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Review Article

Case Report

Original Article

Moisturizing Nanoemulgel of Turmeric (*Curcuma longa*) Rhizome Extract Ameliorates Atopic Dermatitis-like Skin Lesions in Mice Model through Thymic Stromal Lymphopoietin, Interleukin-13, and Interleukin-17

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ABSTRACT

Introduction: Various moisturizers have been developed for mild and moderate atopic dermatitis (AD). Turmeric (*Curcuma longa*), containing a potent anti-inflammatory substance, is one of the promising ingredient for moisturizers. By formulating turmeric into nanoemulgel preparation, cutaneous absorption is enhanced. This study aims to determine the effect of 1% turmeric rhizome extract moisturizing nanoemulgel on thymic stromal lymphopoietin (TSLP), interleukin (IL)-13, IL-17 levels, histopathological feature, transepidermal water loss (TEWL) value, and dermatitis score in an AD-like mouse model induced by dinitrochlorobenzene (DNCB). **Methods:** This study used 35 female BALB/C mice aged 6–8 weeks, weighing 20–30 g. Mice were divided into the treatment group (DNCB and 1% turmeric rhizome extract moisturizing nanoemulgel) and the control group (DNCB and vehicle gel). The DNCB application was carried out twice a week, from day 14 to day 29. On day 30, skin tissue samples were taken to examine TSLP, IL-13, IL-17 levels, and histopathological examination. **Results:** The treatment group showed lower TSLP, IL-13, and skin tissue IL-17 levels than the control group ($P < 0.05$). In addition, applying 1% turmeric rhizome extract, moisturizing nanoemulgel improved the treatment group's dermatitis score and histopathological features compared to the control group ($P < 0.05$). The 1% turmeric extract moisturizing nanoemulgel decreased the TEWL but was statistically insignificant compared to the control group ($P > 0.05$). **Conclusions:** Applying 1% turmeric rhizome extract moisturizing nanoemulgel ameliorates AD-like skin lesions by decreasing TSLP, IL-13, and IL-17 levels in the DNCB-induced BALB/c mouse model.

Keywords: *Atopic dermatitis like, moisturizer, nanoemulgel, turmeric extract*

Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease characterized by skin barrier defects and immune system dysregulation. The skin barrier disruption is the initial step in developing AD because it can increase transepidermal water loss (TEWL) and skin acidity (pH), which will activate serine proteases[1] via a protease-activated receptor-2 (PAR2).[2] Stimulation of PAR2 on keratinocyte cells will increase thymic stromal lymphopoietin (TSLP) production,[1,3] which will induce inflammation through T helper (Th) 2 cytokines such as interleukin (IL) 4, IL-5, and IL-13, and immunoglobulin (Ig) E formation from plasma cells.[3]

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Disruption of the skin barrier will also activate the inflammasome, which controls the activation of IL-1β and IL-1α, resulting in T helper (Th) 1 or Th17-mediated inflammation.[4] The role of IL-17 produced by Th17 cells in AD is unknown; it is thought to be an inducer that moves Th17 cells to Th2 cells in early skin inflammation.[5]

Various therapeutic guidelines consistently recommend moisturizers for skin barrier maintenance and prevention of AD.[6] Recently, anti-inflammatory agents are often added to moisturizers to improve skin barrier function and control skin dryness to reduce mild and moderate AD symptoms.[7] One of the anti-inflammatory ingredients that can be added to a moisturizer is turmeric (*Curcuma longa*).

Curcumin (CUR), the main active ingredient of turmeric, is a nonenzymatic natural antioxidant from the polyphenol group.[8] The limitation of using topical turmeric preparations is the poor solubility of CUR in water and low absorption in the skin,[9] so it is essential to be made in nanosystem preparation. Nanosystem preparation increases the deliverance of drug molecules to specific targets and minimizes side effects.[10] The 1% CUR nanoemulgel in squamous cell carcinoma cases showed that the drug release to the skin was significantly higher with lower toxic effects.[11] The topical application of 1% CUR nanoemulgel was effective in carrageenan-induced mouse models.[12]

Animal models play an essential role in understanding the pathogenesis of AD and finding effective drugs; hence, the mouse model is gaining wider acceptance.[13] The dinitrochlorobenzene (DNCB) application in BALB/c mice represents AD in humans because it shows symptoms similar to AD in humans.[14] The topical application of turmeric rhizome extract is not beneficial in managing AD. However, the preparation of nanoemulgel is expected to overcome the absorption limitation of topical turmeric rhizome extract. According to its anti-inflammatory potential, moisturizing nanoemulgel turmeric rhizome extract is expected to improve the skin barrier, reduce AD symptoms, and minimize topical steroids. To the researcher's knowledge, the mechanism of anti-inflammatory moisturizer nanoemulgel turmeric rhizome extract in a mouse model exposed to DNCB has never been reported. Based on the data above, more research is needed to investigate further the effect of applying 1% turmeric rhizome extract moisturizing nanoemulgel on AD-like mice models exposed to DNCB.

