



Anti-inflammatory effects of Southern Thai red curry paste extracts on an inflamed intestinal cell model

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ABSTRACT

This study aimed to investigate the anti-inflammatory activities of Southern Thai red curry paste extracts in both lipopolysaccharide (LPS)-activated macrophages and an *in vitro* inflamed intestinal epithelium model. The red curry paste, in both fresh and dried forms, was extracted using ethanol (EtOH). The anti-inflammatory effects were evaluated in LPS-stimulated RAW 264.7 murine macrophages and a co-culture model of human intestinal Caco-2 cells and RAW 264.7 cells. Among all extracts, the dried red curry ethanol extract (DR-EtOH) demonstrated the highest potency in inhibiting nitric oxide (NO) production in RAW 264.7 cells, with an IC₅₀ value of 220 ± 60 µg/mL, and significantly suppressed tumor necrosis factor-α (TNF-α) levels. Western blot analysis revealed that the both fresh (FR-EtOH) and dried (DR-EtOH) forms significantly inhibited the expression of inducible nitric oxide synthase (iNOS) protein. In the inflamed intestinal cell model, FR-EtOH at 125 µg/mL effectively attenuated macrophage-induced intestinal epithelial dysfunction, as indicated by maintaining transepithelial electrical resistance (TEER) values and reducing interleukin-8 (IL-8) levels. These findings provide scientific evidence for the anti-inflammatory properties of Southern Thai red curry paste in both fresh and dried forms. This knowledge supports its potential development as functional foods or nutraceuticals for the prevention or mitigation of inflammatory gastrointestinal diseases.

Keywords: Southern Thai red curry, LPS-stimulated macrophages, inflamed intestinal cell model, anti-inflammation, inducible nitric oxide synthase

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1. Introduction

Inflammatory Bowel Disease (IBD), which includes Crohn's disease and ulcerative colitis, is a chronic, relapsing inflammatory disorder of the gastrointestinal tract. It is clinically characterized by abnormal bowel habits, mucoid or bloody stool, severe diarrhea, and abdominal pain. Persistent intestinal inflammation is also associated with an increased risk of colorectal cancer, representing a significant global health burden.¹ Epidemiological studies have reported marked geographical variation in IBD prevalence, with substantially higher incidence in Western populations compared with Asian populations.² This disparity has led to the hypothesis that environmental factors, particularly dietary and lifestyle patterns, may play a protective role against the development of intestinal inflammation.³

The pathogenesis of IBD involves a complex interplay among genetic susceptibility, environmental factors, gut microbiota, and dysregulated immune responses.⁴ A key mechanism underlying intestinal injury is macrophage activation. Disruption of the intestinal barrier facilitates pathogen infiltration, which triggers macrophage activation and leads to excessive production of pro-inflammatory mediators, including tumor necrosis factor- α (TNF- α) and nitric oxide (NO). These mediators promote epithelial cell apoptosis and downregulate tight junction protein expression, thereby compromising barrier integrity and perpetuating chronic inflammation.⁵ In addition, inflamed intestinal epithelia cells secrete chemokines such as interleukin-8 (IL-8), which recruit neutrophils and further exacerbate tissue damage.⁶

Southern Thai red curry paste is unique culinary formulations composed of diverse medicinal herbs and spices, including chilies, garlic, shallots, turmeric, pepper, and lemongrass. These ingredients are rich in bioactive compounds with well-documented antioxidant and anti-inflammatory properties.⁷ Previous studies have demonstrated that Thai

curry paste extracts can inhibit NO production in LPS-stimulated macrophages.^{7,8} Furthermore, our prior work showed that aqueous extracts of Southern Thai curry pastes exhibit anti-inflammatory activity and preserve intestinal barrier function *in vitro*, although a reduction in bioactive compound availability was observed following simulated gastrointestinal digestion.⁹

In traditional Thai cuisine, curry pastes are widely used in both fresh and dried forms, each subjected to distinct processing conditions that can markedly influence their phytochemical composition. Such processing-induced changes may differentially affect their anti-inflammatory activity and capacity to modulate epithelial barrier function. Thermal drying is known to degrade heat-sensitive and volatile bioactive compounds while potentially concentrating more stable constituents.¹⁰ In addition, drying offers practical advantages, including improved stability, extended shelf-life, and suitability for large-scale production and extraction in functional ingredient development. Despite their widespread use, comparative data on the biological activities of fresh versus dried curry pastes remain limited. Therefore, systematic evaluation of both forms is necessary to elucidate the impact of processing on functional properties and to identify the most effective form for nutraceutical applications.

