



## Assessment of Anti-psoriatic Activity of *Cassia fistula* L. Extract Incorporated Cream

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### Authors' contributions

This work was carried out in collaboration between both authors. Author NTT designed the study, conducted the experiments, performed the statistical analysis, and wrote the first draft of the manuscript. Author HLS managed the literature searches and checked the format of the article. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/BJPR/2015/15997

#### Editor(s):

(1) Nawal Kishore Dubey, Centre for Advanced Studies in Botany, Banaras Hindu University, India.

#### Reviewers:

(1) Anonymous, India.

(2) Mutlu Çayırılı, Mevki Military Hospital-Dermatology Service, Ankara-Turkey.

(3) Farhana Tahseen Taj Haveri, Dermatology, Venereology and Leprosy, KLE University, Belgaum, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history.php?iid=884&id=14&aid=7858>

Original Research Article

Received 31<sup>st</sup> December 2014

Accepted 12<sup>th</sup> January 2015

Published 22<sup>nd</sup> January 2015

### ABSTRACT

**Aims:** To investigate the efficacy of topical application of *Cassia fistula* L. fruit extract in hydrophilic cream for psoriasiform dermatitis on mouse model.

**Study Design:** Formulations containing fruit extract were prepared, characterized, and made available for in vivo animal study.

**Place and Duration of Study:** School of Biotechnology, International University, Vietnam National University, Ho Chi Minh City from January to December 2014. Department of Anatomy, Pham Ngoc Thach University of Medicine, Ho Chi Minh City on October 2014.

**Methodology:** *Cassia fistula* fruit methanol extract at varied concentrations of 2.5, 3.75, 5.0, 6.25, and 0% (w/w) was entrapped in oil-in-water topical emulsion to produce different formulations. Chemically induced psoriasis-like skin in mice was performed, and psoriasis-inflicted mice were then individualized for time-tested topical regimens with prepared formulae. Dithranol 0.5% was served as standard anti-psoriatic treatment. Histometric analysis was conducted for outcome measures including degree of orthokeratosis and relative epidermal thickness.

**Results:** Investigation results revealed that most of the formulations containing methanol extract of

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*Cassia fistula* produced significant ( $P<.001$ ) degree of orthokeratosis with respect to control, wherein a medication with formula incorporating 6.25% extract had the highest efficacy ( $40.81\pm 1.19\%$ ). Treatment with this herbal cream also caused significant ( $P<.001$ ) decreases in relative epidermal thickness by  $26.08\pm 1.39\%$ , which was more potent than the standard ( $51.57\pm 3.05\%$ ).

**Conclusion:** Treatment with *Cassia fistula* contained cream ameliorated psoriasis symptoms of the animals and prevented psoriasis worse. These suggest that *Cassia fistula* could have anti-psoriatic activity and could be further explored for psoriasis treatment.

**Keywords:** Anti-psoriasis; *Cassia fistula* L.; PPD-induced psoriasis; skin disease; herbal treatment.

## ABBREVIATIONS

Here is the Definitions section; This is an optional section. Term: Definition for the term.

## 1. INTRODUCTION

Psoriasis is a commonly immune-mediated disease which is more likely to develop chronic skin disorder characterized by the recurring localized, widespread well-demarcated red plaques often topped by silvery scales over the skin lesions. This disease also diversely damages nails, mucous membranes and joints, but not hair [1]. This health problem strongly influents on patient's psychological aspect because of having to cope with the reactions of others, one of the biggest challenges of living with skin disease. Speculations about the cause of psoriasis are many, such the disease as a result of interplay between genetic and environmental factors. The aetiology of psoriasis is believed to be multifactorial; however, this question has not yet been fully resolved and therapeutic intervention for patients with psoriasis is therefore still not standardized [2,3,4]. Therapeutic modalities for psoriasis comprise topical agents, phototherapies, and systemic treatments which play a role as anti-proliferative agents and reduce keratinocyte proliferation [5]. Although these remedies have their merit, there are several lines of evidence suggesting that they sometimes cause a flare because of irritation, photo-toxicity, or hypersensitivity reaction [6]. These adverse effects on treatment for psoriasis and studies on new drugs for the disease came almost to a standstill; therefore, an alternative therapy like natural remedies which can be used as a substitute to treat dermatitis diseases is required [7,8,9].

