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Research Article

Efficacy and Safety of Bacillus clausii UBBC-07 in chronic kidney disease: a double-blind randomized placebo-controlled study

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Chronic kidney disease (CKD) is a progressive disorder characterized by declining renal function, systemic inflammation, oxidative stress, and accumulation of gut-derived protein-bound uremic toxins. The gut–kidney axis has emerged as a therapeutic target, and probiotics may offer a supportive adjunct approach. This study evaluated the efficacy and safety of Bacillus clausii UBBC-07 supplementation in patients with stage III–IV CKD. In this randomized, double-blind, placebo-controlled trial, participants received either Bacillus clausii UBBC-07 (2 × 10^9 CFU/day; two capsules) or matching placebo for six months. Primary outcomes included changes in protein-bound uremic toxins, renal function markers, inflammatory and oxidative stress biomarkers, electrolyte profiles, and health-related quality of life (SF-8), with safety assessed through adverse event monitoring. Compared to placebo, UBBC-07 significantly reduced key protein-bound uremic solutes, including p-cresyl sulfate (PCS), indole-3-acetic acid (IAA), and indoxyl sulfate (IS). UBBC-07 also demonstrated significant reductions in systemic inflammatory biomarkers and improvement in oxidative stress parameters, reflected by decreased lipid peroxidation and enhanced endogenous antioxidant activity. Renal metabolic indices improved with significant reductions in blood urea nitrogen ($p = 0.001$) and uric acid ($p < 0.0001$), along with a significant increase in eGFR ($p < 0.05$). Electrolyte levels remained stable. SF-8 scores improved significantly at Visits 4 and 5, indicating enhanced quality of life. UBBC-07 was well tolerated, with only mild, self-limiting adverse events and no treatment discontinuations. Overall, Bacillus clausii UBBC-07 appears to be a safe adjunct therapy in stage III–IV CKD, warranting further evaluation in larger studies.

Keywords: Bacillus clausii UBBC-07, Chronic Kidney Disease, Probiotics, uremic toxins, systemic inflammation, oxidative stress, renal function, gut microbiota, quality of life.

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INTRODUCTION

Chronic kidney disease (CKD) is a major health concern characterized by decreased kidney function or sustained damage persisting for at least three months, which significantly contributes to morbidity and mortality through progressive renal failure and associated cardiovascular abnormalities (Roth et al., 2017). Even in its early stages, chronic kidney disease (CKD) is linked to a heightened risk of mortality, cardiovascular disease, end-stage renal disease (ESRD), bone loss and fractures, infections, cognitive decline, and frailty. According to the study report of the global burden of disease, it affects 85 crore individuals globally, and is expected to rank fifth most significant contributor to years of life lost by the year 2040 (Dorgelo & Oostrom, 2022). Chronic kidney disease (CKD) impacts over 10 percent of the Indian population. Of those affected, a substantial number advance to kidney failure annually, with more than 100,000 patients initiating renal replacement therapy across the country. An estimated 229 individuals per million Indians develop end-stage renal disease (ESRD), based on age-adjusted data (Ruggenenti et al., 2001; Kher, 2002; Singh et al., 2013; Hu et al., 2024). The risk of developing chronic kidney disease (CKD) increases with age. Chronic kidney disease is associated with underlying medical conditions such as diabetes,

hypertension and dyslipidemia. In addition to these traditional risk factors, many CKD patients also experience nontraditional complications, including chronic inflammation, oxidative stress, malnutrition, and fluid overload. Individuals with CKD are more prone to cardiovascular complications, including left ventricular hypertrophy, cardiomyopathy, atherosclerosis, and vascular calcification, which tend to occur more frequently and progress more rapidly in this population (Sarnak, 2003).

The defining clinical feature of chronic kidney disease (CKD) is uremia, characterized by the accumulation of uremic toxins such as urea, creatinine, guanidines, asymmetric dimethylarginine (ADMA), and phosphate. These uremic toxins can be categorized into three distinct types (i) water-soluble low-molecular-weight compounds (typically <500 Da), (ii) middle molecules (>500 Da), and (iii) protein-bound solutes, which are not eliminated by dialysis due to their affinity towards the plasma proteins (Duranton et al., 2014). Purines, nicotinamides and reactive carbonyl compounds are low molecular weight water soluble compounds, proteins like adiponectin, fibroblast growth factor 3 and leptin, along with the cytokines like interleukins (ILs), tumor necrosis factor (TNF-), and resistin are middle molecules weighing more than 500 Daltons.

The protein-bound uremic toxins (UTs) include advanced glycation end products (AGEs) like indole and its derivatives, such as indoxyl sulfate (IS) and indole-3-acetic acid; phenol derivatives, including p-cresyl sulfate (pCS) and phenols; as well as polyamines, hippurates, and related compounds. These toxins originate not only from disrupted metabolic pathways in organ dysfunction but also from the fermentation of dietary components by gut microbiota, which are commonly imbalanced in CKD, promoting dysbiosis (Rysz et al., 2021). The decline in renal function results in elevated urea levels within the gastrointestinal tract, where microbial urease activity converts urea into ammonia. Elevated ammonia concentrations disrupt intestinal epithelial tight junctions, compromising barrier integrity and increasing permeability. This results in elevation of luminal pH, impairing the intestinal mucosal barrier and a shift in the microbial composition, selectively promoting the growth of bacteria that produce urease, uricase, and enzymes involved in the synthesis of indole and p-cresol. The integrity of the intestinal barrier is progressively compromised in end-stage renal disease (ESRD) due to frequent occurrences of edema and hypervolemia (fluid overload). Excessive ultrafiltration volumes and intradialytic hypotension can induce transient intestinal ischemia, further aggravate epithelial dysfunction and increase gut permeability.

