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Preventive Effect of Crocin in Inflamed Animals and in LPS-Challenged RAW 264.7 Cells

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Gardenia jasminoides Ellis and *Crocus sativus* L. are both traditional Chinese medicines that have significant biologic activities on inflammatory processes. But the active ingredients remain unclear. Crocin, a representative of carotenoid compounds, has now drawn considerable attention not only because it is a natural food colorant but also because it has great potential in medicine. But until now, the systematic anti-inflammatory effect of crocin has not been well established. In the present study, experiments were carried out to evaluate the anti-inflammatory effects of crocin in vitro and in vivo. In vitro, cyclooxygenase (COX) inhibition assays showed that crocin exhibits a dual inhibitory activity against the COX-1 and COX-2 enzymes. Anti-inflammatory activity in vivo was evaluated using two animal edema model tests. Pretreatment with crocin (p.o.) dose-dependently inhibited the xylene-induced ear edema in mice and carrageenan-induced paw edema in rats. In gastric lesion tests, crocin was gastric-sparing in that it elicited markedly fewer stomach lesions as compared to the number of stomach lesions caused by indomethacin in rats. In further studies, crocin was found to significantly inhibit the productions of prostaglandin E_2 (PGE_2) in lipopolysaccharide (LPS)-challenged RAW 264.7, which is parallel to its prevention of the nuclear translocation of the NF-κB p50 and p65 subunits. These data indicate that crocin exhibits obvious anti-inflammatory effects and may be one of the active ingredients in *Gardenia jasminoides* Ellis or *Crocus sativus* L. that can modulate inflammatory processes.

KEYWORDS: Edema; COX; prostaglandin E_2; NF-κB

INTRODUCTION

Gardenia jasminoides Ellis is a traditional herbal medicine used for the treatment of deficient dysphoria, conjunctival congestion, hemorrhage, pathopyretic ulcer, sprain, swelling, pain, and coronary artery disease in China (*1*).

Studies on the isolation and purification of its chemical components showed that *Gardenia jasminoides* Ellis contains terpenoids, crocin, gardenoside, shanzhiside, scandoside methylester, gentiobiose, geniposide and genipin (formed by hydrolysis of geniposide), sitosterin, gardenin, pectin,tannin, D-mannitol, and so on (*2*).

The most noticeable thing about this herb is that the extract of gardenia fruit can exhibit yellow, red, and blue colors and is widely known for its powerful coloring ability in the food industry (*3*) because it is nontoxic and chemically stable compared with many other natural food pigments.

The main colorants in the extract are now identified as carotenoid compounds (*4*−*6*), which are esterified with one or two glucoses and gentibiose sugarmoieties and are known for their unique water-soluble behavior, in contrast to most families of carotenoids.

Crocin, a representative of carotenoid compounds, has now drawn considerable attention not only because it is a natural food colorant but also because it has great potential in medicine. Numerous studies have shown that crocin has a variety of pharmacological effects, such as protection against cardiovascular diseases (*7*−*9*), inhibition of tumor cell proliferation (*10*), neuroprotection (*11*, *12*), and protection of hepatocytes (*13*).

But to our knowledge, the systematic anti-inflammatory effect of crocin has not been well established. As mentioned in the first paragraph, *Gardenia jasminoides* Ellis has significant biologic activities in inflammatory processes. But the active ingredients of *Gardenia jasminoides* Ellis in inflammatory responses remain unclear. In the present study, experiments were carried out to evaluate the anti-inflammatory effects of crocin on animal models of acute inflammation: xylene-induced ear edema in mice, carrageenan-induced paw edema in rats. Furthermore, in vitro cyclooxygenase (COX) inhibition assays, prostaglandin E_2 (PGE_2) measurement, and evaluation of NF-κB activity in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells were undertaken to unravel the underlying mechanism.

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MATERIALS AND METHODS

Animals. Male Kunming mice weighing 25 ± 2 g and Sprague−Dawley rats weighing 150 ± 10 g were used. The animals were housed in a 12 h light/dark cycle in a temperature-controlled room (21−24 °C). Food and water were available ad libitum. Before treatment, animals were subjected to fasting overnight, with free access to water. The experimental protocol was approved by the Institutional Committee for Animal Care of Nanjing Normal University and in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Cell Line and Cell Culture. RAW 264.7 murine macrophages were obtained from American type Culture Collection (ATCC, Rockville, Maryland, USA). Cells were cultured in 75- or 150-cm^2 flasks with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were incubated in a 5% CO_2 incubator at 37 °C.

