Protective Effect of Sweetpotato (Ipomoea batatas L.) Leaf Phenolic Acids Extract on IL-1?-Induced Barrier Injury of Caco-2 Monolayers

Authors:

Yi Zhang, Jian Sun, Lu Zhao, Fuxiang Niu, Ruixue Yue, Hong Zhu, Wenting Zhang, Chen Ma

Date Submitted: 2023-02-20

Keywords: sweetpotato leaf, phenolic acids, Caco-2 monolayers, barrier injure, tight junction protein, anti-inflammatory

Abstract:

The status quo of a high-fat diet can impair the integrity of the intestinal barrier and promote inflammatory bowel diseases. The exploration of natural products as sources of new lead compounds that control such diseases becomes essential. Sweetpotato leaves (SPLs) have strong biological activity, and their phenolic acids were recently reported to be able to impart antioxidative, anti-inflammatory, and neuroprotection. The aim of this study was to investigate the effects and mechanisms of sweetpotato leaf phenolic acids (SPLPAs) extract on interleukin 1 beta (IL-1?)-induced barrier injury of Caco-2 monolayers. The safety from 0.02 to 0.2 mg/mL SPLPA extracts were demonstrated using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method and Trypan blue staining. The 0.2 mg/mL SPLPA extract significantly decreased the release of Nitric oxide (NO) generation and its contribution to the expression of inflammation-related nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNF-?), interleukin 6 (IL-6), and nuclear factor kappa B (NF-?B) were evaluated. Further investigation suggested that the SPLPA extract inhibited the IL-1?-induced decrease in the transepithelial electrical resistance (TEER) value and could upregulate the IL-1? induced decline in tight junction protein levels. The results suggested that the SPLPA extract could enhance the integrity of Caco-2 monolayers. These results clarified the beneficial effects of SPLPA extract on inflammatory Caco-2 monolayers, indicating that the application of SPLPA extract in medicine and functional food has great potential for exploration.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):	LAPSE:2023.0730
Citation (this specific file, latest version):	LAPSE:2023.0730-1
Citation (this specific file, this version):	LAPSE:2023.0730-1v1

DOI of Published Version: https://doi.org/10.3390/pr10112211

processes

MDPI

Article

Protective Effect of Sweetpotato (*Ipomoea batatas* L.) Leaf Phenolic Acids Extract on IL-1β-Induced Barrier Injury of Caco-2 Monolayers

Yi Zhang [1], Jian Sun [1,*], Lu Zhao [2,*], Fuxiang Niu [1], Ruixue Yue [1], Hong Zhu [1], Wenting Zhang [1] and Chen Ma [1]

[1] Xuzhou Institute of Agricultural Sciences in Jiangsu Xuhuai District, Xuzhou 221131, China
[2] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, School of Pharmacy, Xuzhou Medical University, Xuzhou 221004, China
* Correspondence: sjsg9902@126.com (J.S.); zhaolu850813@163.com (L.Z.)

Abstract: The status quo of a high-fat diet can impair the integrity of the intestinal barrier and promote inflammatory bowel diseases. The exploration of natural products as sources of new lead compounds that control such diseases becomes essential. Sweetpotato leaves (SPLs) have strong biological activity, and their phenolic acids were recently reported to be able to impart antioxidative, anti-inflammatory, and neuroprotection. The aim of this study was to investigate the effects and mechanisms of sweetpotato leaf phenolic acids (SPLPAs) extract on interleukin 1 beta (IL-1β)-induced barrier injury of Caco-2 monolayers. The safety from 0.02 to 0.2 mg/mL SPLPA extracts were demonstrated using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method and Trypan blue staining. The 0.2 mg/mL SPLPA extract significantly decreased the release of Nitric oxide (NO) generation and its contribution to the expression of inflammation-related nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and nuclear factor kappa B (NF-κB) were evaluated. Further investigation suggested that the SPLPA extract inhibited the IL-1β-induced decrease in the transepithelial electrical resistance (TEER) value and could upregulate the IL-1β induced decline in tight junction protein levels. The results suggested that the SPLPA extract could enhance the integrity of Caco-2 monolayers. These results clarified the beneficial effects of SPLPA extract on inflammatory Caco-2 monolayers, indicating that the application of SPLPA extract in medicine and functional food has great potential for exploration.

Keywords: sweetpotato leaf; phenolic acids; Caco-2 monolayers; barrier injure; tight junction protein; anti-inflammatory

Citation: Zhang, Y.; Sun, J.; Zhao, L.; Niu, F.; Yue, R.; Zhu, H.; Zhang, W.; Ma, C. Protective Effect of Sweetpotato (*Ipomoea batatas* L.) Leaf Phenolic Acids Extract on IL-1β-Induced Barrier Injury of Caco-2 Monolayers. *Processes* **2022**, *10*, 2211. https://doi.org/10.3390/pr10112211

Academic Editors: Changling Hu, Yao Tang and Jia Xiong

Received: 25 September 2022
Accepted: 20 October 2022
Published: 27 October 2022

1. Introduction

Commonly higher in fat content, most processed foods are easier to obtain, with the development of society and the economy. In addition to popular processed foods, many dishes among different cultures and ethnicities contain ingredients with a large amount of fat to increase flavor and appeal. High-fat diet-induced excess cytokines can result in detrimental outcomes due to the effects of its high-fat content on intestinal epithelial cells, thus damaging the integrity of the intestinal barrier and promoting inflammatory bowel diseases [1,2]. Possible mechanisms by which excessive dietary fats reduce intestinal epithelial cell viability and weaken the intestinal barrier include inflammation triggered by these cellular irritants [3–6]. Therefore, maintaining the function and integrity of the intestinal epithelium can be an important factor in preventing high-fat diet-related diseases [7]. The Caco-2 cell line is a human intestinal epithelial cell model used as a model of the intestinal epithelial barrier and intestinal inflammation [8,9], and the barrier integrity was measured by TEER [10]. It is known that natural products from plant origin can inhibit the TNF-α induced loss of caco-2 cell barrier integrity [11,12].

Sweetpotato (*Ipomoea batatas* L.) is widely produced and consumed in Asia, Sub-Saharan Africa, and Oceania. According to FAO statistics, China's sweetpotato production comprises 54.97% of the world's total [13]. Presently, only a few SPLs are utilized, and most of the SPLs are wasted and pollute the environment. There are high nutritional value and powerful health functions in SPLs [14], such as phenolic acids, flavonoids, anthocyanins, and carotenoids, which are important for health [15]. Phenolic acids (PAs), with a carboxylic acid group, are one of the most important functional components of the variety of plant-based foods, viz. the skins of fruits and the leaves of vegetables contain them in the highest concentrations [16]. Typically, phenolic acids are present in the form of amides, esters, or glycosides, rarely in the free form [17], which are mainly divided into two sub-groups: hydroxybenzoic and hydroxycinnamic acid [18]. (1) Hydroxycinnamic acids (C6-C3): caffeic, p-hydroxycinnamic, and sinapic acids are the most common hydroxycinnamic acids. The most abundant soluble bound hydroxycinnamic acid mainly consists of caffeic acid as well as mono-, di-, and tri-O-caffeoylquinic acids (CQAs), which are ester compounds of caffeic acid with quinic acid [14,19]. (2) Hydroxybenzoic acids (C6-C1): hydroxybenzoic acids are p-hydroxybenzoic, vanillic, and syringic acids are the most common hydroxybenzoic acids. Hydroxybenzoic acid derivatives are found in a soluble form and conjugated with sugars or organic acids [20]. Due to their antioxidative, anti-inflammatory, antimicrobial, and neuroprotective effects, the health benefits of PAs have recently attracted particular interest [21–23]. Most of the studies showed that SPLs are naturally rich in PAs and the identification of 20 different PAs by HPLC-QTOF-MS^2 [24]. Caffeoylquinic acids were quantitatively the major subgroup of PAs detected in the stem and the only subgroup detected by LC-MS^3 in the leaves of sweetpotato cultivated in China [25]. Phenolic acids in leaves are not only affected by the variety but also by environment and climate, etc. A previous study found that temperature affects the content of PAs in sweetpotato leaves; compared with 25 °C, its content increased at 30 °C [26]. The contents of 4-CQA and 5-CQA in sweetpotato leaves are affected by harvest batches and times [27]. Drought and flooding also led to a decrease in PAs in leaves [28].

The objective of this study was to determine and analyze the purification of PAs from sweetpotato leaves and to prove that SPLPAs attenuate the IL-1β-induced barrier injury of Caco-2 monolayers. It is worth noting that high-phenolic-acids-containing food supplements have been developed to a large extent recently, but the other components in the formulations may modify the bioactivity of PAs. In this study, the SPLPA extract contained PAs in the original ratio, did not contain other components, and might be a good and safe source of PAs for these nutritional products. Thus, we investigated the SPLP extract to provide data for applications on whether it preserves the effectiveness of the restoration of intestinal barrier injury and anti-inflammatory activities.