This impairment of colonic epithelial tight junctions facilitates the translocation of bacteria and endotoxins across the intestinal wall into submucosal compartments, where they may activate innate immune responses and initiate localized inflammation. This inflammatory milieu can perpetuate barrier injury, contributing to a cycle of ongoing epithelial damage (Sabatino et al., 2015; Guldris et al., 2017). Modulation of the gut microbiota may facilitate regulation of the colonic nitrogen balance, reduction of colonic pH, deceleration of colonic transit, and attenuation of gut-derived uremic toxin production. Bacillus clausii (Alkalihalobacillus clausii / Shouchella clausii) UBBC-07 is a well-characterized, Gram-positive, spore-forming probiotic with high acid and bile tolerance, enabling it to survive gastric transit and reach the intestine in a viable form. Its spore-forming nature confers exceptional stability during manufacturing, storage, and gastrointestinal passage. The strain exhibits antagonistic activity against pathogenic Gram-positive bacteria through the production of the lantibiotic clausin, while lacking genes for enterotoxins.

hemolysins, and other virulence factors. Extensive genotypic and phenotypic characterization, whole-genome sequencing, and multiple human and animal studies demonstrate a strong safety profile. The in vitro studies demonstrate that spores of UBBC-07 exhibit significantly greater stability and survival than vegetative cells under simulated gastrointestinal conditions, including high gastric acidity, bile salts, gastric juice, and intestinal fluids. The spores are capable of germination and multiplication in intestinal conditions, ensuring functional activity in the host gut. The higher cell surface hydrophobicity and favorable zeta potential of spores enhance adhesion to mucin and intestinal surfaces, promoting colonization and persistence in the gastrointestinal tract.

B. clausii UBBC-07 also modulates cellular stress and apoptosis-related pathways, inducing pro-apoptotic markers (CJUN, CASP-9) and downregulating anti-apoptotic BCL-6, suggesting broader gut-protective and immunomodulatory effects. In an in vivo rat model of acetaminophen-induced uremia, supplementation with B. clausii UBBC-07 spores (1×10^9 CFU/day) significantly reduced serum urea, creatinine, and malondialdehyde (MDA) while restoring antioxidant defenses, including superoxide dismutase (SOD), catalase, and glutathione (GSH). These findings indicate a reduction in oxidative stress and improvement in renal function biomarkers. In silico genome analysis further reveals the presence of key stress-response and survival determinants, including F_0F_1-ATP synthase, amino acid decarboxylases, bile acid symporters, mucin/collagen/fibronectin-binding proteins, heat and cold shock proteins, and universal stress proteins, collectively supporting resilience under physiological stress.

These properties demonstrate that B. clausii UBBC-07 is a robust spore-forming probiotic with strong gastrointestinal survivability, adhesion capacity, antioxidant activity, and protective effects against uremia, supporting its potential role as a novel adjunctive natural therapy for chronic kidney disease-associated complications (Lakshmi et al., 2017; Yenuganti et al., 2021; Ahire et al., 2021). Additionally, UBBC-07 has shown the ability to enhance protein absorption (Tarik et al., 2025) modulate gut microbiota (Bamola et al., 2022), support immune responses (Sudha et al., 2022; Mirza et al., 2024), and improve gastrointestinal health (Sudha et al., 2013; Sudha et al., 2019), supporting its use as a safe and effective probiotic for human consumption.

This study aimed to evaluate whether Bacillus clausii UBBC-07, as an adjunct therapy in stage III-IV CKD patients, can improve biochemical and clinical outcomes compared to placebo by reducing protein-bound uremic toxins (PCS, IS, IAA), lowering inflammation and oxidative stress, improving renal function markers (urea, creatinine, uric acid) and eGFR, enhancing quality of life, and confirming its overall safety and tolerability.

Methodology

Study design

This randomized, double-blind, placebo-controlled trial was conducted at Nizam's Institute of Medical Sciences, Hyderabad, India, to evaluate the efficacy of Bacillus clausii UBBC-07 capsules as an adjunct in the management of chronic kidney disease (CTRI

REF/2019/07/026928). The study received approval from the Institutional Ethics Committee of Nizam's Institute of Medical Sciences before initiation and was conducted in accordance with established ethical standards for research involving human participants, including the principles outlined in the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines, and the Ethical Guidelines for Biomedical Research on Human Subjects issued by the Indian Council of Medical Research.

Participants

Inclusion criteria

Sixty patients of either sex with chronic kidney disease (CKD) were enrolled in this randomized, double-blind, placebo-controlled trial. Eligible participants were 18-65 years old with stage III-IV CKD and documented stable renal function for at least six months before enrolment.

