

Evaluation of Antipsoriatic Activity of Topical Curcumin by using Mouse Tail Model

Assistant Prof. hadaf A. Aljunaeh. F.I.B.M.S dermatology*(hadaf.a@utq.edu.iq)

Dr.hameed N. Mousa F.I.B.M.S pathology*(hamed@utq.edu.iq)

Bassam A. Hassan, pharmacist MSc pharmacology**

Abstract

Background;

Psoriasis is a one of the common dermatological diseases with no curative topical or systemic treatment, Curcumin is a naturally occurring phytochemical present in turmeric; it was traditionally used for the treatment of many skin diseases. This study was designed to evaluate the anti-psoriatic effect of curcumin preparations.

Objectives; this study aimed to examine the probability of anti-psoriatic activity of topical curcumin by using mouse tail model.

Methods; The extracted curcumin was assessed for antipsoriatic activity using a mouse tail model of psoriasis. 18 animals were divided into three groups (6 animals each).

Group A: control group (cream base treated group)

Group B: (treated with curcumin cream 1%)

Group C: (treated with curcumin cream 2 %)

Granular layer formation, epidermal thickness and drug activity were taken as an evaluating parameter to detect the efficacy of the new preparation.

Results; The local application of curcumin 1%, 2% on the mouse tail skin produced a significant differentiation in the epidermis, as seen from its degree of orthokeratosis (43.4 ± 0.75) and (44.4 ± 0.98) respectively when compared with the negative control (17.06 ± 2.5).

Conclusion;

Topical curcumin have anti-psoriatic activity and seems to be well tolerated on skin since the incidence of side effects was limited except staining of skin by an orange color.

*Collage of medicine /Thi-qar University

**Bent Al-Huda Teaching hospital Thi-qar Iraq

1.INTRODUCTION

Psoriasis is one of the most widespread immune-mediated chronic inflammatory skin disorders characterized by hyper-proliferative keratinocytes and massive infiltration of leukocytes (Schon and Boehncke, 2005). The prevalence of psoriasis is estimated to be around 1.3-2.2 % (Parisi, *et al.*, 2011) in the UK, in Iraq the incidence of psoriasis was 1.5 % (Al Nuamy., 1988). Psoriasis hyper-proliferation of the keratinocytes, in the epidermis, with an increase in the epidermal cell turnover rate, the cause of the loss of control of keratinocyte turnover is unknown (Griffiths *et al.*, 2010). However, several factors can trigger an initial episode of psoriasis and these factors also worsen psoriasis and can induce a severe relapse these factors are environmental, genetic, and immunologic factors which appear to play the major role (Griffiths *et al.*, 2010). Many evidences suggested that psoriasis is an autoimmune disease. Studies show high levels of dermal and circulating TNF- α (Etthadi *et al.*, 1999). In addition to the treatment with TNF- α inhibitors is often successful. Psoriasis, a multi-factorial skin disease, was originally thought of as

a disorder primarily of epidermal keratinocytes, but is now recognized as one of the commonest immune-mediated disorders where TNF α , dendritic cells and T. cells all contribute substantially to its pathogenesis (Griffiths *et al.*, 2007). Interestingly, elevated levels of TNF- α specifically are found to correlate with flares of psoriasis. (Nickoloff *et al.*, 1991).

1.1 Aim of study

The present study was performed to Examine the probability of anti-psoriatic activity of topical curcumin by using mouse tail model.

2. Materials and methods

2.1.Plant material

Curcuma longa (Turmeric) rhizome were collected from Iraq market as dried rhizomes and authenticated by Department of Pharmacognacy, College of Pharmacy University of Karbala

2.2 List of chemicals

1. Benzyl alcohol Chemdyes Malaysia
2. Butyl hydroxyl anisole Qualikams fine chemical India
3. Butyl hydroxyl toluene BDH United kingdom

4. Cetostearyl alcohol Chemdyes Malaysia
5. Dibasic potassium phosphate BDH United kingdom
6. Disodium EDTA SCR China
7. Ethanol SDFCL fine chem India
8. Formalin XIAMEN China
9. Glycerin Thornton & Ross United kingdom
10. Hexane SDFCL FINE CHEM India
11. isopropyl myristate Mistral Lab Chemicals United kingdom
12. Light liquid paraffin JT BAKER Netherland
13. Polyethylene glycol 4000 Yash Lab. India
14. Propylene glycol BDH United kingdom
15. Tween-80 BDH United kingdom
16. White soft paraffin Alliance pharmaceuticals United kingdom.