In Vitro Cyclooxygenase (COX) Inhibition Assays. The ability of crocin and indomethacin (0.01, 0.1, 1, 10, 30, and 100 μM) to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (Cayman Chemical, Ann Arbor, Michigan, USA) according to a previously reported method (*14*). The solution of the test compound was prepared immediately before starting the assay.

Xylene-Induced Ear Edema Test. In order to test whether crocin possesses anti-inflammatory properties, the xylene-induced ear edema test was performed as previously described with minor modifications (*15*). Crocin was administered orally, as a finely homogenized suspension in 0.5% carboxymethylcellulose (2 mL per 100 g body weight), at doses of 25, 50, and 100 mg/kg 1 h before the application of xylene. A total of 20 μL of xylene was applied to the inner surface of the right ear of each mouse. The left ear remained untreated. Control animals received the vehicle (0.5% carboxymethylcellulose). Indomethacin (10 mg/kg, homogenized in 0.5% carboxymethylcellulose) was used as the reference drug. The animals were sacrificed by cervical dislocation 1 h later, and two ear plugs (7 mm in diameter) were removed from both the treated ear and the untreated ear. Weights of treated and untreated ear plugs were measured with an electronic balance. The difference in weight of the two ear plugs was taken as a measure of edematous response.

Carrageenan-Induced Edema in Rats. Groups of nine rats each were used. Paw swelling was induced by subplantar injection of 0.05 mL of 1% sterile lambda carrageenan in saline into the right hind paw (*16*). Crocin at doses of 12, 25, and 50 mg/kg was administered p.o. 1 h before carrageenan injection (the dose has been transformed according to the body surface area). Indomethacin (5 mg/kg) was used as described above. The control group received the vehicle only (5 mL/kg p.o). The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (YLS-7A, Zhenghua Biotechnology Co.Ltd., An Hui, China) at times of 1, 2, 3, 4, 5, and 6 h after carrageenan injection. The difference between the left and the right paw volumes (indicating the degree of inflammation) was determined, and the percent inhibition of edema was calculated in comparison to that of the control rats.

PGE_2 Level in Inflamed Paws. After determining the extent of the paw edema (0 h and 6 h), the animals were sacrificed by cervical dislocation. The right hind paws were removed below the ankle and degloved to remove the bone. The tissues were then homogenized in 5 mL of ice-cold saline, and sonicated on an ice bath for 12 s. The tissue homogenates were centrifuged at 2000*g* at 4 °C for 5 min, and aliquots of the supernatant were used to determine the PGE_2 levels by enzyme-linked immunosorbent assay (ELISA) with a microplate reader (Biorad, Hercules, California, USA) according to the manufacturer's instructions.

Gastric Lesion Assessment. Drug preparation was performed as already described in Carrageenan-Induced Edema in Rats. Male rats were fasted overnight and then subjected to a daily oral dose of crocin and indomethacin for seven successive days. The control rats were administered an equivalent volume of the vehicle. Twenty-four hours after the last dose, the rats were sacrificed so that the stomach could be removed, opened along the greater curvature, and cleaned gently by dipping in saline. The mucosal damage was examined by light microscopy. The gastric lesion score is calculated by summing the length of all lesions in a given stomach and expressed in mm of lesion (*17*, *18*). Crocin was tested at an oral dose of 12, 25, and 50 mg/kg.

PGE_2 Measurement in LPS-Stimulated RAW 264.7 Cells. To further investigate the anti-inflammatory mechanism of crocin, PGE_2 productions in LPS-stimulated RAW 264.7 cells were examined. For PGE_2 determination, RAW 264.7 cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated for 18 h. Then, cells were washed twice with phosphate buffered saline (PBS) and pretreated with various concentrations of crocin (1,3,10 μM) and indomethacin (10 μM) for 2 h before being further incubated for 16 h in fresh DMEM with or without 1 μg/mL of LPS. After incubation, supernatants were collected to measure PGE_2 concentration by ELISA with a microplate reader (Biorad, Hercules, CA, USA) as specified by the manufacturer.

