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RESEARCH

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Possible protective role of probiotic and symbiotic to limit the progression of chronic kidney disease in 5/6th nephrectomized albino rats

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Abstract

Background: The unbalanced gut microbiota, poorly ingested enriched fiber foods, leaky gut is connected to the progression of chronic kidney disease (CKD). The leaky gut translocates uremic toxins to the systemic circulation, promote systemic inflammation, worsen CKD. Decreasing the uremic toxins influx from the gut may decrease the progression of CKD. So, we aimed to evaluate the effect of probiotic and symbiotic supplementation on the leaky gut and their role to prevent CKD progression.

Methods: 48 white albino rats were randomly allocated into 6 groups: sham group; CKD rats; probiotic treated and symbiotic treated rats. Treatment started either immediately or 2 weeks after the operation for each treated group. Blood pressure, body weight changes, serum level of urea, creatinine, indoxyl sulphate and CRP were determined. Histological studies of kidney remnants and intestine and renal fibrosis index were calculated. SPSS program was used for statistics.

Results: Serum urea, creatinine, indoxyl sulphate, CRP, fibrosis index and blood pressure significantly increased in CKD rats. Probiotic treatment decreased serum level of urea, creatinine and CRP and fibrosis index. Symbiotic treatment decreased the serum level of urea, creatinine, indoxyl sulphate and CRP compared to CKD rats. Blood pressure and fibrosis index were decreased significantly upon symbiotic treatment.

Conclusions: A strong correlation between the gut microbial ecosystem and CKD has been proved. The use of probiotics and symbiotic to modulate an unhealthy gut microbiome is a promising intervention to delay CKD progression specially in early stages. Symbiotic results were better than probiotic alone.

Keywords: Chronic kidney disease, Indoxyl sulfate, Probiotics, Symbiotics

Background

Microbial colonization of the human gut just begins at birth. The infant's intestine is believed to be sterile or contains a very low level of microbes at birth (Milani et al. 2017). During the first year of life, the microbial diversity increases and the microbiota composition converges towards a distinct adult-like microbial profile with temporal patterns that are unique to each infant. Feeding, type of birth, hygiene, and use of antibiotics affects the formation of the intestinal microbiome in the first years of life (Meijers et al. 2019). In a study comparing the microbiota of a young cohort and an elderly population (70 years), the diversity of the microbiota from a

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cohort of centenarians was significantly reduced (Biagi et al. 2010).

The gut microbiota provides a range of beneficial properties to the host. Some of the most important roles of these microbes are providing the host with nutrients such as vitamins, a wide range of metabolic capabilities such as breakdown of indigestible plant polysaccharides**,** biotransformation of conjugated bile acids, cholesterol metabolism, degradation of dietary oxalates and to protect against pathogens (Plata et al. 2019). In addition, commensal microbiota act as the 'outside-in' modifier of T cell and natural killer cells subsets and to maintain the integrity of the mucosal barrier (Kim and Song 2020; Mertowska et al. 2021).

A healthy gut environment ensures that defense mechanisms impair the translocation of toxic substances and microbes from the intestinal lumen into the bloodstream. The dynamic equilibrium between the microbiota and the host is established by many factors such as the maintenance intestinal barrier integrity, IgA secretion and a balanced immunological response on the intestinal wall (Ondrussek-Sekac et al. 2021).

Chronic kidney disease (CKD) is defined as a "silent epidemic". It is rarely diagnosed in early stages, as clinical symptoms occur only when kidney function has been irreversibly damaged (Chen et al. 2019). In CKD, the colon becomes the major excretory organ to maintain body homeostasis. Increased serum urea during CKD increases urea influx into the intestinal lumen, where urease producing bacteria hydrolyze it into ammonia and ammonium hydroxide, increasing intestinal pH and promoting mucosal irritation and structural alterations to the gut barrier. Such adaptive alterations are associated with bacterial translocation and endotoxemia (Yang et al. 2019).

Furthermore, CKD patients are often under dietary restrictions to avoid hyperkalemia, pharmacological approaches and antibiotics to treat current infections. These measures reinforce the unbalanced biochemical milieu with overgrowth of nitrogen compound-metabolizing bacteria such as urease, p-cresol- and indol-producing microbes in CKD (Wong et al. 2014). These nitrogen compounds such as indoxyl sulfate and p-cresol sulfate are normally excreted into urine. Accumulation of these substances in CKD have deleterious effects on progression of CKD and increase cardiovascular diseases risk (Rukavina Mikusic et al. 2020).