Accordingly, this study aimed to evaluate the anti-inflammatory potential of ethanolic extracts from Southern Thai red curry pastes in both fresh and dried forms using LPS-stimulated RAW 264.7 macrophages and a Caco-2/RAW 264.7 co-culture model of intestinal inflammation. The findings are expected to provide a scientific basis for the development of Southern Thai curry pastes as nutraceutical agents for the prevention and management of inflammatory gastrointestinal disorders.

2. Materials and Methods

2.1 Preparation of Southern Thai red curry paste (RC)

Traditional Southern Thai red curry paste (Kaeng-ped) was prepared according to standardized local recipes with defined ingredient ratios, as presented in Table 1. All fresh ingredients were thoroughly washed, peeled, and sliced prior to processing. For fresh curry paste, the prepared ingredients were immediately blended to obtain a homogenous paste. For dried curry paste, 75 g of the ingredients were subjected to hot-air drying at 50°C for 48 hours, followed by grinding into a fine powder.

2.2 Ethanolic extraction

Extraction was performed for both fresh and dried curry paste samples.

Briefly, 75 g of fresh sample and dried sample obtained from 2.1 were macerated in 300 mL of 80% (v/v) ethanol and subjected to continuous agitation using an incubator shaker at 25°C and 150 rpm for 24 hours. The mixtures were then filtered through a fine muslin cloth to remove solid residues. The filtrates were concentrated under reduced pressure using a rotary evaporator at 45°C to remove the solvent. The crude extracts were weighed to determine the extraction yield (%). The resulting ethanolic extracts from dried and fresh red curry paste were designated as DR-EtOH and FR-EtOH, respectively. All extracts were stored in a desiccator under dark conditions to minimize moisture exposure and light-induced degradation prior to biological activity assays.

Table 1. Ingredients of Southern Thai red curry paste.

Components	Species	Weight (%)
Fresh chilies	<i>Capsicum annuum</i> L.	7
Dry chilies	<i>Capsicum annuum</i> L.	28
Turmeric	<i>Curcuma longa</i> L.	5
Lemongrass	<i>Cymbopogon citratus</i> (DC.) Stapf	40
Garlic	<i>Allium sativum</i> L.	10
Shallots	<i>Allium ascalonicum</i> L.	5
Black pepper	<i>Piper nigrum</i> L.	5

2.3 Cell culture

The murine macrophage cell line RAW 264.7 (ATCC, USA), was used in this study between passages 6 and 20. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin (Gibco, USA). Cultures were maintained in a humidified incubator at 37°C with 5% CO₂ and 95% relative humidity.

The human intestinal epithelial cell line Caco-2 (CLS Cell Lines Service, Germany), was used in this study at passages 47-57. Cells were cultured in DMEM containing 1.0 g/L glucose, supplemented with 10% (v/v) fetal bovine serum and 1%

(v/v) penicillin/streptomycin (Gibco, USA). Cultures were maintained in a humidified incubator at 37°C with 5% CO₂ and 95% relative humidity under sterile conditions, with medium replacement every three days. For experiments, cells were seeded at a density of 3.125×10^4 cells/cm² in 96-well or 24-well plates (Thermo Scientific, USA). To induce full differentiation into enterocyte-like cells, cultures were maintained for 21 days post-confluence with medium changes every three days as previously described.⁹

2.4 Determination of nitric oxide (NO)

RAW 264.7 cells were seeded into 96-well plates at a density of 1.0×10^5 cells per well in 0.2 mL of complete medium and incubated for 24 hours to allow cell

attachment. After attachment, cells were pretreated with DR-EtOH or FR-EtOH at concentrations ranging from 62.5-2,000 µg/mL for 1 hour. Inflammation was subsequently induced by adding lipopolysaccharide (LPS; Sigma-Aldrich, USA) to a final concentration of 10 ng/mL in all groups except the normal control. Cells were then incubated for an additional 24 hours. Cell culture supernatants were collected for NO quantification.

