International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
October - November, 2014, Vol. 3, No.6, pp 1265-1273
ISSN: 2278-0238

Research Article

ANTIPSORIATIC ACTIVITY OF DITHRANOL TRANSDERMAL PRONIOSOMES GEL ON SWISS ALBINO MICE

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(Received: May 08, 2014; **Accepted:** July 12, 2014**)**

ABSTRACT

Psoriasis is distinguished by hyper-proliferation and unusual differentiation of epidermal keratinocytes, lymphocyte infilteration containing mainly of T lymphocytes. Psoriasis may be treated mainly by topical and dermal route because systemic therapy or phototherapy mainly concerns with the possible tissues toxicity and sometimes result in carcinogenicity. Beyond the various presently existing management options of psoriasis, topical therapy is the most widely used. Proniosomes create beneficial choice for transfer of the drug without delay. Simultaneously, its provided better physical and chemical stability than comparison to liposomes and niosomes. Proniosomes is converted to niosomes prior to action and release of drug. Proniosomal transdermal gel of Dithranol optimized preparation was used for antipsoriatic activity on Swiss albino mice give relevant result for treatment of psoriasis, without any tissue toxicity such as redness, erythema in animal skin, as well as convulsion, tremor, circling, depression, hypothermia and mortality. Percent drug activity of Proniosomal transdermal gel (1% w/w) was found to be 79.56% which show a significant antipsoriatic effect in comparison of standard drug Derobin® (1.125w/w) 82.31%. The frequently shown different side effects associated with dithranol, in this research solved related problem of existing preparation of dithranol by using of proniosomes transdermal gel preparation on animal body as tissue toxicity study.

Keywords: Dithranol, Proniosomes, Psoriasis, Proniosomes Transdermal Gel.

INTRODUCTION

DRUG DELIVERY SYSTEM AS A TRANSDERMAL FORM

The chief aim of the research was the discovery of drug delivery system that is acceptable through skin. Transdermal drug delivery system play is vital role because this route is free from invasive methods. Topical preparation is the best patient compliant manner of drug delivery in the body1.Transdermal drug delivery system is related to drug supply as sustained release (i.e. constant plasma drug concentrations), over an extended time period2.

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. The skin is made up of three layers; epidermis, dermis, and subcutaneous tissue. The outermost level, the epidermis, made up of a specific group of cells known as keratinocytes, which function is to produce a long, threadlike protein called keratin, for a protective role. Cornifiede (corneocytes) covering of skin is makes very densely cross linked structure of protein which decreases absorption of drug into the cells3.Skin of human is most significant and targeted area/site for treatment of local skin diseases. Skin acts as rate limiting barrier for entering or penetrating of drugs in body. There are a lot of approaches

or method for penetration enhancement across the skin in which vesicular and provesicular system is one of the most promising system that fulfils this requirement. [4,5]

Psoriasis

Psoriasis is an inflammatory skin disorder characterized by hyperproliferation and abnormal differentiation of the stratified epidermis of skin. This disease also attacks joints in several patients. However, the first-line treatments of psoriasis is performed through, small molecule topical therapies e.g. dithranol (anthralin) and vitamin D analogs as calcipotriol, and topical corticosteroids. [6,7,8.]

Psoriasis is a chronic, long life, skin disease in which body immune system is mainly responsible. It is characterized by hyper proliferation of keratinocytes9. Psoriasis of first type (Type I) starts at or before 40 years age whereas second type (Type II) begins after the age of 40 years. More than 75% of patients are related to Type I disease 10. Treatment of psoriasis is totally depends on following nature of disease as Severity, cost of drug and convenience of patient, relevant co-morbidities, effectiveness, and individualization of patient response [11].

Dithranol or Anthralin (DTH)

Anthralin (dithranol) is still characterized as a significant therapeutic alternative in all age groups. In hospital location, dithranol in petrolatum base is still employed as "long-contact" therapy and it is usually applied in OPD in a "short-contact" treatment to diminish its side effects like irritation, perilesional skin staining, and stable staining of clothing 12. Dithranol 0.1% to 3% dose without problems of washable formulation is use for psoriatic plaques for half an hour and after then washed. The most extensively established topical dosage form as ointment was used but a localized and staining effect of drug such as irritation and pigmentation of skin by short contact period of time and need frequent application of drug. DTH gets collected in mitochondria (cell power house), where it obstructs with the transfer of energy to the cell, possibly by releasing free radicals from oxidation of dithranol therefore delay replication of DNA and slowdowns the extreme cell division that takes place in psoriatic plaques. Additionally, Anthralin may act by decreasing the high levels of cyclic GMP (cGMP) that occurs in psoriasis. The frequently shown side effects associated with DTH are skin rash, itching, peeling, redness, blistering, burning, stinging, swelling, or extra sign of skin irritation etc. In this research work we have overcome these problems and increased patient compliance by design of an alternative drug delivery system in form of proniosomes.

