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Antipsoriatic activity of imiquimod induced wistar model treated with cholecalciferol nanoemulsion with UV therapy

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Abstract

Psoriasis is the most common autoimmune skin condition affecting around 2-3% of world population which in severe cases found associated with comorbidities like arthritis and heart condition, etc. To measure the severity of the illness based on induration, erythema, and scaling, psoriasis area severity index PASI score is used. Present study was performed to analyse PASI score by conducting antipsoriatic activity of cholecalciferol containing nanoemulsion CONE with UV exposure in imiquimod induced psoriatic wistar model. The study was conducted in four groups; namely, Group I control, Group II test, Group III normal and Group IV imiquimod treated. During extensive study of 44 days, including induction of psoriasis under standard conditions as per CPCSEA guidelines, followed by its treatment with fixed doses of 40000 IU of CONE and UV at 395 nm topically starting from 14th day on groups. Out of which, healing rate was high in Group II CONE with UV treatment and showed synergic effect in wistar model and PASI score was found to be low as compared to other groups. Thus, it can be concluded that antipsoriatic activity of CONE had positive effect on imiquimod induced psoriatic wistar model.

1. Introduction

In India, the earliest reference to curative properties of some plant medicines were mentioned in Rigveda (years 3500-1800 BC). Among the four vedas, Rigveda, Yajurveda, Samaveda and Atharveda, the last veda is also considered to be precursor of Ayurveda (Nagaiah, 2023). Many of drugs derived from various sources like natural, synthetic and semisynthetic. Abdallah *et al.* (2011) around 2-3% of people around the world are affected by psoriasis. Out of which, 30% of patients experience illness onset before the age of 18, in some cases, in adults under 20-30 years of age and some at 50-60 years of age. It affects men and women equally, mostly children and young people are affected, and get a miserable existence with diseased conditions. It is an autoimmune condition since our own immune cells mistakenly start attacking healthy skin cells which result in red bumpy and patchy skin covered with silver scales. An average life cycle of human skin is about 7 weeks, migrate from the lower layer of the epidermis to the dead horny layer of the stratum corneum in a healthy person, but due to some risk factors like stress, trauma, inflammation, or infection or genetic predisposition, psoriasis get aggravated and it becomes evident that the cycle gets completed in 3-4 days and forms scaly silver dead cell patch which is made up of keratinocytes. Skin cycle is too fast in psoriasis patients as compared to normal healthy people presented in Figure 1, how normal skin turned into dark color thickened, dried slivery patches in plaque psoriasis, and shaded off in dried form as dead cells.

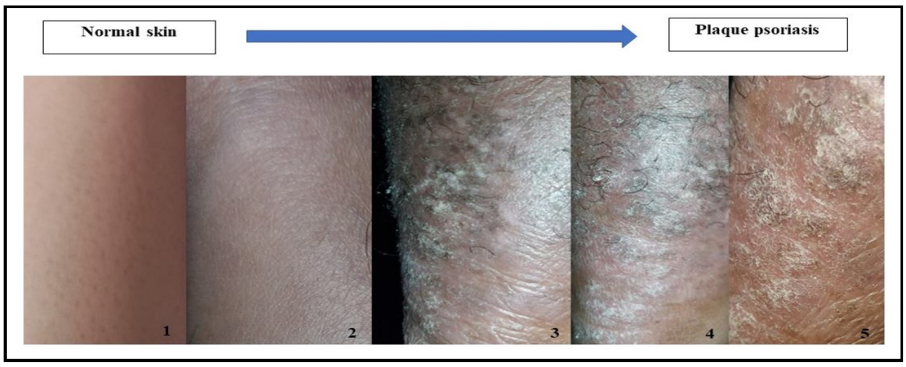

Figure 1: Various skin conditions from normal cell to plaque psoriasis.

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1.1 Types of psoriasis

Langley *et al.* (2005) described and discussed in details about types of psoriasis basically the scalp, elbows, knees, hands, and feet are the most frequently affected locations; however, nails and delicate

genital tissues can also be affected presented in Figure 2. Most people have one type of psoriasisat a time.

1.1.1 Plaque psoriasis/psoriasis vulgaris

The symptoms of this type of psoriasisis inflamed skin with red skin color with silvery patch, causing burning sensation. It is generally seen in the elbow, knees, hands, and back portion of the body, and can grow even on the scalp.

