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ANTIPSORIATIC ACTIVITY OF CURCUMIN-LOADED PHYTOSOMAL GEL ON ALBINO MICE USING MOUSE TAIL MODEL

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ABSTRACT:

The hyper-proliferation and peculiar differentiation of epidermal keratinocytes, as well as lymphocyte infiltration primarily composed of T lymphocytes, are characteristics of psoriasis. Topical therapy is the most often used form of treatment. Plant-derived nutraceutical polyphenols can be delivered using vesicular carriers called phytosomes, which have greater bioavailability and improved drug penetration into the skin than liposomes. A naturally occurring polyphenolic substance, curcumin has been extensively studied for its potential to treat a variety of anti-inflammatory conditions, including psoriasis.

The purpose of the current work is to create an optimized Curcumin-Phytosomes gel formulation and employ a mouse-tail model to conduct an in-vivo investigation on albino mice. The in-vivo results validated the formulation's improved permeability and extended release. In psoriatic lesions, the granular layer of the epidermis is significantly diminished. In the mouse tail test, the topical application of phytosomal gel formulation significantly affects drug-antipsoriatic activity (%), orthokeratosis (%), and relative epidermal thickness (%). The standard drug and phytosomal gel have reduced the epidermal thickness to 44.65 ± 1.09% and 43.78 ± 1.21%, respectively. The anti-psoriatic impact of the Curcumin-laden phytosomal gel (1%) was shown to be considerable when compared to the standard medication, with percent drug activity of 56.43±1.25% and 59.65±1.18%, respectively. The scaly areas displayed parakeratosis, whereas the control group displayed orthokeratosis at a rate of 25.76 ± 1.72%. The standard formulation of Tazarotene and the phytosomal gel of Curcumin exhibited orthokeratosis values of 68.59 ± 1.15% and 67.25±1.27%, respectively. These results demonstrated the phytosomal gel-based method's ability to stimulate psoriasis patients' natural epidermal development.

Keywords: Curcumin, Phytosomes, Curcumin-Phytosomes gel

INTRODUCTION:

Psoriasis is a chronic, recurrent, inflammatory skin disease that affects 2 to 3% of the population worldwide and causes significant morbidity. Classic lesion is a well marginated, redness of the skin due to pathological changes, erythematous plaque with silvery-white surface scale, mainly distributed into extensor surfaces (i.e., knees, elbows, and buttocks), and may also involve palms and scalp. Associated findings include psoriatic arthritic and nail changes. These are inflammation, hyper proliferation of the epidermis, vascular alterations which add to the redness. Its exact etiology is unknown, but it is generally believed to be a complex autoimmune inflammatory disease with a genetic basis [1]. Histologically, psoriasis is characterized by acanthosis (thickened epidermis) and parakeratosis (nucleated cells in stratum corneum) and has been described as showing benign hyperplasia. The dermal blood vessels are abnormally tortuous and dilated, and lymphocytic infiltration is frequently seen in the dermis and occasionally in the epidermis. Therefore, some effective therapies appear to act as antiproliferative agents and diminished rates of either epidermal DNA synthesis, mitosis or both. Treatment of psoriasis includes topical, systemic, phototherapy and biological [3]. But these therapies have many side-effects. So, there is requirement of an alternative therapy like natural remedies.

Phytosomes Drug Delivery System;

The use of phytosomes is a new advanced modern dosage formulation technology to deliver herbal products and drugs by improved better absorption and, as a result, produce better results than those obtained by conventional herbal extracts. This phytosome technology is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefit, assured delivery to the tissues, and without compromising nutrient safety. Certain of the water-soluble phyto-molecules (mainly flavonoids and other polyphenols) can be converted into lipid-friendly complexes by reacting herbal drugs with phospholipids, which are called phytosomes. They are more bioavailable as compared with simple herbal extracts owing to their enhanced capacity to cross the lipid-rich biomembranes and, finally, reach the blood. They have improved pharmacokinetic and pharmacological parameters which are advantageous in the treatment of acute diseases as well as in pharmaceutical and cosmetic compositions. Phytosome is produced by binding individual components of herbal extracts to phosphatidyl choline, resulting in a dosage form that is better absorbed and thus, produces better results than the conventional herbal extracts.

