Food and Chemical Toxicology 48 (2010) 639–643

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Anti-inflammatory activity of seed essential oil from *Zizyphus jujuba*

Sharif M. Al-Reza [a,b,1], Jung In Yoon [a,1], Hyo Jung Kim [c], Jong-Sang Kim [c], Sun Chul Kang [a,*]

[a] Department of Biotechnology, Daegu University, Kyoungsan, Kyoungbook 712-714, Republic of Korea
[b] Department of Applied Chemistry and Chemical Technology, Islamic University, Kushtia 7003, Bangladesh
[c] Department of Animal Science and Biotechnology, Kyungpook National University, Daegu 702-701, Republic of Korea

ARTICLE INFO

Article history:
Received 11 September 2009
Accepted 23 November 2009

Keywords:
Anti-inflammatory activity
Zizyphus jujuba
Essential oil
BALB/c mice

ABSTRACT

This study was undertaken to evaluate the effect of essential oil from seeds of *Zizyphus jujuba* on TPA-induced skin inflammation in experimental mice. Exposure of TPA on the ear of the BALB/c mice caused a marked increase in both ear thickness and skin water content. The ear thickness was measured for TPA-induced ear was 0.54 mm, as compared to control (0.23 mm). Treatment with 1% and 10% of essential oil caused significant decrease in ear thicknesses which were measured to be 0.30 and 0.35 mm, as well as reduce the water content about 51% and 53% in the TPA-induced skin inflammation model, respectively. Furthermore, histological analysis clearly confirmed that *Z. jujuba* essential oil inhibited the inflammatory responses of skin inflammation in animal model. Therefore, our findings demonstrate that the essential oil of *Z. jujuba* seeds might accelerate the development of new drugs for various inflammatory diseases.

1. Introduction

Inflammation is a physiological response of a body to stimuli, including infections and tissue injury. However, excessive or persistent inflammation causes a variety of pathological conditions, such as bacterial sepsis, rheumatoid arthritis and skin inflammation (Dinarello, 1997; Palladino et al., 2003). As the primary interface between the body and the external environment, the skin provides the first line of defense against traumatic injury and invasion by microbial pathogens. In addition to its properties as a physical barrier, the skin has many active defense mechanisms (Kupper and Fuhlbrigge, 2004) and regulation of these mechanisms is crucial, as inappropriate or misdirected immune activity is implicated in the pathogenesis of a large variety of inflammatory skin disorders. While some of these conditions are easily remedied, treatments for chronic inflammatory diseases such as psoriasis and atopic dermatitis are not 100% successful (Chi et al., 2003).

High levels of inflammatory cytokines and reactive oxygen species are proposed to contribute to the pathophysiological mechanisms associated with various inflammatory skin disorders (Trouba et al., 2002). It is widely recognized that cutaneous inflammation is produced and maintained by the interaction of various inflammatory cell populations that migrate to the inflammation site in response to the release of soluble pro-inflammatory mediators such as cytokines, prostaglandins, and leukotriene (Briganti and Picardo, 2003; Lee et al., 2003).

Current therapies focus on treating symptoms of skin disorders with a combination of moisturizers, antihistamines, antibiotics, and corticosteroids, with the aim of repairing barrier function, and reducing itch, secondary infections, and inflammation. However, steroids can disrupt a number of cytokine networks involved in lymphocyte function, resulting in immunosuppression, and long-term topical use can decrease collagen synthesis, leading to skin atrophy (Oikarinen et al., 1998). Because of these risks, new therapeutic approaches are being intensively investigated.

A large number of plant species contain various bioactive compounds exhibiting health beneficial properties, anti-oxidative, anti-inflammatory and mainly antimicrobial effects, and their preventive and therapeutic use increases. Numerous natural products have been already tested in various animal models for the development of new anti-inflammatory therapeutics (Chien-Tsong et al., 2008; Lee et al., 2009).

