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Preclinical evaluation of topical emulgel containing fixed dose allo-herbal combination in imiquimod induced psoriasis

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ABSTRACT

Introduction : Psoriasis is a well-known autoimmune, progressive inflammatory condition having long-term impact on the patient's physical and psychological well-being with worldwide prevalence. The available allopathic and herbal medicines have their own merits and demerits. Allopathic drugs may produce potent beneficial as well as adverse effects while alone herbal drugs may exert slow and less potent effects. Hence the current study attempted to prepare and evaluate fixed dose combination of allo-herbal emulgel in order to minimize adverse effects and improve beneficial effects during the treatment of psoriasis.

Materials and Methods: This study used a well-established, robust, and validated model (Imiquimod (IMQ)) for psoriasis induction. Emulgel was formulated and tested using phytochemical and physicochemical methods. The anti-psoriatic and anti-inflammatory activities of the prepared emulgel were investigated. In addition, percent release and in vivo absorption were done to ensure adequate release and absorption of emulgel content. Progress of psoriasis induction and treatment was analyzed by morphological and histopathological studies.

Results: The formulated emulgel was found to comply with the standard physicochemical test with the desired release pattern. Morphological and histological data showed induction of psoriasis using the IMQ model, and significant improvement was observed after emulgel treatment. All formulations were discovered to significantly reduce formalin-induced inflammation.

Conclusion: The present study provided the rationale for the combination of C. amada and P. pinnata with salicylic acid. The selected allo-herbal combination and optimized dosage form are stable, biocompatible, and effective anti-inflammatory and anti-psoriatic with potentially fewer side effects.

1. Introduction

Psoriasis is an immuno-inflammatory skin condition that affects 1-3% of the population world-wide. It is characterized by infiltration of immune cells, epidermal hyper proliferation and abnormal keratinocyte differentiation, which results in thickening and scaling of the epidermis followed by lesions causing medico-psychological disturbance.[1] Plaque, pustular, guttate, inverse, erythrodermic, arthritic, and psoriasis vulgaris are all kinds of psoriasis. It can be induced in experimental animals by UV radiation and Imiquimod (IMQ) application.[2–6]

A number of synthetic and semi-synthetic preparations are available, but they exhibit some drawbacks such as temporary staining, irritation, redness, and burning. Several plant products have traditionally been used to cure psoriasis.[2] The herbal formulations may be established

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as an alternative to the synthetic/semi-synthetic drugs due to its cost effectiveness, complete accessibility, improved tolerance, good protection, fewer side effects, and optimum efficacy.[1] A large body of evidence suggests that herbal formulations containing Dithranal, Ricinus communis, Curcuma amada (C. amada-(Mango ginger or Ambehalad)), curcumin, Pongamia pinnata (P. pinnata-Karanja), Commiphora mukul, and Psoralea corilyfolia can treat psoriasis-like conditions.[7–12]

C. amada species possess antibacterial, antioxidant, analgesic, anti-inflammatory, anti-hyperglycemic, anti-lipidoxidative, and anti-allergic which may be due to the presence of flavonoids and associated chemicals.[7,13] Salicylic acid is an analgesic, anti-inflammatory, and keratolytic agent which causes softening of the stratum corneum and reduces hyperkeratosis.[14]

Topical treatment of psoriasis has achieved great importance because of its good penetration efficacy and compatibility. Emulgel shows properties of both emulsion and gel which effectively deliver a hydrophobic drug via skin.[15,16] Based on the literature, the current study aimed to formulate and pharmaceutically evaluate the anti-psoriatic potential of C. amada and P. pinnata in conjunction with synthetic salicylic acid.

2. Materials and Methods

2.1. Drugs and chemicals

C. amada powder and P. pinnata oil, IMQ gel (Imiquad, Glenmark Pharmaceuticals Ltd, Goa), Salytar Ointment (3%, Meramini India Pvt. Ltd., Mumbai), Diclomol gel (Win Medicare) and Vicco Turmeric (Vicco Laboratories) were purchased from the local market. Himalaya Drug Company, Bangalore and Unijules Life Sciences Ltd. Kalmeshwar, Nagpur provided standard curcumin and salicylic acid as a gift sample.

