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Short Communication

Topical anti-inflammatory potential of Physalin E from *Physalis angulata* on experimental dermatitis in mice

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ABSTRACT

The anti-inflammatory effect of physalin E, a seco-steroid isolated from *Physalis angulata* L. was evaluated on acute and chronic models of dermatitis induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and oxazolone, respectively, in mouse ear. The changes in ear edema/thickness, production of pro-inflammatory cytokines (TNF-α and IFN-γ), myeloperoxidase (MPO) activity, and histological and immunohistochemical findings were analysed, as indicators of dermal inflammation. Similar to dexamethasone, topically applied Physalin E (0.125; 0.25 and 0.5 mg/ear) potently inhibited the TPA and oxazolone-induced dermatitis, leading to substantial reductions in ear edema/thickness, pro-inflammatory cytokines, and MPO activity. These effects were reversed by mifepristone, a steroid antagonist and confirmed by immunohistochemical and histopathological analysis. The data suggest that physalin E may be a potent and topically effective anti-inflammatory agent useful to treat the acute and chronic skin inflammatory conditions.

Introduction

Physalin E (Fig. 1) is a naturally occurring seco-steroid isolated from the stems and aerial parts of *Physalis angulata* L. (Solanaceae) (Bastos et al., 2008). Extracts/infusions from this plant are popularly used to treat inflammatory and painful conditions like sore throat, rheumatism, hepatitis and cervicitis (Bastos et al., 2008). The anti-inflammatory and immunomodulatory effects of plant-derived physalins B, D, F and G, but not of physalin E have been well documented (Soares et al., 2003; Vieira et al., 2005; Jacobo-Herrera et al., 2006; Magalhães et al., 2006; Damu et al., 2007; Guimarães et al., 2009).

The prevalence of atopic dermatitis and allergic or irritant contact dermatitis has been increasing significantly in the general population, causing considerable economic costs and decreased quality of life (Pónyai et al., 2007). Studies reveal that a large percentage of patients use some form of complementary and alternative medicine for the treatment of atopic and contact dermatitis, which include herbal remedies (Simpson et al., 2003; Nicolaou and Johnston, 2004). Recently, Guimarães et al., 2009 have shown that topical treatment with physalins purified from *P. angulata* reduce the skin lesions in cutaneous leischmaniasis. Physalin E, being a seco-steroid, structurally related to other physalins, an anti-inflammatory action is expected. Thus, this study was aimed to evaluate its topical anti-inflammatory potential, using the TPA and the oxazolone models of cutaneous dermatitis, commonly employed to test the compounds efficacy against contact/atopic dermatitis (Zhang and Tinkle, 2000; Murakawa et al., 2006).

Materials and methods

Plant material, extraction and isolation of Physalin E

The aerial parts of *Physalis angulata* were collected from Pentecoste County, Ceará, Brazil, after its identification by Prof Edson P. Nunes. A voucher sample (No. 33.576) has been deposited at the Herbarium Prisco Bezerra of this University. Air-dried and grounded plant material (4.3 kg) was percolated with hexane (2 x 10 l) followed by EtOH (2 x 10 l) at room temperature. The hexane and EtOH extracts were filtered and concentrated under reduced pressure to yield 1.5 g and 70.0 g of crude extracts, respectively. The EtOH extract was fractioned over

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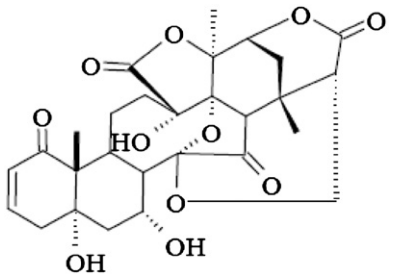

Fig. 1. Structure of physalin E.

silica gel using hexane, CH_2Cl_2, EtOAc and MeOH as eluents. The EtOAc fraction (25 g) was chromatographed over silica gel using increasing amounts of hexane-EtOAc and EtOAc to obtain 9 fractions. Fraction 3 (4.6 g), obtained by elution with hexane-EtOAc (3:7), was rechromatographed over silica gel to afford a white precipitate by elution with CH_2Cl_2-EtOAc (4:6). This material was recrystallized in acetone to yield a pure compound (**1**), which was identified as physalin E (1.2 g, mp 302-305 °C). The ^1H and ^{13}C NMR data are in agreement with the literature (Row et al., 1978; Magalhães et al., 2006).