MATERIALS AND METHODS

Dithranol IP (DTH; M/s Agon Pharma Pvt. LTD. India), As a Gift Sample, Swiss albino mice animals were provided by authorized animal house of Dakash institute of pharmaceutical sciences (DIPS), Chhatarpur MP, DEROBIN® 1.15% Vardhaman Remedies Pvt. Ltd. was purchase from local drug store.

Animal Care and Handling

The animals were carried for experiment from the authorized animal house of Dakash institute of pharmaceutical sciences (DIPS), Chhatarpur MP. All the albino mice were healthy and of 25-30gm body weight. The animals were kept in air conditioning environment and temperature was maintained to 25 °C to 26 °C with conventional laboratory food and fresh drinking water. The bedding of animals was changed every 3rd day. All animals were taken care of under ethical concern as per the guidelines of CPCSEA with approval from the Institutional Animal Ethics Committee (Registration No. DIPS/IAEC/030/2014).

Acute Dermal Toxicity Study

The acute dermal toxicity test (LD50) of Proniosomal transdermal gel was determined according to the OECD (Organization for Economic Corporation and Development) guidelines no. 402 on Swiss albino mice. Healthy young adult albino mice (approx. 25-30g) of either sex were used. Animals were acclimatized to the laboratory conditions for 5 days prior to the test. Animals were divided in to 3 groups, each group consisting of 4 animals (n=4). 24 hours prior the test, fur was removed from the 10% of the body surface area from dorsal area of the back of the test animals by using hair remover cream avoiding any abrasion on skin. Gradually increasing dose (topically) of Proniosomal gel (DNPS6F8) was applied to all three groups (n=4). The treated animals of all groups were examined for 14 days for any change in fur, eyes, sleep pattern, central nervous system activity, behavior pattern, toxic reactions and time of death occurring during the dermal toxicity studies. During overall toxicity study animals were placed at optimum environmental conditions (25-30°C temperature, 30-70%

humidity) with 12:12 hours light and dark cycle with regular supply of drinking water and conventional laboratory diet. The Proniosomal gel was found safe up to the dose of 2000 mg/kg and from results suitable range of dose (1% w/w) was chosen for Anti-psoriatic activity for further study 13.

In-Vivo Anti-Psoriatic Activity

a) Mouse Tail Model

The mouse tail model is widely accepted as a testing method for measurement of anti-psoriatic activity of drugs. Principle of this model is that topical application of a mouse-tail with anti-psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic was used for evaluation of drug efficacy in animal model. The anti-psoriatic activity was executed according to mouse tail model as described in Vogel 2002, with slight modification [14, 15].

Total 15 animals were used in the present research work. Animals were divided in to three groups of five each. The first group was control which was left untreated and the second group was standard group treated with marketed ointment (Derobin®) - 1.15% w/w. The third group was treated with the 1% w/w, optimized Proniosomal transdermal gel (PTG).

b) Experimental Design

Grouping and treatment of animals

Group I: Control

Group II: Standard (Derobin® ointment, 1.15% w/w) Topical,

Group III: Test group (1% w/w, PTG)

Hairs were removed from the 10% of the body surface area from dorsal area of the back portion of all the test animals by using hair remover cream. Psoriasis was induced by a single subcutaneous injection of 0.1 ml of tumor necrosis factor on dorsal area of the back portion of all the test animals 16. After 4 days of administration of tumor necrosis factor there was presence of granular layer in the shaved area of skin which after a period of time was transformed in psoriatic lesion. After the induction of psoriasis animals were treated with respective dose of standard (Derobin® ointment, 1.15% w/w) and test formulation (1% w/w, Proniosomal gel) once daily, for 14 days to evaluate the therapeutic effect. During this period, animals were visualized daily to record the symptomatic effect and the photographs of every animal were taken from each group. Two hours after the last treatment the animals were sacrificed using deep ether anesthesia by cervical dislocation, and the sections of skin were cut from each group and stored in 10 % formalin in saline. Longitudinal sections of about 5 µm thickness were prepared by microtomy and stained with hematoxylin-eosin dye for histological examination.

