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Mucoadhesive role of tamarind xyloglucan on inflammation attenuates ulcerative colitis

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Abstract

Tamarind xyloglucan (TXG) is edible, bioavailable and mucoadhesive polysaccharide. The aim of this study was (i) to investigate molecular docking studies on the interaction of TXG to MUC1 and cytokine receptors and (ii) to assess the mucoadhesive role of TXG in UC. *In vivo* study: C57Bl6 mice were administered with DSS 3% (w/v) in drinking water; TXG 100 or 300 mg/kg/day was given orally for 7 days simultaneously. TXG consistently binds to MUC1 and cytokine receptors in molecular docking studies. TXG decreased the expression of MUC1 and MUC2. The mucoadhesive ability of TXG decreased IL-1 β and IL-6 levels. Furthermore, TXG decreased the expression of TLR4, MyD88, I- κ B and NF- κ B thereby attenuating inflammation via TLR4/NF- κ B signaling pathway. TXG mucoadhesion to MUC1 played a pivotal role in attenuating inflammation. To conclude, the mucoadhesive role of TXG is important in the attenuation of inflammation and healing of UC.

Graphical abstract

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The authors declare no conflict of interest.

Keywords

Tamarind xyloglucan; Ulcerative colitis; Mucin 1; Mucoadhesion; Inflammation; Soluble dietary nanofiber

1. Introduction

Inflammatory bowel disease (IBD) was sporadic in Asia until a couple of decades ago, however, in recent years there was a steady increase in the incidence and prevalence in Asia, especially ulcerative colitis (UC) (Hou, El-Serag, & Thirumurthi, 2009). In Asian countries, India has the highest incidence followed by Japan and Korea. The increase in the UC incidence might relate to diet rich in refined sugars, fats, and animal protein and avoiding traditional fiber-rich diets (Prideaux, Kamm, De Cruz, Chan, & Ng, 2012; Cho et al., 2017).

Mucins are high-molecular-weight glycoproteins. Alterations in the mucin synthesis may be the primary event in UC, secondary to inflammation. Mucins are constantly degraded in equilibrium with synthesis (Niv, 2016). Mucin 1 (MUC1) is a cell surface mucin largely expressed in mucosal tissues. MUC1 is increased in experimental DSS-induced acute colitis and plays a role in the injury repair process (Monk et al., 2016). Mucin 2 (MUC2) is a secretory mucin, highly expressed by goblet cells during and after DSS treatment in mice. MUC2 synthesis and secretion are the key functions of goblet cells in epithelial defense against luminal substances and pathogens as a defensive measure and epithelial repair (Renes et al., 2002). Globally an increased mucin expression is observed in UC patients relative to healthy controls (Niv, 2016). MUC1 is a target for small molecule inhibitors (Nath & Mukherjee, 2014). In addition, MUC1 is hypothesized to be a functional analog of cytokine receptors (Zrihan-Licht, Baruch, Elroy-Stein, Keydar, & Wreschner, 1994).

Elevated oxidative stress, inflammation, and loss of gut barrier function are the characteristic features of UC (Davis et al., 2014; Bitzer et al., 2016). Nuclear factor- κ B (NF- κ B) is a major inflammatory pathway downstream of toll like receptor 4 (TLR4). Inhibition of NF- κ B activation is an effective treatment for the prevention of UC (Wei & Feng, 2010; Zhang et al., 2016). Activation of NF- κ B induces the production of inflammatory cytokines, such as TNF- α and IL-1 β in DSS-induced UC (Wang, Fu, Cai, Sinclair, & Li, 2016). However, the exact molecular mechanism and pathophysiology of UC remain unclear.

Dietary fibers are a group of compounds resistant to digestion in the human small intestine and can be categorized into water soluble dietary fiber and water insoluble dietary fiber. Dietary fiber consumption produces a variety of physiologic functions on our health based on the physiochemical properties of the consumed fiber (Naito et al., 2006). Most of the consumed soluble dietary fiber is spontaneously fermented by colonic bacteria to produce organic acids, including short-chain fatty acids (SCFAs). SCFAs promote mucosal blood flow, colonic epithelial cell proliferation, and colonic motility. In specific, butyrate is the key energy source for colonocytes (Venema, Vanhoutvin, Troost, & Brummer, 2008; Cazarin et al., 2016). It has the potential to remove mutated epithelial cells via apoptosis. These cellular functions contribute to the maintenance of colonic homeostasis. In addition, SCFAs are known to involve in the regulation of the intestinal barrier (Suzuki, Yoshida, & Hara, 2008; Cazarin et al., 2016). Animals deprived of fermentable dietary fibers lead to deprivation of short-chain fatty acid energy substrates ultimately leading to thinning of the inner mucus layer of the colon with increased proximity of colonic microbes to the colon epithelium. A shift in the balance of colonic microbial species in favor of increased mucus-consuming and epithelial barrier weakening bacterial species is also found (Glade & Meguid, 2016).

