European Journal of Pharmacology 662 (2011) 63–69

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and Inflammation

Embelin reduces cutaneous TNF-α level and ameliorates skin edema in acute and chronic model of skin inflammation in mice

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ARTICLE INFO

Article history:
Received 20 November 2010
Received in revised form 31 March 2011
Accepted 14 April 2011
Available online 30 April 2011

Keywords:
Embelin
Skin inflammation
TNF-α
Psoriasis

ABSTRACT

Tumor necrosis factor-α (TNF-α) is known to play a crucial role in the pathogenesis of psoriasis. The present study was designed to investigate the effects of embelin on lipopolysachharide induced TNF-α production in mice and in human keratinocytes *in vitro* and also to study the effect of embelin on acute and chronic skin inflammation in mice. Production of pro-inflammatory cytokines (TNF-α and IL-1β), activation of myeloperoxidase and histological assessment were examined in acute and chronic skin inflammation using 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced mouse ear edema. Embelin inhibited topical edema in the mouse ear, leading to substantial reductions in skin thickness and tissue weight, inflammatory cytokine production, neutrophil-mediated myeloperoxidase activity, and various histopathological indicators. Furthermore, embelin was effective at reducing inflammatory damage induced by chronic TPA exposure. Our data indicate that embelin has anti-inflammatory activities in both acute and chronic irritant contact dermatitis *in vivo* and this effect of embelin may be due, at least in part, to the inhibition of IL-1β and TNF-α and to the subsequent blockade of leukocyte accumulation.

1. Introduction

Psoriasis is a common inflammatory and hyperproliferative skin disease characterized by abnormal keratinocyte proliferation and differentiation, accumulation of polymorphonuclear leukocytes in the skin and T-cell activation (Kemeny et al., 1994). It affects nearly two percent of the world population with no preventive or curative therapy. Psoriasis is a non-contagious skin disorder and does not normally impinge significant threat on the life of the patient. Psoriasis not only has a physical impact such as itching in the skin, pain and restricted motion in their joints, but also adversely affects the psychological feelings of the sufferers leading to anxiety, anger, embarrassment, low self-esteem and even depression. The pathogenesis of psoriasis is not completely understood. It has been demonstrated that epidermal keratinocytes are known to participate in immune and inflammatory reactions by producing a variety of cytokines (Hauser et al., 1986; Kock et al., 1990; Kupper et al., 1986). Among the cytokines, TNF-α has been thought to play a crucial role in various immunological disorders and inflammation in the skin. TNF-α released from keratinocytes would stimulate accumulation of inflammatory cells via induction of expression of adhesion molecules such as ICAM-1 on neighboring endothelial cells (Nickoloff and Turka, 1993). Also, TNF-α could induce production of secondary cytokines (Nickoloff et al., 1991), resulting in expansion of immune/inflammatory reactions. In the dermis, there is also a dilation of the blood vessels and increased angiogenesis. Hence, inhibition of cytokines like TNF-α and angiogenesis could be an attractive strategy for the treatment of psoriasis.

The fruit of the *Embelia ribes* Burm. plant (*Myrsinaceae*) (called false black pepper in English, Vidanda in Sanskrit, and Babrang in Hindi languages) contains a quinone derivative embelin (3-undecyl 2,5-dihydroxy, 1,4-benzoquinone) as its active constituent. Embelin is reported to have antihelmintic, antifertility (Krishnaswamy and Purushothaman, 1980) antitumor, antimicrobial (Chitra et al., 1994), analgesic (Chitra et al., 2003) anti-inflammatory and anti-diabetic (Bhandari et al., 2002) activity. Although the precise mechanism underlying most of these activities is unclear, recent evidence indicates that the antitumor activity can be attributed to the ability of embelin to bind and inhibit XIAP, thereby inducing activation of caspace3, -7 and -9, and apoptosis. Embelin is also reported to block nuclear kappa factor-κB (NF-κB) signaling pathways, thereby leading to down-regulation of a variety of gene products involved in tumor cell cervical, proliferation, invasion, angiogenesis and inflammation. TNF-α can activate NF-κB by degrading IκB, its inhibitory protein. However, the effect of embelin on TNF-α and skin inflammation is not

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doi:10.1016/j.ejphar.2011.04.037

known. Hence, the aim of the present study was to investigate the effect of embelin on LPS induced TNF-α in mice and human keratinocytes *in vitro* and also to study the effect of embelin in acute and chronic skin inflammation in mice.

2. Material and methods

2.1. Plant material

Dry fruits of *E. ribes* Burm.(*Myrsinaceae*) were collected from PERD Centre, Gujarat, India. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Presidency College Madras University Herbarium (Specimen No. PBPB-26). The material was air dried in shade, powdered mechanically and stored in airtight containers. One kilogram of the powdered material was refluxed with ethanol in a soxhlet apparatus for 48 h in batches of 250 g each. The extract was filtered, pooled and the solvent was removed under reduced pressure at 40 ± 5 °C using rotary flash evaporator and the yield was 5.27 g.