Evaluation parameters for Anti-psoriatic activity

I. Measurement of Percent Orthokeratosis (OK)

An antipsoriatic drug that targets the epidermis is a compound that restores skin homeostasis by suppressing keratinocyte hyper proliferation, abnormal differentiation, or both. The granular layer is greatly reduced or almost absent in epidermis of psoriatic lesions. This parakeratosis condition is one of the most important hall marks of psoriasis. Granular layer formation around the epidermis is known as orthokeratosis condition. The main principle behind the mouse tail test is conversion of parakeratosis to orthokeratosis. Percent orthokeratosis in those parts which normally have a parakeratotic differentiation was quantified measuring the length of the continuous granular layer (A) and the length of the scale (B) and expressed as a percentage of total number of scales region per section.

$$\%\ \boldsymbol{Orthokeratosis} = \frac{\boldsymbol{Length\ of\ contineous\ granular\ layer\ (A)} \times \mathbf{100}}{\boldsymbol{Length\ of\ scale\ (B)}}$$

II. Measurement of Epidermal thickness (ET)

It was obtained by measuring the distance between the dermo-epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of the different animals was calculated. The change in epidermal thickness of standard and formulated ointment treated group was then calculated.

$$\boldsymbol{Epidermal\ Thickness}\ (\%) = \frac{\boldsymbol{ET\ of\ treated\ group - ET\ of\ control\ group} \times \mathbf{100}}{\mathbf{100} - \boldsymbol{ET\ of\ control\ group}}$$

ET = Epidermal thickness

III. Measurement of Drug activity

Drug activity is calculated by the percentage increase of orthokeratotic regions.

$$\%\ \boldsymbol{DrugActivity} = \frac{\boldsymbol{Mean\ OK\ of\ treated\ group - mean\ OK\ of\ controlgroup} \times \mathbf{100}}{\mathbf{100} - \boldsymbol{Mean\ OK\ of\ the\ control\ group}}$$

OK = Orthokeratosis

IV. Statistical Analysis

Data obtained in the present study was presented as weighed mean±standard error. In the mouse tail test for statistical comparisons, probabilities were obtained by the Tukey's multiple range test. Statistical calculations were performed using Graph Pad Prism software. Values with $p<0.05$ are considered significant.

RESULTS AND DISCUSSION

Acute dermal toxicity study

Swiss albino mice were selected for acute dermal toxicity study. Animal were divided in to three groups each group consisting of four animals. I, II and III group animals were treated topically with respectively 1000, 1500 and 2000 mg/kg body weight dose of formulated Proniosomal gel. No significant signs of toxicity were noticed in animals like redness, erythema in animal skin, convulsion, tremor, circling, depression, hypothermia and mortality. No mortality was observed up to 2000mg/kg. Hence it was observed that formulated Proniosomal gel was safe up to 2000mg/kg. Therefore 1% w/w Proniosomal gel was selected for further anti-psoriatic activity.

In-vivo anti-psoriatic activity

a) Mouse tail model

Proniosomal gel was screened for its possible anti-psoriatic activity using mouse tail model. PTG (1% w/w) and Derobin® ointment, (1.15% w/w) were applied on the induced psoriatic lesions. Proniosomal gel has increased the orthokeratotic regions by 59.14%, in comparison to control group. The standard drug Derobin® showed the increase the orthokeratotic regions by 65.35% (Table no. 5). Proniosomal gel has decreased the epidermal thickness 62.38% while standard drug decreases the epidermal thickness 69.12% (Table no. 6). Percent drug activity of PTG (1%) was found to be 68.56% which show a significant antipsoriatic effect in comparison of standard drug Derobin® 82.31% (Table no. 7)

b) Histological examination of mouse skin

Proniosomal gel of Dithranol also showed considerable change in epidermal thickness compared to control group's animals. Granular layer of the epidermis is more reduced in psoriatic lesions. Parakeratotic condition is seen in the skin which is one of the hallmarks of psoriasis17. Formation of granular layer in the region of the epidermis is known as orthokeratosis state. The key theory following the mouse tail test is alteration of parakeratosis condition to orthokeratosis. Drugs which show their mechanism of action with multiple mechanisms in the treatment of psoriasis are more significant than other drugs performing by one solitary mechanism14. This is for the reason that psoriasis is a recurring chronic inflammatory skin disorder by way of manifold pathogenic factors & etiologies.