Exclusion criteria

Patients with intolerance to milk or dairy products; history of kidney transplant, severe infections, advanced heart disease, liver disease, malignancy, autoimmune disorders, severe malnutrition, or clinical conditions requiring artificial feeding; pregnant or nursing women; history of gastrointestinal disorders (including inflammatory bowel disease, Crohn's disease, ulcerative colitis, prior bowel resection, or abdominal radiation); recent use (within four weeks) of antibiotics, prebiotics, or probiotics; positive serology for hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus HIV; active alcohol or substance abuse; or any medical, psychiatric, surgical, or lifestyle condition that could interfere with protocol compliance or patient safety were excluded. Participation in another investigational clinical trial within six months before enrolment was also an exclusion criterion. Participation was voluntary, and all patients provided written informed consent before entering the study

Outcomes

The primary outcomes were changes in the concentrations of the protein-bound uraemic toxins p-cresyl sulphate (PCS), indoxyl sulphate (IS), and indole-3-acetic acid (IAA), as well as changes in serum urea, creatinine, and uric acid levels. The secondary outcomes included changes in estimated glomerular filtration rate (eGFR); inflammatory biomarkers, including high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α); and oxidative stress markers, including nitric oxide (NO), malondialdehyde (MDA), and glutathione (GSH). Health-related quality of life (HRQoL) was assessed using the Short Form-8 (SF-8) questionnaire. Safety outcomes included the incidence and severity of adverse events (AEs) and serious adverse events (SAEs).

Randomization

A simple randomization sequence was employed to allocate participants to probiotic or placebo groups. The allocation sequence was concealed from all study personnel and participants until completion of the statistical analyses. Patients were contacted regularly during the study to encourage adherence and to monitor potential side effects. Adherence was assessed by capsule counts, calculated as the number of capsules dispensed minus the number returned, divided by the expected number of capsules taken, and expressed as a percentage.

Sample size calculation

The sample size was determined based on a two-sided Type I error (α) of 0.05 and a Type II error (β) of 0.20, yielding 80% power to detect a clinically meaningful difference. Based on these parameters, 25 subjects were required per treatment group. To account for an anticipated dropout rate of 20% (approximately five subjects per group), the final target enrolment was set at 30 participants per group, for a total of 60 subjects.

Intervention

Bacipro capsules (Bacillus clausii UBBC-07; 2×10^9 CFU) was provided by Unique Biotech Limited (UBL), Hyderabad. The study was partially supported by Unique Biotech Limited. Patients were randomly allocated to receive either probiotic or placebo capsules. The intervention was administered as two capsules per day for six months.

The study comprised a screening visit (Visit 1); randomization/baseline (Visit 1); on-treatment visits at Day 45 ± 4 (Visit 2), Day 90 ± 4 (Visit 3), and Day 135 ± 4 (Visit 4); and the end-of-study visit at Day 180 ± 4 (Visit 5). The total study duration was six months. At baseline, demographic information, medical history, dietary records, and concomitant medications were documented, and a thorough physical examination was performed. At each study visit, levels of blood urea nitrogen, uric acid, serum creatinine, uremic toxins, oxidative stress factors and eGFR were measured. Adverse events were monitored at each visit and through interim telephone follow-ups. Serum concentrations of indole-3-acetic acid (3-IAA), indoxyl sulfate (IS), and p-cresol sulfate (p-CS) were quantified using an in-house validated HPLC method (Reddy et al., 2023).

High-sensitivity CRP was measured using a sandwich ELISA (Sigma-Aldrich; 0.005-0.1 mg/L). TNF-α and IL-6 levels were determined using Invitrogen ELISA kits (Thermo Fisher) (Clark & Engvall, 2018) with sensitivities of 1.7 pg/mL and <1 pg/mL, and ranges of 15.6-1000 pg/mL and 10.24-400 pg/mL, respectively. Total nitric oxide was estimated by a modified Griess reaction (Miranda et al., 2001). MDA levels were assessed via the TBARS assay (Esterbauer & Cheeseman, 1990). Glutathione was quantified using a modified Ellman's method (Ellman, 1959) with DTNB, read at 412 nm after protein precipitation. Serum creatinine was measured using the Jaffe method (Bower's & Wong, 1980) (Cobas c111, Roche), with a range of 0.17-24.9 mg/dL. Sodium and potassium were analysed by Ion-Selective Electrode (ISE) on the same platform. The updated 2006 MDRD four-variable equation was applied to calculate estimated GFR. Health-related quality of life was evaluated at each visit using the SF-8 questionnaire, an 8-item Likert-scale tool where higher scores indicate worse outcomes and lower scores reflect better health status (Borges et al., 2018).

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 9.1). Data were expressed as mean ± standard deviation (SD). Baseline demographic characteristics were compared between groups using independent samples t-tests. Normality of data distribution was assessed using the Shapiro-Wilk test. Repeated-measures analysis of variance (RM-ANOVA) was used to evaluate changes within groups over time and differences between groups. For non-normally distributed variables, the Wilcoxon signed-rank test was used for within-group comparisons, while the Mann-Whitney U test was applied for between-group comparisons. All statistical tests

were two-tailed, and p-values < 0.05 were considered statistically significant.

