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Lupeol as anti-inflammatory compound tested on Balb/c - TPA Model

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Abstract

Lupeol, a pentacyclic triterpene present in many vegetable structures, is a biologically active compound that has been reported to possess a number of pharmacological properties in the in vivo and in vitro studies. In this study, we investigated the antiinflammatory activities of lupeol on 12-O-tetradecanoylphorbol-13-acetate (TPA) induced inflammatory processes on mouse model - skin and ear. The inflammation evolution was tested using Mexameter, Tewameter and skin pH-meter and by histological evaluation.

Keywords: anti-inflammatory activities, lupeol, TPA, mouse model

1. Introduction

Over the past decades, 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 [1-3]. Topical corticosteroids have been the first-choice therapy for treatment of these inflammatory skin diseases such as eczema, atopic and seborrheic dermatitis, and psoriasis. While effective in many patients, this form of therapy carry the concern of local and systemic adverse effects and may induce skin atrophy, especially after long-term use [4, 5].

Triterpenoids represent a very large class of plant secondary metabolites which have been demonstrated to exhibit a variety of biological activities including anti-inflammatory activities [6-8].

Lupeol, a pentacyclic triterpene, is a biologically active constituent that has received much attention due to its wide spectrum of medicinal properties that include strong antiinflammatory effects [9-11].

Lupeol was investigated for its antiinflammatory activity under *in vitro* and *in vivo* conditions [12-16]. Using multiple different inflammation-associated mouse models, several studies led to the idea that the anti-inflammatory potential of lupeol is very important [17-22]. Lupeol application with therapeutic intention has been shown to diminish the inflammation in mouse models of arthritis and bronchial asthma [23]. Comparative studies performed in the case of inflammation rodent models revealed that the antiinflammatory activity of lupeol is higher than indomethacin, dexamethasone or various anti-inflammatory phytochemicals [23-25].

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The studies performed until now revealed an undeniable truth that the efficacy of lupeol as an anti-inflammatory agent is caused to its potential to act on multiple molecular targets associated with inflammation process [19, 20, 25-28]. Lupeol is considered as a modulator of the expression level of several inflammation associated molecules such as soybean 15-lipoxygenase, tumor necrosis factor α (TNFα), Interleukin β (ILβ), prostaglandin E2 (PGE2), cytokines (IL-2, IL-4, IL-5, IL- 6, IL-13, IFN-γ-Th1), myeloperoxidase, macrophages and T-lymphocytes [29, 30].

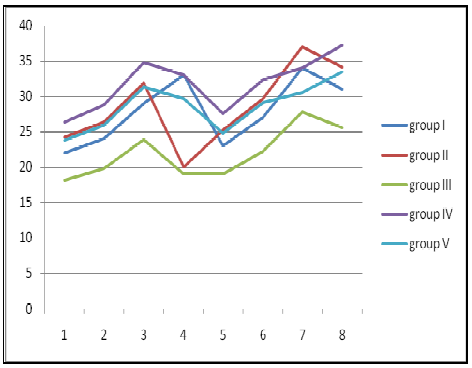
Aim and objectives. In the present study, we investigated the antiinflammatory activities of lupeol on 12-O-tetradecanoylphorbol-13-acetate (TPA) induced inflammatory processes on mouse model -skin and ear.

2. Materials and methods

The work procedure followed all National Institute of Animal Health (NIAH) rules: animals were maintained during the experiment in standard conditions: 12 hours light-dark cycle, food and water *ad libidum*, temperature 24 ºC, humidity above 55 %. It were used 15 BALB/c mice which were divided into five groups: one blank (I), one with solvent only (II), one with TPA applied on skin (III) and one with TPA applied on ear (IV) and a treated group (V). Inflammation was induced in both ears of mice by the topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces. Thirty minutes after the application of TPA, the inner and outer surface of each ear was treated with 2 mg of lupeol. The inflammation evolution was tested using Mexameter, Tewameter and skin pH-meter and by histological evaluation. Mice were killed after 24 hours by cervical dislocation and ears were collected. Ears were weight and histological analyzed. For the histological analyze, tissue samples (skin) were fixed in 10 % formalin solution and were embedded in paraffin and cut at 4 microns. Finally after deparaffinized the samples were stained with H&E (hematoxylin-eosin) and microscopically analyzed.

3. Results and Discussion

The treatment lasted four weeks. In every week, we made topical applications and measurements twice. The data collected from the ear are presented in figure 1, a-d, and those from the skin are presented in figure 2, a-d.



