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Lentinula edodes polysaccharides suppressed pro-inflammatory cytokines expression and colitis in mice

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ABSTRACT – Background – Polysaccharides from edible mushrooms possess immunomodulatory, anti-inflammatory, and anti-tumor activities. Recent studies indicated that necroptosis plays a role in the pathogenesis of inflammatory diseases and mediates increased expression of inflammatory cytokines. **Objective –** Therefore, it is imperative to determine the impact of polysaccharide extract from *Lentinula edodes* (*L. edodes*) on inflammatory cytokines in experimental model of colitis in mice. **Methods –** Female C57BL/6 mice divided into three or four mice per group were used for this study. Polysaccharide sample was orally administered to mice prior to (7 days) and during colitis induction with 2.5% dextran sodium sulfate (7 days), followed by additional 3 days of administration. Changes in body weight and colon length were used as markers for colitis, and pro-inflammatory cytokines and tumor necrosis factor receptor 1 (TNFR1) expressions, as well as necroptosis were analyzed in the colon of colitis mice. Data obtained were analysed by Tukey-Kramer and two-tailed standard *t* tests. **Results –** The results indicated that the polysaccharide sample suppressed colitis in mice using effects on the body weight and colon length as markers. Also, it was demonstrated that necrostatin-1, a specific inhibitor of necroptosis, suppressed the expression of interleukin (IL)-8, a pro-inflammatory chemokine, in Caco-2 cells induced necroptosis induced by zVAD and TNF-α, an indication that necroptosis may be involved in the expression of pro-inflammatory cytokines. Moreover, the polysaccharide sample suppressed the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-6, IL-1β, and interferon (IFN)-γ in the colon of mice. **Conclusion –** These results suggested that the suppressive effects of the polysaccharide sample on inflammatory cytokines expression may contribute to its anti-colitis effect, and so may serve as a potent therapeutic agent against inflammatory bowel disease.

Keywords – Edible mushrooms; polysaccharides; necroptosis; colitis; pro-inflammatory cytokines.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammation of the gut, that has defied every medical intervention. It is characterised by abdominal pain, bloody diarrhea, body weight loss, shortened colon length, and loss of appetite. It is subdivided into Crohn's disease and ulcerative colitis, with Crohn's disease affecting both the small and large intestines, while ulcerative colitis affects the large intestine[1]. The global incidence and prevalence are on the increase[2,3], and considering the rate at which the incidence is rising worldwide with the associated morbidity and mortality, couple with huge economic burden on the victims, it is imperative to search for alternative natural agents, with less adverse effects, to manage/treat the disease. The intestine is constantly harassed by myriads of exogenous substances ingested alongside food and water, and in most cases the exogenous substances include pathogenic organisms and environmental toxicants[4]. The unavoidable interactions of intestinal epithelial cells and exogenous substances sometimes cause serious damage to the cells, and in turn elicits intestinal inflammation. Therefore, intestinal inflammation may result from various interacting factors ranging from genetic predisposition, exposure to environmental toxicant through food and water, dysbiosis of luminal microbiota, and aberrant reaction of innate immune cells to luminal content[5-8].

Necroptosis is a caspase-independent inflammatory form of regulated cell death, mechanistically and morphologically different from apoptosis[9]. Necroptotic cell death is widely reported to play important role in the etiology and pathogenesis of several disease conditions, including IBD, renal disease, cancer, cardiovascular disorder, and neurological diseases[9], as well as in mediating the excessive expression and release of inflammatory cytokines[10,11]. Receptor interacting serine/threonine protein kinase 3 (RIPK3) is an important player in the necroptosis signaling pathway. It plays dual roles upon the initiation of necroptosis, by mediating both necroptosis-dependent damage-associated molecular patterns (DAMPs) release and necroptosis-independent expression of inflammatory cytokines[12]. There are various ways that RIPK3 can up-regulates inflammatory cytokines, which includes stimulation of reactive oxygen species (ROS) production and processing of pro-IL-1β into active IL-1β. ROS and IL-1β, in turn, stimulate other signaling cascades resulting in the expression of more inflammatory cytokines[12,13]. Furthermore, studies have shown that RIPK3 directly contributes to the activation of nuclear fac-

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tor kappa B -mediated inflammatory cytokines expression[13-15]. RIPK3-mediated activation of the mitogen-activated protein (MAP) kinase, extracellular signal regulated kinase (ERK) and C-Jun N-terminal kinase (JNK), has also been reported[12]. DAMPs released from necroptotic cells into the extracellular environment strongly activate and induce the expression of pro-inflammatory cytokines in surrounding cells, thereby promoting the spread of inflammatory signals[16-19].

Edible mushrooms have served as food and medicine for centuries in many parts of the world, including America, Asia, Europe, and Africa. The health benefits of mushrooms consumption cannot be over emphasized. Currently, many reports indicate that mushrooms possess anti-allergic, antiviral, antidiabetic, antioxidant, anti-cancer, anti-ageing, cholesterol-lowering, anti-bacterial, anti-fungal, and anti-inflammatory properties. Most of the health benefits are reported to be triggered by their polysaccharide contents[20-23]. Other low molecular weight bioactive compounds present in mushrooms, ranging from polyphenols, terpenoids, flavonoids, and alkaloids groups, have also been reported to possess health enhancing activities[24]. However, there is little information on the effect of mushroom polysaccharides on necroptosis-mediated inflammatory cytokines expression. This study investigated the effect of polysaccharides from *L. edodes* on colitis and inflammatory cytokines expression in the colon of mice.