Preparation of Nuclear/Cytosolic Fractions and Evaluation of NF-κB Activity. Nuclear and cytosolic fractions from RAW 264.7 murine macrophages (about 5×10^6 cells) were performed by using Nuclear Extract Kit (Active Motif Europe, Rixensart, Belgium), according to the manufacturer's instructions. The supernatant was aliquoted and stored at −80 °C until use for p50/p65 assays. Protein concentration was determined by using a protein assay.

The effect of crocin (1, 3, and 10 μM) and indomethacin (10 μM) on the activation of NF-κB was evaluated by commercially available ELISA kits for p50 and p65 subunits. Nuclear and cytosolic extracts were prepared as described above and evaluated for the presence of p50 and p65/RelA subunits using Trans AM NF-κB p50 Chemi and NF-κB p65 Chemi Transcription Factor Assay kits, according to the manufacturer's instructions. An equal amount (1 μg) of lysate was used for each sample. These assay kits specifically detected bound NF-κB p65 or p50 subunits in human extracts; activities of p50 and p65 were measured by a microplate reader and expressed as RLU (Relative Luminescence Unit). The amount of translocated p50 and p65 subunits is evaluated as the nuclear/cytoplasm (N/C) ratio (*19*).

Materials. Lipopolysaccharide (*Escherichia coli* serotype O_{127}:B_8), (Sigma Chemical Co., St. Louis, Montana, USA); ovine COX-1/COX-2, PGE_2 enzyme inmmunoassay kits (Cayman Chemical, Ann Arbor, Michigan, USA); Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS), indomethacin, crocin, xylene, carrageenan from seaweed (a mixture of λ- and κ-carrageenans) (Sigma Chemical Co., St. Louis, Montana, USA); Trans AM NF-κB p50 Chemi and NF-κB p65 Chemi Transcription Factor Assay kits (Active Motif Europe, Rixensart, Belgium); and penicillin, streptomycin, and carboxymethylcellulose (Sunshine biotechnology, Nanjing, China) were obtained as indicated.

Statistical Analysis. All results were expressed as the mean ± stabdard deviation (SD) of at least three independent experiments or with the number of observations indicated in the text. Statistical analysis was conducted either by oneway analysis of variance (ANOVA) followed by post hoc Tukey's test or, where appropriate, by Student's *t* test. $P < 0.05$ indicated significant difference.

RESULTS

Effect of Crocin on COX activities. Crocin inhibited the activities of COX-1 and COX-2 in vitro to different extents as shown in **Table 1**. Indomethacin used as the reference drug gave the IC_{50} values of 2.1 μM on COX-1, 2.8 μM on COX-2. Crocin showed an inhibition of COX-1and COX-2 with IC_{50} values of 9.7 and 1.2, respectively, exhibiting higher inhibitory effects on COX-2. These data indicated that both crocin and indomethacin have dual COX-1/COX-2 inhibition and that crocin has a larger selective ratio (IC_{50} of COX-1/IC_{50} of COX-2) than indomethacin.

Effect of Crocin on Xylene-Induced Ear Edema in Mice. Anti-inflammatory activity of crocin was evaluated as the inhibition of the xylene-induced ear edema in mice. Topical application of xylene induced cutaneous inflammation at the ears of mice, which caused a significant increase in ear plug weight of the right ear when compared to that of the vehicle-treated left ear. As a reference drug, indomethacin (10 mg/kg) inhibited the change in ear plug weight. Treating the mice with indomethacin resulted in a percent inhibition of 61.8% in ear plug weight (**Figure 1**).

Table 1. In Vitro COX-1 and COX-2 Enzyme Inhibition Assay Data for Crocin and Indomethacin[a]

	IC_{50} (μM)	
drugs	COX-1	COX-2
indomethacin	2.1	2.8
crocin	9.7	1.2

[a] Values are the means of three determinations acquired using ovine COX-1 and COX-2 assay kits, and the deviation from the mean is <10% of the mean value.

