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Original article

Protective effect of selected urease positive *Lactobacillus* strains on acetaminophen induced uremia in rats

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ABSTRACT

Urease positive probiotic *Lactobacillus* strains were tested for oxidative stress and uremic profile on experimental rat (Wister strains) induced by acetaminophen (APAP) overdose. Experimental rats received acetaminophen interperitoneally at the dose of 500 mg/kg/day, continuously for 10 days. From 11th day onwards they were orally fed with *Lactobacillus fermentum* (MTCC 903), *Lactobacillus plantarum* (MTCC 4462) and *Lactobacillus rhamnosus* (MTCC 1408) respectively at the dose of 10^9 CFU/mL/100 g of body weight/day for 15 days continuously. Plasma, kidney, liver and fecal samples were tested for uremic profile of the sacrificed rats after the experiment. In APAP treated rats, plasma urea, creatinine (Cr), glutamate oxaloacetate transaminase (GOT) and malonaldehyde (MDA) level elevated and catalase (CAT) and super oxide diusmutase (SOD) level declined significantly compared to negative control. However, level of plasma urea, Cr, GOT and MDA in tested rats were significantly lower in comparison to positive control. The uremic profile of the probiotic induced rats was very much comparable with the negative control, even better for some parametric values. Prevention of DNA fragmentation in kidney tissues and reduction of enteric pathogens in feces of *Lactobacillus* fed rats were noticed. Electrolytes profile of the tested plasma samples were in acceptable range. To sum up, tested urease positive *Lactobacillus* strains were shown to improve the clinical condition of the acetaminophen induced uremic experimental rats.

1. Introduction

Acetaminophen (APAP), recognized as an analgesic and antipyretic drug, can also result in both hepato- and nephrotoxicity due to overdose, though nephrotoxicity is less common than hepatatoxicity. In APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury [1,2]. APAP on interaction with cytochrome P450 (CYP) produces toxic electrophile N-acetyl p-benzoquinone imine (NAPQI) both in liver and kidney [3]. At therapeutic doses, the quantity of NAPQI formed is relatively small and is detoxified by conjugation with reduced glutathione (GSH) [4]. APAP overdose results in the excess formation of reactive intermediate of NAPQI that effectively causes the depletion of cellular GSH, thereby allowing NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to renal injury [1].

Urea is the predominant nitrogenous waste product of protein catabolism [5]. Although the mechanisms involved in urea's toxicity are poorly understood, it is well established that urea contributes to the synthesis of other toxic moieties including guanidines and carbamylation products [6]. Parallelly it increases the severity of renal dysfunction. Moreover, excess urea induces generation of free radicals and oxidative stress, which has been implicated in cellular damage [7]. Uremia is a precondition to renal failure wherein nitrogenous waste products, chiefly urea, accumulate in the blood.

"Enteric dialysis" is an unconventional analysis for solute elimination in uremia, based on the reality that the intestinal barrier functions as a semi permeable membrane. Concentration gradient makes solutes disperse from plasma into the lumen due to increase amount of uremic toxins in plasma than lumen. This leads to circulation of a large amount of uremic solutes throughout the intestine [8]. This approach requires large quantities of exact sorbents to be ingested daily. Thus to minimize uremic solutes, the use of living bacteria which degrade uremic toxins within the gut has been an acceptable therapy of today [9–11]. Prebiotics are non-digestible compounds beneficially modifying the composition as well as function of the intestinal flora. Whereas probiotics are living bacteria administered as food components or supplements, which provide specific benefits reducing urea alone themselves. Modification of intestinal flora to refrain generation of toxins, either by prebiotics or by probiotics, is cited in various literature [11,12]. Probiotics as well as prebiotics evaluate the impact on solute concentration in