High serum levels of indoxyl sulfate and p-cresol sulfate were found to negatively correlate with kidney function, cause interstitial fibrosis in renal tubular cells and to develop systemic inflammation (Liu et al. 2018). The detrimental effects of high levels of indoxyl sulfate were associated with podocytes dysfunction represented by prominent foot process effacement, reorganization of the actin cytoskeleton inducing a pro-inflammatory phenotype, decreased podocyte viability and impaired its functions (Ondrussek-Sekac et al. 2021).

The current definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit to the host" (Dunne et al. 2001). Probiotic to have therapeutic effects should have certain characteristics including gastric acid and bile salt stability, to colonize the intestinal tract and to adhere to the intestinal mucosa (Cani et al. 2019).

The favorable effects of probiotics are attributed to many mechanisms including to fight for cellular attachments (Fuller 1991), to synthesize antimicrobial compounds Bacteriocins which is a biologically active protein moiety with bactericidal action" (Wieërs et al. 2020), to stimulate the immune response in the form of increased secretion of immunoglobulin-A, elevated numbers of natural killer cells and enhanced phagocytic activity of macrophages (Chi et al. 2021) and to compete for nutrients that would otherwise be utilized by pathogens (Cani et al. 2019).

A prebiotic is a nondigestible (by the host) food ingredient that has a beneficial effect through its selective stimulation of the growth or activity of one or a limited number of bacteria in the colon. Prebiotics include inulin, fructo-oligosaccharides, galacto-oligosaccharides, soya-oligosaccharides, xylo-oligosaccharides, and pyrodextrins (Mafra et al. 2019**).**

Prebiotic promotes growth of certain bacteria species, reduces inflammation, improves metabolic function, decreases serum concentrations of p-cresol and indoxyl sulfate (Meijers et al. 2010**),** decreases cecal indoles and improves renal functions, stress markers and apoptosis (Furuse et al. 2014). Symbiotics are probiotic supplements combined with prebiotics (Cigarran Guldris and González Parra 2017**).**

As CKD continues to impact more and more people, novel therapies will need to be developed to improve patient outcomes and reduce treatment costs in all countries (Mahsa et al. 2021). So, this study was designed to investigate the possible role of probiotics, symbiotic in limiting the progression of CKD in experimental albino rats.

Methods

The present study was performed on 48 adult male albino rats initially weighing 150-250 g. The rats were purchased from Research Institute of Ophthalmology (Giza). They were maintained in the (MASRI) Animal House in animal cages ($50 \times 30 \times 20$ cm) each cage contained 5 rats under controlled conditions of temperature (25 ± 2 °C) and relative humidity of 50–70%. Rats were allowed standard

pelleted chow and tap water ad libitum with normal light/dark cycle. They were acclimatized to the laboratory conditions for a week prior to experimental procedures to decrease the possible discomfort of animals.

Animals were not exposed to unnecessary pain or stress and animal manipulation was performed with maximal care and hygiene. Surgical procedure ran under anesthesia to avoid induction of pain in animals. At the end of experiment, animals were killed by overdose of anesthesia. Animal remains disposal occurred by incineration.

Study design

Animals were randomly allocated into six experimental groups as follows:

Sham-operated control rats (Control, $n=8$) Rats in this group were subjected to all the procedures of 5/6th nephrectomy operation without the removal of the kidneys and scarified after 6 weeks.

5/6th Nephrectomy rats (CKD, $n=8$) Rats in this group were subjected to 5/6th nephrectomy operation (Sugano et al. 2008) and sacrificed after 6 weeks.

Early probiotic-treated 5/6th nephrectomy rats (E-Prob. CKD, $n=8$) Rats in this group were subjected to 5/6th nephrectomy operation and received probiotic treatment immediately after operation, treatment continued for 6 weeks and then scarified.

Late probiotic-treated 5/6th nephrectomy rats (L-Prob. CKD, $n=8$) Rats in this group were subjected to 5/6th nephrectomy operation and received probiotic treatment 2 weeks after operation, treatment continued for 4 weeks and then scarified.

Early symbiotic-treated 5/6th nephrectomy rats (E-Symb. CKD, $n=8$) Rats in this group were subjected to 5/6th nephrectomy operation and received symbiotic treatment immediately after operation, treatment continued for 6 weeks and then scarified.

Late symbiotic-treated 5/6th nephrectomy rats (E-Symb. CKD, $n=8$) Rats in this group were subjected to 5/6th nephrectomy operation and received symbiotic treatment 2 weeks after operation, treatment continued for 4 weeks and then were scarified.