2.2. Experimental animals and IAEC approval

Adult Wistar rats (either sex) weighing 180–200 g were chosen and kept at a temperature of 24±20^{O}C and on a routine light-dark cycle (12/12hrs) with water and a regular pellet diet ad libitum. The animals were cared for in accordance with the ARRIVEs guidelines. CPCSEA, Ministry of Environment and Forests, Government of India, New Delhi, India, authorized the animal study procedures through the Institutional Animal Ethical Committee (IAEC) (Reg. No. 648CO/Ere/S/02/CPCSEA. 30/09/2013; proposal No. PJLCP/2017/04).

2.3. Preparation of oil extract

30g of C. amada powder was mixed with 65 mL of P. pinnata oil and was used in different concentration in formulation.

2.4. Qualitative phytochemical evaluation of extract

The plant materials were phytochemically analyzed using several chemical identification tests for alkaloids, glycosides, flavonoids, tannins, saponins, and phenolic compounds, along with tests for biomolecules like carbohydrates, proteins, starch, and amino acids (Table 1).[17,18]

2.5. Formulation of emulgel

The gel was prepared by dissolving Carbapol 940 in filtered water and swirling regularly while maintaining a pH of 6-6.5 with triethanolamine (TEA). The oil and watery phases of the emulsion were generated by dissolving Span 20 in liquid paraffin and filtered water. Before being mixed with the aqueous phase, methyl and propyl paraben were dissolved in propylene glycol. Separately, the oil and aqueous phases were heated to temperatures ranging from 70 to 80 ^{0}C. Until the aqueous phase reached room temperature, the oil phase was gradually and constantly mixed into the aqueous phase with constant mechanical stirring at 200 Revolution Per Minute (RPM) for 2 min until it reached room temperature (Table 2).[11]

2.6. Qualitative physicochemical evaluation of emulgel

The appearance, pH, skin irritation, viscosity, spreadability, extrudability, drug content, homogeneity, diffusion and stability of the prepared emulgel were all tested (Table 3).[19–21]

2.7. Pharmacological evaluation

2.7.1. Anti-inflammatory activity

The anti-inflammatory activity of the optimized emulgel was tested in rats using the formalin-induced hind paw edema method. Each rat's left hind paw was marked directly below the tibio-tarsal junction, so that the paw could be dipped up to the same point every time to maintain constant paw volume. The animals were divided into five groups (n=5) at random viz. Induced (formalin-emulgel base), Formulation-F1, F2, F3, standard (Diclofenac gel). The formulations were gently applied to the dorsal area (9cm^2) with microspore adhesive 30 minutes prior to formalin injection. Acute inflammation was induced 30 minutes after drug treatment by injecting 0.1 mL of 1 percent (v/v) formalin solution into the sub-plantar region of the left hind paw. The paw edema was measured with a Vernier caliper at 30, 60, 90, 120, and 150 minutes and expressed in millimeters. Using the following formula, the percentage inhibition of edema was estimated for all groups in comparison to the control group.[22]

$$\%\ inhibition = \frac{Volume\ at\ time\ 't' - volume\ at\ time\ '0'}{Volume\ at\ time\ '0'} \times 100x$$

Table 1: Qualitative phytochemical evaluation of extract (test)