1.1.2 Guttate psoriasis

Generally, small pink spots seen in thigh, trunk, upper arm and elbow in children and young adults. In this type, skin seems red, smooth and shiny but it has no scales. It is present generally in the breast area, skin folds, genital and buttocks area ,*etc.* It can occur due to sweating, fungal infection and friction.

1.1.3 Pustular psoriasis

It is found in adults, consisting of pustules (bumps with pus) on hands and legs. Sometimes, it occurs in the whole body and is called generalized pustular psoriasis.

1.1.4 Erythrodermic psoriasis

This is the least common type but poses serious threats. It appears to be burnt skin like patches all over the skin surface. It gives a fast heart rate, burning sensation and peeling appearance with change in body temperature. Severe allergic reactions and sunburn are the triggers for these diseases.

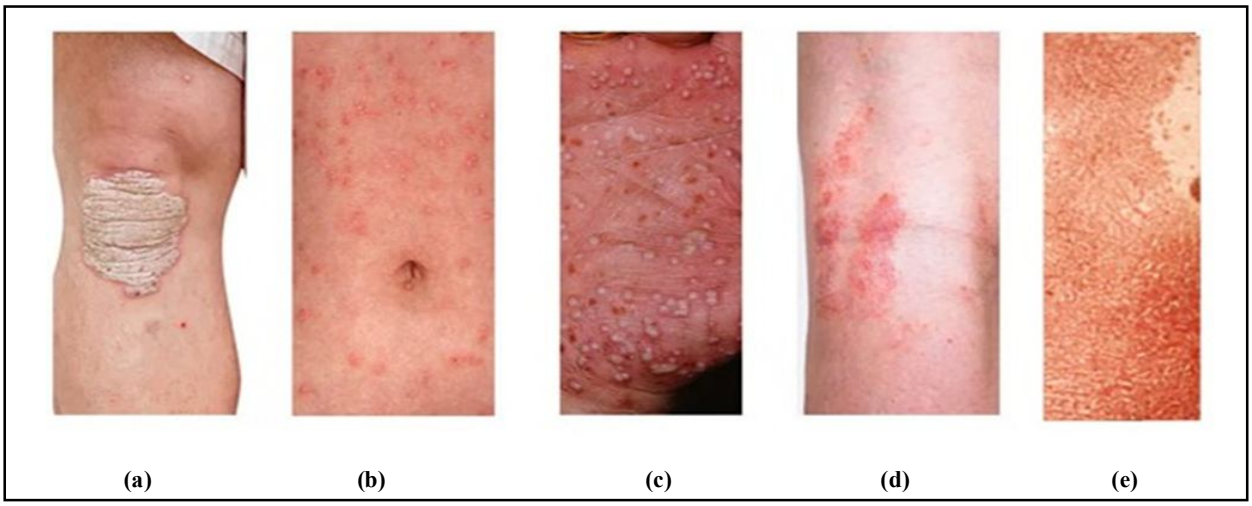

Figure 2: Different types of psoriasis: (a) Plaque psoriasis, (b) Guttate psoriasis, (c) Pustular psoriasis, (d) Inverse psoriasis, (e) Erythrodermic psoriasis in human.

1.1.5 Nail psoriasis

It is generally seen in psoriatic arthritis patients. Structure of the nail changes rapidly causing tendering, pain, separation and color change.

1.2 Kamiya *et al.* (2019) discussed the conditions or factors that make people susceptible which is of two types:

1.2.1 Extrinsic factors

Air pollution, stress, infection, vaccination, alcohol and smoking; sometimes antimalarial drugs, blood pressure medications, mood stabilizers, antibiotics, and non-steroidal anti-inflammatory drugs may sometimes trigger the condition.

1.2.2 Intrinsic factors

Obesity, metabolic syndromes, hypertension, mental stress, diabetes mellitus, dyslipidemia, unhealthy levels of one or more kinds of lipid in blood. Specific pathogens, such as bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*), viruses (endogenous *retroviruses* and human *papillomavirus*), and fungi (*Candida albicans* and *Malassezia*) can cause or aggravate psoriasis (Wang and Jin, 2018; Fry and Baker, 2007). There are following drugs and dosage form available in market used for the treatment of plaque psoriasis as shown in Table 1. There is a need to improve dosage forms for effectiveness and complete cure.