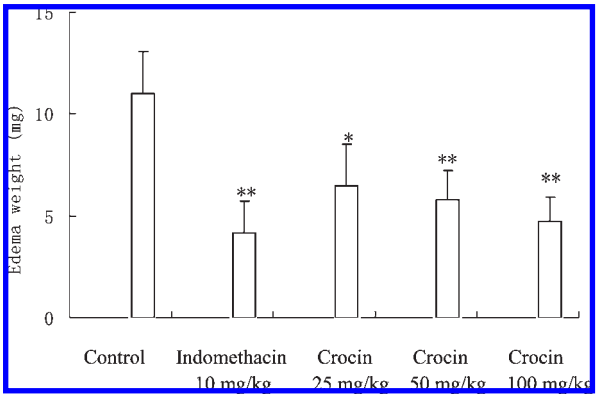

Figure 1. Effect of crocin on xylene-induced ear edema in mice. Crocin (25 mg/kg, 50 and 100 mg/kg, p.o.) was administered 1 h before the application of xylene. A total of 20 μL of xylene was applied to the inner surface of the right ear of each mouse. The left ear remained untreated. Control animals received the vehicle (0.5% carboxymethylcellulose). Indomethacin (10 mg/kg, homogenized in 0.5% carboxymethylcellulose) was used as the reference drug. Weights of treated and untreated ear plugs were measured with an electronic balance. The difference in weight of the two ear plugs was taken as a measure of edematous response. Values represent the mean $\pm$ SD for 10 mice. Inhibition percentages indicate the relative degree of inhibition with respect to the control treated with the vehicle and xylene. * $P < 0.05$, ** $P < 0.01$ compared with control mice.

When crocin was orally administered at 25, 50, and 100 mg/kg, it produced an inhibitory effect in xylene-induced ear edema formation in a dose-dependent fashion (**Figure 1**) and was comparable to that of indomethacin at the highest dose.

Effect of Crocin on Carrageenan-Induced Paw Edema in Rats. In carrageenan-induced paw edema, there was a gradual increase in the edema paw volume in the control group during the whole experiment (6 h). As shown in **Figure 2**, maximal edema formation was observed 6 h after 1% carrageenan injection, and treatment with crocin dose-dependently inhibited carrageenan-induced paw swelling. In particular, treatment with crocin at 50 mg/kg (p.o.) significantly suppressed edema formation 3−6 h after edema induction, the maximal inhibitory percent being 37.1%. As a reference drug, indomethacin (5 mg/kg, p.o.) produced a higher inhibition (40.0%−42.9%) of edema development than crocin 3−6 h after the carrageenan injection (**Figure 2**). The above data indicate that crocin contains reasonable anti-inflammatory activity.

Effect of Crocin on PGE_2 Level in Paw Tissues During Carageenan-Induced Edema. In addition to the inhibition of hind paw swelling, the anti-inflammatory effect of both crocin and indomethacin was also assessed using a range of biochemical assays. Thus, carrageenan-induced hind paw swelling was associated with a pronounced increase in PGE_2 level in the homogenate of an inflamed paw. A nearly 4-fold increase of PGE_2 over the measurements at time 0 was observed 6 h after the carrageenan injection (**Figure 3**). Both Crocin and indomethacin pretreatment

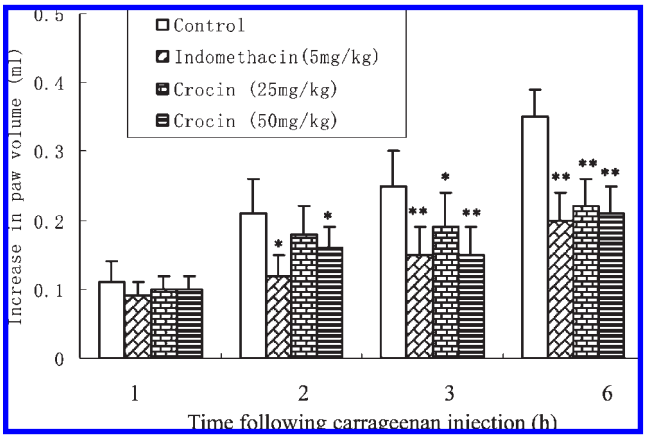

Figure 2. Effect of crocin on carrageenan-induced paw edema in rats. Crocin (25 mg/kg and 50 mg/kg, p.o.; 12 mg/kg is not shown) was administered 1 h min before carrageenan injection. Reference control animals were treated with indomethacin (5 mg/kg, p.o.). Paw edema was induced 1 h later by subplantar injection of 1% carrageenan, 0.05 mL per rat. The volume of the paw was measured at intervals of 1, 2, 3, 4, 5, and 6 h postinjection (data at 4 and 5 h were not shown). The values are expressed as the means $\pm$ SD ($n = 9$). *$P < 0.05$ and **$P < 0.01$ indicate significant differences from the vehicle control group.

reduced the rise in hind paw PGE_2 level observed in carrageenan-injected hind paws. Crocin (at the highest dose) exhibited potency similar to that of indomethacin with respect to PGE_2 formation (**Figure 3**).