METHODS

Reagents

Dextran sulphate sodium (DSS), (36,000–50,000 Da) was purchased from MP Biomedicals (Canada). Diethylaminoethyl (DEAE)-sepharose CL-6B was purchased from GE Healthcare Bio-sciences AB (Sweden). Dulbecco's Modified Eagle Medium (DMEM) containing glutamine and glucose (4.5 g/L) was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). RPMI 1640 medium and MEM non-essential amino acids (NEAA) were purchased from Nissui Pharmaceutical (Tokyo, Japan) and Gibco (Grand Island, NY, USA) respectively. FBS (Fetal Bovine Serum) was purchased from Biological Industries (Beit, Israel). Neutralizing anti-hDectin-1-IgG was purchased from InvivoGen (San Diego, USA). Recombinant human tumor necrosis factor-α (rhTNF-α) was purchased from PeproTech (Rocky Hill, NJ, USA). Pan-caspase inhibitor carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (zVAD-fmk) was purchased from Selleck (Tokyo, Japan). Anti-mouse β-actin antibody, anti-mouse TNFR1 antibody, and anti-human TNFR1 antibody were from Santa Cruz Biotechnology (Delaware Avenue, CA), rabbit monoclonal anti-mouse mixed lineage kinase domain-like pseudokinase (pMLKL) (ab196436) was purchased from Abcam. Mouse HRP-conjugated anti-IgG and rabbit HRP-conjugated anti-IgG were purchased from R&D Systems (Minneapolis, USA) and Cell Signaling Technology (Danvers, MA, USA) respectively. *L. edodes* (Berk) was gifted by a mushroom cultivation company in Japan (Naka Shiitake). All other chemicals and reagents were standard guaranteed commercial products.

Extraction of crude polysaccharides from *L. edodes*

Crude polysaccharides were extracted from *L. edodes* according to the method of Mizuno et al.[25] with little modifications. Briefly, fresh mushroom samples (fruiting body) were pulverized in liquid nitrogen using an Ace homogenizer (AM_7, Nihonseiki Kaisha Ltd) and lyophilized. The dried powdery sample (20 g) was extracted in 600 mL of distilled water at 100°C for 6 h. The extract was centrifuged at 3500 rpm, 25ºC for 20 min, and the pellet was discarded. Crude polysaccharide was precipitated using absolute ethanol at the ratio of 3:1 (Ethanol: extract) and kept at 4°C for 24 h. The precipitate was recovered by centrifugation at 3500 rpm, 4°C for 20 min, followed by twice washing of the pellet with distilled water and centrifugation at 3500 rpm, 4°C for 20 min. Pellet was lyophilized to get the dried crude polysaccharides which was labeled LeA.

Chromatographic separation of crude polysaccharide sample

DEAE-sepharose CL-6B column chromatography was employed in separating the crude polysaccharides (LeA). This was carried out according to the method of Muhidinov et al.[26]. DEAE-sepharose CL-6B slurry was made with distilled water and packed in a column (2.4 cm × 18 cm) at a flow rate of 1 mL/min. The column was equilibrated with distilled water and loaded with 20 mL of 5 mg/mL of the polysaccharide sample. The sample was eluted in a step wise manner with NaCl solution of increasing ionic strength (0.0, 0.5, and 1.0 M) at a flow rate of 1 mL/min and 10 mL/tube of the eluate was collected. Carbohydrate content of the eluate was determined by phenol-sulfuric acid method at 490 nm[27]. The fraction eluted with 0 M NaCl was labeled LeAP1 while fraction eluted with 0.5 M NaCl was labeled LeAP2.

Mice

Female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan) and used for this study. Mice were acclimatized for 7 days with free access to water and food (*ad libitum*), housed in a room with controlled temperature at 23 ± 2ºC, humidity of 50 ± 10%, and 12 h light/ dark cycle. This study was duly approved by the university's Animal Care and Use Committee (approval number: 28-10-04-R1), and was carried out according to the Institution's Animal Experimentation Regulations.

Colitis induction

Colitis was induced in mice using 2.5% of DSS (w/v) in drinking water for 7 days. Polysaccharides sample was orally administered to mice for 7 days prior to and during DSS administration for another 7 days, followed by additional 3 days of polysaccharides administration. Body weights of mice were recorded daily, and mice were sacrificed by cervical dislocation. Colon tissues were excised and used for downstream processes.