Table no.1 Presence of redness on skin of animals of different groups

Group No.	**I**	**II**	**III**
Treatment	Topically treated with Proniosomal gel 1000mg/kg	Topically treated with Proniosomal gel 1500mg/kg	Topically treated with Proniosomal gel 2000mg/kg
Animal No	1,2,3,4	1,2,3,4	1,2,3,4
After 7 days	Not Seen	Not Seen	Not Seen
After 14 day	Not Seen	Not Seen	Not Seen

Table no.2 Presence of erythema on skin of animals of different groups

Group No.	**I**	**II**	**III**
Treatment	Topically treated with Proniosomal gel 1000mg/kg	Topically treated with Proniosomal gel 1500mg/kg	Topically treated with Proniosomal gel 2000mg/kg
Animal No	1,2,3,4	1,2,3,4	1,2,3,4
After 7 days	Not Seen	Not Seen	Not Seen
After 14 day	Not Seen	Not Seen	Not Seen

Table no.3 Behavioural changes in animals of different groups

Group No.	**I**	**II**	**III**
Treatment	Topically treated with Proniosomal gel 1000mg/kg	Topically treated with Proniosomal gel 1500mg/kg	Topically treated with Proniosomal gel 2000mg/kg
Animal No	1,2,3,4	1,2,3,4	1,2,3,4
After 7 days	-	-	-
After 14 day	-	-	-

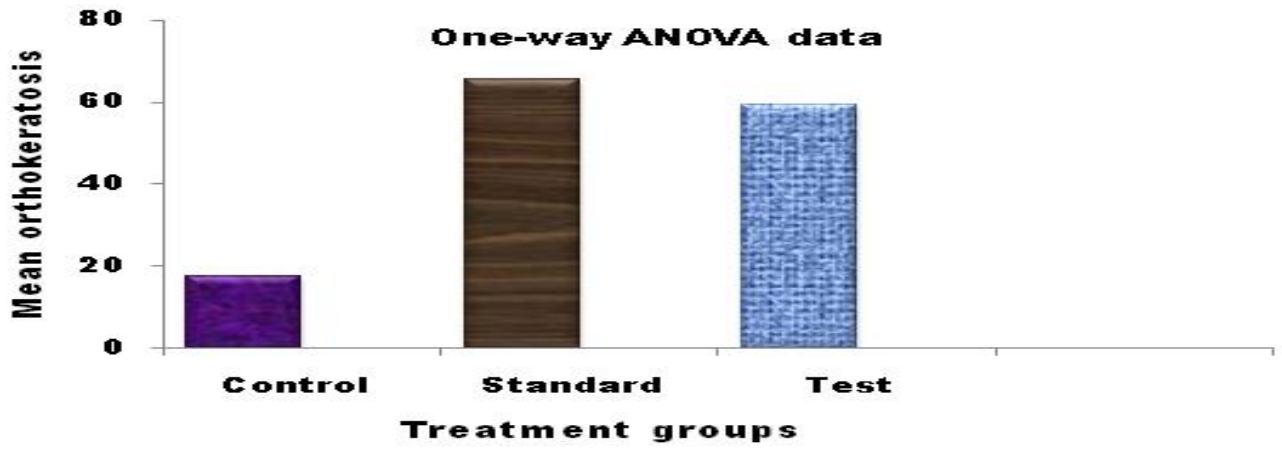
(-Sign) indicate not observed

Table no.4 Mortality during acute dermal toxicity studies of Proniosomal gel

Group No.	**I**	**II**	**III**
Treatment	Topically treated with Proniosomal gel 1000mg/kg	Topically treated with Proniosomal gel 1500mg/kg	Topically treated with Proniosomal gel 2000mg/kg
Mortality at the end of study	All animals were survived	All animals were survived	All animals were survived

Table no.5 Effect of Proniosomal gel on degree of Orthokeratosis (%) (n=5)

