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REVIEW ARTICLE

Therapeutic effects of probiotics and herbal medications on oxalate nephrolithiasis: a mini systematic review

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ABSTRACT

Background and Objectives: The majority of all kidney stone cases are oxalate urolithiasis with a high risk of recurrence. Beside its widespread occurrence, kidney stones are characterized by severe complications and high treatment costs. Probiotics and herbal medications could be forthcoming therapeutic interventions in the management of oxalate kidney stones.
Materials and Methods: The PubMed/MEDLINE database was searched for keywords "*Oxalobacter formigenes*" AND "Oxalate" OR "oxalate degradation" AND "*Lactobacillus*" OR "*Bifidobacterium*" OR "recombinant *Lactobacillus*" OR "*Bacillus subtilis*", and "*urolithiasis*" AND "herbal extract". The search returned 253 results, 38 of which were included in the review.
Results: Most of the oxalate-degrading probiotics belong to the *Oxalobacter formigenes*, *Lactobacillus*, *Bifidobacterium*, and *Bacillus* genus with a minimum dosage of 10^7 CFU in the form of capsules, sachets, and lyophilized powder. Oxalate concentration in media was 5-50mM with an incubation time ranging from 24h to 14 days. The majority of the studies suggested that probiotic supplementation might be useful for reducing urinary excretion of oxalate and urea and alleviation of stone formation. Different herbal extracts were used on murine models of nephrolithiasis (induced by 0.5-3% ethylene glycol) with reduction of renal inflammation and urinary parameters, and calcium oxalate crystals.
Conclusion: Several strains of probiotics and herbal extracts confer protective effects against kidney stone/nephrolithiasis, indicating their promising nature for being considered as elements of preventive / adjuvant therapeutic strategies.

Keywords: Kidney stone; Probiotics; Herbal extract; Nephrolithiasis; Urolithiasis

INTRODUCTION

Kidney stones are extremely painful and cause various symptoms such as infection and hemorrhage. The pain could be controlled by NSAIDs, Tamsulosin, but the greater stones should be removed by surgical techniques such as percutaneous/transureteral nephrolithotomy, ureteroscopy, and wave lithotripsy (1-3).

The surgical methods are complex and expensive with no effects on stone recurrence. Besides, various drugs are used to prevent calculi formation or elimination but their effectiveness are limited with low tolerability. In addition, antibiotics could impact intesti-

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nal and urinary microbiome composition that cause increased prevalence of kidney stones (3). Emerging urolithiasis treatment options include traditional/dietary plants and probiotics due to their negative regulatory effects on crystallization, and aggregation of these crystals, as well as oxalate-degrading activity.

Herbal medicine, as an alternative to modern medicine problems, has gained much attention both in developed and developing countries over the past decade, and extracts from a variety of plants have been studied for their biological effects (4, 5), and also to treat nephrolithiasis (6). Nephrolithiasis, also known as kidney stone, is determined by the presence of different insoluble deposits such as calcium-based stones (calcium oxalate and phosphate), struvite-based (magnesium ammonium phosphate), uric acid, and cysteine-based stones in the urinary tract (7). Development of kidney stones is a multistep process that include crystal aggregation followed by nucleation, and crystal growth (8). The formation of kidney stones is a complex process that depends on various factors such as age, gender, ethnic differences, heredity, diet, intake of fluid/meat/fruit/sodium/calcium, obesity, metabolic disorders, urinary infection, and inadequate physical activity (9). Due to these risk factors, the frequency of nephrolithiasis is increasing (affecting men more than women) with a limited number of effective drugs and preventing/treatment procedures being available.

The aim of this systematic review is to provide a critical account of the standing of herbal medications and probiotics as natural supplements in the management of kidney stones with an emphasis on their mechanisms of actions.

MATERIALS AND METHODS

Search strategy and selection of studies. All original investigations exploring the effects of probiotics and traditional/herbal medicine on the kidney stone prevention/treatment published within the last 10 years (2012-2022) were considered. Our keywords included "*Oxalobacter formigenes*" AND (Oxalate OR oxalate degradation) AND (*Lactobacillus*" OR "*Bifidobacterium*" OR "recombinant *Lactobacillus*" OR "*Bacillus subtilis*") AND "*urolithiasis*" AND "herbal extract".

Inclusion and exclusion criteria. All reports in English concerned with the administration of Probiotics (*Oxalobacter formigenes*, *Lactobacillus* spp., *Bifidobacterium* spp. and *Bacillus subtilis*) and anti-urolithiasis effects of herbal extracts in both *in vitro* and *in vivo* conditions and clinical trials from 2012 to 2022 were included. Other publication types such as review articles, editorial materials, conference proceedings, and papers in languages other than English were excluded.

Data extraction. Each of the included studies was screened for the following data: probiotic and herbal extract administration dose, intervention time, outcome, *in vivo/in vitro* model, and clinical trials.

Quality of study. A checklist, presented in Table 1, was adopted to assess the quality of evidence for each of the included studies, containing 12 yes/no questions, with each affirmative and negative answer being equal to a score of 1 and 0, respectively. Based on this scoring system, only studies with a minimum score of 8 were included.

RESULTS

The search returned 253 studies, 38 of which were ultimately included in the review (Tables 1-3). According to the evidence, most of the oxalate-degrading probiotics used in the studies belonged to the four genera including *Oxalobacter formigenes*, *Lactobacillus*, *Bifidobacterium*, and *Bacillus*.

Oxalate-degrading *Oxalobacter formigenes*. *O. formigenes* is a Gram-negative anaerobic strain with obligate oxalotrophic activity, which can colonize in the human colon, using oxalate as an obligatory energy source. Therapeutically, after colonization into gut, it is able to degrade the ingested oxalate, lower intestinal absorption of oxalate, stimulate the oxalate excretion from the colon, decrease the risk of oxalate stone formation, reduce the urinary oxalate concentration, confer protective effects against hyperoxaluria. It is assumed that 10^7 CFU of *O. formigenes* is able to degrade oxalate efficiently at 0.1-4.4 nmol/h/gr stool (10-14). In addition, *O. formigenes* expresses enzymes such as oxalate oxidase and decarboxylase, oxalyl-CoA decarboxylase (OXC), and formyl-CoA transferase to break down oxalate into formic acid and CO_2. Importantly, this strain is sensitive to some condition such as low pH, oxygen, oxalate-depend