Methods

This research was conducted at the Integrated Biomedical Laboratory, Department of Drug and Experimental Animal Development, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia, after obtaining approval from the research ethics committee of the Faculty of Medicine, Udayana University/Sanglah Hospital Denpasar no: 1275/UN 14.2.2.VII.14/LT/2021.

Turmeric rhizome was obtained from the Technical Implementation Unit of Materia Medika Batu, Malang. The moisturizing nanoemulgel of turmeric rhizome extract and vehicle gel was made at the Mahaganesha College of Pharmacy, Denpasar. Histopathological preparations with Hematoxylin and Eosin (HE) staining were made at the Anatomical Pathology Laboratory of the Sanglah Central General Hospital. The TSLP, IL-13, and IL-17 levels examination using the sandwich enzyme-linked immunosorbent assay (ELISA) method at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Udayana University.

Clinical parameters (dermatitis score and TEWL value) and laboratory (histopathological levels, skin tissue TSLP, IL-13, and IL-17) are assessed in this study. Clinical dermatitis is assessed by a scoring system, including redness (erythema), swelling (edema), scarring (dryness), erosion, or excoriation. The value of each parameter is 0–3, namely a score of 0 (no symptoms), 1 (mild: <20%), 2 (moderate: 20%–60%), and 3 (severe >60%), with a maximum score 12. The TEWL value was assessed at baseline, after DNCB application, and at the end of the study, with the GPSkin Barrier Pro® device.

The histopathological parameters of dermatitis in this study included the epidermal thickness and the number of eosinophil cells in five fields of view. Epidermal thickness was measured from the stratum corneum to the stratum basalis using a microscope equipped with a ruler. The eosinophil cells were counted with an objective magnification of forty times.

Research procedures

This study is an experimental study using female BALB/c mice, aged 6–8 weeks, weighing 20–30 g, with a randomized posttest-only control group design to determine the effect of 1% turmeric rhizome extract moisturizing nanoemulgel compared to controls. BALB/c mice that met the inclusion and exclusion criteria were given sterile food and water and placed in cages with cycles every 12 h in light and 12 h in dark conditions. The room temperature was set at 23°C ± 3°C, and the humidity was 55% ± 15%. Mice were adapted for a week and weighed. The drop-out criteria were BALB/c mice that died during the study.

Manufacture of dinitrochlorobenzene sensitizer material

One percent DNCB solution was prepared by dissolving 100 mg DNCB powder in 10 mL acetone: olive

oil (3:1; v/v), while 0.5% DNCB was prepared by dissolving 50 mg DNCB powder in 10 mL acetone: olive oil (3:1; v/v).

The moisturizing nanoemulgel of turmeric rhizome extract preparation

The method for making nanoemulsion of turmeric rhizome extract is called a self-nano emulsifying drug delivery system (SNEDDS) with a simplex lattice design (SLD). The composition of SNEDDS includes components of sunflower oil, surfactant tween 80, and cosurfactant polyethylene glycol (PEG) 400, with a ratio of 1:8:1. Turmeric rhizome extract nanoemulgel was made using carbopol 934 as a gelling agent.

Treatment protocols

Samples were randomized into two treatment groups: the control group given DNCB and the vehicle gel application (n = 18); the treatment group was given DNCB and 1% turmeric rhizome extract moisturizing nanoemulgel (n = 18 mice). The day before the treatment, the dorsal skin of the mice was shaved. The dorsal skin of BALB/c mice was sensitized with 200 µl DNCB 0.5% (3:1, v/v) with a size of 1 cm × 1 cm once a day for three days (sensitization phase). BALB/c mice were re-exposed (rechallenge) with 20 µL DNCB 1% (3:1, v/v) in both ears and 100 µL DNCB 1% on the dorsal skin on days 14, 17, 20, 23, 26, and 29. On the 30th day, they were sacrificed using ketamine. The moisturizing nanoemulgel of 1% turmeric rhizome extract and vehicle gel was applied to the sensitized dorsal skin on the 14th day. Applications of 1% turmeric rhizome extract moisturizing nanoemulgel in treatment group and vehicle gel in control grup were carried out two times a day for 2 weeks. If DNCB and 1% turmeric rhizome extract moisturizing nanoemulgel and vehicle gel occurred on the same day, then the turmeric rhizome extract moisturizing nanoemulgel and vehicle gel was carried out 4 h before DNCB administration. On the last day of treatment (30th day), mice were sacrificed for examination – the treatment protocol is shown in Figure 1.