2. Materials and Methods

2.1. Chemicals and Materials

Macroporous resin AB-8 was purchased from the Chemical Plant of Nankai University (Tianjin, China). Human Caco-2 intestinal epithelial cells were purchased from the Chinese Academy of Sciences cell bank (Shanghai, China), and IL-1β was provided by Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Minimum Essential Medium (DMEM) and fetal bovine serum were obtained from Thermo Fisher Scientific (Gibco, Waltham, MA, USA). Methanol, acetonitrile, and acetic acid were purchased from ANPEL. All of the solvents were of LC–MS grade. The other chemicals and reagents were purchased from Sinopharm, which were AR Grade (Beijing, China). Additionally, using the Milli-Q water purification system prepared μLtra-pure water. Sweetpotatoes (cultivar 'Simon No. 1′) were planted with standard production practice at the Xuzhou Institute of Agricultural Sciences in Jiangsu Xuhuai District (117.29° E, 34.28° N), and the sweetpotato leaves were harvested at the beginning of August 2020.

2.2. Isolation of the Phenolic Acid Compounds from Sweetpotato Leaves

The sweetpotato leaves were washed, freeze-dried (24D × 48 GPFD 25L ES-53°, SP Scientific, NYS, USA), and pulverized into powder. The resulting powder was passed through a 100-mesh sieve (aperture = 149 μm), packaged in a light-proof package, and stored at 4 °C prior to analysis. The method of extraction, separation, and purification was according to Xi et al. with modifications [29]. Briefly, sodium hydrogen sulfite was used to set the pH values of the 70% ethanol solution to 3.0. The powder was extracted with a 78% (v/v) ethanol solution at a ratio of 1:23 (w/v) for 40 min at 50 °C using ultrasonic assistance (THC-300; Tianhua Ultrasonic Electronic Instrument Co., Jining, China). After the solution was centrifuged (ST16R; Thermo Fisher Scientific Inc., Waltham, MA, USA) at 7000× g for 10 min, the residue was re-extracted twice. The supernatants were combined and concentrated in the rotary evaporator (N-1100D, Pailan Instrument Equipment Co., Ltd., Shanghai, China) at 50 °C to obtain the crude polyphenols. The PAs were purified using dynamic adsorption and desorption on AB-8 macroporous resin. The AB-8 resin was mixed with crude polyphenols extraction at a ratio of 1:25 (w/v), and then the mixture was shaken with the immersion oscillator (DKZ-3B, Yiheng Scientific Instrument Co., Ltd., Shanghai, China) (100 r/min) at 25 °C for 24 h. The resins were washed twice using deionized water and desorbed using 78% (v/v) ethanol, and then the supernatant was adjusted to pH 7.0, concentrated in a vacuum, and freeze-dried to obtain SPLPAs extract.

2.3. Composition Analysis of the Sweetpotato Leaves Phenolic Acid Compounds

The method for the determination of the chlorogenic acid isomers in the SPLPA extract was performed according to Jaiswal et al. with modifications [30]. Qualitative and quantitative analyses of the purified SPLPAs extract were carried out using a UPLC-Orbitrap-MS system (UPLC: Vanquish, MS: Q Exactive, Thermo-Fisher Scientific, Waltham, MA, USA). The samples were extracted with 70% aqueous methanol, and the supernatant was collected and filtered on 0.22 μm nylon membrane filters. The separation was completed using a Waters ACQUITY UPLC® HSS T3 (1.8 μm, 2.1 mm × 50 mm). The mobile phase consisted of ultrapure water containing A: water (0.1% acetic acid) and B: Acetonitrile (0.1% acetic acid). The elution was performed with the following linear gradient: 97:3 v/v at 0 min, 97:3 v/v at 1.0 min, 50:50 v/v at 5.0 min, 10:90 v/v at 6.0 min, 10:90 v/v at 7.0 min, 97:3 v/v at 7.1 min, 97:3 v/v at 9.0 min. The column temperature was 40 °C, the flow rate was 0.3 mL/min, and the injection volume was 2 μL. The HRMS data were recorded on a Q Exactive hybrid Q-Orbitrap mass spectrometer equipped with a heated ESI source (Thermo-Fisher Scientific) utilizing single-ion monitoring (SIM) MS acquisition methods. The ESI source parameters were set as follows: The spray voltage was −2.8 kV; the sheath gas pressure was 40 arb; the aux gas pressure was 10 arb; the sweep gas pressure was 0 arb; the capillary temperature was 320 °C; and the aux-gas heater temperature was 350 °C.

The method for the determination of the other phenolic acid compounds in the SPLPA extract was performed according to Zhang et al. with modifications [31]. The samples were hydrolyzed with NaOH at 40 °C in a gas bath with shaking and protection from light. Then, the pH value was adjusted to 2. The mixture was shaken with n-hexane to remove the n-hexane layer. Ethyl acetate was used to extract the aqueous layer, and the mixed extracts were concentrated. Before analysis, the residue was dissolved in 50% methanol/water. The mobile phase consisted of ultrapure water containing A: water (0.1% acetic acid) and B: Acetonitrile (0.1% acetic acid). The elution was performed with the following linear gradient: 90:10 v/v at 0 min, 90:10 v/v at 2.0 min, 40:60 v/v at 6.0 min, 40:60 v/v at 8.0 min, 90:10 v/v at 8.1 min, 90:10 v/v at 12.0 min. The column temperature was 40 °C, the flow rate was 0.3 mL/min, and the injection volume was 2 μL. The HRMS data were recorded on a Q Exactive hybrid Q-Orbitrap mass spectrometer equipped with a heated ESI source (Thermo-Fisher Scientific) utilizing SIM MS acquisition methods. The ESI source parameters were set as described above.

The data were acquired on the Q-Exactive using Xcalibur 4.1 and processed using TraceFinder™ 4.1 Clinical (Thermo-Fisher Scientific). The quantitation of the SPLPA extract was performed using a comparison with external authentic standards.

2.4. Determination of Cell Viability

The Caco-2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. The medium was changed every two days, and the cells were subcultured every week at a 1:20 split ratio by treatment with trypsin-EDTA solution (0.25% trypsin, 10 mM EDTA).

For the viability experiments, the Caco-2 cells were seeded on 96-well plates (Greiner Bio-One, Monroe, NC, USA) at a density of 3×10^4 cells/cm^2. The cells were treated with different concentrations of the SPLPA extract and incubated for 48 h. After this period, the addition of 20 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, 5.0 mg/mL) was carried out, which was incubated at 37 °C for 4 h. The formazan crystals were dissolved in DMSO, and the optical density of the formazan solution was measured at 490 nm using the Bio-Rad iMark microplate reader.

2.5. Caco-2 Monolayers Culture

The Caco-2 intestinal epithelial cells were grown routinely in DMEM medium supplemented with 10% fetal bovine serum. The cell monolayers were grown on 1.12 cm^2 permeable Transwell® polycarbonate filters with a 0.4 µm pore size (Corning, Lowell, MA, USA), and then used in the experiment after forming a fully differentiated fusion monolayer [32]. These samples were used for the examination of the ELISA, Western Blotting, and TEER.

2.6. Nitric Oxide Inhibition Assay and ELISA Measurements

The Griess Assay was used to measure NO production [33]. After being stimulated by IL-1β (10 ng/mL) for 30 min, the Caco-2 cells were treated with SPLPAs for 48 h. Then, 50 µL of the cell culture media was added to 50 µL of the Griess reagent (Beyotime Institute of Biotechnologies, Nanjing, China). The mixed solution was shaken in the dark for 10 min. The optical density was measured at 570 nm using the Bio-Rad iMark microplate reader.

According to the manufacturer's protocols, the commercially available ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to evaluate the contents of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in the cell culture medium.

2.7. Measurement of Transepithelial Electric Resistance

Transepithelial Electric Resistance was used to measure the integrity of the Caco-2 monolayers [34]. The density of 2×10^5 Caco-2 cells/well was inoculated on Corning Transwell® polycarbonate filters for 21 days to prepare the differentiated cell monolayers. Transepithelial electrical resistance (TEER) was measured with a Millicell ERS resistance system (Millicell ERS-2, Millipore, MA, USA). The TEER values were measured every 6 h after the IL-1β treatment.