Western Blot analysis

Colon tissues (4–5 mg) were homogenized using electric homogenizer in ice-cold radioimmunoprecipitation buffer (50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% Nonidet-P40, 0.5% Sodium deoxycholate, and 0.1% SDS) containing phenylmethylsulfonyl fluoride and a cocktail of protease and phosphatase inhibitors (aprotinin, leupeptin, Na Fluoride, and dithiothreitol). Protein quantification was carried out by Lowry's method[28], and equal amount of total protein (20 µg) was separated with sodium dodecyl sulfate/polyacrylamide gel electrophoresis (10% gels) under reducing conditions (2× Laemmli buffer: 4% SDS, 10% 2-mercaptoethanol, 20% glycine, 0.004% bromophenol blue, and 0.125 M Tris-HCl (pH 6.8)). After electrophoresis, the proteins were transferred onto polyvinylidene fluoride membranes in running buffer (25 mM Tris

base, 190 mM glycine, and 0.1% SDS (pH 8.3)) with 10% methanol. The membrane was washed four times in tris-buffered saline (TBST) (containing 0.1% tween 20) for 5 min each. Non-specific sites were blocked with 4% (w/v) BSA (bovine serum albumin) in TBST for 1 h 30 min at room temperature (25°C). The membranes were then incubated overnight at 4°C with anti-pMLKL antibody (dilution of 1:1000), anti-TNFR1 antibody (dilution of 1:1000), anti-β-actin (dilution of 1:5000). After four washes with TBST for 5 min each, the membranes were incubated with horseradish-peroxidase conjugated secondary antibodies for 2 h in TBST (dilution of 1:5000). After four washes with TBST for 5 min each, protein bands were visualized using enhanced chemiluminescence (ECL) Plus western blot detection kit. ImageJ software was used to determine the relative density of the bands.

Total ribonucleic acid (RNA) isolation

Colon lumen was flushed with ice-cold PBS immediately after mice sacrifice, and 4–5 mg of distal region of colon tissue was collected for total ribonucleic acid (RNA) isolation. Total RNA was isolated using Sepasol-RNA I Super G (Nacalai Tesque, Kyoto, Japan) following the prescribed procedure for total RNA isolation. The concentration of total RNA was determined using NanoDrop 2000 spectrophotometer (Thermo Scientific, U.S.A).

mRNA purification with LiCl

The total RNA obtained with Sepasol extraction was subjected to purification using two-step lithium chloride (LiCl) precipitation, which removes contaminating DSS, other polysaccharides, DNA, tRNA, nucleotides, and precipitates only mRNA[29,30]. Briefly, 0.1 volume of ice-cold 8 M LiCl was added to 1 volume of total RNA sample, and was kept on ice for 2 h, followed by centrifugation at 14,000 × g for 30 min at 4ºC. Supernatant was discarded and pellet was redissolved in 200 µL ribonuclease (RNase) free water. Again, this step was repeated by adding 20 µL of ice-cold 8 M LiCl and kept on ice for 2 h, centrifuged at 14,000 ×g for 30 min at 4ºC, and supernatant was discarded. Again, 200 µL of RNase free water was added to the pellet, followed by addition of 20 µL of ice-cold 3 M sodium acetate (pH 5.2) and 400 µL of -20ºC pre-chilled 100% ethanol, followed by incubation at -20ºC for 30 min. The solution was centrifuged at 14,000 ×g for 30 min at 4ºC, supernatant was discarded and pellet was washed with 100 µL of -20ºC prechilled 75% ethanol, followed by centrifugation at 14,000 ×g for 10 min at 4ºC. The supernatant was removed and the pellet was air-dried, and redissolved in 30 µL (smaller volume than the initial starting volume of total RNA) of RNase free water. The concentration of purified mRNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, U.S.A).

cDNA synthesis and quantitative RT-PCR

Complementary DNA was synthesized from 5 µg of purified mRNA using the High capacity cDNA reverse transcription Kit (Applied Biosystems, Carlsbad, CA) with a T100 Thermal cycler (BIO-RAD, Singapore) programmed at 25ºC for 10 min, 37ºC for 120 min, and 85ºC for 5 sec. The cDNA was then stored at 4ºC for subsequent use. Quantitative real-time polymerase chain reaction was performed on a 7500 Fast-Time PCR System (Applied Biosystems) programmed at 95ºC for 10 min, 95ºC for 10 sec, and 60ºC for 30 sec, using a FastStart Universal Probe Master (ROX) mix (Roche Diagnostics GmbH, Mannheim, Germany). The TaqMan probes used include Mm00607939_sl for mouse *Actb* (housekeeping gene), Hs02786624_g1 for human *GAPDH* (housekeeping gene), Mm00443258_ml for mouse *Tnf*α, Mm00446190_ml for mouse *Il6*, Mm00434228_ml for mouse *Il1b*, Mm01168134_ml for mouse *Ifng*, Mm00441242_ml for mouse *Ccl2*, and Hs00174103_m1 for human *Il8*. Gene expression was normalized using β-actin or GAPDH.

Data analysis

All data are expressed as mean ± SD. Statistical analysis was performed by Tukey-Kramer and two-tailed standard *t* tests. Statistical significance was defined as $P<0.05$ and $P<0.01$.

RESULTS

Crude Polysaccharides from *L. edodes* prevented colitis in mice

To investigate the anti-inflammatory activity of crude polysaccharides (LeA) from *L. edodes*, 500 µg/mouse of LeA was orally administered to colitis mice. The results indicated that LeA significantly prevented DSS-induced colitis in mice (FIGURE 1A-C). DEAE-sepharose CL-6B chromatographic separation of LeA showed two peaks in the elution profile (FIGURE 1D), indicating that LeA possessed two kinds of polysaccharides which were named LeAP1 (eluted with 0.0 M NaCl solution) and LeAP2 (eluted with 0.5 M NaCl solution). However, in a recent study by Alagbaoso and Mizuno[31], it was reported that the anti-inflammatory and anti-necroptosis activities of crude polysaccharides from *L. edodes* was majorly dependent on the carbohydrate-rich fraction (fraction 1) obtained by column chromatographic separation. Therefore, in this study, the fraction 1 (LeAP1) obtained by column chromatographic separation was used for further investigations.