S.No.	Phytochemical constituent	Procedure	Observation
1.	Carbohydrate	2 mL test + 2 drops Molisch's reagent+ Conc. H_2SO_4	Purple color at interface
2.	Protein	3 mL of test + 3% NaOH + few drops of 1% $CuSO_4$	Change of blue color to violet or pink color
3.	Starch	3 mL of test + 2-3drops of Dil. Iodine sol.	Blue color which disappears on boiling
4.	Amino acid	5 mL test + few drops of 40% NaOH & 10% lead acetate + boil	Brick precipitate
5.	Steroid	2 mL of test + 2 mL Chloroform + 2 mL Conc. H_2SO_4 +shake	Red:chloroform layer; acid layer-greenish fluorescence
6.	Glycoside	Test + Glacial acetic acid + few drops of $FeCl_3$ + Conc. H_2SO_4	Bluish green at upper layer and Reddish brown color at the junction
7.	Flavonoid	4 mL test + 1.5mL of 50% methanol + warm + Mg metal + 4-6 drops of Conc. HCl	Flavonoid- Red color; Orange color-Flavones
8.	Alkaloid	0.5 g test + 5 mL of 1% aq. HCl in water bath + filter, 1 mL of filtrate + Mayer's reagent	Yellow color precipitate
9.	Tannin	0.5 mL test + 1 mL water + 1-2 drops of $FeCl_3$	Blue color-Gallic acid; Green black-catecholic acid
10.	Saponin	1 mL test + 2 mL Distilled Water (DW) + shake	Persistent foam
11.	Phenolic compounds	2-3 drops of test + following:	
		5% FeCl3	Deep blue-black color
		Lead acetate solution	White precipitate
		Bromine water	Discoloration

Table 2: Composition of formulated emulgel

S.No.	Content(P. pinnata oil + C. amada powder)	F1	F2	F3
1.	Macerated oil: Salicylic acid (ml: mg)	0.5:1	1:1	1.5:1
2.	Carbopol 940 (g)	1.0	1.0	1.0
3.	Liquid Paraffin (mL)	7.5	7.5	7.5
4.	Tween 20 (mL)	0.5	0.5	0.5
5.	Span 20 (mL)	1.0	1.0	1.0
6.	Propylene glycol (mL)	5.0	5.0	5.0
7.	Methyl paraben (g)	0.003	0.003	0.003
8.	Propyl paraben (g)	0.19	0.19	0.19
9.	Triethanolamine (mL)	q.s	q.s	q.s
10.	Water (mL)	q.s	q.s	q.s

2.7.2. Anti-psoriatic activity

Anti-psoriatic activity was assessed in rats using an IMQ-induced psoriasis model.[4] Animals were divided into different groups viz. control group, psoriasis induced group (IMQ 4mg/kg), F1, F2, F3, standard (Salytar ointment 3%) and treated for 15 and 30 days respectively.

All of the rats in the study had their backs shaved with hair removal lotion. Except for the control group, IMQ cream (5%) at a dose of 80 mg (daily) containing 4 mg IMQ was topically applied on shaved skin for 15 and 30 consecutive days. Control group was treated with a vehicle emulgel base.[6] All the animals were manually observed regularly in the morning to check and confirm the development and progress of psoriasis for 15 and 30 days of treatment.

Psoriasis Area and Severity Index (PASI) score was used to assess the psoriasis, which measured the average thickness, redness, and scaliness of the lesions (0-4 scale for each parameter).[4,12]

The rats were sacrificed after 15 and 30 days, and the dorsal skin was removed, bathed in a 10% buffered formaldehyde solution in ethanol, embedded in paraffin wax, and sliced at a thickness of 3 μm. Haematoxylin and eosin (H&E) were used to stain the samples, which were then photographed under a light microscope.

2.8. In vivo absorption study

The procedure for estimation of in vivo skin absorption was slightly modified according to Schaefer et al.[23] The blood sample was collected from each animal of control, F1, F2, F3, and standards of salicylic acid and curcumin after each