The jujuba fruit has been described as the "fruit of life". The ancient Chinese learned the unique properties over thousands of years. Today's medical practitioners are now finding scientific proof of its exceptional properties. Jujuba or red date is a species of *Zizyphus jujuba* is a thorny rhamnaceous plant in the buckthorn family Rhamnaceae, used primarily for its fruits. Its precise natural distribution is uncertain due to extensive cultivation, but is thought to be in southern Asia, Syria, northern India, southern and central China, and possibly also southeastern Europe though more likely introduced there, Wikipedia said. Fruits of this plant are edible and different parts of *Z. jujuba* possess multiple medicinal properties such as antifertility, analgesic, and antidiabetes (Ambasta, 1986; Erenmemisoglu et al., 1995). The local tribal people use the bark mixture of *Z. jujuba* to prevent the pregnancy

* Corresponding author. Tel.: +82 53 850 6553; fax: +82 53 850 6559.
E-mail address: sckang@daegu.ac.kr (S.C. Kang).
[1] These authors equally contributed in this work.

0278-6915/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2009.11.045

(Souleles and Shammas, 1998). However, there are few scientific studies about the effect of *Z. jujuba* seeds. In traditional medicine, the seed of *Z. jujuba* has been used for its action on insomnia and anxiety (Lee, 1986). As reported by Kim (2002), *Z. jujuba* seeds were effective on the improvement of the blood glucose, lipid compositions in serum of dietary hyperlipidemic rats. In particular, *Z. jujuba* seeds were more effective as a therapeutic regimen for the control of metabolic derangements in adult disease.

However, there is no report available on anti-inflammatory activity of the seed essential oil from *Z. jujuba*. Therefore, the aim of the present study was to assess the anti-inflammatory activity of essential oil from seeds of *Z. jujuba*, as well as its possible mechanism of anti-inflammatory effect.

2. Materials and methods

2.1. Plant material

The seeds of *Z. jujuba* were collected from the local area of Kyoungsan, Republic of Korea, in August 2008. Seeds were cleaned, dried and ground. Initially the seeds were identified by morphological features and in-house data base by Prof. Man Kyu Huh. A voucher specimen number was deposited in the Herbarium of the College of Engineering, Department of Biotechnology, Daegu University, Republic of Korea.

2.2. Chemicals

12-*O*-tetradecanoylphorbol-13-acetate (TPA) and hydrocortisone were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of the highest commercial grade.

2.3. Experimental animals

Five-week-old female BALB/c mice (18–20 g) were purchased from Orient Bio Inc. (Seoul, South Korea). The animals were kept in polypropylene cages (three mice per cage) and maintained on a standard laboratory diet and water *ad libitum*. They were housed in an air-conditioned room with 12:12 h light and dark cycle at least 7-day prior to experiment. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically.

2.4. Isolation of the essential oil

About 250 g ground seeds of *Z. jujuba* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 °C until further analysis.

2.5. Assay of TPA-induced inflammation in mice

12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced a skin inflammation resulting in increase in ear thickness and skin water content in BALB/c mice. TPA was dissolved in AOO (acetone:olive oil = 4:1) and used as an inducer of skin inflammation. A volume of 10 μl was delivered to both the inner and outer surfaces of the ear for inducing skin inflammation. Ten microliters of the sample solution, its vehicle, as a control, was applied topically about 30 min prior to TPA treatment. Hydrocortisone (HC), which is currently used to treat various inflammatory skin diseases, was used as a positive control. Ear thickness was determined with a pocket thickness gauge with a range of 0–9 mm, graduated at 0.01 mm intervals and modified so that the contact surface area was increased to reduce the loading, which was applied to the tip of the ear. The ear thickness was measured before treatment (a) and 4 h after the TPA treatment (b = TPA alone; b' = TPA plus sample). The following values were then calculated:

Edema A induced by TPA alone ($b - a$).
Edema B induced by TPA plus a sample ($b' - a$).
Inhibitory rate (%) = [(edema A − edema B)/edema A] × 100.

After measuring ear thickness, animals were anesthetized and 6 mm^2 diameter ear punch biopsies were collected and individually weighed on a Mettler–Toledo (AE-163) electronic balance and the weight was defined as wet weight. The ears were dried for 24 h in dry oven, weighed again and the weight was defined as dry weight. Skin water content was calculated by subtracting dry weight from wet weight and dividing this by dry weight again, and expressed as mg H_2O/mg dry weight.

2.6. Morphological analysis of mouse ear tissue

For morphological assessment of cutaneous inflammation, biopsies from control and treated ears of mice in each treatment group were collected and fixed in 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) and decalcified. Fixed tissues were serially sliced at a thickness of 5.0 μm using a microtome (LEICA RM 2125RT, Nussloch, Germany). The sections were stained with Harry's hematoxylin-eosin and its length was measured by using light microscopy and a representative area was selected for qualitative light microscopic analysis of the cell mediated inflammatory response.

