Methanol Extract of Xanthium strumarium L. Possesses Anti-inflammatory and Anti-nociceptive Activities

In-Tae Kim, Young-Mi Park, Jong-Heon Won, Hyun-Ju Jung, Hee-Juhn Park, Jong-Won Choi, and Kyung-Tae Lee

* College of Pharmacy, Kyung Hee University; Hoegi-Dong, Seoul 130–701, Korea; b Division of Applied Plant Sciences, Sangji University; Woosan-Dong, Wonju, 220–702 Korea; and c College of Pharmacy, Kyungsung University; Dayeondong, Pusan, 608–736, Korea. Received July 8, 2004; accepted October 5, 2004

As an attempt to identify bioactive natural products with anti-inflammatory activity, we evaluated the effects of the methanol extract of the semen of Xanthium strumarium L. (MEXS) on lipopolysaccharide (LPS)-induced nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor-α (TNF-α) production in RAW 264.7 cells. Our data indicate that MEXS is a potent inhibitor of NO, PGE2 and TNF-α production. Consistent with these findings, the expression levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein and iNOS, COX-2 and TNF-α mRNA were down-regulated in a concentration-dependent manner. Furthermore, MEXS inhibited nuclear factor kappa B (NF-κB) DNA binding activity and the translocation of NF-κB to the nucleus by blocking the degradation of inhibitor of kappa B-α (IκB-α). We further evaluated the anti-inflammatory and anti-nociceptive activities of MEXS in vivo. MEXS (100, 200 mg/kg/d, p.o.) reduced acute paw edema induced by carrageenin in rats, and showed analgesic activities in an acetic acid-induced abdominal constriction test and a hot plate test in mice. Thus, our study suggests that the inhibitions of iNOS, COX-2 expression, and TNF-α release by the methanol extract of the semen of Xanthium strumarium L. are achieved by blocking NF-κB activation, and that this is also responsible for its anti-inflammatory effects.

Key words Xanthium strumarium; nitric oxide; prostaglandin E2; inducible nitric oxide synthase; cyclooxygenase-2; nuclear factor kappa B

Nitric oxides (NOs) and prostaglandins (PGs) are well-known proinflammatory mediators in the pathogenesis of inflammation.1 NO plays a major role in the regulation of vascular tone, neurotransmission, platelet aggregation, and other homeostatic mechanisms.2 NO is synthesized by the three isoforms of nitric oxide synthase (NOS); neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Although nNOS and eNOS are constitutively expressed, iNOS is expressed in response to interferon-γ, lipopolysaccharide (LPS), and a variety of proinflammatory cytokines.3–5 The proinflammatory properties of NO are attributed to the excessive production of NO by iNOS and are influenced by factors such as interactions between NO and other oxidants (e.g., peroxynitrite formation with the superoxide anion), the duration of NO production, and substrate availability. A number of studies have shown that the chronic phase of inflammation in particular, is correlated with an increase in iNOS activity.6 Moreover, elevated levels of NO have been detected in a variety of pathophysiological processes, including circulatory shock, inflammation, and cancer.5,7,8

Cyclooxygenase (COX) is involved in the inflammatory process and catalyzes the rate-limiting step in the synthesis of prostaglandins from arachidonic acid. COX exists in two isoforms; COX-1 and COX-2.9 Like nNOS and eNOS, COX-1 is expressed constitutively in most tissues and appears to be responsible for maintaining normal physiological functions. On the other hand, COX-2 is detected in only certain tissues and is induced transiently by growth factors, proinflammatory cytokines, tumor promoters, and bacterial toxins.9,10

LPS is a major inflammatory molecule that triggers the production of proinflammatory cytokines such as TNF-α in various cell types.12,13 TNF-α plays a key role in the induction and perpetuation of inflammation in autoimmune reactions by activating T cells and macrophages, and by up-regulating other proinflammatory cytokines and endothelial adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, which enhance the recruitment of leukocytes to sites of inflammation.14