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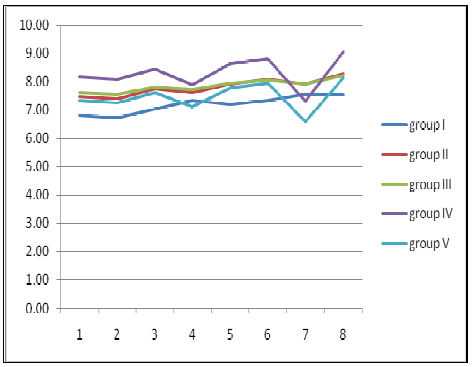

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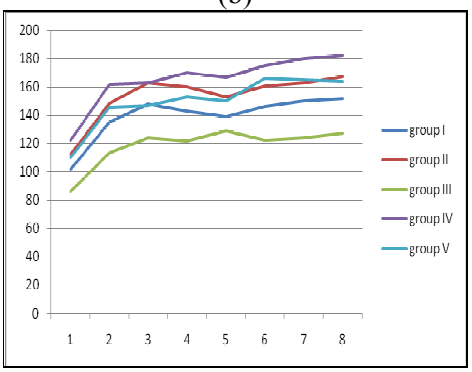

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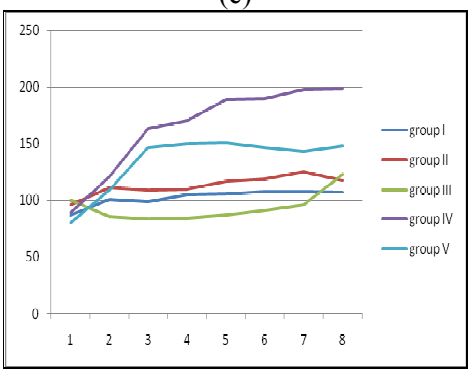
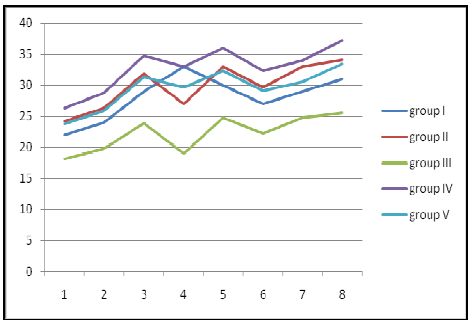
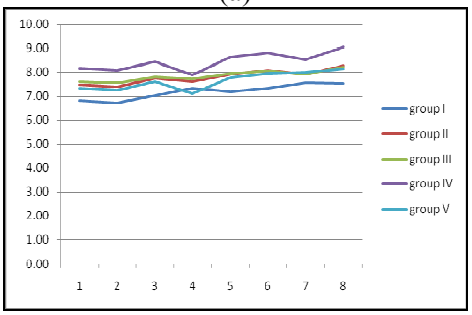


(d)

Figure 1. (a) Tewameter; (b) pH-meter; (c) melanin; (d) erythem values for ears



(a)



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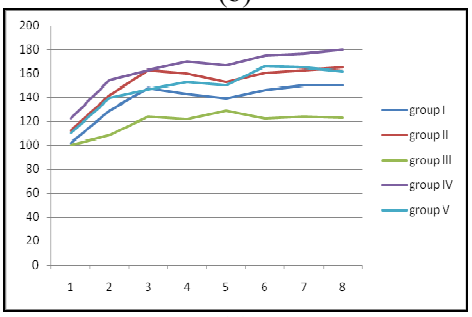

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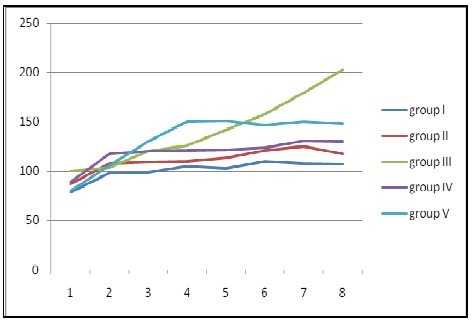

(d)

Figure 2. (a) Tewameter; (b) pH-meter; (c) melanin; (d) erythem values for skin

In the Tewameter and pH-meter determinations it were not recorded a linear increase or decrease or other curved continuously shape. So, we can say that the treatment not affect these values. The melanin values have an easier increase in all groups, but the important part of this study is the differences between the erythem evolutions. The erythem values have a significant increase in TPA treatment group and an easier increase for the last group (lupeol treatment).

Mice were killed by cervical dislocation and ears were collected. Ears were weighed. The average weights of the ears are: 0.041 g group I; 0.047 g group II; 0.043 g group III; 0.101 g group IV; 0.064 g group V. Macroscopically one can observe the difference regarding the size between ears depending of treatment. Increased size was due to the edema cause by inflammation.

