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# The anti-inflammatory effects of apigenin and genistein on the rat intestinal epithelial (IEC-6) cells with TNF-α stimulation in response to heat treatment

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ABSTRACT

The aims of the present study were to investigate the anti-inflammatory function of two flavonoids apigenin and genistein in rat intestinal epithelial (IEC-6) cells stimulated by tumor necrosis factor-alpha (TNF-α) and to clarify whether the heat treatment of the flavonoids might affect flavonoid activity. The flavonoids at lower dosage (e.g. 5 μmol/L) had no toxic effect but growth promotion on the cells. Meanwhile, the flavonoid pretreatment of the cells before TNF-α stimulation could maintain cellular morphology, decrease the production of prostaglandin $E_2$ and two pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-6, but increase the production of two anti-inflammatory cytokines IL-10 and transforming growth factor-β. Additionally, the flavonoids could block off the nuclear translocation of nuclear factor-kappaB (NF-κB) p65, and suppress the expression of phosphorylated IκBα and p65 induced by TNF-α. Meanwhile, the NF-κB inhibitor BAY 11–7082 shared a similar function with the flavonoids to mediate the production of IL-6/IL-10. Furthermore, *in silico* analysis also declared that the flavonoids could interact with the IκBα–NF–κB complex at the binding pockets to yield the binding energies ranging from −31.7 to −34.0 kJ/mol. However, the heated flavonoids were consistently less effective than the unheated counterparts to perform these anti-inflammatory effects. It is thus proposed that both apigenin and genistein have anti-inflammatory potential to the TNF-α-stimulated IEC-6 cells by inactivating the NF-κB pathway, while heat treatment of the flavonoids caused a negative impact on these assessed anti-inflammatory effects.

## 1. Introduction

The small intestine is the digestive and absorptive organ of the human body, while the gastrointestinal tract also represents the largest immune organ. The small intestine also plays a substantial role in health maintenance through the associated innate and adaptive immune systems (Mowat and Agace, 2014). Intestinal epithelial cells (IECs) maintaining barrier and immuno-regulatory functions are capable of acting as the frontline sensors for microbial encounters and integrating commensal bacteria-derived signals into anti-microbial and immuno-regulatory responses (Alenghat et al., 2013; Ganesan et al., 2021). Thus, intestinal health is very important to the body. However, digestive tract is exposed to a wide range of toxic metabolites in daily diet, most of which can be eliminated and excreted by the body's immune function. Increased levels of toxins thus can trigger chronic intestinal inflammation sometimes. The immune response and inflammatory process involving multiple inflammatory factors are the current research hotspots regarding the pathogenesis of inflammatory bowel disease. Current theories on the pathogenesis and progression of inflammatory bowel disease (IBD) mostly focus on the dysregulation of the inflammatory response, including a large uncontrolled release of pro-inflammatory mediators that can trigger a cascade of events even lead to extensive tissue damage (Lee et al., 2018). In particular, tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine exerting a variety of immunological functions such as the regulation of cellular immune response and modulation of the expression of many other cytokines (Hummel et al., 2009). It is accepted that elevated levels of TNF-α and other pro-inflammatory cytokines are associated with the development of several disease states, such as rheumatoid arthritis, IBD, septic shock, multiple sclerosis, and hepatitis (Ma et al., 2014).

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Nowadays, synthetic TNF-α blockers are widely used in the treatment of rheumatoid arthritis, IBD, and psoriasis (van Dullemen et al., 1995; Maini et al., 1998; Tobin and Kirby, 2005). However, purposeful use of these bioactive compounds in the diet to reduce the damage of these toxins to epithelial cells, weaken the inflammatory response, and maintain intestinal health, is also of great significance to the maintenance of body health.

Fruits and vegetables usually occupy a large proportion of our daily diet. As the important phytonutrients distributed in a wide range of fruits, vegetables, nuts, and beverages (like wine and tea), natural flavonoids possess beneficial anti-tumor, anti-oxidant, anti-diabetic, and anti-inflammatory activities (Vera et al., 2018; Sun et al., 2020). Much attention has been paid to examining the biological functions of flavonoids, due to their negligible adverse effects. For example, it was proved that flavonols have anti-cancer activities to human colon cancer HCT-116 cells (Wang et al., 2017), while those from bacopa floribunda were able to suppress microgliosis, oxidative stress, and neuro-inflammation (Oyeleke and Owoyele, 2022). Flavonoids thus are able to alleviate inflammation response in the intestine. However, thermal treatments are necessary for food processing, while powerful heat treatments such as sterilization are usually applied to plant foods to ensure the shelf-lives of final products (Fu et al., 2021). The performed heat treatment can induce adverse changes in food components (*e.g.* acrylamide formation and vitamin loss). In general, flavonoids are chemically unstable, while the influence of heat treatment on flavonoid properties including their anti-inflammatory activity thus is critical for processed foods. Our previous results showed that heat treatment of galangin and quercetin could cause lower anti-inflammatory potential in the lipopolysaccharide (LPS)-injured rat intestinal epithelial (IEC-6) cells (Cai et al., 2021). However, whether or how heat treatment might affect flavonoid bioactivity is still less investigated.

In the present study, the anti-inflammatory effects of two flavonoid members, namely apigenin ($C_{15}H_{10}O_5$, 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) and genistein ($C_{15}H_{10}O_5$, 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), on the rat intestinal epithelial (IEC-6) cells stimulated by TNF-α at 50 ng/mL were evaluated mainly *via* measuring cell viability, the production levels of several pro-inflammatory and anti-inflammatory mediators, and the regulation on two critical proteins namely phosphorylated IκBα and p65 in the NF-κB pathway. Meanwhile, the changes in the anti-inflammatory activity of the flavonoids in response to a heat treatment at 100 °C were also verified. The aim of this study was to clarify how the used heat treatment caused bioactivity change for the assessed flavonoids.

## 2. Materials and methods

### 2.1. Reagents and materials

Both apigenin and genistein (purity >97%) were bought from Dalian Meilun Biological Technology Co. Ltd. (Dalian, Liaoning, China). The Dulbecco's modified Eagle's medium (DMEM), TNF-α, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) were all purchased from Sigma-Aldrich Co. (St Louis, MO, USA), while fetal bovine serum (FBS) was provided by Wisent Inc (Montreal, QC, Canada). The bovine insulin, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), Triton X-100, and trypsin-EDTA were all purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China). All enzyme-linked immunosorbent assay (ELISA) kits were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The NF-κB inhibitor BAY 11–7082, radio immunoprecipitation assay (RIPA) lysis buffer, phenylmethanesulfonyl fluoride (PMSF), and bicinchoninic acid (BCA) protein assay kit were all purchased from Beyotime Institute of Biotechnology (Shanghai, China). Enhance chemiluminescence (ECL) reagent and 4% paraformaldehyde were provided by Biosharp Biotechnology (Hefei, China). Other chemicals used were analytical grade. Ultrapure water generated using Milli-Q Plus system (Millipore Corporation, New York, NY, USA) was used in this study.

The primary antibodies used for phosphor–NF–κB p65 (p-p65) (Bioss bs-0982R) and β-actin (Bioss bs-0061R) were all provided by Biosynthesis Biotechnology Inc. (Beijing, China). The primary antibodies of phosphor-IκB alpha (p-IκBα) (CST 2859) and the goat anti-rabbit horseradish peroxidase (HRP) secondary antibody (CST 7074) were obtained from Cell Signaling Technology (Danvers, MA, USA). The 4,6-diamidino-2-phenyl-indole (DAPI), primary antibodies for NF-κB p65, and goat anti-rabbit secondary antibody were provided by Servicebio Bioscience Co. Ltd. (Wuhan, China).

### 2.2. Sample preparation

Apigenin (or genistein) was dissolved in DMSO to reach a concentration of 20 mmol/L, and then diluted with serum-free medium to four dosages of 2.5–20 μmol/L with final DMSO content less than 0.1%. Afterwards, each solution was divided into two parts. One part was added with FBS and then used in cell experiments immediately. Another part was heated at 100 °C for 15–30 min, cooled in ice, added with FBS, and then used in cell experiments immediately.