2.8. Quantitative Real-Time Polymerase Chain Reaction Analysis

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted as described previously [33]. The total RNA of the Caco-2 cells was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). According to the manufacturer's protocol, reverse transcription was performed using a PrimeScript™ RT Master Mix kit (Takara, Shiga, Japan). qRT-PCR was performed using an SYBR Green Premix Ex Taq kit (Takara Biotechnology) and carried out in the LightCyclers 480 qPCR instrument (Roche, Basel, Switzerland).

2.9. Western Blotting

Briefly, the total proteins were extracted from the collected Caco-2 cells by using RIPA and evaluated using a BCA Protein Quantification Kit (Beyotime Biotechnology, Nanjing, China). The supernatants were then separated by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed using Western blotting. For Western blotting, the samples were separated by denaturing SDS–PAGE and transferred to a polyvinylidene difluoride membrane (0.45μm, Merck Millipore, Burlington, MA, USA) by electrophoretic transfer. The membrane was incubated in blocking buffer for 1 h and incubated overnight with primary antibodies, including Nuclear factor kappa B p65 (NF-κB p65), NF-kappa B inhibitor alpha (IκB-α), Zonula Occludens 1 (ZO-1), Claudin 1, Occludin, Lamin B1, and GAPDH (Abcam, Cambridge, UK). The primary antibodies were finally detected using the corresponding secondary antibodies (Odyssey, New York, NY, USA), and the protein band was visualized and quantified using the Odyssey Infrared Imaging System.

2.10. Statistical Analysis

The experiments were performed at least in triplicate. All of the statistical analysis was performed using SPSS software (version 22.0), and the results were expressed as a mean ± standard deviation (Lenovo, Beijing, China). The data were analyzed with one-way ANOVA followed by Tukey's Honestly Significant Difference post hoc test. Statistical significance was set at $p < 0.05$ for all tests.

3. Results

3.1. Characterisation and Quantification of SPLPAs Extract

The peaks were identified using UPLC-Orbitrap-MS analysis and a comparison of the commercial standards of PAs (Figure 1). Table 1 shows the list of compounds, along with their retention time and identification results, in addition to the quantitative analysis. Up to 25 different PAs were detected in the SPLPA extract. The pure extract mainly consisted of six chlorogenic acid isomers and a portion of caffeic acid, which was similar to several previous reports [14,19,29]. The isochlorogenic acid A (40.78 ± 1.51% DW) content was the highest. The content of isochlorogenic acid B was 8.51 ± 0.08% DW, which was lower than the isochlorogenic acid C (12.43 ± 0.25% DW) but higher than the chlorogenic acid (7.57 ± 0.14% DW) and the cryptochlorogenic acid (3.78 ± 0.02% DW), while the neochlorogenic acid content (1.64 ± 0.02% DW) was the lowest among the chlorogenic acid isomers. The total content of these six chlorogenic acid constituents was 74.70 ± 1.83% DW. Among the other PAs, the caffeic acid had the highest concentration of 10.02 ± 0.09% DW, and the following was the benzoic acid (0.25 ± 0.01% DW), while the other seventeen phenolic acid concentrations were less than 0.2% DW. The total content of these nineteen constituents was 10.95 ± 10.09% DW. The experiment was repeated three times, and 5.67 ± 0.26 g of the purified products were obtained from 500 g of SPL powder under optimal conditions, with polyphenol purifications of 85.65 ± 1.77% DW, and the results had good reproducibility.

3.2. Cytotoxic Effect of SPLPAs Extract on the Caco-2 Cells and the Concentration Dependence of Inhibiting the NO Generation Action

Firstly, the effects on the cell viability of the Caco-2 cells at different concentrations (0.02–5 mg/mL) were determined using the MTT method to screen out the effective concentration of the compounds with inflammation inhibitory activity. As shown in Figure 2a, the SPLPA extracts were not cytotoxic to the Caco-2 cells at concentrations from 0.02 to 0.2 mg/mL, but the cell viability was observably reduced at 1 mg/mL and 5 mg/mL, with 37.0% and 41.8% inhibition, respectively. Staining the Caco-2 cells with trypan blue showed that the cells remained viable at concentrations from a dose of 0.02 to 0.2 mg/mL SPLPA extract. Nitric oxide (NO), which is induced by IL-1β, is an important pro-inflammatory mediator released during the inflammatory process. Thus, doses of the SPLPA extracts at 0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL were used for the subsequent NO-inhibition

assay, and the NO level in the culture media was determined using the Griess assay. The IL-1β treatment for 48 h resulted in a significant increase in the NO level in the Caco-2 cells, and the SPLPA extract dose-dependently suppressed IL-1β-induced NO generation in the Caco-2 cells (Figure 2b). Therefore, 0.1 mg/mL and 0.2 mg/mL of the SPLPA extracts could significantly reduce the NO level ($p < 0.05$), the dose of the SPLPA extract at 0.2 mg/mL performed best, and the dose of SPLPA extract at 0.05 mg/mL showed little obvious NO-inhibitory activity. These data confirmed the NO-inhibitory ability of the SPLPA extract, and its anti-inflammatory activity against Caco-2 monolayers was further studied. In this study, a dose of 0.2 mg/mL SPLPA extract was used for the subsequent experiments.

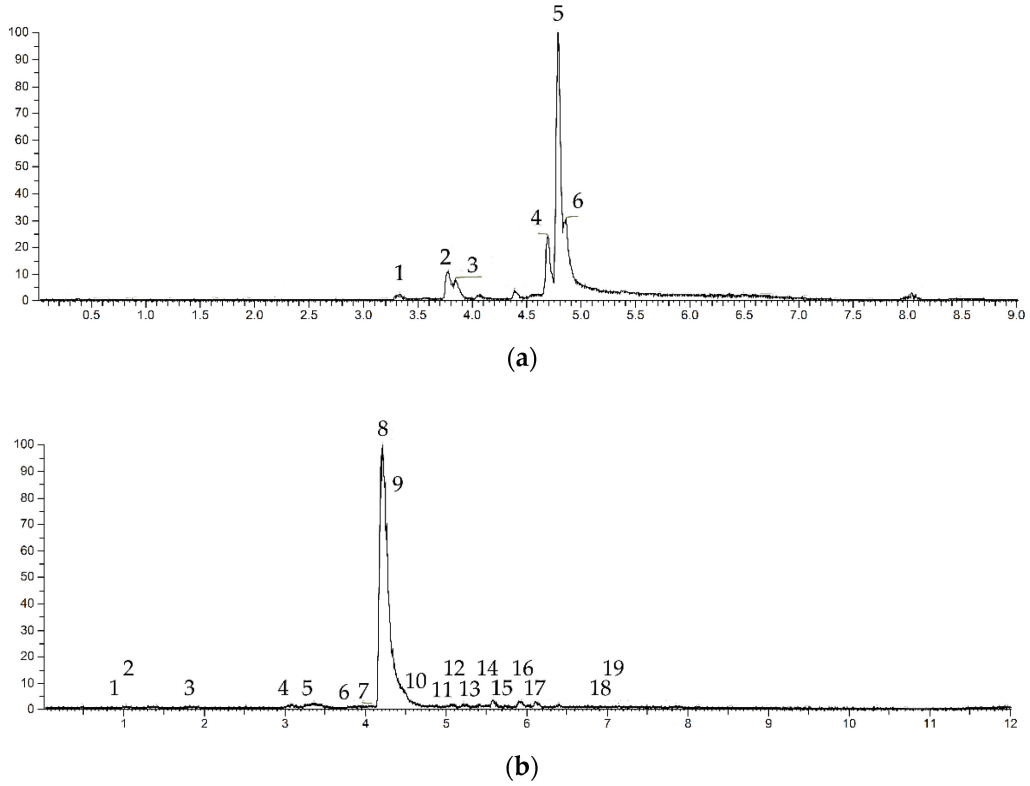

Figure 1. Total ion chromatography of SPLPAs extract. Peaks were identified by UPLC-Orbitrap-MS under negative ion mode. (**a**): The ion chromatograms of chlorogenic acid isomers in SPLPAs extract. (**b**): The ion chromatograms of other phenolic acid compounds in SPLPAs extract.

3.3. Anti-Inflammatory Evaluation of SPLPAs Extract in the IL-1β-Treated Caco-2 Monolayers

The influence of the SPLPA extract on other inflammatory mediators was tested by ELISA and Quantitative Real-time qPCR analyses. Firstly, the regulatory effect of the SPLPA extract on the levels of TNF-α and IL-6 was evaluated, respectively. As shown in Figure 3a,b, IL-1β stimulation significantly increased the contents of endogenous TNF-α as well as IL-6 in the cell culture supernatant, and the influence was significantly inhibited by the SPLPA extract ($p < 0.01$). A similar regulatory effect of the SPLPA extract was also observed through the Quantitative Real-time qPCR evaluation (Figure 3d,e), which tested the mRNA level of TNF-α as well as IL-6 in the IL-1β-treated Caco-2 cells.