2.2. Isolation

The coarse powder of dry fruits of *E. ribes* Burm. was extracted exhaustively with n-hexane and the extract so obtained was chromatographed on silica gel column and eluted successively using chloroform and ethanol in the ratio 1:1. The eluted fractions were collected at an interval of 5 ml each and were monitored by thin layer chromatography and grouped into five fractions. The fraction two recovered in higher concentration was recrystallized from chloroform to get an orange red needle like aromatic compound. The structure and purity of embelin was confirmed by HPTLC, IR, 1H and NMR studies.

2.3. Experimental animals

Female (Balb/C) mice weighing 25–30 g were purchased from C.L. Baid Mehta College, India. All mice were housed 2/cage and fed standard laboratory chow in the animal room with 12 h dark/light cycles and constant temperature of 20 ± 5 °C. All animal experiments were conducted under university guidelines and approved by ethical committee.

2.4. Chemicals and reagents

TPA and Prednisolone were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Micrometer (Mitutoyo Series 293), Embelin from Tocris (Cat No. 2156). Betamethasone dipropionate was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Calcium ionophore was obtained from Sigma-Aldrich India. Bacterial LPS (*Escherichia coli* serotype 055:B5), ATCC-PCS-200-011 (Primary Epidermal Keratinocytes; Normal, Human, Adult), complete growth medium: Keratinocytes-Serum Free medium (GIBCO-BRL 17005–042) with 5 ng/ml human recombinant EGF and 2 mM L-glutamine (without bovine pituitary extract and without serum), atmosphere air 95%; carbon dioxide 5% (CO_2). All other chemical reagents were of the highest commercial grade available.

2.5. LPS-induced TNF-α production in mice

Female (Balb/C) mice 6–8 weeks old were fasted overnight and intraperitoneally injected with LPS (1 mg/kg). Embelin or the vehicle was administered orally 30 min prior to LPS injection. Serum was collected 90 min after LPS injection. The amounts of TNF-α in serum were measured using commercially available ELISA kits (R&D Systems).

2.6. Acute inflammatory model in mice

Acute TPA-induced skin inflammation was examined as described previously (Delescluse et al., 1987) with a slight modification. Briefly, 20 μl of TPA solution (50 μg/ml of TPA in 1% dimethylsulfoxide/99% methanol) was applied topically on both ears of Balb/C mice. Edema was expressed as the increase in ear thickness and ear weight due to inflammatory challenge. Ear thickness was measured with a micrometer (Mitutoyo Series 293) before and after induction of an inflammatory response. Kadoshima-Yamaoka et al. (2009) have previously confirmed that the solvent (1% dimethylsulfoxide/99% methanol) itself has no effect on the ear thickness. To examine the effects of embelin or betamethasone dipropionate, each compound was dissolved in the TPA solution and the solution was painted onto the ears instead of the TPA solution.

2.7. Chronic inflammatory model in mice

Chronic skin inflammation was evaluated using a previously described procedure that was slightly modified (Burke, 2001). Briefly, 20 μl of TPA solution (50 μg/ml of TPA in 1% dimethylsulfoxide/99% methanol) was applied topically on both ears with a micropipette on alternate days. Embelin or betamethasone dipropionate was given topically once a day for 10 days each morning immediately after TPA application. On day 10, the mice were sacrificed at 6 h after treatment and 8 mm diameter ear punch biopsies were collected and weighed.

2.8. Enzyme immunoassay of IL-1β and TNF-α

Ear tissue levels of the cytokine proteins such as IL-1β and TNF-α were determined 6 h after TPA application using a standard sandwich enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The results were expressed as arbitrary units of relative value.

2.9. Determination of MPO activity

MPO activity was assessed from ear tissue after repeated application of TPA for ten days, using the mouse MPO ELISA kit, according to the manufacturer's instructions (Hycult Biotechnology, The Netherlands). The results were expressed as arbitrary units of relative value.

2.10. TNF-α production by human keratinocytes in vitro

Human epidermal keratinocytes were cultured in 96 well collagen I-coated flat bottom plate (5×10^3 cells/well) for 18 h before the experiments. After removing the supernatant and adding the fresh medium, different concentrations of embelin were added to the culture and incubated for 30 min. Then, TPA (100 ng/ml) and calcium ionophore A23187 (1 μg/ml) were added to the culture and incubated for additional 8 h. The contents of TNF-α in the culture supernatants were measured using human TNF-α ELISA kit (Invitrogen Co., Carlsbad, CA).

2.11. Histology

For histological assessment of cutaneous inflammation, ear biopsies of mice were collected from each control and treated groups. This was fixed with paraformaldehyde (0.1 M phosphate buffer, pH 7.4). The ear samples were dehydrated with 15, 20, 25, and 30% serial sucrose solutions. A series of 10 μm ear cross-sections were prepared by a freezing microtome. The sections were stained with hematoxylin and eosin (H&E) for the evaluation of leukocyte accumulation and edema. A representative area was selected for

qualitative light microscopic analysis of the cell mediated inflammatory response. To minimize bias, the sample analysis was blinded.

2.12. Statistics

Results are expressed as mean ± standard error of mean (S.E.M). The statistical significance of the detected differences was calculated by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Differences were considered statistically significant when $P<0.05$.