Like a TNF-α, LPS is known to activate transcription factors such as nuclear factor-kappa B (NF-κB)/Rel, activator protein (AP)-1, NF-IL6 and CREB/ATF. These nuclear factors are important for the expressions of numerous genes, which include IL-1β in macrophages.15,16 Transcription factors belonging to the NF-κB family regulate a range of genes that mediate inflammation and cell survival.17 NF-κB exists in most cells as homodimeric or heterodimeric complexes containing p50 and p65 subunits, and remains inactive in the cytoplasm in association with the NF-κB inhibitory protein IκB. Moreover, the activation of NF-κB is tightly controlled by a series of inhibitory proteins (IκB-α, IκB-β, and IκB-ε) that sequester the NF-κB complex in the cytoplasm and prevent it from binding to nuclear DNA. The pathway of NF-κB induction in response to proinflammatory stimuli involves the activation of IKK, which phosphorylates both IκB-α and IκB-β on serine residues, triggering their ubiquitination and degradation by 26S proteasome. This process releases NF-κB from IκB proteins which is translocated to the nucleus,18 where it increases the expression of genes encoding pro-inflammatory mediators, such as iNOS, COX-2, TNF-α, interleukin (IL)-6 and -8, and others.19,20

Thus, as a part of our on-going screening program to evaluate the anti-inflammatory potentials of natural compounds, we investigated the anti-inflammatory activity of the methanol extract of the semen of Xanthium strumarium L. in...
vitro and in vivo. In previous studies on Xanthium strumarium, carboxyatractyloside, xanthanol, isoxanthenol, hydroquinone, alkaloids, caffeylquinic acids, and thiazinedione were identified.\(^1\) The semen of Xanthium strumarium L. has been used to treat bacterial infections, diabetes, inflammatory diseases like rhinitis, empyema, and rheumatoid arthritis in Orient,\(^2\) but no report has been issued on its anti-inflammatory activity or its mode of action. Therefore, we evaluated the effects of the methanol extracts of the semen of Xanthium strumarium L. (MEXS) on LPS-induced NO, PGE\(_2\), and TNF-α production in the macrophage cell line RAW 264.7, and subsequently evaluated its in vivo anti-inflammatory and anti-nociceptive effects.

**MATERIALS AND METHODS**

**Chemicals** Dulbecco’s modified Eagle’s minimum essential medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Life Technologies Inc. (Grand Island, NY, U.S.A.). iNOS, COX-2, COX-1, 1α,25-[OH]_2D_3, p-Ib-β-α, p65 and β-actin monoclonal antibodies and the peroxidase-conjugated secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). The enzyme immunoassay (EIA) kit for prostaglandin E\(_2\) (PGE\(_2\)) and tumor necrosis factor-α (TNF-α) were obtained from R&D Systems (Minneapolis, MN, U.S.A.). NS-398, a COX-2 enzyme inhibitor, was from Calbiochem (CA, U.S.A.). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), aprotinin, leupeptin, phenylmethylsulfonylfuoride (PMSF), dithiothreitol, caffeic acid, 1-N^\text{6}-((1-iminoethyl)lysine (T-NIL), LPS (from Escherichia coli 055: B5), acetylsalicylic acid (Aspirin), carragheenin, and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

**Cell Culture and Sample Treatment** The RAW 264.7 murine macrophage cell line was obtained from the Korea Cell Line Bank (Seoul, Korea). These cells were grown at 37°C in DMEM medium supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml) in a humidified atmosphere of 5% CO\(_2\). Cells were incubated with MEXS at different concentrations (30, 60, 90 μg/ml) or positive chemical and stimulated with LPS 1 μg/ml for 24 h.