### 2.3. Cell culture

IEC-6 cells that have the characteristics of the stable passage of crypt epithelial cells were obtained from the American Type Culture Collection (Rockville, MD, US). As recommended, the cells were maintained in the DMEM containing 10% FBS, 1% sodium pyruvate, 100 U/L bovine insulin, and 100 mg/mL penicillin/streptomycin, using a humidified atmosphere with 5% $CO_2$. The incubator temperature was set at 37 °C. The same protocol was used in most cell experiments; that is, the cells were starved in a serum-free medium for 12 h, pretreated with the flavonoids for 1 h, and then exposed to TNF-α alone for 24 h.

### 2.4. MTT assay

The cells ($2 \times 10^3$ cells/well) were seeded in 96-well microplates. When the cells were attached, they were starved in a serum-free medium for 12 h. Flavonoids solutions of 100 μL were added to each well, while the cells were incubated at 37 °C for 6, 12, and 24 h, respectively. After medium removal, the cells were washed three times with a phosphate buffered solution (PBS, 10 mmol/L, pH 7.4), followed by incubation with 100 μL MTT reagent (1 mg/mL) for 4 h. After discarding the medium, 150 μL DMSO was added into each well, while the plates were shaken gently for 10 min. The absorbance value of each well was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The cell viability (%) was determined by comparing the absorbance at 490 nm with the control wells containing cell culture medium only.

### 2.5. Assays of the morphology of IEC-6 induced by TNF-α

IEC-6 cells were inoculated into 24-well plates ($8 \times 10^4$ cells/well) and allowed to adhere for 24 h. Afterwards, the cells were starved in a serum-free medium for 12 h, pretreated with the flavonoids for 1 h before adding TNF-α for 24 h. The images of the cells were captured under an optical microscopy (EVOS FL Auto 2, Invitrogen, Massachusetts, USA) using an objective of 20-fold.

### 2.6. Assays of cytokines and prostaglandin $E_2$ production

The levels of the inflammation-related cytokines and prostaglandin $E_2$ ($PGE_2$) were assessed using respective ELISA kits. IEC-6 cells were inoculated into 24-well plates ($8 \times 10^4$ cells/well), allowed to adhere for 24 h, treated in the serum-free medium for 12 h, incubated with the flavonoids at 5 μmol/L (or BAY 11–7082 at 10 μmol/L) for 1 h, and then exposed to TNF-α alone for 24 h. The supernatants were collected and

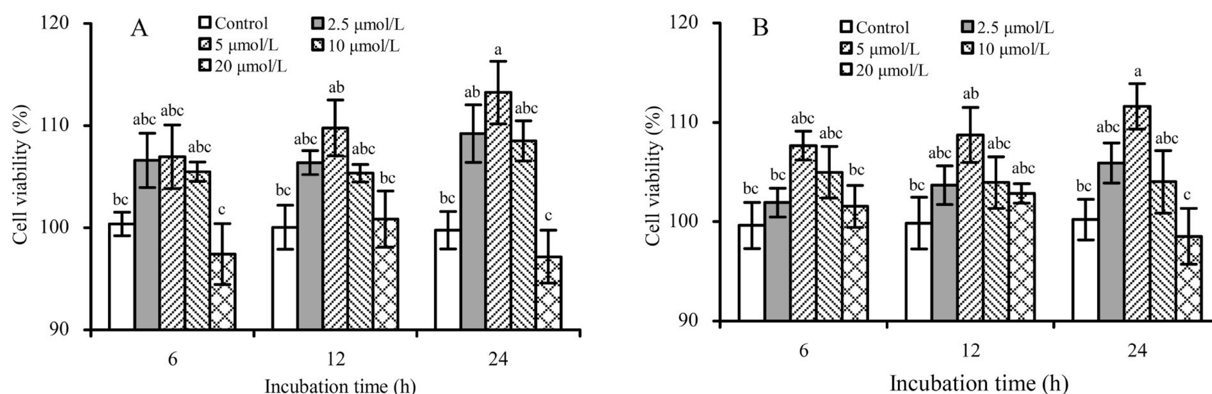


**Fig. 1.** The detected viability values of the IEC-6 cells exposed to apigenin (A) and genistein (B) at the dosages of 2.5–20 μmol/L with treatment times of 6–24 h. Different lowercase letters (a–c) above the columns indicate that the mean values differ significantly ($p < 0.05$).

then measured for the levels of four cytokines and $PGE_2$ using the protocols provided by the kit manufacturers. The target pro-inflammatory cytokines were interleukin-6 (IL-6) and IL-1β, while the measured anti-inflammatory cytokines were IL-10 and transforming growth factor-beta (TGF-β).

### 2.7. Western-blot assay

The cells were subjected to flavonoid or BAY 11–7082 treatments as above, whereas the cell homogenates were obtained after TNF-α exposure of 30 min. The collected cells were washed with the PBS at 4 °C for three times. RIPA cleavage buffer and PMSF were used to lyse the cells on ice for 30 min, followed by centrifugation at 12,000×*g* for 5 min at 4 °C to extract total protein. Quantitative determination of total protein was performed using the BCA protein analysis kit. The extracted proteins were then separated using 4–12% SDS-PAGE and transferred to PVDF membranes, which were placed in 5% skim milk in the TBST containing 0.1% Tween-20 and sealed at 37 °C for 120 min. Afterwards, the membranes were incubated overnight at 4 °C with primary antibodies β-actin (1:10,000 dilution), p-IκBα, and p-p65 (1:1000 dilution for each). After washing three times with the TBST, the membrane was incubated with the HRP-conjugated secondary antibody (1:2000 dilution) at 37 °C for 1 h and then visualized using the ECL kit following a TBST washing of three times. Moreover, the ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Hercules, CA, USA) were used to detect protein bands, while the Image J software (National Institutes of Health, Bethesda, MD, USA) was used for quantitative analysis. The band density was normalized to β-actin.

### 2.8. Immunofluorescence analysis

Immunofluorescence staining was conducted as previously described (Liu et al., 2019). The cells were treated with or without the flavonoids for 1 h, cultured with TNF-α for 30 min, washed with the PBS, fixed with 4% paraformaldehyde for 20 min, membrane-permeabilized by exposing to 0.1% Triton X-100 for 2 min in the PBS, and then placed in blocking serum (5% bovine serum in the PBS) at 20 °C for 2 h. The cells were then exposed to the primary antibody of p65 (1:200) overnight at 4 °C. After washing with ice-cold PBS followed by the treatment with the goat anti-rabbit secondary antibody (1:300) for 50 min at 20 °C and DAPI staining for 5 min, the fluorescence was visualized using a Nikon Eclipse C1 fluorescence microscope (Nikon, Tokyo, Japan).

### 2.9. Molecular docking analysis

The X-ray crystal structures of the IκBα–NF–κB complex (PDB code, 1IKN) were downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/pdb/home/home.do) (accessed on July 10, 2021). The 3D structures of apigenin, genistein, and the inhibitor BAY 11–7082 were all obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) (accessed on September 15, 2021). Further changes such as the addition of polar hydrogen and deletion of water molecules were completed. The docking simulations were performed using the PyMOL Molecular Graphics System (PyMOL, Version 2.4.0, Schrödinger, LLC, New York, USA) and AutoDockTools (Version 1.5.6, Molecular Graphics Laboratory, The Scripps Research Institute, San Diego, CA, USA). The grid spacing was set to 0.0397 nm, and the grid box dimensions were set to 6.0, 6.0 and 6.0 nm, enclosing the binding-site residues of the target proteins. The numbers of genetic algorithm runs were 100, while the docking results were sorted on the basis of the lowest interaction energy.

### 2.10. Statistical analysis

All experiments or assays were performed at least three times, while the data are reported as the means or means ± standard deviations. One-way analysis of variance (ANOVA) was used for the significance analysis among different groups ($p < 0.05$) using the Social Science Statistical Program 16.0 software package (SPSS Inc., Chicago, IL, USA) and Duncan's multiple comparison.