Zhu *et al.* (2022) discussed pathophysiology of psoriasis in which it is characterized by immune related cells like T cells and dendritic cells, genetic predispositions and role of lipoprotein-2, galactosin-3, antimicrobial peptides, human neutrophilic peptides, *etc,* in the progression of psoriasis. They reviewed traditional treatments from corticosteroids, vitamin D3 analogues, calcineurin inhibitors, methotrexate, cyclosporine, acitretin to biologics to phototherapy, *etc*, along with nanotechnological interventions for effective treatment of psoriasis. Gran *et al.* (2020) highlighted the fact that whether psoriasis is considered majorly as T lymphocyte-mediated disease in which innate immune cells and pathogenic T cells gets activated, resulting in skin inflammation and hyperproliferation of keratinocytes; recent findings elaborate role of B cells in inflammatory skin diseases.Treatment of psoriasisis based on the control of symptoms. Topical, systemic therapies as well as phototherapy are available. In practice, a combination of these methods is often used .Marwaha and Dabas (2019) reviewed traditional vitamin D formulations that are fat-soluble have been utilized for medicinal and preventive purposes for years. Recent technological developments have made it possible to provide vitamin D *via* nanoemulsion formulations, which guarantee greater medication delivery and absorption. They discussed difficulties

surrounding the bioavailability of vitamin D nanoemulsion preparations in comparison to traditional fat-soluble preparations. Phototherapy is a good option for treatment of plaque psoriasis. It involves exposure of affected skin to certain types of light in different frequencies alone or sometimes in combination with oral and topical medications. Kaul *et al.* (2018) addressed the emerging advancements of nanotechnology in the field of cosmeceuticals. They reviewed various novel carriers used for the delivery of cosmeceuticals, their pros and cons, its marketed formulations, toxicity, and regulations. (Soleymani *et al.*, 2015). In addition, vitamin D3 has been reported to inhibit keratinocyte growth in culture and controls epidermal differentiation. Kim (2010); Wolverton (2007); Krueger and Griffiths (2005) reviewed how epidemiology, clinical manifestations and plaques of psoriasis adversely impact the quality of life of patients physically, emotionally and, psychosocially.

Table 1: Classification of drugs used in treatment of Plaque Psoriasis

Drugs	Examples	Dosage forms
Topical corticosteroids	Retinoid, betamethasone, tazarotene	
Vitamin D analogs	Calcifediol, calcijex, calcitriol doxercalciferol, hectorol, paricalcitol, rayaldee, rocaltrol, zempler	
Anthralin	Dithranol	
Topical retinoids	Retinol, tretinoin, adapalene, tazarotene, alitretinoin, and bexarotene	Cream, ointment, spray, gel, lotions, liquid forms, foam,,*etc.*
Calcineurin inhibitors	Tacrolimus and pimecrolimus	
Anti-inflammatory	Salicylic acid derivatives	
Petroleum product	Coaltar	
Immune inhibitors	Methotrexate, cyclosporine	
Immune modulators	Glycyrrhizin, leflunomide	

2. Material and Methods

2.1 Drug

The drug cholecalciferol was analytical grade and procured from Sigma Aldrich, Mumbai, India for nanoemulsion preparation. The nanoemulsion of cholecalciferol was pre-optimized by Box Benkhen design prepared by probe Sonication method.

2.2 Excipients

Excipients like soybean oil, methyl paraben, sodium dihydrogen phosphate, potassium hydrogen phosphate, sodium chloride and many more purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India. Imiquimod (imiquad 5% w/w) procured from Glenmark Pharma Private Limited, for inducing plaque psoriasis in animal model. Hair removing cream (veet) and vaseline white petroleum jelly cream, procured from Hindustan Unilever Ltd. purchased from market.

2.3 Approval for study

As per the guidelines of the committee for the purpose of control and supervision of experimental animals (CPCSEA), below experiment was carried out with reference number 779/CPCSEA/IAEC/2022/001, approved by Institutional Animal Ethics Committee (IAEC) of Devi Ahilaya Vishwavidyalaya, Indore, Madhya Pradesh, India.

2.4 Animal study model

All wistar rats (male) selected for study, aged between 7-8 weeks. Study done at Animal house, Devi Ahilaya Vishwavidyalaya, Indore, Madhya Pradesh, India, housed 7 days prior to experimentation. All animals handled according to CPCSEA guidelines and regulation. The study procedure was preapproved by Institutional Animal Ethics Committee (IAEC) of Devi Ahilaya Vishwavidyalaya, Indore (Madhya Pradesh), India. About 20 animals wistar rat having 150-300 g weight, issued for study. The study imiquimod induced antipsoriatic model was performed. To find the antipsoriatic property of cholecalciferol nanoemulsion in combination with ultraviolet light.