5/6th nephrectomy

After isoflurane anesthesia, the rat was put in prone position, a small incision was made on the back of the rat to expose the kidney and the right kidney and two third of the left kidney was removed with one week apart. The incisions were closed using 2-0 chromic catgut for the muscle and silk thread for the skin (Sugano et al. 2008). Asepsis using Baneocin antibiotic powder (Bacitracin + Neomycin, Pharco pharmaceuticals Co., Egypt) was insured during the operation and daily after that till wound healing.

Probiotic treatment

Colonies of Lactobacillus acidophilus were prepared by Dairy and Food Microbiology Department of National Research Center, Cairo, Egypt. The colonies were cultured in yogurt; each 1 ml contained 1×1010 CFU. They were prepared weekly and stored in refrigerator. They were then supplied by gavage daily in the morning at a dose of 1×1010 CFU = 1 ml/kg/day (Cani et al. 2019).

Symbiotic treatment

The probiotic formulation was enriched with inulin fructo-oligosaccharides 2% of yogurt as a prebiotic prepared by Dairy and Food Microbiology Department of National Research Center, Cairo, Egypt (Mehanna et al. 2003).

Methods

Body weight and arterial blood pressure were determined for all groups initially and one day before sacrifice. Arterial blood pressure (SBP, DBP and MAP) was measured using the non-invasive small animal tail blood pressure system (NIBP200A, Biopac systems Inc; USA). On the day of sacrifice, overnight fasted rats were anaesthetized with i.p. injection of pentobarbital, in a dose of 40 mg/kg B.W, then rats were subjected to the following studies:

Collection of blood samples for determination of serum level of creatinine according to the method of Bartels et al. (1972) (CAT. No. CR 12 50), and urea according to Fawcett and Scott (1960) (CAT. NO. UR 21 10), by calorimetric method using kits supplied by Bio-Diagnostic, Egypt. In addition to determination of Indoxyl sulphate by ELISA technique using kits supplied by Shanghahi YL BiotecinCo., Ltd, China (YLA0143CT) and determination of CRP was performed according to Mitra and Panja (2005), using kits supplied by BioVendor-Laboratornímedicínaa.s, Karasek, Brno, Czech Republic (Catalogue number: 740001).

Histological studies Samples of terminal ileum and colon were fixed in 10% formalin, embedded in paraffin, cut into 4-µm sections and stained with hematoxylin and eosin. Samples of remnant kidneys were fixed in10% formalin, embedded in paraffin, cut into 5-µm sections and stained with hematoxylin and eosin. In addition, glomerular sclerosis and renal fibrosis were assessed by Masson-trichrome staining. The proportion of the fibrotic area was measured using Image-Pro Plus 3.0 (Media Cybernetics, Silver Spring, MD, USA) (Kelly et al. 2004). Images were digitized and captured with a CCD camera connected to a personal computer.

Animals from all groups were subjected for morphometric study. Measurements were taken from five

different slides obtained from each animal. Five haphazardly selected non-overlapping fields were examined for each slide.

Statistical analysis

All results in the present study were expressed as mean ± SEM of the mean. Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) program, version 20.0 was used to compare significance between each two groups. One -Way ANOVA (Analysis of Variance) for difference between means of different groups was performed on results obtained in the study. Differences were considered significant by LSD when $p \leq 0.05$.

Results

Serum creatinine, urea, indoxyl sulphate, CRP and renal fibrosis index

As seen in Table 1: CKD group showed significant elevation of serum urea, creatinine indoxyl sulphate and CRP. Upon treatment by probiotic, the level of serum urea, creatinine and CRP significantly decreased compared to CKD group but they were still significantly higher than the levels obtained in the control group. Level of indoxyl sulphate was non-significantly changed after probiotic treatment. All parameters were non-significantly changed in late treated group than in early treated group.

Symbiotic treatment decreased the level of urea, creatinine, indoxyl sulphate and CRP significantly compared to CKD. Symbiotic supplementation succeeded to decrease level of indoxyl sulphate and CRP to control level but not the level of urea and creatinine. Early symbiotic treatment succeeded to decrease level of indoxyl sulphate significantly compared to early probiotic treatment.

Renal fibrosis index was significantly elevated in all groups compared to control group but significantly decreased in all treated rats compared to CKD rats.

Changes in blood pressure and body weight in all groups (Table 2)

5/6th nephrectomized rats showed elevated systolic, diastolic and mean arterial blood pressure. Probiotic treatment either early or late supplementation didn't significantly decrease arterial blood pressure in comparison to nephrectomized rats. While early and late symbiotic treatment succeeded to decrease systolic, diastolic and mean arterial blood pressure near to levels obtained in control group.

Body weight was significantly changed in nephrectomized, late probiotic and late symbiotic treated rats. Early probiotic and symbiotic treatment increased body weight to non-significant levels compared to control group.