Results

The study randomized 60 CKD patients of Stage III and IV who were not on dialysis. Four patients were excluded from the study due to high serum creatinine levels and abnormal liver function. All participants received treatment from the attending nephrologist in accordance with established clinical guidelines.

Forty-four patients completed the intervention protocol, 22 in the placebo group and 22 in the probiotic supplementation group. In both cohorts, diabetes mellitus was the predominant cause of chronic kidney disease. About eight patients were lost to follow-up as they failed to comply with the protocol (Fig.1). The baseline characteristics of the patients in both groups were similar. (**Table 1**)

Table 1. Participant demographics

	Placebo (n=30)	B clausii UBBC-07 (n=30)
Age (years completed) Mean ± SD	42.83 ± 8.49	44.73 ±9.66
Gender (Male: Female)	18:12	20:10
Weight(kg) Mean ± SD	81.53 ± 8.87	77.33 ±10.32
BMI (Kg/m^2) Mean ± SD	26.78±3.18	27.48±3.87
Underweight < 16.0 - 18.4*	Nil	Nil
Normal range 18.5 - 24.9*	9	5
Overweight (pre-obese) 25.0 - 29.9*	15	21
Obese ≥ 30.0*	6	4
DM*	14	12
HTN*	17	14
DM+HTN*	10	7
CAD*	6	11
CVD*	4	3
Heart Failure*	4	3
Stroke*	2	1

Protein-bound uremic toxins

p-cresol sulphate (PCS)

Serum p-cresol sulphate (PCS) levels were comparable between the placebo and Bacillus clausii UBBC-07 groups at baseline (Visit 1), confirming balanced groups before intervention. Over the course of treatment, the placebo group showed persistently elevated PCS levels with no meaningful change across visits, whereas the Bacillus clausii UBBC-07 group demonstrated a progressive and time-dependent reduction in PCS concentrations. While the between-group difference was not statistically significant at Visit 2, a significant reduction in PCS was observed with Bacillus clausii UBBC-07 by Visit 3 ($p = 0.0361$). This effect became more pronounced at Visit 4 and was maintained through Visit 5, with highly significant differences compared to placebo ($p < 0.0001$ at both visits). By the end of the study, Bacillus clausii UBBC-07 treatment resulted in an approximate 50% reduction in PCS levels compared to placebo

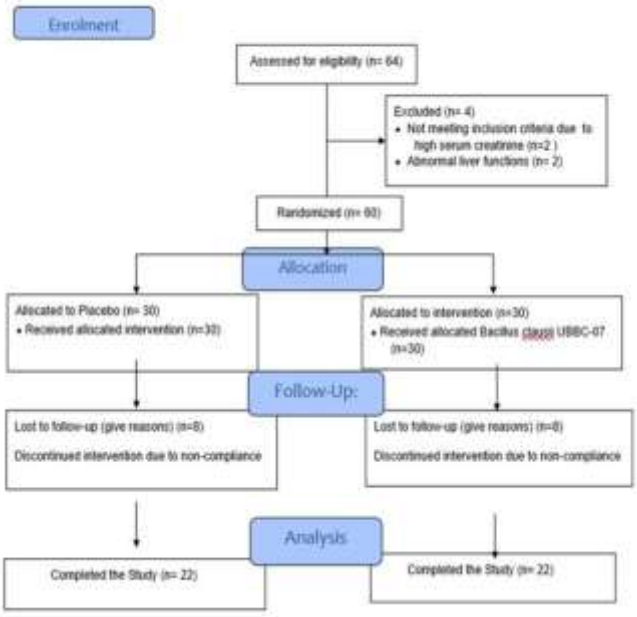

Figure 1. Participant Demographics

Indole 3-acetic acid

At baseline (Visit 1), serum indole-3-acetic acid (IAA) levels were comparable between the placebo and Bacillus clausii UBBC-07 capsule groups, indicating no pre-treatment differences. During follow-up, IAA levels in the placebo group remained relatively high with minimal reduction over time, whereas patients receiving Bacillus clausii UBBC-07 demonstrated a consistent and progressive decline in IAA concentrations. Although the between-group difference was not statistically significant at Visit 2, a significant reduction in IAA levels with Bacillus clausii UBBC-07 compared to placebo was observed by Visit 3 ($p = 0.0049$). This effect was further strengthened at Visit 4 ($p < 0.001$) and remained statistically significant at Visit 5 ($p = 0.0031$). By the end of the study, Bacillus clausii UBBC-07 treatment resulted in a marked reduction in IAA levels relative to placebo.

Indoxyl sulphate

Serum indoxyl sulfate (IS) levels were comparable between the placebo and Bacillus clausii UBBC-07 groups at baseline (Visit 1), indicating balanced treatment groups prior to intervention. Throughout the study period, IS levels in the placebo group showed a gradual increasing trend, whereas patients receiving Bacillus clausii UBBC-07 exhibited a numerical reduction in IS concentrations over time. However, the between-group differences at all post-baseline visits (Visits 2-5) were not statistically significant ($p > 0.05$). Despite the lack of statistical significance, a consistent downward trend in IS levels was observed in the Bacillus clausii UBBC-07 group, with the greatest separation from placebo noted at Visit 5. The data demonstrating the trend toward reduced uremic toxin levels are presented in Table 2 below.