It has been suggested that herbal medicine predominates in the management of skin disease [10]. Medicinal plants which could eliminate a

wide range of side effects that has been found in other remedies, can address principles that are pertinent to skin diseases. Herbs have been highly prized and are coming to realize that it is possible to boost the immune system. Instead of signaling for the memory cells, most herbs generally act as stimulators to trigger the immune response rather than specific to a particular antigen [11]. The genus *Caesalpinia* (*Caesalpinaceae*) has more than 500 species, many of which have not yet been investigated for potential pharmacological activity. Ethnobotanical investigations indicate that a spontaneous flora as *Cassia fistula* Linn. (*C. fistula*) which is indigenous to south Asia, has been used in folkloric medicine in Vietnam for the treatment of skin related autoimmune disease such as leprosy. To date, the clinical efficacy of *C. fistula* has just been investigated for anti-inflammatory, antioxidant, antibacterial, and immunomodulatory activities [12,13]. These suggest that *C. fistula* is a prominent plant source for treatment of psoriasis. However, there is meager information about its mechanisms of action and still none of scientific report deals with the evaluation of *C. fistula* for the anti-psoriatic potential.

The mysterious autoimmune disorder has been studied for psoriasis, and the need of the hour is to seek an effective treatment with plant-derived source which is able to ease the symptoms and therefore bring hopeful outlook for patient, with fewer bothersome side effects. In this study, *C. fistula* extract was examined for its anti-psoriatic activity by the use of oil-in-water emulsion as a vehicle for delivering extract to localized areas of psoriatic lesion, wherein histological parameters were analyzed at the end of experiment.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Methanol and Di-n-Propyl Disulfide (PPD) were purchased from Sigma chemicals. Other ingredients used for cream base include stearic acid, cetyl alcohol, isopropyl myristate, glycerin, potassium hydroxide, sodium hydroxide, and methyl paraben (Kanto Chemical Co., Inc). Distilled water was used throughout the study.

Fruit pulp of *C. fistula* was identified by comparison with the herbarium (CCFL. No. 1309) at the School of Biotechnology, International University, Vietnam. A voucher specimen (PCFL/2014/1309-01) was also deposited at the same department.

### 2.2 Animals

Adult Swiss albino mice weighing  $23 \pm 2$  g and aging 10 weeks were obtained from Pasteur Institute of Ho Chi Minh City. The animals were acclimated to the test conditions for 1 week. The mice were supplied standard pellet diet, water ad libitum, and kept at room temperature under 12 h light-dark cycles. Handling and treatment of experimental animals were complied with the ethical guidelines for animal research.

### 2.3 Preparation of Herbal Extract

The ripe fruits of *C. fistula* were harvested in late autumn from area of Ho Chi Minh City, Vietnam. Fruit pulp of plant was isolated and subjected to dry heat treatment at 55°C before grinding it into small pieces. The dried pulp of fruits was macerated with methanol and kept aside for 5 days with frequent agitation. The hydro-methanol (3:7) extract of *C. fistula* fruit pulp was then derived by rotary evaporation of the solvent from the total extract, at 60°C and 20 mbar. The percentage of yield was 31%. Afterwards, the

dried extract was used in the step of formulated preparation.

### 2.4 Preliminary Phytochemical Screening

Qualitative analysis of the main groups of phytochemicals commonly known as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins, was conducted by the standard protocol [14,15].

### 2.5 Herbal Formulations

#### 2.5.1 Preparation of herbal cream

The appropriate hydrophilic cream base for delivering botanical extract was prepared according to the previously reported procedure [16]. The vehicle constituents were listed in Table 1. The oil phase was prepared by heating the solid lipid materials at 75°C until it liquefied, and the internal phase was apparently dispersed into the external phase. The solution was mixed well using homogenizer at  $200 \pm 25$  rpm until homogenous, which subsequently was quenched to room temperature. Various concentrations (2.5, 3.75, 5.0, 6.25, and 0% w/w) of *C. fistula* extract were separately incorporated into the base cream formula. The proposed formulations were coded as F1 to F5, respectively. The cream base F5 was served as placebo.

#### 2.5.2 Physicochemical evaluation

The prepared formulae were evaluated for the organoleptic characteristics (color, appearance), and physical parameters (spreadability, pH value, apparent viscosity, thermal stability). Spreadability is expressed in units of time in seconds required for a given weight of the cream to separate the two glass slides [17]. Viscosity was measured by Brookfield Viscometer (LVDV-E Model) using spindle LV-II at 30rpm. Thermal stability and pH value was evaluated according to standard guideline [18,19].