Table 3: Test and procedure for physicochemical evaluation of emulgel

S.No.	Test Parameter	Procedure
1	Appearance	Gel was inspected visually for clarity, colour and presence of any particle.
2	pH	2g of gel stirred with DW volume make up to 40 mL pH measured using digital pH meter
3	Skin irritation	Rats were shaved 100mg gel were applied over 2 cm2 area for 6 hrs. cleaned area with acetone and observed using Draze scale (0-no irritation; 1: slight irritation; 2-Severe irritation
4	Viscosity	Brookfield viscometer (Dial type; for 2min at 0.3 RPM), for non-Newtonian spindle no. 4 was used.
5	Spreadability	Multi-timer apparatus is used, An excess of gel (2–5 g) was placed in between two glass slides and then 1000 g weight was placed on slides for 5 min to compress the sample to a uniform thickness. 80 g of weight was added to pan. The time (seconds) required to separate the two slides, was taken as a measure of spreadability. It was calculated using formula, S = M. L / T Where, S = spreadability; M = weight tied to upper slide L = length of glass slide; T = time taken Shorter time interval, to cover distance of 6.5 cm, indicates better spreadability.
6	Extrudability	A one end closed collapsible tube filled with gel was pressed firmly at crimped end. When the cap was removed, gel extrudes until pressure was dissipates. The extrusion pressure (weight in g) required to extrude 0.5 cm ribbon of gel in 10 seconds was determined.
7	Homogeneity	The appearance of any aggregates are inspected using this test
8	Stability Studies	The emulgel filled aluminum collapsible tubes (5g) subjected to stability studies at 5°C, 25°C/60% Relative humidity (RH), 30°C/65% RH, and 40°C/75% RH for a period of 3 months as per ICH guidelines. Samples were withdrawn at 15-day time intervals and evaluated for physical appearance, pH, rheological properties and drug content.
9	Drug content	1g gel mixed gradually in methanol to make up volume 100 mL. The solution was filtered through Whatman filter paper (No. 41). The 5mL filtrate was diluted to 50 mL with methanol and absorbance was taken at 306 nm and 412 nm against blank for calculating the content of salicylic acid and curcumin respectively.

hour for 6 hrs and allowed to coagulate at room temperature. The serum was separated using cooling centrifuged rotating at 3000 RPM for 10 minutes and used for the estimation of curcumin and salicylic acid using UV spectrophotometer.

2.9. In vitro Diffusion study

A fabricated Franz diffusion cell with an effective diffusional area of 3.14 cm 2 and 5 mL of receiver chamber capacity were used. The full-thickness rat skin was excised from the abdominal region to isolate the subcutaneous tissue and the dermal adhering fat was removed by washing with isopropyl alcohol. The cleaned skin of rat was washed with DW and stored in deep freezer at -21°C until further use. At room temperature the skin was mounted between the donor and receiver compartment of the Franz diffusion cell. The donor compartment (empty) and the receiver chamber filled with phosphate buffer (pH 7.4) stirred with a magnetic rotor at a speed of 100 RPM, and kept at 37 ± 1°C temperature. All the receiver fluid was replaced every 30 min to stabilize the skin. After complete stabilization of the skin, 1 ml of emulgel was placed into each donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5 and 6 h) filtered through a 0.45-membrane filter, and analyzed for drug content by UV spectrophotometer at λ_{max} of 306 nm and 412 nm for salicylic acid and curcumin respectively.[24]

2.10. Statistical analysis

The data obtained following the treatments of various agents was represented as a mean ± standard error of mean (SEM). All the data was statistically analyzed by Graph Pad Prism V.5 using one-way analysis of variance (ANOVA) followed by post-hoc Bonferroni multiple comparison test. The value of $P<0.05$ was considered as significant

3. Results

3.1. Qualitative phytochemical evaluation

The result of thin layer chromatography showed the single spot of curcumin in macerated oil (R_f 2.057) which was matched with standard curcumin (R_f 2.057). The amount of curcumin extracted in oil was measured by UV spectrophotometer and was found to be 14 mg/mL. The phytochemical testing of the C. amada powder and P. pinnata oil found to contain following vital constituents (Table 4).

3.2. Physicochemical evaluation of formulations

All the formulations tested for the pH, skin irritation, spreadability, extrudability were found to be within the standard acceptable limit with no skin irritation.