3. Results

3.1. Structural confirmation of embelin

Repeated crystallization and purification of petroleum ether extract of *E. ribes* fruits with methanol resulted in isolation of 722 mg embelin (yield 2.88% w/w). The IR, NMR confirmed the structure assigned to embelin and was further supported by mass spectral studies which results in molecular ion peak at m/z 294 confirm the structure (5-hydroxy-3-undecylcyclohex-5-ene-1,2,4-trione) (Fig. 1). The purity of embelin was confirmed by HPTLC analysis. The methods were validated in terms of precision, repeatability and accuracy. The linear relationship between peak area and amount of embelin applied was found only within the range of 320–960 ng/spot with correlation coefficient 0.993 and standard deviation 4.12%. The limit of detection for embelin was found to be 20 ng and the limit of quantification was found to be 40 ng. The average of percentage recovery at three different levels was found to be 99.47%. The amount of embelin in *E. ribes* fruit was found to be 3.21% w/w as quantified by the proposed method.

3.2. Effects of embelin on LPS-induced TNF-α production in mice

Amounts of serum TNF-α were measured 60 min after LPS injection since TNF-α level was maximal at this time. The average baseline TNFα levels were 6589 pg/ml in mice. The plasma TNF-α level then underwent rapid decline to baseline by about 3 to 4 h post LPS administration. As shown in Table 1, embelin (0.1, 1, 10, 30 and 50 mg/kg, p.o.) resulted in dose-dependent inhibition of LPS-induced TNF-α production, and the ED_{50} of embelin was 9.28 mg/kg mice, statistically estimated using Sigmoidal dose–response (variable slope) by best fit value to calculate ED_{50} using graph prism.

3.3. Effect of embelin on skin edema in TPA-induced ear edema model

We assessed the anti-inflammatory activity of embelin in a mice model of TPA-induced acute irritant contact dermatitis. Increased skin thickening is often the first hallmark of skin irritation and local inflammation. This parameter is one 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 to TPA on the ear of the mouse

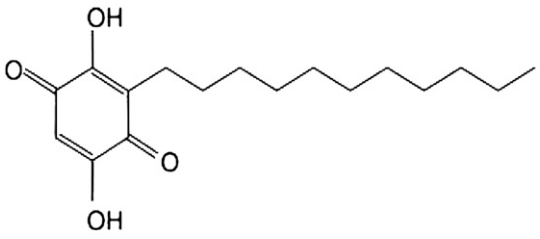

Fig. 1. Structure of embelin.

Table 1
Effects of embelin on LPS-induced TNF-α production in mice. Embelin or prednisolone was administered orally 30 min prior to LPS injection. Serum was collected 90 min after LPS injection. Results are expressed as the mean ± S.E.M. (N = 6).

Treatment	Dose (mg/kg)	Mice TNF-α		ED_{50} (mg/kg)
		Levels (pg/ml)	% inhibition	
Embelin	0.1	6452 ± 456	2.08	9.28
	1	5124 ± 234	22.23	
	10	3214 ± 423	51.22	
	30	2065 ± 376	68.66	
	50	1754 ± 230	73.38	
Prednisolone	3	2421 ± 212	67.98	
LPS		6589 ± 347		