LeAP1 from *L. edodes* suppressed colitis

To investigate the effect of LeAP1 on colitis, 300 µg/mouse of LeAP1 was orally administered to DSS-induced colitis mice. Consistent with previous report, the polysaccharides significantly inhibited DSS-induced colitis in mice (FIGURE 2A-C). However, unlike the crude polysaccharide, LeAP1 significantly prevented DSS-induced body weight loss (FIGURE 2A), suggesting that other components associated with the crude polysaccharides may have contributed to the body weight loss of the mice. These data suggested that the polysaccharides sample exerted anti-inflammatory activity.

LeAP1 from *L edodes* suppressed inflammatory cytokines expression and necroptosis in mice colon

Necroptotic cell death is associated with increased expression of inflammatory cytokines. To investigate the contribution of necroptosis on the expression of inflammatory cytokines, necroptosis was induced in mice using 2.5% DSS (w/v) in drinking water. Mice were pre-treated with polysaccharides (300 µg/mouse) and during necroptosis induction for a total of 17 days. The results indicated that necroptotic cell death was inhibited in the colon of mice treated with polysaccharides (FIGURE 3 A,B). Expression of inflammatory cytokines such as TNF-α, IL-6, IL-1β, IFN-γ, and c-c motif chemokine ligand 2 (CCL-2) in the colon of mice was significantly prevented by polysaccharide sample (FIGURE 3C-G). Furthermore, necroptosis was induced in 200 µg/mL LeAP1-treated Caco-2 cells using 20 ng/mL TNF-α +50 µM zVAD-fmk (pan-caspase inhibitor) for 24 h, and IL-8 mRNA expression was analysed. The data indicated that the polysaccharides sample