Histological results

Kidney of sham operated rats showed normal appearance of healthy glomerulus, Bowman's capsule and capillary tuft (Fig. 1A). Examination of CKD kidney sections revealed abnormal appearance of renal tissue, unhealthy glomerulus, widening of Bowman's capsule and tuft retraction, shedding of epithelial cells inside the tubules, tubular cast formation, visible interstitial damage and

Table 1 Serum level of Urea, creatinine, indoxyl sulphate, c-reactive protein and fibrosis index among different studied groups

	Urea (mg/dl)	Creatinine (mg/dl)	Indoxyl sulphate (µg/ml)	c-reactive protein (ng/L)	Renal fibrosis index (%)
Control ($n=8$)	30.1 ± 2.46	0.6 ± 0.04	2.7 ± 0.41	72 ± 10.54	2.78 ± 0.44
CKD ($n=8$)	118.8[a] ± 10.84	2.1[a] ± 0.17	6.1[a] ± 0.71	201[a] ± 15.73	24.59[a] ± 2.06
E-Prob. CKD ($n=8$)	79.2[a, b] ± 5	1.5[a, b] ± 0.067	5[a] ± 0.27	122[a, b] ± 20.75	10.16[a, b] ± 0.94
L-Prob. CKD ($n=8$)	84.7[a, b] ± 4.93	1.6[a, b] ± 0.058	5.7[a] ± 0.66	153[a, b] ± 26.58	13.39[a, b] ± 1.48
E-Symb. CKD ($n=8$)	84.2[a, b] ± 3.76	1.6[a, b] ± 0.071	2.8[b, c] ± 0.39	84[b] ± 13.65	5.14[a, b] ± 0.64
L-Symb. CKD ($n=8$)	98[a, b] ± 4.89	1.7[a, b] ± 0.067	3.2[b, d] ± 0.35	84[b, d] ± 4	7.65[a, b] ± 1

All data are expressed as Mean ± SEM and significant at $P \leq 0.05$ by LSD

n number of samples

[a] Is significance from control group

[b] Is significance from CKD group

[c] Is significance from E-prop. CKD

[d] Is significance from L-prop. CKD

Table 2 Changes in body weight (Δ BW), systolic (SBP), diastolic (DBP) and mean arterial blood MAP pressure among different studied groups

	Δ BW (%)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
Control ($n=8$)	31.2 ± 2.16	120 ± 2.3	86 ± 2	97 ± 1.54
CKD ($n=8$)	17.6[a] ± 1.91	128[a] ± 1.7	95[a] ± 1.9	107[a] ± 1.53
E-Prob. CKD ($n=8$)	28.5[b] ± 3.15	126[a] ± 0.6	97[a] ± 1	107[a] ± 0.78
L-Prob. CKD ($n=8$)	21.7[a] ± 2.07	126[a] ± 1.7	97[a] ± 1.6	107[a] ± 0.78
E-Symb. CKD ($n=8$)	27.2[b] ± 1.13	122[b] ± 0.9	89[b] ± 1	100[b] ± 2
L-Symb. CKD ($n=8$)	22.3[a] ± 0.88	122[b] ± 0.09	88[b] ± 1.7	99[b] ± 1.72

All data are expressed as mean ± SEM and significant at $P \leq 0.05$ by LSD

n number of samples

[a] Is significance from control group

[b] Is significance from CKD group

increased cellularity with high amount of fibrosis within renal tissue (Fig. 1B).

Kidney remanent of early probiotic treated group showed near normal appearance of kidney tissue: a normal glomerulus, no widening of Bowman's capsule, no tuft retraction, an intact brush border of tubular cells and tubular cast formation there is less visible interstitial damage, and moderate amount of fibrosis within renal tissues (Fig. 1C). Late probiotic treatment group showed more sever affection of kidney tissue as proved by widening of Bowman's capsule, interstitial damage, and more fibrosis (Fig. 1D).

Examination of kidney remanent of early symbiotic treated group revealed normal glomerulus (G), no widening of Bowman's capsule and no tuft retraction, intact brush border of tubular (T) cells and no visible interstitial (I) damage low amount of fibrosis within renal tissue (Fig. 1E). While late symbiotic treated kidney remanent revealed atrophy of glomerulus (G) and widening of Bowman's capsule, intact brush border of tubular (T) cells, no visible interstitial (I) damage and moderate amount of fibrosis within renal tissue (Fig. 1F).