**Table 1. Cream formulation**

Ingredients	Composition (w/w)				
	F1	F2	F3	F4	F5
Stearic acid	15.0	15.0	15.0	15.0	15.0
Cetyl alcohol	0.5	0.5	0.5	0.5	0.5
Isopropyl myristate	3.0	3.0	3.0	3.0	3.0
Glycerin	6.0	6.0	6.0	6.0	6.0
Potassium hydroxide	0.5	0.5	0.5	0.5	0.5
Sodium hydroxide	0.18	0.18	0.18	0.18	0.18
Methyl paraben	0.15	0.15	0.15	0.15	0.15
<i>C. fistula</i> fruit extract	2.5	3.75	5.0	6.25	-
Distilled water q.s. (100 g)	q.s.	q.s.	q.s.	q.s.	q.s.

### 2.5.3 Skin irritation test

As a step to guarantee for safety used formula, skin irritation score was graded according to OECD guidelines for Testing of Chemicals [20] as follows: 0.00: no irritation; 0.04 – 0.99: irritation barely perceptible; 1.00 – 1.99: light irritation; 2.00 – 2.99 mild irritation; 3.00 – 5.99: moderate irritation; and 6.00 – 8.00: severe irritation.

## 2.6 Pharmacological Activity Evaluation

### 2.6.1 PPD-induced psoriasiform mouse model

The spontaneous development of psoriasis in mice was conducted according to the modified method for psoriasiform lesions [21,22]. The shaved dorsal region of mice was carefully applied Di-n-Propyl Disulfide (PPD) with the recommended dose for irritant at 5 µL/cm<sup>2</sup>/day on the following 6 days. The mice were further randomized to seven groups of five mice each. Each of these groups was received different daily topical regimens for 2 weeks as the following: groups I - V received formula F1 – F5, respectively; group VI was served as positive control (dithranol 0.5%) while group VII was regarded as negative control (no additional treatment).

### 2.6.2 Histological examination

At the end of the entire treatment period, skin biopsies from experimental psoriatic mice before and after treatment were subjected to histological examination by haematoxylin and eosin stain. Histopathology slides were analyzed for quantitative values [23] as described below:

- (1) The whole scale length lying in between two adjacent hair follicles
- (2) The horizontal length of fully developed granular layer within an individual scale (n = 10 scales per animal).
- (3) The vertical epidermal thickness identified by the distance between dermoepidermal border line and the beginning of the horny layer. (n = 5 measurements per scale, n = 10 scales per animal).
- (4) Degree of orthokeratosis (OK) of an individual scale. Percentage of orthokeratosis was calculated by dividing (2) by (1)
- (5) Drug activity was measured by the equation:

Drug activity =

$$\frac{\text{Mean OK of treated group} - \text{Mean OK of control group}}{100 - \text{Mean OK of control group}} \times 100\%$$

- (6) The relative epidermal thickness of individual scale as the percentage ratio of the measure under (3), for a given treatment in relation to the mean of controls set to 100%.

All measurements were made with OLYMPUS BX51 microscope using a 40x lens.

## 2.7 Data Analysis

Statistical results were expressed as mean ± S.E.M, and compared using one-way ANOVA followed by Turkey's test to examine the significant differences between experimental data. Values with  $P < .05$  were considered significant. Sigma Plot statistical software package (version 11.0) was used for analysis.

## 3. RESULTS AND DISCUSSION

### 3.1 Preliminary Phytochemical Screening

Qualitative tests for the main secondary metabolites produced in *C. fistula* fruit extract were alkaloids, flavonoids, glycosides, phenols, saponins, steroids, and tannins.

### 3.2 Herbal Formulations

#### 3.2.1 Physicochemical evaluation

Cream base (F5) and the formulae containing *C. fistula* extract (F1 - F4) were analyzed for their consistency at different temperatures and other attributes that presented in Table 2. The oil-in-water cream F5 performed white and smooth in appearance. The remaining formulae (F1 – F4) were creamy with color intensifying from light brown to dark brown. Those colors were resulted from an increasing in the concentration of *C. fistula* extract incorporated with.

Spreadability of all formulations was found to vary from 8.33±0.8 to 13.26±0.4, indicating an easy in spreading over the skin and therefore the active ingredient, which was *C. fistula* extract, would be released for a local effect. Viscosity of the formulae was ranged from 6379±17.2 to 6680±8.65 cps, and rather increased with the increasing amounts of *C. fistula* extract. The pH values were found to be in the range of 6.04±0.07–6.7±0.09, which showed good

compliance with that of mouse skin [24]. With respect to thermal stability, there was no problem encountered in emulsion preparation. The prepared formulae were generally stable under varying temperatures (20°C-40°C) against creaming, cracking and phase inversion. It is likely to be due to globule size of the disperse phase was reduced under shearing forces for small enough to stabilize the emulsion system [25]. In general, all formulations met the acceptable conditions of consistency for application.

