Pharmacological Properties of Fructooligosaccharides Modulates the Lipopolysaccharide-Induced Gastrointestinal Tract Inflammation in Mice

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Abstract

Objective: The gut is a neuroendocrine-immune organ, vulnerable to stress, and toxic agents, including lipopolysaccharide (LPS) leading to gut dysbiosis and inflammation. The aim of present study was to evaluate the pharmacological properties of prebiotic fructooligosaccharides (FOS) against the LPS-induced gut inflammation in mice.

Materials and Methods: The Swiss albino mice (female, 8 weeks) were divided into following four groups (n = 6/group): Group-I/Control: received saline (0.9% NaCl), (II) Group-II/LPS (1 mg/kg for 5 days, intraperitoneal), Group-III/LPS+FOS (LPS 1 mg/kg for 5 days followed by FOS 2 g/kg for 28 days), and Group-IV/FOS (FOS 2 g/kg for 28 days, through oral gavaging).

Results: The LPS exposure significantly decreased the body and gut weight compared to control which, after the FOS treatment, increased to control level. In LPS-exposed mice, the decreased of gut associated superoxide dismutase and catalase activity was enhanced and normalized by FOS. Similarly, LPS-induced the pro-inflammatory cytokines IL-6 and TNF-α level were also decreased to control level after FOS treatment. Moreover, LPS exposure caused various histopathological alterations in gut, such as lesions of epithelial layer, edema of villi, and disruption of goblet cells, in which FOS modulated.

Conclusion: The pharmacological prebiotic FOS shows the anti-oxidative, anti-inflammatory properties which modulated the LPS-induced gut toxicity by decreasing inflammation and oxidative stress and improving histological architecture.

Key words: Fructooligosaccharides, gut toxicity, inflammation, lipopolysaccharide, oxidative stress

INTRODUCTION

The gastrointestinal (GI) tract/gut is considered the neuroendocrine-immune organ consisting of an enteric nervous system, enteroendocrine cells, and GALT, an immune component. The gut mucosal epithelium contains the endocrine and immune cells, which maintain the integrity of the epithelial barrier and gut homeostasis. The GI tract is susceptible to various extraneous toxicants and pathogens, for example, bacteria, viruses, and parasites, that compromise gut epithelial barrier integrity. Under normal circumstances, the GI tract maintains a homeostatic population of a large variety of gut microbiota (GM), which are either beneficial (e.g., Bifidobacterium, Lactobacillus, etc.) or harmful (e.g., Clostridium, Shigella, etc.) to the host’s health. Some recent studies reported that several factors, such as diet, xenobiotic, and overuse of antibiotics, alter the composition of GM, leading to an increase in harmful gut microbial components, including lipopolysaccharide (LPS). LPS is a toxic component of the bacterial cell wall (e.g., Escherichia coli) and acts on various immune cells, such as macrophages and mucosal epithelial cells. In homeostatic conditions, the LPS concentration in the gut and systemic circulation remains low. The increased LPS level triggers inflammatory response cascades to increase pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and induce oxidative free radicals such as superoxide, nitric oxides, etc. Subsequently, these inflammatory mediators impair the epithelial barrier allowing the permeability of toxic substances or pathogens to enhance gut leakage, inflammation, and dysbiosis.

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Several studies reported that the intake of prebiotics has beneficial effects on intestinal homeostasis in animals and humans. Microbiota fermentation of prebiotics produces short-chain fatty acids (SCFAs), stimulating the growth of beneficial bacteria. Prebiotics are non-digestible oligosaccharides that maintain the homeostasis and diversity of the bacterial population in the GI tract. The most widely used prebiotics having health benefits in humans are fructooligosaccharides (FOS) and galactooligosaccharides. The presence of these compounds contributes to the integrity of the gut mucosal barrier and has great potential for improving gut health.

The FOS is a low molecular weight oligosaccharide, made up short fructose chain, and naturally present in many plants such as chicory, onion, blue agave, and garlic. Moreover, a few clinical studies have suggested the anti-inflammatory and anti-oxidative properties roles of FOS, but their mechanism still needs to be better understood. Therefore, the present study hypothesized that the FOS might protect the inflammation and oxidative stress of the gut. The present study aimed to elucidate the therapeutic property of prebiotic FOS against mice’s LPS-induced gut toxicity (inflammation and oxidative stress).

**MATERIALS AND METHODS**

**Drugs and chemicals**

The LPS (E. coli serotype 026: B6, L-2654), FOS (Code: F8052), and ELISA Kits (IL-6: Code: RAB0308; TNF-α: Code: RAB0477) were purchased from Sigma-Aldrich (St. Louis, USA). Chemicals for oxidative stress such as nitro blue tetrazolium chloride (NBT) (Code: MB107), L-methionine (Code: GRM200), riboflavin (Code: CMS181), and coomassie brilliant blue (Code: MB092) were purchased from HiMedia (Mumbai, INDIA).