2.5 Segregation of animals

In Groups1, 2 and 4 wistar rats were shaved dorsally with hair removing cream (veet) and marked properly one day prior, segregated in 4 groups; namely, Group I control, Group II test, Group III normal and Group IV imiquimod treated; each group consists of 5 animals kept in cage on regular diet as per regulation and guidelines.

2.6 PASI scoring

The PASI clinical scoring system was used to assess the inflammatory status of the mice dorsal skin for all the days. It included the visual examination, followed by three parameters: erythema (redness), induration (thickness) and desquamation (scale) on the back skin of each rat. Each parameter was given a score between 0 and 4 (0-none, 1-slight, 2-moderate, 3-marked, 4-very marked). Mean values were calculated.

2.7 Statistical analysis

All data analysed statistically, using Prism GraphPad software, 2021.The mean ± standard deviation of n=5 measurements used to conduct data analysis and the significant difference was set at $p<0.05$.

2.8 Ultra violet radiation source

UV light torch 12 LEDs UV portable flashlight with 395 nm wavelength, the diameter of this torch is 1.18 inches and length was 3.66 inches used for study purchased from Qpets®.

2.9 Experimental evaluation

2.9.1 Antipsoriatic activity

The protocol followed by Vander Fits *et al.* (2009). To measure the effect of optimized nanoformulation with ultra violet radiation treatment on imiquimod induced psoriasis in wistar model. 20 animals taken then segregate in 4 groups. Each group consist of 5 animals (n = 5) presented in Table 2. Zhang *et al.* (2022) the uv light source used in range of 200-395 nm, approx. 395 nm wavelength on short duration of time. 15 numbers of wistar rats at 8 to 11 weeks of age old about 150-300 g weight received a daily topical dose of 62.5 mg of commercially available imiquimod cream (5% w/w) on the shaved back for 5 or 6 consecutive days, translating in a daily dose of 3.125 mg of the active compound. This dose was empirically determined to cause most optimal and reproducible skin inflammation in wistar model. After a consecutive day of imiquimod treatment, the scaling, erythema, inflammation, hardening of skin visible, then treatment with optimized formulation along with phototherapy given and observed the changes. Psoriasis was seen on the 12th day then treatment was started. Control, Group I wistar rats were treated similarly with a control vehicle cream, vaseline cream. Group II, est I cholecalciferol containing nanoformulation applied dorsally on marked shaved area and exposed to UV light over the increased lap of time during each consecutive day started from 10 sec to 5 min. Group III, normal group animals were maintained at a normal diet and no nanoformulation and any other cream applied dorsally.

Table 2: Antipsoriatic activity by various groups

S.No.	Groups	General informations
1	I	Control + IMQ + vaseline
2	II	Test I + IMQ + CONE and UV light
3	III	Normal
4	IV	Imiquimod(IMQ)

Imiquimod: IMQ; CONE: Cholecalciferol nanoemulsion

All treated groups compared to control group as per PASI score and imiquimod treatment. For this study, uv phototherapy system used to determine the effect of UV light with optimized CONE formulations. The ultra violet light source used in the range of 200-395 nm, approx. 390 nm wavelength on short duration of time (10 sec to 5 min) increased day-by-day with fixed doses of 40000 IU of CONE topically applied so that optimal effect achieved in Figures 3 (a and b).