**3.2.2 Skin irritation test**

All tested formulae were well tolerated by experimentally mice. No signs of erythema and edema occurred, and the formulae therefore were evaluated for safety data showed in Table 3.

**3.3 In vivo Anti-psoriatic Activity**

**3.3.1 Induction of psoriasis**

Psoriasisform lesion induced by topically applied allergen, PPD, which belongs to the class of dialkyl disulfides. At about a week, experimental mice expressed markers for acute episode of plaque psoriasis, such as reddish plaques covered with silvery white scales and impaired hair growth (Fig. 1(A)). Histological results of psoriasis-like mouse skin featured the changes in epidermis such as acanthosis, thickening epidermis, proliferation of epidermal keratinocyte and elongation of rete ridges (Figs. 1(B-C)). Abnormal changes in vascular such as increased vascularity, and intra-epidermal microabscess were also observed (Figs. 1(D-E)). Part of the reason for this chemically induced psoriasis is that the smoldering inflammation could lead to instability of genes through recruitment of macrophages and other innate immune cells, thereby altering skin homeostasis. Additionally,

PPD plays a role as a mimic molecular that may give rise to autoimmunity and caused abnormal changes in phenotypes [26,27]. The adverse effects of using PPD to induce psoriasisform lesion prompted us to use such an agent to induce psoriasis in mice.

**3.3.2 Histological results**

*C. fistula* fruit extract was screened for their possible anti-psoriatic activity after 2 weeks of daily topical treatment. The profile of three overall parameters such as degree of orthokeratosis, relative epidermal thickness, and drug activity is given in Table 4. Percentage of orthokeratosis was significantly increased in *C. fistula* extract-treated groups including groups II-IV (from 30.48±1.13% to 40.81±1.19%, *P* < .001) compared with that of control (16.54±1.02%). However, no significant differences were found for group I which was given formula F1 (*P* = .19) and group V treated with cream base F5 (*P* = .99) in comparison with that of control. Among the testing formulae containing extract, formula F4 (40.81±1.19%) was found to produce the highest percent of orthokeratosis. The results were validated using dithranol 0.5% as positive control which showed a potent activity with degree of orthokeratosis value of 52.58±2.0%.

In case of quantifying epidermal thickness, a significant change in epidermal thickness compared to that of control was also found in all groups which received formulae F1 – F5 in daily regimen, ranging from 26.08±1.39% to 84.94±3.49% (*P* < .001). In most of these cases, there was a dramatic decrease in epidermal thickness for group that was given formula F4 (26.08±1.39%). Interestingly, in mice, overall and by groups III-IV had significantly lower value of relative epidermal thickness than dithranol 0.5%-treated group (51.57±3.05, *P* < .05).

**Table 2. Physicochemical characteristics of the formulae**

Parameters	Formulation code				
	F1	F2	F3	F4	F5
<b>Appearance</b>	<b>Light brown</b>	<b>Brownish</b>	<b>Brown</b>	<b>Dark brown</b>	<b>White</b>
Spreadability (g.cm/s)	11.10±0.4	11.86±0.3	12.0±0.5	13.26±0.4	8.33±0.8
Viscosity (cps)	6390±11.7	6455±7.07	6462±4.61	6680±8.65	6379±17.2
pH	6.54±0.05	6.38±0.04	6.18±0.06	6.04±0.07	6.7±0.09
<b>Thermal stability</b>					
20°C	+	+	+	+	+
30°C	+	+	+	+	+
40°C	+	+	+	+	+

(+) denotes stability of formulations. Values are expressed as the mean ± S.E.M.