**Animals and experimental design**

Swiss albino female mice (8 weeks old; Body weight: 22 ± 3 g) procured from the Indian Institute of Toxicology Research, Lucknow, India, and housed in polypropylene cages with 12/12 light-dark cycles at an ambient temperature (23 ± 2°C) and humidity (55 ± 5%) and acclimatized for 2 weeks. Supplied the food and water were ad libitum. After acclimatization, the following four groups of mice were maintained for experimentation (six mice per group, Figure 1).

- **Group-I (Control):** Treated with saline (0.9% NaCl) for 5 days.
- **Group-II (LPS):** Given LPS (1 mg/kg) intraperitoneally (i.p.) for 5 days.
- **Group-III (LPS+FOS):** Exposed to LPS (1 mg/kg for 5 days), after that, FOS (2 g/kg) for 4 weeks through oral gavaging.
- **Group-IV (FOS):** FOS (2 g/kg) for 4 weeks through orally gavaging.

Mice were sacrificed using anesthesia pentobarbital (100 mg/kg) at the end of experiments. The exposure dose of FOS (w/v) is equivalent to the rat oral dose.

**Study of gut oxidative stress**

**Superoxide dismutase (SOD) and CAT activity**

The activity of SOD was measured following the method of Beauchamp and Fridovich with certain modifications. In brief, 10% homogenate of gut tissue was prepared in 0.05 M potassium phosphate buffer (PPB, pH 7.4), followed by centrifugation at 10,000 rpm for 15 min. The 100 µL tissue supernatant was mixed with 900 µL of reaction mixture containing 0.05 M PPB, 0.1 M methionine, 0.1 M Ethylenediaminetetraacetic acid (EDTA), 0.45 M NBT, and 0.01 M riboflavin and incubated in light condition for 1 h. The sample’s optical density (OD) was measured by spectrophotometer against a reference blank at 560 nm. One unit of SOD activity (unit/mg protein) was calculated as the amount of enzyme inhibited by 50% of NBT. The Bradford method was applied to measure protein content in gut tissue.

The CAT activity was studied according to the method of Cohen et al. and Aebi. In brief, 10% homogenate of gut tissue was prepared in 0.05 M PPB (pH 7.4) and centrifuged at 10,000 rpm for 15–20 min. 500 µL supernatant mixed with 5 µL ethanol and kept in ice for 30 min. After this, 450 µL of this aliquot was mixed with 50 µL Tritan-X-100, vortexed, and from this sample, 100 µL taken in a cuvette and mixed 1.4 mL of 13 mM H₂O₂. OD was measured at 240 nm for 1 min with the help of a UV–VIS spectrophotometer (Shimadzu, UV-1800 pharma spec), using extinction coefficient of H₂O₂ (43.6 M⁻¹cm⁻¹) and is expressed as unit/mg of protein.

**Figure 1:** Schematic representation of the experimental design
Measurement of inflammatory cytokines (IL-6 and TNF-α)

Blood samples were immediately collected from the abdominal aorta of anesthetized mice in 0.1% EDTA treated vials, centrifuged at 2500 rpm for 15 min, and kept plasma at −20°C until assay. Plasma IL-6 and TNF-α in duplicate were measured using a commercially available ELISA kit (Sigma-Aldrich, USA). The intra-assay and inter-assay coefficient variation for IL-6 and TNF-α were <10% and 12%, respectively.

Histopathology of GI tract

Histopathology of the GI tract (Jejuno-ileum and Colon) was studied by hematoxylin and eosin (H&E) staining. In brief, tissues were quickly dissected, washed, and weighed. The 4% paraformaldehyde was used for the fixation of gut tissue. After overnight fixation, tissues were thoroughly cleaned, dehydrated through graded alcohols (50%, 70%, 90%, and 100%), and embedded in paraffin wax. Sections of 8 µm thickness were cut and stretched on albumin-coated glass slides. After deparaffinization in xylene and rehydrated in water through graded series of alcohols, gut tissue was stained with H&E. Photomicrography was done by light microscope (Leica DM 2500, Germany). The morphometric analysis of various parameters of gut histology was done using ImageJ 1.32 image analysis software (NIH, Bethesda, USA). The density of enterocytes in villi and goblet cells in the colon was counted in a selected counting frame of 100 × 100 µm (10000 µm²) area. The goblet cell size in the colon was measured at 30 cells from each section (10 sections from each animal).