**MTT Assay for Cell Viability** Cell viability studies were performed in 96-well plates. RAW 264.7 cells were mechanically scraped and plated at 1×10^5/well in 96-well plates containing 100 μl of DMEM medium with 10% FBS and incubated overnight. MEXS was dissolved in DMSO, and the DMSO concentrations in all assays did not exceed 0.1%. After overnight incubation, the test material was added, and the plates were incubated for 24 h. Cells were washed once before adding 50 μl of FBS-free medium containing MTT 5 mg/ml. After 4 h of incubation at 37°C, the medium was discarded and the formazan blue that formed in the cells was dissolved in DMSO 100 μl. The optical density was measured at 540 nm.

**Determination of Nitrite** The nitrite accumulated in culture medium was measured as an indicator of NO production based on the Griess reaction.\(^3\) Briefly, 100 μl of cell culture medium was mixed with 100 μl of Griess reagent [equal volumes of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 0.1% (w/v) naphthylethylenediamine–HCl], incubated at room temperature for 10 min, and then the absorbance at 550 nm was measured in a microplate reader. Fresh culture medium was used as the blank in all experiments. The amount of nitrite in the samples was measured with the sodium nitrite serial dilution standard curve and nitrite production was measured.

**Determination of PGE\(_2\) and TNF-α** PGE\(_2\) and TNF-α level in macrophage culture medium were quantified by EIA kits according to the manufacturer’s instructions.

**Western Blot Analysis** Cellular proteins were extracted from control and MEXS-treated RAW 264.7 cells. The washed cell pellets were resuspended in extraction lysis buffer (50 mM HEPES pH 7.0, 250 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 mM Na fluoride (NaF), 0.5 mM Na orthovanadate) containing 5 μg/ml each of leupeptin and aprotilin and incubated with 15 min at 4°C. Cell debris was removed by microcentrifugation, followed by quick freezing of the supernatants. The protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacturer’s instruction. Forty micrograms of cellular protein from treated and untreated cell extracts was electrophoresed onto a nitrocellulose membrane following separation on a 10% SDS-polyacrylamide gel electrophoresis. The immunoblot was incubated overnight with blocking solution (5% skim milk) at 4°C, followed by incubation for 4 h with a 1:500 dilution of monoclonal anti-iNOS, 1:1000 dilution of anti-COX-2 or COX-1 antibody, 1:1000 dilution of anti-Ib-β-α antibody and 1:500 dilution of anti-p65 antibody (Santa Cruz Biotechnology Inc.). Blots were washed two times with Tween 20/Tris-buffered saline (TTBS) and incubated with a 1:1000 dilution of horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology Inc.) for 1 h at room temperature. Blots were again washed three times with and then developed by enhanced chemiluminescence (Amer sham Life Science, Arlington Heights, IL, U.S.A.). Densities of control, LPS-treated group and β-actin bands were measured using densitometer and density ratio, each group vs. β-actin was calculated.

**RNA Preparation and Polymerase Chain Reaction** Total cellular RNA was isolated using Easy Blue® kits (Intron Biotechnology) according to the manufacturer’s instructions. From each sample, 1 μg of RNA was reverse-transcribed (RT) using MuLV reverse transcriptase, 1 mM dNTP, and oligo (dT\(_{12-18}\)) 0.5 μg/μl. Then PCR analyses were performed on the aliquots of the cDNA preparations to detect iNOS, COX-2, TNF-α and β-actin (as an internal standard) gene expression using a thermal cycler (Perkin Elmer Cetus, Foster City, CA, U.S.A.). The reactions were carried out in a volume of 25 μl containing (final concentration) 1 units of Taq DNA polymerase, 0.2 mM dNTP, ×10 reaction buffer, and 100 pmol of 5’ and 3’ primers. After initial denaturation for 2 min at 95°C, thirty amplification cycles were performed for iNOS (1 min of 95°C denaturation, 1 min of 60°C annealing, and 1.5 min 72°C extension), COX-2 (1 min of 94°C denaturation, 1 min of 60°C annealing, and 1 min 72°C extension) and TNF-α (1 min of 95°C denaturation, 1 min of 55°C annealing, and 1 min 72°C extension). PCR primers used in this study are listed below and were purchased from Bioneer (Seoul, Korea): sense strand iNOS,
5'-ATGGCAACATCGGTCGCAAGTCTACT-3', anti-sense strand iNOS, 5'-GCTGTCAGTACAGTCTGC-AC-TC-3', sense strand COX-2, 5'-GGAGAAGACTCAAGATAGTG-3' anti-sense strand COX-2, 5'-ATGTCATCTAGA-CCTTTACA-3', sense strand TNF-α, 5'-ATGAGCAGACAAGCAGATGC-3', anti-sense strand TNF-α, 5'-TACAGGCTTCTCCTGACTGAAT-3', sense strand β-actin, 5'-TCACTGAGGTGACGTTCATCGATGT-3', anti-sense strand β-actin, 5'-CTTAGAAGCTTTGCGGTGACGATG-3'. After amplification, portions of the PCR reactions were electroforesoed on 2% agarose gel and visualized by ethidium bromide staining and UV irradiation.