Table 1. Oxalate-degrading probiotics: *in vitro* studies

Strains	Oxalate concentration in media	Incubation time	Oxalate degradation rate	Oxalate detection method	Ref
O. formigenes DSM 4420	26 mmol/L of ammonium oxalate	4 days	60.2%	enzymatic oxalate kit method	(15)
13 *Lactobacillus* and 5 *Bifidobacterium* strains	10 mM of ammonium oxalate	24h	65-51%	HPLC method	(22)
L. acidophilus	5 mg/L of sodium oxalate	72 hours	85.3 and 161.9 mg/L	Ion chromatography oxalate-	(23)
L. fermentum	20 and 50 mmol/L of calcium	48h, 72h	increased the Enzymatic	degrading enzyme indexes,	(26)
L. gastricus	oxalate		Activity Index to 6.3 and 6.9	viable bacterial cunts oxalate	
L. paragasseri UBLG-36	10-20 mM of odium oxalate	1,5,10 days	45% degradation	assay kit	(27)
L. fermentum (NPL280)	calcium oxalate	72h	46.33%	oxalate assay kit high	(28)
L. rhamnosus GG	20 mmol/L of sodium oxalate	72h	> 35%	performance liquid chromatography (HPLC)	(24)
L. gasseri	20mM of sodium oxalate, 1g/L	48h	1.8-36% *Lactobacillus*	zone of clearance, oxalate	(25)
L. acidophilus	of calcium chloride		coding oxc gene	decarboxylase gene (oxc)	
L. animalis					
Recombinant oxalate-degrading probiotics					
L. plantarum NC8 express *B. subtilis* OxdC	50 mmol l^{-1} of calcium oxalate and 50 mmol l^{-1} of disodium oxalate	24h	> 90% compared to 15% by the wild type	colorimetric method	(39)
L. plantarum ΔthyA:OxdC	10 mM potassium oxalate and 5 mM manganese chloride	120h	53%	semi-automated analyzer	(41)
Bacillus subtilis 168 (BS168)	MDCK renal epithelial cells	Pre-treatment with ∼ 5×10^3 CFU/ml.(20 min)	prevented increased CaOx crystal adhesion and aggregation	inverted microscope	(43)
Bacillus subtilis YvrK gene	Human Embryonic Kidney 293 (HEK293) cells (DMEM supplemented with 750 μM of potassium oxalate)	48h	cell survival rate increased under oxidative stress restored antioxidant activity, mitochondrial membrane potential Remained less	enzymatic kit	(44)
O. formigenes, strain OxCC13 Oxalo™ product (*O. formigenes*, *Lactobacillus acidophilus, Bifidobacterium lactis*, and *Bacillus coagulans*) ™PRO	20 mM oxalate	14days	than 0.1 mM oxalate (by OXCC13) not by other strains	Ion chromatography, oxalate decarboxylase gene (oxc) analyzing	(58)
Herbal extract					
Bergenia ciliata (Saxifragaceae), rhizomes extract	synthetic urine system	1, 2, 5, 7.5 and 10 mg/mL	-inhibited the nucleation and aggregation of CaOx, with a significant reduction in the number and size of COM crystals	spectrophotometic methods	(47)
14 Kampo extracts	Madin–Darby canine kidney cells	10 μg/mL	- Sanshishi and Takusha exhibited a strong inhibitory effect on the aggregation of calcium oxalate monohydrate crystals	- analysis of the adherent COM crystals by atomic absorption	(48)
5 herbal extracts	synthetic urine system	*F. pyrrosiae* (1.5 g/25 ml), *D. styracifolium* (1.5 g/25 ml), *P. niruri* (3.75 g/25 ml), *O. stamineus* (2.5 g/25 ml) and 2 Tablets *Cystone* (®))	- inhibit calcium oxalate stone formation	Determination of metastable limit (MSL), average particle size, nucleation and growth rates of crystals	(59)

Table 2. Oxalate-degrading probiotics: *In vivo* studies

Probiotic strains	Animal model	Oxalate induced by	Administration time	Outcome	Ref
O. formigenes strains HC-1	male and female C57BL/6 (WT) mice	1.5% oxalate-supplemented diet for 5 days	7-56 days	-bacteria were present in the large intestine, small intestine for varying periods of time - decrease in urinary oxalate excretion	(16)
L. paragasseri UBLG-36 and *Lacticaseibacillus paracasei* UBLPC-87	hyperoxaluria-induced nephrolithiasis rat model	4.5% sodium oxalate	42 days	reduced urinary excretion of oxalate and urea, alleviated stone formation	(29)
L. plantarum secreting OxdC	Male Wistar albino rats	5% potassium oxalate	15 to 28 days	reduced urinary oxalate, calcium, urea, and creatinine levels	(30)
B. animalis subsp. lactis DSM 10140 and *B. adolescentis* ATCC 15703	Mice deficient in the hepatic enzyme alanine-glyoxylate aminotransferase (Agxt-/-)	oxalate-supplemented diet (1, 0.5,1.5 %), oral gavage	22-35 days	colonization into the gut colonization of *B. animalis*: 37% in Agx$^{-/-}$ Decreased urinary oxalate excretion by degrading dietary oxalate not enteric oxalate excretion	(38)
Recombinant oxalate degradation probiotics					
Recombinants strain of *L. plantarum* (WCFS1OxdC)	male wistar albino rats	5% potassium oxalate diet	2 weeks	degradation of oxalate up to 77% reduce excretion of oxalate and deposition of CaOx crystals	(40]
Lactococcus lactis MG1363 (coding the oxalate decarboxylase (ODC) and oxalate *oxidase (OxO) genes)*	*In vitro* (media contain 100 mmol/L of ammonium oxalate) - female Sprague-Dawley (SD) rats	fed high oxalate diet (normal chow mixed with 5% additional ammonium oxalate) + 5CFU of wild-type LAB by intragastric administration	30 days feeding	decrease urinary oxalate levels inhibited oxalate crystals	(42)
Bacillus subtilis 168 (BS168)	Drosophila melanogaster model	lithogenic diet (~108 CFU/ml)	14days	- stably colonized into the D. melanogaster intestinal tract for as long as 5 days - decreased stone burden and fecal excreta	(43)
recombinant *E. coli* named pBy	Transient hyperoxaluric rat model (Eight-week-old Sprague- Dawley male rats)	Received oral oxalate (0.5 ml of 1 mM sodium oxalate + 0.5 ml of *pBy* homogenates)	0-3 h	- increased survival and behavioral markers after 14 days Purified ODC degraded more than 50% of oxalate Urine oxalate concentration was decreased significantly by pBy homogenates compared to control	(45)
Herbal extract	**nephrolithiasis rat model**	**Intervention**	**Administration time**	**Outcome**	**Ref**
Pyrrosia lingua	1% ethylene glycol	300,600, 900 mg/kg of PL	28days	- Reduction of urine oxalic acid, urine calcium, and osteopontin	(49)
Urtica dioica and *Tribulus terrestris* Plus probiotics	3% ethylene glycol	U. dioica : 1400g/kg/body weight T: terrestris to 200mg/kg/body weight Probiotics: (1011~:FU) Sankol: (7.5 -9 ml/kg/b.w)	30 days	- increasing microflora toward the *O. formigenes, Bacteriodetes*, *Bifidobacterium* and *Fecalibacterium* - decreased renal inflammation and urinary parameters - reduce calcium oxalate crystals in shorter time	(50)
Sankol oral drops plus probiotics	3% ethylene glycol		30days	- significantly reduced urinary oxalate - any CaOx crystal	(51)
Rhizomes of *Acorus calamus*	0.75% v/v ethylene glycol in drinking water	250, 500 and 750 mg/kg	28 days	- reduced excretion and deposition of compounds promoting urolithiasis	(52)
Tutukon product	0.5% ethylene glycol	15 ml/day	28 days	- reverted impaired renal function - increased urine oxalate levels - oxalate excretion levels not changed - decrease stone deposition	(53)
Flos carthami (FC)	0.75%	300, 600, and 1200 mg/day	4 weeks	- inhibit crystal deposits	(54)
Bergenia ligulata	0.75%	185mg/kg and 7 mg/kg of dichloromethane (DCM) fraction	21 days	- significant reduction in serum and urine parameters - low calcium oxalate deposits	(55)
Tutukon	0.75%	45 ml three times/daily	28 days	Inhibited renal tubular crystal deposition and apoptotic changes	(56)
Tutukon product	0.5% ethylene glycol	15 ml/day	28 days	- increased urine oxalate levels - oxalate excretion levels not changed - decrease stone deposition	(53)