**Table 3. Irritation scores observed for formulae**

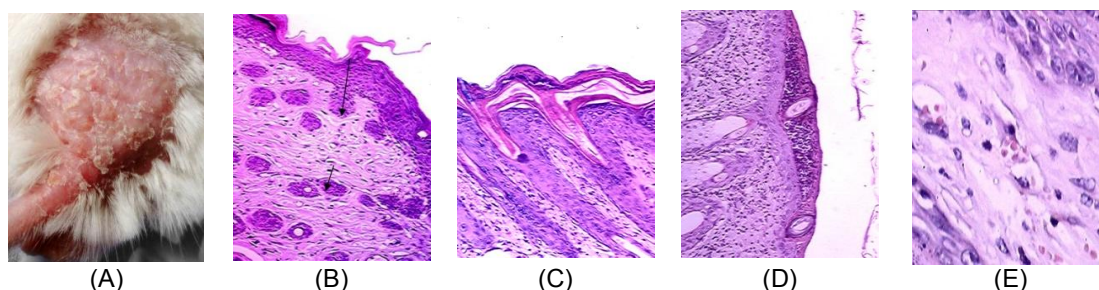
Formulation	Irritation score			Evaluation
	24h	48h	72h	
F1	0	0	0	No irritation
F2	0	0	0	No irritation
F3	0	0	0	No irritation
F4	0	0	0	No irritation
F5	0	0	0	No irritation

In addition, these evaluations were re-emphasized when considering 'drug activity' as an overall parameter related to the control. Drug activity for formulae F1 – F5 given in daily regimen was 10, 16.71, 23.80, 29.08, and 2.23%, respectively. Formula F3 and formula F4 possessed a similar efficacy but each of them was less potent than that of dithranol 0.5% (43.19%).

The histological specimens underlying the histological investigation throughout 14 days of the treatment for skin disorder's parameters such as the change in epidermis (acanthosis,

elongated rete ridges), vascular change (increased vascularity) and inflammatory change (intraepidermal microabscesses), were shown in Fig. 2. Acanthosis was observed after 2 weeks of treatment in all experimental groups (Figs. 2(A-C-E-H-J-L)) except for group IV (Fig. 2G). The severe of acanthosis was gradually reduced from groups I - IV. Elongation of rete ridges, another hallmark for epidermal change, was recorded in some extract treated groups (Figs. 2(B-D-F)) and dithranol 0.5% given group (Fig. 2(J)) but was slightly less than group received cream base and control group (Figs. 2(I-L)). An increase in vascular presented in group VI and VII was not found in the remaining groups (Figs. 2(K-M)). Munro's microabscesses were only detected in negative control (Fig. 2K).

According to the histometrical and histological analyses, formulations containing *C. fistula* were noticeably improved psoriasis-like skin condition. Increasing the amount of added extract concentration increased degree of orthokeratosis but also reduced the epidermal thickness. Accuracies regarding the true factors of herb efficacy have already been discussed. However,



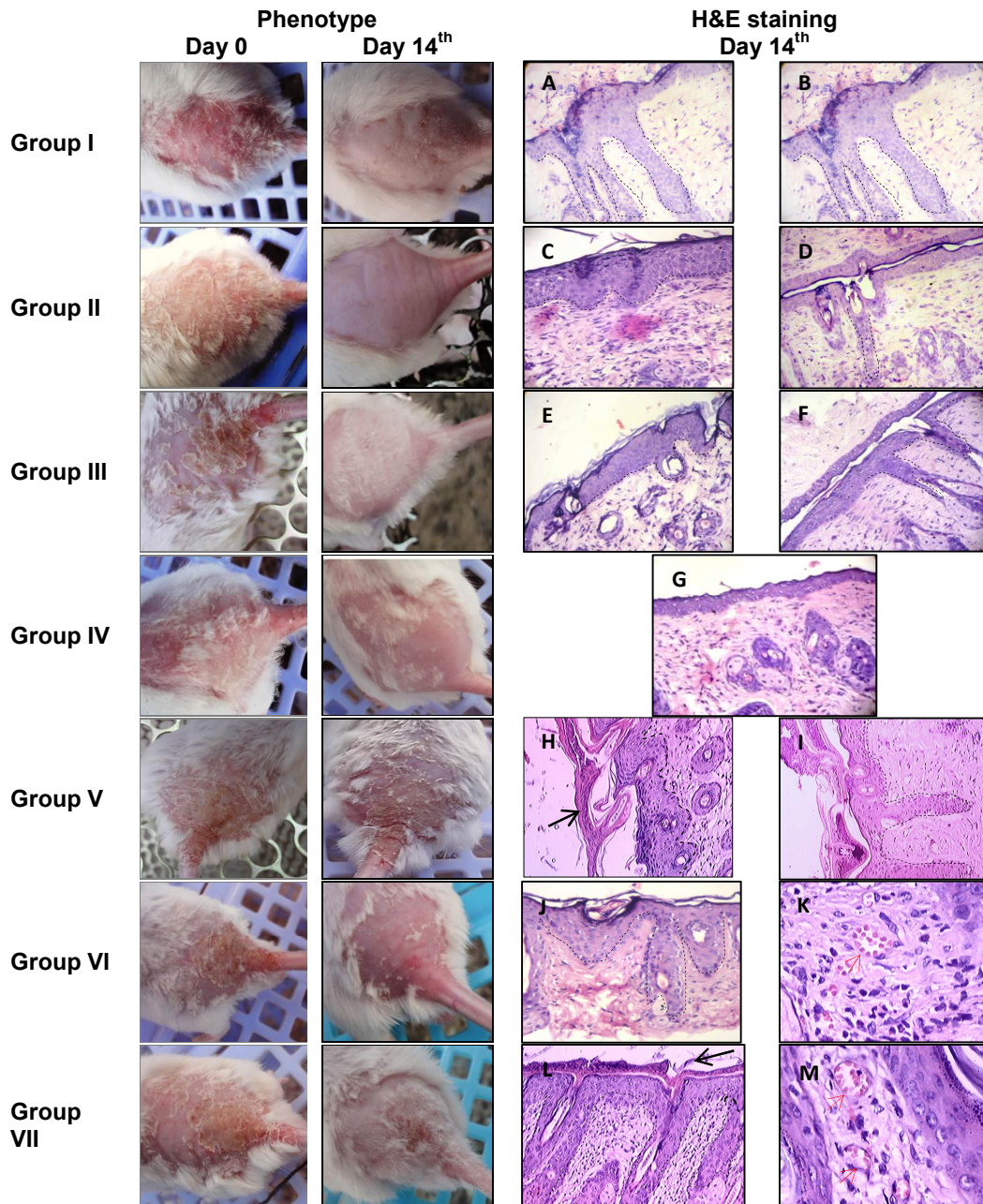
**Fig. 1. Psoriasis-like mouse skin features**

