JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/29734
RESEARCH ARTICLE

Topical Application of Ursolic Acid Cream Ameliorates Imiquimod-induced Plaque Psoriasis in BALB/c Mice

Precious Derera[1], M. Sumithra[2*], V. Chitra[2], R. Mrinalini[2] and Rukaiah Fatma Begum[2]

[1]Department of Pharmacy, Harare Institute of Technology, Harare - BE 277, Zimbabwe
[2]Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur - 603203, Tamil Nadu, India; sumi26379@gmail.com

Abstract

The valued studies of alternative psoriasis treatment options are in a much higher need among the Scientific Community. This study aimed to evaluate the anti-psoriatic activity of ursolic acid cream in imiquimod-induced psoriasis in BALB/c mice. The creams containing ursolic acid, a pentacyclic triterpenoid at percentages of 0.1 and 0.2% were formulated. The pH, spreadability, physical characteristics and acute dermal irritation of the cream were assessed. Animals were grouped into five each having 6 animals. Clobetasol, a topical corticosteroid, was used as the standard. One group was used as control and four groups were treated with the formulated imiquimod cream while receiving treatment. Parameters such as skin inflammation severity, ear thickness, plasma level of interleukins (IL)-17, histology of the back of the skin and spleen weight were evaluated. Erythema and scales were scored on a daily basis with the 0.1 and 0.2% ursolic acid cream significantly ameliorating psoriatic-like symptoms in a manner comparable to clobetasol. Imiquimod-induced epidermal hyperplasia and inflammation were inhibited by topical application of ursolic acid as shown by the results of histopathology. Spleens of the positive control group were larger in comparison with the rest of the groups. BALB/c mice treated with ursolic acid creams exhibited a decrease in the plasma levels of cytokines IL-17 when compared to the positive control group. The result of this study provided an insight that topical application of ursolic acid can be a potential treatment for psoriasis.

Keywords: Imiquimod, Inflammation, Interleukin-17, Psoriasis, Ursolic Acid

1. Introduction

Psoriasis, a chronic inflammatory skin disease, affects a population of approximately 1-2%[1]. It is characterized by an excessively rapid proliferation of skin resulting in plaques of thickened scaling skin[2]. Prevalence of this condition increases with age and usually begins in the third decade[3]. The most common type of psoriasis is plaque psoriasis affecting about 85-90% of the patients. The other types include: guttate psoriasis, erythrodermic psoriasis and pustular psoriasis[4]. The scalp skin, lumbo-sacral regions, knees, glans penis, and intergluteal cleft are mostly affected. The lesions are well dermacarted with salmon-pink-coloured plaques covered with loose adherent silver-white coloured scales[1]. A lot of factors are known to cause or precipitate psoriasis with hereditary being the most common cause. Other factors are physical trauma towards skin, stress, infections, and medications[5]. Smoking has been linked with exacerbating psoriasis[6].

Psoriasis is an immunological disease, occurring due to genetic susceptibility and environmental factors[2]. It is a T-cell mediated autoimmune response to an unknown inciting antigen which can either be self or environmental[7]. There are different mediators in psoriasis including dendritic cells and T-cells. The inflammatory dendritic myeloid cells secrete IL-12 and IL-23 that will trigger

Article Received on: 20.04.2022 Revised on: 26.08.2022 Accepted on: 07.12.2022

the production of Th22 cells, Th1 cells and T cells which will secrete IL-17 and manufacture more cytokines that causes psoriasis such as IL-17, interferons-gamma (INF-gamma), IL-22, and tumor necrosis factor-alpha (TNF-a). The cytokines will mediate effects on keratinocytes to increase the extent of psoriatic inflammation[8].

Co-morbidities linked with psoriasis include psoriatic-arthritis, metabolic syndromes, diabetes, immune-mediated conditions (like Chron's disease), multiple sclerosis, psychological conditions[2] and metabolic syndrome with increased insulin resistance and cardiovascular risk[9,10].

Pharmacological agents employed in the management of psoriasis are topical agents, systemic therapeutics (including biologics), and phototherapeutics[11]. The problem with the conventional therapy available is that some of them are expensive, while some have compliance issues and some are associated with side effects on repeated use. For example, coal tar products used in treatment of mild psoriasis are not preferred by patients because of the odour and staining of skin and clothes after application, thus limiting their use. Topical retinoids are associated with side effects like skin irritation and this can result in the patient being non-compliant since repeated usage is required for the treatment[12].