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plasma or on their fecal or urinary excretion [9]. Various probiotic strains against APAP-induced nephrotoxicity are available in the literature. A few reports are also available about this issue by using different probiotic *Lactobacillus* strains [13,14]. Fermented food based common strains like *Lactobacillus fermentum*, *Lactobacillus planturum* and *Lactobacillus rhamnosus* could be apparently effective in this context, hither to unreported in literature. In this study, these urease positive *Lactobacillus* strains were evaluated for bacteriotherapy of APAP-induced uremia. To achieve this goal, experimental rat model was used considering certain microbiological, biochemical and molecular biological parameters.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Urease positive probiotic *L. fermentum* (MTCC 903), *L. plantarum* (MTCC 4462) and *L. rhamnosus* (MTCC 1408) were cultured in De Man–Rogosa–Sharpe (MRS) broth under anaerobic condition at 37 °C (Difco, Detroit, MI, USA) with 30% (v/v) glycerol. Before experimental use, cultures were subcultured twice in MRS broth (Difco). Indicator bacteria used for antimicrobial assay namely *E. coli* (MTCC443), *V. cholereae* (MTCC 3906), *S. aureus* (MTCC 3160), *S. mutans* (MTCC890), *K. pneumoniae* (MTCC 109) and *C. albicans* (MTCC227) were provided by Microbiological laboratory and clinical detection center, Midnapur (West Medinipur, India). They were cultured in tryptone soy broth or agar (TSB or TSA) in aerobic condition at 37 °C.

2.2. Experimental design for in vivo treatment

2.2.1. Selection of animal and care

The study was conducted on 30 healthy adult male albino rats of Wister strain (supplied from Ghosh Animal, Animal Foods and Animal Cages Supplier, Kolkata 54) having body weight of 105 ± 15 g. They were acclimatized to laboratory condition for 2 weeks prior to the experiment. Animals were housed three rats/cage in a temperature-controlled room (22 ± 2 °C) with 12–12 h dark–light cycles (8.00–20.00 h light, 20.00–8.00 h dark) at a humidity of 50 ± 10%. They were provided with standard food containing all the recommended macro- and micronutrients (56% carbohydrate, 18.5% protein, 8% fat, 12% fiber and adequate levels of minerals and vitamins) along with water in adequate volume [15]. Animal care was provided according to the Guiding Principle for the Care and Use of Animals [16].

2.2.2. Grouping of animals and experimental procedure

Rats were divided into five equal groups as follows: Group I or negative control (NC): six animals were subjected to control group. They were housed at room temperature (25 ± 3 °C) and were fed with standard normal diet with water at regular time of interval. Group II or acetaminophen induced uremic positive control (PC): six animals were randomly placed in cage with normal diet and were injected with acetaminophen at the concentration of 500 mg/kg of body weight/day for 10 days to achieve uremia. Group III or acetaminophen with administration by *L. fermentum*: six animals were randomly placed in cage with normal diet and the probiotic strain. Strain was administered (1 mL/day) forcefully for 15 days at specific time before providing the food. Group IV and V were similar as Group III, except the probiotic strains. *L. plantarum* and *L. rhamnosus* were the probiotic strains respectively for Group IV and V. Concentration of probiotic bacteria dose in all three groups was 10^9 CFU/mL/100 g of body wt.

2.2.3. Collection of blood, kidney and liver from the rat

This experimental design was continued for 25 days, after which the animals were sacrificed by mild chloroform anaesthesia [17]. Blood was collected on decapitation; plasma was separated by centrifugation (3500 rpm × 20 min) and preserved at −20 °C. Livers and kidneys were immediately dissected out, washed and stored in 0.9% ice cold saline for various biochemical evaluations.

2.2.4. Estimation of biochemical parameters of plasma, and tissue

Uremic profile as plasma urea [18] creatinine (Cr) [19,20] were assayed using diagnostic reagent kit (Merk, Japan). Biochemical parameters, catalase (CAT) [21], super oxide diusmutase (SOD) [22], glutamate oxaloacetate transaminase (GOT) [23] and lipid per oxidation marker as malonaldehyde (MDA) level [24] were estimated in plasma, kidney and liver.

2.2.5. DNA fragmentation assay

Extent of DNA fragmentation in the kidney tissues was determined by gel electrophoresis method [25].

2.3. Safety assays in vivo

2.3.1. Influence on fecal microflora of rats

After rats were fed with 1 mL respective probiotic suspensions at 10^9 CFU/mL/100 g of body weight, survival of pathogen as well as *Lactobacillus* spp. during transit through the gastrointestinal tract was determined in fecal samples [26].

