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Therapeutic effect of *Streptococcus thermophilus* (MTCC 1938) on acetaminophen induced uremia in experimental rats

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Uremia is a major feature of chronic kidney diseases (CKD). Studies were conducted with a standard urease producing probiotic strain, *Streptococcus thermophilus* (MTCC 1938) to explore the effectiveness of probiotic organism against uremia. The therapeutic potential of the probiotic bacterium was tested against acetaminophen (APAP) induced uremic rats. Supplementation of *S. thermophilus* in food resulted in improved uremic profile compared to positive control (PC) rats (no probiotic supplementation in food) during 15 d experimental period. Much lower concentration of plasma urea (77%), plasma creatinine (80%), urinary protein (68%) and urine glucose (68%) was found in the earlier. Significant increment of plasma glutathione (GSH) level (58%) in probiotic treated group was also noticed. Moreover, during the feeding with probiotic strain at a dose of 1×10^9 CFU/mL bacteria, reduction of enterobacteria in faeces was observed. Notable biochemical changes were reflected in histopathological examination of kidney sections. The uremic profile of the probiotic induced rats was very much comparable with the negative control (NC), even found better for some parametric values. The present study suggests that *S. thermophilus* could be used as a novel alternative natural therapy for uremia, a major syndrome of CKD.

Keywords: Acetaminophen, chronic kidney disease (CKD), probiotic, rats, *Streptococcus thermophilus (*MTCC 1938), uremia

Introduction

Chronic kidney disease (CKD) is a long term condition caused by damage to both kidneys. It is generally irreversible and in some cases dialysis or transplantation may become necessary[1,2]. The average prevalence has been reported at 11% in USA, Europe and UK (excluding those on dialysis or with a functioning transplant)[3]. Patient number with CKD is increasing worldwide. It is being recognised as a major public health problem that may reach epidemic level over the next decade. Early detection of CKD can be established if kidney disease is likely to be progressive, allowing appropriate treatment in time to its slow progression[4]. Uremia is an illness that accompanies kidney failure and CKD[5]. Uremic illness is considered to be largely due to the accumulation of organic waste products, which are normally cleared by the kidneys. Uremic retention solutes are generated in part in the gastrointestinal tract (GIT). Gut microbiota and the ensuing micro-biometabolome play a significant role in the proliferation of uremic retention solutes. A toxin generated in or introduced into the body *via* the intestine that contributes to CKD originates in the GIT[6].

Over doses of acetaminophen (APAP) is one of the cause of CKD[7]. In early stage of APAP toxicity, formation of the reactive intermediate N-acetyl-P-imino benzoquinone imine (NAPQI) through cytochrome P450 occurs. High dose of APAP ingestion results in the reduction of cellular glutathione (GSH), allows NAPQI to be attached to cellular protein and begins lipid per oxidation along with renal injury[8].

"Enteric dialysis" is an adaptive physiological process for solutes removal from the body. High concentration gradients can facilitate diffusion of solutes from plasma to intestinal lumen. Recent studies report that uremic toxins like urea, creatinine, etc. can be excreted through enteric dialysis[9].

In recent years, both research and consumer interests in probiotics have grown as an alternative management. Increasing clinical evidences support some of the proposed health benefits related to the use of probiotics. The most widely used probiotics include lactic acid bacteria, specifically *Lactobacillus*

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and *Bifidobacterium* species[10]. It is noteworthy that probiotic effects tend to be specific to a particular strain, so a health benefit attributed to one strain is not necessarily applicable to another strain, even within one species. As with most human diseases, animal models have advanced our understanding of the role of GIT microflora in human disease, although the complexity of the human precludes that there are animal models that can mimic all the features of a particular human disease in its entirety[11]. The urease producing bacteria on mucosal layer of intestine may accelerate the dialysis process of uremic toxins. Administration of a probiotic formulation, with or without prebiotics, for bowel-based toxic solute extraction is a biologically plausible pharmacobiotic therapy for CKD. Restructuring the GIT microbial community by rescuing from a pro-inflammatory or dysbiotic state may prove beneficial in reducing the uremic load produced in the gut and its escape to the systemic circulation[12]. In this context, present study was conducted to evaluate potential health beneficial role of a urease positive lactic acid bacteria against uremic rats. Previous studies demonstrated that feeding with probiotics attenuates blood urea-nitrogen levels and improves the life span of uremic animals[13,14].

Streptococcus thermophilus is a thermophilic lactic acid bacterium (LAB) widely used as starter in manufacture of dairy products. It is the second most important industrial dairy starter[15]. The objective of the present study was to evaluate the probiotic, *S. thermophilus* (MTCC 1938) as a remedy for uremia in APAP induced uremic rats.

