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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