

## Anti-inflammatory effects of jojoba liquid wax in experimental models

Ramy R. Habashy<sup>a</sup>, Ashraf B. Abdel-Naim<sup>a,\*</sup>, Amani E. Khalifa<sup>a</sup>, Mohammed M. Al-Azizi<sup>b</sup>

<sup>a</sup> Department of Pharmacology and Toxicology, Ain Shams University, Cairo, Egypt

<sup>b</sup> Department of Pharmacognosy, Ain Shams University, Cairo, Egypt

Accepted 30 April 2004

### Abstract

Jojoba [*Simmondsia chinensis* (Link 1822) Schneider 1907] is an arid perennial shrub grown in several American and African countries. Jojoba seeds, which are rich in liquid wax, were used in folk medicine for diverse ailments. In the current study, the potential anti-inflammatory activity of jojoba liquid wax (JLW) was evaluated in a number of experimental models. Results showed that JLW caused reduction of carrageenin-induced rat paw oedema in addition to diminishing prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) level in the inflammatory exudates. In a test for anti-inflammatory potential utilizing the chick's embryo chorioallantoic membrane (CAM), JLW also caused significant lowering of granulation tissue formation. Topical application of JLW reduced ear oedema induced by croton oil in rats. In the same animal model, JLW also reduced neutrophil infiltration, as indicated by decreased myeloperoxidase (MPO) activity. In addition, JLW ameliorated histopathological changes affected by croton oil application. In the lipopolysaccharide (LPS)-induced inflammation in air pouch in rats, JLW reduced nitric oxide (NO) level and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) release. In conclusion, this study demonstrates the effectiveness of JLW in combating inflammation in several experimental models. Further investigations are needed to identify the active constituents responsible for the anti-inflammatory property of JLW.

© 2004 Elsevier Ltd. All rights reserved.

**Keywords:** Jojoba liquid wax; Anti-inflammatory; Prostaglandin E<sub>2</sub>; Myeloperoxidase

### 1. Introduction

Jojoba [*Simmondsia chinensis* (Link 1822) Schneider 1907] is an arid perennial shrub indigenous to Arizona, California and Northwestern Mexico [1]. It is also grown in Australia [2], Brazil, Argentina and some Middle East countries [3]. Jojoba seed is rich in liquid wax, commonly mistaken for "jojoba oil" [4]. As much as 97% of jojoba liquid wax (JLW) consists of a mixture of esters of long chain fatty alcohols and long chain fatty acids. More than 60% of this mixture of esters contains *cis*-11-eicosenoic (jojobenoic) acid (C20) [5]. JLW contains a natural anti-oxidant postulated to be an allylic derivative of hydroxy-toluene [6]. JLW was used in folk remedies for renal colic, sunburn, chaffed skin, hair loss, headache, wounds and sore throat [7]. Animal studies demonstrate that JLW can be classified as a non-toxic substance [8]. JLW has shown moderate digestibility that increases when the wax is mixed with other oils [9]. In addition, JLW possesses moderate

absorption when applied topically [10]. In human studies, sulfurized JLW was effective in the treatment of acne while the unmodified wax was used for treatment of psoriasis [11]. Furthermore, dermatological research suggests that JLW may help to reduce inflammation [12].

Inflammation is an essential protective process preserving the integrity of organisms against physical, chemical and infective insults [13]. However, it is frequent that the inflammatory response to several insults erroneously leads to the damaging of normal tissues [14]. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is generated from arachidonic acid by the enzyme cyclo-oxygenase (COX) at sites of inflammation in substantial amounts and can mediate many of the pathologic features of inflammation [15]. One of the early cellular events in inflammation is the margination of leukocytes, primarily neutrophils. This response can be measured by using the neutrophil specific enzyme myeloperoxidase (MPO), an indicator of neutrophil accumulation [16]. In addition, nitric oxide (NO) plays an important role in inflammation and NO synthase (NOS) inhibitors can reverse several classic inflammatory symptoms [17]. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), a cytokine, plays an important role in inflammation. TNF- $\alpha$  stimulates neutrophils to transcribe and release cytokines,

\* Corresponding author. Tel.: +20 10 5182741; fax: +20 26831492.

E-mail address: abnaim@yahoo.com (A.B. Abdel-Naim).

and chemokines biosynthesis [18]. The conventionally used therapies for inflammation; non-steroidal anti-inflammatory drugs (NSAID's), have very important role in managing pain and inflammatory conditions [19], though with rather discouraging profile of side effects [20]. Even the newer anti-inflammatory drugs, cyclo-oxygenase 2 (COX-2) inhibitors, are not devoid of adverse effects [21]. Meanwhile research has revealed that oxidative mechanisms are at the origin of inflammation, and has suggested the use of antioxidant substances [22]. This demonstrates the need for new and safe anti-inflammatory drugs. In this regard, natural products have long gained wide acceptance among the public and scientific community [23]. Therefore, the present study was designed to explore the potential anti-inflammatory effects of JLW.

## 2. Materials and methods

### 2.1. Chemicals

Croton oil, dimethylbenzidine, hexadecyltrimethylammonium bromide, *o*-dianisidine, indomethacin, lipopolysaccharide from *E. coli* serotype 0111:B4 (LPS), *N*-1-naphthylethylene diamine dihydrochloride (Griess reagent II), sodium nitrite and sulfanilamide (Griess reagent I) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Inflammatory-grade carrageenin was purchased from FMC (Rockland, ME, USA). PGE<sub>2</sub> and TNF- $\alpha$  kits were purchased from Assay Designs Inc. (Ann Arbor, MI, USA). Acetone, ethanol, ether and pyridine were purchased from SDS (Peypin, France). All other chemicals were of the highest available commercial grade.

### 2.2. *Jobaba liquid wax*

Before any processing, the plant (*S. chinensis*) was authenticated by Department of Pharmacognosy, Ain Shams University, Cairo, Egypt. *Jobaba* seeds were harvested in August by El-Salam Collaborative Agricultural Society, Manayph, Ismaileya, Egypt. Fresh JLW was then prepared by cold compression of the seeds in the facilities of the Egyptian Natural Oil Company, Cairo, Egypt. Physical properties of the oil were as follows (Table 1).

### 2.3. Animals

Throughout the experiments, adult male Sprague–Dawley rats weighing 150–175 g were used. Animals were housed at a temperature of  $23 \pm 2^\circ\text{C}$  with free access to water and standard food pellets (El-Nasr Co., Abo-Zaabal, Egypt). Rats were acclimatized in our animal facility for at least 1 week prior to any experiment. Protocol of the present work was approved by Experiments and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Table 1  
Physical properties of JLW

Freezing point	9 °C
Boiling point	398 °C
Smoke point <sup>a</sup>	195 °C
Flash point <sup>a</sup>	295 °C
Refractive index at 25 °C	1.46
Specific gravity at 25 °C	0.87
Viscosity (25 °C)	50 cP
Iodine value	81
Saponification value	93
Acid value	2
Acetyl value	2
Unsaponifiable matter	51%
Total acids	52%

<sup>a</sup> Determined according to official method, Cc9a-48, of the American Oil Chemists, Society [24].