2.3.Extraction method:

The method of Casey was used for extraction of curcumin by ethyl alcohol as the extraction solvent (Soni *et al.*, 2011). Dried rhizomes were milled with high speed miller to produce *curcuma longa* powder .then 150 ml ethyl alcohol in a 250 ml Round-Bottom flask was attached to a soxhlet extractor; the soxhlet extractor was filled with 50 gr of turmeric powder,

Extraction was started by maintaining the temperature at 50 to 60 degrees. The extraction was continuing for 3 to 4 hours until the solvent became almost colorless then, the soxhlet was removed and concentrate the extract by evaporation of alcohol of, then hexane 50 ml was added to the extract and with stirring solution using a magnetic stirrer, Water was added slowly to the solution. Curcumin was precipitated; Curcumin was filtered using suction filtration and recrystallize from ethanol (Harborne, 1984; Soni *et al.*, 2011).

2.3.1High performance liquid chromatography (HPLC) analysis

Quantitative analysis of the curcumin in the sample was performed to using HPLC system, the test was done by ministry of science and technology directorate of material research.

2.4.Cream formulation.

To prepare oil in water cream , cream was prepared by the addition of oily phase to the aqueous phase with continuous agitation. Oily phase consisted of the following: cetostearyl alcohol (10%), propylene glycol (5%), glycerin (5%), white soft paraffin wax (12%), light liquid paraffin (8%), polyethylene glycol 4000 (5%), tween

80 (5.33%) and butyl hydroxyl anisole (0.001%) (Oily phase contain cetostearyl alcohol 10gram+ propylene glycol 5ml +glycerin 5ml+ white soft paraffin wax 12gram+ light liquid paraffin 8ml+ polyethylene glycol 4000 5 gram + tween 80 5.33ml)are the main oily phase component to prepare 100gram of o/w cream. Aqueous phase was prepared as a follow water (q.s.) was heated to the temperature (70 ± 5 °C) by using water path and then disodium EDTA (0.01%) was added, butyl hydroxyl toluene (0.001%), dibasic potassium phosphate (0.2%) , Curcumin was mixed in benzyl alcohol (1%)and added in it. After that, oily phase was added to the aqueous phase with continuous stirring at slow speed for 1 hour and slowly decrease temperature and meanwhile, while isopropyl myristate (4%) was added to the mixtures of both phases.The prepared creams were transferred into wide mouth containers and stored in cool place. Base was also prepared by the same method with same ingredients but without adding the curcumin. **Evaluation of o/w cream (Saraf, 2009)**

2.4.1 Sensitivity and allergic test

It is tested by "Patch test". Apply product on 1 cm² patch of skin for 10 Minutes, if no any inflammation, edema, redness, itching or rashes then it considered as free from sensitivity.

2.5. Laboratory animals

Adult albino male mice (weight: approx. 25-27 g) were used in this experiment. They were obtained from animal house of Thi-Qar University and were housed in polypropylene mouse cages as 6 animals per cage and wood shaving was used as the bedding material .housing room conditions were maintained with a 12 h lightdark cycle, at temperature of 22 ± 02 °C, humidity 30-70%, and kept on laboratory mouse pellet feed and pure drinking water. The animals were acclimatized to thelaboratory conditions for a period of seven days prior to commencement of treatment.

