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Immunomodulatory effect of thymoquinone on atopic dermatitis

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ABSTRACT

Background: Atopic dermatitis (AD) or atopic eczema is a skin disease characterized with itching, increased serum level of immunoglobulin E, and peripheral eosinophilia. Thymoquinone (TQ) is an important ingredient of *Nigella sativa* seeds having antioxidant and anti-inflammatory effects.

Objective: Present study investigates the immunomodulatory effects of Thymoquinone (TQ) in mice model of atopic dermatitis.

Methods: Ear pinnas of mice were sensitized and challenged with DNCB (2–4 di nitro chloro benzene) to induce AD-like lesions. The mice were then treated with TQ and tacrolimus, both orally and topically. Ear thickness and weight were measured along with gross changes. Total and differential leukocyte counts were measured in blood. Total serum IgE levels were measured by enzyme linked immunosorbent assay (ELISA). The mRNA expression levels of IL-4, IL-5, and IFN-γ in ear tissue were measured using reverse transcription polymerase chain reaction (RT-PCR).

Results: Both oral and topical thymoquinone showed the potential to improve atopic dermatitis by significantly reducing the inflammatory cells infiltration in the blood ($p < 0.001$) and improving the dermatitis score ($p < 0.001$). Significant reduction in ear thickness ($p < 0.001$) and IgE levels ($p < 0.001$) were also observed. TQ and tacrolimus also significantly attenuated mRNA expression levels of IL-4, IL-5 and IFN-γ ($p < 0.001$).

Conclusions & clinical relevance: Taken together, our results showed that oral and topical application of thymoquinone exerts immunomodulatory effects in animal model of atopic dermatitis, suggesting further studies and clinical trials to establish it as a candidate nutraceutical for the treatment of AD.

1. Introduction

Atopic dermatitis or atopic eczema is a continuous itchy state characterized by inflammation which generally starts in infancy and extends into adult life. The disease is genetically susceptible and its expressions are altered by environmental factors. The distinctive characteristics of AD are frequent occurrence, reverting and worsening of condition, a disrupted state of epidermal-barrier function that end up in skin dryness, and IgE -mediated changes to various food and environmental irritant substances (Abdel-Hamid, 2003). AD is considered as biphasic disorder and is also characterized by disequilibrium between Th1/Th2 lymphocytes (Agrawal et al., 2011). The reactions to irritants are elicited by Th2 immune response, while infections are elicited by Th1 immune responses (Romagnani, 2000). IL-4, IL-5, and IL-13 are representative Th2 type cytokines which stimulate the differentiation of Th2 cells and IgE generation by B cells. Whereas, IL-12 and IFN-γ are representative Th1 type cytokines that cause the differentiation of T cells into Th1 type cells (Horikawa et al., 2002).

There are different treatment options available to balance the disequilibrium state in AD, but the success rate is very low. The management does not completely relief AD, and is not free of adverse effects as well. Treatment over time is also affected by genetic variations in FLG and TSLP genotype (Chang et al., 2017).

Topical calcineurin inhibitors are chosen as second-line drugs in patients above 2 year age. They are used as a substitute to topical corticosteroids and are recommended for the limited time. They are also prescribed for off and on chronic treatment in moderate to severe cases of AD (Krakowski et al., 2008). Tacrolimus, a calcineurin inhibitor, is usually suggested to those patients who stop responding to steroids, or due to steroid induced atrophy and various adverse effects. It is an immunosuppressive drug and suppresses the production of associated cytokines (Yamamoto and Nishioka, 2003). The adverse effects

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noticed from the use of tacrolimus include burning sensation and itching on the application spot. Some studies also reported malignancy (i.e., skin cancer and lymphoma) its use (Leung and Bieber, 2003).

Nigella sativa is an attractive alternative for the treatment of allergic/atopic diseases (Badary et al., 2000). Its use in eczema has been accepted worldwide (Goreja, 2003). Many studies showed that the biological activity of *Nigella sativa* seeds were primarily associated with thymoquinone, an essential oil component of plant (Hajhashemi et al., 2004; Burits and Bucar, 2000; Gali-Muhtasib et al., 2006). TQ possesses various antioxidant and anti-inflammatory properties. It is also known to reduce the incidence of arthritis (Budancamanak et al., 2006; Badary et al., 2003; Tekeoglu et al., 2007). It has exhibited positive effects in different clinical conditions including protection against skin inflammation (Kundu et al., 2013). The present study investigates the immunomodulatory effects of TQ on AD-like lesions using BALB/c mice model.