Table 2. Effect of *Bacillus clausii* UBBC-07 on uremic toxins

Parameter	Visit	Placebo (A) Mean ± SD	B. clausii UBBC-07) Mean ± SD	P Value (A vs B)
p-Cresol sulphate PCS (mg/L)	Visit 1	14.94 ± 5.33	15.07 ± 5.20	0.924
	Visit 5	15.58 ± 4.23	7.89 ± 1.88	<0.0001

Indole-3-acetic acid IAA (µg/mL)	Visit 1	361.53 ± 2.219	359.18 ± 1.494	0.456
	Visit 5	356.83 ± 1.008	333.77 ± 0.993	0.0031
Indoxyl sulphate IS (mg/L)	Visit 1	6.817 ± 2.541	6.99 ± 5.898	0.883
	Visit 5	7.95 ± 3.478	5.824 ± 1.031	0.127

Anti-Inflammatory and pro-inflammatory biomarkers

High sensitivity-C-reactive protein

High-sensitivity C-reactive protein (hs-CRP) levels were comparable between the placebo and probiotic formulation groups at baseline (Visit 1), confirming balanced inflammatory status before treatment initiation. During follow-up, hs-CRP levels in the placebo group remained variable with no sustained improvement, whereas patients receiving the Bacillus clausii UBBC-07 probiotic demonstrated a progressive reduction in hs-CRP over time.

While the between-group difference was not statistically significant at Visit 2, a significant reduction in hs-CRP was observed in the probiotic group by Visit 3 ($p = 0.0148$). This anti-inflammatory effect was maintained and further strengthened at Visit 4 ($p = 0.0329$) and Visit 5 ($p = 0.00257$). Bacillus clausii UBBC-07 significantly reduced systemic inflammation.

Interleukin -6

Interleukin-6 (IL-6) levels were comparable between the placebo and Bacillus clausii UBBC-07 probiotic formulation groups at baseline (Visit 1), indicating similar inflammatory status before treatment. Across the study period, IL-6 levels in the placebo group remained relatively stable with minor fluctuations, while the Bacillus clausii UBBC-07 group showed a gradual numerical reduction over time. However, the between-group differences at all post-baseline visits (Visits 2-5) did not reach statistical significance ($p > 0.05$). Although a lower mean IL-6 level was observed in the Bacillus clausii UBBC-07 group at the end of treatment (Visit 5) compared with placebo, this difference was not statistically significant ($p = 0.101$).

TNF- α

Tumor necrosis factor-alpha (TNF-α) levels were comparable between the placebo and probiotic formulation groups at baseline (Visit 1), indicating similar inflammatory profiles before treatment initiation. During follow-up, TNF-α levels in the placebo group remained elevated with no consistent improvement, whereas patients receiving B.clausii UBBC-07 showed a progressive numerical reduction over time. Although formal statistical comparisons were not available for Visits 2 through 4, a clear separation in mean TNF-α levels favoring the probiotic group was observed across these visits. By the end of the study (Visit 5), TNF-α levels were significantly lower in the probiotic group compared with placebo ($p = 0.0141$). The changes in the inflammatory profile are demonstrated in Table 3 below.

Table 3: Effect of *Bacillus clausii* UBBC-07 on anti-inflammatory and pro-inflammatory biomarkers

Parameter	Visit	Placebo (A) Mean ± SD	Bacillus clausii UBBC-07 Mean ± SD	P Value (A vs B)
Highly sensitive C-reactive protein Hs-CRP (mg/L)	Visit 1	3.99 ± 1.641	3.949 ± 1.447	0.9186
	Visit 5	4.16 ± 1.73	2.594 ± 1.497	0.00257
Interleukin 6 IL-6 (pg/L)	Visit 1	6.75 ± 2.06	6.657 ± 3.676	0.904
	Visit 5	7.01 ± 1.42	6.095 ± 2.12	0.101
Tumor Necrosis Factor - α TNF-α (pg/mL)	Visit 1	3.21 ± 1.157	3.275 ± 1.36	0.827
	Visit 5	3.83 ± 1.68	2.589 ± 1.605	0.0141

Oxidative Stress Biomarkers: MDA, GSH, and NO

Across five study visits (Visit 1 to Visit 5), Bacillus clausii UBBC-07 demonstrated clear improvements in oxidative stress and metabolic markers compared with placebo. At baseline (Visit 1), groups were comparable for key oxidative markers, with no significant differences in MDA ($p=0.4144$) or GSH ($p=0.876$). Over time, the placebo showed a worsening profile. MDA increased from 8.34±1.78 to 9.28±2.50 µmol/L, and GSH declined from 563.9±124.9 to 540.1±80.86 µmol/L, whereas Bacillus clausii UBBC-07 produced a strong antioxidant effect, reducing MDA from 9.259±2.209 to 6.311±1.782 µmol/Lwith a highly significant between-group difference at Visit 5 ($p<0.0001$) and increasing GSH from 568.1±79.65 to 663.3±105.1 µmol/L ($p=0.0009$). Nitric oxide levels did not differ significantly between groups at any visit (all $p>0.6$), indicating no measurable treatment effect on NO in this dataset. The data showing changes in oxidative stress status are presented in Table 4.