Table 1. Quantitated PAs from SPLPAs extract. Results expressed as mean ± standard deviation (n = 3).

Peak No.	Retention Time (min)	Identification	Standard Curve	R^2	Peak Area	Content (%, DW)
1	3.33	Neochlorogenic acid	$Y = 6.524 \times 10^4X$	0.9998	$4.43 \times 10^6 \pm 6.62 \times 10^4$	1.64 ± 0.02
2	3.77	Chlorogenic acid	$Y = 4.08 \times 10^4X$	0.9994	$1.28 \times 10^7 \pm 2.43 \times 10^5$	7.57 ± 0.14
3	3.84	Cryptochlorogenic acid	$Y = 6.487 \times 10^4X$	0.9990	$1.02 \times 10^7 \pm 6.34 \times 10^5$	3.78 ± 0.02
4	4.69	Isochlorogenic acid B	$Y = 6.183 \times 10^4X$	0.9977	$2.18 \times 10^7 \pm 2.05 \times 10^5$	8.51 ± 0.08
5	4.78	Isochlorogenic acid A	$Y = 4.576 \times 10^4X$	0.9993	$7.74 \times 10^7 \pm 2.86 \times 10^6$	40.78 ± 1.51
6	4.85	Isochlorogenic acid C	$Y = 6.036 \times 10^4X$	0.9989	$3.11 \times 10^7 \pm 6.28 \times 10^5$	12.43 ± 0.25
Total chlorogenic acid isomers						74.70 ± 1.83
1	0.98	Gallic acid	$Y = 4.272 \times 10^3X$	0.9995	21.42 ± 0.25	$1.73 \times 10^{-3} \pm 2.03 \times 10^{-5}$
2	1.07	Phenylalanine	$Y = 3.716 \times 10^4X$	0.9966	11.78 ± 0.50	$9.50 \times 10^{-4} \pm 4.01 \times 10^{-5}$
3	1.82	3,4-Dihydroxybenzoic acid	$Y = 8.246 \times 10^4X$	0.9997	$1.33 \times 10^3 \pm 26.95$	$0.11 \pm 2.17 \times 10^{-3}$
4	3.08	Protocatechualdehyde	$Y = 1.492 \times 10^5X$	0.9996	$1.37 \times 10^3 \pm 17.49$	$0.11 \pm 1.41 \times 10^{-3}$
5	3.37	p-Hydroxybenzoic acid	$Y = 1.392 \times 10^5X$	0.9992	$1.45 \times 10^3 \pm 58.42$	$0.12 \pm 4.71 \times 10^{-3}$
6	3.88	Catechin	$Y = 1.08 \times 10^5X$	0.9995	0.31 ± 0.01	$2.51 \times 10^{-5} \pm 4.25 \times 10^{-7}$
7	4.07	Vanillic acid	$Y = 9.867 \times 10^4X$	0.9994	488.41 ± 14.12	$3.94 \times 10^{-2} \pm 1.14 \times 10^{-3}$
8	4.21	Caffeic acid	$Y = 1.406 \times 10^5X$	0.9995	$1.24 \times 10^5 \pm 1.11 \times 10^3$	10.02 ± 0.09
9	4.44	Syringic acid	$Y = 9.361 \times 10^4X$	0.9981	18.44 ± 0.24	$1.49 \times 10^{-3} \pm 1.94 \times 10^{-5}$
10	4.74	L-Epicatechin	$Y = 1.292 \times 10^5X$	0.9999	0.43 ± 0.01	$3.44 \times 10^{-5} \pm 8.40 \times 10^{-7}$
11	5.04	Vanillin	$Y = 9.974 \times 10^4X$	0.9993	100.68 ± 0.36	$8.12 \times 10^{-3} \pm 2.92 \times 10^{-5}$
12	5.23	p-Hydroxycinnamic Acid	$Y = 5.275 \times 10^4X$	0.9977	$1.00 \times 10^3 \pm 34.90$	$0.08 \pm 2.81 \times 10^{-3}$
13	5.38	Syringaldehyde	$Y = 1.473 \times 10^5X$	0.9985	6.78 ± 0.08	$5.47 \times 10^{-4} \pm 6.30 \times 10^{-6}$
14	5.47	Salicylic acid	$Y = 2.891 \times 10^5X$	0.9987	116.68 ± 6.67	$9.41 \times 10^{-3} \pm 5.38 \times 10^{-4}$
15	5.58	Trans-Ferulic acid	$Y = 8.546 \times 10^4X$	0.9971	$2.24 \times 10^3 \pm 30.79$	$0.18 \pm 2.48 \times 10^{-3}$
16	5.63	Sinapic Acid	$Y = 1.211 \times 10^5X$	0.9961	122.66 ± 4.69	$9.89 \times 10^{-3} \pm 3.78 \times 10^{-4}$
17	5.93	Benzoic acid	$Y = 7.26 \times 10^4X$	0.9970	$3.15 \times 10^3 \pm 124.93$	0.25 ± 0.01
18	7.04	Hydrocinnamic acid	$Y = 9.492 \times 10^4X$	0.9986	0.91 ± 0.03	$7.38 \times 10^{-5} \pm 2.12 \times 10^{-6}$
19	7.14	Trans-Cinnamic acid	$Y = 7.65 \times 10^4X$	0.9987	82.03 ± 1.89	$6.62 \times 10^{-3} \pm 1.53 \times 10^{-4}$
Total other phenolic acid compounds						10.95 ± 0.09
Total PAs						85.65 ± 1.77

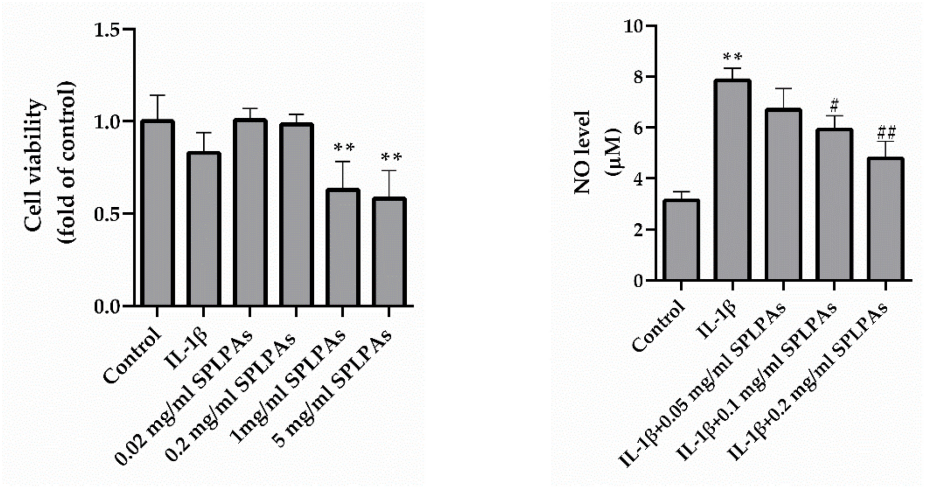

Figure 2. Cytotoxic and NO-inhibitory effect of SPLPA extract. (**a**): Cytotoxic effect of SPLPAs extract at 0.02–5 mg/mL. (**b**): NO inhibitory effect of SPLPAs extract at 0.02 to 0.2 mg/mL. ** $p < 0.01$ vs. the control group. ** $p < 0.01$ vs. the control group. # $p < 0.05$ and ## $p < 0.01$ vs. the IL-1β (10 ng/mL) group.