2. Material and methods

2.1. Animals

A total of 60 healthy female BALB/c mice, weighing 30–35 g, were divided into six groups and each group comprised of ten mice. The animals were kept under standard environmental conditions in the Experimental Research Laboratory of UHS Lahore at controlled room temperature (23 ± 2 °C), humidity (50 ± 5%), and light/darkness conditions. The animals were fed on standard diet and water *ad libitum*. The study was approved by Ethical Review Committee (Pharma/UHS/204) and Advanced Studies and Research Board of University of Health Sciences, Lahore.

2.2. Experimental design

Animals were numbered from 1 to 60 and assigned randomly to groups I, II, III, IV, V, and VI. The groups were then designated as control, diseased, and experimental groups.

2.3. Drugs and chemicals

Tacrolimus was provided from CCL Pharmaceuticals (Lahore, Pakistan). DNCB and thymoquinone (≥98%) were purchased from Sigma Aldrich (US).

2.4. Induction of AD-like skin lesions in the ear

DNCB was used to induce AD-like skin lesions according to the method of Chan et al. (2013) with modification. To induce AD-like skin lesions in diseased and treatment groups, mice were sensitized by applying 20 µl of 0.5% DNCB in acetone / olive oil (3:1) on pinna of each ear at days 1–3. Two weeks later, mice in diseased and treatment groups were challenged by applying 20 µl of 1% DNCB on pinna of each ear at days 14, 17, 20, 23, 26 and 29. These groups were also treated with TQ and tacrolimus during days 14-29.

2.5. Group I (control)

The mice were sham-sensitized and challenged epicutaneously on pinna of each ear with acetone/olive oil (3:1). Ethanol/distilled water (3 ml) in 1:2 ratio was also given orally, one hour before the challenge.

2.6. Group II (diseased)

The mice were sensitized at days 1–3 and then six times epicutaneously challenged starting from day 14 to day 29 on pinna of each ear with DNCB.

2.7. Group III (experimental I/ oral tacrolimus)

The mice were treated with tacrolimus (30 mg/kg body weight) orally dissolved in 3 ml ethanol/distilled water (1:2), one hour before the challenge (Meingassner et al., 2003).

2.8. Group IV (experimental II/ topical tacrolimus)

The mice were treated with tacrolimus (1%) topically dissolved in ethanol (0.2 mg of tacrolimus was dissolved in 0.02 ml of ethanol), one hour before challenge (Hirotaka et al., 2010).

2.9. Group v (experimental III/oral thymoquinone)

The mice were treated with TQ (10 mg/kg body weight) orally, dissolved in 3 ml ethanol/distilled water (1:2), one hour before the challenge (Ammar et al., 2011).

2.10. Group VI (experimental IV/ topical thymoquinone)

The mice were treated with TQ (5 µmol in 0.2 ml acetone), one hour before the challenge. The picked dose was based on the results of Kundu et al. (2013), who used same dose of TQ to elucidate the molecular mechanism of anti-inflammatory activity TQ in mouse skin.

All animals were sacrificed 24 h after the last treatment and challenge at day 30 under light ether anesthesia. Blood sample was collected to measure TLC and DLC. Serum was stored at −20 °C to measure IgE levels. Ears were excised to measure weight and determine mRNA expression level of cytokines.

2.11. Assessment of the ear clinical score/ macroscopic evaluation of ear skin lesions

Clinical symptoms of each mouse were evaluated at day 0, 14 and 29. Briefly, erythema, edema, excoriation and dryness on the ear surface were scored as 0 (not visible), 1 (mild), 2 (moderate) and 3 (severe). Scoring was performed by two independent observers, and the final score was taken as an average for each group (Matsuoka et al., 2003). Skin status of each group was also photographed before and after the treatment.

2.12. Ear thickness

Right and left ear thickness was measured at day 0, 14 and 29, with a dial thickness gauge, before challenge and treatment. For each mouse, both right and left ears were measured, and an average was calculated.

2.13. Analysis of hematological parameters

TLC and DLC in blood were determined by microscopic evaluation. The blood was collected 24 h after the last treatment and challenge in EDTA vacutainer via cardiac puncture at day 30. For TLC, the diluted blood was stained with gentian violet and glacial acetic acid. Then the blood was transferred to Neubauer counting chamber and cells were counted under low power microscope. For DLC, diluted blood was stained with Wright-Giemsa stain and counted under microscope.

2.14. Ear weight

The excised right and left ear was weighed soon after they were sacrificed. The difference of weight represented the severity of inflammation.

2.15. Determination of IgE levels by ELISA

For determination of IgE levels, blood was collected in serum