Material and Methods

Bacterial Strain and Culture Conditions

S. thermophillus (MTCC1938) strain was collected from culture collection centre, IMTECH, Chandigarh, India. The strain was cultured in LAPTg broth (peptone, 15 g/L; tryptone, 10 g/L; yeast extract, 10 g/L; glucose, 10 g/L; & Tween 80, 1 mL/L) and sub-cultured at least twice in reconstituted skim milk (RSM) (100 g/L) prior to experimental use. The strain was maintained at −20°C in RSM containing 100 mL/L glycerol, 10 g/L glucose, and 5 g/L yeast extract[16].

Animal

The study was conducted with 18 Wister strain of male albino rats (weighted between 100-150 g) (Supplied from Ghosh Animal—animal food and animal cage supplier—Kolkata, India) and maintained in a room with a 12 h light/dark cycle at 25±3°C. Animals were individually housed in cages (20×30×15 cm^3) with litter tray (20×30×6 cm^3) and allowed to have free access to conventional balanced diet and water adlibitum. All rats received no food for 24 h before the assays but had free access to water. The principles and guideline of laboratory animal care of National Institute of Health, USA was followed throughout the duration of experiment[14]. The animal study was approved by Institutional Animal Ethical Committee.

Grouping of Animals and Experimental Procedure

Animals were randomized and divided into 3 groups with 6 animals in each group. First group was negative control (NC). It served as untreated control and was injected with distilled water at 1 mL/100 g body wt daily for 10 d. Second group was positive control (PC) and injected with APAP 500 mg/kg body wt/d for 10 d by intraperitoneal injection for inducing uremia. Third group was treatment group (ST) administrated with *S. thermophilus* (MTCC1938) for 15 d after exposing APAP at given dose.

Formulation

Food balls were arranged by casein-based diet with *S. thermophilus* and milk mixture[14]. The formulation was stored in a −20°C freezer in aseptic conditions. While no microbial additives were provided to rats of NC and PC groups, ST group was administered with *S. thermophilus* at a dose 1×10^9 CFU/mL/100 g body wt for 15 d. After 25 d, all experimental animals from all groups were sacrificed as well as blood and kidney samples were collected.

Blood Uremia Profile

Plasma Urea

The collected blood samples were centrifuged and plasma fractions were separated. Urea level of plasma was measured by commercially available standard blood urea kit (Merck, Japan) in semiautoanalyser by standard protocol for photometric determination of urea according to the urease GLDH method[17].

Plasma Creatinine

Creatinine level of plasma was determined by commercially available standard creatinine kit (Merck, Japan) in semiautoanalyser (Merck, Japan) by standard protocol for photometric determination of creatinine, based on Jaffe kinetic method without deproteinization[18].

Urine Glucose and Protein

The periodical quantitative determination of glucose in urine in lower animals (rats and mice), which excretes only a few millilitres, was a tedious job and determined using modified Anthrone method[19]. The protein content in each supernatant sample was estimated according to Lowry method[20].

Plasma Glutathione (GSH)

Enzymatic determination of plasma GSH was performed by Cayman's GSH assay kit using glutathione reductase for the quantification of GSH. Measurement of the absorbance of at 405 or 412 nm provides an accurate estimation of GSH in the sample[21].

Limited Analysis of Faecal Enteric Bacteria

After rats were fed with 1 mL respective probiotic suspensions at10^9 CFU/mL/100 g body wt, survival of pathogen as well as *Lactobacillus* spp. during transit through the gastrointestinal tract was determined in faecal samples[22].

Histopathological Examination

For histopathological evalution of kidney tissues, paraffin-embedded specimens were cut into section and were stained with hematoxylin-eosin for light microscopic examination. All section of kidney samples was examined for characteristic histological changes including tubular epithelial alteration[23].

Statistical Analysis

All the experiments were carried out at least in triplicate. The effect of each treatment was analysed by ANOVA, followed by Duncan's test. The level of significance was defined at $P<0.05$.

Results and Discussion

Body Growth

Body wt of rats increased at the end of experiment in NC and ST compared to their initial body wt (Table 1).

Table 1—Antiuremic effect of *S. thermophilus* on body growth of APAP induced uremic rats.