Medicinal plants have been a vital constituent of traditional medicines. Medicinal plants are significant as they are an abundant source for all-natural drug-associated research and developments. They are sources of bioactive agents and phytopharmaceuticals which are used in the pharmaceutical industry. One of these plant bio actives are the triterpenoids which are attracting a lot of interest due to their anticancer, anti-inflammatory and antioxidant activities[13].

In the developing nations, a huge lot of people show dependence on their respective traditional herbal medicines. Nowadays herbal creams are becoming more acceptable when compared to the synthetic marketed products[13]. Since a large number of people now opt for medicines that are from natural sources, this has led to the formulation of a cream containing ursolic acid which is a plant bioactive.

Ursolic acid (a pentacyclic triterpenoid) has been extracted from a variety of berries, like cranberries (*Vaccinium macrocarpon*)[14], in higher concentration from thyme (*Thymus vulgaris*) leaves, apple *(Malus domestica*) fruit peel, hawthorn (*Crataegus spp*.) leaves and flowers, rosemary (*Rosmarinus officinalis*) leaves, *Sambucus nigra* bark and leaves, eucalyptus bark and leaves, marjoram (*Origanum majoram*), *Origanum vulgare* leaves, coffee (*Coffee arabica*) leaves, lavender (*Lavandula angustifolia*) leaves and flowers, sage (*Salvia officinalis*) leaves and the wax layer of several edible fruits[15]. Ursolic acid has a broad range of biologic activities that includes the following - anti-inflammatory[16], anticancer[17], anti-tumor[18], hypoglycemic, antioxidant[19], antibacterial[19] and hepatoprotective[20]. However, no prior research has been performed globally putting Ursolic Acid to test against psoriasis. Hence, our study aimed to perform the study and determine the efficacy of Ursolic Acid against clinically significant psoriasis condition in psoriasis-induced Balb/c mice.

2. Materials and Methods

2.1 Materials

All reagents were purchased from SRL Chemicals and ursolic acid was purchased from ACROS ORGANICS. Male BALB/C mice (25-30 g) were obtained from King Institute, Guindy, Chennai, Tamil Nadu, India.

2.2 Methods

2.2.1 Formulating the Ursolic Acid Cream[21]

The cream was prepared with both water and oil phase. The oil phase contained White beeswax (16%), Mineral oil (50%) and Ursolic acid 0.1 and 0.2%. The water phase contained Methyl paraben (0.5%), Borax (0.7%) and Water (30%).

2.2.2 Evaluating the Ursolic Acid Cream[22]

2.2.2.1 Physical Properties

The creams formulated were then assessed by checking the colour, odour and appearance.

2.2.2.2 Determination of pH

5 grams of the prepared Ursolic Acid cream was dissolved in 45ml of distilled water. The pH was then measured by employing a digital pH meter at 27°C.

2.2.2.3 Spreadability

3 grams of the sample was applied at the center of a glass slide and another glass slide was placed on top of it. The glass slides were pressed together by placing a weight of 0.1 kg on top for 5 minutes. After that, the time taken to separate the two glass was measured and used to calculate

the spreadability. Determinations were done in triplicates and the average of the three readings were recorded. Spreadability (S) is calculated via the formula:

S= m*l/t

Where,

S – Spreadability

l- Glass slide's length

m- Weight that was tied to the upper glass slide (30 gm)

t- Time taken for the glass slides to separate.

2.2.3 Maintenance of Animals

All animals were housed at relative humidity (55±5%), optimum temperature (25±2°C) and 12 hours light /12 hours dark cycle. The mice were acclimatized to the laboratory conditions for a period of 10 days before the commencement of study. Standard pellet and water was given ad libitum. Experimental protocol was authorized by Institutional Animal Ethical Committee (IAEC) following CPCSEA. The proposal number submitted to CPCSEA was IAEC 178/2017

2.2.4 Acute Dermal Irritation Studies (OECD 404)[23]

As per OECD 404 guidelines, one animal was used for each sample by single dose method. 0.5 gram of 0.1 and 0.2% ointment were applied onto a small area of shaved skin. The cream was applied to a small area. It was covered with a gauze patch. A non-irritating tape was used to stick it. The exposure period was 4 hours; after which the residual cream was removed using water. The mice were then examined for erythema and oedema formation and the outcome was scored at 60 minute intervals during exposure. After the patch was removed, scoring was done at 24 hours, 48 hours and 72 hours. Observations were continued for 14 days. Dermal grading was done using the scale from 0 (no), 1 (very slight), 2 (well defined), 3 (moderate to severe), and 4 (severe).