Enzyme-linked immunosorbent assay methods

Examination of skin tissue TSLP, IL-13, and IL-17 levels using an ELISA kit from the BT Lab Bioassay Technology Laboratory, Jiaxing, Zhejiang, China, with the following protocol: (1) Add 50 µl of standard solution and 50 µl of standard diluents (as blank) into each well; (2) put 40 µl of sample solution into each well; (3) add 10 µl of anti-TSLP/anti-IL-13/anti-IL-17 antibody to each sample well; (4) add 50 µl of Streptavidin-HRP into each of the standard and sample wells, homogenize briefly by tapping by hand; (5) cover and incubate at 37°C for 60 min; (6) open the seal cover, drain the liquid well in the sink, dry it with the well facing down and tap on a tissue towel; (7) fill the 350 µl wash buffer, which is left for 30 s; (8) repeat procedure no. 79 (four times washes in total); (9) add 50 µl of chromogen A and add it to all wells; (10) add 50µl of chromogen B and put it into all wells in a dark room; (11) homogenized, incubated for ten minutes at 37°C in a dark room; (12) add 50 µl stop solution and add to all wells; (13) the absorbance is measured at a wavelength of 450 nm.

Data analysis

The baseline characteristic data, including body weight, TEWL values, and the dorsal skin condition after shaving (erythema, dryness, and erosion), expressed in mean ± standard deviation. Skin tissue TSLP levels, skin tissue IL-13 levels, clinical dermatitis, epidermal thickness, and the number of eosinophil cells on histopathological examination were not normally distributed and not homogeneous ($P < 0.05$) using the Mann–Whitney test. Data on skin tissue IL-17 levels, TEWL values normal distributed and homogeneous ($P > 0.05$), were analyzed by independent t-test.

Results

Characteristics of nanoemulgel turmeric extracts

Turmeric rhizome extract nanoemulgel has good characteristics in terms of adhesion (1.04 s), dispersibility (6.18 cm), pH (6.03), viscosity (26),

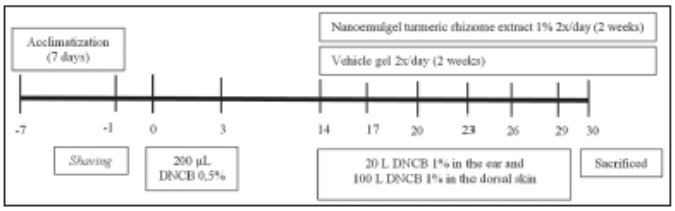

Figure 1: Treatment protocol in this study

particle size (25,193 mm), polydispersity index (0.582), and zeta potential (−58.8 mV), which meet the nanoemulgel criteria.

Characteristics of research subjects

The initial subjects of the study were 36 BALB/c mice, divided into the treatment group and the control group. In the control group, 1 BALB/c mouse dropped out because of death, so the number analyzed in this study was 35. There was no difference in body weight between the treatment group (26.00 ± 2.17 g) and the control group (26.24 ± 3.13 g) (P = 0.799). The TEWL values in the treatment group (10.11 ± 4.56 g/h/m^2) and the control group (11.19 ± 5.44 g/h/m^2) were not statistically deferent (P = 0.530). The condition of the dorsal skin after shaving in the treatment group (0.889 ± 0.32) and the control group (0.765 ± 0.44) was also not statistically different (P = 0.337).

Effect of 1% turmeric rhizome extract moisturizing nanoemulgel application on thymic stromal lymphopoietin, interleukin-13, and interleukin-17 of skin tissue levels

The effect of 1% turmeric rhizome extract moisturizing nanoemulgel on TSLP, IL-13, and IL-17 skin tissue levels is presented in Table 1. Based on Table 1, it was found that skin tissue TSLP levels, skin tissue IL-13 levels, and skin tissue IL-17 levels in the treatment group were significantly lower than in the control group (P < 0.05).