## 3. Results

### 3.1. Effect of apigenin and genistein on the growth of IEC-6 cells

To select a suitable dosage used for the later assays for these anti-inflammatory effects or to avoid possible cytotoxicity caused by higher flavonoid dosage, the cells were treated with apigenin or genistein at four dosages (2.5–20 μmol/L) for three time periods (6–24 h) and then measured for viability changes. The results (Fig. 1) showed that both apigenin and genistein at 2.5–10 μmol/L had growth promotion on the cells, causing respective viability values of 97.2–113.2% and 98.5–111.6% in both dose- and time-dependent manners. However, the flavonoids at 20 μmol/L might cause a slight growth inhibition on the cells, resulting in viability values less than 100%. In addition, the cells with longer treatment times (*i.e.* 24 h) usually exerted higher growth proliferation than the counterparts with shorter treatment time (*e.g.* 6 h). Collectively, the flavonoids at a lower dosage had no toxicity but growth promotion on the cells. The flavonoids at 5 μmol/L mostly caused the highest viability values in the cells. This dosage was thus used in later assays.

### 3.2. Protective effect of apigenin and genistein on IEC-6 cells with TNF-α exposure

When IEC-6 cells were stimulated with TNF-α, the morphology of the stimulated cells could be changed (Islam et al., 2018). Similar results

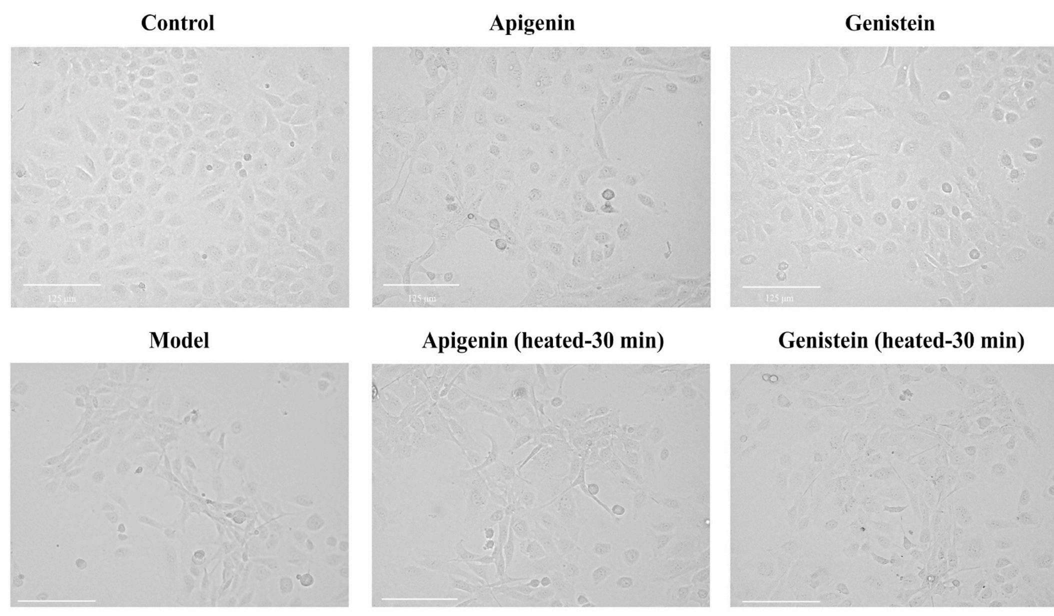


**Fig. 2.** The morphological changes of IEC-6 cells pretreated with apigenin, genistein, and the heated flavonoids (100 °C for 30 min) for 1 h and then exposed to TNF-α (50 ng/mL) alone for 24 h. The used flavonoid dosage was 5 μmol/L, while the labeled bar is 125 μm.

**Table 1**
The detected levels (pg/mL) of two pro-inflammatory cytokines (IL-1β and IL-6) and PGE2 in the cells with or without TNF-α and flavonoid treatments.

| Cell group | IL-1β | IL-6 | $PGE_2$ |
|---|---|---|---|
| Control | 5.8 ± 0.4$^{ef}$ | 87.6 ± 3.3$^{g}$ | 5.0 ± 0.4$^{d}$ |
| Model | 11.6 ± 0.5$^{a}$ | 151.5 ± 6.1$^{a}$ | 17.6 ± 1.6$^{a}$ |
| Apigenin | 4.9 ± 0.7$^{f}$ | 107.7 ± 4.8$^{ef}$ | 5.8 ± 0.6$^{d}$ |
| Heated AP-15 | 6.5 ± 0.3$^{de}$ | 117.1 ± 4.0$^{de}$ | 7.2 ± 0.9$^{cd}$ |
| Heated AP-30 | 7.4 ± 0.5$^{cd}$ | 132.7 ± 6.0$^{bc}$ | 9.7 ± 1.2$^{b}$ |
| Genistein | 6.0 ± 0.3$^{e}$ | 117.7 ± 5.8$^{de}$ | 5.8 ± 0.6$^{d}$ |
| Heated GE-15 | 7.6 ± 0.5$^{c}$ | 123.6 ± 8.1$^{cd}$ | 6.9 ± 0.7$^{cd}$ |
| Heated GE-30 | 8.7 ± 0.3$^{b}$ | 138.2 ± 6.7$^{b}$ | 8.3 ± 0.3$^{bc}$ |
| BAY 11-7082 | Not assessed | 99.7 ± 4.1$^{f}$ | Not assessed |

AP, apigenin; GE, genistein. The numbers following flavonoids indicate the heat time (min) used in the heat treatment of the samples.
Different lowercase letters after the means as the superscripts in the same column indicate that one-way ANOVA of the means differs significantly ($p < 0.05$).

**Table 2**
The detected levels (pg/mL) of two anti-inflammatory cytokines IL-10 and TGF-β in the cells with or without TNF-α and flavonoid treatments.

| Cell group | IL-10 | TGF-β |
|---|---|---|
| Control | 25.0 ± 1.3$^{cd}$ | 36.5 ± 0.9$^{d}$ |
| Model | 11.5 ± 1.3$^{g}$ | 27.9 ± 2.8$^{e}$ |
| Apigenin | 27.4 ± 2.0$^{bc}$ | 54.0 ± 4.1$^{ab}$ |
| Heated AP-15 | 24.2 ± 1.0$^{de}$ | 46.3 ± 1.4$^{c}$ |
| Heated AP-30 | 17.7 ± 1.4$^{f}$ | 32.5 ± 0.7$^{de}$ |
| Genistein | 31.9 ± 1.3$^{a}$ | 56.6 ± 0.9$^{a}$ |
| Heated GE-15 | 28.8 ± 1.9$^{ab}$ | 46.7 ± 1.0$^{bc}$ |
| Heated GE-30 | 21.9 ± 1.8$^{e}$ | 35.4 ± 0.8$^{d}$ |
| BAY 11-7082 | 25.1 ± 1.6$^{cd}$ | Not assessed |

AP, apigenin; GE, genistein. The numbers following flavonoids indicate the heat time (min) used in the heat treatment of the samples.
Different lowercase letters after the means as the superscripts in the same column indicate that one-way ANOVA of the means differs significantly ($p < 0.05$).

were observed in this study (Fig. 2) because the control cells were observed to be round or oval with smooth cell edges without pseudopodia, whereas the model cells were shrunken with polygonal shape and elongated pseudopodia. However, if the cells were pretreated with the flavonoids for 1 h before the TNF-α stimulation, they were observed with normal morphology to a certain extent, demonstrating a protective effect of the flavonoids on the cells with TNF-α exposure. However, the heated flavonoids were observed with a reduced ability to protect the cells, suggesting the heat treatment of the flavonoids caused activity decrease in the cells.