2.2.5 Experimental Design[24,25]

The animals were shaved on back using hair removing cream four days before initiating the experiment. The mice were then isolated into 5 groups each having six mice. 4 groups were given a topical dose of 62.5 mg of 5% Imiquimod (IMQ) cream daily on hair less back and on the right ear of mice to develop the model of IMQ-induced psoriasis. Each mouse was caged individually. The test creams (Ursolic Acid 0.1% and 0.2%) and the standard cream (Clobetasol) were applied to the assigned groups 4 hours after IMQ application once, followed by another dose three hours later from the previous dose. The procedure was continued for seven days. Figure 1 depicts the experimental design followed.

The parameters evaluated were: scoring of severity of skin inflammation, measuring ear thickness, measuring cytokines- IL-17 levels, spleen weight measurement and skin histology.

2.2.6 Psoriasis Area and Severity Index (PASI) Score[26]

The PASI scoring was performed on the mice daily for assessing the extent of psoriasis in Imiquimod-induced mice by noting the extent of scaling and erythema on the clinically affected skin surface. For scoring, the extent of inflammation, scaling and erythema was scored from 0 to 4, where 0 (none), 1 (slight clinical signs), 2 (moderate clinical signs), 3 (marked clinical signs) and 4 (very marked clinical signs).

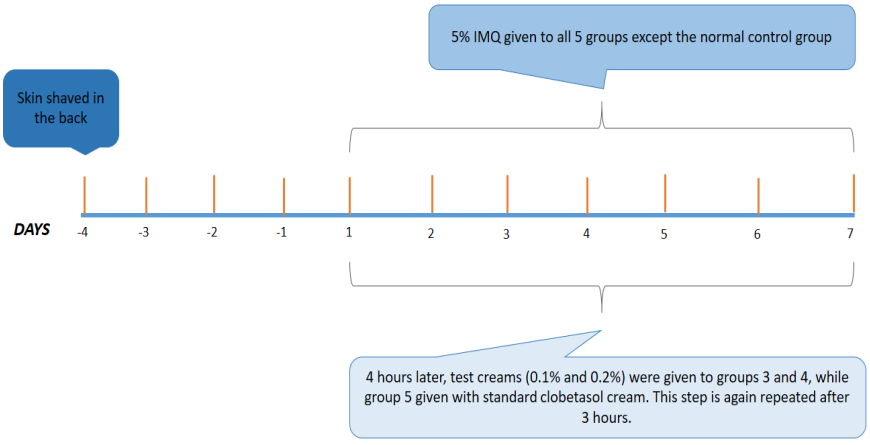

Figure 1. Experimental design of psoriasis induction and treatment.

2.2.7 Measuring Ear Thickness[24]

The ear thickness was measured on Days 0 and 7 using a digital Vernier caliper and was recorded. An elevated ear thickness was employed to indicate the extent of inflammation.

2.2.8 Measuring Cytokines- IL-17 Levels[25]

Blood was collected via the retro-orbital plexus and placed in the Eppendorf tubes having 10% of sodium citrate as the anticoagulant. It was centrifuged at 1500 rpm for 15 minutes at room temperature for plasma collection. The level of IL-17 in culture medium was determined with a commercial IL-17 ELISA kits according to the manufacturer's protocol.

2.2.9 Spleen Weight[27]

The animals were euthanized using Phenobarbitone, 24 hours after the last treatment. The animals were then dissected and the spleens were taken and immediately weighed after excision. The weight was recorded and the average was calculated.

2.2.10 Skin Histology[24]

The animals were euthanized by giving Phenobarbitone and the skin samples from the animals' back were taken and placed in bottles containing 10% formalin. After fixation, the processing as well as embedding in paraffin blocks were performed. The sample sections were prepared by employing a rotary microtome. These sectioned samples were then stained using hematoxylin and eosin stain and was observed microscopically via a digital camera system to capture the images.

2.2.11 Statistical Analysis

All of the obtained data were analyzed through one-way ANOVA prior to Dunnetts 't' test. The results were considered statistically significant if *P < 0.05, **P < 0.01.

3. Results

3.1 Evaluating the Ursolic Acid Cream

3.1.1 Physical Properties

The physical properties were assessed and recorded as shown in Table 1. The cream showed consistency and was homogenous with no grittiness or particulate matter being observed.

Table 1. Physical properties of 0.1% and 0.2% Ursolic Acid cream

Property	0.1% U.A Cream	0.2% U.A Cream
Colour	White	White
Odour	Characteristic	Characteristic
Appearance	Semi-solid	Semi-solid

3.1.2 Determination of pH

The pH of the two creams was measured after formulating, and the pH was found to be 6.3 and 6.1 for the 0.1% Ursolic Acid cream and 0.2% Ursolic Acid cream respectively.