2.3.2. Antimicrobial activity and nature of antimicrobial substances

To characterize the antimicrobial compounds produced by *Lactobacillus* strains, cell free culture supernatants (CFCS) were treated with various substances. Treatment of CFCS was as follows: for organic acids, culture supernatants were neutralized to pH 6.5 with the addition of 1 M NaOH; for bacteriocins, supernatants were treated with either protease (final concentration of 1 mg/mL) or trypsin (final concentration of 1 mg/mL) for 2 h at 30 °C; and for hydrogen peroxide, supernatants were treated with catalase (final concentration of 0.5 mg/mL). Treated CFCS were used for antimicrobial assay by the agar well-diffusion method. Presence or absence of inhibitory zones around wells was determined after incubation for 24 h at 37 °C [27].

2.4. Statistical analysis

The values were expressed as mean ± standard error (SE). Data were analyzed using one-way ANOVA followed by multiple two-tail *t*-test. P value < 0.05 was considered as significant.

3. Results

3.1. Body weight

Body weight increases in groups I, III, IV and V at the end of the experiment were compared with their initial body weight (Table 1). In group II, percentage of body weight increment was noticeably lesser compared to other groups, due to acetaminophen induced oxidative stress.

3.2. Estimation of uremia profile

Urea and creatinine levels were significantly increased in group II animals (the acetaminophen-treated control group) compared to group I. However, in group III, IV and V, significantly lower levels of urea and creatinine were observed, compared to group II; and the values returned to those of the control group (Table 2). Probiotic

Table 1
Body weight of experimental rats: anti-uremic and anti-oxidative effect of *Lactobacillus* strains at the dose 10^9 CFU/mL/100 g of body wt., respectively on body weight of experimental male albino rats. Data are expressed as mean ± SE ($n = 6$). ANOVA followed by multiple two-tail *t*-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($P < 0.05$).

Group	Final body weight (g)	Initial body weight (g)	Increase/decrease in body weight (g %)
I	110.66 ± 10.98^a	100.0 ± 5.0^a	10.66
II	93.33 ± 2.8^b	90.66 ± 1.6^b	0.33
III	136.66 ± 2.88^c	124.66 ± 2.9^c	12
IV	76.6 ± 5.2^d	75.6 ± 3.7^d	1.6
V	145.66 ± 4.19^d	140 ± 4.5^c	5.66

Group I: NC; Group II: PC; Group III, IV and V: administration with *Lactobacillus* strains *L. fermentum*, *L. plantarum* and *L. rhamnosus* respectively at the dose 10^9 CFU/mL/100 g of body weight respectively.

effect to acetaminophen treatment group resulted in lesser blood plasma urea and creatinine levels when compared with positive control.

3.3. Estimation of antioxidant enzymes (CAT and SOD)

After receiving acetaminophen (group II), the antioxidant enzymes activity in blood plasma kidney and liver were decreased significantly, compared to group I. In group III, IV and V, CAT activity in blood plasma, kidney and liver were elevated, in comparison with group II. There was a significant protection in CAT activity after treatment with *Lactobacillus* strains. Administration of *Lactobacillus* strains to group III, IV and V resulted in a significant elevation in the activity of SOD in blood plasma, kidney and liver, compared to the animals in group II (Table 2). Activity of this enzyme was decreased significantly in blood plasma, kidney and liver in group II, in comparison with groups I. Oral administration of probiotic strains into the animals in group III, IV and V, resulted in significant restoration of SOD activity, compared to group II, and the values returned to the control levels. Activity of CAT was increased and SOD was decreased in the acetaminophen-treated control group (PC).

3.4. Activity levels of GOT

Activity levels of GOT were altered significantly in blood plasma, among all test groups, as shown in Table 2.

3.5. Quantification of lipid per oxidation by MDA level

Quantities of MDA altered significantly in blood plasma, kidney and liver among all test groups (Table 2).

3.6. Influence on fecal microflora of rats

The number of lactic acid bacteria increased significantly in Groups III, IV and V, in comparison to control and uremic group. Furthermore, all three examined strains positively influenced the fecal microflora by decreasing the number of *Enterobacteria* spp. and *Staphylococcus* spp. No fungal colony was observed in tested MRS agar plates (Table 3).

3.7. Antimicrobial activity and nature of antimicrobial substances

CFCS of *Lactobacillus* strains displayed antimicrobial activity against all kinds of pathogens in agar well-diffusion assay. Antimicrobial activity was destroyed when CFCS were adjusted to pH 6.5 in all three probiotic strains. In addition to that, the antimicrobial activity remained active after protenase K and H_2O_2 treatment for the probiotic strains (Table 4).