Dextran sodium sulfate (DSS)-induced colitis in mice is known to mimic the morphological and pathophysiologic features observed in human UC. Those include mucosal injury, ulceration, diarrhea, impaired mucus epithelial barrier function, and inflammatory cytokine production (Cooper, Murthy, Shah, & Sedergran, 1993; Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014). Colon barrier function based treatments target reestablishing mucosal barrier integrity in colon and those treatments shows an efficacy in UC therapy (Hwang, Jo, Kim, & Lim, 2017).

Tamarind seeds are the most abundant source of xyloglucan and soluble fiber in nature (Kozioł et al., 2015). Xyloglucans are one of the two major hemicelluloses in plants (Nie & Deters, 2013). TXG is edible, biocompatible, bioavailable and mucoadhesive, highly substituted, food grade and versatile use in foods (Mishra & Malhotra, 2009). It possesses antitumor and immune stimulating activity, healing dry eye syndrome, and cutaneous wounds (Mishra & Malhotra, 2009; Bin Mohamad, Akram, Bero, & Rahman, 2012). It also promotes wound healing, skin regeneration and corneal wound healing (Mishra & Malhotra, 2009; Nie and Deters, 2013). In addition, supramolecular TXG is not digested by the small intestinal enzymes (Sone, Makino, & Misaki, 1992). Thus, it reaches the colon unaltered, later breaks down to oligosaccharides (Hartemink, Van Laere, Mertens, & Rombouts, 1996). The 'mucin-like' molecular structure of TXG is similar to corneal and conjunctival MUC1 (Rolando & Valente, 2007) and known to possess mucomimetic, mucoadhesive and pseudo-plastic properties (Mannucci, Fregona, & Di Gennaro, 2000; Mishra & Malhotra, 2009). Despite mucomimetic, mucoadhesive, wound healing and pseudo-plastic properties of TXG, the mode of action of TXG has never been experimentally demonstrated in DSS-induced UC.

Mucoadhesive TXG may have the potential to protect against UC. IBD therapy is innovative; however, the treatments are mainly focused on modulating inflammation (Vindigni, Zisman, Suskind, & Damman, 2016). Anti-inflammatory agents such as infliximab, adalimumab and certolizumab pegol reduce IBD symptoms. Although anti-TNF-

α agents are successful in treating many patients, remission is found in one third of the IBD cases, and ultimately loosing intestinal function (Hwang, Jo, Kim, & Lim, 2017). Anti-inflammatory drugs such as aminosalicylates, corticosteroids and immunosuppressive agents, are frequently used. However, these agents produce adverse side effects, mainly during long-term therapy, high costs and, in a few cases, unpredictably low response from patients. Therefore, alternative anti-inflammatory agents and dietary supplements are investigated (Sałaga, Zatorski, Sobczak, Chen, & Fichna, 2014). Those agents are often based on natural, plant-derived ingredients, which may be advantageous in IBD therapy (Cho et al., 2017). But, those have not been studied completely. Therefore, there is a need to search for health foods and supplements which are cost effective in nature (Han, Fan, Yao, Yang, & Han, 2017). TXG is a dietary nanofiber and a potential candidate for the research and development into a 'functional food', which is mucomimetic, mucoadhesive with wound healing, and most importantly nontoxic in nature (Mishra & Malhotra, 2009; Periasamy et al., 2018).

We have extracted and structurally characterized TXG as dietary nanofiber (Periasamy et al., 2018). The extraction of TXG yield is $32.17 \pm 1.26\%$ of purified TXG. The scanning electron microscopy of TXG depicts a long fibrous arrangement with substantial interconnection forming a 3-dimensional link. The liquid HR-FE-TEM of TXG shows long branched chain filamentous structures. The core fiber average length is about ~ 950 nm and the branches are ~ 200 to 480 nm. The width of the TXG is found to be 3.77 nm. The FTIR spectral studies reveal the bonding and functional groups of TXG such as carboxyl groups with asymmetrical and symmetrical COO^- , C-C bonded pyranose ring, hydrogen bonded O-H, C-H stretching, anomeric CH of β -galactopyranosyl residues and the skeleton bending of pyranose ring. Thus, TXG possess a β -D-glucose backbone chain with $\beta(1 \rightarrow 4)$ linked glucose units partially substituted with side chains of α -D-xylose at O-6 position by $\alpha(1 \rightarrow 6)$ linkage, these xylose residues are substituted with β -D-galactose at O-2 position $\beta(1 \rightarrow 2)$ glycosidic linkage (Fig. 1).