NO production was determined by measuring nitrite accumulation in the culture supernatants using the Griess assay. The Griess reagent (Sigma-Aldrich, USA), composed of 0.1% (w/v) naphthylethylenediamine dihydrochloride and 1.0% (w/v) sulfanilamide in 5.0% (v/v) phosphoric acid, was prepared in 4% (w/v) in deionized water prior to use. Briefly, 75 µL of Griess reagent was mixed with an equal volume (75 µL) of cell culture supernatant in a 96-well plate and incubated at room temperature for 30 minutes. Absorbance was then measured at 546 nm using a microplate reader. Nitrite concentrations were quantified using a standard curve generated with sodium nitrite (NaNO₂).

2.5 Cytotoxicity test

Cell viability was evaluated using the MTT assay. Briefly, 200 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Thermo Fisher Scientific, USA) at a concentration of 0.5 mg/mL was added to each well and incubated for 3 hours. After incubation, the MTT solution was removed, and the resulting formazan crystals were dissolved in 200 µL of dimethyl sulfoxide (DMSO), yielding a purple solution. Absorbance was measured at 560 nm with a reference wavelength of 670 nm for background correction using a microplate reader (Biochrom EZ Read 400 Microplate Reader, USA). Cell viability was calculated as a percentage relative to the control group.

2.6 Establishment of the *in vitro* inflamed intestinal co-culture model

To simulate an inflamed intestinal epithelium, a Caco-2 and RAW 264.7 co-culture model was established based on the method of Utaipan et al. (2025)⁹ with minor modifications. Caco-2 cells were seeded at a density of 0.5×10^5 cells/well onto the apical side of 12-well Transwell® inserts (0.4 µm pore size; Corning Costar Corp., USA) at a density of 0.5×10^5 cells/well and cultured for 21 days with medium replacement every 2-3 days to allow full differentiation and formation of a confluent monolayer. Subsequently, RAW 264.7 cells (1.0×10^5 cells/well) were seeded into the basolateral compartment, and the co-culture system was maintained for 48 hours. Prior to treatment, the medium in both compartments was replaced with serum-free DMEM. Curry paste extracts were applied to the apical compartment at concentrations of 125, 250, 500 µg/mL. After 2 hours pre-incubation, inflammation was induced by adding LPS (10 ng/mL) to the basolateral compartment, followed by incubation for 24 hours.

2.7 Measurement of transepithelial electrical resistance (TEER)

The integrity of the Caco-2 monolayer in the co-culture system was assessed by measuring transepithelial electrical resistance (TEER) using a Millicell® ERS-2 Volt-Ohm Meter (Millipore, USA). Electrical resistance was recorded between the apical and basolateral compartments of the Transwell inserts. TEER measurements were taken at 0, 12, and 24 hours after LPS-induced inflammation. Values were expressed as $\Omega \times \text{cm}^2$, calculated by subtracting the resistance of a blank insert (without cells) and multiplying by the effective surface area of the membrane.

2.8 Enzyme-linked immunosorbent assay (ELISA)

Proinflammatory cytokine levels were quantified in the supernatants of RAW 264.7 cells and Caco-2 cell cultures. Tumor

necrosis factor- α (TNF- α) was selected as a marker of M1 macrophage-mediated inflammatory response. In the co-culture system, interleukin-8 (IL-8) levels were measured in Caco-2 supernatants to assess intestinal epithelial inflammatory responses. TNF- α and IL-8 concentrations were determined using ELISA MAXTM Standard Sets for mouse TNF- α and human IL-8 (Biolegend, USA), respectively, according to the manufacturer's instructions.