2.5.1 Evaluation for anti-psoriatic activity. (Vogel G.H., 2002; Dhanabal *et al.*, 2012; Dwarampudi *et al.*, 2012) This is accepted as a screening method for measuring anti psoriatic activity of drugs. The basis of this method is that topical treatment of a mouse-tail with antipsoriatic drugs enhances orthokeratotic cell differentiation in the

epidermal scales. This characteristic is used for direct measurement of drug efficacy in an animal model. Topically 0.1g of drug is applied, once daily, 5 times in a week, for 2 weeks. Two hours after the last treatment the animal are sacrificed, longitudinal sections of the tail skin are made and skin tail was prepared for histological examination using hematoxylin- eosin staining the occurrence of orthokeratosis, represented by the number of scale regions with a continuous granular layer was counted and expressed as a percentage of the total number of scale regions per section. Drug activity is defined by an increase in percentage of orthokeratotic region.

2.5.2 Histopathological Evaluation

Longitudinal histological sections from the tail skin were passed in an ascending concentration of alcohol ,embedded in paraffin ,sectioned by microtome 5um prepared and stained with hematoxylin- eosin and specimens were analyzed under microscope for: (1) The individual scale horizontal length in between adjacent hair follicles including sebaceous glands (n = 10 scales per animal, n = 6 animals per treatment

group; i.e. a total of 60 measurements per treatment), (2) the fully developed granular layer horizontal length within an individual scale (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), and (3) The vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum corneum (n = 5 measurements per scale, n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 300 measurements per treatment). Taken together, from these calculations, the following three overall parameters were eventually used for the evaluation of the drug effects: (a) the degree of orthokeratosis, (b) the 'drug activity' and (c) the relative epidermal thickness.

$$\text{Drug Activity} = \frac{\text{OK(s)} - \text{OK(c)}}{100 - \text{OK(c)}} \times 100$$

With OK (i.e. orthokeratosis) as the mean of the parameter explained under for a test substance (s), and the untreated control condition (c), respectively.

2.6.The statistical analysis

Collected data were analyzed using SPSS version 17.0 for windows (SPSS statistics, IBM, USA). Results were

expressed as a mean \pm standard deviation (SD).

3. Result

3.1. Extraction result

Soxhlet extraction of the *Curcuma longa* yield (3.4%) curcumin (1.7gram from 50gram) as yellow to orange powder with melting point 175 °C Two different concentrations of O/W creams (Test 1-1%, and Test 2- 2%) were prepared to evaluate their anti-psoriatic activity. Number of parameters was used to evaluate O/W creams. Physical evaluation revealed that creams having orange to yellow color, characteristic pleasant odor, semisolid in nature and pH ranges from 6.5- 7. Stability of creams (base and formulation) was evaluated on 40 ± 2 °C/75%RH \pm 5%RH for a period of three months. No phase separation was observed during the stability study of creams. No liquefaction is observed during the study period of three months.

3.2. Evaluation of antipsoriatic activity

The degree of orthokeratosis of cream base that used as control was ($17.06\% \pm 2.5$) whereas the formulation of topical curcumin 1% , 2% showed ($43.4\% \pm 0.75$) and ($44.4\% \pm 0.98$) degrees of orthokeratosis respectively in mouse tail as in a table (3-4) ,and the drug activity of topical curcumin was 31.3, 32.5 for the two concentrations respectively.