### 3.3. Effect of the flavonoids on the production of three pro-inflammatory mediators

Both IL-6 and IL-1β are the major inflammatory cytokines that are essential in the initiation and progression of chronic intestinal inflammation, while $PGE_2$ plays a key role in the generation of the inflammatory responses (Cianciulli et al., 2012; Park et al., 2019). The model cells (treated with TNF-α only) showed the highest values in the three inflammation-related mediators IL-1β, IL-6, and PGE2 (Table 1), indicating that TNF-α induced cellular inflammation. As expected, NF-κB inhibitor BAY 11–7082 showed a capacity to inhibit IL-6 production because it blocked the NF-κB signaling pathway. Similarly, the flavonoids-treated cells had a significant decrease in the values of the three mediators ($p < 0.05$), confirming that the flavonoids indeed had an ability to combat against the induced cell inflammation efficiently. Additionally, the results also declared that the unheated flavonoids possessed higher anti-inflammatory efficacies than the heated counterparts (especially those with longer heat time), suggesting that the performed heat treatment had an adverse effect on the anti-inflammatory activity of the flavonoids. Moreover, apigenin had similar anti-inflammatory activity to genistein to reduce the production of IL-6 and PGE2 but showed a higher capacity than genistein to suppress the production of IL-1β.

### 3.4. Effect of the flavonoids on the production of two anti-inflammatory cytokines

Anti-inflammatory cytokines also are a series of immuno-regulatory molecules regulating the immune response (Opal and DePalo, 2000). Thus, two anti-inflammatory cytokines namely IL-10 and TGF-β were detected in these treated cells (Table 2). Compared with the control cells, significant decreases in IL-10 and TGF-β were observed in the model cells, reflecting the cellular inflammation induced by TNF-α. In

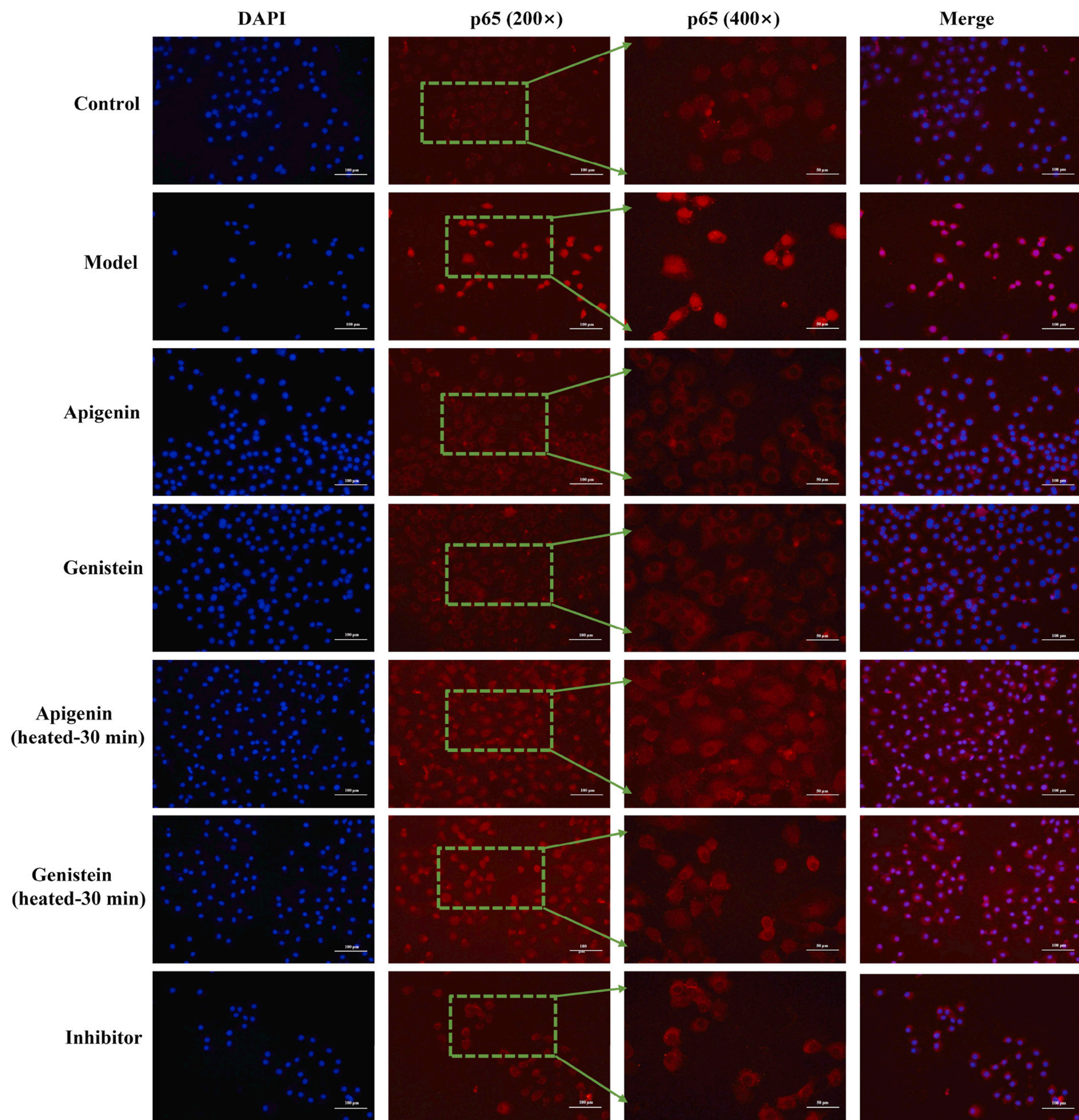


**Fig. 3.** Immuno-fluorescence analysis of NF-κB p65 (red) in the IEC-6 cells in response to the pretreatment with the inhibitor (Bay 11–7082), apigenin, genistein, and the heated flavonoids (100 °C for 30 min) for 1 h and then exposed to TNF-α (50 ng/mL) alone for 30 min. The used flavonoid dosage was 5 μmol/L, while the used inhibitor dosage was 10 μmol/L. The labeled bar is 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

contrast, the flavonoid-treated cells showed higher production of the two cytokines, compared with the model cells. The two flavonoids thus were regarded to exert anti-inflammatory potentials in the stimulated cells. Meanwhile, the inhibitor BAY 11–7082 also alleviated the inflammatory response by enhancing IL-10 production, evidencing the anti-inflammatory potentials of the flavonoids. It was also seen from the data that genistein was more active than apigenin to promote IL-10 production, while the two flavonoids showed similar capacity to enhance TGF-β production. Furthermore, the unheated flavonoids were more potent than the heated counterparts to promote the secretion of IL-10 and TGF-β. In other words, the performed heat treatment led to weakened anti-inflammatory activity for the flavonoids.

### *3.5. Effect of the flavonoids on nuclear translocation of NF-κB p65 and protein expression in NF-κB signaling pathway*

When NF-κB is activated, NF-κB acting as a transcription factor modulating inflammatory cytokines will rapidly transfer from the cytoplasm to the nucleus (Somensi et al., 2019). To find out whether the two flavonoids could block off the nuclear translocation of NF-κB p65, an immuno-fluorescence assay was conducted to observe the distribution of p65 protein (Fig. 3). Compared with control cells in which NF-κB p65 was mainly distributed in the cytoplasm of the cells, the nucleoplasm area of the model cells was observed with stronger fluorescent signals, indicating that a large amount of p65 protein translocated into the nucleus. Compared with the model cells, the cells treated with BAY 11–7082, apigenin, and genistein exhibited a similar phenomenon; that is, a blocked nuclear translocation of p65 protein. Specifically, the