Herbal drug, Curcumin;

Curcumin is the main constituent of turmeric. It is a naturally occurring hydrophobic molecule and considered to be therapeutically safe.it is a naturally present polyphenolic compound, belonging to class of flavonoids glycosides. It is also known as diferuloylmethane and has been widely studied for its therapeutic efficacy for many disorders including several inflammatory diseases. However its application is highly restricted due to its poor aqueous solubility, physicochemical instability and inadequate bioavailability. It has anti-inflammatory, antioxidant, anti-carcinogenic and anti-infectious properties. It aids in the management of oxidative and inflammatory conditions, anti-infectious properties. It may also help in the management of exercise-induced inflammation and muscle soreness, thus enhancing recovery and performance in active people. In addition, a relatively

low dose of the complex can provide health benefits for people that do not have diagnosed health conditions. Most of these benefits can be attributed to its antioxidant and anti-inflammatory effects. Ingesting curcumin by itself does not lead to the associated health benefits due to its poor bioavailability, which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination[4]. There are several strategies that can increase bioavailability. Phytosomes are such kind of technique that has been shown to increase a lot of absorption. Curcumin combined with phytosomes provides multiple health benefits. Supplementation with curcumin reliably lowers some markers of inflammation and increases the levels of endogenous antioxidants in the body.

MATERIALS AND METHODS:

Curcumin and Soya-lecithin were obtained from K Patel Phytoextraction Pvt. Ltd., Mumbai and Konark Herbals & Health Care, Daman respectively. All chemicals and reagents used were of analytical grade.

Methods:

Formation of Curcumin Phospholipid Complex (Cur-PC):

The Curcumin phytosomes were prepared by refluxing Curcumin and Soya lecithin in different ratios of (1:0.5, 1:1.5, and 1:2.5)**.** Briefly, accurately weighed amounts of Curcumin and Soya lecithin were placed into a 100 mL round bottom flask and dissolved in 20 mL of ethanol. The reaction temperature of the reflux was controlled at 50 °C using a water bath for 3 h. The resultant clear solution was dried at 45°C under vacuum to remove traces of solvents in order to obtain the Curcumin phytosomes. The prepared thin layer had been kept overnight in room temperature prior to hydration. This dried film was hydrated with 10 ml distilled water in a rotary at 45°C. The phytosomes were finally sonicated for 4 minutes in a probe sonicator, with 60% amplitude and 5 seconds on-off interval. All phytosomes batches were stored in the refrigerator.

Table 1: Composition of Phytosome formulations containing different ratios of Curcumin and Soya lecithin:

S. No.	Formulation Code	Drug: Phospholipid ratio	Temperature (^{0}C)	Ethanol (ml)
1	F1	0.5	65	20
2	F2	0.5	45	20
3	F3	1.5	65	20
4	F4	2.5	55	20
5	F5	1.5	45	20
6	F6	2.5	45	20
7	F7	1.5	55	20
8	F8	0.5	55	20
9	F9	2.5	65	20

The curcumin-phytosomes complex were characterized for different physiochemical parameters.

In-vitro dissolution study:

The in-vitro dissolution profiles of Curcumin phytosomes were studied in a USP XXIII, six station dissolution test apparatus at 100 rpm and at 37°C. An accurately weighed amount of Cur-PC equivalent to Curcumin 50 mg was put in to 900 ml of pH 6.8 phosphate buffer. Samples (5 ml each) of dissolution fluid were withdrawn at different time intervals and replaced with an equal volume of fresh medium to maintain sink conditions. Withdrawn samples were filtered (through a 0.45 µm membrane filter), diluted suitably and then analyzed spectrophotometrically at 430 nm to determine drug release from the drug phospholipid complex.

Selection of Optimized batch of Curcumin-phytosomes:

Based on the various physiochemical properties, the Expert Design software has suggested a check point batch of which in-vitro drug release was further studied.

Development of Phytosomal gel of Curcumin phytosomes:

For application of Curcumin phytosomes for management of Psoriasis, the developed formulation was converted into a gel. To the above Curcumin dispersion, weigh quantity of 0.5, 1, 1.5% w/v of Carbopol 934 was added to get uniform dispersion. The sample was stored in a dark and cool place to complete the gelling process. Triethanolamine was used to adjust the pH and to give the appearance of a clear, viscous gel. Finally, a preservative, benzalkonium chloride (0.01% w/ w) was added to the gel. The composition of Curcumin laden phytosomal gel was presented in table 3.4.