3.3. Mean thickness of the epidermis

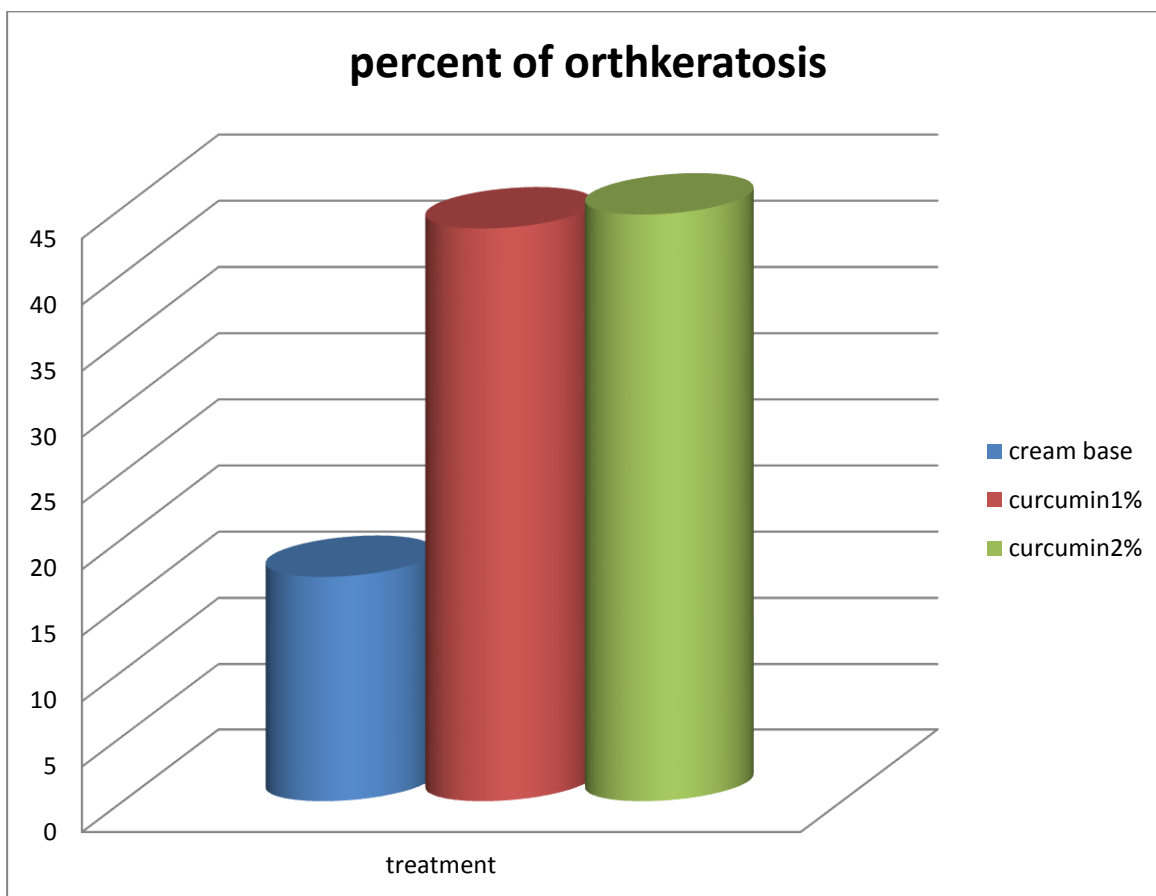
The epidermal thickness was measured as the distance between the dermo-epidermal borderline and the lowest part of the stratum corneum. Mean thickness of the epidermis, in the control and groups treated with curcumin 1%, 2% were found to be 38.32 ± 0.65 , 38.38 ± 0.71 , and 39.6 ± 2.2 micrometer, respectively.

Table ; The effect of curcumin1%and 2% on orthokeratosis (mean \pm SD)

Group	No. of animals	mean \pm SD	Drug activity%
Control (cream base	6	17.066 ± 2.517	0

Curcumin 1%	6	43.400 ± 0.981 *a	31.7 *a
Curcumin 2%	6	44.445 ± 0.755 *a	32.9*a

* P<0.001 in comparison with control, similar letter means not Significant (t-test)



The mean of increase in the percent of orthokeratosis in animal treated with curcumin 1% and 2% in comparison with control (cream base treated group).

Table ;The difference in epidermal thickness

Group	Epidermis thickness in μm (Mean \pm SD)	P value
Control	38.32 ± 0.65	
Treated with curcumin 1%	38.38 ± 0.71	>0.05

Treated with curcumin 2%	39.6 ± 2.2	>0.05
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The difference in epidermal thickness between groups was not statistically significant ($p > 0.05$).