Gastric Lesion Test. To characterize the GI safety profiles of crocin, experiments were performed to measure the extent of crocin-induced stomach lesions. Indomethacin at an oral dose (5 mg/kg) caused significant stomach lesions with a lesion score of >30 mm, while crocin (at the highest oral dose) caused negligible lesions (**Figure 4**). Examination of stomach specimens under light microscopy revealed that in rats treated with this compound there is no injury observed in stomach mucosa. The stomach of indomethacin-treated rats is characterized by appreciable damage of the protective mucosal layer.

Effect of Crocin on PGE_2 Levels in LPS-Challenged RAW 264.7 Cells. The level of PGE_2 was significantly upregulated in LPS-challenged RAW 264.7 cells (**Figure 5**). After the induction of LPS, the accumulation of PGE_2 in RAW 264.7 cells increased nearly 8-fold. However, crocin inhibited the LPS-induced accumulation of PGE_2 in a concentration-dependent manner. At a concentration of 10 μM, crocin significantly decreased almost 73% of the PGE_2 production in LPS-challenged RAW 264.7 cells, while indomethacin exhibited 67% inhibition of PGE_2 production. Our findings suggested that crocin inhibited PGE_2 production stimulated by LPS and had comparable inhibitory effects to those of indomethacin.

Crocin Inhibits NF-κB Activation. To ensure a quantitative evaluation, we assessed the translocation of p65 and p50 subunits in murine macrophage RAW 264.7 cells, by using a commercially available ELISA kit (**Figure 6A**). In unstimulated macrophages, a low basal activation of NF-κB is detected; conversely, LPS at 1 μg/mL potently stimulates p50 (**Figure 6A**) and p65 nuclear translocation (**Figure 6B**). Crocin inhibits, in a concentration-dependent manner (1 to 10 μM), the nuclear translocation of the NF-κB p50 subunit: at the highest 10 μM concentration, LPS-induced p50 translocation is inhibited by about 66.9% in RAW 264.7 cells. Crocin is more effective than indomethacin, which has been used as a reference drug (**Figure 6A**). As depicted in **Figure 6B**, crocin does not significantly affect p65 translocation

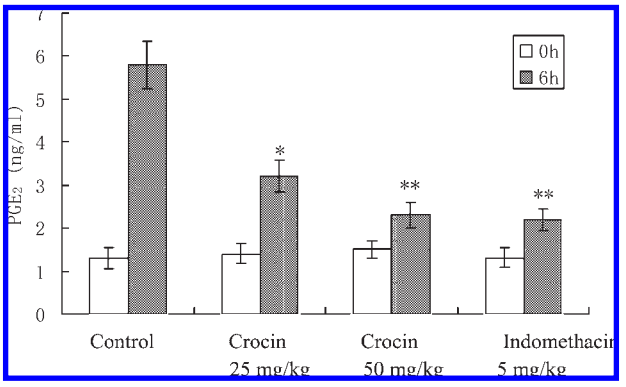

Figure 3. Effects of crocin on PGE_2 level in paw tissues at 6 h after injection of carrageenan (or saline) in the right hind paw. The animals were pretreated with crocin (12 mg/kg was not shown) or indomethacin 1 h before the carrageenan injection. The animals were sacrificed 6 h later, and the contents of PGE_2 were determined in the supernatant prepared from the paws collected at time 0 and 6 h after carrageenan administration. The values are expressed as the means ± SD ($n = 6$). * $P < 0.05$ and ** $P < 0.01$ compared to the control group at the same time point (receive vehicle and saline injection).