Table 2: Composition of Curcumin laden Phytosomal gel formulations

S. No.	Formulation Code	Curcumin Phytosomes	Carbopol 934 (g)	Triethanolamine	Benzalkonium chloride (%w/w)
1	Cur-OPTG1	Equivalent to dose	0.5	q.s to maintain pH equal to 7.4	0.01
2	Cur-OPTG2	Equivalent to dose	1	q.s to maintain pH equal to 7.4	0.01
3	Cur-OPTG3	Equivalent to dose	1.5	q.s to maintain pH equal to 7.4	0.01

The prepared Curcumin laden phytosomal gels were evaluated for in-vitro drug release studies.

Evaluation of Phytosomal gel

The prepared gels were physically examined for pH, consistency, viscosity, and drug content and *in-vitro* drug release. The quantification of the drug in the gel was done by UV-spectrophotometer using Methanol as blank at 430 nm. The prepared sample, 1g of formulation (Phytosomal gel) was dissolved in 10 mL of methanol and evaluated for drug content in a triplicate. From the absorbance, the mean drug content along with SD was calculated.

In-vivo study

The prepared gel formulation

Acute Dermal Toxicity Study

The acute dermal toxicity test (LD50) of Curcumin laden phytosomal gel was determined according to the OECD (Organization for Economic Corporation and Development) guidelines no. 402 on albino mice. Healthy young adult albino mice (approx. 20-25 g) 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 3 animals (n=3). About 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 phytosomal gel was applied to all three groups (n=3). 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 (25-30ºC temperature, 30-70% humidity) with 12:12 hours light and dark cycle with regular supply of drinking water and conventional laboratory diet. The suitable dose of the formulation was determined for anti-psoriatic activity.

Determination of *in-vivo* anti-psoriatic activity of Curcumin laden phytosomal gel using 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. Total 24 animals were used in the present research work (Table 3). Animals were divided into four groups of six (n=6) animals in each group. The first group was the vehicle control which was left untreated, and the second group was the negative control group treated with TNF-α. The marketed ointment (Tazarotene- 0.1% w/w) was applied to third group which was considered as positive group. The fourth group were treated with the 1% phytosomal gel of Curcumin.

Table 3: Study Plan

Group Name (n=6)	**Treatment**	**Dose**	**Route**	**Dose & Frequency**
Vehicle Control	Normal Saline	1-1.5 ml	Topical	14 days
Negative control	TNF- α	0.1 ml	SC	Once
Standard group	Tazarotene cream	0.1%	Topical	14 days
Test group	Phytosomal gel of Curcumin (Cur-OPTG2)	1%	Topical	14 days

Experimental design:

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. After 4 days of administration of tumor necrosis factor, there was the 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 (Tazarotene- 0.1% w/w) and test formulation (Phytosomal gel, Cur-OPTG2-1%) 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 was 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 were stained with hematoxylin-eosin dye for histological examination.

Evaluation parameters for Anti-psoriatic activity:

Measurement of Percent Orthokeratosis (OK)

An anti-psoriatic 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 is 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.

% Orthokeratosis= Length of continuous granular layer (A)/ Length of Scale (b) × 100

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 formulation treated group were then calculated.

$$\textbf{Epidermal Thickness (\%)} = \frac{\text{ET of treated group-ET of Control group}}{\text{100-ET of Control group}} \times 100$$

ET = Epidermal thickness

Measurement of Drug activity:

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

$$\%\ \text{Drug Activity} = \frac{\text{Mean OK of treated group-mean OK of control group}}{\text{100-Mean OK of the control group}} \times 100$$

OK = Orthokeratosis

Result and Discussion:

In-vitro dissolution study:

The prepared Curcumin phytosomes were evaluated for in-vitro dissolution study and results were depicted in the table 4. The in-vitro dissolution study revealed that maximum release was found to be 90.83% from batch F9 at the end of 12 hr.

