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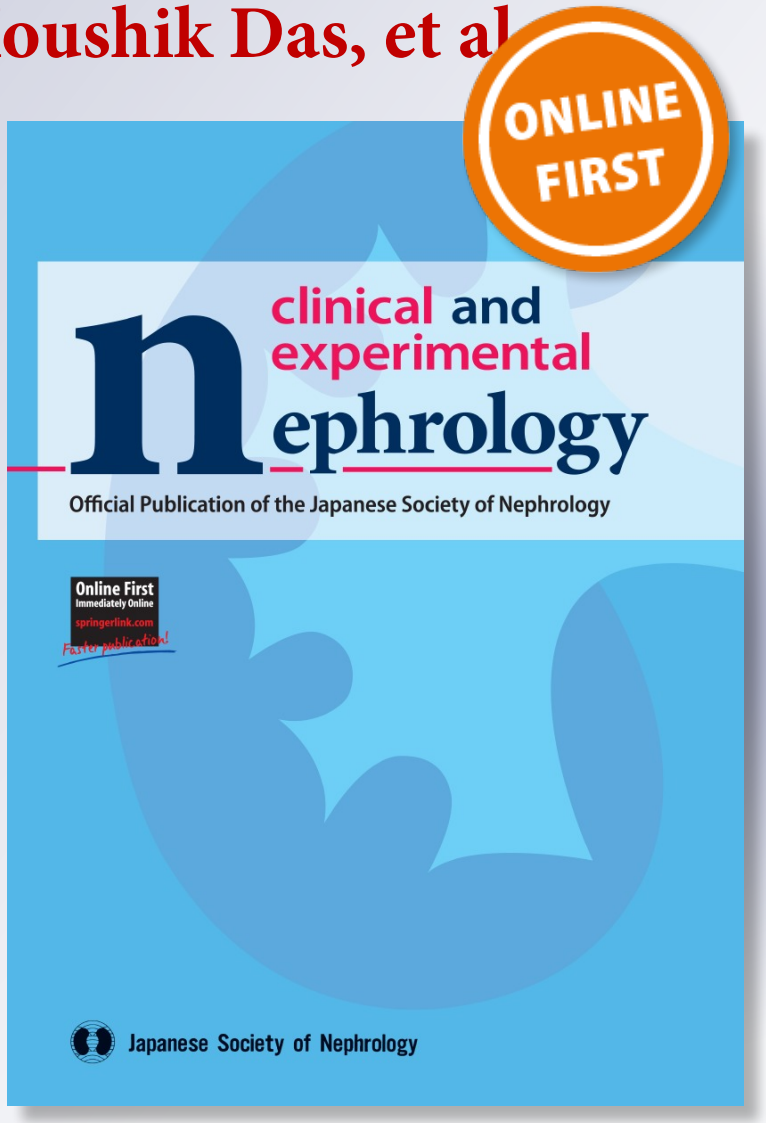

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ORIGINAL ARTICLE

Therapeutic potential of different commercially available synbiotic on acetaminophen-induced uremic rats

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Abstract
Background Currently kidney disease appears a foremost problem across the world. Acetaminophen is a commonly used antipyretic agent, which in high doses, causes uremia and used for experimentally induction of kidney disease. Bacteriotherapy affords a promising approach to mitigate uremic toxins by ingestion of urease positive bacteria, probiotics and symbiotic able to catabolize uremic solutes within the gut. The present study evaluates the effect of seven commercial symbiotic on kidney disease.
Methods Fifty-four albino male rats were randomly divided into nine groups. Control group (Group-I) received distilled water interperitoneally for 7 days. Positive control group (Group-II) received 500 mg/kg acetaminophen interperitoneally for 7 days. Commercially available seven symbiotic combinations at a dose of 10^9cells/day for 3 weeks was administered to the tested groups (Group III–IX) after receiving 500 mg/kg/day acetaminophen interperitoneally for 7 days. Blood, kidney, liver and stool samples were collected after scarification for biochemical tests and DNA fragmentation assay of kidney tissue, kidney histological studies. Limited fecal analysis was conducted.
Result Blood urea nitrogen and toxicity indicators were increased, and antioxidant enzymes were decreased in Group-II. Blood urea nitrogen, toxicity indicators, glomerular necrosis, DNA damage of kidney tissue were reduced, and antioxidant enzymes were increased significantly in the treated Groups IV and IX ($p < 0.05$) in response to Group-II. Number of pathogenic bacteria decreased in synbiotic treated groups than Group I and II.
Conclusion The study demonstrated that some of commercial symbiotic combination can reduce the sever effect of kidney disease.

Keywords Kidney disease · Acetaminophen · Uremia · Synbiotic · Enteric dialysis

Introduction

Kidney diseases rank third among the life-threatening diseases after cancer and cardiac ailments. Hundred people per million of population succumb to kidney disease 90000 kidney transplants per year required in India. Of this, 6 % had stage III kidney disease which necessitates medical attention and, in some cases, costly treatment like dialysis or transplant [1, 2]. At present worldwide statistical data on the incidence and prevalence of kidney disease, the resulting mortality and the high cost of treatment require an effective alternative.