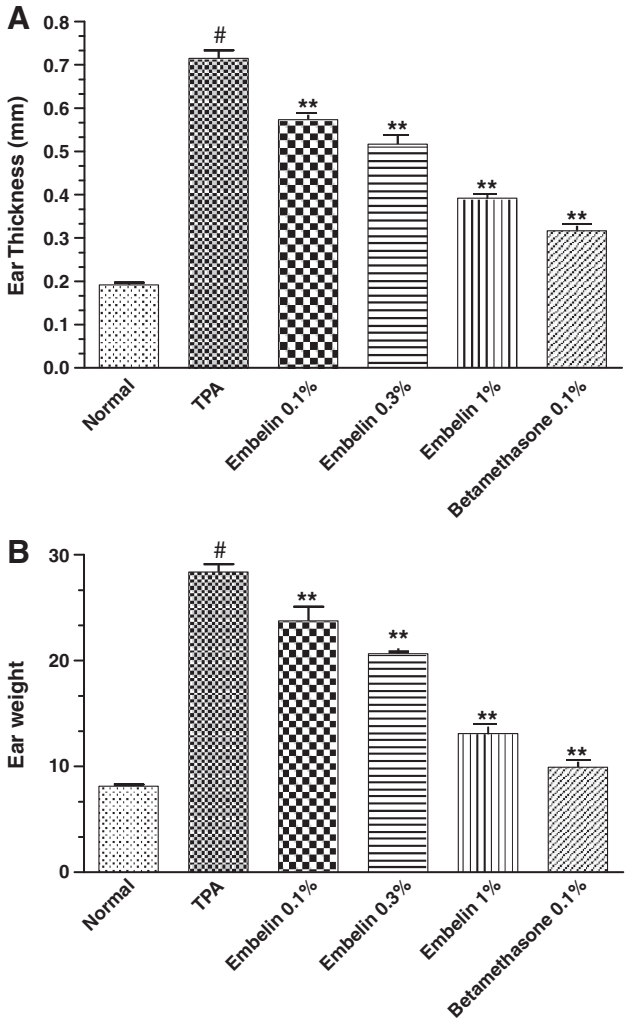

Fig. 2. Effect of embelin on TPA-induced ear edema changes in an acute inflammation model. Mice were treated with normal saline (TPA + S), embelin (TPA + embelin 0.1, 0.3 and 1%) or 0.1% Betamethasone dipropionate (TPA + Beta) for 1 h prior to topical application of 1% dimethylsulfoxide/99% methanol acetone (vehicle). (A) Ear thickness and (B) ear weights were measured at 6 h after TPA treatment. Values are the mean ± S.E.M of the experiment. #$P<0.05$ versus the saline-vehicle treated control group; *$P<0.05$, **$P<0.01$, and ***$P<0.001$ treated versus the TPA induced edema group were determined using ANOVA and Dunnett's post hoc test using graph prism.

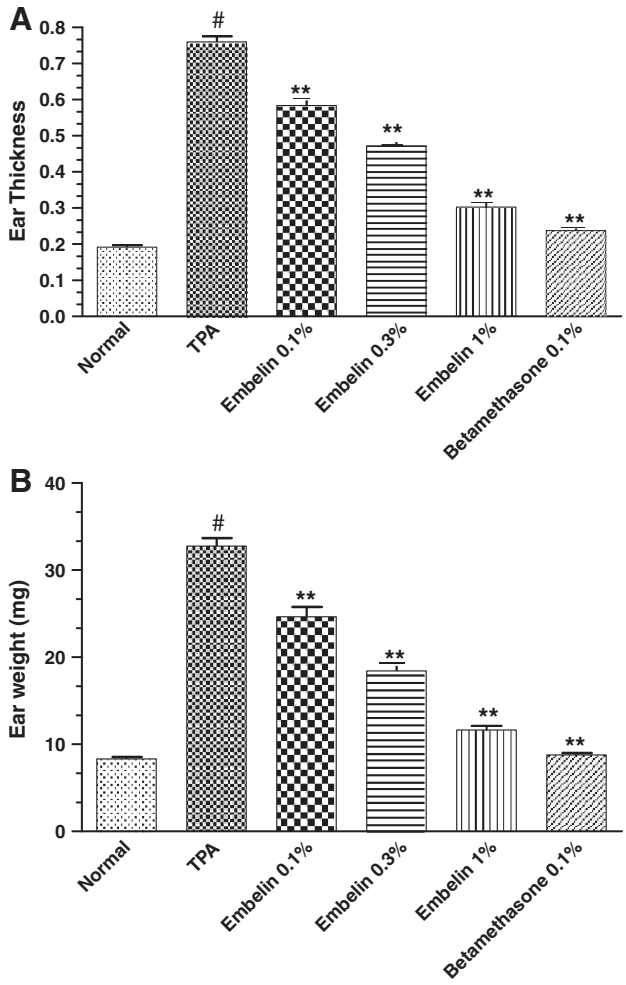

Fig. 3. Effect of embelin on TPA-induced ear thickness and weight changes in the chronic inflammation model. Mice were treated with repeated topical applications of TPA. Ear thickness (A) and ear weights (B) were measured on day 10. Values are the mean ± S.E.M of the experiment. #P<0.05 versus the saline-vehicle treated control group; *P<0.05, **P<0.01, and ***P<0.001 treated versus the TPA induced edema group were determined using ANOVA and Dunnett's post hoc test using graph prism.

resulted in marked increases in both ear thickness and weight (Fig. 2A and B) in acute and chronic model (Fig. 3A and B). Topical application of 1% dimethylsulfoxide/99% methanol (vehicle) alone did not alter the skin thickness significantly. However, embelin significantly reduced the phorbol ester-induced ear edema, indicating the therapeutic effect of embelin.

Next, we investigated H&E-stained ear sections from TPA treated 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 in acute model (Fig. 4A). Treatment with embelin reduced ear thickness and associated pathological indicators to an extent comparable to the positive control betamethasone dipropionate in chronic model (Fig. 4B). These results directly illustrate the effects of embelin within the target tissue, providing further evidence that embelin ameliorates TPA-induced contact dermatitis.

3.4. Examining the effect of embelin on pro-inflammatory cytokine production

To assess the efficacy of embelin at the molecular level, we investigated the pro-inflammatory cytokine inhibitory effect of embelin. As shown in Fig. 5A and B, topical application of TPA caused a dramatic increase in the production of TNF-α and IL-1β at 6 h after challenge. In contrast, treatment with TPA plus embelin or betamethasone reduced both IL-1 β and TNF-α cytokine levels significantly. Thus, embelin may act by reducing the levels of activated cellular infiltrates and secretion of cytokines, thereby reducing cutaneous inflammation.