2.9 Western blot analysis

To investigate protein expression, RAW 264.7 cells (1.0×10^5 cells/mL) were seeded into 60 mm $\times$ 15 mm culture dishes and co-treated with curry paste extracts and LPS (10 ng/mL) for 24 hours. After incubation, the medium was removed and cells were washed with phosphate-buffered saline (PBS). Total proteins were extracted using lysis buffer (Bio-Rad, USA) supplemented with a protease inhibitor cocktail (HiMedia, India). Lysates were collected in 1.5 mL microcentrifuge tubes and centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatant was collected, and protein concentration was determined using a Detergent Compatible (DC) protein assay kit (Bio-Rad, USA). For denaturation, 50 μ g of protein was mixed with 2X Laemmli sample buffer (Bio-Rad, USA) at a 1:1 ratio and heated at 95°C for 5 minutes. Proteins were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad, USA). Membranes were blocked with 5% non-fat dry milk for 1 hour and washed with Tris-buffered saline containing 0.1% Tween-20 (TBST). Subsequently, membranes were incubated overnight (16 hours) at 4°C with primary antibody against iNOS (1:1,000; Cell Signaling Technology, USA). After washing with TBST, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000; Cell Signaling Technology, USA) for 1 hour at room temperature. Protein bands were then visualized using a chemiluminescent HRP substrate (Millipore,

Germany) and detected by exposure to Hyperfilm (Cytiva, USA).

2.10 Statistical analysis

All experiments were conducted in at least three independent replicates. Data are presented as mean $\pm$ standard deviation (SD). Statistical differences among groups were analyzed using One-way Analysis of Variance (ANOVA), followed by Duncan's multiple range test for post-hoc comparisons. All analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). A p -value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1 Extraction yields of DR-EtOH and FR-EtOH

The ethanolic extracts (EtOH), obtained after vacuum evaporation, appeared as dark orange, viscous, and semi-solid materials. The extraction yields of dried red curry ethanol extract (DR-EtOH) and fresh red curry ethanol extract (FR-EtOH) were 14.87% and 15.26%, respectively.

3.2 Cytotoxicity and inhibitory effects of DR-EtOH and FR-EtOH on nitric oxide production

The cytotoxicity and anti-inflammatory effects of ethanolic extracts from dried and fresh red curry paste were evaluated in RAW 264.7 macrophages over a concentration range of 6.25-2,000 μ g/mL (Fig. 1). In the cell viability assay, DR-EtOH exhibited significant cytotoxicity at concentrations of 1,000-2,000 μ g/mL, resulting in reduced cell viability, whereas FR-EtOH showed no significant cytotoxicity effects at any tested concentration (Fig. 1A). These results indicate that cytotoxicity of the dried formulation occurred only at the highest concentration tested. NO production was assessed by measuring nitrite accumulation in culture supernatants. All extracts significantly reduced NO levels compared with the LPS-stimulated control group (Fig. 1B). Notably, DR-EtOH demonstrated strong

NO-inhibitory activity from 62.5 $\mu\text{g/mL}$, significantly greater than that of FR-EtOH ($\#p < 0.05$). IC_{50} analysis further confirmed that DR-EtOH exhibited higher inhibitory potency, with an IC_{50} value of $220 \pm 60 \mu\text{g/mL}$, approximately two-fold lower than that of FR-EtOH ($530 \pm 20 \mu\text{g/mL}$).

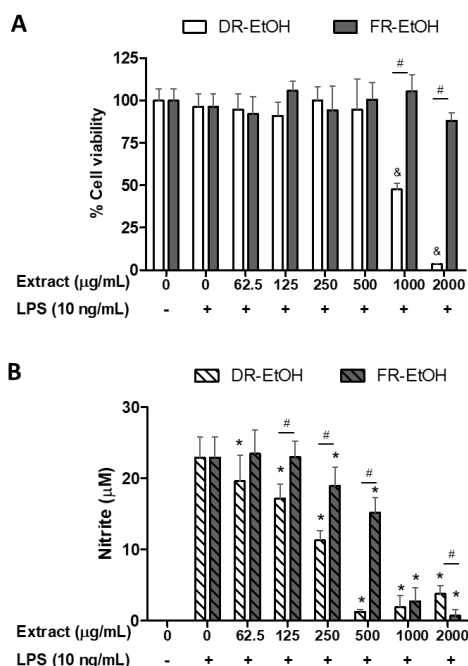


Fig. 1 Effects of DR-EtOH and FR-EtOH on (A) cell viability and (B) NO production in LPS-activated RAW 264.7 cells after treatment with the curry paste extracts for 24 hours. The results are expressed as the mean $\pm$ SD of three independent experiments in triplicate. & $p < 0.05$ vs. normal control group (0 $\mu\text{g/mL}$); * $p < 0.05$ vs. LPS-stimulated group; # $p < 0.05$ vs. between DR-EtOH and FR-EtOH.