Effect of 1% turmeric rhizome extracts moisturizing nanoemulgel application on dermatitis severity on histopathological examination

The histopathological results of the treatment group show milder epidermal hyperplasia, spongiosis, and a dermal infiltrate consisting of lymphocytes, neutrophils, and eosinophils, which were lighter than in the control group [Figure 2]. Histopathological appearances show overlapping of acute and chronic phase dermatitis. The effect of 1% turmeric rhizome extract moisturizing nanoemulgel on histopathologic parameters is presented in Table 1. Based on Table 1, it was found that the epidermal thickness and eosinophil count in the treatment group were significantly lower than in the control group (P < 0.05).

Effect of 1% turmeric rhizome extracts moisturizing nanoemulgel on transepidermal water loss value

The DNCB application increased the mean value of TEWL in the treatment group (38.73 ± 13.52 g/h/m^2) and the control group (38.96 ± 11.24), which were not statistically different (P = 0.676). The application of moisturizer decreased the mean value of TEWL in the treatment groups (22.66 ± 10.32 g/h/m^2) and the control group (28.44 ± 8.82 g/h/m^2), which were not statistically different (P = 0.084).

Effect of 1% turmeric rhizome extracts moisturizing nanoemulgel on dermatitis score

Skin barrier dysfunction caused by DNCB application was assessed with a dermatitis score (erythema, edema, dryness, and erosion or excoriation). The median dermatitis score after DNCB application in the treatment group was 5.00 (1.25), while in the control group, it was 5.00 (1.00), which was not statistically different (P = 0.126). The moisturizing nanoemulgel of 1% turmeric rhizome extract caused the median score of dermatitis in the treatment group (3.50 [2.25]) to be significantly lower than the control group (4.00 [1]) in the AD-like mouse model induced by DNCB (P < 0.05). The effect of DNCB administration and moisturizer on clinical dermatitis is presented in Figure 3.

Multivariate logistic regression analysis was performed to analyze the independent relationship and degree of influence of the TSLP, IL-17, IL-13, and TEWL

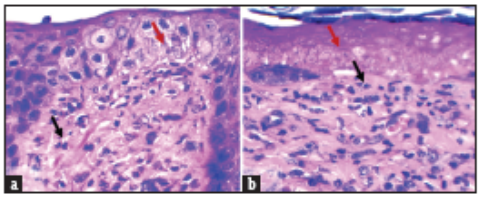

Figure 2: Histopathological features comparison between the treatment groups (a) and control groups (b) with Hematoxylin Eosin staining (×400). Inflammatory cell infiltrates in the dermis (black arrows) and spongiosis (red arrows) in treatment groups were lighter compared to control groups

Table 1: Comparison of laboratory results in the treatment group and the control group

Variable	Groups		Mean difference	95% CI (minimum–maximum)	P
	Treatment (n=18)	Control (n=17)			
TSLP levels (pg/ml), median (IQR)	205.70 (28.72)	211.27 (160.02)	−89.56	−173.76–−5.36	0.038*,a
IL-13 levels (pg/ml), median (IQR)	144.61 (61.19)	164.05 (34.64)	−30.21	−59.53–−0.89	0.032*,a
IL-17 levels (pg/ml), mean±SD	194.79±62.27	244.31±70.23	−49.52	−95.11–−3.94	0.034*,b
Epidermal thickness (mm), median (IQR)	61.28 (45.81)	83.60 (55.97)	−23.01	−45.69–−0.33	0.045*,a
Eosinophil count (cells), median (IQR)	0.40 (0.40)	0.80 (0.60)	−0.52	0.82–−0.22	<0.001*,a

*Statistically significant if P<0.05, [a]Analysis using the Mann–Whitney U [test, [b]Analysis using independent t-test. IQR: Interquartile range, SD: Standard deviation, 95% CI: 95% confidence interval, TSLP: Thymic stromal lymphopoietin, IL: Interleukin

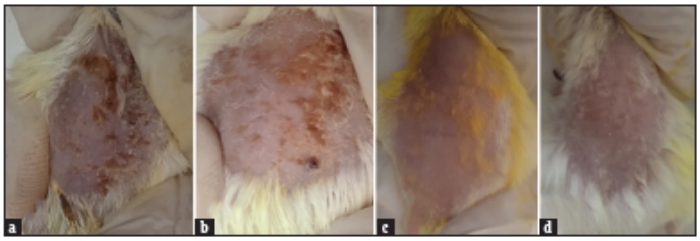

Figure 3: Clinical dermatitis after DNCB application in the treatment group (a) was not significantly different from the control group (b). Clinical dermatitis after application of DNCB and moisturizer in the treatment group (c) was considerably milder than in the control group (d). DNCB: Dinitrochlorobenzene