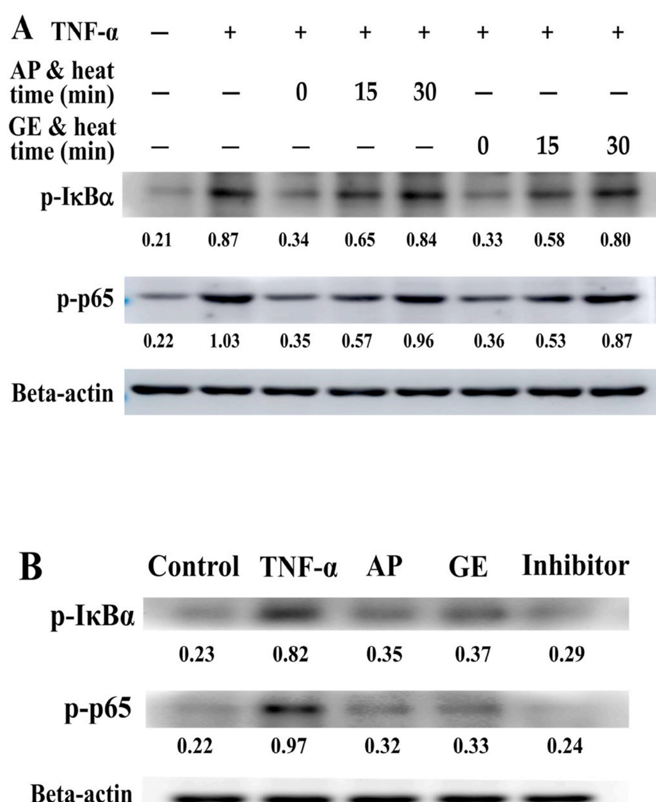


**Fig. 4.** The measured expression levels of the inflammation-related proteins in IEC-6 cells pretreated with the inhibitor (Bay 11–7082), apigenin (AP), genistein (GE), and heated flavonoids (100 °C for 15–30 min) for 1 h and then exposed to TNF-α (50 ng/mL) alone for 30 min. The used flavonoid dosage was 5 μmol/L, while the used inhibitor dosage was 10 μmol/L.

distribution area of p65 protein was around but did not exist in the nuclear. BAY 11–7082, apigenin, and genistein thus possessed anti-inflammatory functions in the stimulated cells. In consistence with other assaying results, the heated flavonoids also were observed with a weaker capacity than their unheated counterparts to block off the nuclear translocation of p65 protein.

It is well-known that TNF-α could induce the activation of the NF-κB signaling pathway and subsequently cause inflammatory responses (Dilshara et al., 2015). Possible changes of the critical inflammation-related proteins like p-IκBα and p-p65 in the cells subjected to different treatments were thus detected. The results (Fig. 4) showed that the model cells had the highest levels of p-IκBα and p-p65, demonstrating that TNF-α activated NF-κB signaling pathway and then induced inflammatory responses. Meanwhile, both apigenin and genistein down-regulated the expression levels of p-IκBα and p-p65 clearly, as the inhibitor BAY 11–7082 did. It thus revealed that both apigenin and genistein could suppress the activation of NF-κB signaling pathway. In addition, the results also proved that the used heat treatment of the flavonoids resulted in weaker potentials for the heated flavonoids to suppress NF-κB activation, demonstrating again that the performed heat treatment inhibited the anti-inflammatory activities of the two flavonoids.

### 3.6. *Interaction potentials of the flavonoids with NF-κB*

The possible interaction between NF-κB and the flavonoids was thus investigated using an analysis with molecular docking. The resultant binding energies and involved binding residues of the targeted proteins with the flavonoids are described in Fig. 5. The results showed that the flavonoids could interact with the IκBα–NF–κB complex, yielding the binding energies of −34.0 (apigenin) or −31.7 kJ/mol (genistein). Meanwhile, the interaction between the inhibitor BAY 11–7082 and IκBα–NF–κB complex caused the binding energy of −29.4 kJ/mol. Furthermore, the hydrogen bonds were produced between the amino acid residues from the backbone residues of IκBα–NF–κB and the flavonoids or BAY 11–7082. It is worth noting that the two flavonoids had similar chemical structures and thus interacted with three same amino acid residues of NF-κB, namely Glu-49, Asp-223, and Gly-259. Collectively, the results confirmed that the flavonoids could bind with IκBα–NF–κB complex at the binding pocket and thus might play a key role to mediate NF-κB signaling pathway. To some extent, these computational results provided persuasive support to the results from cell experiments.

## 4. Discussion

IBD is featured by an exacerbated and uncontrolled intestinal inflammation that leads to poor life quality. A recent study suggested that more than 70% of the drugs introduced over the past 25 years were originated from nature (Gupta et al., 2018). Thus, dietary ingredients show promising potentials to combat against intestinal inflammation. It has been regarded that dietary ingredients can contact the small intestine directly and thereby affect intestinal function (He and Giusti, 2010). Previous studies demonstrated that some diet-derived products had anti-inflammatory properties; for example, a short-chain fatty acid namely butyrate could inhibit the IL-1β-induced inflammation in epithelial H4 cells (Gao et al., 2021), while three different peptides from the dehydrated potatoes showed an anti-inflammatory effect on IEC-6 cell monolayer by inhibiting TNF-α release (Basilicata et al., 2019). Additionally, the water-soluble polysaccharides isolated from purple sweet potato could improve inflammatory lesions by increasing IL-10 levels but decreasing IL-1β, TNF-α, and IL-6 levels (Gou et al., 2019), while the coumarin derivatives also could exert anti-inflammatory activity in the TNBS-induced rats (Witaicenis et al., 2014). In this study, the targeted flavonoids inhibited the release of three pro-inflammatory mediators but enhanced the secretion of two anti-inflammatory cytokines in the stimulated cells. The flavonoids thus were considered to have the anti-inflammatory activity to the stimulated cells.

Inflammation produces several kinds of mediators such as cytokines and chemokines, which are all achieved by activating intracellular signaling cascades that rapidly induce the expression of a variety of overlapping and unique genes involved in the inflammatory and immune responses (Akira et al., 2006; Reuter et al., 2010). Extensive evidence has revealed that dietary ingredients could inactivate numerous signaling pathways involved in the inflammatory responses. It was found that morroniside alleviated the LPS-induced inflammation by mediating TLR4/NF-κB and Nrf2/HO-1 signaling pathways in RAW 264.7 macrophages (Park et al., 2021), while phytosterols suppressed inflammatory mediators via ERK pathway in the LPS-stimulated RAW264.7 macrophages (Yuan et al., 2019). The results provided by Ye and coauthors evidenced that Ginkgo biloba sarcotesta polysaccharide exerted its anti-inflammatory activity by inhibiting the secretion of pro-inflammatory mediators and cytokines including PGF2, TNF-α, IL-6, and IL-1β in the LPS-stimulated RAW264.7 macrophages, or by modulating NF-κB and MAPK signaling pathways (Ye et al., 2019). More importantly, both protectin DX and walnut peptide with anti-inflammatory potentials were proved able to block p65 protein translocation to the nucleus (Liu et al., 2019; Piao et al., 2020). Sharing the same conclusion as these mentioned studies, this study also observed that the flavonoids could exert anti-inflammatory activity to the stimulated cells *via* mediating the NF-κB signaling pathway.

Thermal processing is an effective approach to improve the shelf life and quality of processed foods, however, can induce content changes of flavonoids. For example, it was reported that a significant decrease in total polyphenol content (about 37.8%) was observed during the heat treatment of Chokanan mango juices (Santhirasegaram et al., 2013).