### 2.4. Measurement of paw volume and PGE<sub>2</sub> in carrageenin-induced rat oedema model

Thirty rats were equally divided into five groups assigned Latin numbers I–V. Animals were fasted, with free access to water, 16 h before the experiment. Groups I and II were given saline by intragastric tube, while groups III and IV received JLW in two doses; 5 ml kg<sup>-1</sup> ( $\approx$ 4.35 g) and 10 ml kg<sup>-1</sup> ( $\approx$ 8.7 g), respectively. Animals in group V were orally treated with indomethacin as standard anti-inflammatory drug (10 mg kg<sup>-1</sup>). Dosing volume was kept constant (10 ml kg<sup>-1</sup>) and was completed with saline when required. The choice of the used doses and time of measurement and sampling was based on pilot studies in our laboratory. Thirty minutes after oral treatment, group I received 0.05 ml saline, while groups II–V received 0.05 ml carrageenin (1% solution in saline) sc on the plantar surface of the right hind paw. The right hind paw volume was measured immediately after carrageenin injection by water displacement using UGO-BASILE 7140 plethysmometer (Comerio, Italy) [25]. The volume was measured again 3 h after carrageenin injection and immediately before decapitation.

After decapitation, right hind paws were removed. A volume of 0.1 ml saline containing 10  $\mu\text{M}$  indomethacin was injected to aid removal of the eicosanoid-containing fluid and to stop further production of PGE<sub>2</sub>. Paws were incised with a scalpel and suspended off the bottom of polypropylene tubes with Ependorff pipette tips to facilitate drainage of the inflammatory exudates. For the purpose of the removal of the inflammatory exudates, paws were centrifuged at 1800 g for 15 min [26]. PGE<sub>2</sub> was quantified in the collected exudates using a quantitative binding PGE<sub>2</sub> enzyme immunoassay kit. The kit uses a monoclonal antibody to bind, in a competitive manner, the PGE<sub>2</sub> in the sample as well as alkaline phosphatase-labelled PGE<sub>2</sub> provided in the kit. The enzyme bound, through PGE<sub>2</sub> molecule, to the monoclonal antibodies processes the specific substrate to produce a colour that is measured spectrophotometrically [27].

### 2.5. Chick's embryo chorioallantoic membrane (CAM) test

The chick's embryo CAM test was carried out according to the procedures described by D'Arcy and Howard [28]. Fertile hen's eggs of a local strain (Fayoumi chickens) were purchased from the chicken farm of The Faculty of Agriculture, Cairo University (Giza, Egypt) and Whatmann No. 1 filter paper was purchased from Fisher Scientific Co. (Pittsburgh, PA, USA). Eggs were collected 24 h after being laid. Twenty four fertile eggs, arranged in four groups, were used. Eggs were immediately incubated at a temperature of 37 °C at the time of receipt. Relative humidity was adjusted at 80%. Eggs were rotated four times daily. Eight days later, eggs were implanted with filter paper discs measuring 10 mm in diameter and weighing  $7 \pm 0.04$  mg. The discs were prepared by carefully punching them out from No. 1 Whatmann filter paper using a pneumatic cork borer. After autoclaving, discs were impregnated with sterile drug solutions under aseptic conditions. Each disc was loaded with 14.5  $\mu$ l drug solution. Indomethacin was dissolved in water, while JLW was dissolved in ether and thoroughly dried prior to application to the CAM. JLW was applied at the dose of 3.5 and 7  $\mu$ l per disc (approximately 3.05 and 6.1 mg JLW per disc, respectively). Indomethacin was used as a positive control in concentration of 2.5  $\mu$ g per disc. Group I received only the unmedicated discs while groups II and III were implanted with discs loaded with JLW low and high doses, respectively. Group IV received indomethacin-loaded discs.

Using aseptic techniques, eggshells were opened using a dental drill; loaded discs were placed on the surface of CAM, the removed piece of shell was placed again and the opening was sealed using molten paraffin wax. Eggs were re-incubated under the same conditions of temperature and humidity without rotation. Eggs were opened, 4 days later, by cutting the shells circumferentially along the longer perimeter. CAM membranes were eased out of the shell and the discs along with the adhering, and sometimes infiltrating, granulation tissue were cut with fine scissors. The discs carrying the granulation tissue were dried overnight at 55 °C and weighed individually.

### 2.6. Assessment of ear oedema, tissue myeloperoxidase activity, and histopathology in croton oil-induced ear oedema model in rats

The experiment was performed using a slight modification of the procedure described by Tonelli et al. [29]. An irritant solution was prepared by dissolving 4 parts croton oil (the irritant) in a solvent mixture of 10 parts ethanol, 20 parts pyridine, and 66 parts ethyl ether. JLW and indomethacin, serving as positive control, were dissolved in the same vehicle of the irritant. Two different concentrations of JLW were employed; namely 30 and 50% (v/v). Indomethacin was used in dose of 12.5% (w/v) [30].

Thirty rats were equally arranged in five groups numbered I–V. Irritant, JLW and indomethacin solutions were

applied in volume of 20  $\mu$ l topically on both sides of the right ears. The left ear was kept untreated to serve as a control. Group I served as negative control hence received only the irritant-free solvent mixture. Third and fourth group received JLW solutions and group V received indomethacin solution. One hour later, groups II–V received croton oil solution and group I was re-administered the croton oil-free solvent mixture again. After 4 h, animals were decapitated. An 8-mm cork borer was used to punch out discs from both the treated as well as the control ears. The two punches were weighed immediately after decapitation and the difference in weight was used to assess the inflammatory response.

The entire tissue of the right ear was homogenized for 10 min in ice bath (10%, w/v) in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide with a Glas-Col® homogenizer. Tissue suspensions were centrifuged at  $40000 \times g$  for 15 min. An aliquot of 0.1 ml of the supernatant was added to 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg ml<sup>-1</sup> *o*-dianisidine dihydrochloride, serving as MPO substrate, and 0.0005% hydrogen peroxide. The change in absorbance was measured at 460 nm with a SHIMADZU® UV-1601 spectrophotometer at 25 °C. MPO activity was quantified kinetically; change in absorbance was measured over a period of 2 min, sampled at intervals of 15 s [31]. The maximal change in absorbency per minute was used to calculate the units of myeloperoxidase activity based on the molar absorbency index of oxidized *o*-dianisidine dihydrochloride which equals  $1.13 \times 10^4$  M<sup>-1</sup> cm<sup>-1</sup>. One unit of myeloperoxidase is defined as that degrading one micromole of peroxide per minute at 25 °C [32]. Results were expressed as units of activity per milligram protein. Protein content was determined according to Lowery et al. [33].