3.1.3 Spreadability

Spreadability test was done for the test cream and standard cream. The spreadability was calculated by noting the time taken to separate the two glass slides. The time taken by 0.1% and 0.2% Ursolic Acid cream was 10 and 9 seconds respectively. Hence, the calculated spreadability of the 0.1 and 0.2 % creams was 22.5g/cm/sec and 25g/cm/sec. For the standard cream time taken was 9 seconds and the spreadability was 25.0g/cm/sec. The spreadability test showed that less time was taken to separate the glass slides, which implies that the cream is easy to spread on the skin.

3.2 Acute Dermal Irritation Studies (OECD 404)

Acute dermal irritation studies were done and the score was 0 after evaluation, during the time the patch was attached. After patch removal at 24, 48 and 72 hours, the scoring was 0 for erythema and oedema. Hence, no erythema or oedema formation was noticed after application of the finished product.

3.3 Psoriasis Area and Severity Index (PASI) score

PASI score was used in assessing severity of the skin inflammation and the results were tabulated as in Tables 2 and 3.

The scaling scoring was performed on a scale ranging from 0 (No alteration) to 4 (Very marked or distinct alteration). Erythema was noticed in positive control animals from day 3 onwards and the score was higher when compared to the other animals receiving IMQ and treatment at the same time. Erythema was marked on the positive control group, having a score of 3.5 on day 7.

Table 2. Study of erythema formation in different groups

DAY	Control	Positive control	0.1% ursolic acid cream	0.2% ursolic acid cream	Clobetasol
1	0	0	0	0	0
2	0	0	0	0	0
3	0	1	0	0	0
4	0	2	1	0	0
5	0	2.5	1.5	1	0
6	0	3	2	1	0.5
7	0	3.5	2.5	1.5	0.5

Table 3. Study of scaling scoring in different groups

DAY	Control	Positive control	0.1% ursolic acid cream	0.2% ursolic acid cream	Clobetasol
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0.5	0	0	0
5	0	1.0	0.5	0	0
6	0	2.0	1.0	0.5	0
7	0	3.0	1.0	0.5	0

The 0.1 and 0.2% Ursolic acid had a score of 2.5 and 1.5 on day 7 which was less when compared to the positive control group. Scaling was marked in the positive control group with the scoring being 3 on Day 7. In the animals receiving the treatment it was less with the 0.1% and 0.2% Ursolic acid cream having a score of 1 and 0.5. The 0.2 % Ursolic acid cream was comparable to the standard cream which did not cause any scaling.

3.4 Measuring Ear Thickness

The ear thickness was measured with a Vernier caliper, the results of which are depicted in Figure 2. The ear thickness of the animals in Group 2 increases from 0.27mm to 0.48 mm, because of the inflammation that occurs as a consequence of IMQ application. The ear thickness for groups receiving 0.1% and 0.2% Ursolic acid cream was 0.36 mm and 0.27 mm respectively.

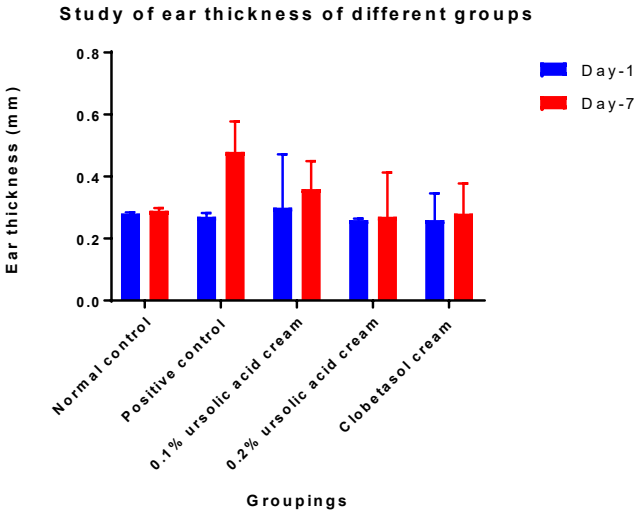

Figure 2. Graph showing the ear thickness measurements.

3.5 Measuring Cytokines- IL-17 Levels

Interleukins 17 play an important part in the pathogenesis and they result in the influx of inflammatory mediators thus causing hyper proliferation of the epidermis. The positive control group level of IL-17 was 556.14 pg/ml. For the 0.1% U.A cream, 0.2% U.A cream and clobetasol, the levels were 278.86 pg/ml, 282.24 pg/ml and 240.85pg/ml respectively. The results showed decreased levels of IL-17 in the groups 3, 4 and 5 on comparison with group-2 as depicted in Figure 3.