Table 4: Phytochemical evaluation in C. amada powder and P. pinnata oil

S.No.	Phytochemical constituent	Curcuma amada Powder	P. pinnata oil
1.	Carbohydrate	+	+
2.	Protein	+	_
3.	Starch	_	_
4.	Amino acid	_	_
5.	Steroid	+	_
6.	Glycoside	+	+
7.	Flavonoid	_	+
8.	Alkaloid	+	+
9.	Tannin	_	+
10.	Saponin	_	_
11.	Phenolic compounds	+	-

Table 5: Physicochemical evaluation of formulations

Formulation	pH	Skin Irritation	Viscosity (Cp)	Spread ability (cm)	Extrudability (g/)
F1	6.23 ± 0.06	No	30587 ± 201.3	7.59 ± 0.64	79 ± 1.00
F2	5.97 ± 0.06	No	29517 ± 277.7	6.37 ± 1.05	67.7 ± 1.53
F3	6.5 ± 0.10	No	38067 ± 138.6	5.94 ± 0.65	62 ± 1.73

3.3. Drug content of formulated emulgel

Drug content analysis was done to find out the actual content of the salicylic acid and curcumin in F1, F2, and F3 (Figure 1). UV absorption for both constituents at 306 nm and 412 nm showed no significant [One-way ANOVA; P > 0.05, F (2, 12= 0.01)] difference among F1, F2, F3 for % content of salicylic acid and curcumin.

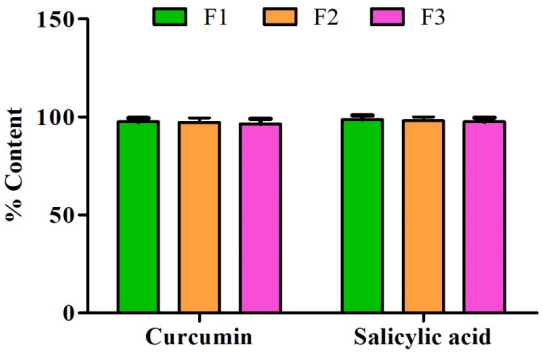

Figure 1: % drug content of formulated emulgel. Values are expressed as mean ± SD and data were analyzed by the one-way ANOVA followed post hoc Bonferroni multiple comparison test P > 0.05 (n=3).

3.4. Cumulative drug release from the formulation (in vitro diffusion study

The cumulative release of salicylic acid and curcumin from F1, F2, and F3 was quantified using a Franz diffusion cell for 6 hours and evaluated for drug content using a UV spectrophotometer at 306 nm and 412 nm, respectively. Release of both constituents from all the three formulations were linearly increased with time [Two-way ANOVA; P<0.0001, F (6, 28)=2.01] but no significant (P> 0.05) difference was observed among F1, F2 and F3 (Figure 2).

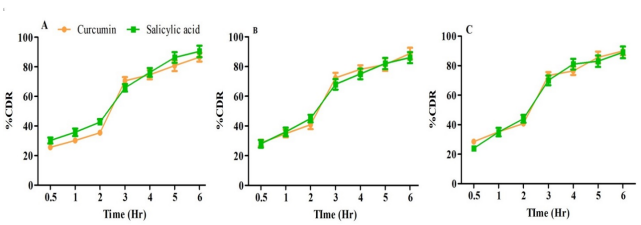

Figure 2: % cumulative drug release of curcumin (CDR) and salicylic acid from the formulated emulgel. Values are expressed as mean ± SD; data was analyses by Two-way ANOVA, %CDR, P<0.0001 versus time and P > 0.05 versus Formulation (F1, F2, F3) (n=3).

3.5. Effect of formulations in formalin induced inflammation

In separate groups (n=6), the injected formalin rat paw caused inflammation. A two-way ANOVA was used to analyze the data, followed by a post-hoc Bonferroni multiple comparison test, which demonstrated that F1, F2, F3, and standard were significantly lowered [two-way ANOVA; P<0.001, F (4, 150)=198] the paw edema as compared with formalin group at the 60 min (Figure 3). F1 and standard prevented the effect of formalin in 30 min while F2 and F3 failed to do so. The F3 showed significant

effect at 60, 120 and 150 min and completely reduced edema produced by formalin. All the treatments' including the formalin showed similar results (% inhibition appox 50%) at the 90 min and no significant difference was seen.