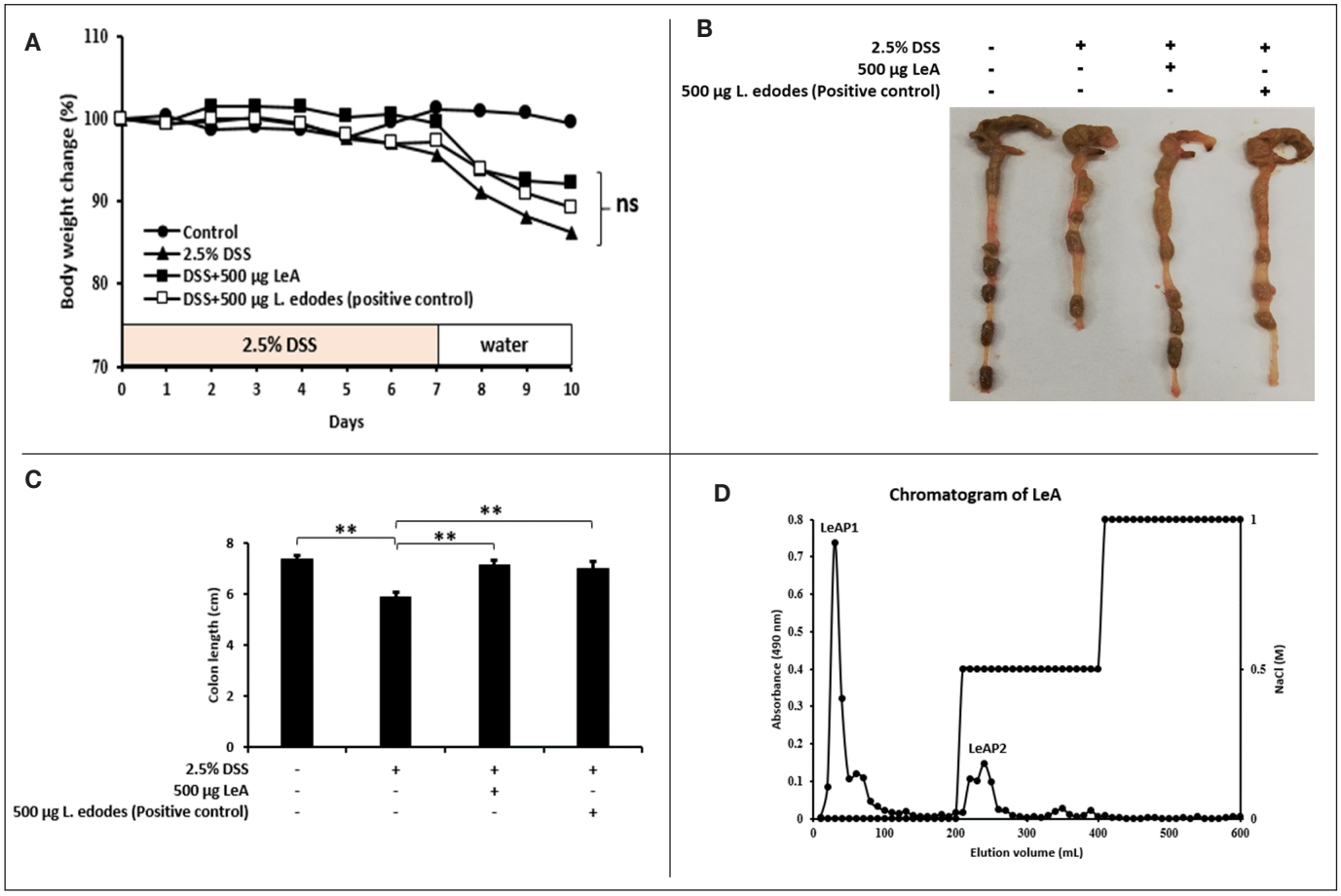

FIGURE 1. Crude polysaccharides from *L. edodes* suppressed colitis.
Crude polysaccharides (LeA: 500 µg/mouse) was administered to mice prior to colitis induction (with 2.5% DSS (w/v) in drinking water) for 7 days and during colitis induction for another 7 days, followed by additional 3 days of administration. Crude polysaccharide sample from a different strain of *L. edodes* (500 µg/mouse) that possessed anti-inflammatory activity was used as positive control. (A) Body weight of colitis mice treated with LeA. (B and C) Colon length of colitis mice treated with LeA. Crude polysaccharides sample was separated using DEAE-sepharose CL-6B column chromatography and NaCl solution of increasing ionic strength (0.0, 0.5, and 1.0 M) was used to elute the column. (D) Elution profile of LeA showing two peaks, fractions LeAP1 (eluted with 0.0 M NaCl) and LeAP2 (eluted with 0.5 M NaCl). Values are expressed as Mean ± SD (n=5). $^{**}P<0.01$.

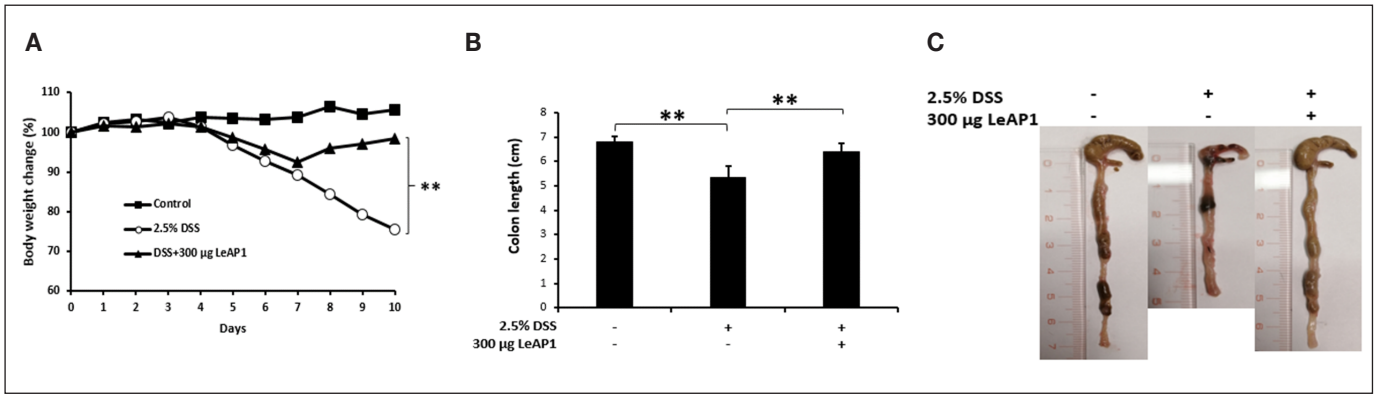

FIGURE 2. LeAP1 from *L. edodes* suppressed colitis in mice.
Colitis was induced in mice with 2.5% DSS (w/v) in drinking water for 7 days. Polysaccharide sample (300 µg/mouse) was orally administered to mice prior to (7 days) and during colitis induction (7 days), followed by additional 3 days of administration. (A) Body weight of mice treated with polysaccharide sample. (B and C) Colon length of mice treated with polysaccharide sample. The dose of LeAP1 administered was determined from the percentage yield after chromatographic separation and lyophilization (LeAP1: 59.5% of 500 µg = 297.5 ≈ 300 µg) to represent the equivalence of 500 µg/mouse crude polysaccharides previously administered. Values are expressed as mean ± SD (n=4–5). $^{**}P<0.01$.