Acetaminophen overdose may result into potentially fatal hepatic and renal necrosis in humans and experimental animals. In early step of acetaminophen toxicity, formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) through cytochorom P450 occurs. At therapeutic doses, NAPQI is removed by conjugating with glutathione sulfhydryl (GSH). High doses of acetaminophen when ingested resulted in the reduction in cellular GSH and allow NAPQI being attached to cellular proteins and begin lipid

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peroxidation, along with renal injury [3, 4]. Paracetamol overdose induces endoplasmic reticulum (ER) stress, characterized by GADD153 upregulation and translocation to the nucleus, as well as caspase-12 cleavage. Interestingly, after treatment of murine tubular cells with paracetamol and calpain inhibitors, the caspase-12 cleavage product was still detectable, and calpain inhibitors were unable to protect tubular cells from paracetamol-induced apoptosis [5].

"Enteric dialysis" is an alternative approach by live microbes for solute removal in uremia is based on the reality that concentration gradient makes solutes disperse from plasma into the lumen when concentration of uremic solute become larger in plasma than lumen and a major parts of uremic solutes become distributed throughout the intestine [6] by binding to large amount of ingestible solute-specific sorbents within the gut. To mitigate uremic solutes using live bacteria which degrade uremic toxins within the gut has been preferable approach of today [7, 8]. Spore-forming urealytic gram-positive *Bacillus*, lactic acid bacteria, and yeast were tested for bacteriotherapy of azotemia by some researchers and demonstrate that feeding with *Bacillus pasteurii* and *Lactobacillus sporogenes* minimize blood urea levels and slow the progression of ESRD and increases the life span of experimental rats [9, 10]. Supplementation of probiotics for 16 weeks lower BUN levels in nephrectomized rats, slowed the progression of azotemia observed in the placebo group, and prolonged the life of uremic rats [11]. *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. *Sporosarcina pasteurii* can be consider for management of uremic patients as it use urea as sole nitrogen source [12, 13].

Our present study wants to evaluate the seven markedly available synbiotics to ameliorate the severity of uremia in acetaminophen-induced uremic rats.

Materials and methods

Selection of animals and care

The study was conducted on fifty-four healthy, adult, male albino rats of Wistar strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata-54) having a body weight of 100 ± 2.51 g (standard deviation value is 2.51). 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 °C) with 12–12 h dark-light cycle (8.00–20.00 h light: 20.00–8.00 h dark) at a humidity of 50 ± 10 %. They were provided with standard food (pellet diet) and water ad libitum. The principle of laboratory animal care National Institute of Health USA guideline was followed throughout the duration of experiment [14] and our Institute Ethics committee approved (IRB approval number-03/dt-24/01/2012) the experimental protocol. All animals in every group were free from any type of infection.

Grouping of animals and experimental procedure

Animals were randomized and divided into nine groups of six animals in each group.

For dosage specifications of different groups, see the Table 1.

Hematological study

After 3 weeks, animals were killed by chloroform anesthesia. Blood samples were collected by hepatic artery punche under diethyl ether anesthesia, using 21-gauge (21 G) needles mounted on a 5-ml syringe (Hindustan syringes and medical devices ltd, Faridabad, India.) into heparin-coated sample bottles for analyzed hematological parameters like 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 plasma fraction was separated. Urea level of plasma was measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyser (Merck, Japan) by standard protocol for photometric determination of urea according to the Urease GLDH method [15].

Biochemical estimation of blood creatinine

The collected blood was centrifuged, and plasma fraction was separated. Creatinine level of plasma was measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyser (Merck, Japan) by standard protocol for photometric determination of creatinine based on Jaffe kinetic method without deproteinization [16].

Antioxidant enzymes

Biochemical assay of catalase (CAT) activity

CAT activity was measured biochemically. In spectrophotometric cuvette, 0.5 ml of hydrogen peroxide (H_2O_2) and 2 ml of phosphate buffer (PBS) and tissue supernatants or plasma were added at volume of 20 µl separately the

Table 1 Dosage specifications of different groups

Groups	Acetaminophen injection concentration-mg/kg of body weight	Microbial additive	No. of rats	Cfu/Dose/Day for 3 weeks (given every day before food forcefully by gavages
Group-I	–	No microbe(s)	6	–
Group-II	500	No microbe(s)	6	–
Group-III	500	BECELAC composition *Lactobacillus acidophilus*, calcium pantothenate, niacinamide, vitamin B12, vitamin C, folic acid, vitamin B6, vitamin B2, thiamine mononitrate. Manufacturer: Dr. Reddy's	6	1×10^9
Group-IV	500	DAROLAC Forte *Lactobacillus acidophilus*, *Lactobacillus rhamnosus, Lactobacillus sporogenes*, *Bifidobacterium longum, Saccharomyces boulardii*. Manufacturer: Aristo	6	1×10^9
Group-V	500	VIBACT composition *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus, mesentericus* Manufacturer: USV	6	1×10^9
Group-VI	500	ECONORM composition *Saccharomyces boulardii.* Manufacturer: Dr. Reddy's	6	1×10^9
Group-VII	500	Lactobacillus Plus *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus longum, Bifidobacterium bifidum, Saccharomyces boulardii,* Fructo- oligo-saccharide, Manufacturer:Infra	6	1×10^9
Group-VIII	500	Ecobion: *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium bifidum, Saccharomyces boulardii, Streptococcus thermophilus,* Fructo- oligo-saccharide Manufacturer:MERCK	6	1×10^9
Group-IX	500	VSL-3 cap: *Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii spp bulgaricus*. Manufacturer: SUN	6	1×10^9