**Nuclear Extraction and Electrophoretic Mobility Shift Assay (EMSA)** RAW 264.7 macrophages were plated in 100-mm dishes (1×10⁶ cells). The cells were treated with various MEXS concentrations (30, 60, 90 µg/ml), stimulated with LPS for 1 h, washed once with PBS, scraped into 1 ml of cold PBS, and pelleted by centrifugation. Nuclear extracts were prepared as described previously with slight modification. The cell pellet was resuspended in hypotonic buffer (10 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl, 0.2 mM PMSF, 0.5 mM DTT, 10 µg/ml aprotinin) and incubated on ice for 15 min. Then the cells were lysed by the addition of 0.1% Nonidet P-40 and vigorous vortexing for 10 s. The nuclei were pelleted by centrifugation at 12000×g for 1 min at 4°C and resuspended in high salt buffer (20 mM HEPES, pH 7.9, 25% glycerol, 400 mM KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 1 mM NaF, 1 mM sodium orthovanadate). Nuclear extract 10 µg was mixed with the double-stranded NF-κB oligonucleotide. 5'-AGTGGAGGGACTCTCCACTTGACAAGCTTTACCTTTACA-3' end-labeled by [γ-³²P] dATP (underlying indicates a binding site for NF-κB/cRel homodimeric and heterodimeric complex). Binding reactions were performed at 37°C for 30 min in 30 µl of reaction buffer containing 10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 4% glycerol, 1 µg of poly (dl-d), and 1 mM DTT. The specificity of binding was examined by competition with the 80-fold unlabeled oligonucleotide. DNA-protein complexes were separated from the unbound DNA probe on native 5% polyacrylamide gels at 100 V in 0.5xTBE buffer. The gels were vacuum dried for 1 h at 80°C and exposed to X-ray film at −70°C for 24 h.

**Animals** ICR male mice weighing 20—25 g and Sprague-Dawley male rats weighing 100—120 g were purchased from Korean Experimental Animal Co. and maintained in a constant condition (temperature: 20±2°C, humidity: 40—60%, light/dark cycle: 12 h for two weeks or more. Twenty-four hours before the experiment, only water was offered to the animals. Considering the variation of enzyme activity during one day, the animals were sacrificed at fixed time (10:00 a.m.—12:00 a.m.). These experiments were approved by the University of Kyungsung Animal Care and Use Committee. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals” published by the National Institutes of Health.

**Carrageenin-Induced Edema Test in Rats** The initial hind paw volume of the Sprague-Dawley strain rats was determined volumetrically. A 1% solution of carrageenin in saline (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the test substances had been administered orally. Test samples were, first, dissolved with 10% tween 80 and diluted by saline. The same volume of solvent only was administered for the normal group. The test solution (each fraction: 100 and 200 mg/kg) had been orally administered for 7 d prior to the injection of carrageenin. The control group received the vehicle. Paw volumes were measured up to 5 h at intervals of 60 min, and the volume of the edema was measured with a plethysmometer. Ibuprofen, an anti-inflammatory drug, was used as a standard drug.