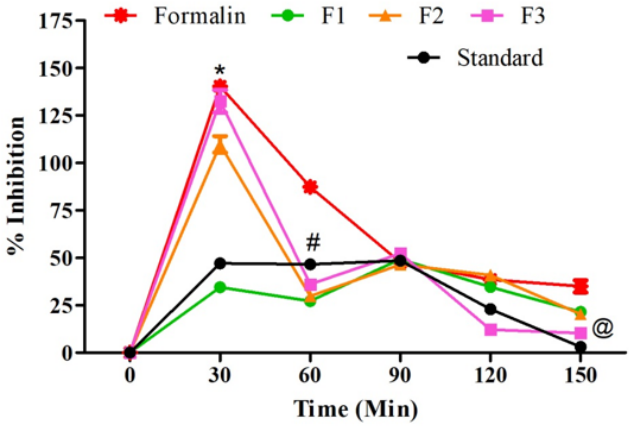

Figure 3: Effect of formulated emulgel and standard drug on inflammation by formalin induced inflammation in rat paw edema. Data expressed in mean ± SD and analyzed by two-way ANOVA followed by post hoc Bonferroni multiple comparisons *P<0.001 formalin versus self-control 0 reading; #P<0.001 formalin versus treatments; P>0.05 formalin versus F1 & F2; @P<0.001 formalin versus standard and F3.

3.6. *Anti-psoriatic activity*

The changes in morphological features were observed daily, and the PASI score analysis results of the 15th and 30th day are shown in Figures 4 and 5 (a and b). On day 15, IMQ treated group showed the extreme increase (P<0.001) in the PASI score parameters like scaliness, redness and skin thickness as compared with the control group and confirm the induction of the psoriasis. All the formulations F1-F3 and standard significantly [one way ANOVA; P<0.001, F (5, 36)=376.44] restricted the elevation of the PASI score parameters on day 15 and 30 as compared to the IMQ-induced group. On day 30, F2 and F3 treated group showed significant inhibition (P<0.001) of scaliness elevation which is also significant than F1. The F3 treated group exhibited significant reversal (P<0.001) of the redness to normal as compared to the F1, F2, and standard treatment. F3 treatment found to significantly reverse the elevation of skin thickness than the F1 and F2 but not significant than standard group. F3 showed protective effects on day 30 in IMQ induced psoriasis.

3.6.1. *Effect on histopathological parameters*

Histopathological examinations revealed that the skin structure in the control group animals was intact. Microscopic inspection of the IMQ-treated group revealed a rise in scaliness, epidermal thickness, and an increase

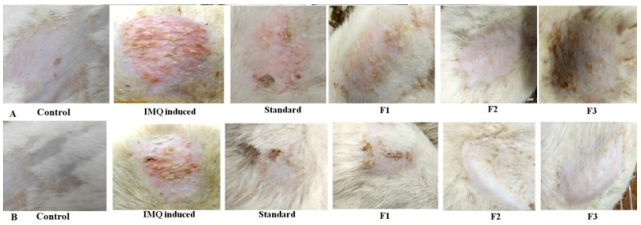

Figure 4: Morphological changes in rat skin after A. 15 days; B. 30 days

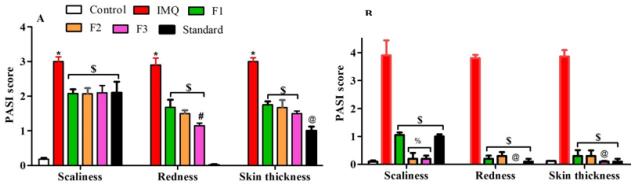

Figure 5: Effect of formulated emulgel and standard drug on morphological features with PASI score. Data expressed in mean ± SD and analyzed by two-way ANOVA followed by post hoc Bonferroni multiple comparisons A (15 days). *P<0.001 versus control; $P<0.001 versus IMQ-induced group, #P<0.001 versus F1&F2, and @ P<0.001 versus Control, F1-F3. B (30 days) $P<0.001 versus IMQ induced, %P<0.001 versus F1, @ P<0.001 versus F1 and F2.