3.3 Effects of DR-EtOH and FR-EtOH on TNF- α Secretion

The anti-inflammatory activity of ethanolic curry paste extracts was further evaluated by measuring tumor necrosis factor-alpha (TNF- α) secretion in RAW 264.7 macrophages. As shown in Fig. 2, stimulation with LPS (10 ng/mL) significantly increased TNF- α production to approximately 27,000 pg/mL compared with the normal

control (& $p < 0.05$), confirming successful induction of inflammation. Pre-treatment with both DR-EtOH and FR-EtOH significantly reduced TNF- α levels at all tested concentrations (125, 250, and 500 $\mu\text{g/mL}$). Notably, FR-EtOH exhibited the strongest inhibitory effect at 125 $\mu\text{g/mL}$, decreasing TNF- α levels to approximately 7,500 pg/mL. These results indicate that both fresh and dried curry paste extracts effectively suppress pro-inflammatory cytokine production in activated macrophages.

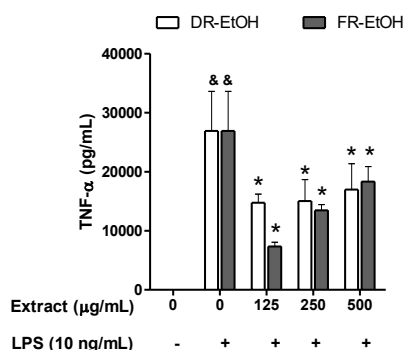


Fig. 2 TNF- α levels in LPS-stimulated RAW 264.7 cells following treatment with curry paste extracts. Data are presented as mean $\pm$ SD from three independent experiments performed in triplicate. & $p < 0.05$ vs. normal control; * $p < 0.05$ vs. LPS-stimulated control.

3.4 Effect of DR-EtOH and FR-EtOH on iNOS protein expression

To elucidate the molecular mechanism underlying nitric oxide inhibition, the effects of dried (DR-EtOH) and fresh (FR-EtOH) ethanolic curry paste extracts on inducible nitric oxide synthase (iNOS) protein expression were evaluated by Western blot analysis (Fig. 3). LPS stimulation (10 ng/mL) significantly upregulated iNOS expression compared with the untreated control (& $p < 0.05$), confirming activation of the inflammatory pathway. Pre-treatment with DR-EtOH and FR-EtOH (125-500 $\mu\text{g/mL}$) for 24 hours resulted in a concentration-dependent reduction in iNOS protein levels, as shown in the immunoblot (Fig. 3, upper panel). Densitometric analysis, normalized

to β -actin, confirmed that both extracts significantly downregulated iNOS expression compared with the LPS-treated group (* $p < 0.05$) (Fig. 3, lower panel). Notably, DR-EtOH exhibited greater inhibitory potency than FR-EtOH, in suppressing iNOS expression., DR-EtOH, with near-complete suppression of iNOS expression at 500 $\mu\text{g/mL}$, restoring levels comparable to the normal control. These findings suggest that the observed reduction in NO production is primarily mediated through downregulation of iNOS expression.

3.5 Effect of DR-EtOH and FR-EtOH on intestinal barrier integrity

The protective effects of red curry paste extracts against inflammation-induced intestinal barrier dysfunction were evaluated using a Caco-2/RAW 264.7 co-culture model. Barrier integrity was assessed by measuring Transepithelial Electrical Resistance (TEER). As shown in Fig. 4, LPS stimulation (10 ng/mL) significantly reduced TEER values from 336.7 ± 11.02 to $232.0 \pm 10.15 \Omega \times \text{cm}^2$ (& $p < 0.05$), indicating disruption of epithelial barrier function. Pre-treatment with FR-EtOH at 125 and 500 $\mu\text{g/mL}$ significantly attenuated this decline, as evidenced by a partial recovery of TEER values (* $p < 0.05$ vs. LPS control). In contrast, FR-EtOH at 250 $\mu\text{g/mL}$ and all tested concentrations of DR-EtOH did not produce significant protective effects, with TEER values remaining reduced. These results suggest that FR-EtOH, at specific concentrations, can preserve intestinal barrier integrity under inflammatory conditions.