3.6 Spleen Weight

After harvesting the spleen, the weight of the spleens was measured and the results were as shown in Figure 4**.** From the results observed, the average spleen weight of the positive control group was 0.2414±0.54 gram (gm) whilst 0.1% Ursolic Acid cream, 0.2% Ursolic Acid cream and Clobetasol was 0.1478±0.01 gm, 0.1180±0.02 gm and 0.13440±.06 gm respectively which is lower when compared to the positive control. The normal control had a spleen weight of 0.1054±0.12 gm.

3.7 Skin Histology

The histology results as depicted in Figure 5, showed epidermal thickness in the positive control group when compared to the normal animal. The epidermal thickness was reduced in groups 3, 4 and 5 on comparison with group 2.

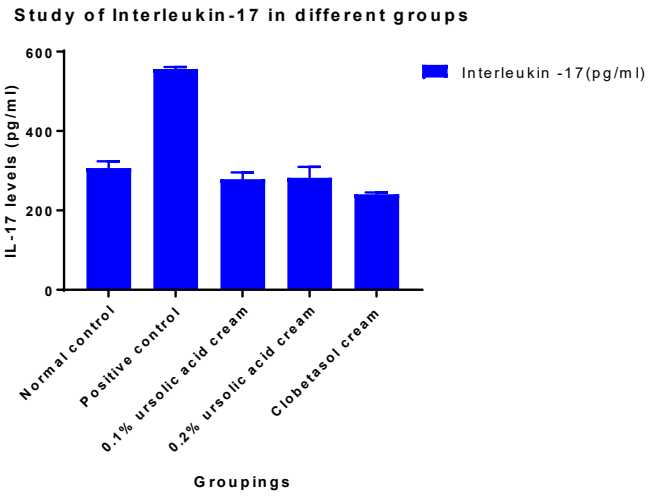

Figure 3. Graph showing the levels of IL-17 in plasma.

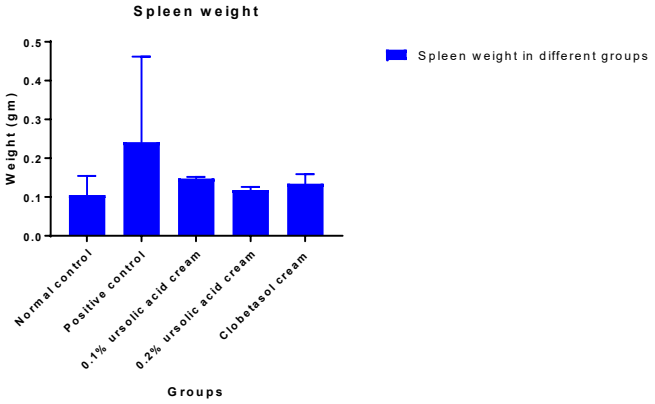

Figure 4. Graph showing the spleen weight measurements.

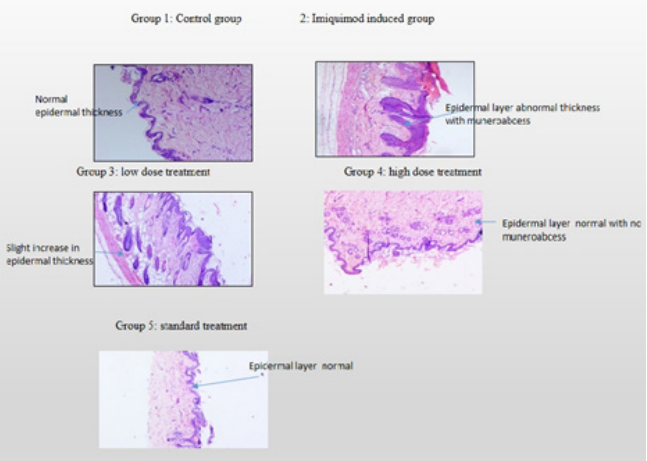

Figure 5. Skin histology results of all 5 groups.

4. Discussion

Psoriasis is a chronic inflammatory skin disease, in which skin cells build up and form scales and itchy, dry patches. This disease has come under some extensive spotlight for research, because of the social taboo associated with it. As humans, no one would want to be seen with the characteristic features of psoriasis in social gatherings and the lack of aesthetic appeal makes psoriasis treatment a necessity amongst the affected population. Although only 2-3% of the Global population is affected with psoriasis as of now, it is expected to increase by 8-9% by 2030.