A representative ear tissue from each group was fixed in 10% formalin solution and kept until the time of preparation of sections. Ear tissue was embedded in paraffin; sections were cut using the microtome at a thickness of 5  $\mu$ m. Sections were stained using hematoxylin and eosin. Then the specimens were observed under a light microscope and photographed. Specimens were examined regarding leukocytic infiltration, oedema as indicated by collagen fiber dispersion and extravasations.

### 2.7. Measurement of NO and TNF- $\alpha$ in rat air pouch model

Thirty rats uniformly divided in five groups were used in the study. Air pouches were formed by subcutaneous injection of 20 ml sterile air in the suprascapular area of the back of the animal. Three days later, the pouches were re-inflated with 10 ml sterile air. Three days later, 100  $\mu$ g ml<sup>-1</sup> solution of LPS in physiological saline (1 ml kg<sup>-1</sup>) was administered intrapouch to groups II–V. Group I received only saline. Thirty minutes later, drugs were injected also intrapouch. JLW dose was 5 and 10 g kg<sup>-1</sup> animal body weight. Indomethacin concentration used was 10 mg kg<sup>-1</sup>. The volume of administered solutions was kept constant at three

ml. Groups I and II received only saline, groups III and IV received JLW low and high doses, respectively and group V received indomethacin. The doses of JLW in this model were based on a pilot trial in our laboratory. Eight hours later animals were sacrificed [34]. Each pouch was lavaged using 1 ml of sterile physiological saline. The lavage fluid was then centrifuged at  $3000 \times g$  for 5 min. Supernatant was used immediately for analysis of NO and TNF- $\alpha$ .

NO was assayed by measuring nitrite accumulation utilizing Griess reaction [35]. Briefly 100  $\mu$ l of the air pouch exudates was combined with 100  $\mu$ l of a 1:1 mixture of Griess reagent I (1% sulfanilamide in 5% phosphoric acid) and Griess reagent II (0.1% *N*-1-naphthylethylene diamine dihydrochloride in water) in a flat-bottomed microtiter plate. The reaction mixture was incubated for 40 min at 37 °C, in a shaking water bath. In the presence of nitrite, azo-dye reaction proceeds in an acid medium producing scarlet color, which can be measured spectrophotometrically at 540 nm in an ELISA reader. The concentrations of nitrite samples were calculated from sodium nitrite standard curve run in the same plate. Results were expressed as  $\mu$ M nitrite. TNF- $\alpha$  was assayed using rat TNF- $\alpha$  enzyme immunometric assay kit. Rat TNF- $\alpha$  was immobilized on polyclonal antibody bound to microtiter plate. Excess sample was washed. A monoclonal antibody specific to rat TNF- $\alpha$ , coupled to horseradish peroxidase, was added. The monoclonal antibody binds specifically to the immobilized rat TNF- $\alpha$ . Excess monoclonal antibody was washed and the substrate, tetramethyl benzidine, was added. After incubation period, the developed colour was measured spectrophotometrically at 450 nm [27].

### 3. Statistical analysis

Results are reported as mean  $\pm$  S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *F*-value was found statistically significant ( $P < 0.05$ ), further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using SPSS statistical

Table 2

Effect of JLW on carrageenin-induced rat paw oedema

Group	Paw volume (ml)	Oedema inhibition (%)
Control	0.85 $\pm$ 0.02	–
Carrageenin-induced	1.38 $\pm$ 0.033 <sup>a</sup>	–
JLW (5 ml kg <sup>-1</sup> )	1.24 $\pm$ 0.02 <sup>a,b</sup>	26.4
JLW (10 ml kg <sup>-1</sup> )	1.20 $\pm$ 0.03 <sup>a,b</sup>	34
Indomethacin (10 mg kg <sup>-1</sup> )	1.15 $\pm$ 0.02 <sup>a,b</sup>	43.4

<sup>a</sup> Statistically significant difference from control group at  $P < 0.05$ .

<sup>b</sup> Statistically significant difference from carrageenin-induced group at  $P < 0.05$ .

software package (SPSS<sup>®</sup> Inc., USA) version 8. Graphs were sketched using GraphPad Prism (ISI<sup>®</sup> software, USA) version 2 software.

## 4. Results

### 4.1. Effect of JLW on paw volume and PGE<sub>2</sub> in carrageenin-induced rat oedema

Intraplantar injection of carrageenin to rats resulted in severe discernible inflammation and significant increase in the mean volume of the challenged paw compared to that of the untreated paws (162.3% of the untreated paws) (Table 2). Pretreatment of rats with JLW in doses of 5 and 10 ml kg<sup>-1</sup> significantly inhibited the carrageenin-induced increase in the oedema volume of the paws by 26.4 and 34%, respectively. Similarly, indomethacin-treated group showed significant anti-oedema effect (43.4% of the induced paws).

Carrageenin challenge resulted in more than five-fold increase in PGE<sub>2</sub> concentration in inflammatory exudates in group II compared to unchallenged animals in group I (Fig. 1). Animals receiving JLW showed significant reduction of the PGE<sub>2</sub> concentration in exudates that was dose-related in nature (reduction by 58.15 and 77.4% of the carrageenin-treated animals, respectively). The higher dose of JLW as well as indomethacin could lower PGE<sub>2</sub> level in carrageenin-challenged animals approaching normal levels.

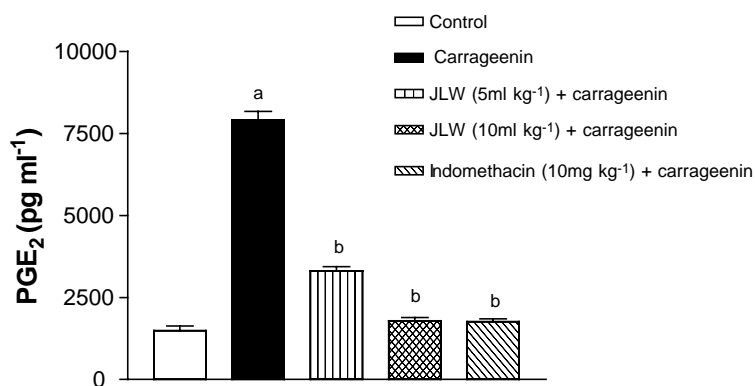


Fig. 1. Effect of JLW on PGE<sub>2</sub> production in exudates from carrageenin-treated rats. <sup>a</sup>Statistically significant from the control group at  $P < 0.05$ . <sup>b</sup>Statistically significant from the carrageenin-induced group at  $P < 0.05$ .