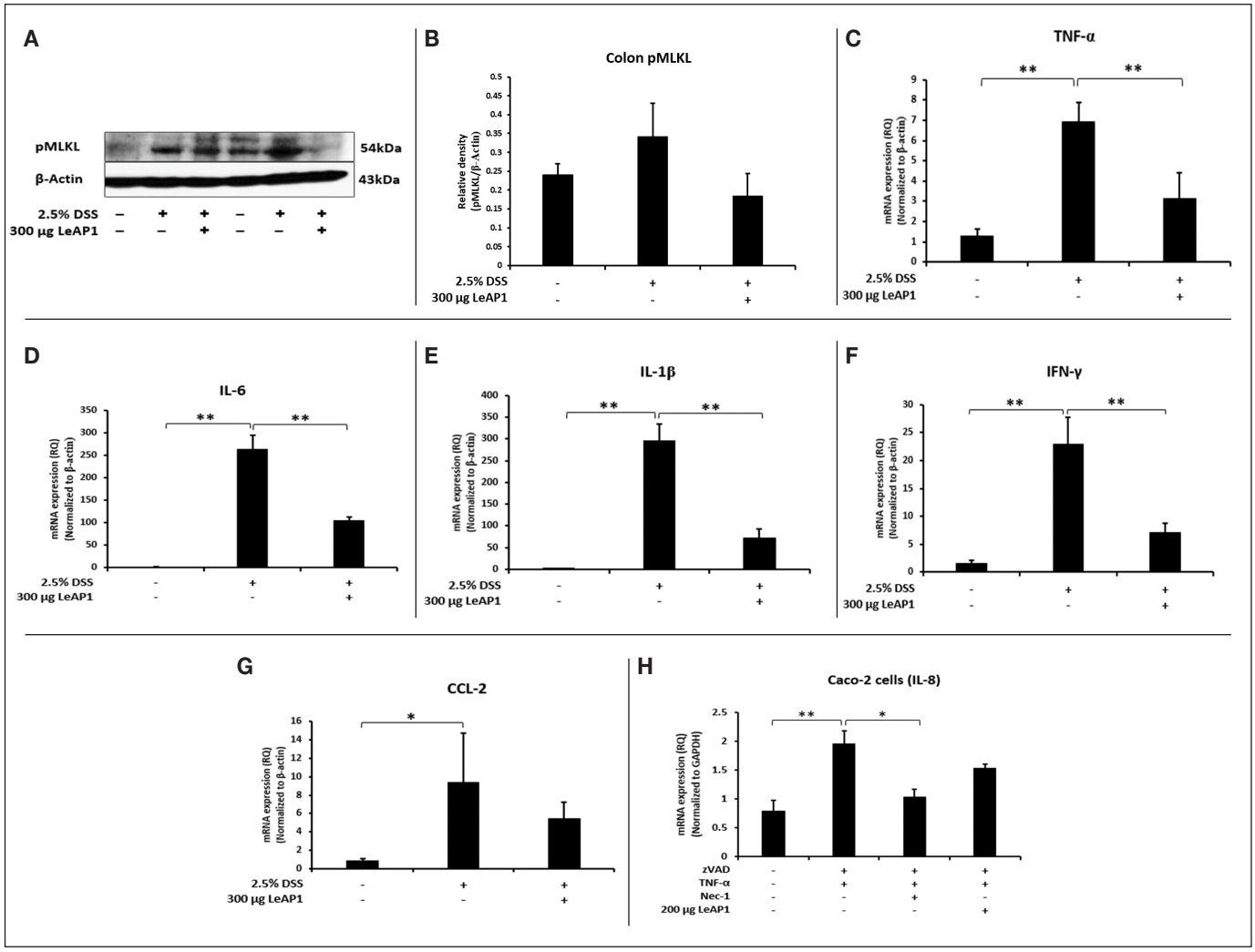

FIGURE 3. LeAP1 from *L. edodes* inhibited necroptosis and inflammatory cytokines in mice. Polysaccharides sample (300 µg/mouse) was pre- (7 days) and co-administered to DSS-treated mice for 7 days, followed by additional 3 days administration of polysaccharide sample. (A and B) Western blot detection of necroptosis executor, pMLKL "in mice colon". (C-G) mRNA expression of inflammatory cytokines in the colon of mice treated with polysaccharide sample. (H) mRNA expression of IL-8 in Caco-2 cells treated with 200 µg/mL LeAP1 or Nec-1 (20 µM) and necroptosis inducer (20 ng/mL TNF-α+50 µM z VAD-fmk) for 24 h. The dose of LeAP1 administered was determined from the percentage yield after chromatographic separation and lyophilization (LeAP1: 59.5% of 500 µg =297.5 ≈ 300 µg) to represent the equivalence of 500 µg/mouse crude polysaccharides previously administered. Values are expressed as mean ± SD (n=3–4). **P*<0.05, ***P*<0.01.

suppressed IL-8 expression (FIGURE 3H). In addition, the data indicated that necrostatin-1 (Nec-1) (20 µM), a specific necroptosis inhibitor, prevented the up-regulation of IL-8 expression (FIGURE 3H), suggesting that necroptosis may play an active role in mediating the expression of pro-inflammatory cytokines. These data suggested that the polysaccharides sample inhibited inflammatory cytokines expression in the colon of mice.

Effect of *L. edodes* polysaccharides (LeAP1) on TNFR1 expression in mice colon and Caco-2 Cells.

Recent reports indicated that TNF-α played important role in activating necroptosis in vivo through binding to its cell surface receptor, TNFR1. To determine the effect of polysaccharides from *L. edodes* on the expression of TNFR1 in the colon of colitis mice, polysaccharide sample was orally administered to colitis mice and the expression of TNFR1 was determined. The results indicated that the polysaccharide sample did not affect TNFR1 expression in mice colon (FIGURE 4A, B).

DISCUSSION

Studies in the last two decades on cell death have improved our understanding of cell deaths and their role in disease conditions. Necroptosis, a regulated form of pro-inflammatory cell death, has been widely shown to play active roles in the etiology and pathogenesis of IBD[9]. Recent reports indicated that inhibitors of necroptosis prevented ulcerative colitis in mice, thereby implicating necroptosis as a cause of IBD rather than a consequence of it[11]. In this study, polysaccharide extract administered to colitis mice showed suppressive effect on colitis at both its crude