Group No.	**Group Name**	**% Orthokeratosis±S.E.M**
I	Control group	16.29±0.008
II	Standard group (Derobin® ointment, 1.15% w/w)	65.35±0.640****
III	Test group (1% w/w, Proniosomal Transdermal gel)	59.14±0.218****



Each bar represents % Orthokeratosis ± SEM (n= 5): *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 as compare to control group (One way ANOVA multiple comparison followed by Tukey's multiple range test)

Fig.1 Effect of Proniosomal gel on degree of orthokeratosis

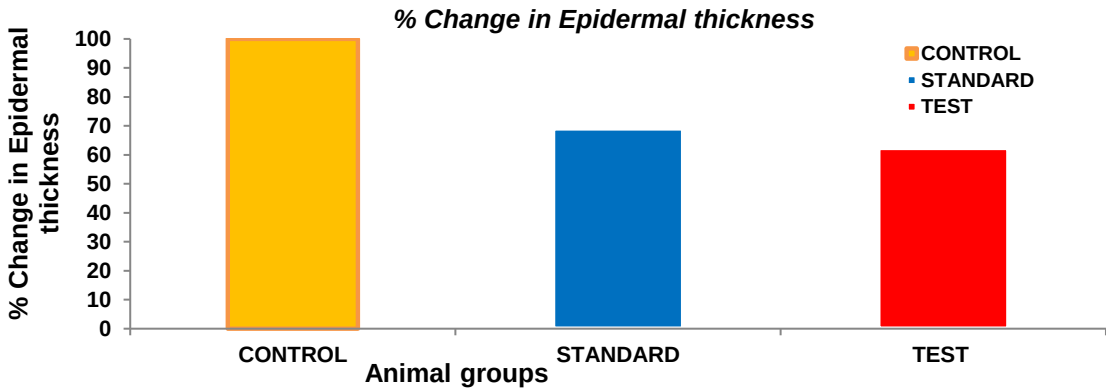


Fig.2 Effect of Proniosomal gel on change in epidermal thickness (%)

Table no. 6 Effect of Proniosomal gel on change in epidermal thickness (%) (n=5)

Group No.	Group Name	Change in epidermal thickness (%)
I	Control group	100
II	Standard group **(Derobin® ointment, 1.15% w/w)**	69.12
III	Test group **(1% w/w, PTG)**	62.38

Table no. 7 Drug activity of Proniosomal gel on skin in mouse tail model

Group No.	Group Name	Drug activity (%)
I	Control group	-
II	Standard group Derobin® ointment, 1.15% w/w	82.31
III	Test group **(1% w/w, PTG)**	79.56