3.6 Effects of DR-EtOH and FR-EtOH on LPS-induced IL-8 secretion in a co-culture model

The anti-inflammatory effects of DR-EtOH and FR-EtOH on intestinal epithelial inflammation were further evaluated by measuring IL-8 secretion in a Caco-2/RAW 264.7 co-culture system. As shown in Fig. 5, LPS stimulation (10 ng/mL) of the basolateral compartment significantly increased IL-8

levels from approximately 15 pg/mL to over 60 pg/mL (& $p < 0.05$), confirming induction of an inflammatory response in the epithelial monolayer. Pre-treatment with FR-EtOH significantly attenuated IL-8 secretion, with the strongest effect observed at 125 $\mu\text{g/mL}$, reducing IL-8 levels (* $p < 0.05$) to approximately 40 pg/mL. This reduction was consistent with the improved barrier integrity indicated by increased TEER values (Fig. 4). Moreover, FR-EtOH at 125 and 500 $\mu\text{g/mL}$ exhibited significantly greater inhibitory effects than DR-EtOH at corresponding concentrations (# $p < 0.05$). In contrast, DR-EtOH did not significantly reduce IL-8 secretion at any tested concentration. These findings suggest that FR-EtOH possesses greater efficacy in mitigating intestinal epithelial inflammation.

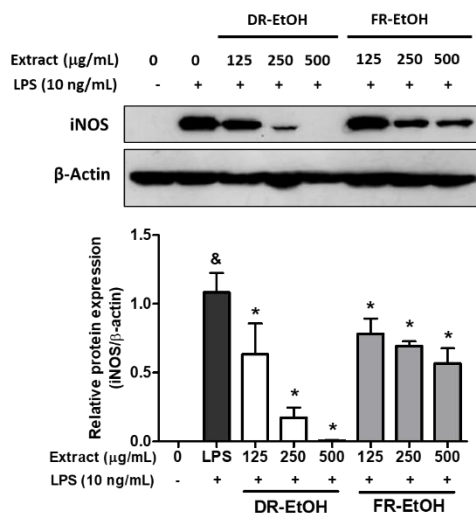


Fig. 3 Effects of DR-EtOH and FR-EtOH (125–500 $\mu\text{g/mL}$) on inducible nitric oxide synthase (iNOS) protein expression in LPS-stimulated after 24 hours treatment. Representative Western blot images of iNOS and β -actin are shown (upper panel), and relative protein expression levels quantified by densitometric analysis normalized to β -actin are presented (lower panel). Data are expressed as mean $\pm$ SD from three independent experiments. & $p < 0.05$ vs. normal control group (0 $\mu\text{g/mL}$); * $p < 0.05$ vs. LPS-stimulated group.

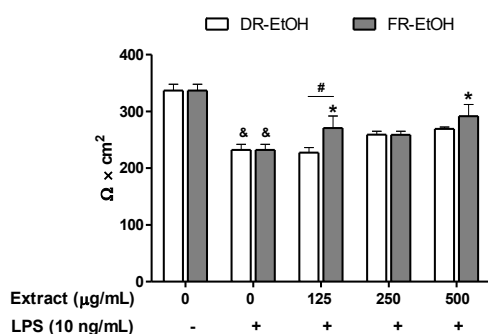


Fig. 4 TEER of Caco-2 intestinal monolayer determined by Millicell Electrical Resistance System after being co-cultured with LPS-activated RAW 264.7 treated with DR-EtOH and FR-EtOH for 24 hours. Each value indicates the mean $\pm$ SD of triplicate sample. & $p < 0.05$ vs. normal control group; * $p < 0.05$ vs. LPS-stimulated group; # $p < 0.05$ between DR-EtOH and FR-EtOH.