The gel permeation chromatography (GPC) of TXG reveal the number average molecular weight (M_n) of TXG is 659 kDa, weight average molecular weight (M_w) is 1331 kDa, higher average molecular weights (M_z) is 2145 kDa, peak molecular weight (M_p) is 1149 kDa and polydispersity index is 2.026 . Thus, TXG is a high molecular weight polysaccharide with a weight average molecular weight (M_w) of 1331 kDa (Periasamy et al., 2018). Regardless of voluminous research, the precise cause of UC remains vague. In specific, the aberrant mucus defect is an important cause of UC. TXG nanofiber is a novel antioxidant and plays an important role in attenuating DSS-induced UC in mice (Periasamy et al., 2018). In this study, we employed molecular docking studies to investigate the efficiency of TXG binding to MUC1 and cytokine receptors. We concurrently focused the role of TXG on mucoadhesion, and its modulating effect on inflammation and TLR4/NF- κ B pathway in healing of UC in mice.

2. Materials and methods

2.1. Molecular modeling studies of TXG

The structure of TXG was built using SYBYLX 2.1 (Tripos Associates, St. Louis, MO). Energy minimization was performed using the Tripos force field with Gasteiger-Huckel charges, a fixed minimization was carried out for a maximum of 1500 iterations subject to a termination gradient of 0.05 kcal/(mol, Å). The coordinates of the crystal structure of MUC1 (PDB ID: 2ACM), IL-6R (1N26) and IL-1βR (3O4O) was downloaded from the Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>). Using the protein preparation step in Sybyl, water molecules were removed, hydrogen atoms were added and protonation states were set to the protein molecule. The structure was energy minimized with fixed heavy-atom coordinates using the Tripos force field for 10,000 iterations subject to a termination gradient of 0.05 kcal/(mol, Å). GOLD v5.2 (Jones, Willett, Glen, Leach, & Taylor, 1997) were used for molecular docking experiments. Analysis of the docked poses was carried out using PyMol and Chimera.

2.2. Extraction of TXG

TXG was extracted and purified in three batches on a laboratory scale. Tamarind seeds were procured from a local market in Salem, Tamilnadu. The outer coats of the tamarind seeds were removed by soaking in water for three days to dehull. The white colored seed kernels were dried in shade and milled to powder. Tamarind seed kernel powder (20 g) was added to 200 mL of cold distilled water and made a slurry. The slurry was slowly added to 800 mL of boiling (100–110 °C) distilled water and stirred in a water bath for 20 min. The slurry was allowed to stand overnight, the upper thin clear solution formed. The thin clear upper supernatant was then centrifuged at 5000 rpm for 20 min. On a magnetic stirrer with continuous stirring of the supernatant, added double the volume of absolute ethyl alcohol slowly. Xyloglucan curdles out as precipitates out, which was separated, washed twice with absolute ethyl alcohol, diethyl ether, petroleum ether and dried at 60 °C under vacuum. Xyloglucan was ground, sieved and tested for cytotoxicity using intestinal epithelial cell-6 which showed non-toxic and cytoprotective in nature (Periasamy et al., 2018).

2.3. Animals

Female C57BL/6 mice, aged 7-8 weeks were obtained from and housed in the Institutional Laboratory Animal Center of National Cheng Kung University. Randomly thirty mice were grouped into six groups, housed individually in a room 12-h dark/light cycle and with central air conditioning (25 °C, 70% humidity). They were given Laboratory Autoclavable Diet 5010* from *LabDiet*® (Richmond Standard; PMI Feeds, Inc., St. Louis, MO, USA). Nutrient composition (%) of the feed was protein–24.6, fat (ether extract) –5.0, fat (acid hydrolysis) –6.4, fiber (crude) –4.2, carbohydrates (Nitrogen-free extract) –50, total digestible nutrients–74.8, minerals–6.1 and vitamins–2610.7 (ppm) and water *ad libitum*. The animal studies are done according to ethical procedures and experimental protocols were approved in accordance with nationally approved guidelines for “The Institutional Animal Care and Use Committee” (IACUC No.104068).