in keratinocytes in the basal layer, resulting in hyper-proliferation and acanthosis. After 30 days of treatment, the epidermal thickness in the IMQ-treated group was nearly four times that of the control group. In addition to the lymphocyte infiltration, elongation of rete ridges, and micro-abscesses, were seen in the IMQ-treated group. The sections of skin treated with formulated emulgel showed decreased epidermal thickness, keratosis, and other inflammatory changes (Figure 6 a and b).

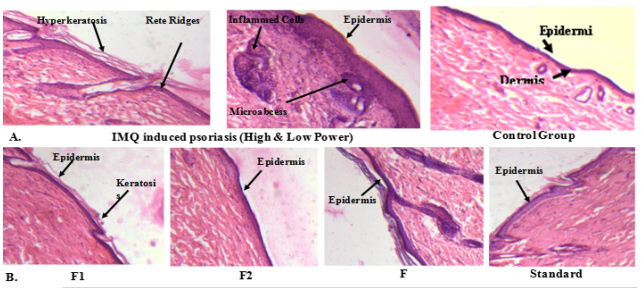

Figure 6: Results of histopathological studies treatment groups A. IMQ induced psoriasis and control group; B. Formulated emulgel and standard on IMQ induced psoriasis

3.7. *Results of in vivo absorption study*

The absorbance of a serum sample was recorded during each hr for 6 hrs using UV spectrophotometer. As shown

in Figure 7, there is linear increase in percent absorption of salicylic acid and curcumin in all the formulation viz. F1, F2, F3. As compared to standard no significant [Two-way ANOVA; P>0.05, F (15, 120=2.83)] difference was noted amongst them.

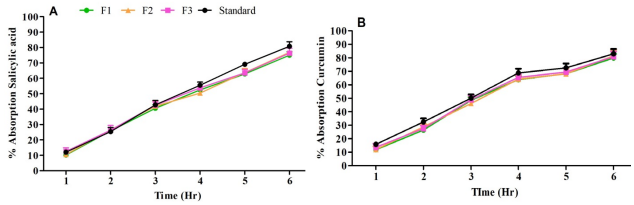

Figure 7: Values are expressed as mean ± SD and data were analyzed by the two-way ANOVA followed by Bonferroni multiple comparison test P>0.05 among the formulations.

4. Discussion

Psoriasis has an impact on the patient's physiological and psychological well-being.[12] Among the numerous therapies available for psoriasis, ayurvedic preparations and topical dosage forms are gaining prominence.

Salicylic acid in topical dosage form is used for its keratolytic, antibacterial, fungicidal, and photo-protective potential, but its continuous use may be harmful, i.e., salicylism.[14] It also possesses analgesic and anti-inflammatory effects, but at a dose much greater than the anti-psoriatic. A fixed dose combination (Diprosalic and Nerisalic) of corticosteroids and salicylic acid is more promising for anti-psoriatic treatment than a single drug.[25]

Curcumin (turmeric) and P. pinnata oil, are known to be used traditionally and recently been described as a valid and safe phytoconstituent as anti-psoriatic because of antioxidant, anti-proliferative, anti-inflammatory, antiviral, antibacterial, and antifungal effects.[7,10,12]

Among the dosage forms, emulgel become popular because of its key qualities such as thixotropic, greaseless, readily spreadable, quickly removed emollient, non-staining, and compatibility with a variety of excipients.[26–28] Hence, to reduce the adverse effects of salicylic acid and improve the anti-psoriatic therapy, emulgel formulations containing various concentrations of macerated oil and a fixed quantity of salicylic acid were prepared.[25]