3.5. Effect of embelin on prolonged inflammation induced by repeated TPA application

As a second *in vivo* measure of the anti-inflammatory activity of embelin, the extract was administered in a chronic skin inflammation model induced by repeated exposure to phorbol ester. Skin inflammation is persistent in this model (Burke, 2001; Stanley et al., 1991), which makes it useful for assessing whether embelin resolves existing inflammatory lesions. Exposure to TPA resulted in marked increase in both skin thickness (Fig. 3A) and tissue weight (Fig. 3B). Interestingly, embelin significantly inhibited these phorbol ester-induced increases, indicating a therapeutic effect of this extract in the chronic model. Consistent with the edema parameters, embelin reduced the level of MPO activity, an indicator of polymorphonuclear leukocyte influx, by 76% in the chronic inflammation model (Fig. 6). These findings support the ability of embelin to resolve an existing, persistent inflammatory lesion induced by multiple topical TPA applications, with an efficacy comparable to that of betamethasone (0.1%).

3.6. Effect of embelin on TNF-α production by human keratinocytes in vitro

Human keratinocytes produced 94.04 pg/ml of TNF-α without stimulation, but the TNF-α production was markedly induced when the cells were activated with TPA plus calcium ionophore A23187. Embelin inhibited the TPA/A23187-induced TNF-α production, with a significant effect at 9.18 μM and higher (Fig. 7). Embelin also inhibited the TNF-α production by keratinocytes.

4. Discussion

Previously embelin has been reported to have anti-inflammatory, analgesic and antitumor activity in animal models (Chitra et al., 1994). However, the direct effect of embelin on skin inflammation was not reported. To study this we used phorbol ester-induced mouse ear inflammation model. Since this model mimics several aspects of psoriasis, it is a reliable *in vivo* model system to evaluate compounds for both acute and chronic effects in skin inflammation (Stanley et al., 1991). In the present study, embelin inhibited TPA stimulated TNF-α in human keratinocytes with an IC_{50} of 9.18 μM. Embelin also showed dose dependent decrease in LPS induced TNF-α level in mice with an ED_{50} of 9.28 mg/kg. These preliminary results made us to study the effect of embelin on TPA induced skin inflammation.

In the present investigation, we evaluated the efficacy of embelin on skin inflammation in mouse models. Increased skin thickening is often the first hallmark of skin irritation and local inflammation. This parameter is one indicator of a number of processes that occur during skin inflammation, including increased vascular permeability, edema, and swelling within the dermis and proliferation of epidermal keratinocytes. In both acute and chronic irritant contact dermatitis mouse models, embelin significantly inhibited the TPA-induced increase in ear thickness and weight. In addition, embelin ameliorated several histopathological indicators, decreased release of proinflammatory cytokines and diminished neutrophil activation and migration of polymorphonuclear leukocytes, following application of TPA. Results from our study demonstrate that embelin inhibits phorbol ester-induced increases in ear edema. These findings improve our understanding of the pharmacological mechanisms of embelin used for the treatment of inflammatory skin diseases.