Table 3  
Effect of JLW on weight of granulation tissue in chick's embryo CAM experiment

Group	Weight of granulation tissue (mg)	Inhibition of the induced discs (%)
Induced	12.92 ± 0.06	0
JLW (3.05 µg per disc)	10.87 ± 1.08 <sup>a</sup>	15.86
JLW (6.1 µg per disc)	8 ± 0.23 <sup>a</sup>	38.08
Indomethacin (2.5 µg per disc)	4.3 ± 0.40 <sup>a</sup>	66.7

<sup>a</sup> Statistically significant difference from the induced discs at  $P < 0.05$ .

#### 4.2. Effect of JLW on granulation tissue formation in the chick's embryo CAM experiment

The data in Table 3 show that introduction of filter paper discs to CAM resulted in accumulation of granulation tissue on discs that mounted to 12.9 mg per disc. Preloading of the filter paper discs with JLW in concentration of 30% (v/v) and 50% (v/v) resulted in statistically significant reduction of granulation tissue weight by 15.8 and 38%, respectively compared to plain discs. Similarly, indomethacin produced significant lowering (66.7%) of the granulation tissue weight per disc compared to untreated discs.

#### 4.3. Effect on croton oil-induced ear oedema, MPO tissue activity and histopathological changes

Application of croton oil to rat ears caused massive increase in the weight of the ear punch (216%) compared to unchallenged ears. Pretreatment of rat ears with the two dose levels of JLW, 30% (v/v) and 50% (v/v) significantly reduced the increase of punch weight by 28 and 43.6%, respectively. In addition, indomethacin produced significant reduction of oedema by 25.6% (Table 4).

Assay of MPO activity in rat ears indicated that application of croton oil increased the enzyme activity by 83-fold. JLW [30% (v/v) and 50% (v/v)] pretreatment, however, reduced MPO activity by 29 and 53.5% of the induced ear, re-

Table 4  
Effect of JLW on weight of croton oil-induced ear oedema in rats

Group	Ear-punch weight (g)	Un-induced ear-punch weight (%)
Control	0.018 ± 0.0004	100
Croton oil-induced	0.039 ± 0.001 <sup>a</sup>	216.7
JLW (30%, v/v)	0.028 ± 0.0006 <sup>a,b</sup>	155
JLW (50%, v/v)	0.022 ± 0.0005 <sup>a,b</sup>	122
Indomethacin (12.5%, w/v)	0.029 ± 0.0004 <sup>a,b</sup>	161

<sup>a</sup> Statistically significant from the control group at  $P < 0.05$ .

<sup>b</sup> Statistically significant from the croton oil-induced group at  $P < 0.05$ .

spectively. Topical application of 1% indomethacin resulted in significant protection against croton oil-induced enhancement of MPO activity (Fig. 2).

Histopathological examination of the ear tissue confirmed those results obtained by assessing MPO activity. Fig. 3, Plate A shows normal histological characteristics of the epidermal, dermal as well as subcutaneous layers with no obvious neutrophil infiltration. The croton oil-treated rats showed massive neutrophil infiltration with extravasations of RBC's as well as oedema in the dermal layer (Fig. 3, Plate B). Fig. 3, Plate C, corresponding to JLW 30% (v/v), shows less neutrophil infiltration and less hyperemia. Fig. 3, Plates D and E, which represent the ear tissue treated with 50% v/v JLW solution and indomethacin, respectively showed almost normal dermal tissue with mild degradation of discrete cells of sebaceous glands.

#### 4.4. Effect of JLW on NO and TNF- $\alpha$ in air pouch model

Injection of LPS into the air pouch caused about 60-fold increase of NO production compared to that untreated animals (group I). JLW injection in doses of 5 and 10 ml kg<sup>-1</sup> showed significant reduction of NO level by 31.4 and 32.8%, respectively compared to group II (Fig. 4). However, the

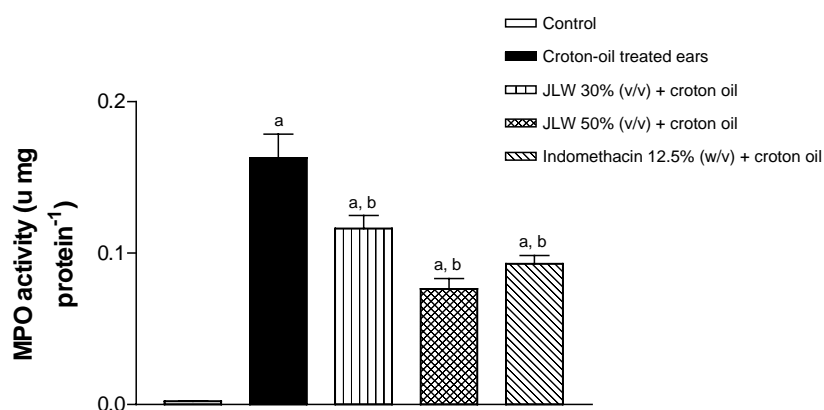


Fig. 2. Effect of JLW on MPO activity in croton oil-induced oedema. <sup>a</sup>Statistically significant from the control group at  $P < 0.05$ . <sup>b</sup>Statistically significant from the croton oil-induced group at  $P < 0.05$ .