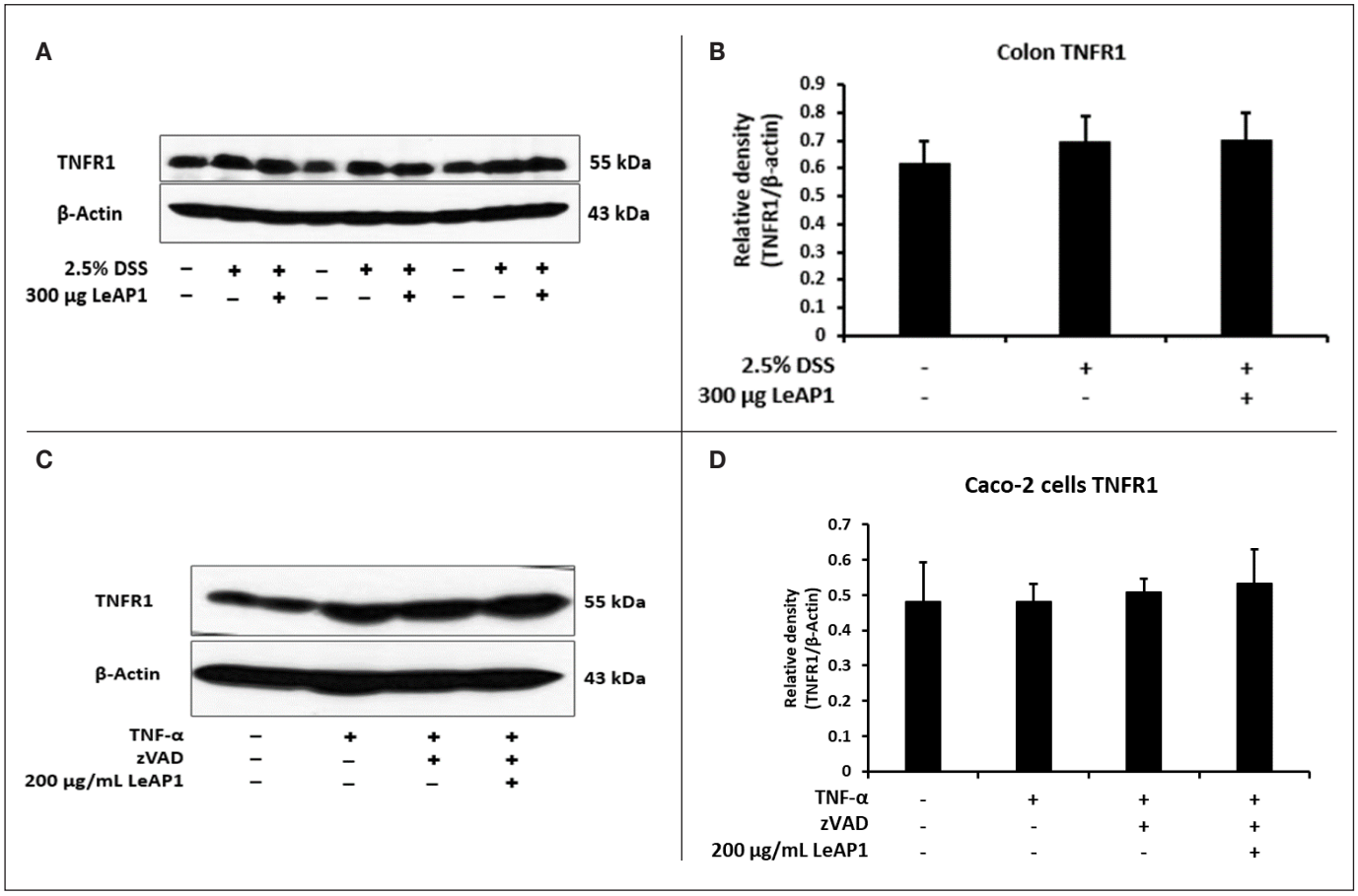

FIGURE 4. Effect of LeAP1 from *L. edodes* is independent of its effect on TNFR1 expression. Polysaccharide sample (300 µg/mouse) was pre- (7 days) and co-administered to colitis mice for 7 days, followed by additional 3 days administration of polysaccharide sample. (A and B) Protein expression of colon TNFR1 of colitis mice treated with 300 µg/mouse of *L. edodes* polysaccharide. To induce necroptotic cell death, Caco-2 cells were incubated with 50 µM zVAD or 50 µM zVAD+200 µg/mL LeAP1 for 2 h prior to TNF-α (20 ng/mL) stimulation for an additional 24 h. (C and D) Protein expression of TNFR1 in Caco-2 cells treated with necroptosis inducer and *L. edodes* polysaccharides. The dose of LeAP1 administered was determined from the percentage yield after chromatographic separation and lyophilization (LeAP1: 59.5% of 500 µg =297.5 ≈ 300 µg) to represent the equivalence of 500 µg/mouse crude polysaccharides previously administered. Values are expressed as mean ± SD (n=3). $P<0.05$.

state and when chromatographically partially purified. This data supports the reports of previous studies by Alagbaoso CA and Mizuno M [31], and suggests that the polysaccharide extract possessed anti-inflammatory activity (FIGURE 1). In the report of Alagbaoso and Mizuno[31], fraction 1 component from column chromatographic separation of polysaccharide extract from *Lentinus edodes*, which was rich in carbohydrate, was shown to be responsible for its anti-inflammatory activity. In agreement, this study demonstrated that the fraction 1 component of our polysaccharide sample obtained by column chromatographic separation with 0.0 M NaCl, exerted anti-inflammatory activity as seen in the body weight changes and colon length of colitis mice (FIGURE 2).