Table 4. Drug release from Curcumin Phytosomes formulation batches F1-F9

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	12.85	10.52	20.84	16.92	11.96	18.44	15.79	13.15	22.89
2	25.93	22.83	30.68	28.46	24.69	29.37	27.47	26.81	31.65
3	34.41	31.92	39.27	37.28	33.24	38.83	36.52	35.73	41.36
4	43.79	40.75	48.63	46.69	42.86	47.29	45.31	44.55	50.62
5	56.36	54.42	61.91	59.37	55.47	60.46	58.48	57.84	62.84
6	60.21	58.64	65.79	63.46	59.83	64.58	62.83	61.72	66.95
8	64.49	62.17	69.46	67.83	63.79	68.15	66.42	65.25	71.31
10	75.72	72.33	81.37	79.48	74.51	80.56	78.65	76.91	81.83
12	86.74	85.21	89.01	87.92	86.28	88.15	87.59	86.97	90.83

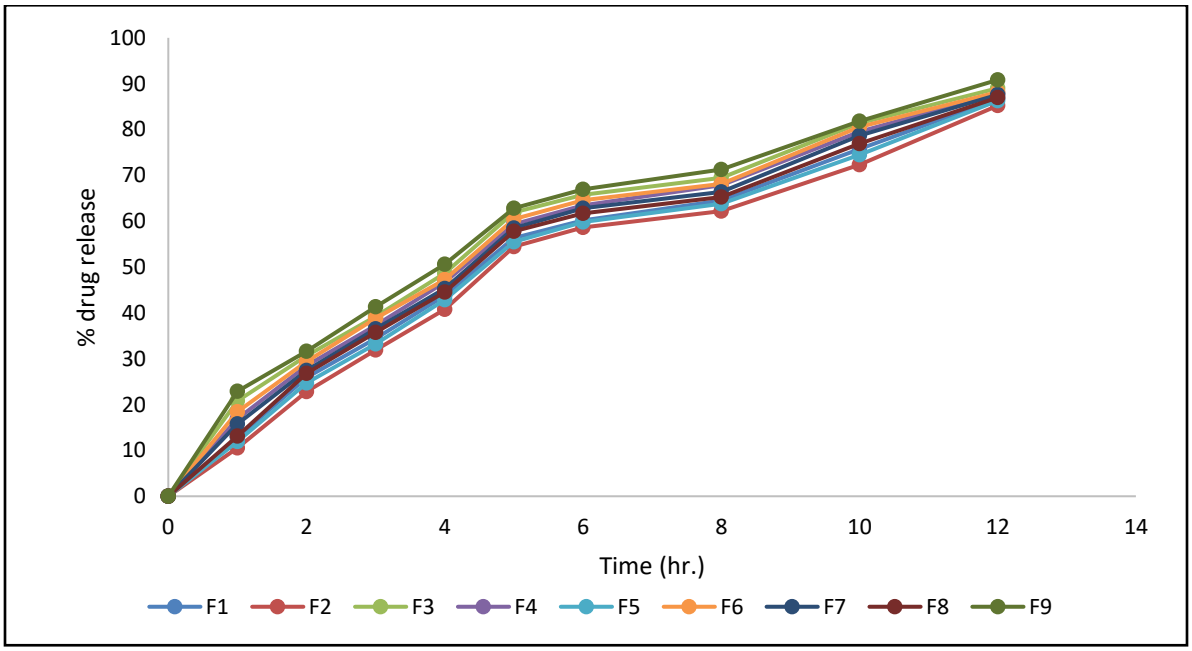

Figure 1: In-vitro dissolution profile of Curcumin phytosomes formulations (F1-F9)

In-vitro drug release of optimized batch of Curcumin phytosomes formulation (Cur-OPT):

In-vitro drug release study of optimized batch of Curcumin phytosomes formulation was conducted.

Table 5: *In-vitro* release study of optimized batch of Curcumin phytosomes (Cur-OPT)

S. No	Time (hr.)	Optimized formulation (Cur-OPT)
1.	0	0.00±0.00
2.	1	23.65±0.041
3.	2	32.83±0.032
4.	3	42.56±0.019
5.	4	51.75±0.028
6.	5	63.41±0.047
7.	6	67.29±0.051
8.	8	72.83±0.066
9.	10	82.64±0.048
10.	12	91.77±0.023