subsequent, and twelve readings were noted at 30 s. interval at 240 nm because absorbance of colorless substance (H_2O_2 and PBS) is measured at 240 nm according to Beers, 1952 [17, 18].

Biochemical assay of superoxide dismutase (SOD)

Kidneys were homogenized in ice-cold 100 mM Tris-cacodylate buffer (LOBA Chem, India) to give a tissue concentration of 50 mg/ml centrifuged at 10,000 g for 20 min at 4 °C. The SOD activity of these supernatants was estimated by measuring the percentage of inhibition of the pyrogallol (HIMEDIA, India) autooxidation by SOD [18, 19].

Measurement of GSH

GSH was quantified using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) according to the protocols as described by Ellman (1959). The reaction between GSH and DTNB formed a yellow-colored complex that was measured by spectrophotometry at 420 nm. [18, 20].

Estimation of lipid peroxidation from the levels of malondialdehyde (MDA)

The 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 4 °C for 5 min individually, and the supernatants were used for the estimation of MDA. The MDA in sample was calculated using the extinction coefficient of 1.56 × 105 M/cm and expressed in the unit of nM/mg of tissue or nM/ml of plasma [18, 21].

Toxicity study

Biochemical estimation of glutamate oxaloacetate transaminase (GOT) and biochemical estimation of glutamate pyruvate transaminase (GPT)

For the assessment of toxicity in serum, liver, kidney GOT and GPT were measured according to the method of Goe [18, 22].

Limited analysis of fecal Limited analysis of fecal microbiota was conducted for rats from all groups. Fresh fecal samples were obtained from individual animals after 3 weeks of feeding. Samples were stored at −20 °C for analysis. Fecal mass was re-suspended in physiological peptone saline at 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 MRS (Man, Rogosa, Sharpe agar, Highmedia, India) agar to obtain lactic acid bacteria counts, thiosulfate-citrate-bile-sucrose agar for *Vibrio* sp., MaConkey agar for total count of enteric bacteria and xylose lysine deoxycholate agar for *Salmonella* and *Shigella* sp. Plates were incubated at 37 °C for 48 h [11].

DNA fragmentation assay The extent of DNA fragmentation in the kidney tissue was determined by the method as described by Lin [23]. Briefly, kidney tissue homogenates were treated with 100 mM Tris buffer, pH 8.0, 1 mM EDTA and 0.5 % triton X-100 and centrifuged. The supernatant was transferred carefully in a tube, and 1 ml of 25 % TCA was added to it, the mixture was vortexed vigorously and incubated overnight at 4 °C.

Histological studies

Kidneys from the normal and experimental mice were fixed in 10 % buffered Bruins reagent and were processed for paraffin sectioning. Sections of about 5 μm thickness were stained with hematoxylin and eosin to evaluate under light microscope. Histologic scoring system was used to quantitate renal pathology. The scoring system was as follows: 0, absent of deformed glomerulus; 1, present deformed glomerulus and 2 marked deformed glomerulus. The parameters assessed were tubular necrosis score and total histological score [24].

Statistical analysis

Analysis of variance (ANOVA) followed by a multiple two-tail '*t*' test with Bonferroni's modification was used for statistical analysis of the collected data. Differences were considered significant when $p < 0.05$.

Results

Body weight

Body weight increased at the end of experiment in all groups compared with their initial body weight (Table 2). In Group-II, the percentage of elevation in body weight was slightly higher than the other groups, due to the uremia induced edema. After administration of the synbiotics in Group IV and IX, body weight was resettled toward the control level (Group-I).

Levels of hemoglobin and total RBC

Hemoglobin level and total RBC count were significantly decreased in Group-II animals (the acetaminophen treated control group), compared with Group-I. But in two Groups IV and IX significantly higher levels of hemoglobin and total RBC count were observed, compared with Group-II (Table 2).