Group	Final body wt (g)	Initial body wt (g)	% increase/decrease in body wt
I	110.6±10.9[a]	100.0±5.0[a]	10.6±6.8[a]
II	73.7±5.20[b]	75.6±3.7[b]	-1.9±3.2[b]
III	145.6±4.1[c]	140.0±4.5[c]	5.6±8.0[c]

Group I, NC; Group II, PC; Group III, ST

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 ($p<0.05$)

In PC rats, the percentage of the increase in body growth was dramatically lesser than the other groups due to the toxicity of APAP.

Effects of *S. thermophilus* on Plasma Urea and Creatinine Levels

Plasma urea and creatinine supplementation levels were considered to be nephrotoxicity biochemical markers[24,25]. In the present study, administration of nephrotoxic dose of APAP into rats (PC) resulted in a significant elevation of plasma urea and creatinine when compared with untreated rats (NC). Moreover, oral administration of probiotic *S. thermophilus* to the APAP-treated rats (ST) decreased the levels of plasma urea and creatinine by 77 and 81%, respectively as compared to the PC rats. Interestingly, there were significant ($p<0.05$) differences in the levels of plasma urea and creatinine among the NC, PC and ST group (Table 2). In renal disease, the plasma urea accumulates because the rate of plasma urea production exceeds than the rate of clearance. Elevation of urea and creatinine levels in the plasma is taken as the index of nephrotoxicity. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown[26]. Thus plasma urea concentration is often considered as a more reliable renal function predictor than plasma creatinine.

Effect of *S. thermophilus* on GSH

As shown in Table 2, treatment with the analgesic and antipyretic drug, such as, APAP, caused a significant decrease in plasma GSH content as

Table 2—Effect of APAP and probiotic *S. thermophilus* (MTCC1938) on experimentally induced uremic male albino rats

Parameter	Group I (NC)	Group II (PC)	Group III (ST)
Plasma urea (mg/dL)	33.1± 0.46[a]	69.3±1.6[b]	43.56 ±0.56[c]
Creatinine (mg/dL)	0.43±0.004[a]	1.47± 0.02[b]	0.49± 0.01[a]
Plasma GSH (mg%)	39.51±0.51a	24.22±0.71b	37.55±0.54a
Urine protein (mg/dL)	0.65±0.14[a]	50.1±0.26[b]	24.21±1.3[c]
Urine glucose (mg/dL)	0	85±5.72a	12±0.5b

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 ($p<0.05$)

compared to the NC group rats. Current evidence suggests that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP-induced toxicity in the liver and kidney[27,28]. The generation of the reactive oxygen species appears as an early event, which precedes intracellular GSH depletion and cellular damage in APAP toxicity[29]. High dose of APAP saturates the metabolic pathway, decreases the liver clearance of APAP and allows higher amounts of the un-metabolized drug to come in contact with the kidney[30]. It is also reported that intracellular GSH plays an essential role in detoxification of APAP and prevention of APAP induced toxicity in liver and kidney[31]. GSH is an important non-enzymatic cellular antioxidant, which functions in preventing the oxidation of protein sulfhydryl groups by free radicals generated during oxidative stress. In the present study, oral administration of probiotic *S. thermophilus* was found to prevent GSH depletion after APAP administration (Table 2).

Effect of *S. thermophilus* on Urine Glucose and Protein

The significant depletion of urinary protein ($P<0.05$) and urinary glucose ($P<0.05$), which were rendered non-traceable in the urine of ST group of rats, indicated a successful renoprotective impact of probiotic *S. thermophilus*. Urinary glucose and protein are the early biomarkers of altered glomerular permeability[32]. APAP exposure significantly increased the urine glucose level (Table 2). The appearance of glucose and protein may be attributed to the dysfunction of the proximal convoluted tubules because glucose and proteins are completely absorbed from the proximal convoluted tubules under normal conditions. However, *S. thermophilus* treatment (ST) reduced urinary glucose and protein level compared to APAP exposed animals (PC). This phenomenon points to the declined progression of end stage renal dysfunction for the earlier.