4. Discussion

The potent biological activities of Southern Thai red curry paste can be attributed to its complex matrix of secondary metabolites derived from multiple herbal components, including chilies, garlic, lemongrass, shallots, pepper, and turmeric. Rather than acting as isolated compounds, these extracts comprise a synergistic mixture of phytochemicals that collectively contribute to their bioactivity. Alcoholic solvents, particularly ethanol, have been reported to be more efficient than aqueous solvents in extracting phenolic and flavonoid compounds.¹¹ This is consistent with the present findings, where ethanolic extracts exhibited superior anti-inflammatory activity compared to water-based extracts.⁹ Previous studies have identified key flavonoids in ethanolic red curry paste extracts, including quercetin, kaempferol, luteolin, and apigenin, along with carotenoids such as lutein, β -carotene, and α -carotene, which are largely responsible for anti-inflammatory effects in macrophages.¹² In addition, the presence of bioactive compounds from individual ingredients further enhances biological efficacy, including allicin from garlic,¹³ capsaicin

from chilies¹⁴ and linalool oxide from lemongrass.¹⁵ Notably, the inclusion of turmeric (*Curcuma longa*) distinguishes Southern Thai curry formulations, providing curcuminoids¹⁶ that have been shown to exert protective effects on intestinal epithelial integrity under inflammatory conditions in similar co-culture models.¹⁷

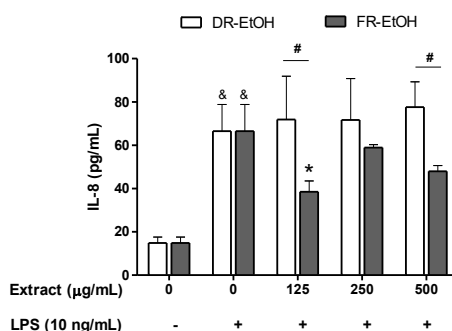


Fig. 5 IL-8 secretion in the Caco-2/RAW 264.7 co-culture system. The LPS-activated RAW 264.7 cells at the basolateral side were treated with DR-EtOH and FR-EtOH for 24 hours. Cell culture supernatants from the apical side were collected and IL-8 levels were determined by ELISA. Each value indicates the mean $\pm$ SD of triplicate samples. & $p < 0.05$ vs. normal control group; * $p < 0.05$ vs. LPS-stimulated group; # $p < 0.05$ between DR-EtOH and FR-EtOH.

Notably, the functional efficacy of these extracts is influenced by processing conditions, as thermal drying can significantly alter phytochemical profiles. For examples, hot-air drying of turmeric rhizomes (60 – 80°C) has been reported to increase total phenolic content,¹⁸ while drying of chili peppers can enhance capsaicin levels by up to 6-fold compared with fresh samples.¹⁹ In contrast, thermal processing of garlic reduces allicin content, thereby diminishing its capacity to inhibit NO and pro-inflammatory cytokine production.¹³ These observations indicate that thermal drying may degrade heat-sensitive compounds or volatile compounds while concentrating more heat-stable constituents.^{10,18} Consequently, fresh and dried curry paste extracts are enriched with distinct profiles of bioactive

compounds. Dried forms are likely enriched in heat-stable compounds such as curcuminoids and capsaicin, whereas fresh forms retain volatile and labile constituents. Consistent with this, the present study demonstrated distinct differences in anti-inflammatory efficacy between fresh and dried extracts in both RAW 264.7 macrophages and inflamed intestinal epithelial co-culture models.

Nitric oxide (NO) is a key mediator of inflammation in gastrointestinal disorders, including ulcerative colitis (UC) and Crohn's disease (CD). Clinical studies have reported that iNOS expression and NO production elevated by more than eight-fold in intestinal tissues of patients with IBD, with a stronger association observed in UC than in CD.²⁰ Although NO is considered a non-specific biomarker, it remains a reliable indicator of inflammatory status in *in vitro* models. In the present study, DR-EtOH at concentrations of 250–500 µg/mL significantly suppressed iNOS expression and NO production in LPS-stimulated RAW 264.7 macrophages. These findings are consistent with previous reports demonstrating that ethanolic extracts of Thai chili paste inhibit inflammatory mediators in macrophages.¹² The greater inhibitory effect on NO/iNOS pathway of DR-EtOH compared to FR-EtOH may be attributed to higher levels of heat-stable bioactive compounds, such as curcuminoids and capsaicin.^{18,19} These compounds are known to reduce intracellular reactive oxygen species (ROS) levels, thereby suppressing activation of nuclear factor-kappa B (NF-κB), a key transcription factor regulating the expression of pro-inflammatory genes, including iNOS, cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines.^{21,22}