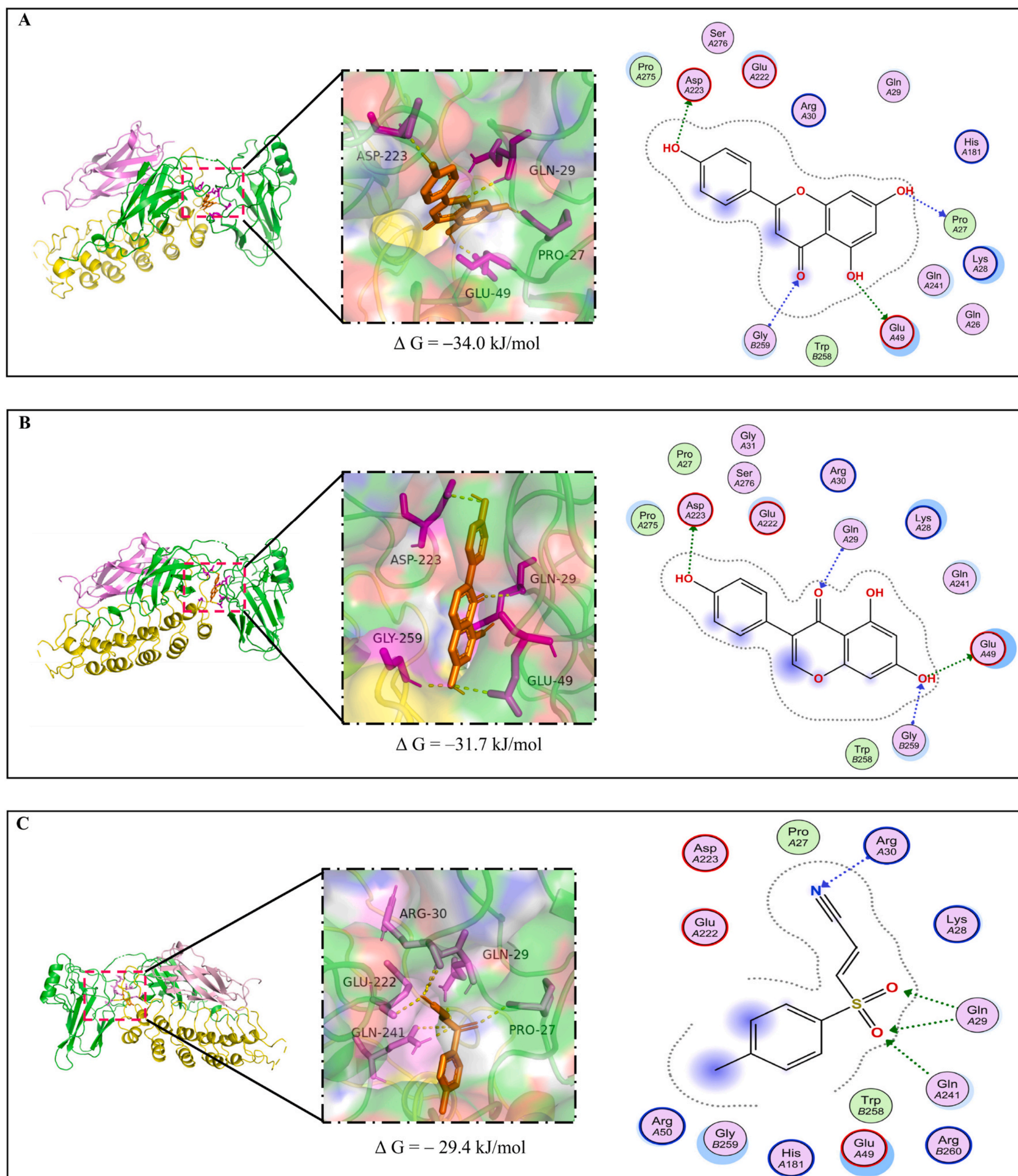


**Fig. 5.** Molecule docking 2D (right) and 3D (left) diagrams describing the interaction between apigenin (A), genistein (B) or the inhibitor BAY 11–7082 (C) and the targeted protein IκBα–NF–κB (PDB code, 1IKN).

Another study also pointed out that the frying treatment of onion led to decreased flavonoid content by 33%, while boiling and steaming reduced flavonoid content by 14–20% (Lee et al., 2008). Additionally, heat treatment also could induce structural damage of flavonoids (Granito et al., 2007), via a serial reaction such as oxidation, hydroxylation, and especially ring-cleavage. For example, the chalcan-trione structure of quercetin can be opened by a nucleophilic attack at C3 (Barnes et al., 2013). It was verified that heat treatment of flavonoids under alkaline conditions triggered cleavage of the heterocyclic ring (*i.e,* the C-ring) via the oxidation of the ether bond (Chen et al., 2017). Therefore, heat treatment of flavonoids might cause activity change. It has been identified that the heated vegetable juices had decreased anti-proliferative activity against the HL-60 cell line (Roy et al., 2007), while the cold-processed surf clam extracts showed better anti-tumor activity in seven cancer cell lines than the heat-processed extracts (Odeleye et al., 2020). In addition, the thermal processing of lactoferrin had an impact on its ability to inhibit the LPS-induced NF-κB activation (Goulding et al., 2021). It was reasonable that the heated flavonoids in this study contained some degraded products and subsequently possessed a reduced anti-inflammatory capacity in the cells. Based on these mentioned facts, the possible impact of heat treatment on other bioactive components in foods should be emphatically an interesting topic in the future.

Flavonoids are an important class of bioactive polyphenols in foods, and their structural diversity endows them with different activity potential. A previous study had shown that the –OH group positions, double bonds, and B-ring structure are critical to the anti-inflammatory activity of flavonoids (Comalada et al., 2006). Moreover, the A-ring was crucial for the anti-inflammatory effect of the flavonoid members namely flavones and isoflavones in human vascular endothelial EA.

hy926 cells; for example, the substituted position of the B-ring at C3 rather than C2 was important for NO release (Yi et al., 2011). It was clarified that the B-ring position, a C2=C3 double bond in the C-ring, and an OH-group at C5 and C7 of the A-ring were associated with higher anti-inflammatory effect (Zhang et al., 2019). Thus, different flavonoid compounds had different anti-inflammatory potentials. A past study had found that galangin had higher anti-inflammatory potential than quercetin, due to different chemical features in the B-ring (Cai et al., 2021). It was reasonable that apigenin and genistein having differences in their chemical structures were measured with different regulatory effects on the release of the targeted cytokines (e.g. IL-1β and IL-10). However, the relationship between flavonoid structure and their anti-inflammatory efficiencies is still not clarified sufficiently, and further research should be conducted to close the gap.

Molecular docking studies have been commonly used to predict the interactions between ligands and targets. In silico studies had revealed that chebulagic, chebulinic, and gallic acids were capable of binding TNF-α receptor-1 to mediate an anti-TNF-α activity (Shanmuganathan and Angayarkanni, 2018), while ferulic acid exhibited significant ligand efficiency towards pro-caspase-1 and NF-κB (Doss et al., 2016). To support the anti-inflammatory activity of the flavonoids, the potential interaction between the targeted flavonoids and IκBα–NF–κB complex was explored. In agreement with the mentioned results, it was also found in this study that the molecular events happened at the binding interface of ligand (apigenin/genistein)-protein (IκBα–NF–κB), indicating that apigenin and genistein might be potential inhibitors of NF-κB. Hence, the *in silico* results demonstrated again the anti-inflammatory activity of the flavonoids towards the stimulated cells.

## 5. Conclusion

The present study declared that pre-incubation with two flavonoids apigenin and genistein could attenuate the TNF-α-induced cellular inflammation in IEC-6 cells, through inhibiting the release of three pro-inflammatory mediators (PGE2, IL-1β, and IL-6) but enhancing the production of two anti-inflammatory cytokines (IL-10 and TGF-β). The anti-inflammatory effect was achieved by inhibiting the phosphorylated IκBα and p65 and blocking the nuclear translocation of p65 protein. Molecular docking results also confirmed that the flavonoids might interact with the signal factor IκBα–NF–κB in the NF-κB pathway. The above anti-inflammatory potentials of the flavonoids could be verified by the similar function of NF-κB inhibitor BAY 11–7082 in the targeted cells. However, heat treatment of the flavonoids consistently reduced the assessed anti-inflammatory activity. Regarding potential healthcare functions of phytochemicals, excessive heat treatment of plant foods might have disadvantages and should be concerned about in the future.

## CRediT authorship contribution statement

**Shi-Qing Cai:** Formal analysis, Methodology, Writing – original draft. **Zhi-Mei Tang:** Formal analysis. **Cen Xiong:** Formal analysis. **Fei-Fei Wu:** Formal analysis. **Jun-Ren Zhao:** Formal analysis. **Qiang Zhang:** Writing – original draft. **Li Wang:** Methodology. **Xiao-Nan Zhang:** Methodology. **Xin-Huai Zhao:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801. https://doi.org/10.1016/j.cell.2006.02.015.