Figure (A): Longitudinal histological sections through the skin of mouse tails treated vehicle control topically for 2 weeks; H&E staining (original magnification 40X) showed dermis, Basal cells layer, Prickle cells layer and Horny layer

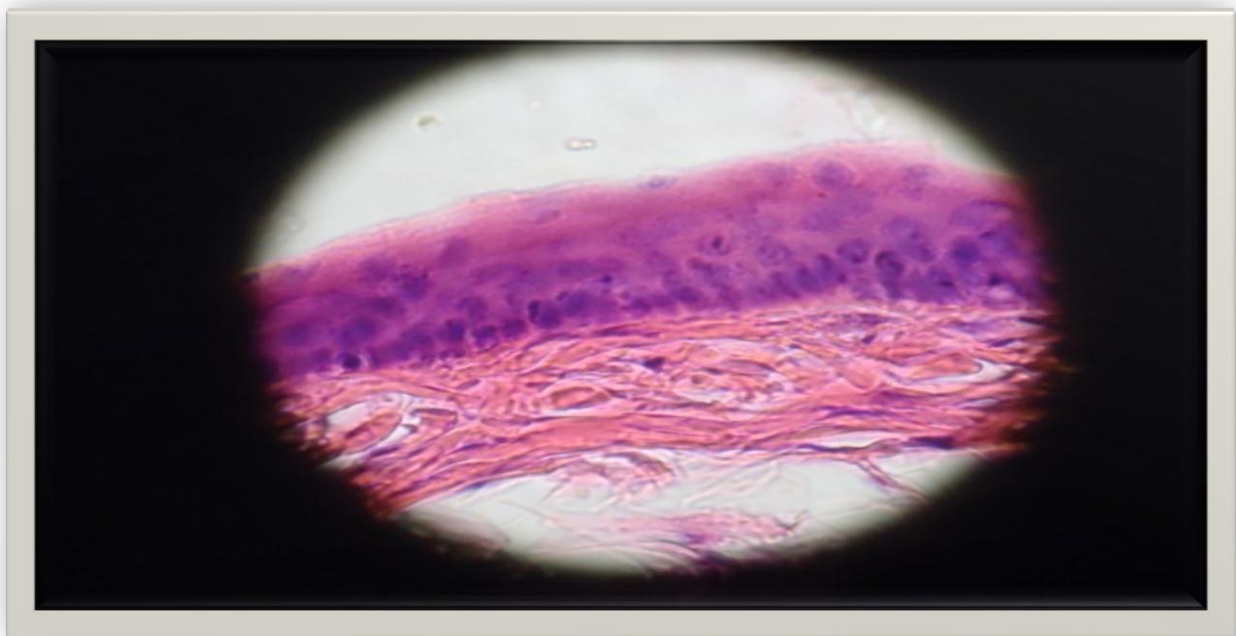
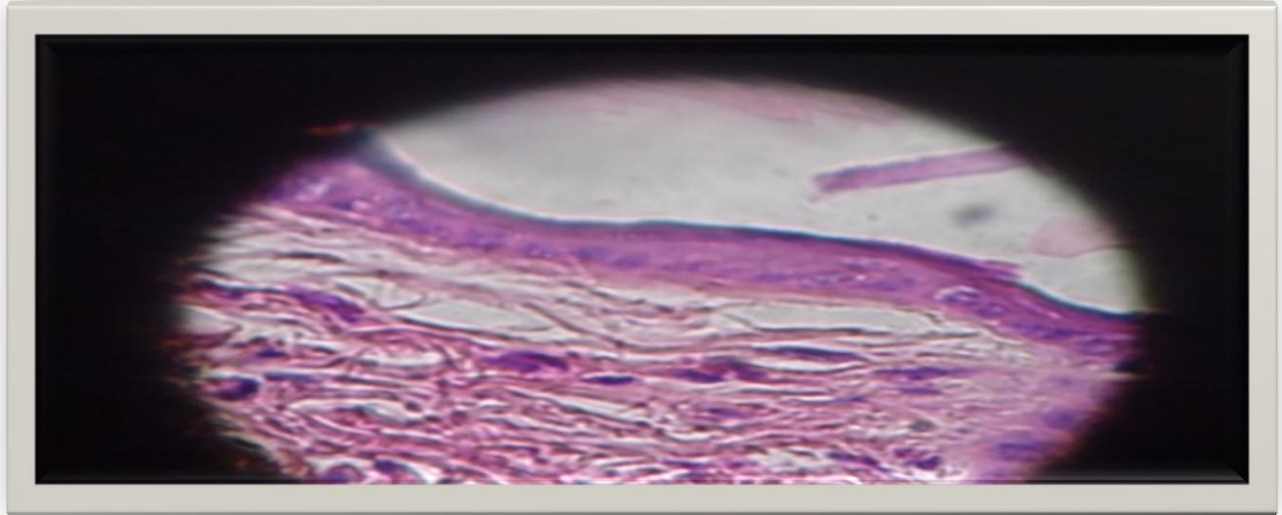
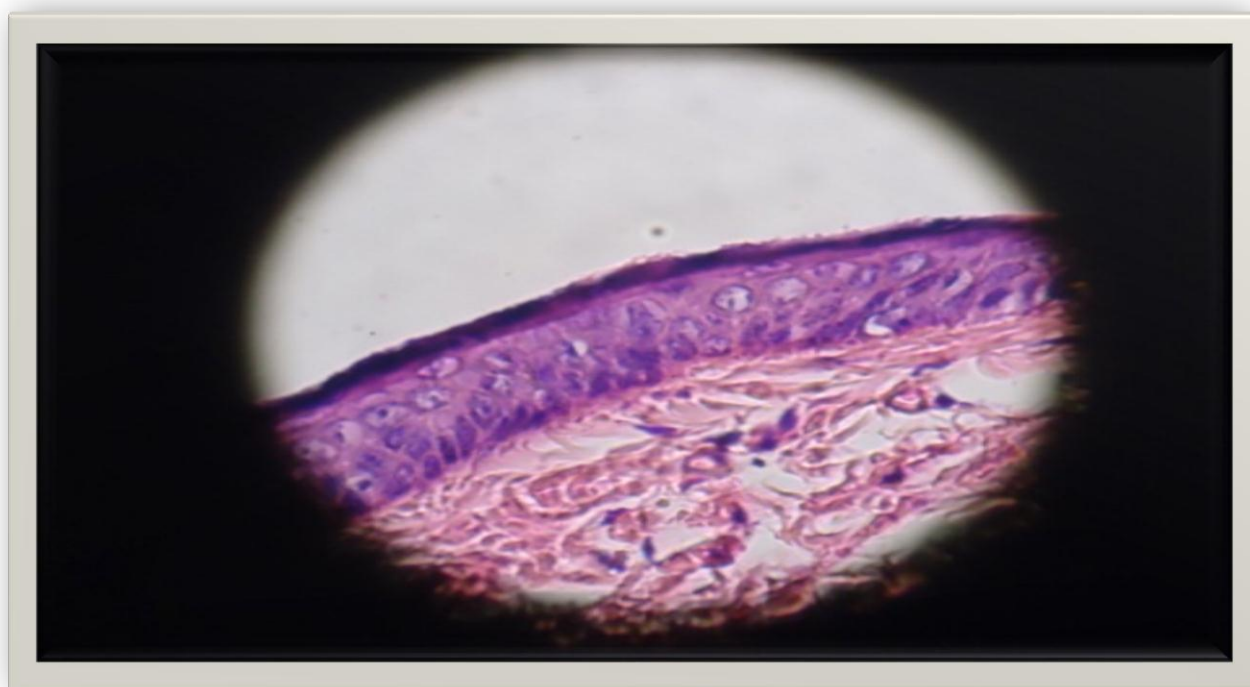