Effects of *S. thermophilus* on Histology of Kidney

Kidney histological study was used to determine the protective effect of probiotic *S. thermophilus* on APAP-induced injury as shown in Fig. 1. APAP treatment (in PC) caused several visible histological changes. The renal sections showed extensive tubular damage by presence of necrotic epithelial cells. Tubular degeneration, necrosis, cell swelling, mononuclear cell infiltration and degenerated organelles were also observed in the kidney following the APAP exposure. Some epithelial cells were found damaged in the tubular lumen (Fig. 1b). However, *S. thermophilus* significantly alleviated the kidney damage in APAP-exposed rats. No obvious difference was observed in the kidneys between the ST and NC group samples (Fig. 1a & c). APAP causes a reduction in blood flow. Since blood reaches the renal cortex (outside) first and then the renal medulla (inside), the deeper structures of the kidney are most sensitive to decreased blood flow. Thus the innermost structures of the kidney, called the renal papillae, are especially dependent on prostaglandin synthesis to maintain adequate blood flow. Inhibition of cyclooxygenases, therefore rather selectively damages the renal papillae, increasing the risk of renal papillary necrosis[33]. Most healthy kidneys contain enough physiologic reserve to compensate for this APAP-induced decrease in blood flow. However, those subjected to additional injury from phenacetin or paracetamol may progress to analgesic nephropathy. Treatment with *S. thermophilus*, (ST) however, reduced the tubular damages, tissue necrosis and degeneration of organelles. Different lactic acid bacteria, such as, *L. ingluvi, Sporosarcina pasteurii* are reported to lessen APAP induced renal tubular degeneration[13,14].

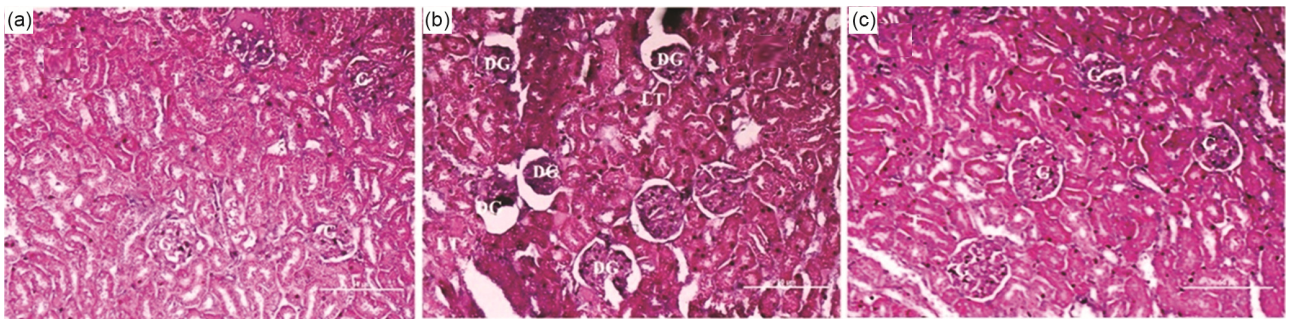

Fig. 1 (a-c)—Haematoxylin and eosin stained kidney sections: (a) Normal rats (20×), showing appearance of glomeruli; (b) APAP exposed rats, showing multiple foci of hemorrhage, necrosis and cloudy swelling of tubules (marked with arrows) (20×); & (c) Kidney section of the rats post treated with *S. thermpphilus* (20×), showing almost normal appearance of glomeruli and tubules in kidney.

Table 3—Antiuremic effect of *S. thermophilus* on faecal analysis of APAP induced uremic rats

Types of microbes	Log of cfu/g of feces								
	Group I			Group II			Group III		
	1st d	7th d	14th d	1st d	7th d	14th d	1st d	7th d	14th d
A	8.55±0.21[a]	8.66±0.24[a]	8.76±0.20[a]	8.58±0.14[a]	6.54±0.07[b]	5.59±0.84[c]	8.45±1.12[a]	9.37±0.32[b]	10.79±0.26[b]
B	5.05±0.25[a]	5.15±0.24[a]	5.28±0.30[a]	5.74±0.11[a]	5.21±0.86[b]	6.96±0.54[c]	5.12±0.26[a]	5.01±0.34[a]	4.21±0.54[b]

Group I, NC; Group II, PC; Group III, ST

A: Total lactic acid bacteria on MRS and streptococcal specific media (LPTg), & B: Total enteric bacteria on Macon key-agar

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 ($p<0.05$)

Effects of *S. thermophilus* on Faecal Analysis

Significant decrease in enteric bacterial population was found in the faecal sample of ST group of rats in comparison to the NC group (Table 3). *S. thermophilus* are reported to exhibit antimicrobial activity *in vitro*[9]. The decrease in enteric bacteria may be due to daily intake of *S. thermophilus,* which upon adhering to the intestinal membrane creates worse conditions for enteric bacterial survival.

In conclusion, the present study showed that the suitable bacteriotherapy of probiotic, *S. thermophilus* (MTCC 1938) had the ability of glomerular and tubular repair and recovery of the kidney from nephrotoxicity. It was found efficient for lowering the uremic toxins in APAP induced uremic rats. Thus, in future, *S. thermophilus* (MTCC1938) can be used as a therapeutic food supplement for kidney failure patients.

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