The physicochemical properties of C. amada powder, P. pinnata oil, and salicylic acid were found to be within the standard acceptable range. Phytochemical screening revealed the presence of phenolic compounds, carbohydrates, proteins, steroids, glycosides, and alkaloids in C. amada powder, as well as carbohydrates, glycosides, alkaloids, tannins, and flavonoids in P. pinnata oil. The F1, F2, and F3 were complied with the official standards in terms of appearance, pH, skin irritation, viscosity, spreadability, extrudability, stability, and homogeneity tests. The contents and cumulative release of salicylic acid and curcumin in F1, F2, and F3 were found to be above 97 % and followed a linear release pattern for 6 hrs.

Inflammation significantly contributes to the pathology of psoriasis.[29] Hence, an anti-psoriatic drug must possess an anti-inflammatory property. Intra planter formalin induced edema within 30 min of injection in control animals. The F1 and standard formulation inhibited the formalin-induced edema up to 50% in 30 min, while F2 and F3 failed. On the other hand, F3 took more time, i.e., 120 and 150 min, but completely (90–95%) reversed the edema produced by the formalin, which may be due to slow but steady release of the medicament. Hence, F3 is a better anti-inflammatory combination than F1 and F2 and was comparable with the standard.

The skin changes like scales, redness, and thickening were prominent with IMQ treatment, whereas all the treatments were found to reduce the elevated parameters of the PASI score. A histopathological study showed increased epidermal cell proliferation that causes significant epidermal thickening (acanthosis) and rete ridge downward elongation, mitotic figures above the basal cell layer, stratum granulosum is thinning or missing, and parakeratosis scale is visible; this is typical of psoriasis plaques with supra-papillary plates.[6] The increased epidermal thickness, hyperkeratosis, and inflammatory changes including lymphocyte infiltration, Munro's micro abscesses, and elongation of rete ridges disappeared significantly with treatment of F2 and F3 as compared to standard salicylic acid gel. This indicates that curcumin and P. pinnata oil have enhanced the anti-psoriatic action and showed a synergistic effect with salicylic acid incorporated in the formulated emulgel.

The in vivo absorption study revealed that over 6 hrs, curcumin and salicylic acid absorption in the blood is only up to 75%, which is sufficient to exert the desired local protective effect. Curcumin and P. pinnata oil may have anti-psoriatic properties due to the presence of phytochemical ingredients such as phenolic compounds, flavonoids, and tannins. Curcumin inhibits phosphorylase kinase, which is associated with the remission of human psoriasis.[12] As a result, salicylic acid's keratolytic, antibacterial, and photo-protective properties; curcumin's antioxidant, anti-proliferative, and anti-inflammatory properties; and P. pinnata's anti-psoriatic potential are all involved in the improved anti-psoriatic action. The formulated fixed combination emulgel may also reduce the dose and associated adverse effects of the salicylic acid alone treatment. Further studies are needed to find the molecular mechanism and pathway by which the pharmacological effects of curcumin and P. pinnata oil are mediated and how salicylic acid and curcumin interact with each other.

4.1. Study Limitations

Present study is limited to the physicochemical, pharmacognostic, animal behavioral morphological, and histopathological data but exact interaction and molecular mechanism of the allo-herbal could provide scope for further research**.**

4.2. Conclusion

The present study provided the rational combination of C. amada and P. pinnata with salicylic acid for psoriasis treatment. The formulated dosage form was stable, homogeneous, non-irritant, aesthetic, and easily spreadable on the skin and complied with standard physicochemical parameters. Pharmacological evaluation confirmed the anti-inflammatory and anti-psoriatic activities of the emulgel. Hence, it can be concluded that selected allo-polyherbal combinations and optimized dosage forms are stable, biocompatible, and efficacious as anti-psoriatic. After a suitable cell line study, and molecular investigation the combination can be established as an effective medication for psoriasis treatment.

5. Source of Funding

None.

6. Conflict of Interest

There are no any potential interests to declare.

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