Intestinal tissue section of sham-operated control group showed normal tall crypts (arrow) and parallel lined with goblet cells and columnar cells (Fig. 2A). Examination of intestinal section of CKD group revealed atrophy and shedding of the villi (arrow) (Fig. 2B). These changes disappeared upon treatment. As the intestinal tissue section of both CKD early and late probiotic treated group showed normal crypts (arrow) lined by goblet cells and columnar absorptive cells (Fig. 2 C&D). The same results were obtained in intestinal tissue section of CKD early and late symbiotic treated (Fig. 2E, F).

Discussion

The CKD group had successful induction of chronic renal failure as proved by increased level of serum urea, creatinine and indoxyl sulphate. Indoxyl sulphate is a protein-bound uremic solute, produced in the large intestine by the bacteria from tryptophan, absorbed from the colon, metabolized in liver into sulphur-conjugated substances and then excreted in the urine from the kidney (Takada et al. 2018**).** The decreased clearance of indoxyl sulphate toxic product with renal impairment was previously attributed to the inhibition of ATP-binding cassette transporter subfamily G member 2, the primary apical transporter that mediates the excretion of indoxyl sulphate from the body through kidney (Mutsaers et al. 2011**).**

Once accumulated by renal impairment, indoxyl sulphate causes renal tissue damage through oxidative stress (Yoshifuji et al. 2018**).** Thus, the high level of indoxyl sulphate in non-treated CKD group could be considered as a result as well as a cause for further renal impairment.

In addition, the CKD groups in this study showed a significant increase in plasma C–reactive protein in comparison to the control group which points to presence of systemic inflammation. The occurrence of a state of chronic systemic inflammation in CKD is suggested to be due to several factors including increased production of proinflammatory cytokines and/or their decreased clearance, oxidative stress, acidosis in addition to intestinal dysbiosis (Tang et al. 2019**).**

The intestinal dysbiosis (microbiota imbalance inside the body) which is also named "The leaky gut" is believed to be caused by the influx of urea and other retained toxins associated with CKD, impairing the intestinal barrier functions, promoting inflammation throughout the gastrointestinal tract and allows translocation of bacteria and toxins into the systemic circulation (Lau et al. 2018). So, renal impairment could lead to leaky intestine and disturbance in intestinal microbiota which in turn could be another factor in increased production of indoxyl sulphate toxin and hence more renal impairment in a positive feed- back like cycle.

It is of interest to refer to the histological findings in this study in non-treated CKD group which confirmed the liability for intestinal dysbiosis where the intestinal villi are showed to be atrophied with absent crypts and inflammatory cells invasion, in addition to the significantly high renal fibrotic score in CKD compared to control group.

The histological findings as well as higher renal fibrotic index could be attributed to high level of indoxyl