Table 2: The logistic regression analysis result between thymic stromal lymphopoietin, interleukin-17, and transepidermal water loss with dermatitis scores

Variable[a]	*B*	SE	*P*	Exp (B)	95% CI	
					Lower limit	Upper limit
TSLP	0.022	0.010	0.025*	1.022	1.003	1.041
IL-13	0.019	0.011	0.039*	1.019	1.010	1.042
IL-17	0.015	0.008	0.038*	1.015	1.008	1.031
TEWL	0.010	0.048	0.047*	1.010	1.007	1.053
Constant	−4.010	3.890	0.021*	0.018		

[a]Treatment variables are excluded from the equation because they are shown to be significant. TEWL: Transepidermal water loss, 95% CI: 95% confidence interval, TSLP: Thymic stromal lymphopoietin, IL: Interleukin, SE: Standard error. *Significant if *P*-value <0.005

with dermatitis score as a clinical outcome, presented in Table 2. The logistic regression analysis showed that changes in TSLP, IL-13, IL-17, and TEWL were significantly associated with changes in dermatitis score (score 4 vs. score <4).

Discussion

TSLP is an upstream cytokine that induces Th2 cells (IL-4, IL-5, and IL-13) activation and triggers the formation of immunoglobulin (Ig) E from plasma cells.[3] Recent studies have concluded that IL-13 is a crucial peripheral Th2 cell cytokine in tissues, while IL-4 significantly affects lymph nodes.[15] IL-4 and IL-13 activate the STAT3 signaling pathway and interfere with keratinocyte differentiation causing skin barrier disruption. In contrast, IL-4 and IL-13 activation in STAT6 will cause an increase in chemoattractants that play a role in inflammation.[16]

The essential function of IL-17 is to induce local tissue inflammation through the upregulation of proinflammatory cytokines, chemokines, and neutrophil activator cells, which enable activated T cells to migrate through the extracellular matrix.[17] During the acute phase of AD, IL-6, IL-1β, and TGF-β will promote Th17 differentiation. Th17 cells will be mobilized to skin lesions, interacting with Th2 cells, keratinocytes, and AMP.[18] Nakajima *et al*. found that IL-17 deficiency weakens Th2 cell induction during the acute inflammatory phase, so it can be concluded that IL-17 plays a role in moving Th17 cells to Th2 cells in early skin inflammation.[5] IL-17 production is suppressed by IL-4 and IL-13, which explains the decreased Th17 response in AD patients.[19] Evidence suggests that the percentage of Tregs was increased in peripheral blood AD patients. However, a previous study revealed that Th17 cells and Tregs present mutually antagonistic functions, and the percentage of Tregs negatively correlated with Th17 cell frequency in both the skin specimens and peripheral blood of patients with AD.[20]

In this study, the topical application of 1% turmeric rhizome extract nanoemulgel caused significantly lowered TSLP levels, IL-13 levels, and IL-17 tissue levels. These results are in agreement with the previously reported CUR study. *In vitro* studies have shown that CUR suppresses TSLP expression and production through the caspase-1 and NF-κB pathways.[21] Oral supplementation of CUR in ovalbumin-sensitized AD-like mouse models was reported to decrease the expression of TSLP/IL-33 cytokines and Th2 cytokines (IL-4, IL-5, IL-13, and IL-31), repair skin lesions characterized by reduced epidermal thickness, and decreased inflammatory cell infiltration in the dermis via the STAT6 and GATA3 activation pathways.[22] In a mouse model of psoriasis induced by imiquimod cream, it was found that the application of 1% CUR gel decreased the production of cytokines IL-17A, IL-17F, IL-22, IL-1β, and TNF-α. Reduced expression of IL-17A and IL-17F is thought to be indirectly inhibiting IL-1β/IL-6 production.[23] Anti-inflammatory effect of CUR against IL-13, possibly through the STAT6, GATA3 pathway, suppresses chemoattractant activity that plays a role in inflammation.[22] The anti-inflammatory effect of CUR on IL-17 is thought to be through the mechanism of CUR inhibition on NF-κB and STAT3.[24]

