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Oral administration of indigenous oxalate degrading lactic acid bacteria and quercetin prevents calcium oxalate stone formation in rats fed with oxalate rich diet

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ABSTRACT

Beneficial effects of lactic acid bacteria (LAB) and quercetin have been used as ingredients of functional foods to promote health and prevention of disease. Dietary oxalate and oxalate mediated oxidative stress are the major predisposing factor for calcium oxalate (CaOx) stone formation. Thus, the efficacy of indigenous oxalate degrading LAB and QE on urinary oxalate excretion, CaOx crystal deposition, antioxidant activity and histopathology were evaluated in rats fed with a potassium oxalate (KOx) diet. The results indicated that LAB and LAB + QE administered rats significantly reduced the urinary oxalate level when compared to KOx fed rats. QE, LAB and QE + LAB supplemented group rats significantly altered the increased lipid peroxidation and antioxidant depletion as compared with rats fed with KOx. Combined effect of QE + LAB supplementation decreased the CaOx aggregation in urine and kidneys than other groups. The rats fed with a combination of QE + LAB significantly altered the expression of CaOx modulator genes (Osteopontin, renin and angiotensin converting enzyme) and antioxidant genes (glutathione peroxidase and superoxide dismutase). The results suggest that the probiotic LAB and QE combination could be used as ingredients of functional food to reduce oxidative stress and prevent CaOx crystal deposition.

1. Introduction

Functional foods are necessary for healthy living and serve as a source of dietary material for mental and physical function and in reducing the risk of specific pathologies by modulating the immune, secretion, nerve, circulating or digestive systems (Kumalasari, Nishi, Harmayani, Raharjo, & Sugahara, 2013). Probiotics and antioxidants are commonly used as functional food ingredients to promote human health. Probiotics

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Abbreviations: CaOx, Calcium Oxalate; LAB, lactic acid bacteria; QE, quercetin; KOx, potassium oxalate; MDA, malondialdehyde; GSH, glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; OPN, osteopontin; ACE, angiotensin converting enzyme

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are defined as selected live microbes used as dietary supplements that are believed to be maintaining intestinal microflora and consequently providing beneficial effects for human health and in disease prevention (Schrezenmeir & de Vrese, 2001). Lactic acid bacteria (LAB) are normal inhabitants of the gastrointestinal tract of humans and animals (Shu et al., 1999) which is widely used as probiotics because of their health promoting properties. A recent report suggested that LAB strains are important functional food ingredients to alleviate allergic symptoms (Takeda et al., 2014). Several evidence confirmed the application of potential probiotic LAB resulted in the prevention of gastrointestinal diseases, type-2 diabetes, and ability to degrade luminal oxalate in the gut (Chen et al., 2014; Fang et al., 2014; Gill & Guarner, 2004).

Hyperoxaluria is an elevated urinary oxalate excretion (Asplin, 2002), resulting from high dietary oxalate intake, intestinal oxalate absorption, fat malabsorption, variations in the intestinal oxalate degrading bacteria and genetic modification of oxalate transporters (Robijn, Hoppe, Vervaet, D'Haese, & Verhulst, 2011). This also induces oxidative stress due to free radical generation, which causes renal tubular injury become a major factor in the development of CaOx stone formation (Huang, Ma, Chen, & Chen, 2002; Khan, 2004; Selvam, 2002). Although many modern therapeutic technologies have emerged for kidney stones, none of the methods have reduced the recurrence rate. Treatment with extracorporeal wave lithotripsy (ESWL) often causes persistent stone residuals, renal damage and still stone recurrence about 50%.

An intestinal oxalate degrading bacterium *Oxalobacter formigenes* is used as an alternative therapeutic approach for the prevention of CaOx stone disease. Studies have demonstrated that the absence of intestinal oxalate degrading bacterium *O. formigenes* is a risk factor in CaOx stone patients (Allison, Dawson, Mayberry, & Foss, 1985; Hatch, Gjymishka, Salido, Allison, & Freel, 2011; Siener, Bangen et al., 2013). However, application of this bacterium as probiotics is restricted due to lack of colonization stability and antibiotics sensitivity (Lange et al., 2012). The detection of *Lactobacillus* as a gut inhabitant and their oxalate degrading potential has attracted urologists to utilize these strains as probiotics for the prevention of hyperoxaluria and kidney stone disease.