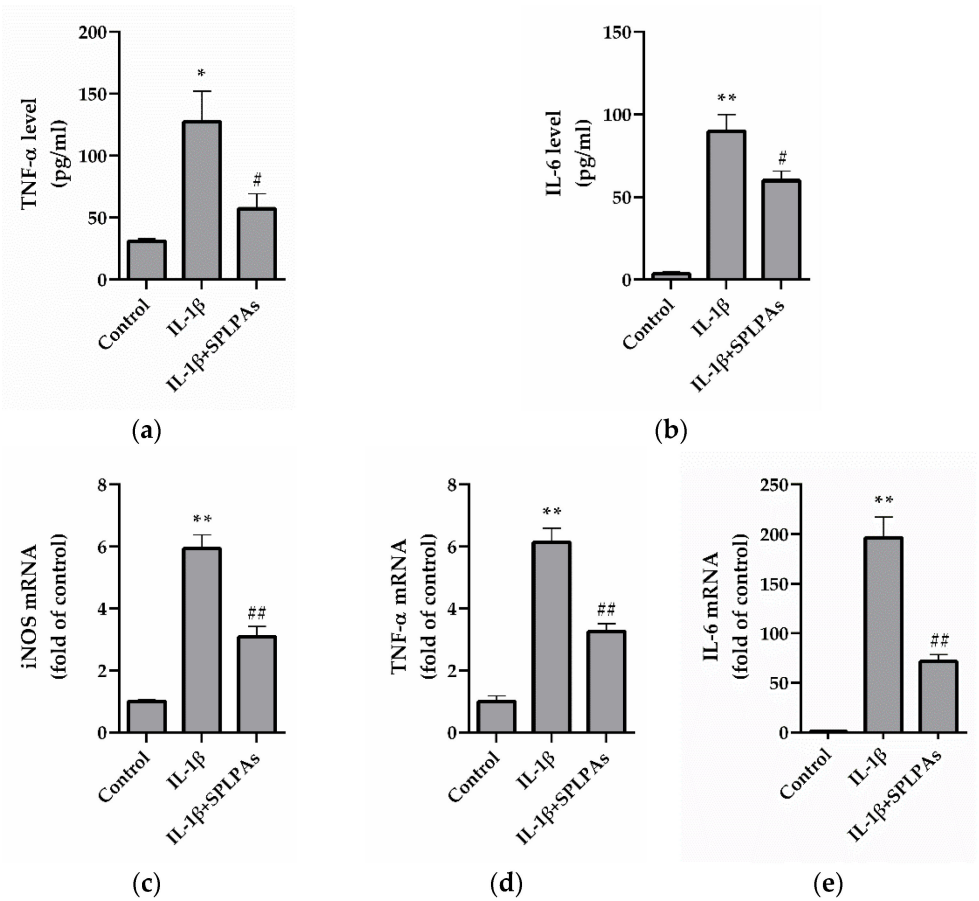

Figure 3. The influence of SPLPA extract on other inflammatory mediators by ELISA and Quantitative Real-time qPCR analyses. (**a**,**b**): Effect of SPLPA extract on the level of TNF-α and IL-6 at 0.2 mg/mL. (**c**–**e**): Effect of SPLPAs extract on the mRNA levels of iNOS, TNF-α, and IL-6 at 0.2 mg/mL. * $p < 0.05$ and ** $p < 0.01$ vs. the control group; # $p < 0.05$ and ## $p < 0.01$ vs. the IL-1β (10 ng/mL) group.

Inflammation-related nitric oxide synthase (iNOS), an isoform of nitric oxide synthase, is known to regulate the generation of NO in cells, and the mRNA level of iNOS was detected by qPCR. As shown in Figure 3c, the increased mRNA level of iNOS after IL-1β

stimulation was significantly reversed by the SPLPA extract ($p < 0.01$), which was consistent with the NO-inhibitory activity of the SPLPA extract as described above.

3.4. Effect of SPLPAs Extract on the Protein Expression of NF-κB and Tight Junctions

Nuclear factor kappa B (NF-κB) is a key transcription factor that plays a central role in inflammation by regulating the expression of various inflammatory cytokines such as NO and TNF-α, and nuclear translocation and activation of NF-κB are negatively regulated by IκB. As shown in Figure 4a–c, IL-1β treatment significantly increased the protein level of p65 in the nucleus and reduced the level of IκBα in the cytoplasm at the same time. SPLPAs extract observably reversed the increased p65 nuclear translocation and IκBα degradation. These results suggested that SPLPAs extract possesses comparatively strong inflammation effect.

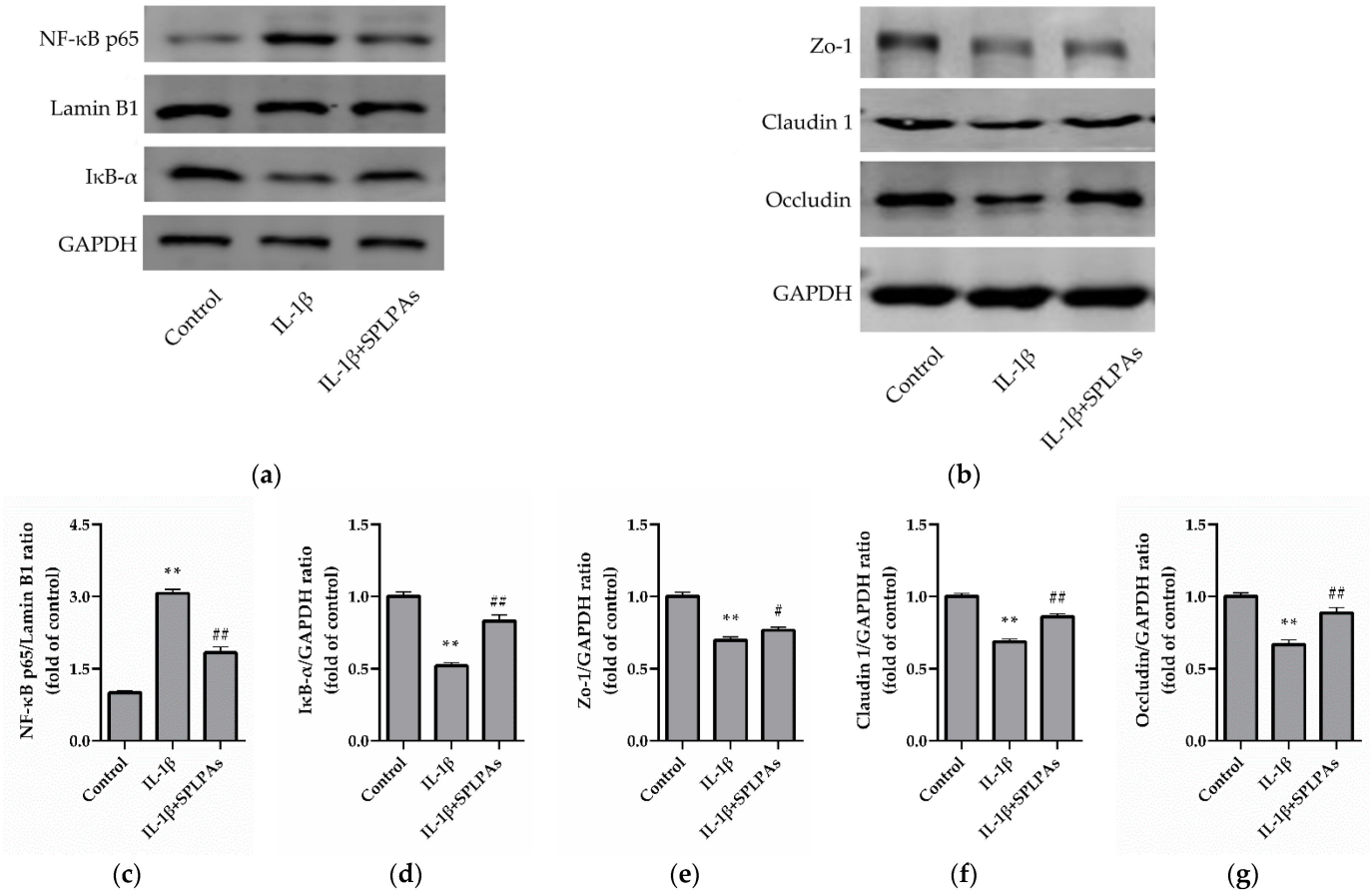

Figure 4. Effect of SPLPAs extract on the protein expression of NF-κB and tight junctions. (**a**–**c**): Effect of SPLPAs extract on the protein expression of p65 in the nucleus and IKB1 in the cytoplasm. Caco-2 cells were treated with 1 for 48 h after IL-1β stimulation, and the protein level was visualized and quantified by Western blotting. (**d**–**g**): Effect of SPLPAs extract on the expression of Zo-1, Claudin 1 and Occludin in Caco-2 monolayers. Caco-2 cells were treated with 1 for 48 h after IL-1β stimulation, and the protein level was visualized and quantified by Western blotting. ** $p < 0.01$ vs. the control group. # $p < 0.05$ and ## $p < 0.01$ vs. the IL-1β (10 ng/mL) group.

Tight junctions are integral components of the intestinal barrier, and dysregulation of tight junctions leads to the defect of epithelial barrier function. To determine whether SPLPAs extract may regulate the tight junction proteins, the protein levels of tight junctions -related proteins ZO-1, Claudin 1 and Occludin were detected by Western blot. These results showed that SPLPAs extract treatment for 48 h significantly upregulated ZO-1, Claudin 1 and Occludin protein levels in Caco-2 cells (Figure 4d–g).