Alenghat, T., Osborne, L.C., Saenz, S.A., Kobuley, D., Ziegler, C.G.K., Mullican, S.E., Choi, I., Grunberg, S., Sinha, R., Wynosky-Dolfi, M., Snyder, A., Giacomin, P.R., Joyce, K.L., Hoang, T.B., Bewtra, M., Brodsky, I.E., Sonnenberg, G.F., Bushman, F.D., Won, K.J., Lazar, M.A., Artis, D., 2013. Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. Nature 504, 153–157. https://doi.org/10.1038/nature12687.

Barnes, J.S., Foss Jr., F.W., Schug, K.A., 2013. Thermally accelerated oxidative degradation of quercetin using continuous flow kinetic electrospray-ion trap-time of flight mass spectrometry. J. Am. Soc. Mass Spectrom. 24, 1513–1522. https://doi.org/10.1007/s13361-013-0698-6.

Basilicata, M.G., Pepe, G., Rapa, S.F., Merciai, F., Ostacolo, C., Manfra, M., Di Sarno, V., Autore, G., De Vita, D., Marzocco, S., Campiglia, P., 2019. Anti-inflammatory and antioxidant properties of dehydrated potato-derived bioactive compounds in intestinal cells. Int. J. Mol. Sci. 20, e6087 https://doi.org/10.3390/ijms20236087.

Cai, S.Q., Zhang, Q., Zhao, X.H., 2021. The in vitro anti-inflammatory activities of galangin and quercetin towards the LPS-injured rat intestinal epithelial (IEC-6) cells as affected by heat treatment. Molecules 26, e7495. https://doi.org/10.3390/molecules26247495.

Chen, L.Y., Wu, J.Y., Liang, J.Y., 2017. Using chromatography and mass spectrometry to monitor isomerization of catechin in alkaline aqueous with thermal processing. J. Food Process. Preserv. 42, e13365 https://doi.org/10.1111/jfpp.13365.

Cianciulli, A., Calvello, R., Cavallo, P., Dragone, T., Carofiglio, V., Panaro, M.A., 2012. Modulation of NF-kappa B activation by resveratrol in LPS treated human intestinal cells results in downregulation of PGE(2) production and COX-2 expression. Toxicol. Vitro 26, 1122–1128. https://doi.org/10.1016/j.tiv.2012.06.015.

Comalada, M., Ballester, I., Bailón, E., Sierra, S., Xaus, J., Gálvez, J., Medina, F.S.D., Zarzuelo, A., 2006. Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structureactivity relationship. Biochem. Pharmacol. 72, 1010–1021. https://doi.org/10.1016/j.bcp.2006.07.016.

Dilshara, M.G., Kang, C.H., Choi, Y.H., Kim, G.Y., 2015. Mangiferin inhibits tumor necrosis factor-alpha-induced matrix metalloproteinase-9 expression and cellular invasion by suppressing nuclear factor-kappa B activity. Bmb Rep. 48, 559–564. https://doi.org/10.5483/BMBRep.2015.48.10.003.

Doss, H.M., Dey, C., Sudandiradoss, C., Rasool, M.K., 2016. Targeting inflammatory mediators with ferulic acid, a dietary polyphenol, for the suppression of monosodium urate crystal-induced inflammation in rats. Life Sci. 148, 201–210. https://doi.org/10.1016/j.lfs.2016.02.004.

Fu, Y., Liu, W.N., Soladoye, O.P., 2021. Towards innovative food processing of flavonoid compounds: insights into stability and bioactivity. LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 150, e111968. https://doi.org/10.1016/j.lwt.2021.111968.

Ganesan, K., Quiles, J.L., Daglia, M., Xiao, J.B., Xu, B.J., 2021. Dietary phytochemicals modulate intestinal epithelial barrier dysfunction and autoimmune diseases. Food Front. 2, 357–382. https://doi.org/10.1002/fft2.102.

Gao, Y.N., Davis, B., Zhu, W.S., Zheng, N., Meng, D., Walker, W.A., 2021. Short-chain fatty acid butyrate, a breast milk metabolite, enhances immature intestinal barrier function genes in response to inflammation in vitro and in vivo. Am. J. Physiol. Gastrointest. Liver Physiol. 320, G521−G530. https://doi.org/10.1152/ajpgi.00279.2020.

Gou, Y.R., Sun, J., Liu, J., Chen, H., Kan, J., Qian, C.L., Zhang, N.F., Jin, C.H., 2019. Structural characterization of a water-soluble purple sweet potato polysaccharide and its effect on intestinal inflammation in mice. J. Funct.Foods 61, e103502. https://doi.org/10.1016/j.jff.2019.103502.

Goulding, D.A., Vidal, K., Bovetto, L., O'Regan, J., O'Brien, N.M., O'Mahony, J.A., 2021. The impact of thermal processing on the simulated infant gastrointestinal digestion, bactericidal and anti-inflammatory activity of bovine lactoferrin - an *in vitro* study. Food Chem. 362, e130142 https://doi.org/10.1016/j.foodchem.2021.130142.

Granito, M., Brito, Y., Torres, A., 2007. Chemical composition, antioxidant capacity and functionality of raw and processed *Phaseolus lunatus*. J. Sci. Food Agric. 87, 2801–2809. https://doi.org/10.1002/jsfa.2926.

Gupta, S.C., Kunnumakkara, A.B., Aggarwal, S., Aggarwal, B.B., 2018. Inflammation, a double-edge sword for cancer and other age-related diseases. Front. Immunol. 9, e2160 https://doi.org/10.3389/fimmu.2018.02160.

He, J.A., Giusti, M.M., 2010. Anthocyanins: natural colorants with health-promoting properties. Annu. Rev. Food Sci. Technol. 1, 163–187. https://doi.org/10.1146/annurev.food.080708.100754.

Hummel, M., Kurian, S.M., Lin, S., Borodyanskiy, A., Zhang, Z., Li, Z., Kim, S.J., Salomon, D.R., Abecassis, M., 2009. Intragraft TNF receptor signaling contributes to activation of innate and adaptive immunity in a renal allograft model. Transplantation 87, 178–188. https://doi.org/10.1097/TP.0b013e3181938971.

Islam, M.S., Kaji, N., Mikawa, S., Yang, Q., Kusabe, M., Hori, M., Ozaki, H., 2018. Induction of myosin light chain kinase and CPI-17 by TGF-beta accelerates contractile activity in intestinal epithelial cells. J. Vet. Med. Sci. 80, 977–984. https://doi.org/10.1292/jvms.17-0684.

Lee, S.H., Kwon, J.E., Cho, M.L., 2018. Immunological pathogenesis of inflammatory bowel disease. Int. Res. 16, 26–42. https://doi.org/10.5217/ir.2018.16.1.26.

Lee, S.U., Lee, J.H., Choi, S.H., Lee, J.S., Ohnisi-Kameyama, M., Kozukue, N., Levin, C.E., Friedman, M., 2008. Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial onion products. J. Agric. Food Chem. 56, 8541–8548. https://doi.org/10.1021/jf801009p.

Liu, C.L., Guo, Y., Zhao, F.R., Qin, H.X., Lu, H.Y., Fang, L., Wang, J., Min, W.H., 2019. Potential mechanisms mediating the protective effects of a peptide from walnut (*Juglans mandshurica* Maxim.) against hydrogen peroxide induced neurotoxicity in PC12 cells. Food Fuct. 10, 3491–3501. https://doi.org/10.1039/c8fo02557f.

Ma, L., Gong, H.Y., Zhu, H.Y., Ji, Q., Su, P., Liu, P., Cao, S.N., Yao, J.F., Jiang, L.L., Han, M.Z., Ma, X.T., Xiong, D.S., Luo, H.R., Wang, F., Zhou, J.X., Xu, Y.F., 2014. A novel small-molecule tumor necrosis factor alpha inhibitor attenuates inflammation in a hepatitis mouse model. J. Biol. Chem. 289, 12457–12466. https://doi.org/10.1074/jbc.M113.521708.