Drugs

12-O-tetradecanoyl-phorbol-13-acetate (TPA), oxazolone, dexamethasone, hexadecyltrimethylammonium bromide, *o*-dianisidine dihydrochloride, hydrogen peroxide, formaldehyde, Tween 80 were from Sigma Chemical Co. (St. Louis, MO, U.S.A).

Animals

Male Swiss mice (20-25 g) obtained from the central Animal House of this University, were maintained in propylene cages, under standard conditions (24 ± 2 °C; 12 h light-12 h dark cycle, and relative humidity ~55%) with free access to food and water. Experimental protocols were approved by the Institutional Committee on Care and Use of Animals for experimentation in accordance with the ethical guidelines of National Institute of Health, Bethseda,U.S.A.

TPA-induced acute ear edema

Mice in groups (n=8) were treated on their right ear with physalin E (0.125; 0.25 and 0.5 mg/ear, 20 µl), vehicle (2% Tween 80, 20 µl) or dexamethasone (0.05 mg/ear, 20 µl) prior to topical application of 12-O-tetradecanoyl-phorbol-13-acetate (TPA, 2.5 µg/ear, 20 µl) (Recio et al., 2000). To verify the possible involvement of glucocorticoid receptor, animals were pre-treated with mifepristone (25 mg/kg, s.c.) 15 minutes prior to Physalin E (0.25 mg/ear) or dexamethasone. Ear thickness/swelling as an indicator of skin inflammation/edema was measured using a Digital Caliper (100.174B/DIGIMESS®) before TPA application and 4 h after. Ear punch biopsies (5 mm) were collected, weighed, and were fixed in 10% buffered formalin solution and processed for histological or immunohistochemical analyses.

Oxazolone-induced chronic dermatitis

Contact dermatitis was induced in mouse ear according to Shin et al. (2005). Groups of animals (n=8) were sensitized by application of 100 µl of 1.5% oxazolone to the abdomen. Then a total of 20 µl of 1% oxazolone was applied to the mouse ear, once in 3 days starting from day-7 after sensitization till the day-19. Ear thickness was measured using a digital caliper, 72 h after each application of the oxazolone. Physalin E, vehicle or dexamethasone were topically applied (at concentrations described earlier), 30 min before and 3 h after each application of oxazolone. Ear punch biopsies (5 mm) were collected, weighed, and processed for immunohistochemical study.

Assays

The degree of neutrophil infiltration was quantified by the measurement of ear myeloperoxidase (MPO) activity (Bradley et al., 1982). The ear tissue TNFα and INF-γ were measured with an ELISA kit (Quantikine®, R&D Systems, Minneapolis, USA).

Immunohistochemistry

In brief, paraffin sections were deparaffinized and hydrated, and then followed with antigen reparation. After blocking with endogenous peroxydase by 3% hydrogen peroxide, sections were