2.4. Animal experimentation

Experimental UC was induced by administration of DSS (average molecular weight, 35,000–50,000 Da) 3% (w/v) in drinking water for 7 days. Mice were divided into five groups of six: Control group (CTL) mice were given only saline orally and free access to drinking; DSS group (DSS) mice were given only DSS (3% w/v) in drinking water for 7 days; DX1 and DX2 group, mice were given 2% TXG (150 or 300 mg/kg) orally with DSS (3% W/V) in drinking water for 7 days; X2 group, mice were given only 2% TXG (300 mg/kg) orally and free access to drinking water. Colon tissue from each mouse was harvested for histopathological, immunofluorescence studies, inflammatory cytokines and protein expressions were assessed.

2.5. Sample collection

At the end of the experiments, the animals were sacrificed. Colon tissue was collected and kept on ice. The colons were excised, cut open longitudinally washed using isotonic solution. One portion of each distal colon was cut and fixed in 10% formalin for histopathological and immunostaining examination; the remaining was used for inflammatory cytokines and protein analysis.

2.6. Staining neutral and acidic mucins

The colon tissue expression of the neutral and acidic mucins was determined individually using the modified histochemical technique of Periodic Acid-Schiff (PAS) staining and Alcian blue-PAS staining. The tissue sections were deparaffinized, rehydrated, and stained in Alcian blue solution for 15 min, rinsed and PAS-stained using the standard protocol using Schiff's reagent for 10 min; and then counterstained using hematoxylin for 30 sec. The sections were dehydrated, cleared, and mounted using DPX. The stained sections were observed under a microscope and photomicrographs were taken (OLYMPUS BX51).

2.7. Measurements of IL-1 β and IL-6

A 96-well immunoassay plate was coated with 100 μ L/well capture antibody overnight at room temperature, followed by a blocking step. Recombinant cytokines ranging from 6.25 to 4000 pg/mL were used as standards. One hundred μ L of sample and serial standards diluted in sample buffer (PBS containing 1% (w/v) BSA, pH7.6) were incubated at room temperature for 2h. The samples and standards were incubated with 100 μ L of biotinylated rabbit anti-mouse IL-1 β and IL-6 antibody, streptavidine-conjugated reaction was initiated by the addition of 100 μ L of TMB for 30 min, and then stopped by adding 50 μ L of 0.5 M H₂SO₄. The absorbance was measured at 450 nm with ELISA reader.

2.8. Immunofluorescence

Tissue sections were deparaffinized, rehydrated, through a series of graded ethanol (100, 95, 70, 50, and 30%) and water, followed by an antigen retrieval step by heating the sections in microwave apparatus using a glass container containing 10 mM sodium citrate buffer, pH 6.0 for 10 min. Sections were then cooled at room temperature, incubated in blocking solution (3% bovine serum albumin (BSA) with 0.2% Tween 20 in PBS for 1 h, followed by incubation overnight at 4 °C with primary antibodies: MUC1 (1:400 dilution, Abcam),

MUC2 (1:200 dilution, Abcam), iNOS (1:200 dilution, BD Bioscience), p47^{PHOX} (1:500 dilution, Santa Cruz), TRL4 (1:200 dilution, Santa Cruz) and NF- κ B (1:100 dilution, EMD Millipore) diluted in 3% blocking solution. Next day, sections were washed and incubated with secondary antibody conjugated to Alexa Fluor 488 (1:400 dilution, Abcam) and/or Alexa Fluor 647 (1:400 dilution, Abcam) for 1 h at room temperature in the dark chamber. The sections were washed in PBS and rinsed in distilled water and mounted using Fluoroshield mounting medium with 4', 6-diamidino-2-phenylindole (DAPI) (Abcam). Images were captured with a microscope (OLYMPUS BX51) under fluorescence setup with appropriate filters.

2.9. Western blot Analysis

Cytoplasmic protein was extracted from the tissues by homogenizing in 200 μ L of protein lysis buffer and then kept on ice for 30 min. After centrifugation (12000 rpm, 30 min), the protein concentration in the supernatant was determined by the protein assay dye (Bio-Rad Laboratory, Hercules, CA, USA) using BSA as the standard. Twenty to fifty μ g protein was separated by electrophoresis in 10% SDS-PAGE, and transferred to nitrocellulose sheets (NEN Life Science Products, Inc, Boston, MA, USA) with a Western blot apparatus (Bio-Rad Laboratories, Hercules, CA, USA) run at 1.2 A for 2 h. After blocking in 5% non-fat skim milk, blots were incubated with rabbit anti-Muc1 (1:1000), rabbit anti-TLR4 (1:1000), rabbit anti-MyD88 (1:1000), rabbit anti-NF- κ B (1:1000), rabbit anti-p-I κ B α (1:1000), rabbit anti-GAPDH (1:1500), mouse anti- β -tubulin (1:1500) antibodies. Following washing with TBST, blots were incubated with anti-mice or rabbit IgG conjugated with alkaline phosphatase (dilution 1:3000) (Jackson ImmunoResearch Laboratories, Inc., Philadelphia, PA, USA). Immunoblots were developed using BCIP/NBT solution (Kirkegard & Perry Laboratories, Inc., Baltimore, MD, USA).