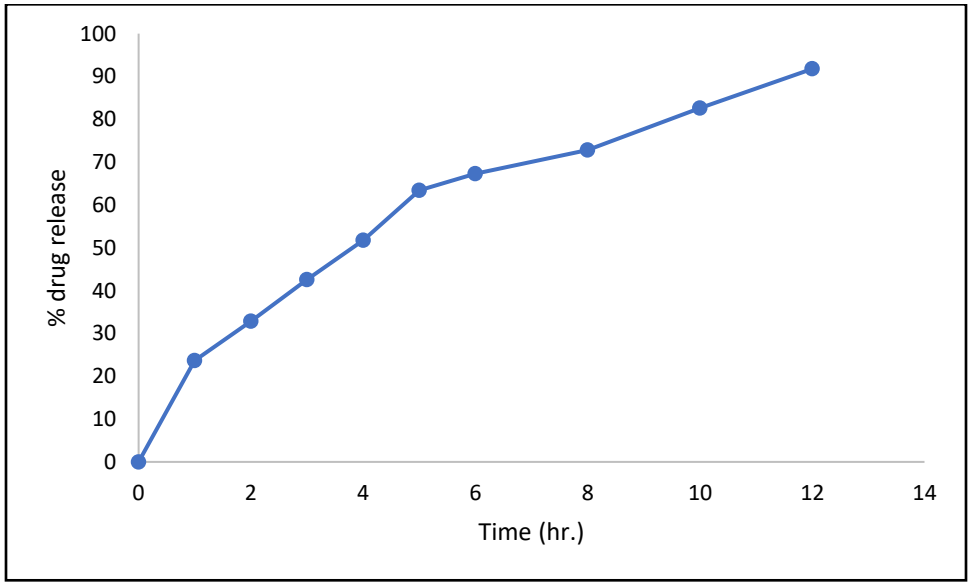

Figure 2: *In-vitro* release study of optimized batch of Curcumin (Cur-OPT) phytosomes

3. Formulation and evaluation of Phytosomal gel of Curcumin

Various combinations of Curcumin laden phytosomal gels (Cur-OPTG1–Cur-OPTG3) were primarily inspected for color, pH, homogeneity, viscosity, phase separation, and drug content for the development of suitable gel formulation. Many advantages of phytosomal gel include enhancing dosage and promoting bioavailability of the active molecule. It was found that the Cur-OPTG2 (1% Carbopol gel) was transparent comprising of good homogeneity, consistency, and no phase separation, as shown in Table 4.23. It was observed that the prepared gel was safe for the skin and no irritation was found after its application. Thus, the Cur-OPTG2 gel was then subjected to further in-vivo evaluation. The percent drug release from the Curcumin phytosomal gel was found to be

maximum from Cur-OPTG2 in comparison to other two gel formulations (Table 4.23 & Figure 4.37). Thus, the Cur-OPTG2 has undergone stability study.

Table 6: Characterization parameters of Curcumin laden Phytosomal gels (batches Cur-OPTG1-Cur-OPTG3)

Gel Formulation code	pH (mean±S.D)	Viscosity (Pa) (mean±S.D)	Visual appearance (mean±S.D)	Drug content (%) (mean±S.D)	Drug release (%) at 12h
Cur-OPTG1	6.68±0.21	823.127±1.16	Clear	95.82±1.58	87.75%
Cur-OPTG2	6.73±0.25	885.264±2.39	Clear	96.45±1.23	90.69%
Cur-OPTG3	6.71±0.22	934.532±2.51	Clear	97.68±1.36	85.42%