3.8. Assessment of DNA fragmentation

DNA isolated from acetaminophen induced uremic animals indicated random DNA degradation – a hallmark of necrosis. No ladder formation was found indicating majority of cells undergone necrosis under the high nephrotoxic dose of acetaminophen. Probiotic *Lactobacillus* strains treatment was found to be effective to prevent this acetaminophen induced smear formation. Quantitative measurement of DNA fragmentation (by the colorimetric diphenylamine reaction) is represented in Fig. 1A. In agreement with the earlier findings, DNA fragmentation increased in case of uremic group, compared to control. That was effectively prevented by treatment with probiotic *Lactobacillus* strains (Fig. 1B).

Table 2
Effect of APAP and urease positive probiotic *Lactobacillus* strains on acetaminophen induced uremia on experimental rats.

Parameter	Group I (NC)	Group II (PC)	Group III (Lf)	Group IV (Lp)	Group V (Lrh)
BUN (mg/dL)	33.1 ± 0.46^a	69.3 ± 1.6^b	43.56 ± 0.56^c	43.56 ± 0.56^a	43.73 ± 0.70^d
Creatinine (mg/dL)	0.43 ± 0.004^a	1.47 ± 0.02^b	0.49 ± 0.01^c	0.45 ± 0.004^d	0.55 ± 0.01^c
Blood CAT (mmol/mL/min)	0.48 ± 0.01^a	0.145 ± 0.002^b	0.38 ± 0.05^c	0.42 ± 0.03^d	0.32 ± 0.043^e
Kidney CAT (mmol/mL/min)	0.51 ± 0.02^a	0.15 ± 0.005^b	0.36 ± 0.007^c	0.48 ± 0.02^a	0.25 ± 0.01^d
Liver CAT (mmol/mL/min)	0.57 ± 0.01^a	0.14 ± 0.001^e	0.41 ± 0.03^b	0.37 ± 0.04^d	0.42 ± 0.03^b
Blood SOD (mmol/mL/min	1.78 ± 0.024^a	0.26 ± 0.017^b	0.85 ± 0.018^c	0.81 ± 0.01^d	0.98 ± 0.026^e
Kidney SOD (mmol/mg/min)	2.78 ± 0.02^a	0.36 ± 0.017^b	1.56 ± 0.15^c	1.78 ± 0.10^d	1.93 ± 0.13^e
Liver SOD (mmol/mg/min)	0.96 ± 0.014^a	0.27 ± 0.008^b	0.76 ± 0.007^c	0.82 ± 0.013^d	0.07 ± 0.007^c
Plasma GOT (U/L)	21.65 ± 0.14^a	50.1 ± 0.26^b	24.21 ± 1.3^c	27.9 ± 0.58^d	26.6 ± 0.4^e
Blood MDA (nM/mL)	34.5 ± 0.6^a	64.0 ± 1.9^b	40.1 ± 0.79^c	47.0 ± 2.8^d	45.5 ± 2.3^d
Kidney MDA (nM/mL)	50.21 ± 3.9^a	98.82 ± 0.79^b	57.99 ± 2.06^c	58.07 ± 2.06^c	48.25 ± 2.8^d
Liver MDA (nM/mL)	46.62 ± 4.8^a	74.78 ± 5.76^b	46.94 ± 4.89^a	53.55 ± 2.44^c	50.21 ± 3.2^d

Estimation of uremia profile: anti-uremic and anti-oxidative effect of three *Lactobacillus* strains at the dose 10^9 CFU/mL/100 g of body wt., respectively on experimental male albino rats. Data are expressed as mean ± SE ($n = 6$). ANOVA followed by multiple two-tail *t*-test (Dunkan's multiple test) and data with different superscripts (a, b, c, d and e) in a specific vertical column differ from each other significantly with respect to negative control ($P < 0.05$).
NC: Negative control; PC: Positive control (Acetaminophen treatment with); Lf, Lp and Lrh: treatment with *L. fermentum*, *L. plantarum* and *L. rhamnosus* respectively; BUN: plasma urea level as blood urea nitrogen; MDA: malonaldehyde; CAT: catalase; SOD: superoxide dismutase; GOT: glutamate oxaloacetate transaminase.

