withdrawal study (Tab. V) showed significantly increased counts of alkalophilic bacteria in group 2 even after 15 days, in comparison with group 1. This result showed that the increased Sp number in the form of alkalophilic bacteria may be due to Sp adhering to the intestinal membrane or the intestinal environment serving to create better survival conditions for bacteria, and bacteria being retained within gut after the cessation of feeding. Thus urease enzyme production through Sp within the rat intestine was not hampered by stopping Sp administration, and the return of uremia was not possible. Although Sp is occasionally isolated from human feces and no pathological reactions have been associated with this bacterium, it is hard to exclude the possibility that ingested Sp may cause infections in some patients with compromised immune systems. These findings suggest the potential use of the Sp as a novel therapeutically useful antiuremic agent.

Acknowledgements

The authors are grateful to Prof. Ananta Kr. Ghosh, IIT Khargapur, and Prof. Antony Gomes, Calcutta University, for their valuable interaction for this type of work.

Financial support: The Department of Science and Technology (DST), Government of India, provided funding for this work, in the form of an INSPIRE Fellowship.

Conflict of interest statement: None.

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References

1. Ayodele OE, Alebiosu CO. Burden of chronic kidney disease: an international perspective. Adv Chronic Kidney Dis. 2010; 17(3):215-224.
2. Agarwal SK, Dash SC, Irshad M, Raju S, Singh R, Pandey RM. Prevalence of chronic renal failure in adults in Delhi, India. Nephrol Dial Transplant. 2005;20(8):1638-1642.
3. Nelson SD. Mechanisms of the formation and disposition of reactive metabolites that can cause acute liver injury. Drug Metab Rev. 1995;27(1-2):147-177.
4. Jones AF, Vale JA. Paracetamol poisoning and the kidney. J Clin Pharm Ther. 1993;18(1):5-8.
5. Hart SG, Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD. Acetaminophen nephrotoxicity in CD-1 mice: Part I: evidence of a role for in situ activation in selective covalent binding and toxicity. Toxicol Appl Pharmacol. 1994;126(2): 267-275.
6. Sparks RE. Review of gastrointestinal perfusion in the treatment of uremia. Clin Nephrol. 1979;11(2):81-85.
7. Hida M, Aiba Y, Sawamura S, Suzuki N, Satoh T, Koga Y. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. Nephron. 1996;74(2):349-355.
8. Patel BG, Zelenaia OZ, Dhree RS, Natarajan R, Friedman EA. Oral bacteriotherapy effectively reduces severity of azotemia in 5/6th nephrectomized rats [abstract]. J Am Soc Nephrol. 2003;14:765A (SU-PO1042).
9. Dunn SR, Marczely J, Ranganathan P, Michael LS, Friedman EA. Probiotics extend survival in untreated 5/6th nephrectomized rats: possible use for probiotics as an adjunct in chronic renal failure (CRF) [abstract]. J Am Soc Nephrol. 2003;14: 765A (SU-PO1043).
10. Ranganathan N, Patel B, Ranganathan P, et al. Probiotic amelioration of azotemia in 5/6th nephrectomized Sprague-Dawley rats. ScientificWorldJournal. 2005;5:652-660.
11. Olert ED, Cross BM, McWilliam AA. Guide to care and use of experimental animals. In: Olert ED, McWilliam BM, eds. Canadian Council on Animal Care. Vol. 1; 2nd ed. Ottawa: US National Institutes of Health;1993:40-93.
12. Ranganathan N, Patel BG, Ranganathan P, et al. In vitro and in vivo assessment of intraintestinal bacteriotherapy in chronic kidney disease. ASAIO J. 2006;52(1):70-79.
13. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. 3rd ed. Philadelphia, PA: WB Saunders; 1999:809-861.
14. Sabbagh M, Rick W, Schneider S. A kinetic method for the direct determination of creatinine in serum with 3,5-dinitrobenzoic acid without deproteinization. J Clin Chem Clin Biochem. 1988;26(1):15-24.

15. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195(1):133-140.
16. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3): 469-474.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358.
18. Goel BK. Routine biochemical test. In: Mukherjee KL, ed. Medical laboratory technology. Vol. 3. New Delhi: Tata McGraw-Hill; 1988:985-1079.
19. Bennett WM, Parker RA, Elliott WC, Gilbert DN, Houghton DC. Sex-related differences in the susceptibility of rats to gentamicin nephrotoxicity. J Infect Dis. 1982;145(3):370-373.
20. Guyton AC. Red blood cells, anemia, and polycythemia. In: Textbook of Medical Physiology. 8th ed. Philadelphia: W.B. Saunders; 1991:56-64.
21. Mayne PD. The kidneys and renal calculi. In: Clinical chemistry in diagnosis and treatment. 6th ed. London: Edward Arnold; 1994:2-24.
22. Ali BH, Ben Ismail TH, Basheer AA. Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity: influence of gonadectomy and hormonal replacement therapy. Indian J Pharmacol. 2001;33:369-373.
23. Somani SM, Husain K, Whitworth C, Trammell GL, Malafa M, Rybak LP. Dose-dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. Pharmacol Toxicol. 2000;86(5):234-241.
24. Abdel-Zaher OA, Abdel-Rahman MM, Hafez MM, Omran FM. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. Toxicology. 2007;243(1-2):124-134.
25. Pande M, Flora SJ. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. Toxicology. 2002;177(2-3):187-196.
26. Manov I, Hirsh M, Ianccu TC. Acetaminophen hepatotoxicity and mechanisms of its protection by N-acetylcysteine: a study of Hep3B cells. Exp Toxicol Pathol. 2002;53(6):489-500.
27. Corcoran GB, Racz WJ, Smith CV, Mitchell JR. Effects of N-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther. 1985;232(3): 864-872.

Accepted: February 06, 2012