**Acetic Acid-Induced Abdominal Constriction Test in Mice** The acetic acid-induced abdominal constriction test was performed as described by Whittle. Vehicle, aminopyrine (100 mg/kg) and test solution (100, 200 mg/kg) were orally administered 30 min before the experiment, and 0.1 ml/10 g of 0.7% acetic acid-saline was then injected i.p. 10 min after the injection, the frequency of abdominal constriction in mice was counted for the succeeding 10 min. Aspirin was used as a standart drug.

**Hot Plate Test** The hot plate test was used to measure the response latencies according to the method described previously by Eddy and Leimbach with minor modifications. In these experiments, the hot plate (Ugo Basile, model-DS 37) was maintained at 56±1°C. The reaction time was noted by observing either the licking of the hind paws or the jumping movements before and after drug administration. The cut-off time was 10 s and morphine sulphate 10 mg/kg (Kuju Pharmaceutical CO), administered intraperitoneally, was used as reference drug.

**Statistical Analysis** Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett’s test for multiple comparisons. The data are reported as mean±S.D. The numbers of independent experiments assessed are given in the legends for the figures.

**RESULTS**

**Effects of MEXS on LPS-Induced NO Production and iNOS Protein and mRNA Expression** NO has been associated with many diseases conditions including inflammation and cancer. To examine the effect of MEXS on LPS-induced NO production in RAW 264.7 cells, cells were treated or not treated with MEXS for 1 h and then treated with LPS (1 µg/ml) for 24 h. Both LPS and sample were not added to control (con.) group. The cell culture medium was then harvested and NO levels were determined using the Griess reaction. LPS induced approximately 10-fold more NO than the control group and MEXS inhibited this NO production in a concentration-dependent manner (Fig. 1B). RT-PCR analysis showed that expression of iNOS mRNA correlated with its protein expression (Fig. 1C). The cytotoxicity of MEXS was evaluated in the presence or absence of LPS by MTT assay. MEXS alone and in the presence of LPS did not affect RAW 264.7 cell viability even at 200 µg/ml for 24 h.
Inhibition of the LPS-Induced Expressions of PGE₂ and COX-2 Protein and mRNA by MEXS

PGE₂ is over-produced in inflammation and this overexpression is related to pain and inflammation. To confirm the effect of MEXS on LPS-induced PGE₂ production, cells were pre-incubated with MEXS for 1 h, and then activated with 1 µg/ml LPS for 24 h. As shown in Fig. 2A, the production of PGE₂ was significantly inhibited by MEXS in a concentration-dependent manner. Moreover, the expressions of COX-2 protein and mRNA were also inhibited in a similar manner (Figs. 2B, C).

Effects of MEXS on LPS-Induced TNF-α Production and mRNA Expression

To investigate the effect of MEXS on LPS-induced TNF-α release, we investigated its effect on TNF-α production using EIA and RT-PCR. Pretreatment of cells with MEXS at 30, 60, and 90 µg/ml reduced TNF-α production (Fig. 3A) and mRNA expression (Fig. 3B) in a concentration-dependent manner.

Effects of MEXS on the Inhibition of NF-κB...
B DNA Binding Activity, on the Degradation of IκBα, and on the Nuclear Translocation of p65

To further investigate the mechanism of the MEXS-mediated inhibition of iNOS, COX-2 and TNF-α transcription, we investigated the status of the transcription factor NF-κB, which is known to transactivate iNOS, COX-2, and tumor necrosis factor-α.10 EMSA demonstrated reduced NF-κB DNA binding activity in nuclear extracts obtained from LPS-activated RAW macrophages treated with MEXS (30, 60, 90 μg/ml) (Fig. 4A). To determine whether MEXS inhibits NF-κB via IκB-α degradation in RAW 264.7 cells, cytosolic extracts were prepared and assayed for IκB-α degradation by western blotting. As shown in Fig. 4B, MEXS inhibited LPS-induced IκB-α degradation in a concentration-dependent manner. In addition, to determine whether IκB-α degradation is related to IκB-α phosphorylation, we examined the effect of MEXS on the LPS-induced phosphorylated form of IκB-α by western blotting, using an antibody that specifically detects only the serine-phosphorylated form of IκB-α. LPS-induced IκB-α phosphorylation was also found to be reduced by MEXS in a concentration-dependent manner. We also investigated whether MEXS prevented the translocation of the p65 subunit of NF-κB from the cytosol to the nucleus after its release from IκB. Western blot analyses showed that the level of LPS-induced p65 in the nuclear fraction was reduced by MEXS in a concentration-dependent manner (Fig. 4B).