Levels of blood urea and creatinine

Urea and creatinine levels were significantly increased in Group-II animals (the acetaminophen treated control group), compared with Group-I. But in Groups IV and IX significantly low levels of urea and creatinine were

Table 2 Anti-uremic effect along with anti anemic effect of different Probiotic (doses 1×10^9/kg of body weight) on acetaminophen-induced uremic male albino rats

Groups	Increases in Body Weight average value (g)	Urea (mg/dL of blood plasma)	Creatinine (mg/dL of blood plasma)	Uric acid (mg/dL of blood plasma)	Hb (mg/dL)	Total RBC %
I	12.63^{a}	18.16 ± 0.93^{a}	0.51 ± 0.22^{a}	5.27 ± 0.3^{a}	11.27 ± 0.3^{a}	10.27 ± 0.3^{a}
II	13.71^{a}	60.61 ± 3.12^{b}	1.30 ± 1.44^{b}	19.87 ± 0.3^{b}	7.87 ± 0.3^{b}	7.87 ± 0.3^{b}
III	9.15^{c}	57.12 ± 10.99^{b}	0.93 ± 0.07^{d}	11.80 ± 0.7^{c}	8.73 ± 0.7^{c}	8.80 ± 0.7^{c}
IV	11.9^{a}	40.22 ± 9.03^{a}	0.99 ± 0.01^{d}	5.91 ± 0.5^{a}	10.02 ± 0.5^{a}	10.31 ± 0.5^{a}
V	9.29^{c}	56.15 ± 2.65^{c}	0.9 ± 0.21^{d}	10.35 ± 0.3^{c}	8.75 ± 0.3^{c}	8.35 ± 0.3^{c}
VI	8.93^{c}	61.6 ± 1.13^{b}	1.48 ± 0.26^{b}	10.55 ± 0.3^{c}	8.91 ± 0.3^{c}	8.55 ± 0.3^{c}
VII	9.8^{c}	59.8 ± 3.92^{c}	0.83 ± 0.02^{d}	11.99 ± 0.6^{c}	9.59 ± 0.6^{c}	8.99 ± 0.6^{c}
VIII	9.4^{c}	61.17 ± 7.56^{c}	0.86 ± 0.28^{d}	10.14 ± 1.73^{c}	9.8 ± 0.71^{c}	9.14 ± 1.73^{c}
IX	11.88^{a}	39.17 ± 7.56^{a}	0.39 ± 0.28^{a}	5.19 ± 0.8^{a}	10.19 ± 0.8^{a}	9.99 ± 0.8^{a}

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 ($p < 0$ 0.05)

Group I—control, Group II—acetaminophen-induced uremia, Group III-IX–acetaminophen + oral administration of seven types of synbiotics

observed, compared with Group-II and the values resettled to the control group (Table 2). In other symbiotic treated groups also, both levels of urea and creatinine were decreased sometime than Group-II (Table 2).

Activities of catalase, SOD and glutathione

After receiving acetaminophen (Group-II), the activity of CAT, SOD and glutathione in blood and kidney was decreased significantly, compared with Group-I. In Groups IV and IX, CAT, SOD and glutathione activities in blood, liver and kidney were elevated, compared to Group-II (Table 3).

Quantification of MDA

Quantities of MDA altered significantly in blood, liver and kidney, among the test groups (Table 4).

Activities of GOT and GPT

The activities of GOT and GPT altered significantly in serum, among the test groups in Table 5.

Assessment of DNA fragmentation

Figure 1 represents the results of DNA fragmentation analysis. A smear on agarose gel has been observed in the DNA isolated from Group-II animals. Two commercial synbiotics post-treatments were found to be effective to prevent this APAP-induced smear formation (Group IV and IX).

Histological assessment

Histological assessments of different renal segments of control and experimental animals have been presented in Fig. 2. Sections from acetaminophen unexposed animals (I) showed normal histology shown in Fig. 2a. On the other hand, renal sections from acetaminophen-exposed animals (II) showed extensive tubular damage by swollen and necrotic epithelial cells shown in Fig. 2b. Treatment with two commercial synbiotics, (IV&IX), however, reduced these alterations (Fig. 2c, d). In Fig: 2e histological score of respective groups prove the fact.

Result of limited fecal analysis

Significant differences in the counts of different pathogenic bacteria were observed after 3 weeks of daily feeding with commercial synbiotics in all groups as compared with Group I and II. Data are expressed as Mean ± SE ($n = 6$) in Table 6.

Table 3 Anti-oxidative effect of different probiotic (doses 1×10^9/kg of body weight) on acetaminophen-induced uremic male albino rats