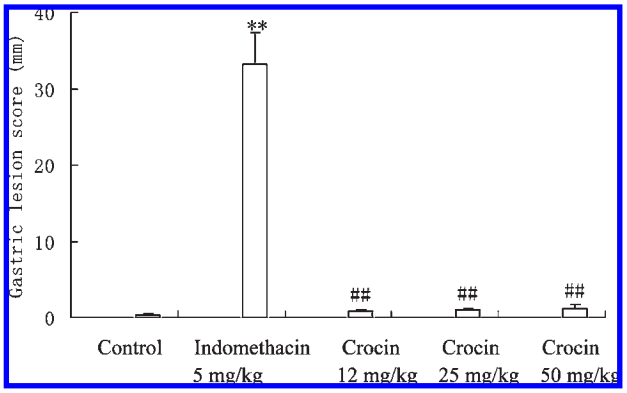

Figure 4. Gastric lesion studies of crocin in rats. Male rats were subjected to fasting overnight and then subjected to a daily oral dose of crocin and indomethacin for seven successive days. The control rats were administered an equivalent volume of the vehicle. Twenty-four hours after the last dose, the rats were sacrificed so that the stomach could be removed, opened along the greater curvature, and cleaned gently by dipping in saline. Values represent mean ± SD for nine rats. The gastric lesion score is calculated by summing the length of all lesions in a given stomach and expressed in mm of lesion. Crocin was tested at the doses of 12, 25, and 50 mg/kg p.o., and indomethacin was tested at 5 mg/kg.* $P < 0.01$ compared with the control group (receive the vehicle). # $P < 0.05$ and ## $P < 0.01$ compared with the indomethacin group.

in unstimulated cells, but it dose-dependently inhibits the LPS-induced one. At the maximum 10 μM concentration, crocin is less effective than indomethacin. (**Figure 6B**).

DISCUSSION

Gardenia jasminoides Ellis and *Crocus sativus* L. are both used in traditional Chinese medicine to treat inflammatory conditions. However, the active ingredients have not been well understood. Phytochemistry analysis demonstrated that both plants have many identical constituents such as crocin, gardenoside, gentiobiose, geniposide, and genipin. We think there may exist some similar substances in these two plants that can play an important role in modulating inflammatory processes.

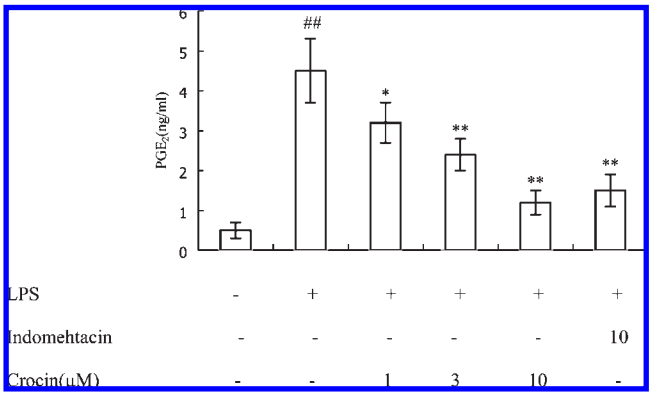

Figure 5. Effect of crocin on PGE_2 production in LPS-challenged RAW 264.7 cells. Cells were treated with 1 μg/mL LPS, 10 μM indomethacin, and the indicated concentrations of crocin. The amounts of PGE_2 in the culture medium were analyzed by enzyme immunoassay. The data represent the means ± SD of three-independent experiments performed in triplicate. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the control group, while $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the LPS-challenged groups.