Table 3
Influence on fecal microflora of rats. Anti-uremic and anti-oxidative effect of *Lactobacillus* strains at the dose 10^9 CFU/mL/100 g of body wt. Comparison of the bacterial count in feces of mice before and after feeding with respective *Lactobacillus* strains on experimental male albino rats. Data are expressed as mean ± SE ($n = 6$). ANOVA followed by multiple two-tail *t*-test and data with different superscripts (a, b, c) in a specific vertical column differ from each other significantly ($P < 0.05$).

Growth media	Log CFU/g feces				
	Group I	Group II	Group III	Group IV	Group V
A	8.68 ± 0.12^a	8.23 ± 0.25^b	7.98 ± 0.11^a	7.21 ± 0.15^a	7.45 ± 0.14^a
B	8.58 ± 0.14^a	6.98 ± 0.22^b	7.89 ± 0.13^a	6.76 ± 0.13^a	7.58 ± 0.16^a
C	7.95 ± 0.21^a	2.58 ± 0.14^b	9.77 ± 0.32^c	9.48 ± 0.30^c	12.25 ± 0.24^a
D	3.79 ± 0.35^a	5.58 ± 0.19^b	3.45 ± 0.25^c	3.15 ± 0.28^c	3.59 ± 0.22^c
E	5.05 ± 0.25^a	6.74 ± 0.11^b	4.21 ± 0.34^a	4.02 ± 0.32^c	4.51 ± 0.30^c
F	4.56 ± 0.38^a	5.54 ± 0.23^b	4.01 ± 0.12^c	4.58 ± 0.15^c	4.25 ± 0.36^a
G	1.10 ± 0.22^a	2.12 ± 0.11^b	–	–	–

Group I: NC; Group II: PC; Group III, IV and V: administration with *Lactobacillus* strains *L. fermentum*, *L. plantarum* and *L. rhamnosus* respectively at the dose 10^9 CFU/mL/100 g of body wt. Total aerobic (A) and anaerobic bacteria (B) on Peptone yeast extract glucose agar; total lactic acid bacteria (C) and *E. coli* (D) on Macon key-agar; E: *Enterobacteriaceae* on Violet red bile glucose agar; F: *Staphylococcus* spp. on Mannitol salt agar. Mean and G: fungal spp on YPD agar (± SE) of results from separate experiments; (–) colonies are not detected.

4. Discussion

In the current study, the potential effect of three different urease positive probiotic *Lactobacillus* strains to reduce the acetaminophen induced uremic toxicity in experimental rats was assessed. Probiotics have been shown to exhibit therapeutic efficacy in a number of experimental animal models of uremia [13,28]. Present study indicated that three tested probiotic strains were effective as they were able to reduce disease activity, prevent the APAP-induced uremia and increase the body weight gain. Acetaminophen (APAP) is usually taken chronically as an analgesic and antipyretic drug. However, overdose of APAP was reported to aid in kidney damage in human [29] and in experimental rats [30]. In this experiment, dosage of APAP of 500 mg/kg of body weight/day for 7 days with de-ionized water was set to achieve uremic condition in rat model. Urea and creatinine levels in plasma were significantly increased in the experimental group treated with APAP alone, in comparison to controlled and APAP + probiotic group. Administration of probiotic strains essentially decreased the plasma urea and creatinine levels into acceptable range in all the tested rats (Table 2).

During kidney injury, superoxide radicals generate at the site of damage. That modulates SOD and CAT, ensuing in the loss of activity and accumulation of superoxide radicals, eventually leading to kidney damage. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism [31]. In the present study, administration of all tested *Lactobacillus* strains increased the level of CAT and SOD activity of plasma, kidney and liver of the uremic rats. The mechanism can be attributed to the nephroprotective effect of respective probiotic *Lactobacillus* strains (Table 2).

After probiotic treatment, GOT levels of plasma in APAP-induced uremic rats were significantly decreased compared to the untreated uremic group (Groups II) (Table 2) due to the removal of toxic agents using probiotic strains.

DNA fragmentation analyses were performed for further verification of APAP-induced renal damage and its protection by probiotic strains. APAP induced random fragmentation of genomic DNA along with subsequent formation of a DNA smear on agarose gel without ladder formation. This observation suggested that APAP-induced renal cell damage occurred through necrotic pathway with apoptosis also took place alongside. Nonetheless, the later one was of lesser significance (Fig. 1).