These facts combined with the established truth that there is no permanent psoriasis cure and the expensive anti-psoriasis treatments with unwanted side effects and non-patient friendly formulations, made us choose psoriasis as our disease of interest. Ursolic acid was chosen as our test compound, since there were quite a few studies proving the anti-inflammatory activity of Ursolic acid by

regulating the Treg/Th17 axis, which plays a major role in psoriasis pathology. Also, the recent trend of going green, with medicines based on herbal formulations under the term of organic medicines, has gained much traction among users globally.

Ursolic acid is a pentacyclic triterpenoid and a hydroxy monocarboxylic acid found at high percentages in apple peels, leaves of olives and the holy basil or tulsi and also in berries. The exact amount of ursolic acid found in each of those sources is listed in this table towards your right. The possibility of the benefits ursolic acid would reap both in a supplemental or therapeutic formulation as well as, as a diet staple, made us wanted to explore its ability against psoriasis.

A cream formulation was considered in this study, because it is administered via the topical route, which has the following advantages like convenience, avoiding first pass metabolism, easy administration, ability to increase the when needed and the minimized side effects and toxicity to other organs[22,28]. The formulated cream passed the physical tests. The pH of the 0.1 and 0.2% ursolic acid cream was 6.1 and 6.3 respectively which is slightly acidic, thus good for the skin as the skin pH ranges between 4 to 6. The creams had a good spreadability, as less time was taken to separate slides, inferring that the cream will be easy to apply and spread with minimum force being used.

Acute dermal irritation studies showed that there was no erythema and no oedema to the animal skin after application of the 0.1 and 0.2% Ursolic acid cream. This shows that the cream is safe to use on the human skin without causing irritation.

Imiquimod was chosen as the inducing agent in this study. It is a ligand for the toll-like receptors (TLR) such as TLR 7 and TLR 8 and it is also a potent immune system activator[29]. IMQ has anti-tumor and anti-viral properties which are a result of TLR 7 and 8 activations. Such activation of these receptors, result in pro-inflammatory chemokines and cytokines production. Scaling, blisters, burning sensation, skin flaking and redness which are side effects of IMQ have made it to be used as a model for induction of human psoriasis in mice as it shows same histological and phenotypical properties[30].

The extent of psoriasis was assessed by the use of PASI scoring to determine erythema as well as scaling which was graded on a scale ranging from 0 to 4. The results showed that the IMQ induced psoriasis that can be considered clinically significant. The treatment and standard groups showed significant combat against the disease. The groups receiving treatment with 0.1% and 0.2% Ursolic Acid cream had reduced erythema and scaling with the 0.2% cream was found to be more effective. Even the ear thickness was reduced in the treatment groups than in the positive control group.

The pathology of psoriasis strongly depends on IL-17A[31], Researches have revealed that the pro-inflammatory cytokines like IL-17 have a role in the pathogenesis of psoriasis. Recently, clinical trials were conducted for a secukinumab which is a drug targeting IL-17A and the drug has been approved for psoriasis treatment[32]. Our study demonstrated that application of the topical cream containing ursolic acid can decrease levels of cytokines such as IL-17 which was less when compared to the group receiving IMQ only. The results suggested decreased levels when compared to the positive control group, however, the standard group had the least value.

The spleen is an immune system organ that plays an important role in the storage of white blood cells and fighting against disease, as it is a lymphatic organ too. It is observed that, in the positive control group, the spleen size had increased because of the immunoreaction occurring, resulting in a large influx of the number of cells in spleen. In the groups receiving treatment with the ursolic acid, the spleen weight was reduced since the ursolic acid was able to inhibit the induction of psoriasis, thus immunoreaction was decreased. The standard groups also had reduced spleen weight.

Histopathological studies of the back skin samples showed that application of topical ursolic acid decreased epidermal thickness which was comparable to the group being treated with clobetasol (standard). In the positive control group hyperkeratosis, parakeratosis and micro abscess were observed. These observations were not present in the control group and groups receiving treatment with clobetasol and ursolic acid creams showed decreased levels of IL-17 in groups 3, 4 and 5 on comparison with group 2.

The creams containing ursolic acid inhibited significant epidermal hyperplasia and scaling. The study showed that ursolic acid cream was effective in reducing erythema and scaling. The study demonstrated that ursolic acid cream reduced the development of imiquimod-induced psoriasis-like skin inflammation in the mice by preventing epidermal alteration as displayed from the histopathological studies and also inhibited Th-1 mediated pro-inflammatory cytokines like IL-17.