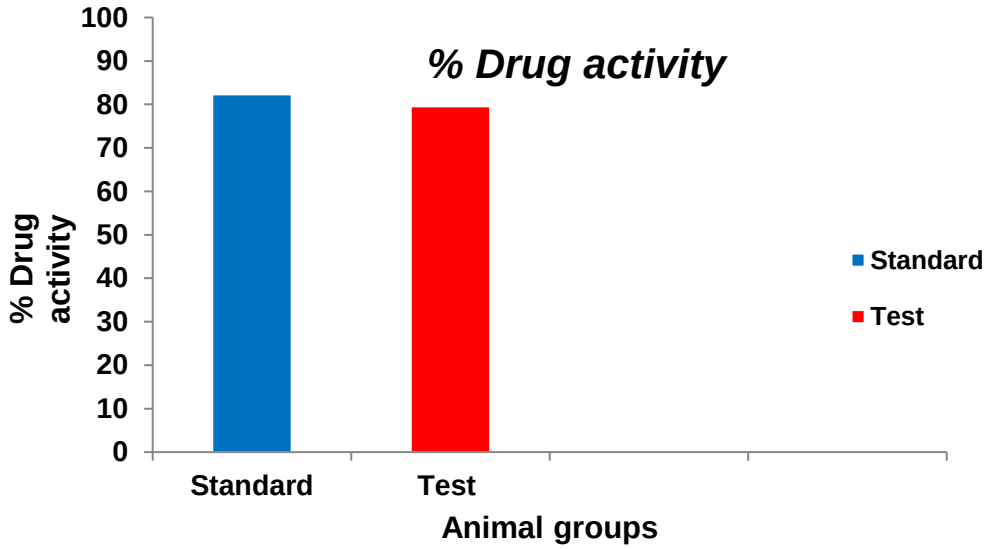
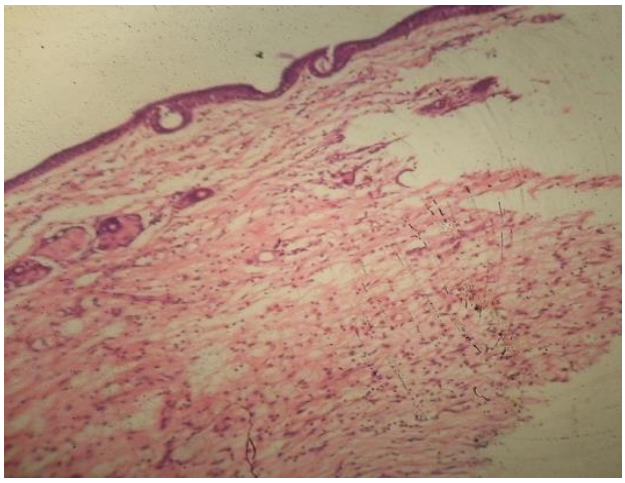
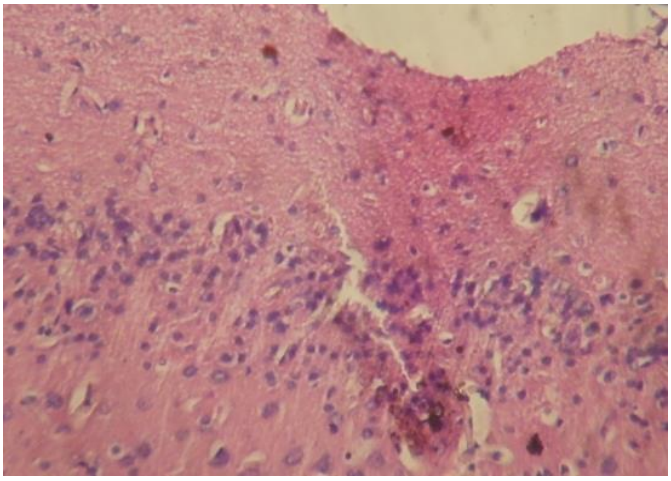


Fig.3 Percentage Drug activity on different groups



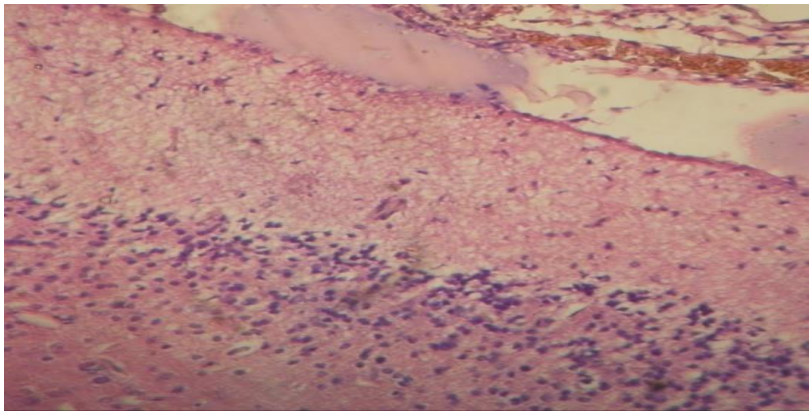
Control group

(Untreated)



Standard group

(Derobin® ointment, 1.15% w/w)



Test group (1% w/w, PTG)

Fig. 4 Histology of psoriasis with control, standard and test group treatment

CONCLUSION

Proniosomal transdermal gel (PTG) of dithranol offers an enormous potential to diminish the side effects of drugs and amplify the therapeutic value, as a semisolid liquid crystal preparation by nonionic surfactants cholesterol and lecithin. The present research investigation decidedly prove the huge potential of provesicular system in accomplishment of elevated skin permeation flux along with high skin retention of dithranol and with improved pharmacodynamically, antipsoriatic activity without any skin irritation.

Acknowledgement

Authors are thankful to Mr. Amit Srivastava Bhopal, MP, Mr.Prashant Gupta from Dakash institute of pharmaceutical sciences, Chhatarpur MP, India for their suggestion and motivation during the work. The author is also thankful to M/s Agon Pharma Pvt. Ltd., India providing the gift samples of dithranol, Dakash institute of pharmaceutical sciences, provided lab facilities for completion of research work India.

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