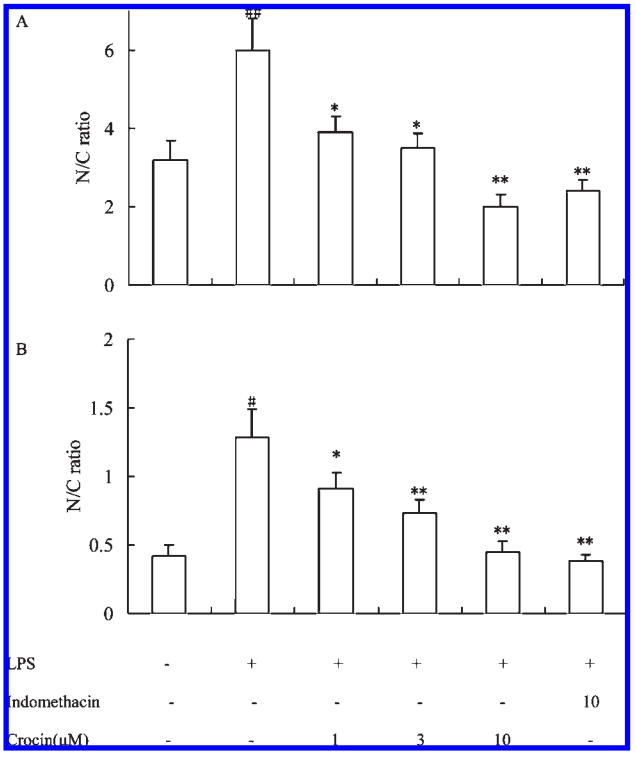

Figure 6. Crocin inhibits NF-κB translocation in murine macrophage RAW 264.7 cells. Crocin inhibits, in a concentration-dependent manner, the nuclear translocation of activated p50 subunit (**A**) and p65 subunit (**B**) in cells challenged by 1 μg/mL LPS. The effects of indomethacin (10 μM) are demonstrated for comparison. Results are expressed as nuclear/cytoplasmic (N/C) ratio. Data are the mean ± SD; $n = 5$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared to control cells; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared to LPS-challenged cells.

Under the illumination of the limited literature and our previous accumulating research work, we determined that crocin may be one of the substances we are looking for. In order to test this hypothesis, we designed this experiment and investigated the possible anti-inflammatory effects of crocin.

First, crocin was subjected to two well-known animal models. In the xylene-induced ear edema test, crocin at 25, 50, and

100 mg/kg significantly suppressed xylene-induced ear edema formation in a dose-dependent fashion (**Figure 1**). In another inflammatory model, the paw edema induced by carrageenan (in which peak edema is characterized by the presence of prostaglandins (*20*)), crocin had potent inhibitory effects on paw swelling and the production of PGE_2 in a homogenate of the inflamed paw. In **Figure 2**, crocin displayed its effect a little later than the reference drug indomethacin. Furthermore, we found that crocin appeared to be less effective than indomethacin. Although the above findings indicated that crocin has a promising anti-inflammatory effect, its underlying mechanism remains unclear and needs to be further elucidated by in vitro experimental data.

Crocin was then subjected to in vitro COX inhibition assays by an enzyme immuno assay (EIA) kit, and its effects on macrophage functions related to inflammation were also investigated. In COX assays, inhibitions of COX-1 and COX-2 by crocin were evidenced by its IC_{50} of 9.7 and 1.2 μM, respectively. In addition, crocin has a higher inhibitory effect on COX-2 than on COX-1 and demonstrated a higher selective ratio than indomethacin. In macrophage functions test, we found that crocin dose-dependently inhibited the LPS-induced proinflammatory molecule PGE_2. This inhibition of the release of PGE_2 may be explained by the inhibition of COX activity as shown in in vitro COX inhibition assays.

NF-κB is a redox-sensitive transcription factor that comprises RelA (p65), NF-κB1 (p50 and p105), NF-κB2 (p52 and p100), c-Rel, and RelB. Although different homo- and heterodimeric forms of this factor have been described, NF-κB is usually composed of the p50/p65 heterodimer (*21*, *22*). Growing evidence has demonstrated that NF-κB is known to play a critical role in the regulation of genes involved in cell survival and in the coordination of the expressions of pro-inflammatory enzymes including LOX and COX (*23*, *24*). Therefore, we examined the p50 and p65 (**Figure 6**) nuclear translocation of NF-κB to confirm whether the releasing inhibition of PGE_2 was influenced by the NF-κB signaling pathway. The results obtained indicate that the p65 and p50 NF-κB subunits to the nucleus are simultaneously inhibited by crocin in a concentration-dependent manner and that this inhibition corresponds to the inhibition of PGE_2 production.

One thing worthy of being mentioned was that in the gastric lesion test crocin did not cause significant gastric ulceration at the highest dose of 50 mg/kg for seven successive days. While in the indomethacin group, the stomach of rats was characterized by complete damage of the protective mucosal layer. These findings indicate that crocin not only exerts remarkable anti-inflammatory activity but also shows a more favorable toxicity profile compared with that of indomethacin treatment.