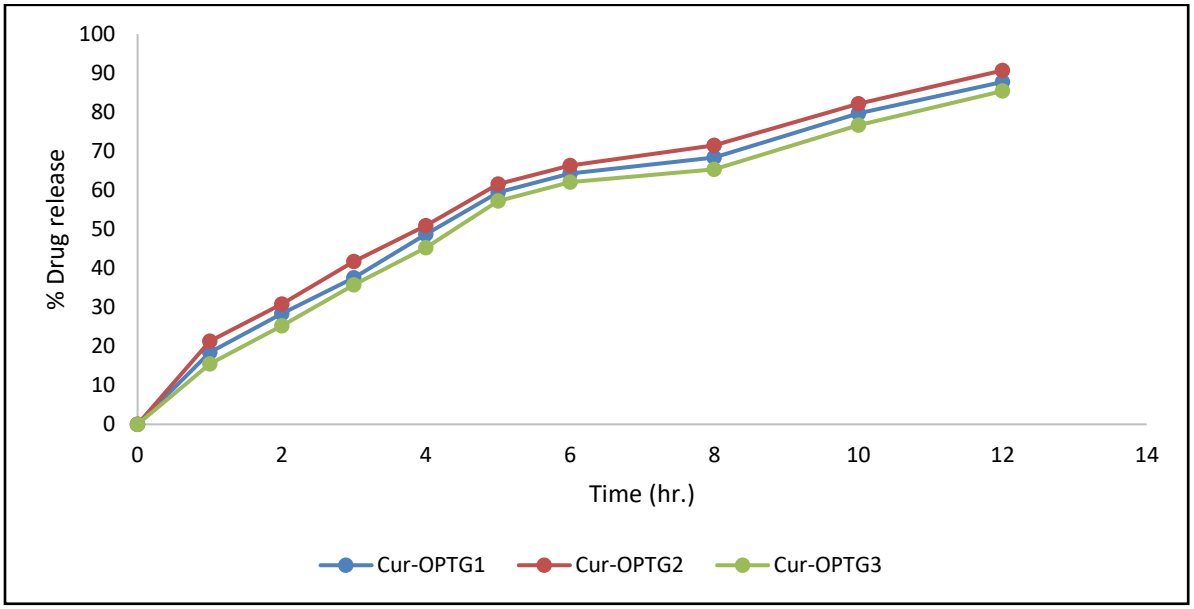

Figure 3: In-vitro drug release from the Curcumin loaded phytosomal gel formulations (batches Cur-OPTG1-Cur-OPTG3)

In-vivo Anti-psoriatic activity:

Acute Dermal Toxicity Study

From the acute dermal toxicity study, it was found that no appreciable changes in fur, eyes, sleep pattern, central nervous system activity and behavior pattern were seen in the treated animals after 14 days.

In-vivo anti-psoriatic activity

The mouse tail test is employed widely for psoriatic investigations because it is simpler model, smooth to carry out and displays good reproducibility, fine sensitivity and high correlation with the activity of oral or topical anti-psoriatic therapeutics at present clinically. Additionally, this model facilitates the quantitative and histometric investigations of fabricated formulation response on epidermal differentiation.

a) Mouse tail model

Phytosomal gel was screened for its possible anti-psoriatic activity using mouse tail model. Curcumin phytosomal formulation (1% w/w) and standard drug (0.1% w/w) were applied on the induced psoriatic lesions. Phytosomal gel has increased the orthokeratotic regions by 67.25±1.27%, in comparison to control group. The standard drug showed an increase in the orthokeratotic regions by 68.59±1.15%. Phytosomal gel has decreased the epidermal thickness upto 43.78±1.21%, while standard drug decreased the epidermal thickness upto 44.65±1.09%. Percent drug activity of Curcumin laden phytosomal gel (1%) was found to be 56.43±1.25% which showed a significant anti-psoriatic effect in comparison to standard drug which has shown the drug activity of about 59.65±1.18%. The results were represented in table 4.25.

Table 4.25: Anti-psoriatic potential of Curcumin Phytosomal gel using mouse tail model

S.NO.	Formulation	Relative epidermal thickness (%)	% Orthokeratosis	Drug activity (%)
1.	Vehicle Control	100.00±0.00	25.76±1.72	0.00±0.00
2.	Negative control	100.00±0.00	0.00±0.00	0.00±0.00
3.	Tazarotene- 0.1% w/w	44.65±1.09*	68.59±1.15*	59.65±1.18
4.	Curcumin Phytosomal gel-1% (Cur-OPTG2)	43.78±1.21*	67.25±1.27*	56.43±1.25

The results were presented in mean ± S.D and results were significant in comparison to control group at $p<0.05$ (*)

Histological examination of mouse skin

Curcumin laden phytosomal gel 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 psoriasis. 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 mechanism.

In the present work, Figure 4.37, illustrates the histological appearance of haematoxylin and eosin-stained sections of mouse tail skin on treatment with various samples. From control group (Group 1), distinct pieces of evidence associated with parakeratosis, a higher density of nucleated keratinocytes and thinning of the granular layer were noticed. Fourteen days of treatment therapy with test formulation (Cur-OPTG2) exhibited considerable histological changes in sections of tail skin with respect to marketed product. Throughout this time, the orthokeratotic stratum corneum provinces spread longitudinally, in the previous parakeratotic condition. The influence of topical application of phytosomal gel formulation on relative epidermal thickness (%), orthokeratosis (%)

and drug antipsoriatic activity (%) in the mouse tail test was displayed in table 4.25. The control group showed 25.76±1.72% orthokeratosis, while the scaly areas presented parakeratosis. Tazarotene formulation (standard) showed 68.59±1.15% orthokeratosis, while phytosomal gel of Curcumin showed an almost 67.25±1.27% orthokeratotic differentiation, respectively. These findings presented the potency of phytosomal gel-based approach to provoke normal epidermal differentiation in psoriasis.