2.7. Statistical analysis

The results are expressed as mean ± SD. One-way ANOVA and Dennett's t-test was used for multiple comparisons using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). The criterion for statistical significance was set at $p < 0.05$.

3. Results

3.1. In vivo anti-inflammatory effect of Z. jujuba essential oil

To investigate whether the seed essential oil of *Z. jujuba* is able to attenuate the inflammation in the skin, we attempted TPA-induced skin inflammation model to assess the potential anti-inflammatory effect of topically applied essential oil of *Z. jujuba in vivo*. Increased skin thickening is often the first hallmark of skin irritation and local inflammation. This parameter is an indicator of number of processes that occur during skin inflammation, including increased vascular permeability, edema and swelling within the dermis, and proliferation of epidermal keratinocytes. Exposure of TPA on the ear of the mouse resulted in marked increases in both

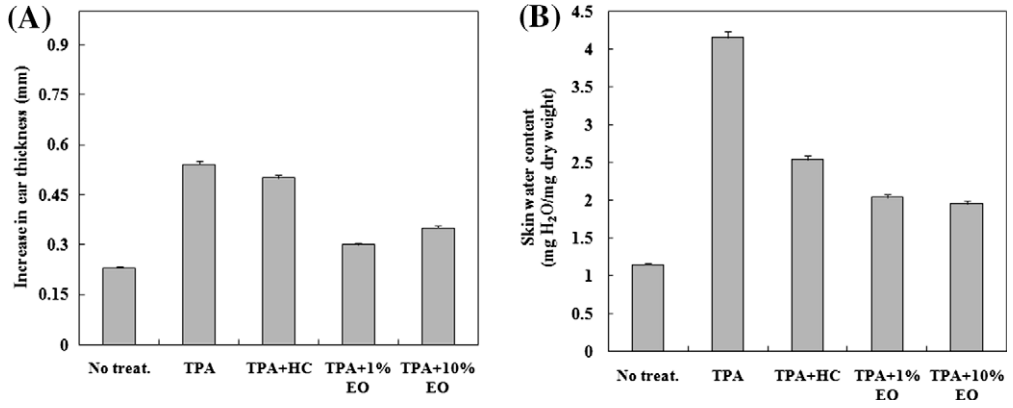

Fig. 1. Effects of *Z. jujuba* essential oil on TPA-induced ear thickness and water content in BLAB/c mice. Mice were pretreated with indicated concentrations of essential oil (EO) and 1% hydrocortisone (HC) for 30 mm and TPA (4 mM) was applied to induce skin inflammation. After 4 h the increase in ear thickness (A) and skin water content (B) was measured. Each column shows the mean ± SD of triplicate determinations.

skin thickness (Fig. 1A) and skin water content in BALB/c mice (Fig. 1B).

Fig. 1A shows that TPA (4 mM) caused a substantial increase in ear thickness, while the topical application of essential oil of *Z. jujuba* significantly inhibited this increase in ear thickness. When the ear thickness was measured, more than twice increase was observed for TPA-induced ear (0.54 ± 0.04 mm) with those of non-treated mice (0.23 ± 0.01 mm). Treatment with 1% and 10% of essential oil caused significant decrease in ear thicknesses which were measured to be 0.30 ± 0.02 and 0.35 ± 0.01 mm, respectively. The effect of 1% and 10% of essential oil on TPA-induced ear was also comparable to that of 1% of HC (0.50 ± 0.1 mm), which was used as a positive control. Increase in skin water content induced by TPA was also suppressed by essential oil treatment in a concentration-dependent manner (Fig. 1B). Treatment with 1% and 10% of essential oil caused about 51% and 53% reduction of water content in TPA-induced skin inflammation model, respectively. However, treatment with hydrocortisone caused about 39% reduction of water content in TPA-induced ear. As the results shown in Fig. 1A and 1B, the inhibitory effect of 1% and 10% of essential oil on TPA-induced skin inflammation was better than that of 1% of HC. Thus, these results demonstrate that essential oil of *Z. jujuba* exerted potential anti-inflammatory effects *in vivo*.