Table 3. Oxalate-degrading probiotics: clinical studies

Probiotics	***Oxalobacter formigenes***			
	Single strain			**Multi-strain**
Type of study	Phase I/II clinical trials (28 patients)	Phase I/III clinical trials (36 patients)	Phase II clinical trial (12 patients)	40 patients with calcium oxalate stones
Treated states	Oxabact® OC5 product	OC3 formulation	OC5	Ochek: 700 million *O. formigenes* + *L. acidophilus* 400 million+ *L. rhamnosus* 300 million+ *B. lactis* 300 million
Study period	-8- to 10-week treatment period -Took oral doses of OC5 or placebo twice daily with meals	24 weeks	-OC5 capsule twice daily - 24-36 months	- Twice a day - One month
Objectives/ measurements	-plasma oxalate concentration (µmol/L) (Pox) -urinary oxalate excretion (Uox). - counting the faecal *O. formigenes* cells	-Urinary oxalate excretion - Urinary creatinine - Total plasma oxalate concentration - Stone events - Creatinine clearance and eGFR	- Pox concentration	- urinary metabolic abnormalities: Oxalate, Calcium, Citrate, Magnesium, Uric acid
Form of treatment	-Enteric-coated, size 4, gelatine capsules (lyophilized bacteria $\geq 10^9$ CFU). -placebo product (microcrystalline cellulose)	lyophilized *O. formigenes* HC-1 (not less than 10^7 CFU)	One capsule of OC5 contains concentrated *O. formigenes* with an oxalate degrading capacity 100 mmol/capsule/day	One capsule of Ocheck
Outcomes	-Uox after treatment did not differ significantly in both groups - number of *O. formigenes* in faecal after treatment was greater in the OC5 group -Pox was not significantly different between the groups	- no significant difference was found between the groups in urinary oxalate per creatinine ratio - stone events were similar in two groups - no significant difference for Pox was found	- decreased Pox concentrations - improved cardiac function and clinical status, without increasing dialysis frequency	Reduced Hyperoxaluria significantly
Ref	(59)	(18)	(19)	(35)
Type of study	Lactic acid bacteria 40 subiects with normal oxalate in diet	Randomized, controlled trial on 11 non-stone formers		
Treated states	Oxadrop: *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Bifidobacterium* infantis, *Streptococcus thermophilus*	VSL#3: *Streptococcus thermophilus*, *B. breve*, *B. longum*, and *B. infantis*, and *L. acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* subsp. *Bulgaricus*		
Study period	-oxalate-rich diet (600 mg oxalate/d) for six weeks	daily ingestion for four weeks of one packet of VSL#3®		
Objectives/ measurements	- Urinary oxalate excretion - Plasma oxalate concentration	urinarv oxalate		
Form of treatment	a daily dose of 3.6×10^{11} CFU (4g/day) for 4 days	1-2 packet of VSL#3®		
Outcomes	No significant effects on the concentration of oxalate in test and control groups	-reduce gastrointestinal oxalate absorption -reduce urinary oxalate and increase absorption on non-stone formers with high oxalate diet (176mg /d)		

growth, and the probiotics producing process (from manufacture, storage and distribution). As this strain is an extensively investigated species with oxalate degrading potential, the searching for strains that be resistant to probiotic/functional food production process is more important. Moreover, administration of prebiotics/probiotic could change the gut microbiota, which affect the activity of this bacterium to degrade oxalate and finally reduce the oxalate exertion and stone formation (15).

In an *in vitro* study, Karamad et al. (2019) investigated oxalate biodegradative activity of *O. formigenes* DSM 4420 and estimated 60.2% degradation in the presence of insulin, used as prebiotics, (1.35 g/L), glucose (36.56 g/L), and ammonium oxalate (26 mmol/L) (15).

Hatch and co-workers (2013) artificially colonized *O. formigenes* HC-1 into male and female C57BL/6 (WT) mice which were feeding by 1.5% oxalate-supplemented diet for 5 days. Sampling at different intervals showed that HC-1 could colonize in the colon and more proximal sections of the gut, promoting intraluminal secretion of oxalate, hence, the decrease in renal excretion of oxalate. Accordingly, HC-1 might actually be a probiotic of choice for counteracting hyperoxaluria (16).