2.10. Statistical analysis

Data were expressed as mean $\pm$ SD. Comparison between groups was made by using SPSS statistical software (SPSS, USA) or Microsoft Excel (Microsoft, USA). Significant differences of measurement traits were analyzed using one-way analysis of variance (ANOVA) followed by Tukey honestly significant difference (HSD) post hoc or Student's *t*-test analysis. The significance was set at $P < 0.05$.

3. Results

3.1. Molecular docking of TXG to MUC1 and to cytokine receptors IL-6R and IL-1 β R

Molecular docking of TXG to MUC1 was performed with GOLD v5.2. Electrostatic potential of the protein molecule (shown in Fig. 2a (i)) was calculated using APBS tool in PyMol for the identification of the most potential binding site where the ligand (TXG) can bind and interact with the target protein MUC1. Using the identified potential binding sites, a radii of 20 Å was set for molecular docking. Other parameters in GOLD were left at default during the docking runs. The docking was performed using no speed-up and a genetic algorithmic search with 100,000 iterations for each 300 GA runs.

In the case of TXG the number of rotatable bonds is 48, which is difficult for most of the molecular docking packages to handle. So both rigid and flexible docking of the ligand was done. The GOLDScore for both runs were 35.6 and 54.3, respectively. The GOLDScore fitness function is the scoring function provided with GOLD. It has been optimized for the prediction of ligand binding positions and takes into account of factors such as hydrogen bonding energy, van der Waals energy, metal interaction and ligand torsion strain. The best sampled docked pose with higher GOLDScore from flexible docking (Fig. 2a (ii)) was taken for further analysis. The hydrogen bond interactions were calculated using Chimera. Residues involved in the hydrogen bond interactions are Gln1070, Arg1071, Leu1089, Ile1092, Lys1093, Arg1095 and Leu1103 (Fig. 2a (iii)).

MUC1 is hypothesized to be a functional analog of cytokine receptors. Based on the above fact, in addition to MUC1, we studied the molecular docking of TXG to IL-6R and IL-1 β R. The same method as described above was used for molecular docking. From molecular docking experiments we identified that TXG bound to IL-6R and IL-1 β R with a GOLDScore of 67.51 and 61.64. The electrostatic potential, docked poses of TXG to IL-6R and IL-1 β R and hydrogen bond interactions are shown in Fig. 2b and Fig. 2c, respectively. Amino acid residues involved in the hydrogen bond interactions between TXG to IL-6R were Lys244, Ser243, Arg237, Thr248, Glu286 and Arg274 (Fig. 2b (iii)). TXG to IL-1 β R were Gln127, Pro126, Tyr125, Ile123, Arg24, Phe26, Lys23, Glu25 and Glu144 (Fig. 2c (iii)).

3.2. TXG protected mucin barrier of colon in the DSS-induced ulcerative colitis model

To examine the protective effect of TXG on DSS-induced mucin barrier injury, the neutral and acidic mucin staining along with expression of MUC1 and MUC2 were assessed. PAS and Alcian blue-PAS staining revealed a significant increase in the both neutral and acidic mucin in DSS group relative to the CTL group. However, the colon section illustrated significant decrease in the both neutral and acidic mucin in DX1 and DX2 groups compared to DSS group. Further, analysis revealed a concomitant decrease in acidic mucin in most of the section relative to neutral mucins. CTL and X2 groups showed no change in the staining of PAS and Alcian blue-PAS (Fig. 3).

The expression and immunofluorescence of MUC1 were significantly increased in the DSS group compared with those in the CTL group. In CTL group, MUC1 immunofluorescence studies revealed expression only on the mucosal villi, because MUC1 is a membrane-associated type of mucin. However, in DSS group the MUC1 expression was found all over the colon, including lamina propria mucosa, glandular epithelium, and the crypt. In DX1 and DX2, the expression of MUC1 (Fig. 4a) and immunofluorescence (Fig. 4b) were significantly decreased dose dependently. MUC2 is the secretory type mucin, the immunofluorescence of MUC2 were significantly increased in the DSS group compared with those in the CTL group. In DSS group the MUC2 immunofluorescence studies showed significant expression only above the surface of the mucosal villi, indicating MUC2 is a secretory type of mucin. In DX1 and DX2, the expression of MUC2 was significantly decreased (Fig. 5).