3.2. Examining the mouse ear tissue morphology

We investigated H&E-stained ear sections from TPA-induced animals. TPA application resulted in a marked increase in ear thickness with clear evidence of edema, epidermal hyperplasia, and substantial inflammatory cell infiltration in the dermis with accompanying connective tissue disruption (Fig. 2A and B). By histological comparison, 1% of essential oil treatment reduced ear thickness and associated pathological indicators to an extent comparable to eugenol and the positive control hydrocortisone (HC) (Fig. 2C–E). These results directly illustrate the effects of essential oil within the target tissue, providing further evidence that *Z. jujuba* essential oil ameliorates TPA-induced contact dermatitis.

To further elucidate the effect of *Z. jujuba* essential oil on TPA-induced skin inflammation, we measured the thickness of epidermal and dermal area of mice ear tissue. As shown in Fig. 3, the thickness of epidermal area of essential oil treated ear tissue (15.2 ± 2.9 μm^2) was found to be nearly same as control (12.3 ± 2.1 μm^2), whereas, the thicknesses of epidermal area for TPA, HC and eugenol were 19.9 ± 3.7, 17.8 ± 3.6 and 16.2 ± 3.1 μm^2, respectively. Further, the thickness of dermal area of essential oil treated ear tissue provides the evidence that *Z. jujuba* essential oil exhibited highest anti-inflammatory activity among all the tested samples (Fig. 4). The thicknesses of dermal area for essential oil, TPA, HC and eugenol were 185.5 ± 20.4, 249.7 ± 40.1, 309.7 ± 28.6 and 204.8 ± 28.2 μm^2, respectively.

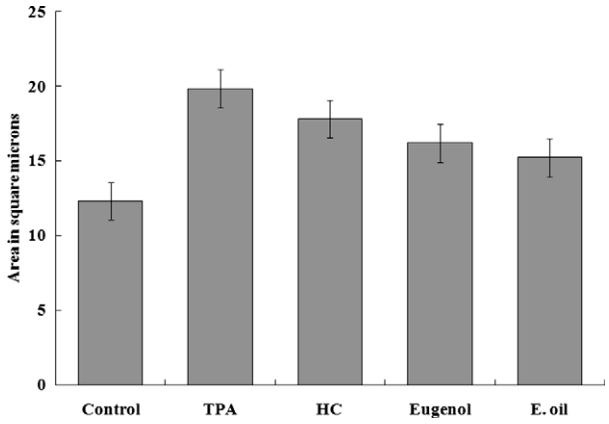

Fig. 3. Area measurements to estimate thickening of epidermis.

4. Discussion

Inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) (Langman, 1998). Unfortunately, both of these widely-prescribed drug classes have significant negative side effects, reducing their use in certain segments of the population (Juni et al., 2005; Pathak et al., 2005). Hence, there is a need to develop new drugs with novel modes of

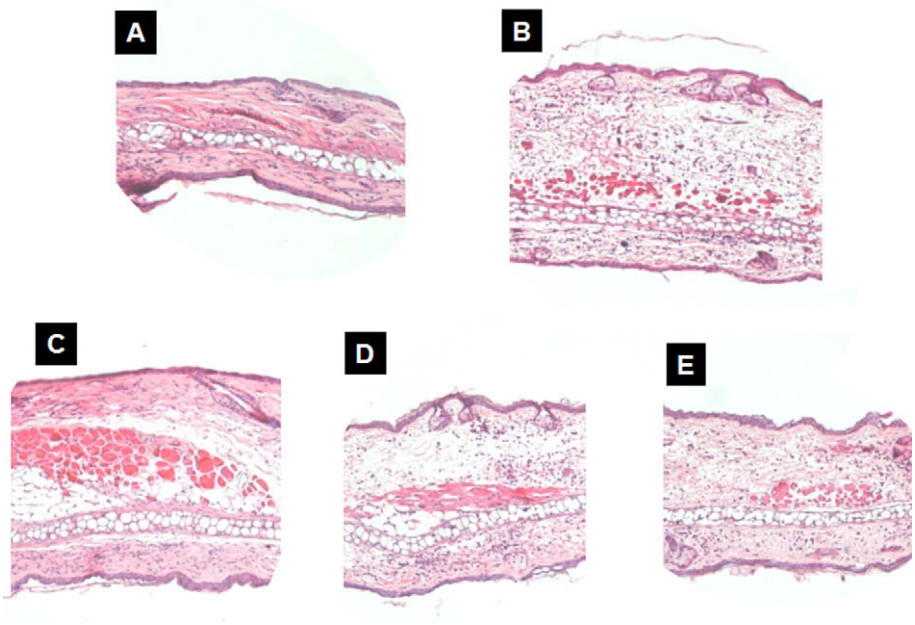

Fig. 2. Histological sections of mouse ear skin biopsies showing epidermal, dermal, and cartilage layers (magnification ×l00). (A) No treatment; (B) TPA; (C) HC; (D) eugenol; (E) essential oil.