Table 4. Effect of *Bacillus clausii* UBBC-07 on oxidative stress markers

Parameter	Visit	Placebo (A) Mean ± SD	*Bacillus clausii* UBBC-07 (B) Mean ± SD	P Value (A vs B)
Malondialdehyde MDA µmol/L	Visit 1	8.34 ± 1.783	9.259 ± 2.209	0.4144
	Visit 5	9.28 ± 2.499	6.311 ± 1.782	<0.0001
Reduced Glutathione GSH (µmol/L)	Visit 1	563.9 ± 124.9	568.1 ± 79.65	0.876
	Visit 5	540.1 ± 80.86	663.3 ± 105.1	0.0009
Nitric Oxide NO (µmol/L)	Visit 1	13.34 ± 8.029	13.42 ± 2.657	0.9590

Renal function biomarkers

Although the placebo group also showed significant reductions in BUN at Visits 4 and 5 relatives to baseline, the B. clausii UBBC-07 group demonstrated consistent and statistically significant decreases across all visits from 33.13±13.42 to 54.33±8.864 mg/dL but decreased with Bacillus clausii UBBC-07 from 34.7±14.15 to 28.3±3.29 mg/dL, with significance between-group emerging from Visit 3 onward (Visit 3 $p=0.005$; Visit 4 $p=0.01$; Visit 5 $p=0.001$). Uric acid followed a similar pattern, increasing in placebo (6.39±1.189 to 7.31±0.603 mg/dL) but decreasing with Bacillus clausii UBBC-07 (6.078±1.437 to 5.406±0.746 mg/dL), becoming significant by Visit 3 ($p=0.0213$) and highly significant at Visits 4 and 5 ($p<0.0001$). Serum creatinine showed a favorable trend with Bacillus clausii UBBC-07 (1.50±0.13 to 1.39±0.51 mg/dL) compared with a gradual rise in placebo (1.49±0.57 to 1.61±0.11 mg/dL), though the between-group difference at Visit 5 remained borderline ($p=0.06$).

Renal function, reflected by eGFR, declined steadily in placebo (45.9 to 42.4 mL/min/1.73m²) but improved in Bacillus clausii UBBC-07 (45.1 to 48.7 mL/min/1.73m²), with significant differences from Visit 2 onward ($p=0.0205$ at Visit 2; $p<0.0001$ at Visit 3; $p<0.05$ at Visits 4–5). Changes in renal function markers, including estimated glomerular filtration rate, are summarized in Tables 5 and 6.

Table 5. Effect of *Bacillus clausii* UBBC-07 on renal function markers

Parameter	Visit	Placebo (A) Mean ± SD	*B. clausii* UBBC-07) Mean ± SD	P Value (A vs B)
BUN (mg/dL)	Visit 1	33.13 ± 13.42	34.70 ± 14.15	0.661
	Visit 5	54.33 ± 8.864	28.30 ± 3.29	0.001
Serum Creatinine (mg/dL)	Visit 1	1.49 ± 0.57	1.50 ± 0.13	0.937
	Visit 5	1.61 ± 0.11	1.39 ± 0.51	0.06
Uric Acid (mg/dL)	Visit 1	6.39 ± 1.189	6.078 ± 1.437	0.363
	Visit 5	7.31 ± 0.603	5.406 ± 0.746	<0.0001

Table 6. Effect of *Bacillus clausii* UBBC-07 on glomerular filtration rates

Parameter	Visits	Placebo (A) (Mean ± SD)	B.clausii UBBC-07 (B) (Mean ± SD)	P value (A vs B)
eGFR (mL/min/1.73m²)	Visit 1	45.9 ± 7.26	45.1 ± 8.37	0.396
eGFR (mL/min/1.73m²)	Visit 5	42.4 ± 5.06	48.7 ± 5.33	<0.05

Electrolyte levels

Serum electrolyte levels (sodium and potassium) are summarized in Table 7 below. No significant changes in serum electrolyte levels were observed from baseline to the end of the study (Visit 5) in either group. At Visit 5, sodium levels were 137.0 ± 7.18 mmol/L in the placebo group and 141.1 ± 1.81 mmol/L in the B. clausii UBBC-07 group. Potassium levels measured 4.920 ± 0.645 mmol/L and 4.597± 0.682 mmol/L, respectively. There were no statistically significant differences between the groups at the end of the study.