Repeated exposure to DNCB on mouse skin causes histopathological changes characterized by hyperkeratosis, epidermal hyperplasia, and infiltration of lymphocytes, eosinophils, and mast cells, similar to the acute phase of AD lesions in humans.[25] The application of moisturizer can restore stratum corneum hydration, reduce cytokine production, mast cell hypertrophy and degranulation, and epidermal hyperplasia.[26] In the 1% turmeric rhizome extract nanoemulgel application group, the epidermis thickness was significantly thinner than the control group, thought to be through the activity of turmeric rhizome extract in inhibiting the IL-13 cytokine in the STAT3 pathway, thereby reducing the occurrence of epidermal hyperplasia.

In the AD-like mouse model, the number of eosinophil cells increased in the group of mice with oxazolone-induced dermatitis. Heo stated that the recruitment of eosinophils in the skin would produce IL-1β, IL-6, and IL-17, increasing the number of Th2 cells and the production of Th2 cytokines.[18] In this study, it was found that the mean number of eosinophil cells on histopathological examination in the group of mice with the application of 1% turmeric rhizome extract moisturizing nanoemulgel was significantly lower than the control group, which was thought to be through the activity of CUR in inhibiting IL-13 in the STAT6 pathway, which will suppress the activity of chemoattractants that play a role in the occurrence of inflammation in AD.

The TEWL result in this study is consistent with a study in a DNCB-sensitized model of AD mice, which showed an increase in TEWL and decreased skin hydration.[27] This result shows that applying moisturizer can improve the skin barrier, characterized by a decrease in the TEWL value. The use of moisturizers has been reported to increase skin hydration and decrease TEWL values during the recovery phase of the skin barrier, which is essential in managing AD.[28,29] Studies show that the application of moisturizers containing anti-inflammatory agents shows inconsistent results concerning skin TEWL values.[29] The value of TEWL *in vivo* can be influenced by several factors such as humidity, temperature, moisture content in the skin, sweat gland activity, metabolism, circadian rhythm, and stress.[30,31] In this study, the topical application of 1% turmeric rhizome extract moisturizing nanoemulgel was not proven to cause lower TEWL values than the control group. This result may be because both test materials contain the same base of moisturizing components, which can improve the skin barrier. Adding 1% turmeric rhizome extract to moisturizer could reduce the skin inflammatory response due to the application of DNCB.

The application of DNCB in experimental animal models can cause AD-like clinical trials, shown by an increase in inflammatory responses such as an increase in the severity of skin lesions, ear edema, and scratching behavior in experimental animals.[32] The use of moisturizers can improve the skin barrier, prevent transdermal sensitization, reduce TSLP levels, and reduce the symptoms and severity of AD.[33,34] Anti-inflammatory in moisturizers is thought to work by blocking cyclooxygenase activity and downregulating pro-inflammatory cytokines, repairing the skin barrier, and calming effect on dry and irritated skin.[35]

This study showed that 1% turmeric rhizome extract moisturizing nanoemulgel reduced AD-like lesions in DNCB-induced mouse models through TSLP, IL-13, and IL-17. This study also found the role of moisturizing nanoemulgel turmeric rhizome extract in repairing skin barrier damage assessed from clinical dermatitis (dermatitis score) and histopathological examination (epidermal hyperplasia and eosinophil cell infiltrate in the dermis). Multivariate analysis indicates that changes in TSLP, IL-13, IL-17, and TEWL due to the 1% CUR gel impact clinical improvement in dermatitis scores. This result confirms the theoretical basis of this study that TSLP, IL-13, IL-17, and TEWL interact with each other in producing dermatitis morphology. The analysis found that the Nagelkerke R-square was 0.460, which means that the effect of the TSLP, IL-13, and IL-17 variables on changes in the dependent variable was 46.0%, with 54.0% effects of other factors that were not researched.

The limitation of this study is that the hapten (DNCB)-induced AD-like mouse model can activate an immune response, which may be difficult to conclude for the incidence of AD in humans. Further research is needed to investigate the effect of applying nanoemulgel turmeric rhizome extract moisturizer in human AD cases.

Conclusions

One percent (1%) turmeric rhizome extract moisturizing nanoemulgel significantly affects clinical improvement in dermatitis through decreasing TSLP, IL-13, and IL-17 tissue levels, strengthening moisturizers' role by repairing the skin barrier and reducing AD lesions theory.

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Conflicts of interest

There are no conflicts of interest.

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