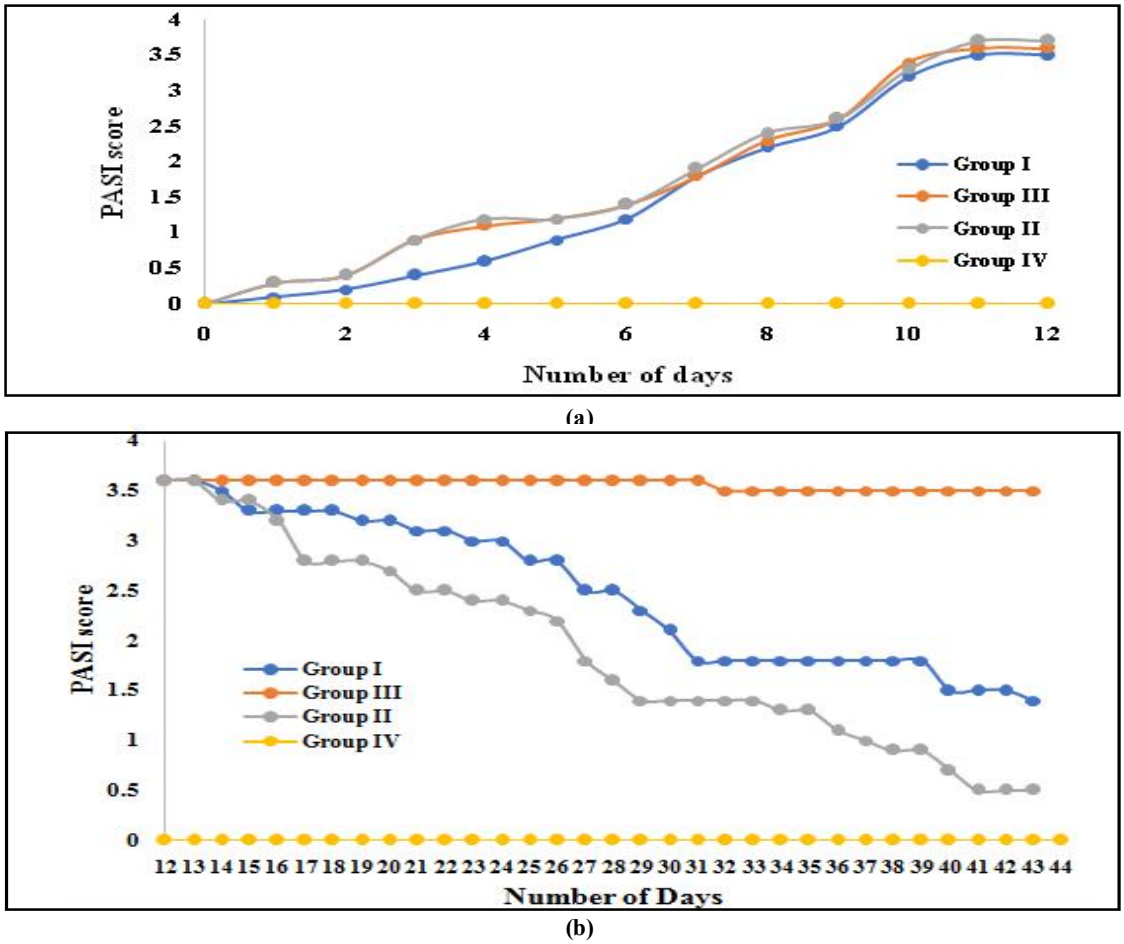

Figure 3: (a) Psoriatic induction by imiquimod in animal model, (b) Effect of optimized CONE formulation with UV on plaque psoriasis after 14 days.