3.3. TXG inhibited pro-inflammatory cytokine production in the DSS-induced ulcerative colitis model

The effect of TXG on DSS-induced IL-1 β and IL-6 in colon tissue were assessed. The DSS group showed a significant increase in the level of IL-1 β (Fig. 6a) and IL-6 (Fig. 6b) compared with CTL group. In DX1 and DX2 groups, the level of IL-1 β and IL-6 were significantly decreased compared to DSS group, indicating a decrease in the inflammation. No significant alterations in the level of pro- and anti-inflammatory cytokines were observed between CTL and X2.

3.4. TXG suppressed the activation of the TLR4/NF- κ B signaling pathway in the DSS-induced ulcerative colitis model

To elucidate the possible mechanism relating to the protective effects of TXG against ulcerative colitis, we studied the activation of the TLR4/NF- κ B pathway. The expression of TLR4, p-I κ B, MyD88, and NF- κ B p65 subunit were significantly increased in DSS group compared to CTL group. However, TLR4, MyD88, p-I κ B, NF- κ B p65 subunit levels were significantly decreased in DX1 and DX2 groups. No significant difference between CTL and X2 in the proteins of TLR4/NF- κ B signaling pathway (Fig. 7). Immunofluorescence studies on TLR4 and NF- κ B revealed a significant increase in DSS group compared to CTL group. In DX1 and DX2, the expression of TLR4 and NF- κ B were significantly decreased relative to DSS alone group (Fig. 8).

4. Discussion

The mucoadhesion of TXG nanofiber played an important role in the attenuation of DSS-induced UC. In spite of advances in treatment of IBD, few IBD cases do not respond to therapies by which cause major adverse side effects and complications. Therefore, studies are focusing on the use of dietary supplements and natural products as alternative treatment for patients who are unresponsive to, or unwilling for standard routine medications (Sun et al., 2016). In the present study, molecular docking studies revealed consistent binding of TXG with MUC1 and cytokine receptors IL-6R and IL-1 β R. Therefore, TXG binding to TXG with MUC1, IL-6R and IL-1 β R might play a pivotal role in decreasing the inflammation, thereby enhancing the healing of UC. *In vivo* studies revealed oral feeding of TXG decreased the expression of MUC1 and MUC2. In addition, it reduced IL-1 β and IL-6. Furthermore, it inhibited the expression of TLR4, MyD88, p-I κ B, and NF- κ B in DSS-induced UC.

Molecular docking studies of MUC1 and TXG revealed that TXG bound consistently to MUC1 indicating TXG was mucoadhesive in nature. TXG possesses good mucoadhesive properties as it has a backbone chain with branching xylose and galactoxylose substituent which imparts its mucin like configuration (Madgulkar et al., 2016). To be more specific the 'mucin-like' structure of TXG is similar to corneal and conjunctival MUC1. In addition, it is also known to possess mucomimetic, mucoadhesive and pseudo-plastic properties (Mishra & Malhotra, 2009). MUC1 is a target for small molecule inhibitors (Nath & Mukherjee, 2014). In addition, MUC1 is hypothesized to be a functional analog of cytokine receptors (Zrihan-Licht, Baruch, Elroy-Stein, Keydar, & Wreschner, 1994). Based on the above fact, we

studied the molecular docking of TXG to MUC1, IL-6R and IL-1 β R. In both rigid and flexible docking runs the ligand (TXG) took the same binding center in MUC1. In rigid docking, the TXG was more constrained, whereas in flexible docking the TXG was extended. The hydrogen bond interactions were calculated using Chimera. Residues involved in the hydrogen bond interactions are Gln1070, Arg1071, Leu1089, Ile1092, Lys1093, Arg1095 and Leu1103 of MUC1. TXG was found to bind MUC1 consistently via hydrogen bond interactions.

TXG nanofiber attenuated the MUC1 and MUC2 expression in DSS-induced ulcerative colitis. DSS administration caused aberrant expression of MUC1 and MUC2 in the colon. This increased expression of MUC1 and MUC2 might be the host response to the colonic injury by DSS. The exact mechanism of induction of colitis by DSS is unknown. The possible mechanism of action might be the alteration of gut permeability (Arrieta, Bistritz, & Meddings, 2006). In addition, the toxicity of DSS affects the integrity of the mucosal barrier through gut epithelial cells of the basal crypts (Kong et al., 2008). Secretion of mucin involves two pathways, e.g. basal and stimulated secretion in response to toxins, pro-inflammatory cytokines, and growth factors causing inflammation in the colonic mucosa leading to the alterations in the expression of mucins.