(A) Clinical phenotype of psoriasis. (B) Acanthosis (black arrow). (C) Elevation of epidermal layer. (D) Intra-epidermal microabscess. (E) Increased vascularity. Images were taken at 20x (B-D), and 40x magnification (E).

**Table 4. Effect of *C. fistula* extract on the degree of orthokeratosis, relative epidermal thickness and the 'drug activity' in the mouse test**

Experimental groups	Degree of orthokeratosis (%)	Relative epidermal thickness (%)	Drug activity (%)
Group I	24.89±1.61 <sup>b</sup>	60.79±3.46 <sup>ac</sup>	10.00
Group II	30.48±1.13 <sup>abc</sup>	42.52±2.68 <sup>ac</sup>	16.71
Group III	36.40±2.75 <sup>abc</sup>	39.53±2.32 <sup>abc</sup>	23.80
Group IV	40.81±1.19 <sup>abc</sup>	26.08±1.39 <sup>abc</sup>	29.08
Group V	18.40±1.12 <sup>b</sup>	84.94±3.49 <sup>ab</sup>	2.23
Group VI	52.58±2.0	51.57±3.05	43.19
Group VII	16.54±1.02	100±2.51	-

Values are expressed as the mean ± S.E.M  
*P* < .05 with respect to <sup>a</sup>control; <sup>b</sup>dithranol 0.5%, <sup>c</sup>cream base



**Fig. 2. Gross morphology and histological features of mouse skin treated topically for 2 weeks.**  
 Images were taken at 20x (A-J, L), and 40x magnification (K-M).

plant extract show good activity in one method cannot be said to have more anti-psoriatic activity as its mechanism of anti-psoriatic activity may be different. In this study, psoriasiform arose as a result of contact with highly irritating chemicals that induce activation of the innate immune system through hyper-production of cytokines and chemokines and an infiltration of inflammatory cells [28]. Immunostimulating

agents from *C. fistula* [14] thereby stimulate natural and adaptive defense mechanisms, such as cytokines, which enables the body to help itself.

#### 4. CONCLUSION

The results of this investigation demonstrate that the herbal cream impregnating *C. fistula* fruit

extract showed good activity in the psoriasis-like skin mouse model by exposing antiproliferant activity, reducing relative epidermal thickness, and inducing orthokeratosis. It is believed that the phytochemicals in the fruit extract play a key role in correcting the immune problems. At a dose of 6.25% (w/w) fruit extract, psoriasiform was ameliorated well without dermal irritation. Formulae containing extract denoted the good outcome of psoriasis; hence, clinical trials are needed to validate the potential of these galenic formulations.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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