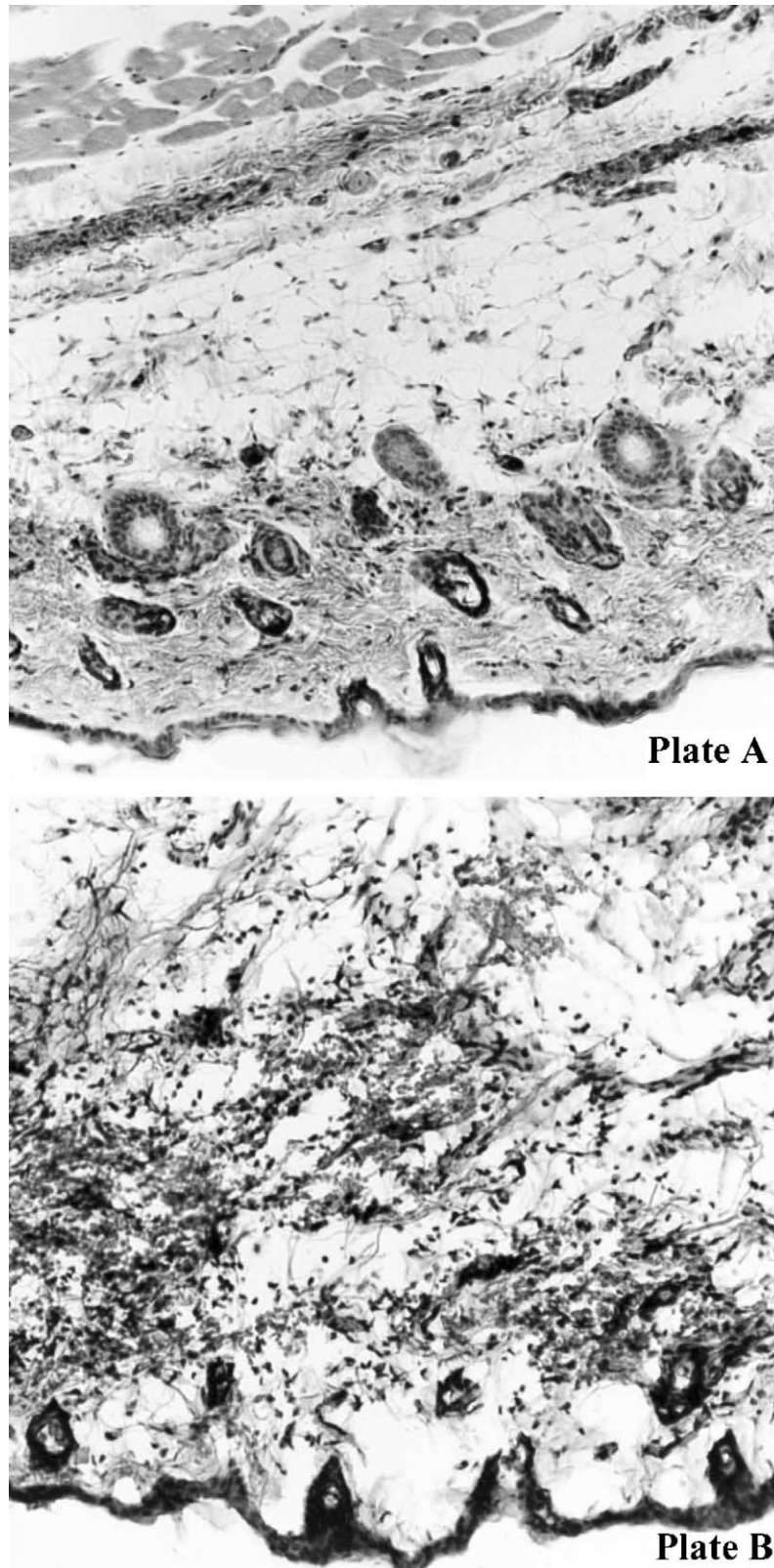


Fig. 3. Effect of JLW on histopathological changes in croton oil-induced ear oedema experiment. Plate A shows normal architecture of the covering dermal and epidermal layers as well as subcutaneous tissue of the skin. Plate B shows ear tissue from croton oil-alone treated rats exhibiting massive neutrophil infiltration with extravasations of red blood cells and oedema in the dermal layer. Plates C–E; corresponding to JLW 30% (v/v), 50% (v/v) and indomethacin 12.5% (w/v), respectively; show less neutrophil infiltration, collagen fiber dispersion and oedema (40 $\times$ ).

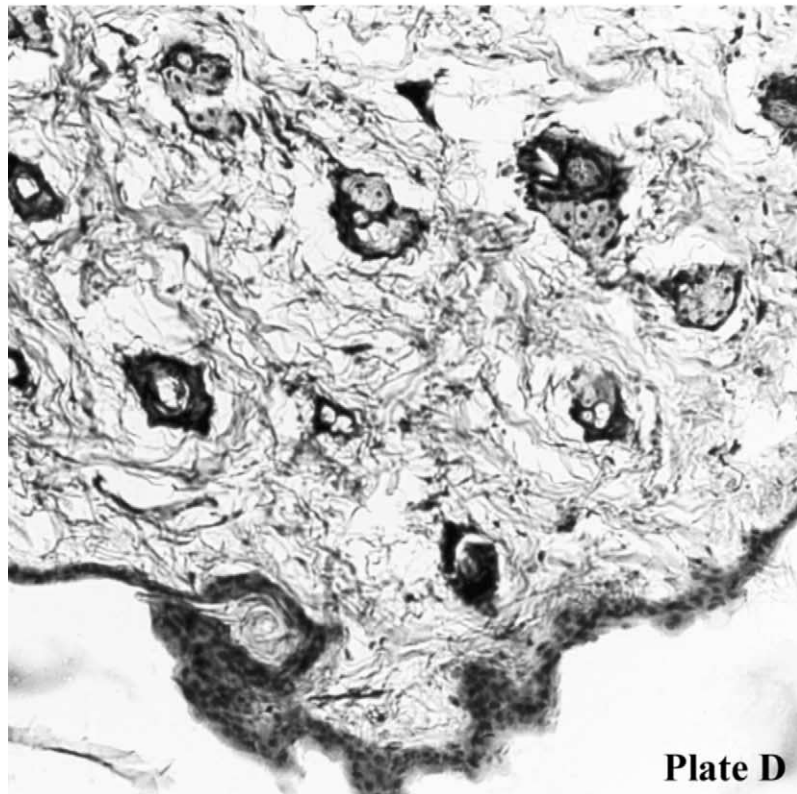
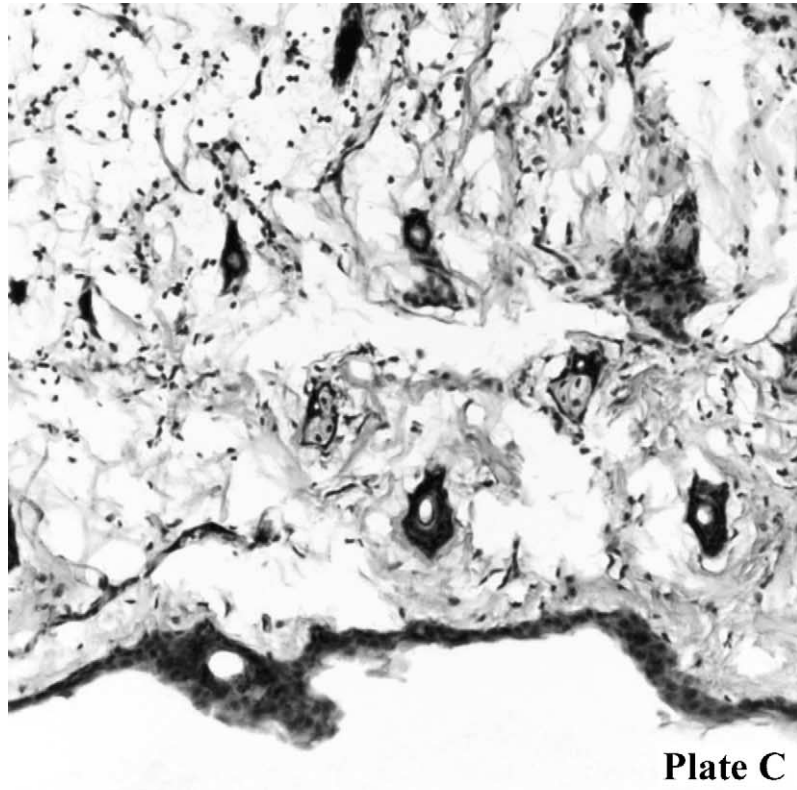