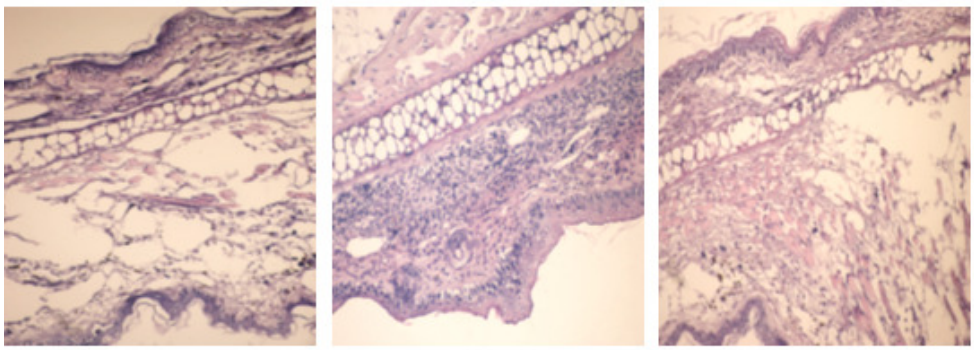

Figure 3. Morphopathological aspects of an ear in (a) group II - discrete edema and inflammatory infiltrate; (b) group IV- subepithelial massive inflammation, epithelial dysplasia; (c) group V - subepithelial edema, collagenization and discrete inflammation

After ears were weighed, morphopathological analyze were made. Results from the morphopathological analyze can be seen in figure 3, a-c. Obtained data suggest that topical application of 2 µg TPA dissolved in 20 µL of acetone to both the inner and outer ear surfaces is a good, reproducible model for initiating acute inflammation in animal model. It can be observed morphopathological aspects of an ear in the group II, epithelial tissue and cartilage with discrete under epithelial edema present because of the physical aggression at the moment of tissue collection.

The parameters analyzed showed an anti-inflammatory action of lupeol in external application both on skin and ear model. It reduced erythema and local inflammation clear detectable by non-invasive methods and histology evaluations.

The application of TPA to mouse skin leads to oedema, cellular infiltration and skin tumor promotion. It is also suggested the participation of arachidonate metabolites in the oedema formation caused by TPA, and TPA has been shown to induce PGE2 production from epidermal cells. We have now demonstrated that TPA induces PGE2 production by the cells of the ear, and PGE2 content increased with the same time course as oedema formation. Since cyclo-oxygenase inhibitors such as indomethacin, aspirin and naproxen have been shown to suppress TPA-induced ear oedema, PGE2 is presumably a mediator of TPA-induced oedema. [31].

Except for the cases that exhibited ulcers or erosions, repetitive treatment of skin with promoting doses of TPA produces a steady-state epidermal hyperplasia with an increased dermal cellularity. Most dermal cells were fibroblasts and endothelial cells, resulting in a very dense and hyper-vascularised dermis. It is interesting to note that most authors have focused on the hyperplasiogenic effects of TPA on the epidermis and have overlooked cell proliferation in dermis [32].

Lupeol has been extensively studied for its inhibitory effects on inflammation under in vitro and in animal models of inflammation [33]. A comprehensive study conducted by Fernandez et al. showed that topical application of Lupeol (0.5 and 1 mg/ear) alleviated 12-o-tetradecanoyl-phorbol acetate (TPA)-induced inflammation in an ear mouse model [20]. This study showed that topical application of Lupeol decreases myeloperoxidase levels, a neutrophilic specific marker, thus causing reduction in cell infiltration into inflamed tissues in mice. The anti-inflammatory potential of Lupeol could be assessed from the observation that Lupeol pretreatment significantly reduced prostaglandin E2 (PGE2) production in A23187-stimulated macrophages [20].

Several studies were carried out to understand the molecular mechanism through which Lupeol inhibits or abrogates the inflammatory processes under in vitro and in vivo situations and such studies provided several mechanistic facets of anti-inflammatory action of Lupeol. Lupeol was reported to modulate several molecules which directly or indirectly play a role in inflammatory process. Lupeol was shown to inhibit the activity of soybean lipoxygenase- 1 (15-sLO) with IC50 equal to 35 lM [34]. Lupeol treatment (10–100 lM) is also shown to decrease the generation of pro-inflammatory cytokines such as tumor necrosis factor a (TNFa and Interleukin b (ILb in lipopolysaccharide- treated macrophages [20]. Recent report by Yamashita et al. suggested that superoxide generation induced by arachidonic acid (AA) is suppressed by Lupeol in N-formyl-methionyl-leucyl-phenylalanine (fMLP)-treated human neutrophils [35].

4. Conclusion

The results of the present study demonstrate that lupeol is effective in combating inflammatory injury of mouse model and protective for skin and external regions that suffered local damages.

These compelling evidences suggest that the therapeutic usefulness of lupeol for inflammatory conditions is attractive and warrants further investigation.

Acknowledgements

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