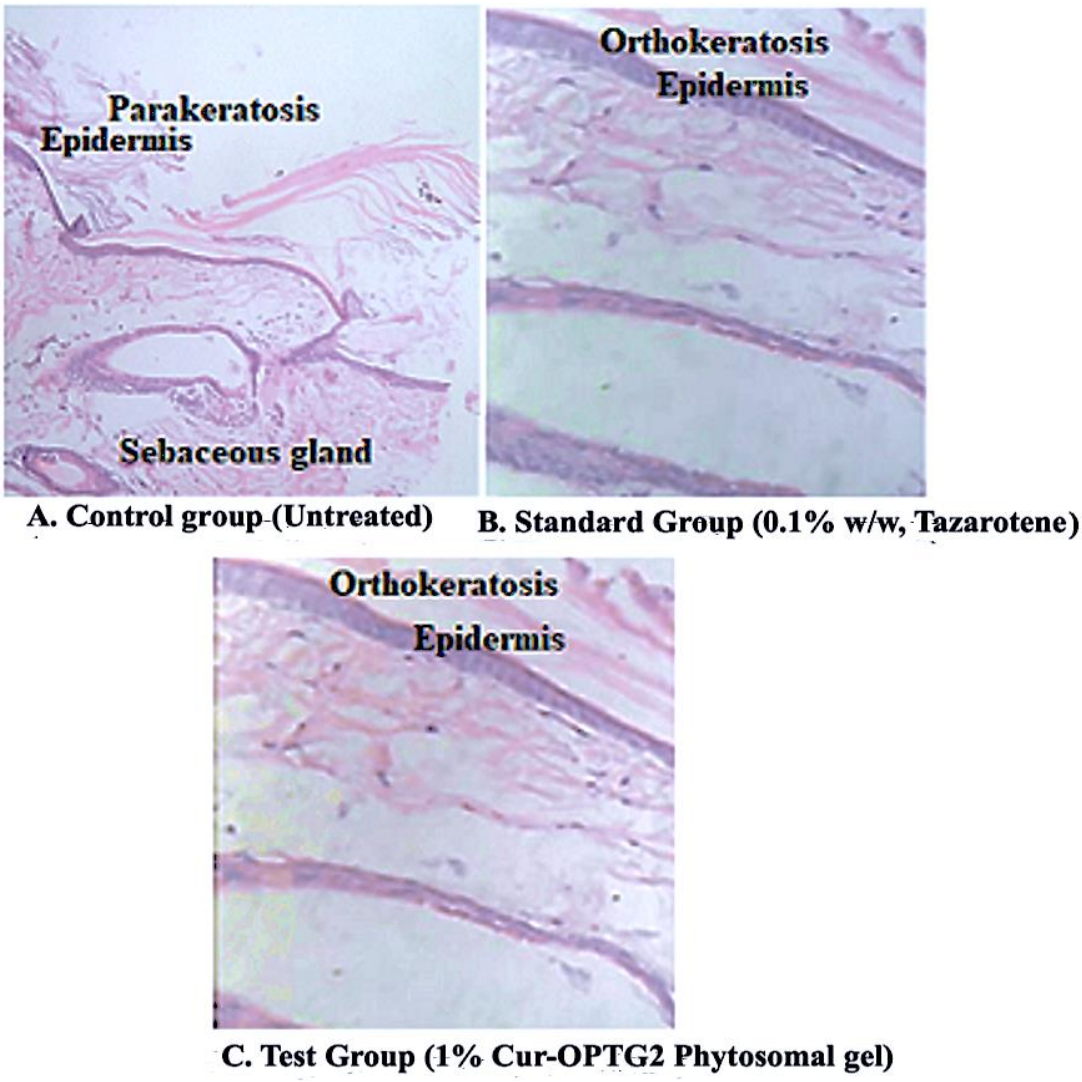

Figure 4: Histopathological evaluation of mice tail skin after various treatments. A. Control; B. Standard drug formulation; C. Phytosomal gel of Curcumin (Cur-OPTG2)

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