Hoppe et al. (2017) assayed probiotic product treatment (Oxabact® OC5) involving *O. formigenes* on 28 primary hyperoxaluria (PH) patients in a randomized (1:1) clinical trials for 8 weeks. The patients took two daily oral doses of OC5 with meals as enteric-coated, size 4, gelatin capsules which contained lyophilized bacteria $\geq 10^9$ CFU and had oxalate-reducing capacity of ≥100 mmol/capsule/19h. According to the results, a non-significant difference was found in urinary oxalate excretion (Uox) and plasma oxalate concentration (Pox) after the treatment between the test and control groups, while Uox per urinary creatinine excretion was increased in the OC5 group significantly. In addition, the number of *O. formigenes* in feces after treatment was greater than placebo group (17). Milliner et al. (2018) investigated the effect of *O. formigenes* (Oxabact OC3) in PH patients for 24 weeks administration as a randomized (1:1) clinical trials on 36 patients. Patients were given one sachet (500mg dose) of lyophilized *O. formigenes* HC-1 (minimum 10^7 CFU/dose) which was able to metabolize 30 mmol (2640 mg) oxalate within 19 h. Both OC3 and placebo groups showed a non-statistically significant reduction in Uox/urinary creatinine ratio. Also, no differences were found for Pox concentration, occurrence of renal caliculi, and the proportion of responsive patients. Pox was increased in the placebo group compared to the OC3 group significantly (3.25 µmol/L vs -1.7 µmol/L) in patients with estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73 m^2, but after 24 weeks, eGFR declined in the placebo group compared to the OC3 group. In addition, OC3 treatment did not decrease urinary oxalate over 24 weeks in comparison to placebo in PH patients (18). In another clinical trial, Hoppe et al. (2021) used Oxabact OC5 (twice-daily for 24 months) in 12 primary hyperoxaluria Type 1 (PH1) to analyses the effect of *O. formigenes* (100 mmol/capsule/day) and found that OC5 administration could substantially decrease Pox level, and improve cardiac function and without negatively affecting dialysis efficacy (19).

Oxalate-degrading *Lactobacillus* spp. *Lactobacillus* species are determined as facultative oxalotrophs because, in contrast to *O. formigenes*, different strains of *Lactobacillus* are regarded as generally-recognised-as-safe (GRAS) probiotics by United States Food and Drug Administration (FDA), and considered to be a crucial constituent of the gut microbiota (20). Evidence is now suggesting various health effects for certain genera of *Lactobacillus*, especially prevention of kidney stones (21). *Lactobacillus* strains can express OXC-like gene with oxalate-degrading activity, which is further discussed below as *in vitro, in vivo,* clinical trials, and recombinant strains.

***In vitro* studies.** Mogna et al. (2014) screened the oxalate-degrading activity of lactobacilli (13 strains) after culturing in the media with 10 mM ammonium oxalate by HPLC method with *O. formigenes* (DSM 4420) as positive reference. The results confirmed that the highest oxalate degradation level belonged to *L. paracasei* LPC09 (DSM 24243) (68.5%) followed by *L. gasserii* LGS01 and LGS02, and *L. acidophilus* LA02 and LA07, the activity level of which was measured to be 68.4%, 66.2%, 54.3%, and 51.4%, respectively. While, the three strains including *L. reuteri* LRE02 and LRE03, and *L. rhamnosus* LR06 showed decomposing oxalate over 20% in the medium (22).

Cho et al. (2015) analyzed the oxalate-degrading potential of two commercial probiotics products. These products contained fructo-oligosaccharides and 5×10^9 CFUs/capsule. Probiotic 1 comprised *L. acidophilus, L. plantarum, Bifidobacterium bifidum, L. casei, L.*

brevis, Bifidobacterium longum, and *Enterococcus thermophiles.* Probiotic 2 contained *L. acidophilus, L. plantarum, B. bifidum, L. casei, L. bulgaricus, E. thermophilus,* and *Enterococcus faecium.* Strains were cultured in media containing 5 mg/L sodium oxalate for 72h, with *L. acidophilus* (ATCC: 53544) being used as positive oxalate-degrading isolate. Following the detection of oxalate concentrations using ion chromatography, it was found that *L. acidophilus* (ATCC 53544) and *L. acidophilus* reduced oxalate level significantly by 85.3 and 161.9 mg/L, respectively. On the contrary, *L. plantarum* significantly increased oxalate level by 36.1-56.1 mg/L, respectively, while *L. casei* did not alter oxalate level (23). Murru et al. (2017) screened the 79 strains LAB from different origin with respect to their oxalate degrading abilities on media containing 20 mmol/L of sodium oxalate using HPLC after 72h. Among these strains, *L. rhamnosus* GG exhibited the highest level of oxalate-degrading activity, and oxalate degradation was highest when glucose was available in the environment (24). Miller et al. (2014) isolated oxalate-degrading using brain heart infusion (BHI) agar supplemented with 20 mM sodium oxalate and 1 g/liter calcium chloride and OXC gene amplification. *Lactobacillus* strains (*L. gasseri, L. acidophilus,* and *L. animalis*) expressed the OXC gene and degraded oxalate from 1.8 to 36% (25). Soliman et al. (2021) isolated 88 LAB and investigated oxalate degrading potential using the enzymatic activity index (in agar plate containing 50 mmol/L calcium oxalate) and measured the count of viable bacteria (in media supplemented with 20 mmol/L of calcium oxalate) after incubation of 72 and 48h. They found that *L. fermentum* (five strains) had EAIs of 2, 5, 6.7, 7, and 7.5 and *L. gastricus* (two strains) had 5.8 and 7.5. Additionally, among the isolates, *L. fermentum* showed the best growth in the media containing oxalate, which along with *L. gastricus* were suggested as suitable probiotics for managing hyperoxaluria (26). Mehra et al. (2021) analyzed the probiotic potential of *L. paragasseri* strain UBLG-36 by genome sequencing and found essential genes for bacteriocin production, adhesion to epithelium, resistance to stress, modulation of immune responses, carbohydrate metabolism, as well as genes for formyl coenzyme A transferase (frc) and oxalyl coenzyme A decarboxylase (oxc) which are involved in oxalate catabolism. In vitro oxalate degradation assay was done in media containing 10 and 20 mM sodium oxalate for 1, 5 and 10 days using oxalate assay kit and presented highly effective degradation more than 45% (27). Aziz et al. (2021) investigated the potential of LAB isolated from camel milk, suggesting that one particular strain, *L. fermentum* named NPL280, was safe and selected due to effective probiotics potential with 46.33% oxalate degradation (28).