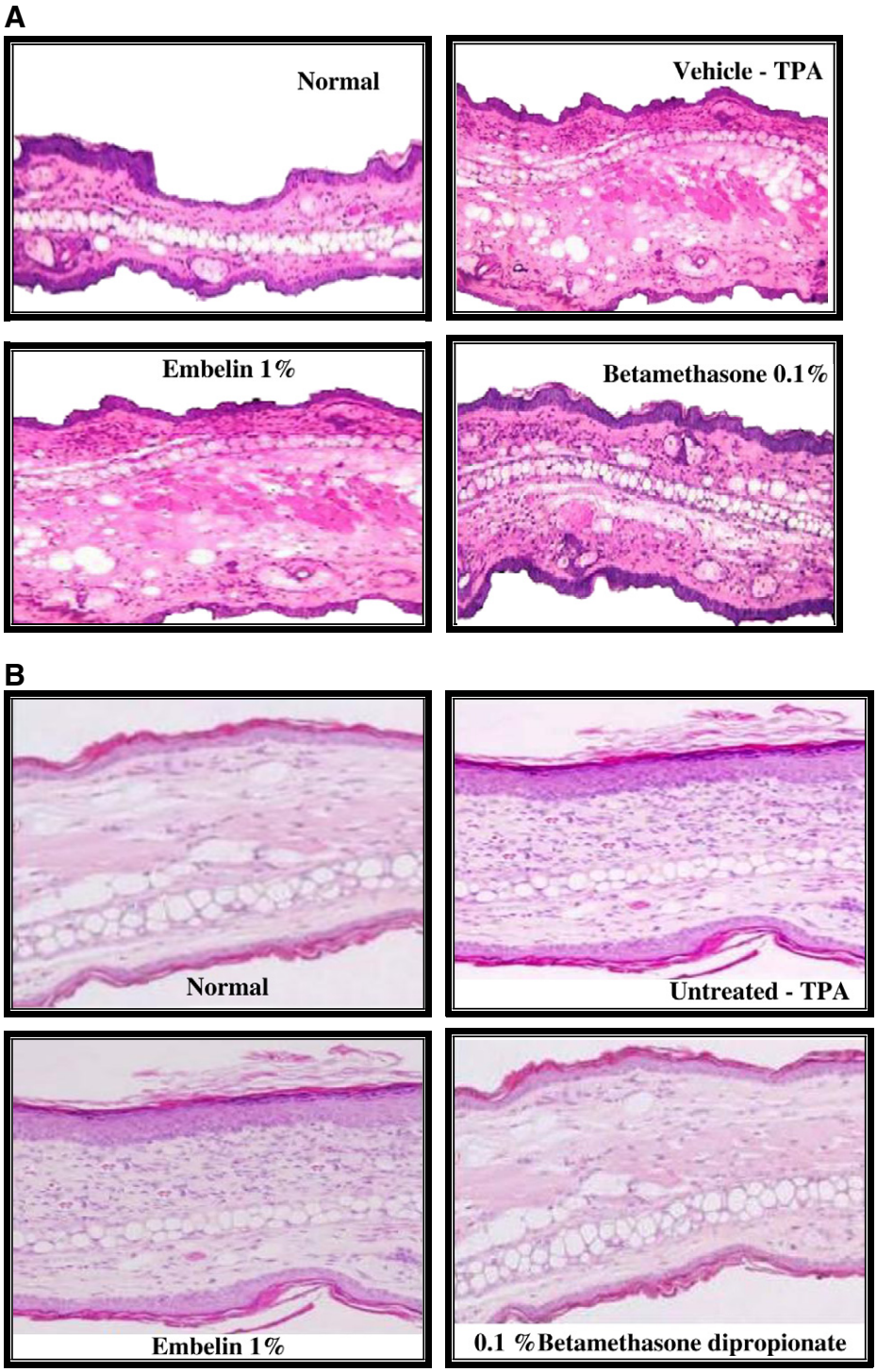

Fig. 4. A) Representative micrographs of H&E-stained mouse ear cross-sections in the acute TPA model. Ears were harvested 6 h post-treatment with normal vehicle, TPA, embelin 1%, or 0.1% betamethasone dipropionate. Note the edema, polymorph nuclear cell influx and epidermal hyperplasia in TPA-treated ears and the reduction in inflammatory cells and edema in ears following embelin 1% treatment. Representative sections from each group are shown (400× magnifications). B) Representative micrographs of H&E-stained mouse ear cross-sections in the TPA applied every other day on the ears for 10 days and thereby a chronic irritant contact dermatitis is evolved. Normal vehicle, TPA, embelin 1%, or 0.3% betamethasone dipropionate. The lesions are characterized by erythema, oedema and scaling and histologically the lesions are characterized by epidermal hyperplasia, infiltration with monocytes and lymphocytes.

Our study showed that TPA exposure results in increased secretion of IL-1β and TNF-α, suggesting that both cytokines mediate inflammatory signaling and play pivotal roles in TPA-induced acute irritant contact dermatitis (Otuki et al., 2005; Ueda et al., 2004). Embelin negatively interfered with these cytokines, which are known to play important roles in the inflammatory process of tumor progression. In general, the longer the inflammation persists, the higher the risk of cancer (Ahn et al., 2007) Therefore, these inhibitory activities of embelin against suppression IL-1β and TNF-α suggest that embelin may be a promising candidate for the suppression of tumor

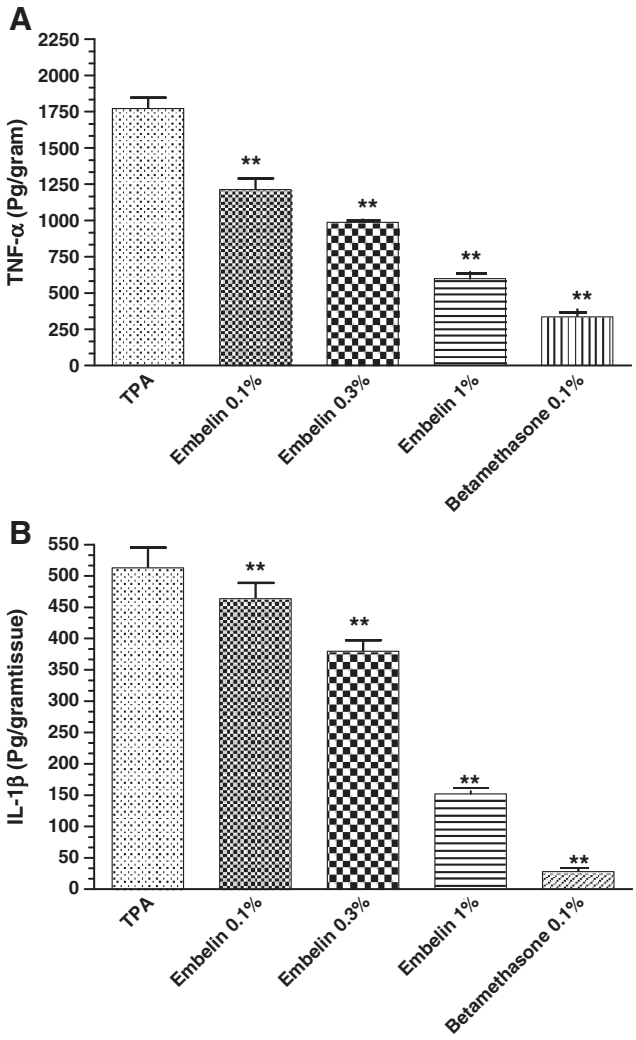

Fig. 5. In acute inflammation model serum was taken 6 h after TPA treatment and examined for the production of the pro-inflammatory cytokines TNF-α (A) IL-1β (B) using ELISA. Data represents the mean ± S.E.M (N = 10) mice per group. **P<0.01 compared to TPA group and were determined using ANOVA and Dunnett's post hoc test using graph prism.