Groups	Plasma			Kidney			Liver		
	Catalase (m mol of H_2O_2 consumption/dl of Plasma/min)	SOD (m mol of H_2O_2 consumption/dl of Plasma/min)	Glutathione (μmol/mL)	Catalase (m mol of H_2O_2 consumption/mg of tissue/min)	SOD (m mol of H_2O_2 consumption/mg of tissue/min)	Glutathione (μmol/g of tissue)	Catalase (m mol of H_2O_2 consumption/mg of tissue/min)	SOD (m mol of H_2O_2 consumption/mg of tissue/min)	Glutathione (μmol/g of tissue)
I	5.71 ± 0.04^a	5.65 ± 0.04^a	5.67 ± 0.05^a	4.87 ± 0.05^a	5.89 ± 0.14^a	4.57 ± 0.05^a	3.37 ± 0.05^a	3.71 ± 0.05^a	3.17 ± 0.05^a
II	0.94 ± 0.02^b	0.89 ± 0.01^b	0.91 ± 0.03^b	0.92 ± 0.03^b	0.98 ± 0.13^b	0.92 ± 0.03^b	0.92 ± 0.03^b	0.22 ± 0.03^b	0.22 ± 0.03^b
III	0.90 ± 0.04^a	1.89 ± 0.24^c	1.46 ± 0.09^c	1.46 ± 0.09^c	1.55 ± 0.05^c	1.46 ± 0.09^c	1.46 ± 0.09^c	1.46 ± 0.09^c	1.46 ± 0.09^c
IV	3.76 ± 0.03^a	4.33 ± 0.40^d	4.09 ± 0.01^d	4.09 ± 0.01^d	3.01 ± 0.05^d	3.09 ± 0.01^d	2.09 ± 0.01^d	5.09 ± 0.01^d	4.09 ± 0.01^d
V	1.79 ± 0.01^a	1.93 ± 0.47^e	2.33 ± 0.02^d	2.33 ± 0.02^d	0.75 ± 0.49^a	2.33 ± 0.02^d	2.41 ± 0.02^d	2.75 ± 0.02^d	2.63 ± 0.02^d
VI	1.98 ± 0.13^a	1.25 ± 0.07^d	2.98 ± 0.02^e	2.98 ± 0.02^e	1.14 ± 0.15^c	2.98 ± 0.02^e	2.78 ± 0.02^e	2.28 ± 0.02^e	2.12 ± 0.02^e
VII	1.11 ± 0.04^a	1.45 ± 0.25^a	0.67 ± 0.03^a	0.67 ± 0.03^a	1.11 ± 0.06^c	0.67 ± 0.03^a	0.67 ± 0.03^a	0.67 ± 0.03^a	0.67 ± 0.03^a
VIII	2.21 ± 0.04^a	1.25 ± 0.25^a	1.67 ± 0.03^a	1.21 ± 0.03^a	1.31 ± 0.06^c	0.54 ± 0.03^a	1.24 ± 0.03^a	1.66 ± 0.03^a	0.37 ± 0.03^a
IX	4.55 ± 0.03^c	5.13 ± 0.9^d	4.93 ± 0.03^a	4.37 ± 0.03^a	4.33 ± 0.07^d	4.18 ± 0.03^a	5.97 ± 0.03^a	4.71 ± 0.03^a	4.00 ± 0.03^a

Data are expressed as mean ± SE ($n = 8$). 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 ($p < 0$ 0.05)

Group I—control, Group II—acetaminophen-induced uremia, Group III-VIII–acetaminophen + oral administration of seven types of synbiotics

Discussion

In early stages, bodyweight decreases in uremic patients. During last stage of kidney disease (ESRD), excess fluid part cannot exit from body. In the experiments, the Group-II animals remain without any treatment for 4 weeks. Thus, the fluid which they intake cannot pass through damaged tubular part of kidney. The cellular debris in the proximal tubules was prominent. Deformed glomerulus, extensive tubular casts inhibit liquid passing through it and limited urine formation. Thus body weight increase in Group-II. After administration of the synbiotics in Group IV and IX, body weight was resettled toward the control level (Group-I).Acetaminophen overdose is often linked to many metabolic disorders of urea and creatinine derangements. Higher concentration of serum urea and creatinine is considered for drug and toxins induced uremia in animals and human [25]. 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 this values can be distorted in certain physiological or pathological states during hemolytic anemia or suppressive inflammation [26]. In the present study, receiving acetaminophen interperitoneally for 7 days significantly decreased the Hb levels and also lowered total RBC count. Decrease in hemoglobin is supposed due to destruction on RBC and lower total RBC count due to hemolytic anemia or suppressive inflammation. Edema or increased fluid level during kidney disease, in body does not dilute Hb percentage because excess fluid in edema stage store in tissue and muscle not in blood vessels [26]. After administration of symbiotics specially Dorolac and VSL-3 for 3 weeks, hemoglobin levels become significantly high with improved level RBC count compare to the acetaminophen-induced Group-II. However, this study shows that the probiotics and prebiotics present in the symbiotics could reverse the hematotoxic effect of acetaminophen, with ensuing improvement of hematopoiesis. Elevation of urea and creatinine levels in serum was taken as an index in nephrotoxicity [27]. 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 nephrotoxic dose of acetaminophen to rats resulted in progress of oxidative stress harmful 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 the Group-II (acetaminophen induced) rats when compared to the normal group (Group-I). Moreover, oral administration of Dorolac and VSL-3 significantly ($P < 0.05$) decreased plasma urea and creatinine in Groups IV and IX when compared to the Group-II. The intestinal mucosal surface functions as a semi-permeable membrane. Driven by 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 *Lactobacillus sporogenes*, *Streptococcus thermophilus* present in Dorolac and VSL-3 when ingested, catabolize uremic solutes within 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 [28]. Previous studies have clearly demonstrated that acute acetaminophen overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissues [29]. However, in the acetaminophen-treated animals, the MDA levels are increased significantly, when compared to normal control rats. On administration of VIBACT and VSL-3, the levels of MDA decreased significantly when compared to acetaminophen-induced rats. During kidney injury, superoxide radicals are generated at the site of damage, which modulate CAT, SOD and glutathione, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. CAT, SOD and glutathione are the most important enzymes involved in ameliorating the effects of oxygen metabolism [30]. The present study also demonstrated that acute acetaminophen overdose resulted in a decrease in the CAT, SOD and glutathione activities, when compared with normal control rats. On administration of Dorolac and VSL-3, the levels of CAT, SOD and glutathione increased significantly when compared to acetaminophen-induced uremic rats. The antioxidant effects of probiotics, containing lactobacilli, are due to their ability to capture and make free radicals harmless, to inhibit the lipid peroxidation; chelation of transient metal ions; stimulation of cellular antioxidant system; protection or potentiation of antioxidants. Hepatoprotective effect of lactobacilli is probably due to the increased by them concentration of glutathione in the liver, which is involved in detoxification of endogenous and exogenous carcinogens and free radicals and modulates the immune function. Thus, lactobacilli improve the health of their host. [31, 32] However, acetaminophen was found to increase the microsomal superoxide and hydrogen peroxide production in mice. The generation of the reactive oxygen species appears as an early event which precedes cell damage in acetaminophen hepatotoxicity [33]. Acetaminophen-induced nephrotoxicity was evidenced by biochemical measurements, and