Increased level of hepatic and renal lipid peroxidation by APAP induction and its remediation by different probiotic administration is reported earlier [32]. This is ascribed to the free radical mediated chain reaction that damages cell membranes. MDA is an indicator of the degree of lipid peroxidation [30]. In this study, significant increase in MDA levels was observed in plasma cell, kidney and liver tissue of rats treated with APAP alone compared to control. Probiotic treatment inhibited the increase of lipid peroxidation. It is likely that action of probiotic strains in reducing the membrane damage is mainly related to its ability to scavenge lipid peroxidation.

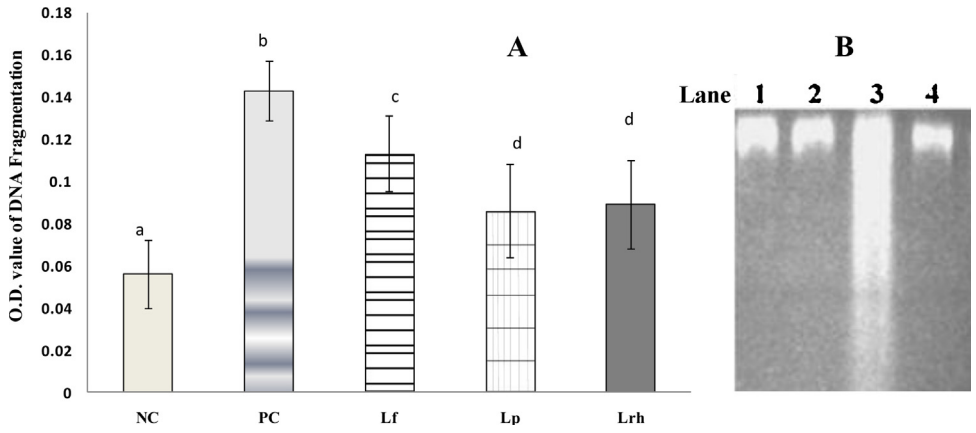

Fig. 1. DNA fragmentation assay. A. Effect of APAP on the extent of DNA, (NC) Normal control rats, (PC) APAP exposed rats Uremic control, L.f, L.p and L.rh-treated with *L. fermentum*, *L. plantarum* and *L. rhamnosus*, respectively. Each column represents mean ± SE ($n = 6$). ANOVA followed by multiple two-tail *t*-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ($P < 0.05$). B. DNA isolated from experimental kidney tissues was loaded on 1% (w/v) agarose gels. Lane 1 and lane 2: DNA isolated from probiotic *L. fermentum* and *L. plantarum* treated kidney samples; Lane 3: DNA isolated from APAP exposed uremic kidney (PC); Lane 4: DNA isolated from probiotic *L. rhamnosus* treated kidney DNA.

Table 4
Antimicrobial activity of probiotic strains against enteric pathogen and nature of antimicrobial substances.

Indicator strains	Inhibition of zone (mm)[a]											
	No treatment of CFCS			Proteinase K treated			NaOH adjusted to pH6.5			H_2O_2 treated		
	1	2	3	1	2	3	1	2	3	1	2	3
E. coli	++	+++	++	++	+++	++	–	–	–	++	+++	++
S. aureus	++	++	+	++	++	+	–	–	–	++	++	+
V. cholereae	+	+++	++	+	+++	++	–	–	–	+	+++	++
K. pneumoniae	++	+++	++	++	+++	++	–	–	–	++	+++	++
S. mutans	+	+	++	++	++	++	–	–	–	++	++	++
Candida albicans	++	++	+	++	++	+	–	–	–	++	++	+

A symbol to the size of the inhibition zone of diameter observed with growing cells: 1 = *L. fermentum*, 2 = *L. plantarum* and 3 = *L. rhamnosus*, + (2–3) mm, ++ 4 mm and +++ (> 5.5) mm and – absence of an inhibitory zone.

Increased *Lactobacillus* quantity may be due to their adhesion into the intestinal environment, which served to create better survival conditions for indigenous bacteria, retained within gut after the cessation of feeding. Although urease positive *Lactobacillus* strains have no pathologic reactions associated with the indigenous intestinal population [13], it is hard to exclude the possibility that ingested strains may cause infections in some patients with compromised immune system. Selected strains presented different levels of antagonistic activities against tested strains (Table 3).