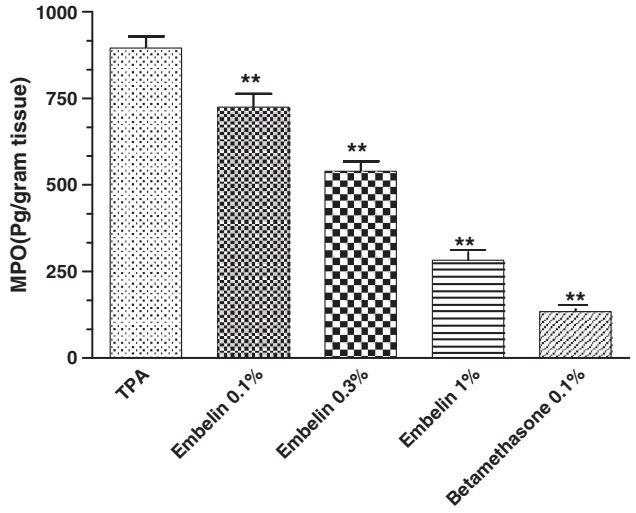

Fig. 6. Effect of embelin on MPO activity in the chronic inflammation model. Mice were treated with repeated topical applications of TPA. On day 10 Neutrophil activation levels were determined by an MPO activity assay of mouse plasma. Data represents the mean ± S.E.M. (N = 10) mice per group. **P<0.01 compared to TPA group and were determined using ANOVA and Dunnett's post hoc test using graph prism.

progression, in addition to regulating the inflammatory response. Furthermore, we demonstrated that embelin inhibits MPO activation in a mouse model of chronic skin inflammation. MPO is an enzyme found in the azurophilic granules of neutrophils and other cells of myeloid origin and is commonly used as an index of granulocyte infiltration. The inhibition of MPO activity is indicative of anti-inflammatory activity in the chronic inflammation model (Fantini and Pallone, 2008).

Importantly, our histological analysis of the ear clearly confirms that embelin inhibits the influx of polymorphonuclear leukocytes to the mouse ear skin following TPA application. We propose that the marked inhibition of ear edema, MPO activity, and activation and migration of leukocytes in response to TPA, may be related to the ability of embelin to inhibit proinflammatory cytokine release. This concept correlates well with previous findings that those cells in the injured skin, such as dermal dendritic cells, epidermal Langerhans cells, melanocytes, fibroblasts, and leukocytes, are known to be sources and targets of cytokines (Grone, 2002). Moreover, embelin has shown inhibition in TNF-α in both LPS and TPA induced inflammation. Taken together, these data suggest that amelioration of TPA-induced skin edema by embelin might be, at least in part, due to the reduction of TNF-α production. However, the mechanism behind this effect of embelin needs to be established. Taken together, these results support the notion that embelin possesses anti-inflammatory properties. Indeed, neutrophil accumulation plays a critical role in cutaneous inflammatory diseases, such as psoriasis, and is related to the pathological mechanism of disease.

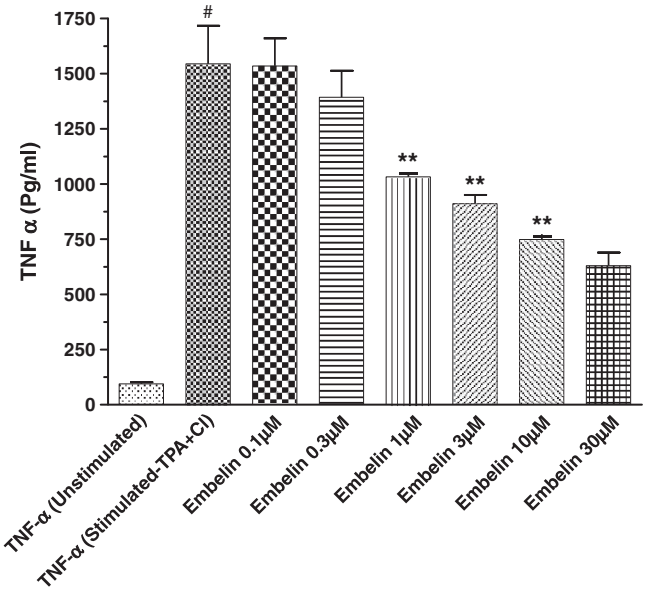

Fig. 7. Effect of embelin on TNF-α production by human epidermal keratinocytes (HEK) stimulated with TPA and calcium ionophore. Human keratinocytes were incubated with various concentrations of embelin for 30 min in 96-well collagen I-coated flat bottom plate, and TPA (100 ng/ml) and embelin (1 μg/ml) were added to the culture. After incubation for additional 8 h, the concentration of TNF-α in the culture supernatant was determined by ELISA. The data are expressed as mean ± S.E.M. of triplicate cultures. #$P<0.01$ compared with the group in which keratinocytes were not activated with TPA plus calcium ionophore (Student's *t*-test). **P<0.01, compared with the group in which keratinocytes were activated and no test compound was added (Dunnett's test).