Inhibitory Effect of MEXS on Carrageenin-Induced Edema in Rats As shown in Table 1, we observed the anti-inflammatory activities of MEXS (100, 200 mg/kg, p.o.) at 2 h after carrageenin injection. Maximal edema inhibition was observed 2—3 h after edema induction. In particular, treatment with MEXS (200 mg/kg, p.o.) reduced edema by 21.2% at 4 h, and the positive control drug, ibuprofen (100 mg/kg, p.o.) reduced edema by 61.4%. The significance of edema reduction by MEXS was maintained for at least 5 h after edema induction.

Anti-nociceptive Effects of MEXS with Acetic Acid-Induced Abdominal Constriction and Hot Plate Test in Mice The anti-nociceptive effects of test samples were assayed using two different models: the acetic acid-induced abdominal constriction test and a hot plate test in mice. MEXS significantly reduced the number of writhings induced by 0.7% acetic acid solution. The percentage protection afforded
by Dunnett’s multiple range test.

Diseases.30) In this experiment, MEXS inhibited NO production. Furthermore, LPS stimulation well known to induce inducible nitric oxide synthase (iNOS) transcriptions and transduction, and subsequent nitric oxide (NO) synthesis. The results of the hot plate testing in Table 2 show that MEXS significantly increased jumping response latency when treated at 100 and 200 mg/kg without affecting the animal’s pain threshold (licking response) to heat, suggesting that MEXS has a central analgesic property. Morphine (10 mg/kg) also exerted a significant effect in this response. The anti-nociceptive activities shown by MEXS in these models suggest that it possesses centrally and peripherally mediated anti-nociceptive properties.

DISCUSSION

In murine macrophage RAW 264.7 cell, LPS stimulation alone induced inducible nitric oxide synthase (iNOS) transcription and transduction, and subsequent nitric oxide (NO) production. Furthermore, LPS stimulation well known to induce IkB proteolysis and NF-κB nuclear translocation.39 Therefore, this cell system presents an excellent model for drug screening and for evaluating the effects of potential inhibitors on induction of iNOS and NO production. The reactive free radical NO synthesized by iNOS is a major macrophage-derived inflammatory mediator, and is also reported to be involved in the development of inflammatory diseases.30 In this experiment, MEXS inhibited NO production, and iNOS protein and mRNA expression in a concentration-dependent manner in LPS-stimulated RAW 264.7 cells (Fig. 1).

Cyclooxygenase (COX) is a key enzyme in the biosynthesis of prostaglandin from arachidonic acid, and has two isotypes. COX-1 is responsible for producing the basal levels of prostaglandin needed for gastrointestinal tract homeostasis, whereas COX-2 is an inducible enzyme which is involved in inflammatory events. Well known nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen inhibit COX.31 Therefore, we evaluated the effects of MEXS on LPS-induced PGE2 production, and COX-2 protein and mRNA expression. Our results indicate that MEXS has concentration-dependent inhibitory effects on PGE2 production and on COX-2 protein and mRNA expression (Fig. 2).