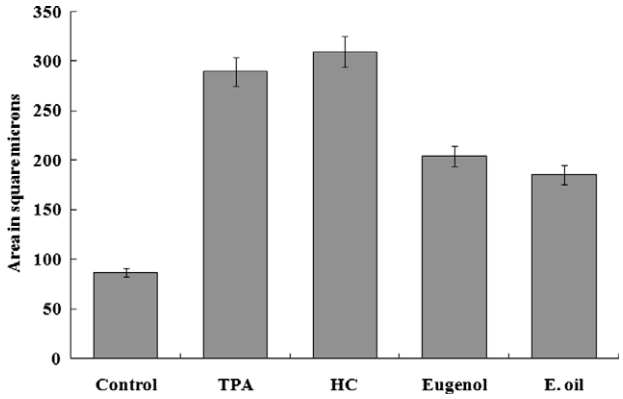

Fig. 4. Area measurements to estimate thickening of dermis.

action that do not produce considerable side effects. Natural product-based anti-inflammatory agents with a transcriptional mode of action, good efficacy and lower risk of side effects offer promising treatment and prevention of inflammation-related conditions.

It is well known that topical application of TPA induces cutaneous inflammation and epidermal hyperplasia (Clark et al., 1985). The TPA application results in a series of events of numerous cellular, biochemical, and molecular changes that eventually lead to the pathological alterations of the mouse skin (Kensler et al., 1987; Nakamura et al., 1998, 2000). The TPA treatment stimulates infiltration of inflammatory cells, which release large amount of H_2O_2 causing oxidative stress (Wei and Frenkel, 1993; Bhasin et al., 2003). The oxidative stress may further amplify the TPA-induced skin injury. Depending on animal models of skin inflammation employed, there are substantial differences in the nature of inflammation produced. For instance, phenol treated contact dermatitis in mice produced an acute inflammation accompanied by dermal edema, and an animal model of delayed hypersensitivity induced an infiltration of inflammatory cells in the lesion (Mitsui et al., 2003; Lim et al., 2004).

This research work describes the complex effect of essential oil on TPA-induced skin inflammation. We examined the effect of *Z. jujuba* essential oil on TPA-induced skin inflammation in a dose-dependent manner. At preliminary stage, we use 1% and 10% of oil for *in vivo* experiment, but the results showed nearly same for reducing TPA-induced ear thickness and skin water content. On the basis of the *in vivo* results, we use only 1% of essential oil during histological analysis. In histological analysis, we also compared the effect of preventive and therapeutic effect of eugenol as it was identified as a major component in *Z. jujuba* essential oil (Al-Reza et al., 2009), and these findings are in agreement with previous study (Thakur and Pitre, 2009). In this study, it was found that the inhibitory effect of essential oil on TPA-induced skin inflammation was better than eugenol (Fig. 2E and D). It may be due to the presence of some other minor active components in the essential oil which might be involved in some type of synergism. It is interesting to note that the positive control hydrocortisone did not show the marked effect on TPA-induced ear (Fig. 2C). Although we know that hydrocortisone (HC), which is currently prescribed to treat various inflammatory skin diseases, as a positive control, but within very short time *Z. jujuba* essential oil showed potent anti-inflammatory effect than hydrocortisone. This investigation has clearly proven that *Z. jujuba* essential oil inhibited the inflammatory responses an animal model of chronic skin inflammation.

To further investigate the anti-inflammatory effect, we measured the thickness of dermal and epidermal area of ear tissue by using ear biopsies. These results also showed that *Z. jujuba* essential oil significantly suppressed the TPA-induced increase in ear thickness of both epidermal and dermal area as compared to control (Figs. 3 and 4). Therefore, histological analysis clearly confirmed that *Z. jujuba* essential oil might be beneficial as a good therapeutic agent for the treatment of various inflammatory diseases.