5. Conclusion

In the present investigation, for the first time, we report that embelin has anti-inflammatory activities in both acute and chronic models of psoriasis. Our results indicate that this inhibitory effect of embelin may be due, at least in part, to the inhibition of IL-1β and TNF-α and to the subsequent blockade of leukocyte accumulation. This suggests that embelin may be a good candidate for the treatment of inflammatory skin diseases. Further studies are needed to evaluate the molecular mechanism behind anti-inflammatory activity of embelin.

References

Ahn, K.S., Sethi, G., Aggarwal, B.B., 2007. Embelin, an inhibitor of X chromosome-linked inhibitor-of-apoptosis protein, blocks nuclear factor-κappaB (NF-κappaB) signaling pathway leading to suppression of NF-κappaB-regulated antiapoptotic and metastatic gene products. Mol. Pharmacol. 71, 209–219.

Bhandari, U., Kanojia, R., Pillai, K.K., 2002. Effect of ethanolic extract of Embelia ribes on dyslipidemia in diabetic rats. Int. J. Exp. Diabetes Res. 3, 159–162.

Burke, J.R., 2001. Targeting phospholipase A2 for the treatment of inflammatory skin diseases. Curr. Opin. Investig. Drugs 2, 1549–1552.

Chitra, M., Sukumar, E., Suja, V., Shyamala Devi, C.S., 1994. Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. Chemotherapy 40, 109–113.

Chitra, M., Devi, C.S., Sukumar, E., 2003. Antibacterial activity of embelin. Fitoterapia 74, 401–403.

Delescluse, C., Vaigot, P., Bailly, J., Hensby, C.N., 1987. Effects of anti-inflammatory agents on edema and DNA synthesis induced by 12-O-tetradecanoylphorbol-13-acetate in the guinea pig. Agents Actions 21, 290–292.

Fantini, M., Pallone, F., 2008. Cytokines: from gut inflammation to colorectal cancer. Curr. Drug Targets 9, 375–380.

Grone, A., 2002. Keratinocytes and cytokines. Vet. Immunol. Immunopathol. 88, 1–12.

Hauser, C., Dayer, J.M., Jaunin, F., de Rochemonteix, B., Saurat, J.H., 1986. Intracellular epidermal interleukin 1-like factors in the human epidermoid carcinoma cell line A431. Cell. Immunol. 100, 89–96.

Kadoshima-Yamaoka, K., Murakawa, M., Goto, M., Tanaka, Y., Inoue, H., Murafuji, H., Hayashi, Y., Nagahira, K., Miura, K., Nakatsuka, T., Chamoto, K., Fukuda, Y., Nishimura, T., 2009. Effect of phosphodiesterase 7 inhibitor ASB16165 on development and function of cytotoxic T lymphocyte. Int. Immunopharmacol. 9, 97–102.

Kemeny, L., Michel, G., Dobozy, A., Ruzicka, T., 1994. Cytokine system as potential target for antipsoriatic therapy. Exp. Dermatol. 3, 1–8.

Kock, A., Schwarz, T., Kirnbauer, R., Urbanski, A., Perry, P., Ansel, J.C., Luger, T.A., 1990. Human keratinocytes are a source for tumor necrosis factor alpha: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. J. Exp. Med. 172, 1609–1614.

Krishnaswamy, M., Purushothaman, K.K., 1980. Antifertility properties of *Embelia ribes*: (Embelin). Indian J. Exp. Biol. 18, 1359–1360.

Kupper, T.S., Ballard, D.W., Chua, A.O., McGuire, J.S., Flood, P.M., Horowitz, M.C., Langdon, R., Lightfoot, L., Gubler, U., 1986. Human keratinocytes contain mRNA in distinguishable from monocyte interleukin 1 alpha and beta mRNA. Keratinocyte epidermal cell-derived thymocyte-activating factor is identical to interleukin 1. J. Exp. Med. 164, 2095–2100.

Nickoloff, B.J., Turka, L.A., 1993. Keratinocytes: key immunocytes of the integument. Am. J. Pathol. 143, 325–331.

Nickoloff, B.J., Karabin, G.D., Barker, J.N., Griffiths, C.E., Sarma, V., Mitra, R.S., Elder, J.T., Kunkel, S.L., Dixit, V.M., 1991. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. Am. J. Pathol. 138, 129–140.

Otuki, M.F., Vieira-Lima, F., Malheiros, A., Yunes, R.A., Calixto, J.B., 2005. Topical anti-inflammatory effects of the ether extract from *Protium kleinii* and alpha-amyrin pentacyclic triterpene. Eur. J. Pharmacol. 507, 253–259.

Stanley, P.L., Steiner, S., Havens, M., Tramposch, K.M., 1991. Mouse skin inflammation induced by multiple topical applications of 12-O-tetradecanoylphorbol-13-acetate. Skin Pharmacol. 4, 262–271.

Ueda, H., Yamazaki, C., Yamazaki, M., 2004. A hydroxyl group of flavonoids affects oral anti-inflammatory activity and inhibition of systemic tumor necrosis factor-alpha production. Biosci. Biotechnol. Biochem. 68, 119–125.