Most effective antagonistic metabolites produced by the tested strains were found to be organic acids (Table 4). Organic acid secretion by *Lactobacillus* strains are reported to inhibit the indigenous population, thereby alter the population structure [30]. These findings suggest the potential use of the urease positive probiotic *Lactobacillus* strains as a novel therapeutically useful anti-uremic agent.

Probiotics may have a substantial impact on human health by supporting the anti-oxidative system which breaks down during acetaminophen overdose. A few studies are there about the protective effect of *Lactobacillus* strains against uremia [13,33–35]. A comparative result on the above ground is cited in Table 5. This study clearly demonstrated that oral administration of 3 tested urease positive probiotic *Lactobacillus* strains namely *L. fermentum*, *L. planturum* and *L. rhamnosus* would prevent acetaminophen induced uremia and improve the health of experimental rats. The efforts have been undertaken to mitigate uremia in animals and humans by administration of live cultures of naturally existing microbes [11]. Taken together, these studies not only proved the concept of in situ continuous "enteric dialysis", but also provided some promises to the millions of people with kidney dysfunction of different etiology.

In conclusion, this pilot study found that probiotic dietary supplements used in groups III, IV and V enhanced survival of rats. Our results support the view that dietary supplements will prefer advantageous application for uremia either as food, food addition, or pharmaceutical preparation. Imitative trials of probiotic

Table 5
Studies with probiotic *Lactobacillus* strains against uremia.

Strain	Mode of study	Effect	Reference
Lactobacillus delbruekii	In vitro	Remove urea level (from 51.5 ± 5.2^b to 44.3 ± 3.9^b mg/dL)	Chow et al. (2003) [33]
Lactobacillus acidophilus	In vitro	Urease positive strain remove uremic toxin from artificial intestinal fluid completely	Friedman et al. (2001) [34]
Lactobacillus acidophilus (formulated)	In vivo (in rat model)	Remove urea and creatinine from blood Count of *Lactobacillus* spp. increase (from 9.00 ± 0.24^a to 9.31 ± 0.07^a CFU/g of feces)	Ranganathan et al. (2005) [13]
Lactobacillus acidophilus	In vivo (in rat model)	46% serum dimethyl amine (DMA)	Dunn et al. (1998) [35]
Lactobacillus sporogens	In vitro	Remove urea 50% Serum creatinine 80%	Friedman et al. (2001) [34]
Lactobacillus sporogens (formulated)	In vivo (in rat model)	Remove serum urea (from 122 ± 7.1^a to 39.7 ± 27.6^a mg/dL) Creatinine (from 89.0 ± 36.5^a to 14.8 ± 15.2^a mg/dL)	Ranganathan et al. (2005) [13]
Lactobacillus fermentum	In vivo (in rat model)	Remove serum urea (from 69.3 ± 1.6^a to 43.56 ± 0.56^a mg/dL of urea) Creatinine (from1.47 ± 0.02^a to 0.49 ± 0.01^a mg/dL) Count of lacticacid bacteria increase (from 7.95 ± 0.21^a to 9.77 ± 0.32 CFU/g of feces)	This study
Lactobacillus plantarum	In vivo (in rat model)	Remove serum urea (from 69.3 ± 1.6^a to 43.56 ± 0.56^a mg/dL) Creatinine (from1.47 ± 0.02^a to 0.45 ± 0.004^a mg/dL) Count of lacticacid bacteria increase (from 7.95 ± 0.21^a to 9.48 ± 0.30^a CFU/g of feces)	This study
Lactobacillus rhamnosus	In vivo (in rat model)	Remove serum urea (from 69.3 ± 1.6^a to 43.73 ± 0.70^a mg/dL) Creatinine, (from1.47 ± 0.02^a to 0.55 ± 0.01^a mg/dL) Count of lacticacid bacteria increase (from 7.95 ± 0.21^a to 12.25 ± 0.24^a CFU/g of feces)	This study

[a] and [b] denote $P < 0.05$ and $P < 0.02$, respectively.

treatment of larger animal and human will further evaluate the potential role of probiotic strains in delaying the onset and clinical severity of clinical illness at different stages of renal failure.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Ethical approval

All animal studies were approved by the Institutional Animal Ethics Committee of Raja N.L. Khan Women's College (West Midnapur, West Bengal, India). The animals were handled according to the guidelines of NIH (United States, 1985) and CPCSEA (New Delhi, India).

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