***In vivo* studies.** Mehra et al. (2021) characterized the ability of *L. paragasseri* UBLG-36 and *Lacticaseibacillus paracasei* UBLPC-87 to degrade oxalate *in vitro* and in the rat model of nephrolithiasis induced by 4.5% sodium oxalate (NaOx). Pre-treatment with these probiotics before inducing nephrolithiasis could protect rats from pathophysiological manifestations of hyperoxaluria. Also, reduced urinary excretion of oxalate and stone formation with significantly mitigated renal histological damage were observed in rats treated with probiotics compared to those which had not been given such treatment (29).

Paul et al. (2018) evaluated the oxalate-degrading capability of OxdC-expressing *L. plantarum* using lactococcal group II intron, Ll. LtrB. Male Wistar albino rats were given 5% potassium oxalate-containing diet for 28 days, with one group receiving *L. plantarum* and the other recombinant *L. plantarum* for 15 to 28 days, the latter of which was found to have markedly reduced urinary oxalate, calcium, urea, and creatinine compared to rats receiving *L. plantarum.* Furthermore, recombinant probiotics able to colonize into the gastrointestinal tract, degrading intraluminal oxalate, and thus, reverting CaOx stone formation in the experimental animals (30).

Besides the oxalate-degrading ability of *Lactobacillus* strains, the modulation of inflammation responses associated with oxalate accumulation was confirmed for some other strains such as *L. plantarum* PBS067, *L. acidophilus* LA-14, *B. breve* PBS077, and *B. longum* PBS108 through the upregulation of IL-4, IL-10, IFN-γ and IL-12 p70 levels (31). Therefore prevention/treatment of kidney stone could be achieved with these probiotics not only with active oxalate degradation, but also the regulation of inflammatory events. An *in vivo* research by Wei et al. in 2021 investigated the effect of *Lactiplantibacillus plantarum* N-1 (LPN1) on hyperoxaluria when administrated for four weeks. The results suggested that LPN1 could prevent the oxalate deposits formation in rat kidney, reduce urinary concentration of oxalic acid, renal osteopontin and CD44, improve enteritis and barrier function by lowering the lipopolysaccharide (LPS) and TLR4/

NF-κB signaling pathways in serum, and up-regulate colon claudin -2. Also, this probiotic could regulate the gut microflora by increasing the short-chain fatty acids (SCFA) production and frequency of SCFA-producing bacteria (*Lachnospiraceae* and *Ruminococcaceae families*) (32).

Clinical trials. The *in vitro* and *in vivo* results confirm the *Lactobacillus oxalate* degrading potential for clinical application. Al-Wahsh and co-workers (2012) administrated oral daily doses of oxalate-degrading probiotic bacteria (VSL#3) with a 176 mg oxalate load to 11 healthy non-stone formers (8 females, 3 males) for four weeks. Freeze-dried live probiotics, each sachet contain 45×10^{10} live strains of *Streptococcus thermophilus, B. breve, B. infantis, B. longum,* and *L. acidophilus, L. delbrueckii* subsp. *Bulgaricus, L. paracasei,* and *L. plantarum.* The study demonstrated that both the single and double doses of the probiotic compound being tested was effective at reducing the concentration of oxalate in the urine, with no remarkable difference in the rate of reduction between the two doses. Else, it was also found that VSL#3 exerted most of its effect by regulating the intestinal absorption of oxalate, and thus, might be most useful in the treatment of patients with high oral intake of oxalate-containing foods (33).

Siener et al. (2013) conducted a study on 20 healthy individuals (10 men and 10 female) with normal oxalate in diet (100 mg oxalate/d) which were then placed on groups with high oxalate in diet (600 mg oxalate/d) for 6 weeks. Along with high oxalate diet in the second week, the participations received LAB (2.6 g/day) for 5 weeks. The subjects were evaluated after being treated for 4 consecutive weeks while receiving a diet containing a normal level of oxalate compounds. Each gram of the LAB product, named Oxadrop, contained 3.6×10^{11} CFU of *L. acidophilus*, *Bifidobacterium* infantis, *Streptococcus thermophilus* and *L. brevis.* After treatment urinary oxalate excretion was found to have significantly increased, which remained upregulated until the end of treatment. The concentration of oxalate in plasma was remarkably higher during a 5-week period of treatment, four weeks after which the urinary oxalate excretion and relative super-saturation of calcium oxalate were found to have decreased to reach the initial levels. This product was not able to reduce urinary oxalate excretion or plasma oxalate concentration, necessitating to alter or select the highest oxalate-degrading LAB strains (34). Jairath et al. (2015) as prospective randomized controlled study, administrated 30 mEq of potassium magnesium citrate (KMgCit) and one capsule of Ochek product (*O. formigenes* 7×10^8, *L. acidophilus* 4×10^8, *L. rhamnosus* 3×10^8, *Bifidobacterium lactis* 3×10^8 CFU) twice a day on the metabolic pattern of 80 patients with calcium oxalate stones. The patients had abnormal hyperoxaluria and hypercalciuria. After one month of treatment, the hyperoxaluria incidence was decreased in both groups. Compared with KMgCit, probiotic supplementation was more effective at mitigating the incidence of hyperoxaluria (15% vs. 37.5%) (35).

Oxalate-degrading *Bifidobacterium* spp. Another probiotic is *Bifidobacterium* species, colonized into the human gut and have been approved as GRAS by the FDA. Besides many health effects, *Bifidobacterium* can modulate oxalate degradation in the gut tract. The potential of oxalate degradation depends on harboring the oxalyl-coenzyme A (CoA) decarboxylase gene, the product of which prompts decarboxylation of oxalyl-CoA to formyl-CoA (36). The homologues genes for OXC and FRC are found in some *B. animalis* subsp. *Lactis, B. dentium, B. gallicum, B. pseudocatenulatum,* and *B. pseudolongum,* which may be able to break down the calcium oxalate by these *Bifidobacterium* strains. Interestingly, in addition to oxalate degradation by *B. breve* PBS077 and *B. longum* PBS078, these bacteria could modulate the immune response linked with oxalate accumulation followed by production of pro or anti-inflammatory cytokines and reduction of inflammatory events (31).