3.5. Effect of SPLPAs Extract on the Integrity of Caco-2 Monolayers

To undoubtedly confirm the intestinal epithelial barrier function, a TEER assay was performed to evaluate whether the SPLPA extract may enhance the integrity of the Caco-2 cell monolayers. In the beginning, the TEER value of each group fluctuated at a baseline value (440 Ω cm^2) (Figure 5a). The treatment with IL-1β decreased the resistance of Caco-2 monolayers to 53.22% ($p < 0.01$) after 6 h, 53.78% ($p < 0.01$) 12 h and 53.64% ($p < 0.01$) 24 h later compared to the TEER changes in the control group (Figure 5b-d). However, compared with the IL-1β group, the TEER values of the SPLPA extract treatment group increased by 85.91%, 81.40%, and 79.17%, respectively. The results showed that the SPLPA extract inhibited the IL-1β-induced decrease in the TEER value, indicating that the SPLPA extract reduced intercellular permeability and enhanced the integrity of Caco-2 monolayers.

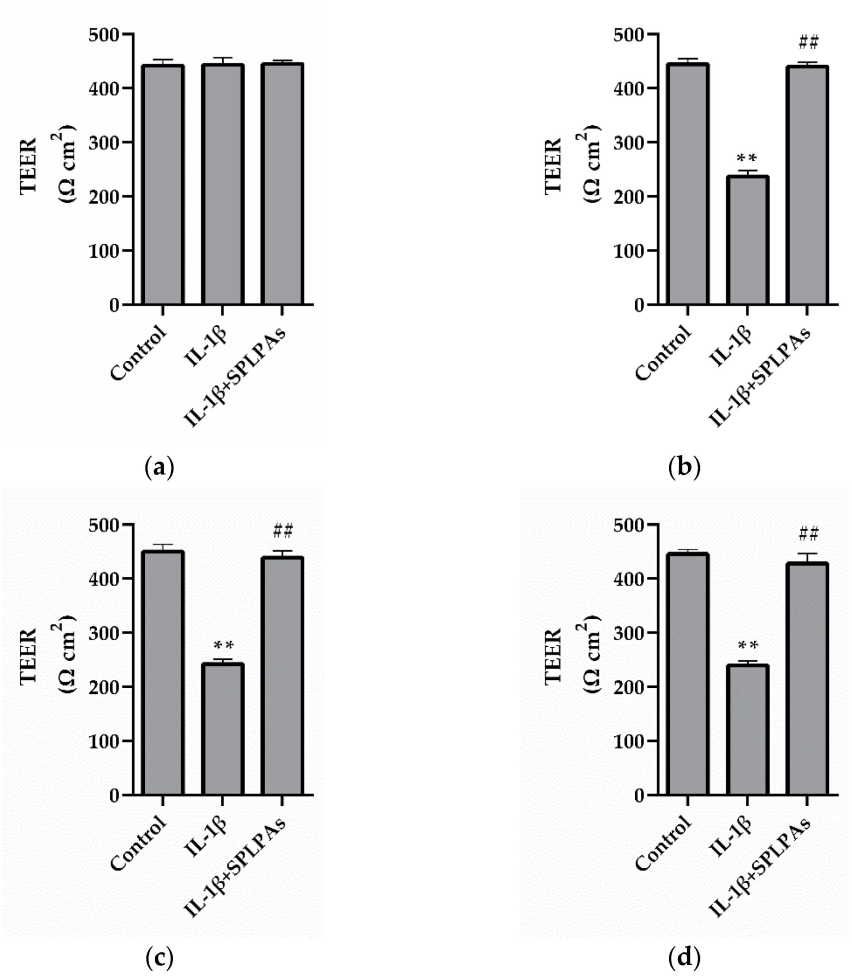

Figure 5. The barrier function of the Caco-2 cell monolayer was investigated with TEER values were recorded at 0 h (**a**), 6 h (**b**), 12 h (**c**), and 24 h (**d**) after IL-1β treatment. ** $p < 0.01$ vs. the control group. ## $p < 0.01$ vs. the IL-1β (10 ng/mL) group.

4. Discussion

The leaves of sweetpotatoes are rich in polyphenols, and the average total polyphenol content was 7.08 g/100 g DW [35], which was 2–3 times that of the reference vegetables (spinach, cabbage, etc.). The SPLPA extract purifications of 85.65 ± 1.77% DW, accumulated form two sub-group types of hydroxybenzoic and hydroxycinnamic acid. The most important phenolic acid compounds were identified using UPLC-Orbitrap-MS analysis and were the following: isochlorogenic acid A (40.78%), caffeic acid (10.02%), isochlorogenic acid C (12.43%), and isochlorogenic acid B (8.51%).

Intestinal barrier integrity is of primary importance to prevent the passage of noxious agents from the intestinal lumen into the mucosa and blood circulation [36]. Dietary components have the most significant impact on the intestinal environment because they regulate the intestinal barrier and exert other biofunctions [37]. A high-fat diet has been shown to damage intestinal cells and increase gut permeability [7], and the Caco-2 monolayers model is an effective tool for the research of barrier dysfunction. In previous studies, phenolic extracts from grape have been used in the 0.1–10 mg/mL concentration range on Caco-2 cells [38]. Thus, to investigate the SPLPA extract's cytotoxic effect on Caco-2 cells, 5, 10, 0.2, and 0.02 μM concentrations of SPLPA extracts were applied. According to the experimental results of cell viability and NO-inhibitory activity, a dose of SPLPA extract at 0.2 mg/mL was used for the subsequent experiments. The enzyme-inducible nitric oxide synthase (iNOS), which is not expressed under normal physiological conditions, is responsible for the localized over-production of NO under pathological conditions. High concentrations of NO can induce inflammation, apoptosis, and oxidative stress as it is recognized as a pro-inflammatory mediator and marker for oxidative stress status [39]. The current results found that the increased expression of the gene coding for iNOS after IL-1β stimulation was significantly reversed by SPLPAs.

The level of the major pro-inflammatory cytokine IL-1β is markedly increased in the Caco-2 cells, which results in an increased level of other inflammatory mediators and further contributes to the progress of inflammatory bowel diseases. Previous studies showed that preselected natural polyphenolic extracts pre-treatment reduces the inflammatory mediators IL-8 and NO, while pomegranate extract is considered the strongest polyphenolic plant extracts inhibitors of NF-κB [40]. According to our results, IL-1β promotes TNF-α and IL-6 secretion, while the SPLPA extract treatment reduces the content and mRNA level of TNF-α and IL-6. All of these suggested that the isolated SPLPA extract protected the Caco-2 cells against the inflammatory activation induced by IL-1β. NF-κB is one of the important mediators in cytokines secretion that controls the expression of various inflammatory cytokines such as NO and TNF-α. The increased p65 nuclear translocation and IκBα degradation were significantly reversed by the SPLPA extract. These results suggested that the SPLPA extract could suppress the IL-1β-induced activation of the NF-κB signaling pathway, and this might be responsible for the anti-inflammatory activity of the SPLPA extract, as mentioned above.

The activation of the NF-κB pathway is a key event in inflammatory reactions, leading to pro-inflammatory mediator expression, resulting in damage to the intestinal barrier. The exposure of Caco-2 cells to cytokines induces the NF-κB pathway, which has effects on the membrane permeability and tight junction redistribution [41,42]. The decreased TEER of the monolayers has been established, indicating epithelial barrier opening and inflammatory response [43]. Previous studies have shown exposure to fatty acids or bile acids to decrease TEER in differentiated Caco-2 cell monolayers and thereby reduce the integrity of a differentiated Caco-2 cell monolayer [44,45]. Exposure to TNF-α was also shown to increase the paracellular permeability of a differentiated Caco-2 cell monolayer [11,46]. The SPLPA extract treatment group increased by 79.17% after 24 h compared to the TEER changes in the IL-1β group later suggest that the SPLPA extract protected the Caco-2 cells against inflammatory barrier dysfunction induced by IL-1β.

The intestinal epithelial barrier consists of IECs and the paracellular apical junction complex, which includes tight junctions, which are integral components of the intestinal barrier and the dysregulation of tight and adherence junctions [47]. The dysregulation of the tight junctions and destruction of intestinal epithelial cells will lead to defects of intestinal epithelial barrier function and inflammation [48]. The tight junctions complex is mainly comprised of occludin, claudins, junctional adhesion molecules (JAMs), and the ZO protein family. In previous studies, several compounds were shown to increase barrier integrity in Caco-2 cells [49]. The current results showed that SPLPA extract administration significantly upregulated the expression levels of ZO-1, Claudin 1, and Occludin in Caco-2 monolayers. Previous studies showed that quercetin has been demonstrated to enhance barrier function

by improving claudin-4 in Caco-2 cells [50]. Recent animal studies have also shown protection by dietary non-flavonoid polyphenol compound (resveratrol) against intestinal barrier dysfunction and inflammation induced by dietary fat, mainly through upregulating tight junction proteins and decreasing inflammation [51]. These results support those of the present study that upregulating the expression of tight junction proteins can improve the intestinal barrier function.