Fig. 3. (Continued)

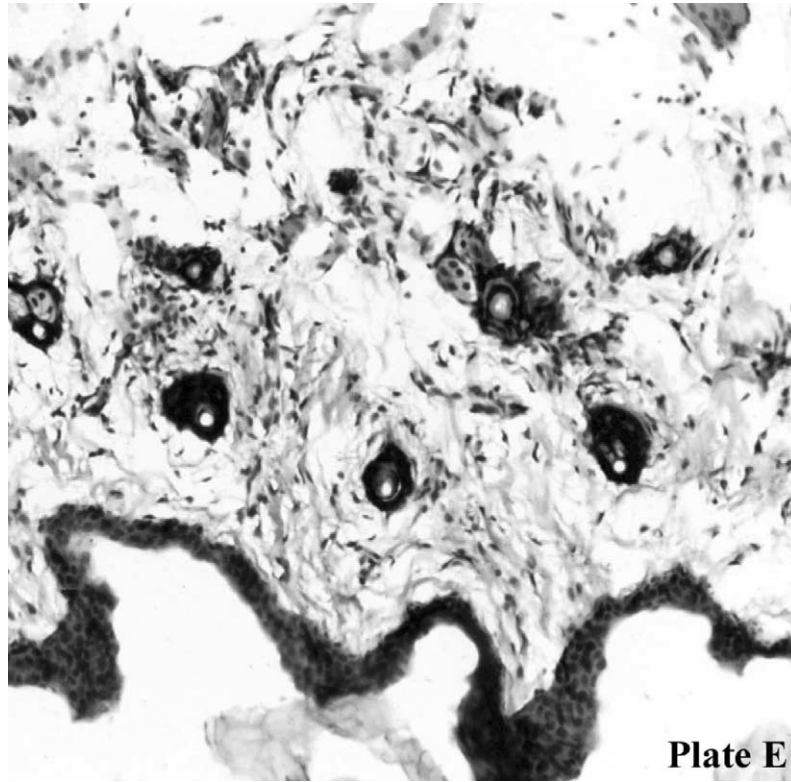


Fig. 3. (Continued).

effect was not dose related. Similarly, indomethacin treatment significantly lowered NO level by 36.6%.

In addition, LPS injection caused about 8-fold increase in TNF- $\alpha$  level in the pouch exudates compared to untreated animals. JLW treatment in doses of 5 g kg<sup>-1</sup> and 10 ml kg<sup>-1</sup> lowered TNF- $\alpha$  release by 62.2 and 75.8%, respectively (Fig. 5). Indomethacin, serving as positive control caused a reduction mounting to 43.4% of TNF- $\alpha$  level in rats of group II.

### 5. Discussion

Jojoba [*Simmondsia chinensis* (Link 1822) Schneider 1907] has been long used as a folk medicine for a variety of conditions, including inflammatory diseases, by the ancient natives of the American continents [7]. Inflammation is the most common aspect of tissue pathology and has always taken a central role in medical practice. The aim of the present work was to evaluate the potential anti-inflammatory effect of JLW as well as the underlying possible mechanism.

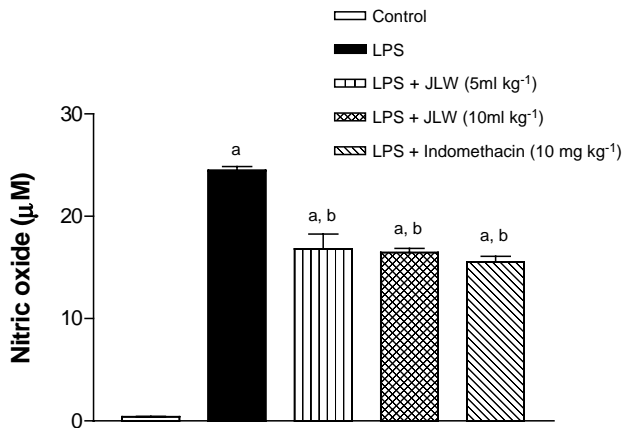


Fig. 4. Effect of JLW on NO level in air pouch experiment in rats. <sup>a</sup>Statistically significant from the control group at  $P < 0.05$ . <sup>b</sup>Statistically significant from the LPS-induced group at  $P < 0.05$ .

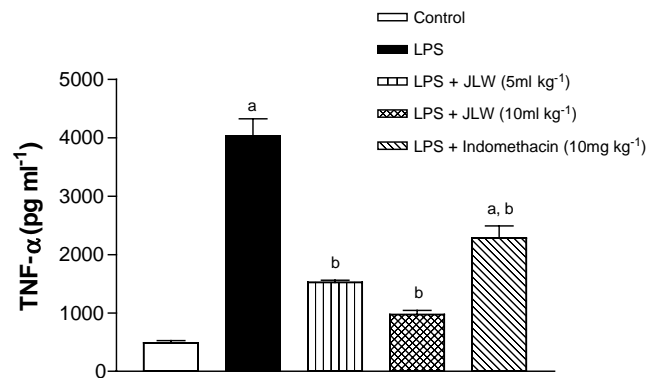


Fig. 5. Effect of JLW on TNF- $\alpha$  release in rat air pouch experiment. <sup>a</sup>Statistically significant from the control group at  $P < 0.05$ . <sup>b</sup>Statistically significant from the LPS-induced group at  $P < 0.05$ .



The results of carrageenin-induced paw oedema [25] showed that JLW reduces carrageenin-induced paw oedema. This finding could be interpreted as a potential anti-inflammatory activity of JLW. Such conclusion was further substantiated by assessing PGE<sub>2</sub> level in the inflammatory exudates of rat paws in the same model. Prostaglandins have been long recognized as a major mediator of inflammation. They are arachidonic acid metabolites synthesized by COX-1 and COX-2 isozymes [36]. Our results indicate that JLW caused statistically significant reduction of PGE<sub>2</sub> content. This finding clearly underlines the anti-inflammatory effect of JLW. Such activity may be explained on the basis of JLW content of long chain fatty acid esters. These esters include erucyl jojobenoate and jojobenyl jojobenoate, which constitute 61% of the total esters present. Jojobenoic acid; *cis*-11-eicosenoic acid, is produced by hydrolysis of their esters by lipases [37] and constitutes 71% of the total fatty acids [5]. The hydrolysis of these esters by lipases results in liberation of free fatty acids which are anticipated to compete with arachidonic acid for the binding site on COX isozymes [38]. It may be suggested that this competition results in decreased synthesis of PGE<sub>2</sub>.