Statistical analysis

All values were represented in mean ± SD using GraphPad Prism 5 software and by one-way analysis of variance (ANOVA). Tukey’s post hoc test was used further to determine the significant level at *P < 0.05, **P < 0.01, and ***P < 0.001.

RESULTS

Effect of LPS and FOS on body and gut-weight

In one-way ANOVA, a significant effect was observed on body weight (F (3, 111) = 22.36, P < 0.001) and gut-weight (F (3, 23) = 118.1, P < 0.001) in the experimental groups compared to the control. The body weight was significantly decreased in LPS (P < 0.001) and FOS co-treated (LPS+FOS; P < 0.01) mice as compared to control but substantially increased in both the FOS co-treated (LPS+FOS; P < 0.01) as well as in only FOS supplemented (FOS: P < 0.001) mice as compared to LPS-challenged mice. The gut weight was significantly decreased in mice exposed to LPS (P < 0.001) than the control. Supplementation of FOS substantially increased the gut weight in LPS+FOS (P < 0.001) and FOS (P < 0.001) exposed mice compared with the LPS treatment group to make that equivalent to the control level [Figure 2].

Effect of LPS and FOS on SOD and CAT activity

In one-way ANOVA, the treated mice showed a significant effect on SOD (F (3, 23) = 9.15, P < 0.001) and CAT (F (3, 23) = 7.29, P < 0.01) activity of the gut. Administration of LPS to mice significantly reduced the SOD and CAT activities (P < 0.001 for both) in the GI tract compared to the control group. Both SOD and CAT activities of gut tissue increased on FOS supplementation (LPS + FOS) compared to LPS, but not significant. In only the FOS group, the activity of SOD was increased significantly (P < 0.01) [Figure 3].

Effect of LPS and FOS on IL-6 and TNF-α

The effect of LPS exposure and FOS treatment on plasma cytokines IL-6 and TNF-α level is shown in Figure 3. The one-way ANOVA analysis revealed substantial changes in IL-6 (F (3, 15) = 57.20, P < 0.001) and TNF-α level (F (3, 15) = 75.97, P < 0.001) in the experimental groups compared to the control. Tukey’s post hoc analysis further showed high levels of both IL-6 and TNF-α in the LPS (P < 0.001) as well as in LPS+FOS (P < 0.05) treated groups compared to the control. As compared to LPS, plasma levels of both IL-6 and TNF-α were significantly less (P < 0.001 for both) in FOS co-treated (LPS+FOS) mice. In only FOS mice, plasma levels of IL-6 and TNF-α remained equivalent to the control.

Histopathological evaluation of GI tract

The result of gut histopathological changes (Jejuno-ileum and Colon) is shown in Figures 4 and 5. In one-way ANOVA, the significant changes in the density of enterocytes (F (3, 23) = 6.44, P < 0.01) in the jejunum were observed in treatment groups. In control, all the layers, including epithelium, lamina propria (LP), submucosa, and muscularis externa (ME) of the intestinal mucosa, were integrated, smooth, and healthy. In LPS-exposed mice, the epithelial barrier becomes damaged, and LP was condensed, exhibiting migration of immune cells to inflammatory regions. In addition, disrupted mucosal goblet cells and mucin deposition to the extent of epithelial cells were also observed. Crypt architecture was also distorted, indicating severe inflammation [Figure 4a-d]. In LPS-exposed mice, the damage of the epithelial barrier exhibited reduced enterocyte density (P < 0.01) compared to the control. Compared to the LPS-treated group, enterocyte density was more both in LPS+FOS and only FOS-treated groups, significantly (P < 0.05) later [Figure 5a].
The colon of LPS-induced mice also revealed similar kinds of histopathological aberrations as found in jejunum-ileum. The lesion in the mucosal epithelium, goblet cell disruption, migration of inflammatory cells, and condensation of LP was observed [Figure 4e-h]. In one-way ANOVA, significant changes in goblet cell density (F (3, 55) = 5.86, $P < 0.01$) and size (F (3, 119) = 3.46, $P < 0.05$) in the colon were observed in treatment groups. The density of the goblet cells was significantly decreased ($P < 0.01$), but not the size in the LPS group. In the cotreated (LPS+FOS) group, goblet cells number/density and size remained equivalent to the control. The goblet cell density increased in only the FOS treated group but not significantly compared to the control [Figure 5b]. However, the increase was significant ($P < 0.01$) compared to LPS. The goblet cell size was significantly increased in only FOS treated group ($P < 0.05$) compared to the control group [Figure 5c].