MUC1, membrane-associated mucin expressed in the apical membrane of goblet cells in the colon, is responsible for epithelial restitution (Dorofeyev, Vasilenko, Rassokhina, & Kondratiuk, 2013). MUC1 offers protective and lubricates epithelial cells. Its cytoplasmic tail is an active pivot point for multiple signaling interactions and functions (Singh & Hollingsworth, 2006; Mehla & Singh, 2014). MUC2, secretory glycoprotein is expressed by crypt and goblet cells in both the proximal and distal colon. It forms a gel-like mucus layer and serves as a wall to shield the epithelium from mechanical stress, noxious agents and microbes. MUC2 expression and secretion are increased during DSS-induced ulcerative colitis (Renes et al., 2002). In the present study, TXG feeding significantly decreased the overall mucins. In addition, it decreased the expression of MUC1 indicating that TXG attaches to MUC1 thereby aiding in epithelial restitution of the colonic mucosa and attenuating further expression and secretion of MUC1. It ultimately led to the decreased MUC2 expression indicating the healing of inflamed colon. Thus, we suggest that TXG protects and repairs the mucous barrier and helps healing colon.

TXG nanofiber decreased pro-inflammatory cytokine IL-1 β and IL-6 release in DSS-induced UC. MUC1 expression is also induced by inflammatory cytokines, including TNF- α and IL-6 (Ahmad et al., 2009). MUC1 is hypothesized to be a functional analog of cytokine receptors (Zrihan-Licht, Baruch, Elroy-Stein, Keydar, & Wreschner, 1994). Pro-inflammatory mediators, including cytokines and neutrophil infiltration have been implicated in the pathogenesis of UC. In DSS-induced UC, crypt loss and increased permeability of the colon usually precede inflammation (Cooper Murthy, Shah, & Sedergran, 1993). In the DSS induced UC, during the course of inflammation, neutrophils accumulate in epithelial crypts and stimulate pro-inflammatory cytokines such as IL-1 β and IL-6 (Yang et al., 2016). As MUC1 functional analog of cytokine receptors, there is a possibility of TXG binding to cytokine receptors, thereby attenuating the release of cytokines and decreasing the further inflammatory process.

TXG nanofiber decreased the expression of TLR4, MyD88, I- κ B, and NF- κ B in DSS-induced UC. MUC1 might play a role in cellular signaling and prominent sensor mechanism in response to damage of epithelia and enhance proliferative signaling (Zrihan-Licht, Baruch, Elroy-Stein, Keydar, & Wreschner, 1994; Carraway, Ramsauer, Haq, & Carothers Carraway, 2003). MUC1 joins in outside-in signaling, sensing the extracellular environment and reprogramming the transcription of cells in response to the extracellular signals (Mehla & Singh, 2014). MUC1 cytoplasmic tail has been associated with the activation of NF- κ B, thus increasing the expression of pro-inflammatory cytokines (Ahmad et al., 2009; Macha et al., 2015). In addition, it signals increase expression of COX-2 that converts arachidonic acid to prostaglandin, a strong immune modulator and immune suppressor thereby favoring inflammation (Macha et al., 2015).

The processes of TLR4/NF- κ B activation include several key links, such as MyD88 availability, phosphorylation, degradation of I- κ B and the subsequent nuclear translocation of NF- κ B (Fitzgerald et al., 2001). TLR4 functions as a mediator of inflammation and blockage of TLR4 ameliorates DSS-induced colitis (Zhang et al., 2014). Activation of NF- κ B plays a key role in the colon inflammation of IBD. Thus, the TLR4/NF- κ B pathway has become a major target for the therapy of inflammatory diseases, including UC (Yang et al., 2016). TXG attenuated the expression of proteins involved in the TLR4/NF- κ B signaling pathway. TXG might bind to MUC1 thereby blocking the signal for the activation of NF- κ B signaling ultimately inhibits the transcription of genes for inflammatory cytokines ultimately attenuating inflammation and aid colon healing. Therefore, TXG might be involved in the attenuation of secondary inflammation through the activation of TLR4/NF- κ B signaling pathway in UC.