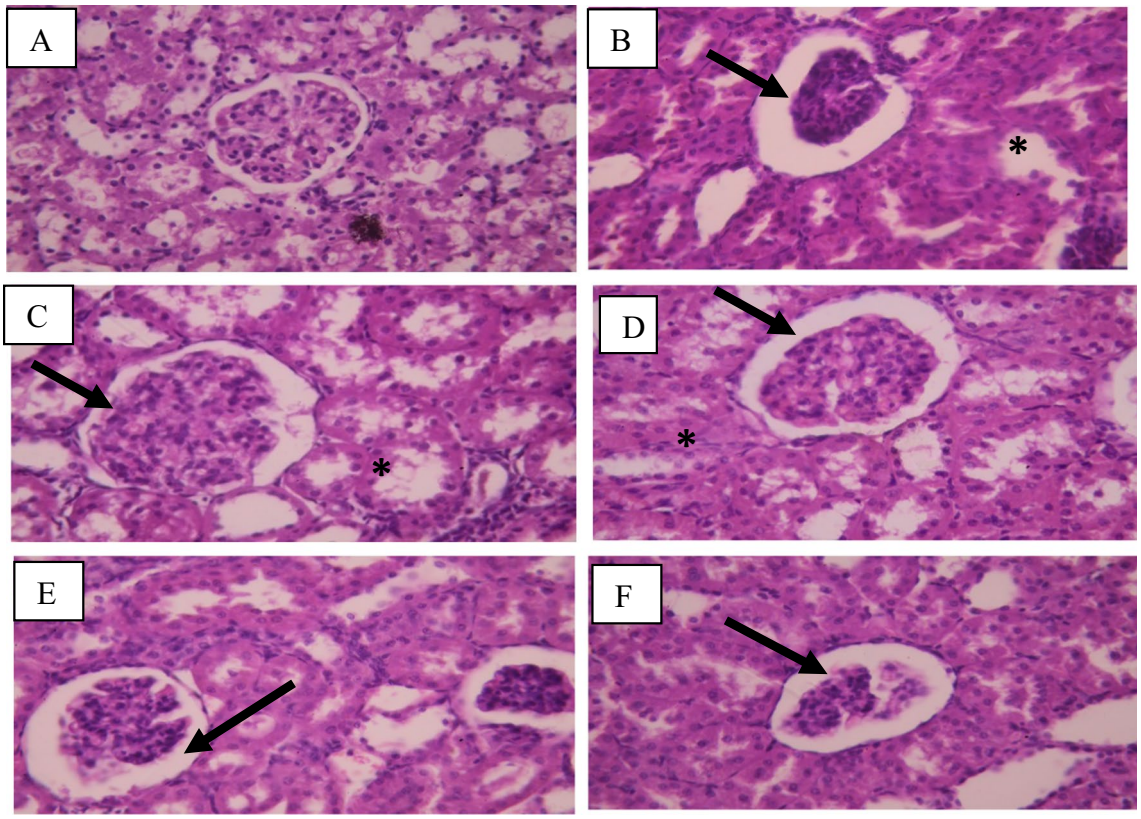

Fig. 1 Sections of kidney remanent of all groups (× 400, H&E): **A** Control group: normal appearance of renal tissue; **B** CKD group: Widening of Bowman's capsule and tuft retraction (↑), shedding of epithelial cells (*); **C** Early probiotic CKD: No widening of Bowman's capsule (↑) and intact brush border of tubular cells (*); **D** Late probiotic CKD: Widening of Bowman's capsule (↑) and interstitial damage (*); **E** Early symbiotic CKD: normal glomerulus, no widening of Bowman's capsule (↑) and no tuft retraction, and **F** Late symbiotic CKD: atrophied glomerulus and widening of Bowman's capsule (↑)

sulphate. This concept is supported by the effects of indoxyl sulphate on podocytes where it causes downregulation of structural actins, integrins, and collagen with formation of cytoplasmic vacuoles (Ondrussek-Sekac et al. 2021). Indoxyl sulphate accumulation was found also to cause interstitial fibrosis in renal tubular cells (Liu et al. 2018**).** Moreover, in this study, positive correlation between indoxyl sulphate and level of both urea and creatinine was noted.

In comparison with control group, there was significant reduction in weight gain in CKD untreated rats. This was known to be due to the imbalance between anabolism and catabolism. Increased levels of circulating proinflammatory cytokines, including IL-6, TNF-α, serum amyloid A and C-reactive protein in CKD was reported to cause muscle wasting (Hung et al. 2011**).** CKD was also found to impair the ability of IGF-1 to regulate muscle protein synthesis (Wang and Mitch 2014**).** Earlier, metabolic acidosis in CKD stimulates protein catabolism in muscle (May et al. 1986).

The significant increase in systolic and diastolic blood pressure in CKD untreated rats compared to control rats could be explained by the uraemia and increased level of indoxyl sulphate. Hypertension and vascular dysfunction induced by uremia is mediated by atherosclerosis, arterial stiffness, vascular calcification, intimal thickening and vascular smooth muscle proliferation (Brunet et al. 2011). Indoxyl sulfate was reported to stimulate proliferation of rat vascular smooth muscle cells proliferation (Yamamoto et al. 2006) and human aortic smooth muscle cells (Barreto et al. 2009). Indoxyl sulphate showed to increase free radicle release (Muteliefu et al. 2009) and to inhibit NO production in human vascular endothelial cells (Tumur and Niwa 2009).

In a trial to manipulate the gut-kidney axis by probiotics, this study aimed to produce a less pathogenic microflora and thus reduce generation of uremic toxins in CKD by administration of probiotic.

In this study, CKD early and late probiotic treated groups showed significant decrease in serum urea and