5. Conclusion

Ursolic acid can be a potential topical treatment in patient with psoriasis, which overcomes the long-term effects associated with topical application of corticosteroids. Ursolic acid cream prevented IMQ-induced psoriasis-like skin inflammation on the basis of the following effects observed, such as a decrease in erythema and scaling, inhibition of hyperkeratosis and reduction in the epidermal thickness, as well as, a decrease in the spleen weight and reduction in the levels of IL-17. The results of this study can be used for further researches as mentioned ahead. Ursolic acid is found to possess both anti- as well as pro-inflammatory properties. Further extensive research is needed to check, when and how Ursolic acid regulates the kind of immunological effect it produces. Food Science and Technology Research can flourish by studying if altering the UA-containing food consumption in a regular diet for a working-class lifestyle, to check the natural efficacy. Phytochemical industry can gain loads by employing Ursolic acid in studies related to Neuroinflammation (Alzheimer's Disease, Parkinson's Disease), Auto-immune disorders with inflammation etiology, and Non-Alcoholic Steatohepatitis, etc.

6. Acknowledgments

We thank Prof K.S. Lakshmi, M.Pharm., Ph.D., Dean, SRMIST College of Pharmacy and staff for providing the necessary facilities for the project to be successful. We thank Prof. Gane Venkata Sudhaka Rao, Head, Department of Pathology, Madras Veterinary College and staff for assisting and providing the facilities for conducting histopathology experiments. We express our sincere gratitude to Dr. Savithiri Shivakumar, Head, Aaranya Biosciences Private Limited for the assistance and equipping with the facilities to carry out assays.

7. References

1. Thomas PS, Pathology VK. Philadelphia, Pa. Blakiston's son; 2007.
2. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: A Pathophysiologic Approach, ed. Connecticut: Appleton and Lange. 2014; 4:141-2.
3. Walker R. Clinical pharmacy and therapeutics E-Book. Elsevier Health Sciences; 2011; Oct 24.
4. Hammer GD, McPhee SJ, Education MH, editors. Pathophysiology of disease: an introduction to clinical medicine. McGraw-Hill Education Medical; 2014.
5. Schon MP, Boehncke WH, Brocker EB. Psoriasis: clinical manifestations, pathogenesis and therapeutic perspectives. Discov Med. 2009; 5(27):253-8.
6. Naldi L. Psoriasis and smoking: links and risks. Psoriasis (Auckland, NZ). 2016; 6:65. https://doi.org/10.2147/PTT.S85189
7. Casciano F, Pigatto PD, Secchiero P, Gambari R, Reali E. T cell hierarchy in the pathogenesis of psoriasis and associated cardiovascular comorbidities. Front Immunol. 2018; 9:1390. https://doi.org/10.3389/fimmu.2018.01390
8. 8. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature. 2007; 445(7130):866-73. https://doi.org/10.1038/nature05663
9. Vena GA, Vestita M, Cassano N. Psoriasis and cardiovascular disease. Dermatol. Ther. 2010; 23(2):144-51. https://doi.org/10.1111/j.1529-8019.2010.01308.x
10. Fernandez-Armenteros JM, Gomez-Arbones X, Buti-Soler M, Betriu-Bars A, Sanmartin-Novell V, Ortega-Bravo M, Martínez-Alonso M, Gari E, Portero-Otín M, Santamaria-Babi L, Casanova-Seuma JM. Psoriasis, metabolic syndrome and cardiovascular risk factors. A population-based study. J Eur Acad Dermatol Venereol. 2019; 33(1):128-35. https://doi.org/10.1111/jdv.15159
11. Kim WB, Jerome D, Yeung J. Diagnosis and management of psoriasis. CFP. 2017; 63(4):278-85.
12. Afifi T, de Gannes G, Huang C, Zhou Y. Topical therapies for psoriasis: Evidence-based review. CFP. 2005; 51(4):519-25.
13. Kang SY, Yoon SY, Roh DH, Jeon MJ, Seo HS, Uh DK, Kwon YB, Kim HW, Han HJ, Lee HJ, Lee JH. The anti-arthritic effect of ursolic acid on zymosan-induced acute inflammation and adjuvant-induced chronic arthritis models. J Pharm Pharmacol. 2008; 60(10):1347-54. https://doi.org/10.1211/jpp.60.10.0011
14. Murphy BT, MacKinnon SL, Yan X, Hammond GB, Vaisberg AJ, Neto CC. Identification of triterpene hydroxycinnamates with in vitro antitumor activity from whole cranberry fruit (Vaccinium macrocarpon). J Agric Food Chem. 2003; 51(12):3541-5. https://doi.org/10.1021/jf034114g
15. Wozniak L, Skapska S, Marszalek K. Ursolic acid-a pentacyclic triterpenoid with a wide spectrum of pharmacological activities. Molecules. 2015; 20(11):20614-41. https://doi.org/10.3390/molecules201119721
16. Ikeda Y, Murakami A, Ohigashi H. Ursolic acid: An anti- and pro-inflammatory triterpenoid. Mol Nutr Food Res. 2008; 52(1):26-42. https://doi.org/10.1002/mnfr.200700389
17. Liu W, Tan X, Shu L, Sun H, Song J, Jin P, Yu S, Sun M, Jia X. Ursolic acid inhibits cigarette smoke extract-induced human bronchial epithelial cell injury and prevents development of lung cancer. Molecules. 2012; 17(8):9104-15. https://doi.org/10.3390/molecules17089104
18. Sultana N. Clinically useful anticancer, antitumor, and antiwrinkle agent, ursolic acid and related derivatives as

