Myristica fragrans Seed Extract Protects Against Dextran Sulfate Sodium–Induced Colitis in Mice

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ABSTRACT Nutmeg (seed of Myristica fragrans [MF]) is one of the most commonly used spices in the world and also a well-known herb for the treatment of various intestinal diseases, including colitis in traditional Korean medicine. The purpose of the current study was to investigate whether water extract of MF (MFE) can protect against dextran sulfate sodium (DSS) induced colitis in a mouse model. Colitis was induced by 5% DSS in balb/c mice. MFE (100, 300 or 1000 mg/kg) was orally administered to the mice twice a day for 7 days. Body weight, colon length, clinical score, and histological score were assessed to determine the effects on colitis. Proinflammatory cytokines (interferon-γ, tumor necrosis factor-α, interleukin [IL]-1β, and IL-6) were measured to investigate the mechanisms of action. MFE dose dependently inhibited the colon shortening and histological damage to the colon. However, it did not prevent weight loss. MFE also inhibited proinflammatory cytokines. The current results suggest that MFE ameliorates DSS-induced colitis in mice by inhibiting inflammatory cytokines. Further investigation, including the exact mechanisms is needed.

KEY WORDS: colitis • dextran sulfate sodium • inflammation • Myristica fragrans • proinflammatory cytokine
stained with hematoxylin and eosin. In a blind fashion, we analyzed the histological damage according to inflammatory cell infiltration and ulceration, and histological score was obtained by averaging all scores. Isolated colons were snap frozen and stored at \(-70^\circ C\). The mucosa was scraped from the colon. Next, 10 mg mucosa was dissolved in triple-detergent lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1% NP-40, 0.02% sodium azide, 0.5% sodium deoxycholate and 1 mM phenylmethylsulfonyl fluoride) and homogenized. Interferon (IFN)-\(\gamma\), tumor necrosis factor (TNF)-\(\alpha\), interleukin (IL)-1\(\beta\), and IL-6 in the mucosa samples were analyzed according to the manufacturer’s manual of biometric multiplex cytokine assay (Millipore, Billerica, MA, USA).

All results were expressed as mean \(\pm\) SEM and analyzed using one-way ANOVA followed by Dunnett’s test (GraphPad Prism version 5.01; GraphPad Software, San Diego, CA, USA) for comparative analysis with control group. \(P\)-value <.05 was regarded as statistically significant.

Body weight in the control group continuously decreased. MFE treatment did not show any effects on body weight (Fig. 1B). While control group showed colon shortening compared with the normal group, the MFE-treated groups exhibited less shortened and edematous colons with less damage to the cecums than in the control group (Fig. 1C). MFE treatment significantly inhibited the colon shortening in a dose-dependent manner (Fig. 1D). The clinical score of the control group began to decrease rapidly from day 3. MFE-treated groups had better clinical scores from day 3 to 5 compared to the control group. However, it did not show the effects from day 6 (Fig. 1C). MFE-treated groups had better clinical scores from day 3 to 5 compared to the control group. However, it did not show the effects from day 6 (Fig. 1C). MFE-treated groups showed relatively intact surface epithelia and cryptal glands, whereas the control group showed disruptions of the cryptal glands and infiltration of inflammatory cells (Fig. 2A). MFE showed dose-dependent protective effects against histologically evaluated damage (Fig. 2A). MFE-treated groups significantly inhibited IFN-\(\gamma\), TNF-\(\alpha\), IL-1\(\beta\), and IL-6 productions in colon mucosa (Fig. 2B).
In the current study, oral administration of MFE showed dose-dependent protective effects against colon shortening, clinical symptoms, and histological observed damage in DSS-induced colitis model. MFE also downregulated mucosal inflammatory cytokines.

MFE treatment showed dose-dependent protection against colon shortening. DSS induces various gastrointestinal or systemic symptoms. Among them, colon shortening is the most representative finding, which is known to be caused by inflammation-induced structural changes, including ulceration and erosion of the colon mucosa, finally leading to colon shortening.6,8,9

MFE treatment improved the histological score that reflects cryptal damages, and the severity and extent of inflammation.8 DSS is known to induce cryptal depletion, epithelial damage, ulceration of mucosal layer, inflammatory cell infiltration, muscle thickening, and edema.8 Thus, the protective effects of MFE might be caused by inhibiting inflammation-derived structural changes.

MFE also improved clinical scores at day 3, 4, and 5, but not day 6 and 7. Clinical score is known to reflect the state and symptoms of human diseases.10 Thus, it could suggest that MFE improves the symptoms in colitis patients. However, ineffectiveness of clinical scores at day 6 and 7 might be originated from severe damages of intestine.

MFE did not inhibit weight loss in DSS-induced colitis. DSS is known to influence on whole body system by penetration through intestinal mucosa and distribution to other organs, including liver and kidney, besides colon.8,9 Thus, the evidence suggests that the effects of MFE might be due to direct anti-inflammatory effects on colon.

In the current study, DSS treatment up-regulated proinflammatory cytokines in inflamed mucosa.11 MFE inhibited representative proinflammatory cytokines (IFN-γ, TNF-α, IL-1β, and IL-6) in the colon mucosa. Delerious effects of proinflammatory cytokines in IBD have been well documented and cytokine inhibition could be the most important target for mechanistic studies.11 Thus, the inhibitory...
effects of MFE on cytokines could be the mechanisms of action.

In conclusion, MFE showed protective effects against DSS-induced colitis by inhibiting proinflammatory cytokines in the colon mucosa. This suggests the potential for using MFE in a preventive application against intestinal inflammation. Further investigation, including the exact mechanism is needed.

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AUTHOR DISCLOSURE STATEMENT

There are no existing conflicts of interest.

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