Macrophage-derived TNF-α is a critical mediator of intestinal epithelial barrier dysfunction, primarily through the downregulation of tight junction proteins and increased epithelial permeability.²³ In the present study, FR-EtOH exhibited greater

inhibitory effects on TNF-α production in LPS-stimulated RAW 264.7 macrophages compared with DR-EtOH. This suppression of TNF-α was associated with preservation of epithelial barrier integrity, as evidenced by improved (TEER) values, and a concomitant reduction in IL-8 secretion, indicating attenuation of intestinal inflammatory responses. Importantly, RAW 264.7 cell viability remained above 90% relative to the control group, confirming that the observed anti-inflammatory effects were not attributable to cytotoxicity or experimental artifacts.

The effects of FR-EtOH on intestinal barrier integrity may be attributed to preservation of heat-sensitive and volatile phytochemicals that are susceptible to degradation during thermal drying.^{10,24,25} FR-EtOH is likely enriched with labile bioactive compounds, including organosulfur constituents from garlic and shallots, as well as volatile essential oils such as citral from lemongrass. These compounds have been reported to modulate key inflammatory signaling pathways, including NF-κB and mitogen-activated protein kinase (MAPK), thereby suppressing the expression of pro-inflammatory mediators such as IL-8.²⁶ In addition, these phytochemicals contribute to maintenance of intestinal epithelial barrier integrity through preservation of tight junction proteins, including zonula occludens-1 (ZO-1) and occludin, resulting in sustained TEER values under inflammatory conditions.²⁷⁻²⁹ Several of these compounds have also been associated with gastrointestinal protective effects.^{30,31} Therefore, the present findings suggest that FR-EtOH exerts direct modulatory effects on epithelial inflammation and barrier function, whereas degradation of these labile compounds during drying may account for the reduced protective activity observed in DR-EtOH. However, the protective effects of FR-EtOH on the TNF-α–TEER–IL-8 axis did not demonstrate a clear dose-dependent pattern, unlike the concentration-dependent

inhibition observed for NO production and iNOS expression. This discrepancy may be attributed to differences in the underlying intracellular signaling mechanisms as well as the phenomenon of hormesis. The regulation of NO production and iNOS expression is primarily mediated through the NF- κ B signaling pathway³² which often exhibits a predictable dose-dependent response following modulation by bioactive compounds. In contrast, the TNF- α –TEER–IL-8 axis involves multiple interconnected signaling pathways, including MAPK, NF- κ B, and oxidative stress-related pathways, which may collectively contribute to non-linear biological responses.²⁶ Furthermore, the observed response may reflect hormetic behavior, whereby phytochemicals exert biphasic effects, producing optimal protective activity at specific concentrations while showing reduced efficacy at higher or lower doses.^{33,34} Despite these promising findings, the absence of comparative phytochemical profiling between fresh and dried extracts represent an important limitation of the present study. Future investigations should focus on comprehensive characterization of both volatile and non-volatile bioactive constituents to better elucidate the relationship between processing conditions and biological activity, thereby facilitating optimization of extraction strategies for functional food and nutraceutical applications.

5. Conclusion

This study demonstrates the significant anti-inflammatory potential of Southern Thai red curry paste extracts and highlights their promise as functional food ingredients for gastrointestinal health applications. The findings revealed that fresh and dried ethanolic extracts exert distinct anti-inflammatory activities depending on the processing form. The dried extract (DR-EtOH) showed greater efficacy in suppressing macrophage-derived pro-inflammatory mediators, particularly NO production and iNOS expression, whereas

the fresh extract (FR-EtOH) provided superior protection against inflammation-induced intestinal epithelial dysfunction in the Caco-2/RAW 264.7 co-culture model through modulation of the TNF- α –TEER–IL-8 signaling axis. Collectively, these findings provide scientific evidence supporting the development of Southern Thai curry paste-based nutraceuticals as potential dietary interventions for the prevention or mitigation of IBD and other inflammatory gastrointestinal disorders.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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