Maini, R.N., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Davis, D., Macfarlane, J.D., Antoni, C., Leeb, B., Elliott, M.J., Woody, J.N., Schaible, T.F., Feldmann, M., 1998. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. 41, 1552–1563. https://doi.org/10.1002/1529-0131(199809)41:9.

Mowat, A.M., Agace, W.W., 2014. Regional specialization within the intestinal immune system. Nat. Rev. Immunol. 14, 667–685. https://doi.org/10.1038/nri3738.

Odeleye, T., Zeng, Z., White, W.L., Wang, K.S., Li, H., Xu, X., Xu, H., Li, J., Ying, T., Zhang, B., Feng, T., Lu, J., 2020. Effects of preparation method on the biochemical characterization and cytotoxic activity of New Zealand surf clam extracts. Heliyon 6, e04357. https://doi.org/10.1016/j.heliyon.2020.e04357.

Opal, S.M., DePalo, V.A., 2000. Anti-inflammatory cytokines. Chest 117, 1162–1172. https://doi.org/10.1378/chest.117.4.1162.

Oyeleke, M.B., Owoyele, B.V., 2022. Saponins and flavonoids from Bacopa floribunda plant extract exhibit antioxidant and anti-inflammatory effects on amyloid beta 1-42-induced Alzheimer's disease in BALB/c mice. J. Ethnopharmacol. 288, e114997 https://doi.org/10.1016/j.jep.2022.114997.

Park, C., Cha, H.-J., Lee, H., Kim, G.-Y., Choi, Y.H., 2021. The regulation of the TLR4/NF-kappa B and Nrf2/HO-1 signaling pathways is involved in the inhibition of lipopolysaccharide-induced inflammation and oxidative reactions by morroniside in RAW 264.7 macrophages. Arch. Biochem. Biophys. 706, e108926 https://doi.org/10.1016/j.abb.2021.108926.

Park, J.S., Choi, J., Hwang, S.H., Kim, J.K., Kim, E.K., Lee, S.Y., Lee, B.I., Park, S.H., Cho, M.L., 2019. Cottonseed oil protects against intestinal inflammation in dextran sodium sulfate-induced inflammatory bowel disease. J. Med. Food 22, 672–679. https://doi.org/10.1089/jmf.2018.4323.

Piao, S., Du, W., Wei, Y.L., Yang, Y., Feng, X.Y., Bai, L.H., 2020. Protectin DX attenuates IL-1 beta-induced inflammation via the AMPK/NF-kappa B pathway in chondrocytes and ameliorates osteoarthritis progression in a rat model. Int. Immunopharm. 78, e106043 https://doi.org/10.1016/j.intimp.2019.106043.

Reuter, S., Gupta, S.C., Chaturvedi, M.M., Aggarwal, B.B., 2010. Oxidative stress, inflammation, and cancer How are they linked? Free Radic. Biol. Med. 49, 1603–1616. https://doi.org/10.1016/j.freeradbiomed.2010.09.006.

Roy, M.K., Takenaka, M., Isobe, S., Tsushida, T., 2007. Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: effects of thermal treatment. Food Chem. 103, 106–114. https://doi.org/10.1016/j.foodchem.2006.08.002.

Santhirasegaram, V., Razali, Z., Somasundram, C., 2013. Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. Ultrason. Sonochem. 20, 1276–1282. https://doi.org/10.1016/j.ultsonch.2013.02.005.

Shanmuganathan, S., Angayarkanni, N., 2018. Chebulagic acid, chebulinic acid and gallic acid, the active principles of Triphala, inhibit TNFalpha induced pro-angiogenic and pro-inflammatory activities in retinal capillary endothelial cells by inhibiting p38, ERK and NF-kB phosphorylation. Vasc. Pharmacol. 108, 23–35. https://doi.org/10.1016/j.vph.2018.04.005.

Somensi, N., Rabelo, T.K., Guimaraes, A.G., Quintans-Junior, L.J., de Souza Araujo, A.A., Fonseca Moreira, J.C., Gelain, D.P., 2019. Carvacrol suppresses LPS-induced pro-inflammatory activation in RAW 264.7 macrophages through ERK1/2 and NF-κB pathway. Int. Immunopharm. 75, e105743 https://doi.org/10.1016/j.intimp.2019.105743.

Sun, C.D., Zhao, C., Guven, E.C., Paoli, P., Simal-Gandara, J., Ramkumar, K.M., Wang, S.P., Buleu, F., Pah, A., Turi, V., Damian, G., Dragan, S., Tomas, M., Khan, W., Wang, M.F., Delmas, D., Portillo, M.P., Dar, P., Chen, L., Xiao, J.B., 2020. Dietary polyphenols as antidiabetic agents: advances and opportunities. Food Front. 1, 18–44. https://doi.org/10.1002/fft2.15.

Tobin, A.M., Kirby, B., 2005. TNF alpha inhibitors in the treatment of psoriasis and psoriatic arthritis. BioDrugs 19, 47–57. https://doi.org/10.2165/00063030-200519010-00006.

van Dullemen, H.M., van Deventer, S.J., Hommes, D.W., Bijl, H.A., Jansen, J., Tytgat, G.N., Woody, J., 1995. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterol. 109, 129–135. https://doi.org/10.1016/0016-5085(95)90277-5.

Vera, M., Torramade-Moix, S., Martin-Rodriguez, S., Cases, A., Cruzado, J.M., Rivera, J., Escolar, G., Palomo, M., Diaz-Ricart, M., 2018. Antioxidant and anti-inflammatory strategies based on the potentiation of glutathione peroxidase activity prevent endothelial dysfunction in chronic kidney disease. Cell. Physiol. Biochem. 51, 1287–1300. https://doi.org/10.1159/000495540.

Wang, B., Wang, J., Zhao, X.H., 2017. In vitro Activities of the four structurally similar flavonols weakened by the prior thermal and oxidative treatments to a human colorectal cancer line. J. Food Biochem. 41, e12310 https://doi.org/10.1111/jfbc.12310.

Witaicenis, A., Seito, L.N., da Silveira Chagas, A., de Almeida Jr., L.D., Luchini, A.C., Rodrigues-Orsi, P., Cestari, S.H., Di Stasi, L.C., 2014. Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives. Phytomedicine 21, 240–246. https://doi.org/10.1016/j.phymed.2013.09.001.

Ye, J., Ye, C.Q., Huang, Y.Y., Zhang, N., Zhang, X.Q., Xiao, M.T., 2019. Ginkgo biloba sarcotesta polysaccharide inhibits inflammatory responses through suppressing both NF-kappaB and MAPK signaling pathway. J. Sci. Food Agric. 99, 2329–2339. https://doi.org/10.1002/jsfa.9431.

Yi, L., Jin, X., Chen, C.Y., Fu, Y.J., Zhang, T., Chang, H., Zhou, Y., Zhu, J.D., Zhang, Q.Y., Mi, M.T., 2011. Chemical structures of 4-oxo-flavonoids in relation to inhibition of oxidized low-density lipoprotein (LDL)-induced vascular endothelial dysfunction. Int. J. Mol. Sci. 12, 5471–5489. https://doi.org/10.3390/ijms12095471.

Yuan, L.L., Zhang, F., Shen, M.Y., Jia, S., Xie, J.H., 2019. Phytosterols suppress phagocytosis and inhibit inflammatory mediators via ERK pathway on LPS-triggered inflammatory responses in RAW264.7 macrophages and the correlation with their structure. Foods 8. https://doi.org/10.3390/foods8110582 e582.

Zhang, P., Mak, J.C.W., Man, R.Y.K., Leung, S.W.S., 2019. Flavonoids reduces lipopolysaccharide-induced release of inflammatory mediators in human bronchial epithelial cells: structure-activity relationship. Eur. J. Pharmacol. 865, e172731 https://doi.org/10.1016/j.ejphar.2019.172731.