Previously, oxalate-degrading level for *B. infantis* was determined to be 5% in an *in vitro* analysis. Moreover, only *B. infantis* presented suitable degradation potential and growth rapidly in an oxalate-containing medium compared to other probiotics including *L. acidophilus, L. plantarum, L. brevis,* and *S. thermophilus* (37). In another screening in vitro study by Mogna et al. (2014), it was found that *B. breve* BR03, *B. animalis, B. longum* BL03, and *B. lactis* BA05 could degrade 28.2%, 27.7%, 25.3%, and 15.5%, respectively (22). Like *Lactobacillus* species, the oxalate-degrading ability of *Bifidobacterium* under *in vitro* conditions does not necessarily indicate an equal level of activity in the living body. Therefore, subsequent trials, animal and clinical studies, should be conducted to appraise the exact effectiveness of candidate probiotic- *Bifidobacteria* for reducing the urolithiasis development. A study by Klimesova et al. (2015) on mouse model for

primary hyperoxaluria ($Agxt^{-/-}$, deficiency in the hepatic alanine-glyoxylate aminotransferase) and wild-type groups administrated with *B. animalis* subsp. *lactis* DSM 10140 showed increased oxalate degradation ability after feeding by oxalate-supplemented diet. These strains after colonization in knockout mice could limit the intestine absorption, diminish urinary oxalate excretion than wild-type mice without administration of DSM 10140, while not being able to promote enteric oxalate excretion (38).

Recombinant oxalate-degrading probiotics. Colonization of the intestinal tract with recombinant probiotic bacteria expressing oxalate-degrading genes such as OxdC might prove to be an effective intervention for reducing the risk of recurrence in the case of CaOx renal caliculi.

Anbazhagan et al. (2013) prepared recombinant *L. plantarum* NC8 to constitutively express heterologous *B. subtilis* OxdC and examined oxalate-degrading *in vitro* (media contain 50 mmol/L of calcium oxalate and disodium oxalate). They discovered that the recombinant probiotic were able to degrade oxalate more than 90% compared to the wild type (15%) and managed to tolerate higher concentration of oxalate about 500 mmol/L (39).

Sasikumar et al. (2014) engineered OxdC expressing *L. plantarum* to evaluate its ability for preventing CaOx stone formation in rats. Male wistar albino rats received 5% potassium oxalate to induce hyperoxaluria and then orally administered with 5×10^{10} CFU/mL/day of non-recombinant and recombinant *L. plantarum* for 14 days. This strain was able to degrade oxalate up to 70-77% at *in vitro* conditions. The experimental group exhibited a marked reduction of urinary oxalate and compared to the control group receiving the wild type strain. Oxalate levels in the kidney homogenates were significantly reduced with no microscopic crystal observations (40).

In addition, Paul et al. (2019) assayed the oxalate degradation potential of recombinant *L. plantarum* ΔthyA:OxdC, and wild-type *L. plantarum* WCFS1 through the oxalate oxidase method (semi-automated analyzer). The results showed that recombinant strain breaks down 53% of the oxalate available in the environment, while the wild-type strain did not degrade any oxalate (41).

Other oxalate-degrading bacteria. Zhao et al. transferred the oxalate decarboxylase (ODC) and oxalate oxidase (OxO) genes into *Lactococcus lactis* MG1363 and evaluated oxalate degradation in both *in vivo* and *in vitro* study. According to the results, strains with ODC gene were able to decrease oxalate level in the media (100 mmol/L of ammonium oxalate) and rat urine (received high oxalate diet) after 24 h as well as inhibit calcium oxalate formation. But, strains encoding the OxO gene were less effective in oxalate degradation process (42). Al et al. (2020) established urolithiasis model using *Drosophila melanogaster* to assess the therapeutic potential of oxalate-degrading bacteria, *Bacillus subtilis* 168 (BS168). This strain is able to survive at high oxalate concentrations and colonize into *D. melanogaster* intestinal tract for as long as 5 days, preventing the oxalate-induced gut dysbiosis. Single-dose of BS168 ($\sim 10^8$ CFU/ml) conferred beneficial effects for 14 days, i.e., decreased stone burden and fecal excreta and increased survival and behavioral markers. In addition, these finding were evaluated by in vitro tests using MDCK renal cell line which were pre-treated with $\sim 5 \times 10^3$ CFU/ml for 20 min. Inverted microscope analysis showed BS168 prevent increased CaOx crystal adhesion and aggregation (43). Albert et al. (2017) cloned the pcDNAOXDC producing *Bacillus subtilis* YvrK gene in HEK293 cells, noting a 28% increase in the survival rate of OxdC-expressing cells treated with oxalate. This was further confirmed by the downregulation of caspase 3, a pro-apoptotic protein, in OxdC-expressing HEK293 cells (44). Lee et al. (2014) analyzed oxalate-degrading activity of purified OxdC enzyme overexpressed by recombinant *Escherichia coli* named pBy. The results showed that purified enzyme could degrade oxalate more than 50% when incubated with 5 mM $MnCl_2$ and 1.5 mM sodium oxalate for 24h at 28°C and pH=5 as optimal condition. Sprague-Dawley male rats as hyperoxaluric rat model received oral 0.5 ml of 1 mM sodium oxalate solution along with 0.5 ml of pBy and control TOP 10 *E. coli* for 3 h. The findings indicated that pBy treatment effectively resulted in reduced concentrations of oxalate in urine (45).

Combination of oxalate degrading probiotic and herbal extracts: *in vivo* and *in vitro* studies. The oxalate-degrading activity of probiotics might restrict the absorption and excretion of oxalate. In addition, application of probiotics and herbal products could alleviate nephrolithiasis as well. Because of the popularity of herbal products, people like to use herbal derivatives especially in the developing countries

(46). Saha et. al. (2013) evaluated the effectiveness of *Bergenia ciliata* (Saxifragaceae), rhizomes extract, for inhibiting CaOx crystallization in vitro, reporting that the extract of *B. ciliata* was a more effective inhibitor of nucleation and aggregation of CaOx monohydrate (COM) crystals than Cystone, as it reduced the number of size of these crystals in a dose-dependent fashion (47).

Nishihata et al. (2013) tested the effect of 14 Kampo extracts (10 μg/mL) on urinary stone formation. Madin-Darby canine kidney cells was used to assess crystal aggregation and crystal adhesion after exposing to 3 mL of calcium oxalate monohydrate crystal suspension for 5 min. The negative regulatory effect of the extracts on stone formation was tested on a murine model (induced with 5% ethylene glycol). The rats were given 10, 20, and 50 mg/body/day of products for 14 days. Of these, Sanshishi, Jiou, Takusha, Kinsensou showed an anti-aggregation activity of 84.5, 64.2%, 64.2% and 63.0%. Sanshishi and Takusha highlighted strong inhibitory effect of the probiotic in question on crystal adhesion to Madin-Darby canine kidney cells by 88.2% and 54.6%, respectively. Also, Sanshishi reported that the probiotic had a prophylactic effect against renal deposition of calcium oxalate crystals (48).