Plant essential oils are plant secondary metabolites possessing various pharmacological properties, primarily anti-oxidative, antimicrobial or immunomodulatory ones. It has been demonstrated that various essential oils display marked anti-inflammatory effects in several different models of inflammation (Yoon et al., 2007). It is often quite difficult to compare the results obtained from different studies, because the compositions of the essential oils can vary greatly depending upon the geographical region, the variety, age of the plant, the method of drying and the method of extraction of the oil. In brief, the hydrodistillation of the seeds of *Z. jujuba* gave pale yellow oil with major components of the oil having phenolic compounds, oxygenated mono- and sesquiterpenes, and their respective hydrocarbons (Al-Reza et al., 2009). In recent years, several researchers have reported that mono- and sesquiterpene hydrocarbons and their oxygenated derivatives as the major components of essential oils of plant origin, which have potent anti-inflammatory effect, and such findings were also confirmed in the present investigation (Peana et al., 2002; Fernandes et al., 2007; Ko et al., 2008).

5. Conclusions

The results presented in this report suggest possible applications of essential oil from seeds of *Z. jujuba* as a useful anti-inflammatory agent. Moreover, the anti-inflammatory effects of the major pharmacological components present in the essential oil of *Z. jujuba* seeds might accelerate the development of new drugs for various inflammatory diseases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was supported by the Daegu University Research Grant, 2009.

References

Al-Reza, S.M., Bajpai, V.K., Kang, S.C., 2009. Antioxidant and antilisterial effect of essential oil and organic extracts from *Zizyphus jujuba*. Food Chem. Toxicol. 47, 2374–2380.

Ambasta, S.P., 1986. Useful Plants of India. Publications and Information Directorate, CSIR, New Delhi, India. pp.703.

Bhasin, G., Kauser, H., Athar, M., 2003. Progressive iron overload enhances chemically mediated tumor promotion in murine skin. Arch. Biochem. Biophys. 409, 262–273.

Briganti, S., Picardo, M., 2003. Antioxidant activity, lipid peroxidation and skin diseases. What's new. J. Eur. Acad. Dermatol. Venereol. 17, 663–669.

Chi, Y.S., Lim, H., Park, H., Kim, H.P., 2003. Effects of wogonin, a plant flavone from *Scutellaria* radix, on skin inflammation: *in vivo* regulation of inflammation associated gene expression. Biochem. Pharmacol. 66, 1271–1278.

Chien-Tsong, L., Chi-Jung, C., Ting-Yu, L., Tung, J.C., Sheng-Yang, W., 2008. Anti-inflammation activity of fruit essential oil from *Cinnamomum insularimontanum* Hayata. Bioresour. Technol. 99, 8783–8787.

Clark, S.D., Wilhelm, S.M., Stricklin, G.P., Welgus, H.G., 1985. Coregulation of collagenase and collagenase inhibitor production by phorbol myristate acetate in human skin fibroblasts. Arch. Biochem. Biophys. 241, 36–44.

Dinarello, C.A., 1997. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest 112, 321–329.

Erenmemisoglu, A., Keletimur, F., Koker, A.H., Utsuol, H., Tekol, Y., Ustdal, M., 1995. Hypoglycemic activity of *Zizyphus jujuba*. J. Pharm. Pharmacol. 47, 72–74.

Fernandes, E.S., Passos, G.F., Medeiros, R., Cunha, F.M., Ferreira, J., Campos, M.M., Pianowski, L.F., Calixto, J.B., 2007. Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur. J. Pharmacol. 569, 228–236.

Juni, P., Reichenbach, S., Egger, M., 2005. COX2 inhibitors, traditional NSAIDs and the heart. Br. Med. J. 330, 1342–1343.

Kensler, T.W., Egner, P.A., Moore, K.G., Taffe, B.G., Twerdok, L.E., Trush, M.A., 1987. Role of inflammatory cells in the metabolic activation of polycyclic aromatic hydrocarbons in mouse skin. Toxicol. Appl. Pharmacol. 90, 337–346.

Kim, H.S., 2002. Effects of the *Zizyphus jujuba* seed extract on the lipid components in hyperlipidemic rats. J. Food Sci. Nutr. 7, 72–77.

Ko, H.H., Hung, C.F., Wang, J.P., Lin, C.N., 2008. Anti-inflammatory triterpenoids and steroids from *Ganoderma lucidum* and *G. tsugae*. Phytochemistry 69, 234–239.