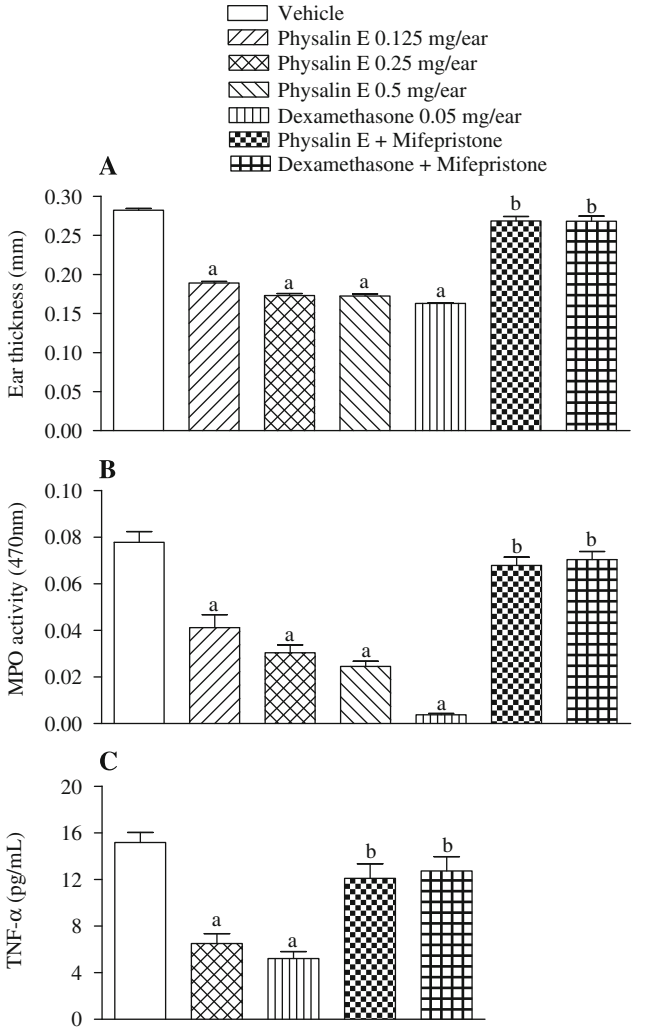

Fig. 2. Effect of topical application of Physalin E on TPA-induced acute ear edema (A), myeloperoxidase activity (B) and on the levels of TNFα (C), and its reversal by mifepristone. Physalin E and dexamethasone were applied immediately before TPA. Mifepristone (25 mg/kg, s.c.) was administered 15 minutes prior to physalin E (0.25 µg/ear) or dexamethasone. Each column represents mean ± SEM of 8 animals per group. $^a p < 0.05$ vs. vehicle group; $^b p < 0.05$ vs. respective control group.

incubated with primary rabbit anti-TNFα antibody (1:100) or anti-NF-κB p65 antibody (1:50). The slides were then incubated with biotinylated goat anti-rabbit secondary antibody (1:400) and incubated with avidin-biotin-horseradish peroxidase conjugate (Vectastain®ABC kit, Vector Laboratories, Burlingame, CA, USA). TNF-α and NF-κB were visualized with the chromogen 3,3 'diaminobenzidine (DAB) **a**nd counterstained with Harry's hematoxylin, mounted, and assessed by light microscopy.

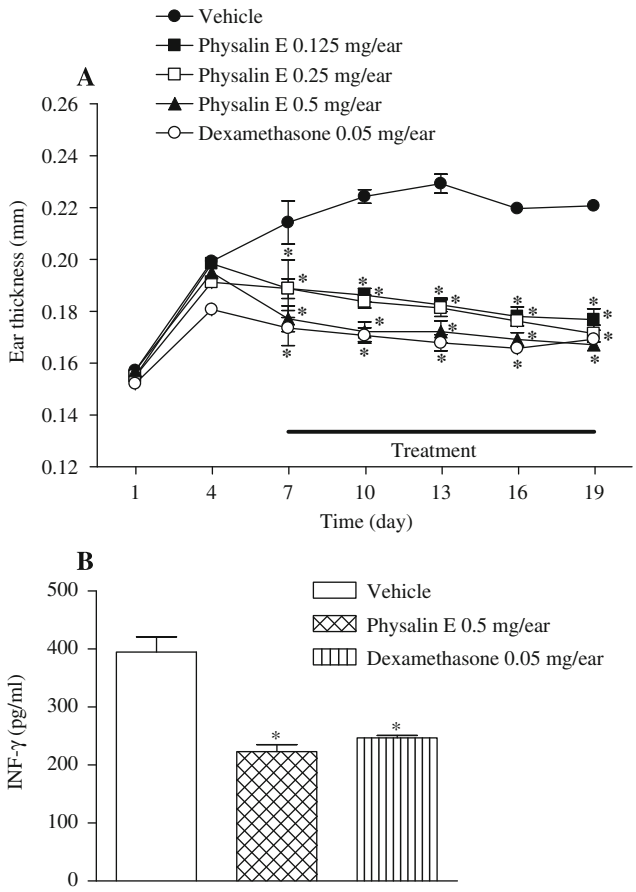

Fig. 3. The effect of topical physalin E and dexamethasone on the ear thickness (A) and levels of INF-γ in ear tissue homogenates (B). Each symbol and vertical bar represents the mean ± SEM for 8 animals. [a]$p < 0.05$ vs. vehicle group; [b]$p < 0.05$ vs. respective control group.

Statistical analysis

Results are expressed as mean ± SEM. Multiple comparisons were performed using one-way ANOVA followed by Student Newman Keul's test. Differences were considered significant at $p < 0.05$.