The above-mentioned protective effects of the pure SPLPA extract support its possible nutraceutical application for irritable bowel syndrome and inflammatory bowel diseases; a protective delayed-release formula should be developed to deliver SPLPAs into the small intestine to protect intestinal function.

5. Conclusions

To investigate the bioactivity of SPLs, the purification of PAs was derived by AB-8 macroporous resins in the presence of low temperatures. The purified SPLPA extract mainly consisted of caffeic acid and chlorogenic acid isomers, especially three types of isochlorogenic acid. The protective effects of the SPLPA extract include inhibiting inflammatory cytokines production and release and restoring the distribution and levels of tight junction proteins. These results illustrated the potential of the SPLPA extract as an excellent raw material for further applications.

Author Contributions: Y.Z.: Data curation, formal analysis, validation, visualization and writing. J.S.: Funding acquisition, project administration, and supervision. L.Z.: Conceptualization, investigation, and supervision. Resources, F.N.; methodology, R.Y. and H.Z.; validation, W.Z. and C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the earmarked fund for CARS-10-Sweetpotato, National Key R&D Program of China (2020YFD1001401), and Scientific Research Fund Project of Xuzhou Academy of Agricultural Sciences (XK2020007).

Institutional Review Board Statement: This study did not require ethical approval.

Informed Consent Statement: Not applicable.

Data Availability Statement: This study did not report any data.

Acknowledgments: The authors would appreciate China Agriculture Research System (CARS-10-Sweetpotato), National Key R&D Program of China (2020YFD1001401), and Scientific Research Fund Project of Xuzhou Academy of Agricultural Sciences (XK2020007).

Conflicts of Interest: All authors declare no conflict of interest.

References

1. Lee, C.Y. The effect of high-fat diet-induced pathophysiological changes in the gut on obesity: What should be the ideal treatment? *Clin. Transl. Gastroenterol.* **2013**, *4*, e39. [CrossRef] [PubMed]
2. Francescangeli, F.; De Angelis, M.L.; Zeuner, A. Dietary factors in the control of gut homeostasis, intestinal stem cells, and colorectal cancer. *Nutrients* **2019**, *11*, 2936. [CrossRef] [PubMed]
3. Sun, C.; Zhao, Y.; Gao, X.; Yuan, Y.; Wang, C.; Wang, Y.; Zhang, L.; Gu, Y.; Zhang, F.; Hu, P.; et al. Cideb deficiency aggravates dextran sulfate sodium-induced ulcerative colitis in mice by exacerbating the oxidative burden in colonic mucosa. *Inflamm. Bowel Dis.* **2017**, *23*, 1338–1347. [CrossRef] [PubMed]
4. Bashllari, R.; Molonia, M.S.; Muscarà, C.; Speciale, A.; Wilde, P.J.; Saija, A.; Cimino, F. Cyanidin-3-O-glucoside protects intestinal epithelial cells from palmitate-induced lipotoxicity. *Arch. Physiol. Biochem.* **2020**, 1–8. [CrossRef] [PubMed]
5. Laugerette, F.; Vors, C.; Géloën, A.; Chauvin, M.A.; Soulage, C.; Lambert-Porcheron, S.; Peretti, N.; Alligier, M.; Burcelin, R.; Laville, M.; et al. Emulsified lipids increase endotoxemia: Possible role in early postprandial low-grade inflammation. *J. Nutr. Biochem.* **2011**, *22*, 53–59. [CrossRef]
6. Park, M.-Y.; Kim, M.Y.; Seo, Y.R.; Kim, J.-S.; Sung, M.-K. High-fat diet accelerates intestinal tumorigenesis through disrupting intestinal cell membrane integrity. *J. Cancer Prev.* **2016**, *21*, 95–103. [CrossRef] [PubMed]
7. Moreira, A.P.B.; Texeira, T.F.S.; Ferreira, A.B.; do Carmo Gouveia Peluzio, M.; de Cássia Gonçalves Alfenas, R. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br. J. Nutr.* **2012**, *108*, 801–809. [CrossRef]

8. Sambuy, Y.; De Angelis, I.; Ranaldi, G.; Scarino, M.L.; Stammati, A.; Zucco, F. The caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on caco-2 cell functional characteristics. *Cell Biology.Toxicol.* **2005**, *21*, 1–26. [CrossRef]
9. Van De Walle, J.; Hendrickx, A.; Romier, B.; Larondelle, Y.; Schneider, Y.-J. Inflammatory parameters in Caco-2 cells: Effect of stimuli nature, concentration, combination and cell differentiation. *Toxicol. Vitr.* **2010**, *24*, 1441–1449. [CrossRef] [PubMed]
10. Chen, S.; Einspanier, R.; Schoen, J. Transepithelial electrical resistance (TEER): A functional parameter to monitor the quality of oviduct epithelial cells cultured on filter supports. *Histochem. Cell Biol.* **2015**, *144*, 509–515. [CrossRef]
11. Cremonini, E.; Mastaloudis, A.; Hester, S.N.; Verstraeten, S.V.; Anderson, M.; Wood, S.M.; Waterhouse, A.L.; Fraga, C.G.; Oteiza, P.I. Anthocyanins inhibit tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity. *Food Funct.* **2017**, *8*, 2915–2923. [CrossRef]
12. Contreras, T.C.; Ricciardi, E.; Cremonini, E.; Oteiza, P.I. (−)-Epicatechin in the prevention of tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity. *Arch. Biochem. Biophys.* **2015**, *573*, 84–91. [CrossRef] [PubMed]
13. FAOSTAT. Crops and Livestock Products Data [OB/OL]. 2020. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 1 August 2022).
14. Islam, M.S.; Yoshimoto, M.; Yahara, S.; Okuno, S.; Ishiguro, K.; Yamakawa, O. Identification and characterization of foliar polyphenolic composition in sweet potato (Ipomoea batatas L.) genotypes. *J. Agric. Food Chem.* **2002**, *50*, 3718–3722. [CrossRef] [PubMed]
15. Johnson, M.; Pace, R.D. Sweet potato leaves: Properties and synergistic interactions that promote health and prevent disease. *Nutr. Rev.* **2010**, *68*, 604–615. [CrossRef] [PubMed]
16. Kumar, N.; Goel, N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol. Rep.* **2019**, *24*, e00370. [CrossRef] [PubMed]
17. Pereira, D.M.; Valentão, P.; Pereira, J.A.; Andrade, P.B. Phenolics: From chemistry to biology. *Molecules* **2009**, *14*, 2202–2211. [CrossRef]
18. Clifford, M.N. Chlorogenic acids and other cinnamates-nature, occurrence, and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362–372. [CrossRef]
19. Jung, J.-K.; Lee, S.U.; Kozukue, N.; Levin, C.E.; Friedman, M. Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (Ipomoea batata L.) plants and in home processed roots. *J. Food Compos. Anal.* **2011**, *24*, 29–37. [CrossRef]
20. Khoddami, A.; Wilkes, M.A.; Roberts, T.H. Techniques for analysis of plant phenolic compounds. *Molecules* **2013**, *18*, 2328–2375. [CrossRef] [PubMed]
21. Sasaki, K.; Han, J.; Shimozono, H.; Villareal, M.O.; Isoda, H. Caffeoylquinic acidrich purple sweet potato extract, with or without anthocyanin, imparts neuroprotection and contributes to the improvement of spatial learning and memory of SAMP8 mouse. *J. Agric. Food Chem.* **2013**, *61*, 5037–5045. [CrossRef] [PubMed]
22. Han, J.; Miyamae, Y.; Shigemori, H.; Isoda, H. Neuroprotective effect of 3,5-di-O-caffeoylquinic acid on SH-SY5Y cells and senescence-accelerated-prone mice 8 through the up-regulation of phosphoglycerate kinase-1. *Neuroscience* **2010**, *169*, 1039–1045. [CrossRef] [PubMed]
23. Sato, Y.; Itagaki, S.; Kurokawa, T.; Ogura, J.; Kobayashi, M.; Hirano, T.; Sugawara, M.; Iseki, K. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.* **2011**, *403*, 136–138. [CrossRef] [PubMed]
24. Zhang, L.; Tu, Z.; Wang, H.; Fu, Z.; Wen, Q.; Chang, H.; Huang, X. Comparison of different methods for extracting polyphenols from Ipomoea batatas leaves, and identification of antioxidant constituents by HPLC-QTOF-MS2. *Food Res. Int.* **2015**, *70*, 101–109. [CrossRef]
25. Zheng, W.; Cliffordb, M.N. Profiling the chlorogenic acids of sweet potato (Ipomoea batatas) from China. *Food Chem.* **2008**, *106*, 147–152. [CrossRef]
26. Islam, M.S.; Yoshimoto, M.; Ishiguro, K.; Okuno, S.; Yamakawa, O. Effect of artificial shading and temperature on radical scavenging activity and polyphenolic composition in sweetpotato (Ipomoea batatas L.) leaves. *J. Am. Soc. Hortic. Sci.* **2003**, *128*, 182–187. [CrossRef]
27. Sasaki, K.; Oki, T.; Kai, Y.; Nishiba, Y.; Okuno, S. Effect of repeated harvesting on the content of caffeic acid and seven species of caffeoylquinic acids in sweet potato leaves. *Biosci. Biotechnol. Biochem.* **2015**, *79*, 1308–1314. [CrossRef]
28. Lin, K.-H.; Chao, P.Y.; Yang, C.-M.; Cheng, W.-C.; Lo, H.-F.; Chang, T.-R. The effects of flooding and drought stresses on the antioxidant constituents in sweet potato leaves. *Bot. Stud.* **2006**, *47*, 417–426.
29. Xi, L.-S.; Mu, T.-H.; Sun, H.-N. Preparative purification of polyphenols from sweet potato (Ipomoea batatas L.) leaves by AB-8 macroporous resins. *Food Chem.* **2015**, *172*, 166–174. [CrossRef]
30. Jaiswal, R.; Müller, H.; Müller, A.; Karar, M.G.E.; Kuhnert, N. Identification and characterization of chlorogenic acids, chlorogenic acid glycosides and flavonoids from Lonicera henryi L. (Caprifoliaceae) leaves by LC-MSn. *Phytochemistry* **2014**, *108*, 252–263. [CrossRef]
31. Zhang, L.Q.; Li, Y.; Liang, Y.; Liang, K.; Zhang, F.; Xu, T.; Wang, M.; Song, H.; Lu, B. Determination of phenolic acid profiles by HPLC-MS in vegetables commonly consumed in China. *Food Chem.* **2019**, *276*, 538–546. [CrossRef]
32. Le Phuong Nguyen, T.; Fenyvesi, F.; Remenyik, J.; Homoki, J.R.; Gogolák, P.; Bácskay, I.; Fehér, P.; Ujhelyi, Z.; Vasvári, G.; Vecsernyés, M.; et al. Protective Effect of Pure Sour Cherry Anthocyanin Extract on Cytokine-Induced Inflammatory Caco-2 Monolayers. *Nutrients* **2018**, *10*, 861. [CrossRef]