The production of TNF-α is crucial for the synergistic induction of NO synthesis in IFN-γ and/or LPS-stimulated macrophages.32–34 Therefore, we examined the DNA binding activity of NF-κB to confirm that the expressional inhibitions of iNOS and COX-2 proteins and iNOS, COX-2, and TNF-α mRNAs by MEXS are due to the suppression of NF-κB. NF-κB is associated with an inhibitory subunit called IκB. NF-κB is present in the cytoplasm in an inactive form and is tightly controlled by IκB. However, when IκB is phosphorylated and it is subsequent proteolysis, which allows the translocation of NF-κB to the nucleus, where it activates the transcriptions of NF-κB-responsible genes.35 Our results indicate that translocation and DNA binding activity of NF-κB are inhibited in a concentration-dependent manner by MEXS (Fig. 4A), and that these events correspond to the inhibition of iNOS, COX-2, and TNF-α expression. We also examined effect of MEXS on the phosphorylation and degradation of IκB-α. MEXS was found to exert an inhibitory effect on the phosphorylation and degradation of IκB-α. MEXS has concentration-dependent inhibitory effects on PGE2 production and on COX-2 protein and mRNA expression (Fig. 2).

NF-κB is known to play a critical role in the regulation of genes involved in cell survival, and to coordinate the expressions of pro-inflammatory enzymes including iNOS, COX-2, and TNF-α.34–38 Therefore, we examined the DNA binding activity of NF-κB to confirm that the expressional inhibitions of iNOS and COX-2 proteins and iNOS, COX-2, and TNF-α mRNAs by MEXS are due to the suppression of NF-κB. NF-κB is associated with an inhibitory subunit called IκB. NF-κB is present in the cytoplasm in an inactive form and is tightly controlled by IκB. However, when IκB is phosphorylated and it is subsequent proteolysis, which allows the translocation of NF-κB to the nucleus, where it activates the transcriptions of NF-κB-responsible genes.35 Our results indicate that translocation and DNA binding activity of NF-κB are inhibited in a concentration-dependent manner by MEXS (Fig. 4A), and that these events correspond to the inhibition of iNOS, COX-2, and TNF-α expression. We also examined effect of MEXS on the phosphorylation and degradation of IκB-α. MEXS was found to exert an inhibitory effect on the phosphorylation and degradation of IκB-α in a concentration-dependent manner (Fig. 4B).

Whilst investigating the anti-inflammatory and anti-nociceptive effects of MEXS in vivo, we found that MEFF mildly reduced the edema induced by carrageenin, during which peak edema was characterized by the presence of PGs.25 The anti-nociceptive effects of test samples were assayed using two the acetic acid-induced abdominal constriction test and the hot plate test in mice. From the results obtained from the acetic acid-induced abdominal constriction test, it was observed that MEXS significantly increased the latency of the jumping response, when treated at 100 and 200 mg/kg, without affecting the oral administration of MEXS (100, 200 mg/kg) ranged from 11.8—26.5%. Aspirin (100 mg/kg) also had a significant protective effect, affording 68.6% protection (Table 2).
the ability to detect the thermal pain threshold (licking response). These results suggest that MEXS has possible central analgesic properties. The anti-nociceptive activities shown by MEXS in these models indicate that it may possess peripherally and centrally mediated anti-nociceptive properties. Although the toxicity of Xanthium strumarium has been reported to the animals which might be either due to a mixture of unidentified alkaloids present in the aerial parts of the plant or due to poisonous water soluble toxic kaurene glycosides, we did not find any toxic syndromes based on the treatments. Although the toxicity of peripherally and centrally mediated anti-nociceptive properties. The anti-nociceptive activities shown by MEXS in these models indicate that it may possess anti-inflammatory fractions are, therefore, required to be undertaken.

In summary, the results of the present study demonstrate that MEXS effectively inhibits LPS-induced iNOS and COX-2 protein and iNOS, COX-2, and TNF-α gene expression by blocking NF-kB activation via inhibiting the degradation of IκB-α in RAW 264.7 macrophages. MEXS also showed anti-inflammatory and anti-nociceptive activities in vivo. Our results suggest that MEXS is a potential therapeutic drug for the treatment of inflammatory and pain syndrome.

REFERENCES