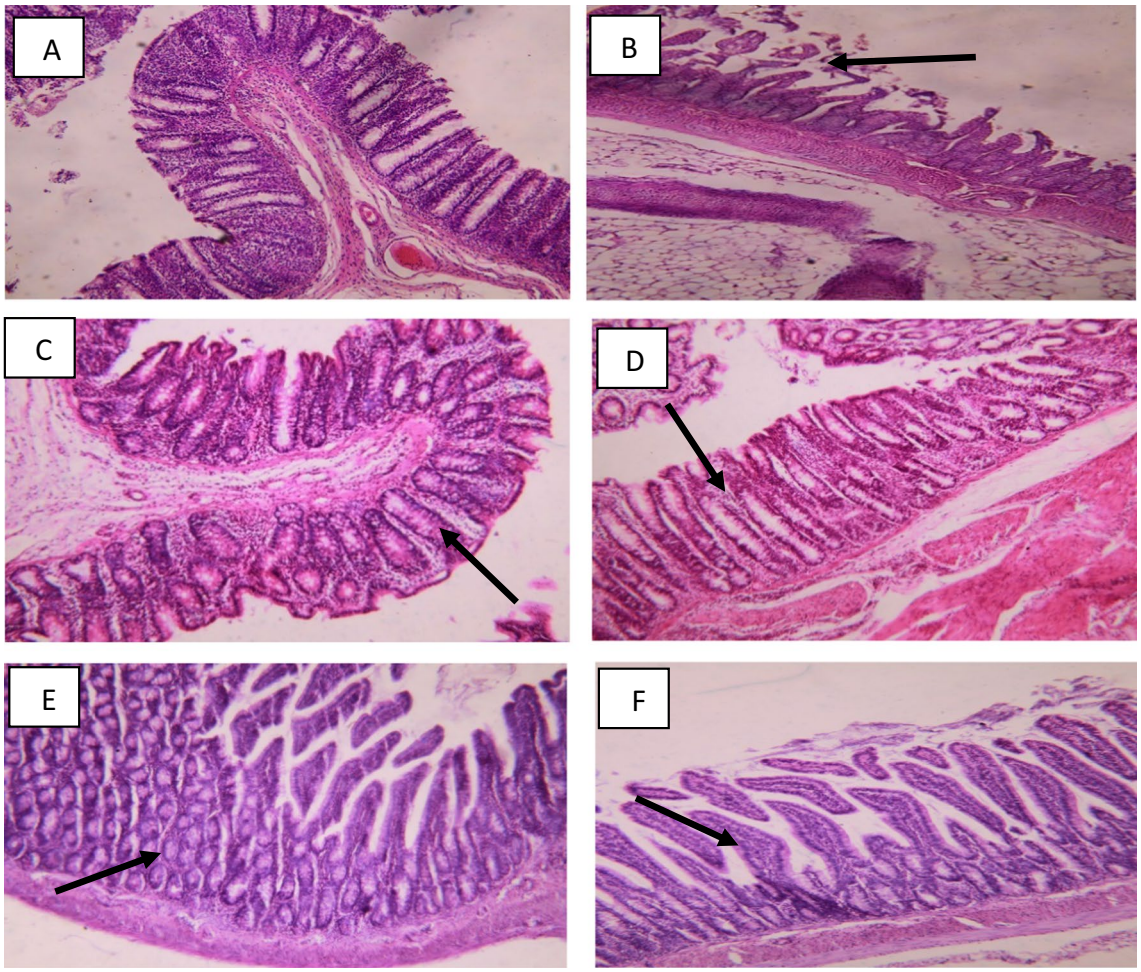

Fig. 2 Sections of intestine of all groups (× 400, H&E): **A** Control group: Normal appearance of intestinal crypts, villi and goblet cells; **B** CKD group: Atrophy and shedding of the villi; **C** Early probiotic CKD: normal crypts (↑) lined by goblet cells and columnar absorptive cells; **D** Late probiotic CKD: normal crypts (↑) lined by goblet cells and columnar absorptive cells; **E** Early symbiotic CKD and **F** late symbiotic CKD: normal crypts (↑) lined by goblet cells and columnar absorptive cells

creatinine level in comparison to CKD untreated rats. The result in this study comes in accordance with other studies (Mahsa et al. 2021; Yoshifuji et al. 2018; Lau et al. 2018**),** but in contrast to Alatriste et al. (2014) and this could be due to longer duration in our study.

From clinical point of view, the effect of the combination of probiotic and prebiotic therapies over a course of 6 weeks in pre-dialysis CKD patients was studied and had same results as our study. The authors showed lowered serum *p*-cresol sulphate and gut microbiome alterations (Lopes et al. 2018). While McFarlane and Ramos (2019) stated that there is limited evidence to support the use of prebiotics, probiotics, and/or synbiotics in CKD management based on systematic review and meta-analysis of 16 clinical studies investigating 645 adults with inclusion criteria.

A possible explanation for the observed reduction in uremia by probiotics is related to the ability of certain anaerobic bacteria to degrade urea and uric acid through the production of enzymes, such as uricase, allantoinase, and urease. In an in vitro study, *Lactobacillus* exposure to urea-enriched environment induced the production of enzymes responsible for urea reduction (Lopes et al. 2018**).**

Probiotic produce bacteriocins (antimicrobial molecules) which further inhibits the growth of pathogens and increase the growth of beneficial bacteria to normalize intestinal microbiota in CKD patients (Ramezani et al. 2016**).** In addition, Probiotic have been shown to produce short-chain fatty acids, in particular butyrate that stimulates the production of antimicrobial peptides, increase the expression and activity of intestinal alkaline

phosphatase, maintaining intestinal homeostasis and hence preventing bacterial translocation and decrease both oxidative stress and inflammation (Leccioli et al. 2017**).**

In this study, both CKD early probiotic treated group and late probiotic treated group showed significant decrease in serum C reactive protein as well as decreased indoxyl sulphate levels in comparison with CKD untreated group. This effect could be correlated to the less progression of CKD in these treated groups proved by less urea and creatinine levels and the ameliorated tubulointerstitial injury evidenced by less fibrotic index.