Table 4 Anti-lipid peroxidation effect of different synbiotics (doses 1×10^9/kg of body weight) on acetaminophen-induced uremic male albino rats

Group	Plasma Kidney	
	MDA (*n* mol/dl of plasma)	MDA (*n* mol/mg of tissue)
I	25.80 ± 1.34[a]	40.27 ± 1.69[a]
II	45.78 ± 1.58[b]	98.82 ± 3.79[b]
III	48.12 ± 0.52[c]	71.18 ± 2.3[c]
IV	37.60 ± 0.25[d]	41.9 ± 1.4[d]
V	36.34 ± 0.83[c]	78.42 ± 1.9[c]
VI	36.6 ± 0.27[c]	74.49 ± 2.6[c]
VII	37.56 ± 0.64[c]	78.46 ± 2.1[c]
VIII	25.91 ± 0.39[a]	76.9 ± 3.9[d]
IX	35.60 ± 0.25[d]	41.9 ± 1.4[a]

Data are expressed as mean ± SE ($n = 8$). 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 ($p < 0$ 0.05)

Group I—control, Group II—acetaminophen-induced uremia, Group III-VIII–acetaminophen + oral administration of seven types of synbiotics

Table 5 Effect of acetaminophen and after then synbiotics treatment on SGPT and SGOT activities in acetaminophen-induced oxidative stress and uremia in rat

Groups	SGOT U/L	SGPT(U/L)
I	17.35 ± 0.83[a]	26.85 ± 0.67[a]
II	72.67 ± 9.43[b]	65.75 ± 9.34[b]
III	42.1 ± 3.1[c]	44.1 ± 3.58[c]
IV	27.25 ± 1.8[d]	33.75 ± 1.99[d]
V	42.1 ± 3.11[c]	41.1 ± 6.58[c]
VI	52.1 ± 3.12[e]	51.1 ± 0.4358[e]
VII	45.1 ± 3.12[c]	43.1 ± 0.43[c]
VIII	59.1 ± 3.14[e]	47.1 ± 0.48[c]
IX	32.1 ± 3.41[d]	31.1 ± 0.58[d]

Data are expressed as mean ± SE ($n = 6$). ANOVA followed by multiple two-tail *t* test and data with different superscript (a, b, c) in specific horizontal column differ from each other significantly ($p < 0.05$)

Group I—control, Group II—acetaminophen-induced uremia, Group III-IX–acetaminophen-induced uremia then oral-administration of nine commercial synbiotics

histopathological changes that coincide with the observations of other investigators [34]. After commercial synbiotics treatment, blood GPT and blood GOT, kidney GOT and kidney GPT, liver GOT and Liver GPT level in acetaminophen-induced rat are decreased than uremic group which may due to toxic compound removal by the bacteria in all treated groups. A smear on agarose gel has been observed in the DNA isolated from acetaminophen-