Chick's embryo CAM test is an accurate method for the screening of anti-inflammatory effect of an agent directly on the developing CAM [28]. JLW demonstrated an anti-inflammatory effect as indicated by significant reduction of the weight of the granulation tissue.

The results of croton oil-induced ear oedema support this finding [29]. A reduction in ear punch weight was shown after application of the two dose levels of JLW compared to the croton oil-alone condition. This activity could be justified by the remarkable cutaneous absorption of JLW. Sebum consists of several lipids, the major constituents of which are wax, free fatty acids and other lipid esters [39]. Owing to the similarity of JLW composition to that of sebum, JLW is efficiently absorbed which may account for its powerful anti-inflammatory activity.

The findings of the same experiment demonstrated that JLW reduced MPO activity in challenged ear tissue. This could be explained on the basis of inhibition of neutrophil infiltration which was confirmed visually by microscopic examination of the ear tissue. JLW reduced neutrophil infiltration, red cell extravasation, oedema and hence collagen fiber dispersion. Cutaneous inflammation is characterized by the infiltration of polymorphonuclear leukocytes such as neutrophils [40]. MPO, a heme protein, is a major component of azurophilic granules of neutrophils. Oxygen-dependent microbicidal activity depends on MPO as the critical enzyme for the generation of hypochlorous acid and other toxic oxygen products [41]. The primary function of neutrophils is the release of MPO into the phagosome containing the ingested microorganism leading to a rapid microbicidal effect. MPO can be released to the outside of the cell inducing damage to adjacent tissue and, thus, contribute to the pathogenesis of inflammation [42].

NO is a highly fat soluble, relatively stable free radical, having numerous promiscuous roles [43]. NO synthesis is greatly amplified by LPS [44]. Several studies have demonstrated that inflammation correlates with level of NO [45]. In this study JLW have shown remarkable, though not dose related, reduction of nitrite accumulation in the air pouch model. This finding may be attributed to the anti-oxidant property of JLW reported to protect the oil itself against rancidity [6]. Furthermore, lowering of NO level after JLW administration may account for the reduction in PGE<sub>2</sub> level since many reports have shown that NO rapidly and strongly stimulates COX enzymes [46]. In addition, NO synthase inhibitors reduce PGE<sub>2</sub> biosynthesis [47].

TNF- $\alpha$  is a pleiotropic cytokine which plays a critical role in both acute and chronic inflammation [48]. TNF- $\alpha$  promotes an acute phase reaction [49]. Several inflammagens have the ability of inducing the synthesis of TNF- $\alpha$ . The formation of a number of small molecular mediators of inflammation is linked with TNF- $\alpha$  and thus contributes to the range of mediators that critically control inflammation [50]. TNF- $\alpha$  facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells [51]. When TNF- $\alpha$  effect is specifically blocked, the severity of inflammation is reduced [52]. More importantly, TNF- $\alpha$  induces the synthesis of PGE<sub>2</sub> [49]. JLW; in particular the higher dose, strongly inhibited TNF- $\alpha$  release. The reduction of TNF- $\alpha$  release by JLW is in agreement with the PGE<sub>2</sub> finding obtained in the current study. An alternative explanation of the resultant reduction of PGE<sub>2</sub> level in inflammatory exudates may be through interference with TNF- $\alpha$  release.

In conclusion, JLW exerts anti-inflammatory activity in several animal models. This is evidenced by decreased paw oedema, PGE<sub>2</sub> content in exudates, CAM granulation tissue formation, ear oedema, MPO activity, NO generation and TNF- $\alpha$  formation as well as amelioration of inflammatory histopathological changes. These results lend support to the effectiveness of JLW in combating inflammation via multilevel regulation of inflammatory mediators. Further investigations are needed to identify the active constituents responsible for the anti-inflammatory property of JLW.

## Acknowledgements

The authors would like to thank Prof. Adel B. Kholoussy, Professor of Pathology, Cairo University for his help in performing the histopathological examinations. Also the authors appreciate the valuable help offered by SEDICO Pharmaceutical Co., 6 October City and the Egyptian Oil Company, Cairo, Egypt.

## References

- [1] Hogan L. Jojoba: new crop for arid regions of the world? *Crops Soils Mag* 1978;31(2):18–9.