Kupper, T.S., Fuhlbrigge, R.C., 2004. Immune surveillance in the skin: mechanisms and clinical consequences. Nat. Rev. Immunol. 4, 211–222.

Langman, M.J.S., 1998. Ulcer complications and NSAIDs. Am. J. Med. (Suppl.) 84, 15–19.

Lee, S.J., 1986. Pen-Tsao-Kun-Mu, vol. 18. Chinese Book Store, Bejium, pp. 57–60.

Lee, J.L., Mukhtar, H., Bickers, D.R., Kopelovich, L., Athar, M., 2003. Cyclooxygenases in the skin: pharmacological and toxicological implications. Toxicol. Appl. Pharmacol. 192, 294–306.

Lee, D.Y., Choi, G., Yoon, T., Cheon, M.S., Choo, B.K., Kim, H.K., 2009. Anti-inflammatory activity of *Chrysanthemum indicum* extract in acute and chronic cutaneous inflammation. J. Ethnopharmacol. 123, 149–154.

Lim, H., Park, H., Kim, H.P., 2004. Inhibition of contact dermatitis in animal model and suppression of proinflammatory gene expression by topically applied flavonoid, Wogonin. Arch. Pharm. Res. 27, 442–448.

Mitsui, G., Mitsui, K., Hirano, T., Ohara, O., Kato, M., Niwano, Y., 2003. Kinetic profiles of sequential gene expressions for chemokines in mice with contact hypersensitivity. Immunol. Lett. 86, 191–197.

Nakamura, Y., Murakami, A., Ohto, Y., Torikai, K., Tanaka, T., Ohigashi, H., 1998. Suppression of tumor promoter induced oxidative stress and inflammatory responses in mouse skin by superoxide generation inhibitor 10-acetoxychavicol acetate. Cancer Res. 58, 4832–4839.

Nakamura, Y., Torikai, K.T., Ohto, Y., Murakami, A., Tanaka, T., Ohigashi, H., 2000. A simple phenolic antioxidant protocatechuic acid enhances tumor promotion and oxidative stress in female ICR mouse skin: dose- and timing-dependent enhancement and involvement of bioactivation by tyrosinase. Carcinogenesis 21, 1899–1907.

Oikarinen, A., Haapasaari, K.M., Sutinen, M., Tasanen, K., 1998. The molecular basis of glucocorticoid-induced skin atrophy: topical glucocorticoid apparently decreases both collagen synthesis and the corresponding collagen mRNA level in human skin *in vivo*. Br. J. Dermatol. 139, 1106–1110.

Palladino, M.A., Bahjat, F.R., Theodorakis, E.A., Moldawe, L.L., 2003. Anti-TNF-alpha therapies: the next generation. Natl. Rev. Drug Discov. 2, 736–746.

Pathak, S.K., Sharma, R.A., Steward, W.P., Mellon, J.K., Griffiths, T.R., Gescher, A.J., 2005. Oxidative stress and cyclooxygenase activity in prostate carcinogenesis: targets for chemopreventive strategies. Eur. J. Cancer 41, 61–70.

Peana, A.T., Aquila, P.S.D., Panin, F., Serra, G., Pippia, P., Moretti, M.D.L., 2002. Antiinflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 9, 721–726.

Souleles, C., Shammas, G., 1998. Flavonoids from the leaves of *Zizyphus jujuba*. Fitoterapia 59, 154–156.

Thakur, K., Pitre, K.S., 2009. Anti-inflammatory activity of extracted eugenol from *Ocimum sanctum* L. leaves. RASĀYAN J. Chem. 2, 472–474.

Trouba, K.J., Hamadeh, H.K., Amin, R.P., Germolec, D.R., 2002. Oxidative stress and its role in skin disease. Antioxid. Redox Signal. 4, 665–673.

Wei, H., Frenkel, K., 1993. Relationship of oxidative events and DNA oxidation in SENCER mice to in vivo promoting activity of phorbol ester-type tumor promoter. Carcinogensis 14, 1195–1202.

Yoon, W.K., Han, M.H., Lee, H., Han, S.B., Lee, K., Park, S.K., Lee, S.H., Yang, K.H., Moon, E.Y., Kim, H.M., 2007. Topical application of a novel ceramide derivative, K6PC-9, inhibits dust mite extract-induced atopic dermatitis-like skin lesions in NC/Nga mice. Int. Immunopharmacol. 7, 1589–1597.