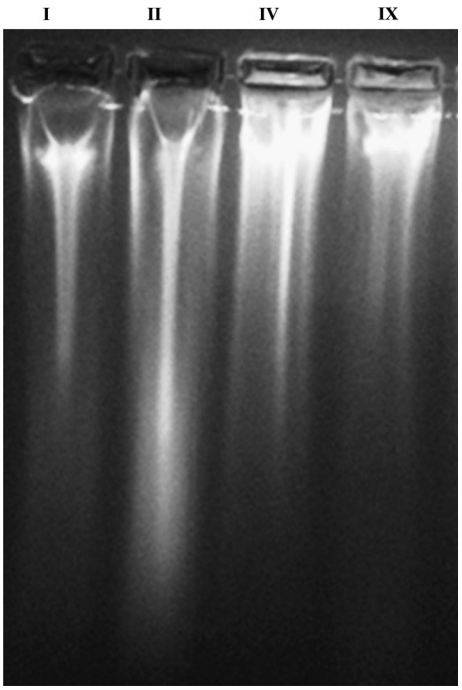

Fig. 1 DNA fragmentation pattern of the experimental rats' kidney. DNA isolated from experimental rat kidney tissues was loaded onto 1 % (w/v) agarose gels. *Lane I* DNA isolated from Group-I kidney samples; *Lane II* DNA isolated from Group-II kidney; *Lane IV* DNA isolated from Group-IV rat kidney and *Lane IX* DNA isolated from Group-IX kidney

exposed animals, indicating random DNA degradation [35]; previous observation shows that acetaminophen-induced random fragmentation of genomic DNA, with subsequent formation of a DNA smear on agarose gel without ladder formation, suggesting that acetaminophen-induced renal cell damage occurs through necrotic pathway. All these studies show that apoptosis also takes place side by side with necrosis; although it is a less significant secondary phenomena in addition to necrosis [36]. Because blood flowing to the kidney first reaches the renal cortex (outside) 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 [25]. Most healthy kidneys contain enough physiologic reserve to compensate for this NSAID-induced decrease in blood flow. However, those subjected to additional injury from phenacetin or paracetamol may progress to analgesic nephropathy. Treatment with two commercial synbiotics (Group IV to IX), however, reduced these alterations. Dorolac and VSL-3 post-treatment were found to be effective to prevent this acetaminophen-