Hye-Jin Koo and colleagues (*25*) studied some pharmacological actions of genipin and reported that genipin showed concentration-dependent inhibition of lipid peroxidation induced by Fe^{2+}/ascorbate in rat brain homogenate and exhibited significant topical anti-inflammatory effect showing an inhibition of croton oil-induced ear edema in mice. Genipin exhibits an inhibitory effect on NO production through the inhibition of nuclear factor-κB (NF-κB) activation. Together with the results in the present study, we conclude that crocin and geniposide may be two of the main active components which may contribute to the regulative effect of inflammatory processes by *Gardenia jasminoides* Ellis or *Crocus sativus* L.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world because of their demonstrated efficacy in reducing pain and inflammation (*26*). Although effective at relieving pain and inflammation, NSAIDs are associated with a significant risk of serious adverse events when used chronically. As for classical NSAIDs (aspirin, indomethacin, diclofenac, etc.), they are reported to be associated with an increased risk of gastrointestinal ulcers, including gastrointestinal hemorrhage, perforation, and obstruction. Treatment of these complications has brought a heavy burden on social fortune. Thus, NSAID application in clinics has been used with caution, weighing the benefits and disadvantages. In this study, crocin has an improved gastric safety profile suggesting that crocin may have advantages over the traditional NSAIDs when used chronically. But how crocin can antagonize gastric injury remains unclear and needs to be further studied.

Evidence from several large scale randomized clinical trials and epidemiologic studies has indicated that selective COX inhibitors can elevate the risk of myocardial infarction and stroke, which is related to thrombosis formation (*27*). This evidence led to the subsequent worldwide withdrawal of rofecoxib and valdecoxib, recently. In this study, crocin has presented characteristics of selective COX inhibitors, although its selective ratio is not high. Can this problem be encountered with crocin application?

It has been proposed that the inhibition of one or both COX enzymes, while reducing the levels of gastrotoxic PGs, may result in alternative processing of amino acids (AAs) via the 5-LOX pathway. This increases the production of harmful byproducts (leukotrienes) that can promote the migration of leucocytes and may damage the arterial wall and induce arterial blood clotting (*28*).

But this problem may not occur in crocin application: (1) Crocin can defend the arterial wall through preventing endothelial cells from attack by noxious stimuli, especially reactive oxygen species. This protective effect may be attributed to its well-known antioxidative activity. (2) Crocin showed a dose-dependent inhibition of platelet aggregation and prolonged the occlusive time in electrical stimulation-induced carotid arterial thrombosis, and these effects may be related to the inhibition of Ca^{2+} elevation in stimulated platelets (*29*). The above results suggest that crocin may directly antagonize thrombosis formation which is related to the cardiovascular risks. (3) Crocin may have some unknown mechanisms that can regulate inflammatory processes such as the prevention of AA metabolism via the 5-LOX pathway or direct interaction with leukotrienes (LTs). (4) Crocin has been shown to have protective activity against cardiovascular diseases that can antagonize the cardiovascular adverse effects caused by the selective COX inhibitors. Till now, no cardiovascular event has been reported in basic or clinical studies.

Recently, clinical and experimental studies have revealed the link between inflammation and cancer risk. The mediators and cellular effectors of inflammation are important constituents of the local environment of tumors. In some types of cancer, inflammatory conditions are present before a malignant change occurs. Conversely, in other types of cancer, an oncogenic change induces an inflammatory microenvironment that promotes the development of tumors. Regardless of its origin, smoldering inflammation in the tumor microenvironment has many tumor-promoting effects. It aids in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immune responses, and alters responses to hormones and chemotherapeutic agents (*30*).

The molecular pathways of this cancer-related inflammation are now being unravelled, resulting in the identification of new target molecules. Molecular pathology studies have revealed that COX-2 is expressed by cancer cells and cells of the tumor stroma during tumor progression and in response to chemotherapy or radiation therapy. Experimental studies have demonstrated that COX-2 overexpression promotes tumorigenesis,

and that NSAIDs and COX-2 inhibitors suppress tumorigenesis and tumor progression (*31*). It has been reported that crocin has anticarcinogenic and antitumor (*32*−*34*) activities. The results in the present study show that crocin can exhibit COX-2 inhibition activity, which may contribute to its anticancer activity.

In conclusion, crocin exhibited obvious anti-inflammatory effects and was gastric-sparing relative to the behavior of indomethacin in this study. The results suggest that crocin is one of the active ingredients in *Gardenia jasminoides* Ellis that can modulate inflammatory processes and may present a potential promising safer chemical alternative for classical NSAIDs in chronic use for the treatment of inflammatory diseases.

LITERATURE CITED

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