Results and discussion

Topical application of TPA is a valid model to screen compounds effective for potential topical anti-inflammatory therapy (Murakawa et al., 2006). In the present study, TPA induced an acute ear edema response associated with an increase in TNFα and myeloperoxidase (MPO) activity, at 4 h following its application (Fig. 2), effects consistent with the observations of Murakawa et al. (2006). Physalin E (0.125; 0.25 and 0.5 mg/ear) significantly reduced the ear edema response by 33%, 38% and 39%, and the MPO activity by 47%, 61% and 68%, respectively. Dexamethasone (0.05 mg/ear) also significantly suppressed the ear edema and MPO activity by 42% and 95%, respectively. Pretreatment with mifepristone, a glucocorticoid antagonist was able to abolish these effects of Physalin E and dexamethasone, suggesting a possible blockade of glucocorticoid receptor. Our histological analysis of the ear tissue clearly confirmed these findings (Fig. 5, upper panel).

Repeated application of oxazolone to sensitized mice caused an increase in ear thickness with a concomitant increase in tissue levels of IFN-γ. This increase was apparent from day 4 onwards and stayed all throughout the experimental period. Both physalin E and dexamethasone significantly suppressed the oxazolone-induced increase in ear thickness and INF-γ level (Fig. 3). Pretreatment with mifepristone could almost abolish these effects, suggesting their usefulness in skin diseases such as psoriasis or atopic dermatitis wherein IFN-γ plays a crucial role. Histological analysis of the ear tissue further confirmed these findings (Fig. 5, lower panel).

In the immunohistochemical study, control mice demonstrated very mild pattern of TNF-α and NF-κB immuno-staining (Fig. 4). On the other hand, while there was a high intensity immuno-staining for TNF-α and NF-κB in TPA untreated group,

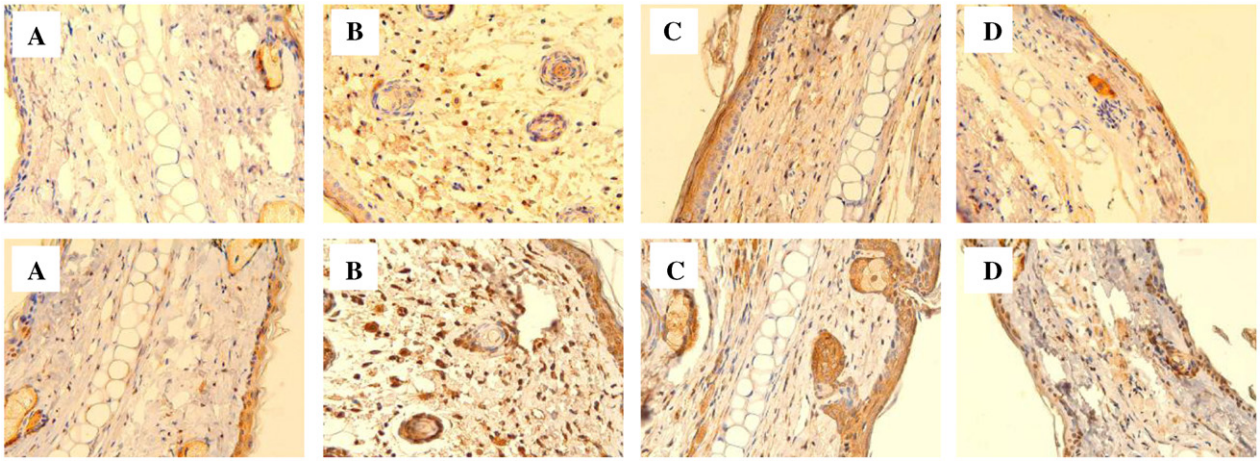

Fig. 4. Effect of Physalin E on TNFα immunoreactivity (upper panel) and NF-κB immunoreactivity (lower panel) in mice on TPA-induced acute dermatitis. A: normal control group showing basal immunostaining; B: vehicle + TPA showing increased dermis and epidermis immunostaining; C: physalin E (0.25 mg/ear) + TPA and D: dexamethasone (0.05 mg/ear) + TPA showing immunostaining comparable to control group.