Rodgers et al. (2014) investigated the effect of 5 herbal extracts (*Folium pyrrosiae* (1.5 g/25 ml), *Desmodium styracifolium* (1.5 g/25 ml), *Phyllanthus niruri* (3.75 g/25 ml), *Orthosiphon stamineus* (2.5 g/25 ml) and 2 tablets *Cystone*) on the deposition rate of CaOx in synthetic urine system. Three of the herbal compounds (*D. styracifolium*, *O. stamineus* and *Cystone*) led to a marked reduction in the average size of the precipitated crystals in relation to that of the untreated group. Additionally, all extracts were found to decrease the growth rate, while increasing the rate of nucleation, proposing these herbal compounds as potential inhibitors of calcium oxalate deposition.

Xu et al. (2021) investigated the effect of *Pyrrosia lingua* (PL) on nephrolithiasis in order to clarify its role in the regulating of intestinal flora and oxalic acid. The rats were grouped and fed with a high, medium and low dose of PL, 900 mg/kg, 600 mg/kg, 300 mg/kg, respectively for 28 days. The rat model of nephrolithiasis was prepared using 1% ethylene glycol solution plus normal fed and water drink. It was found that PL was able to mediate toll-like receptor signaling pathway via the IL-6, TNF (tumor necrosis factor), MAPK8 (mitogen activated protein kinase 8), and SPP1 (secreted phosphoprotein 1) based on quercetin and kaempferol components. The urinary concentrations of oxalate, calcium and osteopontin were markedly reduced in the murine models of nephrolithiasis was reduced significantly. Furthermore, the frequency of microflora was changed toward the *O. formigenes*, *Bacteriodetes*, *Bifidobacterium* and *Fecalibacterium* (49).

Afkari et al. (2019) used simultaneously oxalate-degrading probiotics ($\sim 10^{11}$ CFU) and ethanol extract of *Urtica dioica* (1400g/kg/body weight) and *tribulus terrestris* (200mg/kg/body weight) to evaluate their reducing urinary oxalate potential. Four *Lactobacillus* strains (*L. acidophilus*, *L. Delbrukii*, *L. plantarum*, *L. casei*), two *Bifidobacterium* (*B. bifidum*, *B. animalis* subsp. *Lactis*), *Streptococcus salivarius* subsp *thermophilus*, and two strains of *L. paracasei* AKPL-IR were administrated for 30 days. Rat model of nephrolithiasis was induced with 3% ethylene glycol. Consumption of plant extracts (without probiotic) for 30 days decreased renal inflammation and urinary parameters. The simultaneous administration of herbal compounds with probiotics able to reduce the inflammation and renal calcium oxalate deposition in shorter periods (20 days) is associated with immunosuppressive and inflammatory potential of *L. plantarum* and *L. paracasei* as strains with high power in oxalate degradation (50). In line with this study, Afkari et al. (2019) used the same probiotic strains plus Sankol oral drops (7.5 -9 ml/kg/body weight) in rat model. Treatment with Sankol (9 ml/kg/body weight as maximum concentration) and probiotics markedly reduced urinary oxalate ($P = .0001$). Rats receiving both herbal extracts and probiotics did not show any CaOx crystal in compared to negative control (51).

The anti-urolithiatic effects of ethanolic extract of rhizome derived from *Acorus calamus* rhizome (EEAC) was also investigated. The urolithiasis was induced by 0.75% ethylene glycol in rats and three doses (250, 500 and 750 mg/kg) of EEAC was administrated in the study for 28 days. After treatments, results showed the significant reduction of excretion and precipitation of various promoters of urolithiasis in rats treated with EEAC (52).

Yuruk et al. (2016) investigated the protective effects of Tutukon (15 ml/day) on rat models which were treated with 0.5% ethylene glycol for 4 weeks. This product (100ml) is composed of *Enguisetumarvensis* stem (570 mg, dried parts), *Spergularia rubra* (330 mg,whole plant), *Peumus boldus* (280 mg, leaves),

Opuntia ficus-indica (170 mg, flowers), *Sideritis angustifolia* (170 mg, flowers), *Rozmarinus officinales* (170 mg, leaves), *Cynodon dactylon* (170 mg, rhizomes) and of *Melissa officinalis* (170 mg, leaves). Following a four-week treatment program, while the urine oxalate levels in the control rates were lower compared to that of the groups treated with herbal groups, similar oxalate excretion levels were found in rats treated with herbal extract (non-significant). Tutukon was found to decrease crystal precipitation on zinc disks implanted in the bladder of rats but need more detained studies to understand its preventive effect (53).

Lin et al. (2012) aimed to investigate the effects of *Flos carthami* (FC), also known as *Carthamus tinctorius* on CaOx formation in ethylene glycol (0.75%)-fed rats. The FC received rats (300, 600, and 1200 mg/day) for 4 weeks. Histopathological analysis showed that in the groups of 600 and 1200 mg/day could inhibit crystal deposits in FC treated rats significantly but the main side effects is considerable such as bleeding (54).

Sharma et al. (2017) used dried rhizome of *Bergenia ligulata* (pashanbhed) as therapeutic herbal for urolithiasis. The fractions were prepared successively from aqueous extract of *B. ligulata*, as mother extract, using hexane, toluene, dichloromethane (DCM), n-butanol, and water. Urolithiasis model in albino Wistar rats were induced by 0.75% ethylene glycol for 21 days. Rats in mother extract received 185mg/kg of *B. ligulata* and DCM (greater inhibitor at *in-vitro* test) was administrated with a dose of 7 mg/kg once daily for 21 days. Treated rats with these doses presented resulted in significant reduction in serum and urine parameters and low calcium oxalate deposits (55).

Sahin et al. (2015) evaluated the effect of Tutukon (45 ml three times/daily) on the extent of crystal deposition and the renal tubular apoptotic rate associated with hyperoxaluria in rats (induced with 0.75% ethylene glycol) for 28 days. Evaluation of Caspase-3 positivity in tissue sections showed that while the majority of animals undergoing ethylene glycol treatment exhibited apoptotic changes, administration of Tutukon was found to result in significantly reduced rate of apoptosis. Similar observations were noted for TNF alpha expression. In animals receiving Tutukon limited changes of crystal deposition was found (56).