The limitations of the present study are the effect of TXG dietary nanofiber fermentation by the microbes in the colon may produce short-chain fatty acids (SCFAs) mainly acetate, propionate, and butyrate. These SCFAs are the essential energy source for colon cells, maintains homeostasis and preserves mucosal integrity. TXG may improve or alter the population of prebiotics, those involved in the production of SCFAs and their role in mucosal barrier have to be studied. Therefore, we planned our future investigations are to elucidate the role of TXG involvement in colon microbes and their role mucosal barrier parameters, and junctional complexes. Thus, TXG binding to MUC1 might lead to the reduction of MUC1 and MUC2 expression and promote healing; ultimately modulating inflammation via TLR4/NF- κ B signaling pathway subsequently attenuating DSS-induced UC.

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Highlights

- Molecular docking studies revealed consistent binding of tamarind xyloglucan (TXG) to Mucin1, a functional analogue of cytokine receptors.
- TXG attenuated inflammation via mucoadhesion by decreasing cytokines.
- TXG attenuated inflammation via TLR4/NF- κ B signaling pathway in UC model

Fig. 1.

Representative scientific name and chemical structure of TXG. β -D-glucose backbone chain with $\beta(1 \rightarrow 4)$ linked glucose units partially substituted with side chains of α -D-xylose at O-6 position by $\alpha(1 \rightarrow 6)$ linkage, these xylose residues are substituted with β -D-galactose at O-2 position $\beta(1 \rightarrow 2)$ glycosidic linkage. Glu-glucose, Xyl-xylose, Gal-galactose and *p*-pyranosyl residues.

Fig. 2.

Molecular docking of (a) MUC1 to TXG: (i) MUC1 electrostatics, (ii) Docked pose of MUC-1 to TXG, (iii) Hydrogen bond interaction of MUC-1-TXG. (b) IL-6R to TXG (i) IL-6R electrostatics, (ii) Docked pose of IL-6R to TXG, (iii) Hydrogen bond interaction of IL-6R to TXG. (c) IL-1 β R to TXG (i) IL-1 β R electrostatics, (ii) Docked pose of IL-1 β R to TXG, (iii) Hydrogen interaction of IL-1 β R to TXG. Electrostatic potential was calculated using the APBS plugin in PyMOL. Protein molecules are shown both in cartoon and surface representation. Structure of TXG is shown as sticks (blue color by atom). The hydrogen bond interactions are shown as dotted lines (black).

Fig. 3.

Effect of oral TXG on DSS-induced UC mouse model. Colitis was induced by administration of DSS in drinking water for 7 days. Control group (CTL), gavaged saline and free access to drinking water. Colitis group, DSS in drinking water (DSS); DX1 and DX2 group, mice were given TXG (150 or 300 mg/kg, p.o.) for 7 days with DSS in drinking water; X2 group, mice was given TXG (300 mg/kg, p.o.) for 7 days with tap water. Effect of TXG on mucin analyzed by PAS and Alcian blue-PAS staining in DSS-induced UC mouse model. Neutral mucin (magenta color), acidic mucin (blue color) (n = 6 per group).

Fig. 4. Effect of TXG on (a) MUC1 expression (b) MUC1 immunofluorescence in DSS-induced UC mouse model. MUC1 (green color), DAPI nuclear stain (blue color). Data expressed as means $\pm$ SD. ^{a,b,c} The differences between treatments with different letters are significant ($P < 0.05$). (See groups and treatment details in the Figure 2 legend).

Fig. 5. Effect of TXG on MUC2 expression in DSS-induced UC mouse model. MUC2 (green color), DAPI nuclear stain (blue color). (See groups and treatment details in the Figure 2 legend).

Fig. 6. Effect of TXG on cytokine levels in DSS-induced UC mouse model. (a) IL-1 β , (b) IL-6 (c) TNF- α and (d) IL-10. Data expressed as means $\pm$ SD. ^{a,b,c} The differences between treatments with different letters are significant ($P < 0.05$). (See groups and treatment details in the Figure 2 legend)

Fig. 7.

Effect of TXG on the expression of protein involved in TLR4/NF-κB signaling pathway in DSS-induced UC mouse model. (a) TLR4 expression, (b) MyD88 expression, (c) I-κB expression, and (d) NF-κB expression. Data expressed as means $\pm$ SD. ^{a,b,c} The differences between treatments with different letters are significant ($P < 0.05$). (See groups and treatment details in the Figure 2 legend)

Fig. 8. Effect of TXG on TRL4-NF-κB expression in DSS-induced UC mouse model. TRL4-NF-κB expression by double immunofluorescence staining. TRL4 (green color), NF-κB (red color), DAPI nuclear stain (blue color). (See groups and treatment details in the Figure 2 legend).