Figure (B): Longitudinal histological sections through the skin of mouse tails treated with curcumin 2% topically for 2 weeks, H&E staining (original magnification 40X) showed



induced orthokeratosis

Figure (C): Longitudinal histological sections through the skin of mouse tails treated with curcumin 1% topically for 2 weeks, H&E staining (original magnification 40X) showed induced orthokeratosis



4. Discussion

Curcumin was extracted from *Curcuma longa* plant by using Soxhlet. The ethanolic extracted curcumin was tested by HPLC. The amount of curcumin extracted and its HPLC analysis was similar to that recorded by physiochemical properties of the curcumin and also similar to that of pervious investigations by Harborne,

1984; Rajpal, 2005 and Soni *et al.*, 2011. an antipsoriatic drug that targets the epidermis should be a compound that ideally restores skin homeostasis by suppressing keratinocyte hyper

proliferation, abnormal differentiation, or both e.g. substances like dithranol and vitamin D analogues that affect keratinocyte differentiation are effective in bringing homeostasis of the epidermis in psoriasis conditions (Pol *et al.*, 2003).

Many new drugs used for treating psoriasis have been evaluated by using mouse tail model test and were found to show good efficacies (Danilenko, 2008). The mouse tail test first described by Jarrett and Spearman (1961) as a model mimicking human psoriasis, as the normal mouse tail is characterized by all the histological manifestations of psoriasis. The

granular layer is greatly reduced or almost absent in epidermis of psoriatic lesions (Lowes *et al.*, 2007). This parakeratosis condition is one of the most important hall marks of psoriasis. Granular layer formation around the epidermis is known as orthokeratosis (Danilenko, 2008). The main principle behind the mouse tail test is conversion of parakeratosis to orthokeratosis (Camisa, 1998). Many herbs used in the treatment of psoriasis have been evaluated by this method e.g. aloe Vera (Dhanabal *et al.*, 2012) and nigella sativa seeds (Dwarampudi *et al.*, 2012). In one study by Bosman 1994 Curcumin, showed a favorable effect on a mouse model of psoriasis, and this agreement with result that found in the present study where the appearance of granular layer formation indicates the anti-psoriatic activity of Topical curcumin 1% and 2% concentration, showed significant orthokeratosis 43% and 44% respectively in the mouse tail test when compared to control 17%. Also there were no significant differences in the induction of orthokeratosis between curcumin 1% and 2% concentration. In addition the absence of the significant change in epidermal thickness at the

end of treatment time this indicates curcumin is not an irritant agent.

conclusion

Topical curcumin have anti-psoriatic activity and seems to be well tolerated on skin since the incidence of side effects was limited except staining of skin by an orange color.

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تقييم الفعالية المضادة للصدفية لمادة الكركمين باستعمال نموذج ذيل الفأرة

د.هدف عبد الأمير الجنيح استاذ مساعد*

أ.م.د. حميد نعيم دكتوراه باثولوجي*

الصيدلي بسام عبد الصاحب حسن ماجستير أدوية**

الخلاصة:

الخلفية: الصدفية هي واحد من الأمراض الجلدية الشائعة والتي لا يوجد لها علاج شاف بشكل نهائي. الكركمين يمثل المادة الفعالة الموجودة في نبات الكركم وقد تم تصميم الدراسة الحالية لتقييم تأثير الكركمين في علاج مرضى الصدفية.

الأهداف: تقييم تأثير الاستعمال الموضعي للكركمين بشكل تجريبي وباستخدام نموذج ذيل الفأرة

الطرق: الدراسة التجريبية تمت باستخدام ١٨ فأرة قسمت إلى ثلاثة مجاميع (٦ حيوانات لكل مجموعة)

مجموعة ١ المجموعة الضابطة عولجت بالروزة كريم

مجموعة ب عولجت بالكركمين ١% كريم

مجموعة ج عولجت بالكركمين ٢% كريم

تم اعتماد فحص تشكل الطبقة الحبيبية وسمك البشرة وفعالية المادة كأساس لتقييم فعالية الكركمين .

النتائج: إن نتائج الدراسة التجريبية بعد الاستعمال الموضعي لمادة الكركمين ١% و ٢% على جلد ذيل الفأرة أنتجت تغيرا معتدا به في تشكل الطبقة الحبيبية $P < 0.001$ عند مقارنته بالمجموعة الضابطة.

الاستنتاج: إن استعمال الكركمين بشكل موضعي يمتلك خصائص مضادة لمرض الصدفية.

* كلية الطب \ جامعة ذي قار

** مستشفى بنت الهدى التعليمي