- [2] Davidson S. Jojoba: cautious optimism. *Rural Res* 1983;119:21–5.
- [3] Borlaug N, Baldwin AR, Estefan R, Harris M, Plucknett DL. Jojoba: new crop for arid lands. New raw material for industry. Washington, DC: National Academy Press; 1985. p. 6, 13.
- [4] Van Boven M, Daenens P, Maes K, Cokelaere M. Content and composition of free sterols and free fatty alcohols in jojoba oil. *J Agric Food Chem* 1997;45:1180–4.
- [5] Miwa TK. Structural determination and uses of jojoba oil. *J Am Oil Chem Soc* 1984;61(2):407–10.
- [6] Kampf A, Gringberg S, Galun A. Oxidative stability of jojoba wax. *J Am Oil Chem Soc* 1986;63:246–8.
- [7] Yaron A. Metabolism and physiological effects of jojoba oil. In: Wisniak J, editor. The chemistry and technology of jojoba oil. Champaign, IL: American Oil Chemists' Society Press; 1987. p. 251–65.
- [8] Taguchi N, Kunimoto T. Toxicity studies on jojoba oil for cosmetic uses. *Cosmet Toilet* 1977;92:53–62.
- [9] Heise C, Decombaz J, Anantharaman K. Energy value of jojoba oil for the growing rat. *Int J Vitam Nutr Res* 1982;52:216–21.
- [10] Yaron A, Benzioni A. Absorption and distribution of jojoba wax injected subcutaneously into mice. *Lipids* 1980;15:889–94.
- [11] Mosovich B. Treatment of acne and psoriasis. In: Wisniak J, Zabicky J, editors. Proceedings of the Sixth International Conference on Jojoba and its Uses. Beer Sheva, Israel: University of the Negev; 1985. p. 393–7.
- [12] Borlaug N, Baldwin AR, Estefan R, Harris M, Plucknett DL. Jojoba: new crop for arid lands. New raw material for industry. Washington, DC: National Academy Press; 1985. p. 53.
- [13] Walport MJ, Duff GW. Cells and mediators of inflammation. In: Maddison PJ, Isenberg DA, Woo P, Glass DN, editors. Oxford textbook of rheumatology, vol. 1. Oxford, NY: Oxford University Press; 1993. p. 285–95.
- [14] Majno G, Joris I. Cells, tissues and disease: principles of general pathology. Cambridge, MA: Blackwell Science; 1996. p. 293.
- [15] Serhan CN, Levy B. Success of prostaglandin E2 in structure-function is a challenge for structure-based therapeutics. *Proc Natl Acad Sci USA* 2003;100(15):8609–11.
- [16] Goulet JL, Snouweart JN, Latour AM, Coffman TM. Altered inflammatory responses in leukotriene-deficient mice. *Proc Natl Acad Sci USA* 1994;91:12852–6.
- [17] Amin AR, Vyas P, Attur M, Leszczynska-Piziak J. The modes of action of aspirin-like drugs: effect on inducible nitric oxide synthase. *Proc Natl Acad Sci USA* 1995;92:7926–30.
- [18] Marucha PT, Zeff RA, Kreutzer DL. Cytokine-induced IL-1 $\beta$  gene expression in the human polymorphonuclear leukocyte: transcriptional and post-transcriptional regulation by tumor necrosis factor and IL-1. *J Immunol* 1991;147:2603–8.
- [19] Davies NM, Saleh JY, Skjoldt NM. Detection and prevention of NSAID-induced enteropathy. *J Pharm Pharm Sci* 2000;3(1):137–55.
- [20] James MW, Hawkey CJ. Assessment of non-steroidal anti-inflammatory drug (NSAID) damage in the human gastrointestinal tract. *Br J Clin Pharmacol* 2003;56(2):146–55.
- [21] Wallace JL. Selective cyclooxygenase-2 inhibitors: after the smoke has cleared. *Dig Liver Dis* 2002;34(2):89–94.
- [22] Bermond P. Analgesic and anti-inflammatory properties of vitamins. *Int J Vitam Nutr Res Suppl* 1989;30:153–60.
- [23] Bauer BA. Herbal therapy: what a clinician needs to know to counsel patients effectively. *Mayo Clin Proc* 2000;75(8):835–41.
- [24] Sallee EM. Official and tentative methods of the American oil chemists' society, 3rd ed. Champaign, IL: American Oil Chemists' Society Press; 1980. Method Cc9a-48.
- [25] Winter CA, Risley EA, Nuss GW. Carrageenin-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med* 1962;111:544–7.
- [26] Mnich SJ, Veenhuizen AW, Monahan JB, Sheehan KC, Lynch KR, Isakson PC, et al. Characterization of a monoclonal antibody that neutralizes the activity of prostaglandin E<sub>2</sub>. *J Immunol* 1995;155:4437–44.
- [27] Virella G. Immunoserology. In: Virella G, editor. Medical immunology, 4th ed. New York: Marcel Dekker Inc.; 1998. p. 259–81.
- [28] D'Arcy PF, Howard EM. A new anti-inflammatory test, utilizing the chorio-allantoic membrane of the chick embryo. *Br J Pharm Chemother* 1967;29:378–87.
- [29] Tonelli G, Thibault L, Ringler I. A bio-assay for the concomitant assessment of the antiphlogistic and thymolytic activities of topically applied steroids. *Endocrinology* 1965;77:625–30.
- [30] Escriv V, Ubeda A, Ferrandiz ML, Darias J, Sanchez JM, Alcaraz MJ, et al. Variabilin: a dual inhibitor of human secretory and cytosolic phospholipase A2 with anti-inflammatory activity. *J Pharmacol Exp Ther* 1997;282(1):123–31.
- [31] Bradely PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982;78(3):206–9.
- [32] McVey DC, Vigna SR. The capsaicin VR1 receptor mediates substance P release in toxin A-induced enteritis in rats. *Peptides* 2001;22:1439–46.
- [33] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265–75.
- [34] Edwards JCW, Sedgwick AD, Willoughby DA. The formation of a structure with the features of synovial lining by subcutaneous injection of air: an in vivo tissue culture system. *J Pathol* 1981;134:147–56.
- [35] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [<sup>15</sup>N] nitrate in biological fluids. *Anal Biochem* 1982;126:131–8.
- [36] Herschman HR. Prostaglandin synthase 2. *Biochim Biophys Acta* 1996;1299(1):125–40.
- [37] Bizzi A, Cini M, Bracco U. Absorption and distribution of jojoba oil after oral administration to rats. In: Wisniak J, Zabicky J, editors. Proceedings of the Sixth International Conference on Jojoba and its Uses. Beer Sheva, Israel: University of the Negev; 1985. p. 333–6.
- [38] Ringbom T, Huss U, Stenholm A, Flock S, Skattebol L, Perera P, et al. COX-2 inhibitory effects of naturally occurring and modified fatty acids. *J Nat Prod* 2001;64:745–9.
- [39] McGinley KJ, Webster GF, Ruggieri MR, Leyden JJ. Regional variations in density of cutaneous propionibacteria: correlation of propionibacterium acnes populations with sebaceous secretions. *J Clin Microbiol* 1980;12(5):672–5.
- [40] Lawley TJ, Kubota Y. Cell adhesion molecules and cutaneous inflammation. *Semin Dermatol* 1991;10(3):256–9.
- [41] Nauseef WM, Olsson I, Arnljots K. Biosynthesis and processing of myeloperoxidase—a marker for myeloid cell differentiation. *Eur J Haematol* 1988;40(2):97–110.
- [42] Klebanoff SJ. Myeloperoxidase. *Proc Assoc Am Physicians* 1999;111(5):383–9.
- [43] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43:109–42.
- [44] Kou PC, Schroeder RA. The emerging multifaceted roles of nitric oxide. *Annal Sur* 1995;221(3):220–35.
- [45] Miller MJS, Grisham MB. Nitric oxide as a mediator of inflammation? You had better believe it. *Mediators Inflamm* 1995;4:387–96.
- [46] Sautebin L, Ialenti A, Ianaro A, Di Rosa M. Modulation by nitric oxide of prostaglandin biosynthesis in the rat. *Br J Pharmacol* 1995;114(2):323–8.
- [47] Salvemini D, Settle SL, Masferrer JL, Seibert K, Currie MG, Needleman P. Regulation of prostaglandin production by nitric oxide: an in vivo analysis. *Br J Pharmacol* 1995;114(6):1171–8.
- [48] Holtmann MH, Schuchmann M, Zeller G, Galle PR, Neurath MF. The emerging distinct role of TNF-receptor 2 (p80) signaling in chronic inflammatory disorders. *Arch Immunol Ther Exp (Warsz)* 2002;50(4):279–88.
- [49] Arai KI, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: coordinators of immune and inflammatory responses. *Annu Rev Biochem* 1990;59:783–836.

- [50] Harada A, Sekido N, Akahoshi T, Wada T, Mukaida N, Matsushima K. Essential involvement of interleukin-8 (IL-8) in acute inflammation. *J Leukoc Biol* 1994;56(5):559–64.
- [51] Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci: USA* 1985;82(24):8667–71.
- [52] Dinarello CA. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest* 1997;112(Suppl 6):321S–9S.