We et al. (2014) investigated 80 antilithic medicinal plants in *Drosophila melanogaster* model which induced with 0.5% ethylene glycol for 3 weeks feeding. The crystal formation rate by ethylene glycol was 100.0% and the 16 herbal drugs under study led to termination of crystal formation, that included *Salviae miltiorrhizae, Paeonia lactiflora,* and *Carthami flos.* In addition, CaOx crystal formation was increased by *Scutellaria baicalensis.* In addition, *Commiphora molmol* and *Natrii sulfas* induced death in all flies (57).

DISCUSSION

Adhesion of CaOx crystals to renal cells is a consequential event in the case of hyperoxaluria that might lead to increased formation of renal stones. As the surgical techniques can only eliminate the caliculi without having any effect on the rate of recurrence, probiotics and herbal extracts could be useful as an adjuvant therapy. This systematic review investigated the effect of various strains of probiotics on kidney stone prevention. The six clinical trials included here explained the effectiveness of single strain and multi-strains of probiotics, and the experimental studies reported significant alterations in reduction of oxalate concentration and oxalate crystal formation. Probiotics containing *O. formigenes* displayed considerable efficacy due to their inherent content of oxalate-degrading enzymes. As with the *in vitro* studies, different strains of *Lactobacillus* and *Bifidobacterium* species including *L. acidophilus , L. fermentum, L. gastricus, L. paragasseri , L. fermentum, L. rhamnosus* GG, *L. gasseri, L. acidophilus,* and *L. animalis* were able to degrade 1.8-65% of oxalate (10-26 mmol/L) in the media. In addition, functional recombinant oxalate degrading enzymes expressed by recombinant strains of *L. plantarum* and *B. subtilis* were shown to degrade 50% of oxalate (5-50 mmol/L), and were capable of preventing CaOx crystal adhesion and aggregation in about 90% of cases. On the other hand, results provided by *in vivo* studies on murine models of nephrolithiasis showed reduction of urinary oxalate excretion, alleviation of stone formation, and probiotics colonizing into intestinal. Considering the favorable safety profile of probiotics already used as therapeutics aids and in fermented foods, these findings suggest the potential of these friendly microorganisms as adjunct/preventive treatment to reduce CaOx nephrolithiasis. Also, these bacteria could affect the oxalate metabolism via colonizing into intestinal and production of safe and efficacious products with single strain or multi-

strain of probiotics is more important in preventing urolithiasis. Of note, evaluation of the effectiveness and functional ramifications of mixture of probiotics need studies on much larger size population (56-59).

Besides the probiotics, the health effect and preventive potential of different form of plants (as food additive, fruits, herbal extracts) against kidney stone have been examined (59). In this review, the efficacy of various plants in the prevention/ management of kidney stone was presented. Extracts obtained from *Bergenia ciliate*, Kampo extracts, and 5 different extracts were assayed via synthetic urine system and cell line studies and their inhibitory effect on nucleation and aggregation of CaOx crystals was reported. In addition, the different plants were examined on the nephrolithiasis rat models (induced by 0.5-3% ethylene glycol) for 21-30 days intervention. The majority of studies confirmed the reduction of excretion and deposition of various urolithiatic parameters, oxalate crystals, inflammatory responses and prevention of renal function impairment. In two studies, the simultaneous administration of herbal extract and probiotics were investigated on rats induced with 1-3% ethylene glycol for 28-30 days. The results showed the reduction of urine oxalic acid, urine calcium, and osteopontin, renal inflammation, and calcium oxalate crystals in shorter time compared to when rats received the herbal extract alone. In addition, the microflora population was increased toward the *O. formigenes, Bacteriodetes, Bifidobacterium* and *Fecalibacterium*, considered as oxalate degrading probiotics. However, limited human studies on the efficacy of herbal extract on management of kidney stone were reported.

CONCLUSION

With the increase in the incidence of nephrolithiasis the need to develop preventive methods using probiotics and herbal extracts is the most emphasized recommendation. In addition, stone-forming patients are advised to use an appropriate diet, hence probiotic bacteria with different forms could be an effective method to degrading oxalate and reducing recurrence of stone formation. The focus of studies has mostly been on probiotic bacteria with oxalate degrading potential as single strain, mixture strains, or recombinant strains. Nonetheless, *O. formigenes* has the highest oxalate-degrading capacity, albeit, with disadvantages including sensitivity to antibiotics, preference for low pH, and conditions under which the probiotic product is formulated. A possible solution to its antibiotic sensitivity is using a probiotic mixture to overcome the intestinal colonization issue.

The results showed the positive effect of multi-strain probiotics (*Lactobacillus, Bifidobacterium*, and *O. formigenes*) on oxalate degradation and changing the intestinal microflora due to high concentration of oxalate in urolithiasis patients and the capacity of probiotics growth in different oxalate concentrations. On the other hand, to our knowledge, the human studies were conducted on limited/small groups and future studies need to be conducted under controlled diet conditions with larger populations. In addition, previously available literature presented the preventive effect of medicinal plants on urolithiasis.

Moreover, the *in vitro* and *in vivo* studies revealed the potential inhibitory effect of the herbal extract on nucleation and aggregation of deposits, reduction of renal inflammation, and urinary parameters. The present systematic review denotes the beneficial effects of probiotics and herbal extracts on kidney stones. Therefore, it is of utmost importance to recommend a beneficial regime based on probiotics, herbal extracts, or a combination of them in patients with kidney stones.

They are also known to have immune-modulatory effects on the host, making them a promising therapeutic and preventive option for a variety of chronic diseases like kidney stones. Probiotics are chosen for their ability to: 1) benefit the host, 2) survive transit through the intestines, 3) adhere to the intestinal epithelial cell membrane, 4) produce antibiotic compounds to fight infections, and 5) stabilize the intestinal microbiota. Because probiotic effects differ based on dose, situation, and strain, understanding the probiotics' genus and species is necessary to accomplish the desired effects on the host.

Evidence in this review, suggests that lactobacilli and bifidobacteria, have all-around positive anti-nephrolithiasis effects. The evidence also suggests that probiotics and herbal extracts detailed in the results and discussion positively impact renal inflammation. It is clear from these findings that prebiotics/probiotics appear to have some benefit in certain circumstances – with limitations. The evidence reflects the benefit of probiotics and herbal extracts in animal and, *in vitro* models extensively, and these

findings were present, but not as obvious from the human studies. The evidence reported in the animal studies describing the positive anti- nephrolithiasis effects of probiotics and herbal extracts is similar to the human studies in establishing, positive changes in cytotoxicity, genotoxicity, release of kidney stone and removed them which impart positive immunomodulatory effects on the patients.

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