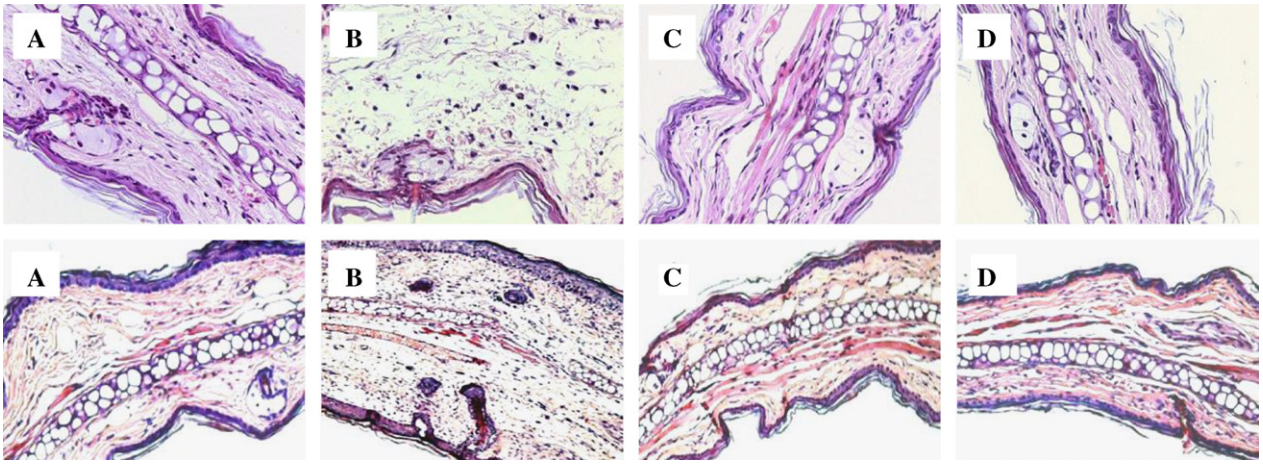

Fig. 5. Histological sections of mouse ear from TPA-treated (upper panel) or oxazolone-treated (lower panel). Vehicle-treated mice (A) showing normal.structure and TPA or oxazolone-treated mice (B) showing intense dermal edema, hemorrhage, inflammatory cell infiltration with the presence of mononuclear and polymorphonuclear cells. Treatment with physalin E (0.5 mg/ear) (C) or dexamethasone (0.05 mg/ear) (D) prevented the dermal edema, hemorrhage, and the inflammatory cell infiltration. Hematoxylin-eosin, x100.

mice pretreated with physalin E (0.25 mg/ear) or dexamethasone (0.05 mg/ear) showed far less immunostaining for both TNF-α and NF-κB. Physalins B and F that possess a double band and an epoxy ring between carbons 5 and 6 are said to function as modulators of NF-kB cascade (Jacobo-Herrera et al., 2006). Since physalin E has a similar structural epoxy ring, and inhibited NF-kB immunoreactivity, its anti-inflammatory action may, in part, involve the NF-kB modulation.

Physalin E being a seco-steroid is likely to interact with glucocorticoid receptor and exert an anti-inflammatory action. Pretreatment with glucocorticoid receptor antagonist mifepristone (RU 486) completely reversed the inhibitory effect of physalin E and dexamethasone on TPA-induced ear edema, increase in MPO activity and on TNFα levels. Our results are in agreement with the findings of Vieira et al. (2005) who described the mifepristone preventable anti-inflammatory action of physalins B and F in the ischemia/reperfusion model. The glucocorticoids involve actions on circulating cellular and cytokine mediators of inflammation as well as on the peripheral vasculature. Unlike physalins B, D, and F, which exhibit both anti-inflammatory and cytotoxicity effects (Damu et al., 2007), physalin E is free from cytotoxicity (Magalhães et al., 2006) and therefore may have an advantage over currently used therapeutic agents against inflammatory dermatoses.

Psoriasis and atopic dermatitis are chronic and relapsing inflammatory diseases of the skin associated with various immunologic abnormalities (Bowcock and Cookson, 2004). Two universal features of psoriasis are epidermal hyperplasia and inflammation and drugs that inhibit these events are useful to treat psoriasis. The results of this investigation provide the first time evidence of anti-proliferative and anti-inflammatory actions of physalin E, suggesting its usefulness to combat inflammatory dermatoses, including psoriasis and atopic dermatitis. However, a detailed study is warranted on its safety pharmacology.

References and further reading may be available for this article. To view references and further reading you must *purchase* this article.

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