The decrease in CKD progression with probiotic therapy in this study is supported by the findings in other previous studies. L acidophilus was well documented to reduce levels of uremic toxins like indoxyl sulphate and to modulate inflammation by CRP level reduction (Lee et al. 2020), decrease oxidative stress and inflammation (Thongprayoon et al. 2018). In addition, probiotics decrease phosphate load on the kidney as it increases dietary fibres fermentation, decrease intestinal pH, increase ionized calcium that act as intrinsic phosphate binder causing its loss in stool (Wong et al. 2014; Iwashita et al. 2018).

Thus, although indoxyl sulphate was non significantly reduced with probiotic but another mechanism could be suggested for the improved kidney function as the better intestinal barrier and less intestinal PH and reduced pi load. The latter was in need for measurement of serum pi.

Probiotic treated groups showed an increase in weight gain in comparison to CKD untreated group. The improvement in weight gain could be explained by the decrease of serum inflammatory mediators and improve kidney function. The probiotic also failed to decrease blood pressure this could be due to the non-significant changes in serum indoxyl sulphate.

When probiotic was combined with prebiotic, symbiotic regimen was obtained. The prebiotic components in this product namely inulin has various benefits. Inulin increase stability during storage, increase gastrointestinal passage, increase probiotic growth by providing better growth conditions (Meijers et al. 2010).

In the present study levels of urea, creatinine, C-reactive protein and indoxyl sulphate in symbiotic treated groups were significantly decreased in comparison with both CKD and CKD probiotic treated rats. One of the mechanisms by which this supplementation can potentially benefit the kidneys is by stimulating growth of gut microbial biomass by increased consumption of dietary fibres; this subsequently decreases ammonia production and facilitates the use of nitrogenous wastes by bacterial cells. Thus, more ammonia is excreted through the feces, and the potentially damaging forms of nitrogen, such as urea, uric acid, and creatinine is formed at lower rates (García-Arroyo et al. 2018).

These findings were supported previously by Lopes et al. (2018) who found that symbiotic intake in different stages of CKD decreased p-cresol serum concentration and normalized bowel habits. In addition, the authors showed a marked reduction of uremic toxins, the fermentation of the fibres by the probiotic bacteria was suggested to increases the production of short chain fatty acids and reduces colonic pH. The modified environment favoured the growth of beneficial bacteria, inhibited the enzymes involved in generation of p-cresol sulphate and indoxyl sulphate, in addition to improving epithelial barrier function via induction of mucin production, strengthening epithelial tight junctions and reduces the influx of uremic toxins.

The better intestinal microscopic picture and the lowest fibrotic index with symbiotic is a new documentation for what were suggested by the previous study.

Upon treatment with symbiotic, arterial blood pressure was reduced both systolic and diastolic this could be explained by significant decrease of indoxyl sulphate level in comparison to CKD and probiotic treated rats. Its level nearly reached the level of control rats.

Conclusions

A strong correlation between the gut microbial ecosystem and CKD has been proved. The use of probiotics and probiotic enriched with prebiotic to modulate an unhealthy gut microbiome could be a promising intervention to delay CKD progression specially in early stages. Probiotic enriched with prebiotic results was better than probiotic alone.

Abbreviations

CKD: Chronic kidney disease; CRP: C-reactive protein; MASRI: Faculty of Medicine, Ain Shams University Research Institute; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial blood pressure.

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Author contributions

EG was responsible for manuscript elaboration and for conducting the project and worked on all its stages. DS assisted in animal handling and in discussions regarding the results. MS assisted in animal handling and in discussion regarding the results. NS revised the manuscript. BK is the main investigator, planned, designed and supervised the project. NM provided us with probiotic and symbiotic preparation needed for this work. All authors approved the final manuscript.

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Availability of data and materials
The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All animal experiments were performed according to the Ethics Committee of Faculty of Medicine, Ain Shams University (Reg. No. FWA 000017585), Ain Shams University, Cairo, Egypt.

Consent for publication
Not applicable.

Competing interests
The authors declare they have no competing interests.

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