Necroptosis signaling stimulates the expression of inflammatory cytokines which together with DAMPs are released into the extracellular environment upon cell membrane rupture, and which results in the promotion and worsening of inflammatory conditions. TNF-α is known to stimulate necroptosis upon binding to its receptor, TNFR1, in the absence or deficiency of caspase-8. In this study, necroptosis and high expression of TNF-α were observed in the colon of DSS-treated mice, which is an indication that DSS-induced necroptotic cell death may play a role in the expression and release of TNF-α which further contribute to initiation of necroptosis in neighbouring cells and exacerbation of the inflammatory process.

Necroptosis actively plays a role in the pathogenesis and promotion of ulcerative colitis, which suggests that necroptotic signaling molecules play crucial roles in the expression of inflammatory cytokines in ulcerative colitis conditions[9,10,32]. It was demonstrated that necrostatin-1, a specific inhibitor of necroptosis supressed the expression of IL-8 in Caco-2 cells, thereby suggesting that necroptosis play a role and contributed to the expression of IL-8, a chemokine (FIGURE 3H). More so, it was shown that the polysaccharide sample from *L. edodes* prevented necroptosis in the colon of mice using the level of phosphorylated MLKL (pMLKL) as a marker (FIGURE 3A, B). Therefore, suppression of necroptosis by the polysaccharide sample may have contributed to the amelioration of colitis. Furthermore, in this study, it was observed that the expressions of inflammatory cytokines such as TNF-α, IL-6, IL-1β, IFN-γ, and CCL-2 were increased in the colon of mice. However, this study showed that polysaccharide sample from *L. edodes* sup-

pressed the expression of inflammatory cytokines in the colon of mice (FIGURE 3A-F). The suppressive effect on the expression of inflammatory cytokines may have resulted from the effect of the polysaccharide sample on necroptosis. These results are consistent with the reports of other researchers who reported that necroptosis inhibitors ameliorated colitis and prevented increased expression of inflammatory cytokines[33,34].

Furthermore, the effect of the polysaccharide sample on the expression of TNFR1 was investigated. In contrast to the report of Nishitani et al.[1] who reported the endocytotic effect of lentinan on TNFR1 expression on mice small intestine and Caco-2 cells as its anti-inflammatory mechanism, the polysaccharide sample in this study did not show similar effect on the expression of TNFR1 in the colon of DSS-treated mice and Caco-2 cells, probably due to differential experimental design.

CONCLUSION

These data, therefore, suggested that the polysaccharide sample possessed anti-inflammatory properties which was demonstrated by its suppressive effects on colitis and expression of pro-inflammatory cytokines, and therefore may serve as a potent therapeutic agent against IBD.

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Authors' contribution

Alagbaoso CA and Mizuno M conceptualized the study. Alagbaoso CA carried out the experiments, collected and analyzed the data, and also drafted the manuscript. Alagbaoso CA and Mizuno M proofread the final draft and approved it.

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RESUMO – Contexto – Polissacarídeos de cogumelos comestíveis possuem atividades imunomodulatórias, anti-inflamatórias e anti-tumorais. Estudos recentes indicaram que a necroptose desempenha um papel na patogênese de doenças inflamatórias e regula o aumento da expressão de citocinas inflamatórias. **Objetivo –** Torna-se imprescindível determinar o impacto do extrato de polissacarídeo de *Lentinula edodes* (*L. edodes*) em citocinas inflamatórias em modelo experimental de colite em camundongos. **Métodos –** Foram utilizados para este estudo os camundongos C57BL/6 femininos divididos em três ou quatro camundongos por grupo. A amostra de polissacarídeo foi administrada oralmente em camundongos antes (7 dias) e durante a indução de colite com sulfato de dextran sulfato de sódio (7 dias), seguido por 3 dias adicionais de administração. Alterações no peso corporal e comprimento do cólon foram utilizadas como marcadores para colite, e citocinas pró-inflamatórias e tumores receptor fator 1 (TNFR1), bem como necroptose foram analisadas no cólon de camundongos colite. Os dados obtidos foram analisados por testes Tukey-Kramer e testes padrão t de duas caudas. **Resultados –** Os resultados indicaram que a amostra de polissacarídeo suprimiu colite em camundongos usando efeitos sobre o peso corporal e o comprimento do cólon como marcadores. Além disso, foi demonstrado que a necrostatina-1, inibidora específica da necroptose, suprimiu a expres-são de interleucina (IL)-8, uma quimiocina pró-inflamatória, em células caco-2 induzidas necroptose induzidas por zVAD e TNF-α, uma indicação de que a necroptose pode estar envolvida na expressão de citocinas pro-inflamatórias. Além disso, a amostra de polissacarídeo suprimiu a expressão de citocinas pró-inflamatórias, como o fator de necrose tumoral (TNF)-α, IL-6, IL-1β e interferon (IFN)-γ no cólon dos camundongos. **Conclusão –**Esses resultados sugeriram que os efeitos supressivos da amostra de polissacarídeo na expressão de citocinas inflamatórias podem contribuir para o seu efeito anti-colite, podendo, portanto, servir como um potente agente terapêutico contra doença inflamatória intestinal.

Palavras-chave – Cogumelos comestíveis; polissacarídeos; necroptose; colite; citocinas pró-inflamatórias.

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