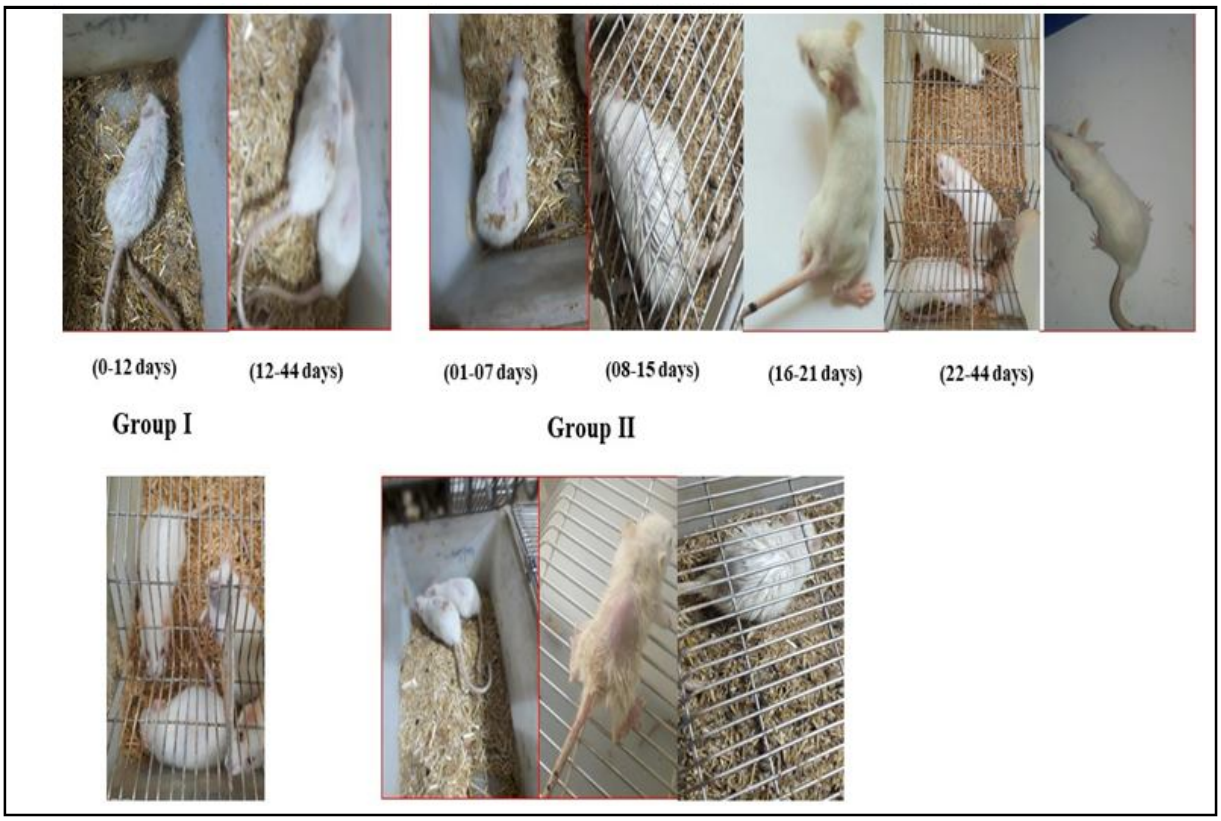

Figure 4: Antipsoriatic activities of CONE with uv radiation inimiquimod induced wistar model.

3. Results

Test cholecalciferol nanoemulsion Group II compared with disease control Group I and imiquimod treatment as per PASI score. PASI score gave the rate and extend of severity of psoriasis referred Figures 3 and 4 for more details. Antipsoriatic activity performed using CONE and the effects observed after 10 days of disease induction. The treatment begins at 11th day along with UV radiation. The duration of UV treatment was 10 sec to 5 min increased day-by- day. High exposure of UV light leads to chances of skin cancer in animal. The erythema began on 10th day, induration, thickness seen on 13th day desquamation and redness reduction begins. The treatment began on 14th day. The keratin depletion begins at 16th day and complete healing seen on 40th day only in Group II. Vitamin D help in depletion of keratinocyte layer and boost immunity stop triggering mechanism. Recovery rate was faster observed as compared to Groups I and IV.

4. Discussion

Imiquimod inducing wistar model treated with cholecalciferol containing nanoemulsion CONE with wavelength (395 nm) ultra violet radiation source had positive effect on imiquimod induced psoriatic wistar model. The Psoriasis Area Severity Index score is used to measure the severity of the illness based on induration, erythema, and scaling. Antipsoriatic activity performed with CONE and the effects observed after 10 days of disease induction. The treatment begins on 14th day along with UV therapy. The ultra violet light source used in range of 200-400 nm, approx. 395 nm wavelength on short duration of time. The duration of radiation exposure (10 sec to 5 min) increased day-by-day with fixed doses of 40000 IU International unit of CONE. Group II topically so that optimal effect achieved, high exposure of UV light leads to chances of skin cancer in rats. The erythema began on 10th day, induration thickness seen at 13th day desquamation began. Combinational therapy of CONE with UV radiation on short duration may help and effective. Complete healing seen in 44th day in Group II. There is a need to explore combination of cholecalciferol nanoemulsion with cold plasma therapy which can be substitute of UV therapy and finding new treatment of plaque *psoriasis* in future. The study clearly showed the less and minimum effect seen in IMQ treated group. Both groups Group I and Group IV, the redness, erythema but cure was delayed and lack of hairs on shaved surface seen on 44 days. Group II showed promising results.

6. Conclusion

It can be concluded from present study that cholecalciferol containing nanoemulsion in combination with UV radiation was found to be more effective in treating psoriatic lesions in wistar rat model. Although, the efficacy on psoriatic lesions might differ in response to duration of topical or systemic therapy and immune condition; hence, further studies experimenting different intensities of UV are needed. Various clinical studies suggest potential of cold plasma therapy in treating psoriatic lesion, which is in developing phase in India; hence, CONE with cold plasma exposure will also be tested in further studies.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article

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