Table 7. Effect of Bacillus clausii UBBC-07 on serum electrolytes (sodium and potassium)

	Follow-up Visits	Electrolytes		p- Value
		Placebo	*B clausii* UBBC-07	
Sodium mmol/L	Visit 1	138.8 ± 3.70	137.0 ± 7.18	> 0.306
	Visit 5	138.8 ± 3.70	141.1 ± 1.81	> 0.05
Potassium mmol/L	Visit 1	4.920 ± 0.645	4.920 ± 0.645	> 0.05
	Visit 5	4.673± 0.500	4.597± 0.682	> 0.05

Assessment of Quality of Life

From baseline to end of study (EOS; Visit 5), scores decreased to 12.71 ± 3.33 in the placebo group and 30.271 ± 1.201in the treatment group. The mean change in SF-8 scores was significantly greater in the Bacillus clausii group at Visits 4 and 5 ($p < 0.05$). SF-8 scores improved across all visits in the treatment group compared to placebo, reflecting enhanced quality of life (QoL), represented in Table 8. This improvement is attributed to the reduction of uremic toxins, inflammation, oxidative stress biomarkers, and slowing of CKD progression. Both treatment groups showed changes in SF-8 scores across visits.

Table8. Effect of Bacillus clausii UBBC-07 on quality of life through HRQL

Parameter	Visit	Placebo Mean ± SD	B. clausii UBBC-07 Mean ± SD	P Value (A vs B)
SF-8 Score	Visit 1	18.44 ± 5.538	18.32 ± 4.511	0.9386
SF-8 Score	Visit 5	12.71 ± 3.33	30.271 ± 1.201	<0.0001

Safety and Tolerance Profile of Bacillus clausii UBBC-07

All adverse events were mild in nature and resolved spontaneously within two to three days without the need for medical intervention. No participants discontinued or were withdrawn from the study due to adverse events. No serious adverse events were reported, and all symptoms resolved within a short period without treatment. The absence of discontinuations due to adverse events further supports the good tolerability of Bacillus clausii UBBC-07

Discussion

The kidneys play a central role in maintaining uric acid homeostasis through secretion and excretion. In chronic kidney disease (CKD), impaired renal clearance is a major contributor to hyperuricemia. In addition, gut dysbiosis in CKD promotes the growth of pathogenic microbes that generate uremic toxins. Prasad (2017) emphasized that probiotic and prebiotic interventions may offer a promising strategy for managing hyperuricemia by influencing purine bioavailability and regulating microbial metabolism of purine-derived compounds. Likewise, Prasad (2018) reported that the rising prevalence of hyperuricemia in obesity-related metabolic dysregulation highlights the potential of dietary interventions incorporating fiber, prebiotics, and probiotics, even for asymptomatic individuals, as hyperuricemia is a frequent metabolic complication. Furthermore, progressive decline in estimated glomerular filtration rate (eGFR) is associated with increased accumulation of gut-derived uremic toxins such as indoxyl sulphate (IS), p-cresol, and p-cresol sulphate (PCS) (Pignanelli et al., 2019). These protein-bound uremic retention solutes are poorly eliminated by conventional dialysis and contribute to CKD progression by promoting renal fibrosis, inflammation, oxidative stress, and functional renal impairment (Lekawanvijit et al., 2012). These findings collectively support microbiome-directed interventions in CKD populations who are at risk of hyperuricemia. Bacillus clausii UBBC-07 supposedly competes with the pathogens and reduces the production of these uremic toxin precursors. In this study, the primary outcome was reduction in gut-derived uremic toxins, and UBBC-07 significantly reduced PCS and IAA. Supplementation with Bacillus clausii UBBC-07 for 6 months resulted in a significant reduction in gut-derived uremic toxins by the end of the study. The observed reductions in PCS and indole-3-acetic acid suggest improved gut microbial metabolism and toxin elimination and that UBBC-07 may play a role in modulating gut-derived uremic toxins. Although indoxyl sulphate (IS) demonstrated a favourable numerical decline in the Bacillus clausii UBBC-07 group, the between-group difference at Visit 5 did not reach statistical significance. Indoxyl sulphate. This may be explained by the clearance characteristics of IS in CKD. The protein-bound indoxyl sulphates are normally eliminated by active pumping (active tubular secretion). In CKD, circulating IS levels increase predominantly due to reduced renal clearance. Therefore, even if UBBC-07 supplementation reduced intestinal generation of IS precursors, the impaired tubular secretion capacity in CKD may have limited the extent of measurable serum IS reduction, resulting in the elevated

circulating levels of indoxyl sulphates. This pattern has been observed in several probiotic-based CKD intervention studies suggesting that reductions in certain uremic toxins may require longer treatment duration, higher dosing or combination strategies. Probiotic and prebiotic approaches have been suggested as sustainable strategies for hyperuricemia management through modulation of purine metabolism and gut microbial pathways (Prasad et al., 2017; Prasad & Wei, 2018).