medicinally important natural product. J Enzyme Inhib Med Chem. 2011; 26(5):616-42. https://doi.org/10.3109/14756366.2010.546793
19. Do Nascimento PG, Lemos TL, Bizerra A, Arriaga A, Ferreira DA, Santiago GM, Braz-Filho R, Costa JG. Antibacterial and antioxidant activities of ursolic acid and derivatives. Molecules. 2014; 19(1):1317-27. https://doi.org/10.3390/molecules19011317
20. Jin YR, Jin JL, Li CH, Piao XX, Jin NG. Ursolic acid enhances mouse liver regeneration after partial hepatectomy. Pharm Biol. 2012; 50(4):523-8. https://doi.org/10.3109/13880209.2011.611143
21. Nema RK, Rathore KS, Dubey BK. Textbook of cosmetics. CBS Publishers and Distributors; 2009.
22. Dhyani A, Chander V, Singh N. Formulation and evaluation of multipurpose herbal cream. J Drug Deliv Ther. 2019; 9(2):341-3. https://doi.org/10.22270/jddt.v9i2.2540
23. OECD Guideline: OECD 404.
24. Sun J, Zhao Y, Hu J. Curcumin inhibits imiquimod-induced psoriasis-like inflammation by inhibiting IL-1beta and IL-6 production in mice. PloS one. 2013; 8(6):e67078. https://doi.org/10.1371/journal.pone.0067078
25. Dou R, Liu Z, Yuan X, Xiangfei D, Bai R, Bi Z, Yang P, Yang Y, Dong Y, Su W, Li D. PAMs ameliorates the imiquimod-induced psoriasis-like skin disease in mice by inhibition of translocation of NF-κB and production of inflammatory cytokines. PLoS One. 2017; 12(5):e0176823. https://doi.org/10.1371/journal.pone.0176823
26. Zhao J, Di T, Wang Y, Wang Y, Liu X, Liang D, Li P. Paeoniflorin inhibits imiquimod-induced psoriasis in mice by regulating Th17 cell response and cytokine secretion. Eur J Pharmacol. 2016; 772:131-43. https://doi.org/10.1016/j.ejphar.2015.12.040
27. Chen YH, Wu CS, Chao YH, Lin CC, Tsai HY, Li YR, Chen YZ, Tsai WH, Chen YK. Lactobacillus pentosus GMNL-77 inhibits skin lesions in imiquimod-induced psoriasis-like mice. J Food Drug Anal. 2017; 25(3):559-66. https://doi.org/10.1016/j.jfda.2016.06.003
28. Kuchekar S, Bhise K. Formulation and development of antipsoriatic herbal gelcream. J Sci Ind Res. 2012; 71(4):279-284.
29. Gilliet M, Conrad C, Geiges M, Cozzio A, Thürlimann W, Burg G, Nestle FO, Dummer R. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. Arch Dermatol. 2004; 140(12):1490-5. https://doi.org/10.1001/archderm.140.12.1490
30. Van Der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, Lubberts E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol. 2009; 182(9):5836-45. https://doi.org/10.4049/jimmunol.0802999
31. Sonja Moos, Alma N Mohebiany, Ari Waisman Florian C. Kurschus. Imiquimod-Induced Psoriasis in Mice Depends on the IL-17 Signaling of Keratinocytes. J Invest Dermatol. 2019; 139(5):1110-1117. https://doi.org/10.1016/j.jid.2019.01.006
32. Thaci D, Humeniuk J, Frambach Y, Bissonnette R, Goodman JJ, Shevade S, Gong Y, Papavassilis C, STATURE Study Group. Secukinumab in psoriasis: randomized, controlled phase 3 trial results assessing the potential to improve treatment response in partial responders (STATURE). British Journal of Dermatology. 2015; 173(3):777-87. https://doi.org/10.1111/bjd.13814