Currently, food product enriched with probiotics and plant derived antioxidants are used as ingredients of functional foods have emerged to enhance the health and reduce the risk of chronic disease. The prevention of CaOx stones could be achieved by utilizing potential oxalate degrading bacterial species and oxidative scavenging effect of antioxidants. Since dietary oxalate makes a significant contribution to calcium oxalate stone disease (Holmes, Goodman, & Assimos, 2001), the potential association of *Lactobacillus* to luminal oxalate degradation and urinary oxalate excretion has attracted considerable attention. Several reports have suggested the reduction of urinary oxalate excretion associated with the consumption of *Lactobacillus* species in humans and animals (Kwak et al., 2006; Okombo & Liebman, 2010). In addition, Al-Wahsh, Wu, and Liebman (2012) demonstrated that acute ingestion of VSL#3® freeze dried live LAB with oxalate in individuals, increased the oxalate degradation and significantly reduced the urinary oxalate excretion. A recent report by Siener, Bade, Hesse, and Hoppe (2013) suggested that the effect of LAB preparation Oxadrop was ineffective in hyperoxaluria induced healthy subjects and suggested that the preparation may be altered by selecting efficient oxalate degrading LAB.

Quercetin (QE) is a flavonoid naturally abundant in fruits and vegetables, it is a well-known bioactive flavonoid owing to its antioxidative, anti-inflammatory and cardioprotective properties (Bischoff, 2008; Maciel et al., 2013). Park et al. (2008) reported that quercetin reduced lipid peroxidation in MDCK kidney epithelial cells induced by oxalate and also showed the inhibitory effect on urinary crystal deposition in rats. Thus, the potential oxalate degrading LAB along with antioxidants QE preparation could be promising suitable functional food ingredients for the prevention of CaOx stone disease and recurrent stone formation.

Primarily, the indigenous oxalate degrading LAB strains *Lactobacillus salivarius* AB11 (*L. salivarius* AB11), *Lactobacillus fermentum* TY5 (*L. fermentum* TY5) and *Lactobacillus fermentum* AB1 (*L. fermentum* AB1) were isolated from human faeces and fermented foods and the probiotic potential of these strains were confirmed (Gomathi et al., 2014). Therefore, the present study was intended to evaluate the combined effect of indigenous oxalate degrading LAB and quercetin against CaOx stone formation in rats fed with high oxalate diet.

2. Methods

2.1. Chemicals

Quercetin, nitrobluetetrazolium (NBT), 5, 5′ dithio-bis (2 nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), thiobarbituric acid (TBA), 1,1,3,3, tetraethoxypropane, 1-chloro-2,4-dinitrobenzene (CDNB) and all fine chemicals including Taq polymerase, SYBR® Green Quantitative RT-qPCR Kit, primers were purchased from Sigma Aldrich, St. Louis, MO, USA. Other chemicals and De Man Rogosa media (MRS) were purchased from Himedia, India. The experimental diet containing 5% potassium oxalate (KOx) was purchased from National Institute of Nutrition (NIN), Tarnaka, Jamai-Osmania, Hyderabad, India (Wiessner, Garrett, Hung, Wille, & Mandel, 2011).

2.2. Preparation of LAB mixture

The strains *L. salivarius* AB11 (NCBI Accession No: KF588360), *L. fermentum* TY5 (NCBI Accession No: KF588358), *L. fermentum* AB1 (NCBI Accession No: KF588356) were grown aerobically in MRS medium at 37 °C. The rifampicin resistant (Rif^R) strains were isolated by plating on MRS agar plate containing 100 μg/ml rifampicin and incubated at 37 °C in aerobic environment for 72 h. The Rif^R strains were evaluated for oxalate degrading efficiency in animal model. The rats were administered with the mixture of bacterial strains in a ratio of 1:1:1 approximately 5×10^8 CFU/ml.

2.3. Animals and experimental protocol

A total of 30 male Wistar albino rats weighing 150–180 g from our own breeding colony were divided into five (n = 6) groups.