DISCUSSION

The present study demonstrated that prebiotic FOS supplementation protects the GI tract from bacterial endotoxin LPS-induced inflammation. The LPS is a potent immune stressor that can activate innate immunity through TLR-4 present on immunocompetent cells such as monocytes,
macrophages, and mucosal epithelial cells. Furthermore, LPS stimulates the production of a wide range of inflammatory substances, such as pro-inflammatory cytokines, chemokines, and oxidative free radicals have been largely reported. The elevated levels of these inflammatory cytokines and oxidative free radicals are potent biomarkers of inflammation. In the present study, the pro-inflammatory cytokines level of IL-6 and TNF-α were increased on LPS exposure, amplifying the inflammatory responses, and initiating gut inflammation, as reported by others. The histopathological disruptions of both the jejuno-ileum and colon might corroborate gut inflammation. The LPS exposure caused lesions of the gut mucosal barrier and leakage and depletion of enterocytes and goblet cells, mucin secretion, and deposition on epithelial layer and condensation of LP, as reported by others. Infiltration of leukocytes in the LP indicated activation of immune cells, a restitution mechanism of inflammation. The condensation of LP may be due to LPS-induced chemokines production, such as cell adhesion molecule ICAM from fibroblast. LPS exposure significantly decreased SOD and CAT in the gut. That the LPS disrupts the homeostasis of the cell’s antioxidant defense system and substantially reduces the SOD and CAT in the liver, kidney, and intestinal mucosa has been reported as observed in this study. Oxidative stress characterized by reduced gut SOD and CAT activity plays an essential role in inflammation, pathogenesis, progression, and severity. The present study measured that the prebiotic FOS supplementation for 28 days to LPS-exposed mice reduced plasma levels of pro-inflammatory cytokines (IL-6 and TNF-α). FOS supplementation attenuated the histopathological alterations of the gut caused by LPS that reflected in the jejuno-ileum and colon. In the jejunum, enterocyte density was increased by FOS and maintained the density and size of the goblet cell in the colon. FOS ameliorated gut inflammation due to the anti-inflammatory properties of different prebiotics (soybean oligosaccharides, lactulose, and polyphenols) has been reported. A recent study has proposed that the prebiotics may directly affect inflammatory cells, maintain the intestinal mucosal barrier integrity, and reduce the severity of lesions in the colon. FOS ameliorated gut inflammation due to the anti-inflammatory properties of different prebiotics (soybean oligosaccharides, lactulose, and polyphenols) has been reported. A recent study has proposed that the prebiotics may directly affect inflammatory cells, maintain the intestinal mucosal barrier integrity, and reduce the severity of lesions in the colon. FOS ameliorated gut inflammation due to the anti-inflammatory properties of different prebiotics (soybean oligosaccharides, lactulose, and polyphenols) has been reported. A recent study has proposed that the prebiotics may directly affect inflammatory cells, maintain the intestinal mucosal barrier integrity, and reduce the severity of lesions in the colon. FOS ameliorated gut inflammation due to the anti-inflammatory properties of different prebiotics (soybean oligosaccharides, lactulose, and polyphenols) has been reported. A recent study has proposed that the prebiotics may directly affect inflammatory cells, maintain the intestinal mucosal barrier integrity, and reduce the severity of lesions in the colon.
The prebiotics neutralized oxidants in the intestinal tract by expressing antioxidant enzymes and reducing inflammation in the gut.[31]

The studies have reported that the consumption of prebiotics leads to the growth of beneficial/good microbiota, for example, bifidobacteria and lactobacilli in the GI tract. The fermentation product of FOS by beneficial bacteria in the large intestine is SCFAs that indirectly scavenge ROS.[28,32] Further, it could be suggested that FOS supplementation might have increased the beneficial bacterial population that protected the gut epithelium from oxidative stress. As reported earlier, this study’s significantly decreasing body and gut weight indicates LPS-induced systemic toxicity.[33,34] FOS supplementation reduces systemic toxicity to maintain body weight. This finding suggests that FOS in an appropriate amount can protect the gut from LPS damage, repair tissue cells, and keep the gut healthy.

CONCLUSION

The FOS supplementation has shown efficacy in protecting histological damage of the gut through the modulation of inflammatory cytokines and oxidative stress. Prebiotic FOS intake may help maintain microbiota homeostasis and helps promote GI health. The supplementation of prebiotic FOS thus could be explored more as a therapeutic adjunct for treating gut illnesses such as inflammatory bowel disease, and ulcerative colitis, and restoring gut health.

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ETHICS STATEMENT

The handling and maintenance of animals were according to CPCSEA, MoEFCC, Government of India guidelines. The experimental protocols (IAEC/AU/2019(1)/01) were approved and certified by the Institutional Animal Ethics Committee (IAEC), University of Allahabad, India.

REFERENCES


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