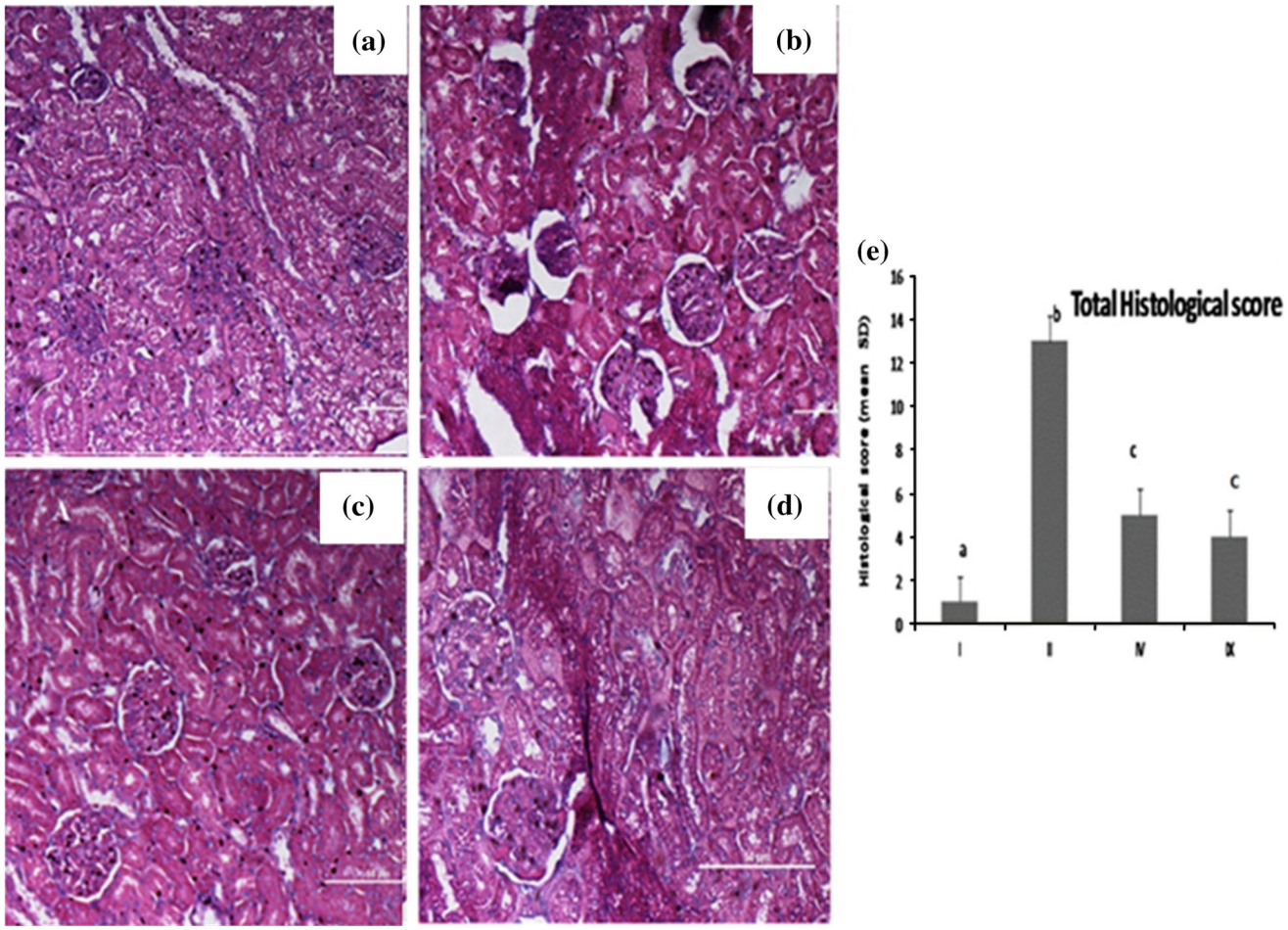

Fig. 2 Hematoxylin and eosin stained kidney section of **a** normal rats (Group-I), showing appearance of glomeruli and normal tubular part, **b** APAP-exposed rats (Group-II), showing deformed glomerulus and large sweled tubular part, **c**, **d** kidney section of the rat post-treated with synbiotic Dorolac (IV) and VLS-3 (IX) showing almost normal appearance of glomeruli and normal tubular part. **e** The scoring system was as follows: 0, absent of deformed glomerulus; 1, present of deformed glomerulus and 2, marked deformed glomerulus. Each column represents mean ± SD, ($n = 6$). "*c*" indicates that there is no significant difference between the Dorolac (Group-IV) and VLS-3 (Group-IX) post-treated and significant difference between other groups. "*b*" indicates the significant difference between the acetaminophen-induced uremic Group (II) and other group. Data of Group-IV and IX are significant lower than APAP-induced uremic Group (II)

Table 6 Result of limited fecal analysis

Data are expressed as Mean ± SE ($n = 6$). ANOVA followed by multiple two-tail t test and data with different superscript (a, b, c) in specific horizontal column differ from each other significantly ($p < 0.05$)

Group I—control, Group II—acetaminophen-induced uremia, Group III-IX–acetaminophen-induced uremia then oral-administration of nine commercial synbiotics

Groups	Thiosulfate Citrate Bile Sucrose Agar for *Vibrio* sp. (dilution-10^7)	MaConkey agar for total count of enteric bacteria(dilution-10^7)	Xylose lysine deoxycholate agar for *Salmonella* and *Shigella* sp. (dilution-10^7)	MRS for lactic acid bacteria(dilution-10^7)
I	27.711 ± 0.04^a	89.711 ± 0.04^a	17.711 ± 0.04^a	60.711 ± 0.04^a
II	28.243 ± 0.02^b	91.243 ± 0.02^b	20.243 ± 0.02^b	60.243 ± 0.02^b
III	11.903 ± 0.04^a	55.903 ± 0.04^a	1.903 ± 0.04^a	99.903 ± 0.04^a
IV	10.762 ± 0.03^a	40.762 ± 0.03^a	3.762 ± 0.03^a	89.762 ± 0.03^a
V	10.791 ± 0.01^a	50.791 ± 0.01^a	3.791 ± 0.01^a	90.791 ± 0.01^a
VI	10.989 ± 0.13^a	49.989 ± 0.13^a	5.989 ± 0.13^a	102.989 ± 0.13^a
VII	11.11 ± 0.04^a	57.11 ± 0.04^a	6.11 ± 0.04^a	111.81 ± 0.04^a
VIII	11.55 ± 0.03^c	51.55 ± 0.03^c	4.55 ± 0.03^c	97.55 ± 0.03^c
IX	10.711 ± 0.04^a	43.711 ± 0.04^a	2.711 ± 0.04^a	110.711 ± 0.04^a

induced smear formation, thus stop necrosis producing of glutathione like substances [33]. This fact is proved when we measured the quantity of glutathione in blood, kidney and liver. Different antioxidant compounds such as taurine treatment, functional foods and probiotic bacteria effectively lessened acetaminophen-induced DNA damage and tubular degeneration [25]. All the tested commercial probiotics showed antagonistic activity against potential human pathogens. Thus, in result of limited fecal analysis, pathogenic bacterial count was significantly low. It was noted that lactic acid and acetic acid are the major acidic compounds in the culture broth. Probiotics are known for their production of various antimicrobial compounds such as organic acids, ammonia, hydrogen peroxide and bacteriocins [37]. The production of these compounds by lactic acid bacteria and other intestinal micro flora is probably one of the most important mechanisms responsible for the antagonistic phenomenon against pathogenic organisms [38], and therefore, it is vital to check this property in probiotic candidates.

In conclusion, the present study demonstrates that two symbiotic composition Dorolac and VSL-3 post-supplementation have curative role against acetaminophen-induced nephrotoxicity, probably through its antioxidant property as well as removing of uremic toxins through enteric dialysis. Dorolac and VSL-3 might, therefore, be considered as potential protective agent against renal injury caused by acetaminophen overdose.

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Conflict of interest All the authors have declared no competing interest.

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