Administration of one billion CFU of Bacillus clausii UBBC-07 to acetaminophen-induced uremic rats resulted in a significant reduction in glutathione (GSH) activity, along with marked decreases in serum urea, creatinine, and malondialdehyde (MDA) levels (Patel et al., 2020). Lipopolysaccharide (LPS)-induced endotoxemia led to elevated expression of pro-inflammatory cytokines, including TNF-α and IL-6, in kidney tissues (Kandil, 2021). The concurrent reductions in inflammatory markers, including hs-CRP and TNF-α, point toward attenuation of systemic inflammation, a key contributor to CKD progression and associated metabolic complications. Markers of oxidative stress and antioxidant status further reinforce the potential mechanistic role of UBBC-07. The reduction in lipid peroxidation alongside enhancement of endogenous antioxidant defences suggests that the intervention may mitigate oxidative injury, which is closely linked to both renal dysfunction and chronic inflammation. Since oxidative stress and inflammation in CKD are closely linked with cardiometabolic dysfunction, probiotic or synbiotic interventions that improve obesity- and lipid-associated risk factors may indirectly contribute to renal protection (Kong, 2022). The lack of significant change in nitric oxide levels suggests that the antioxidant benefits of UBBC-07 may be primarily mediated through reduced oxidative injury rather than direct modulation of nitric oxide–dependent pathways. In a metabolomics pilot study, Saggi et al. (2017) observed that probiotic supplementation reduced blood urea nitrogen (BUN) in a subset of CKD patients, and the responders were characterized by unique metabolic phenotypes potentially influenced by gut microflora. Lower levels of BUN, serum creatinine and uric acid in the UBBC-07 group suggest a favorable effect on nitrogenous waste handling, potentially mediated through gut microbiota modulation. This suggests that UBBC-07 supplementation may improve purine metabolism and reduce hyperuricemia. These observations support the potential role of microbiota-targeted interventions in improving metabolic disturbances associated with CKD progression.

Electrolyte balance, particularly sodium homeostasis, plays a key role in blood pressure regulation through modulation of extracellular fluid volume and vascular resistance. In CKD, impaired sodium excretion promotes fluid retention and contributes to hypertension; therefore, electrolyte stability in the present study supports the cardiovascular safety of UBBC-07 supplementation. In addition to maintaining electrolyte stability, probiotic interventions have also been associated with direct blood pressure -lowering effects. A meta-analysis by Lugito et al. (2022) demonstrated that Lactobacillus-containing probiotics exerted significant reductions in both systolic and diastolic blood pressure compared to placebo. These findings suggest that microbiota-targeted therapies may contribute to cardiovascular risk reduction through multiple complementary mechanisms. Although eGFR is often not emphasized in short- to mid-term probiotic studies due to the slow progression of CKD, the present six-month evaluation provided insight into renal function trends. The favourable eGFR trajectory observed in the UBBC-07 group suggests potential preservation of renal function over the study period, although longer follow-up and larger studies are required to confirm sustained benefits. The observed improvement in quality-of-life scores further suggests that the biochemical and physiological benefits may translate into meaningful functional outcomes for patients. To date, clinical evidence evaluating Bacillus clausii supplementation specifically in CKD populations remains limited; however, the observed reductions in protein-bound uremic toxins, reduction in oxidative stress and improvement in glomerular filtration rate in the present study are consistent with previous reports demonstrating beneficial effects of probiotic interventions on uremic toxin burden and systemic inflammation in CKD.

Despite these encouraging findings, certain limitations should be considered. The dropout rate was relatively high, with only 44 out of 60 randomized participants completing the study, which may limit statistical power and introduce attrition bias. Furthermore, the lack of statistically significant changes in serum creatinine, IL-6, and nitric oxide suggests that larger, longer-term studies may be required to fully elucidate the effects of the intervention. Overall, Bacillus clausii UBBC-07 supplementation demonstrated significant reductions in key gut-derived uremic toxins and improvements in renal function markers, inflammatory status, and oxidative stress parameters at study end, indicating its potential as a supportive therapeutic approach in CKD management.

Conclusion

The present findings support a biologically plausible role for Bacillus clausii UBBC-07 in modulating key pathophysiological pathways involved in chronic kidney disease (CKD). The most pronounced effects were observed in the gut-derived uremic toxin profile, with a marked reduction in p-cresol sulfate and a significant decline in indole-3-acetic acid, suggesting modulation of gut microbial metabolism and reduced generation and/or absorption of harmful metabolites. These reductions in uremic toxins were accompanied by significant improvements in systemic inflammation and oxidative stress, as evidenced by lower hs-CRP and TNF-α levels, reduced lipid peroxidation, and enhanced antioxidant capacity. This pattern is biologically coherent, given the established role of uremic toxins in driving oxidative and inflammatory pathways in CKD.

The concurrent improvements in uremic toxins, inflammatory markers, and oxidative stress provide a mechanistic basis for the favourable changes observed in renal biochemical parameters, supporting the potential utility of B. clausii UBBC-07 as an adjunctive approach to managing CKD-associated metabolic and inflammatory burden via modulation of the gut-kidney axis. Findings suggest that the B. clausii UBBC-07 was well tolerated over the six-month intervention period, with no serious adverse events or study discontinuations, and that may represent a safe and effective adjunct strategy.

Conflict of Interests

Probiotic and placebo samples were provided by Unique Biotech Ltd., Hyderabad, Telangana. CPR is affiliated with NIMS, Hyderabad. MKS and RSM are employed by Unique Biotech Ltd., a manufacturer of probiotics. The authors affirm that the study was carried out independently. Employees of Unique Biotech Ltd. did not participate in the study design, data collection, analysis or interpretation of results

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