33. Zhao, L.; Chen, X.F.; Shao, X.; Wang, Z.Y.; Du, Y.; Zhu, C.C.; Du, W.; Tang, D.Q.; Ji, S. Prenylated phenolic compounds from licorice (Glycyrrhiza uralensis) and their anti-inflammatory activity against osteoarthritis. *Food Funct.* **2022**, *13*, 795. [CrossRef] [PubMed]
34. Ershad, M.; Shigenaga, M.K.; Bandy, B. Differential protection by anthocyanin-rich bilberry extract and resveratrol against lipid micelle-induced oxidative stress and monolayer permeability in Caco-2 intestinal epithelial cells. *Food Funct.* **2021**, *12*, 2950. [CrossRef]
35. Sun, H.-N.; Mu, T.-H.; Xi, L.-S.; Zhang, M.; Chen, J.-W. Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods. *Food Chem.* **2014**, *156*, 380–389. [CrossRef]
36. Hering, N.A.; Fromm, M.; Schulzke, J.D. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J. Physiol.* **2012**, *590*, 1035–1044. [CrossRef] [PubMed]
37. Zhao, X.; Xu, X.X.; Liu, Y.; Xi, E.Z.; An, J.J.; Tabys, D.; Liu, N. The In Vitro Protective Role of Bovine Lactoferrin on Intestinal Epithelial Barrier. *Molecules* **2019**, *24*, 148. [CrossRef]
38. Wang, S.; Mateos, R.; Goya, L.; Amigo-Benavent, M.; Sarriá, B.; Bravo, L. A phenolic extract from grape by-products and its main hydroxybenzoic acids protect Caco-2 cells against pro-oxidant induced toxicity. *Food Chem. Toxicol.* **2016**, *88*, 65–74. [CrossRef]
39. Sies, H.; Berndt, C.; Jones, D.P. Oxidative stress. *Annu. Rev. Biochem.* **2017**, *86*, 715–748. [CrossRef] [PubMed]
40. Romier-Crouzet, B.; Van De Walle, J.; During, A.; Joly, A.; Rousseau, C.; Henry, O.; Schneider, Y.-J. Inhibition of inflammatory mediators by polyphenolic plant extracts in human intestinal Caco-2 cells. *Food Chem. Toxicol.* **2009**, *47*, 1221–1230. [CrossRef] [PubMed]
41. Al-Sadi, R.; Guo, S.; Ye, D.; Dokladny, K.; Alhmoud, T.; Ereifej, L.; Said, H.M.; Ma, T.Y. Mechanism of il-1beta modulation of intestinal epithelial barrier involves p38 kinase and activating transcription factor-2 activation. *J. Immunol.* **2013**, *190*, 6596–6606. [CrossRef] [PubMed]
42. Al-Sadi, R.; Guo, S.; Ye, D.; Ma, T.Y. TNF-α modulation of intestinal epithelial tight junction barrier is regulated by erk1/2 activation of elk-1. *Am. J. Pathol.* **2013**, *183*, 1871–1884. [CrossRef] [PubMed]
43. Ramadan, Q.; Jing, L. Characterization of tight junction disruption and immune response modulation in a miniaturized caco-2/u937 coculture-based in vitro model of the human intestinal barrier. *Biomed. Microdevices* **2016**, *18*, 26809386. [CrossRef] [PubMed]
44. Araki, Y.; Katoh, T.; Ogawa, A.; Bamba, S.; Andoh, A.; Koyama, S.; Fujiyama, Y.; Bamba, T. Bile acid modulates transepithelial permeability via the generation of reactive oxygen species in the Caco-2 cell line. *Free. Radic. Biol. Med.* **2005**, *39*, 769–780. [CrossRef] [PubMed]
45. Aspenström-Fagerlund, B.; Ring, L.; Aspenström, P.; Tallkvist, J.; Ilbäck, N.-G.; Glynn, A.W. Oleic acid and docosahexaenoic acid cause an increase in the paracellular absorption of hydrophilic compounds in an experimental model of human absorptive enterocytes. *Toxicology* **2007**, *237*, 12–23. [CrossRef] [PubMed]
46. Cui, W.; Li, L.X.; Sun, C.M.; Wen, Y.; Zhou, Y.; Dong, Y.L.; Liu, P. Tumor necrosis factor alpha increases epithelial barrier permeability by disrupting tight junctions in Caco-2 cells. *Braz. J. Med. Biol. Res.* **2010**, *43*, 330–337. [CrossRef]
47. Slifer, Z.M.; Blikslager, A.T. The integral role of tight junction proteins in the repair of injured intestinal epithelium. *Int. J. Mol. Sci.* **2020**, *21*, 972. [CrossRef]
48. Martini, E.; Krug, S.M.; Siegmund, B.; Neurath, M.F.; Becker, C. Mend Your Fences: The epithelial barrier and its relationship with mucosal immunity in inflammatory bowel disease. *Cell. Mol. Gastroenterol. Hepatol.* **2017**, *4*, 33–46. [CrossRef]
49. Takenaka, T.; Harada, N.; Kuze, J.; Chiba, M.; Iwao, T.; Matsunaga, T. Application of a human intestinal epithelial cell monolayer to the prediction of oral drug absorption in humans as a superior alternative to the Caco-2 cell monolayer. *J. Pharm. Sci.* **2016**, *105*, 915–924. [CrossRef]
50. Amasheh, M.; Schlichter, S.; Amasheh, S.; Mankertz, J.; Zeitz, M.; Fromm, M.; Schulzke, J.D. Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. *J. Nutrition* **2008**, *138*, 1067–1073. [CrossRef]
51. Chen, M.; Hou, P.; Zhou, M.; Ren, Q.; Wang, X.; Huang, L.; Hui, S.; Yi, L.; Mi, M. Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. *Clin. Nutr.* **2020**, *39*, 1264–1275. [CrossRef]