UNICORN
REDIFINING RESEARCH

Journal of
CURRENT PHARMA
Research
www.jcpronline.in/

Journal of Current Pharma Research 6 (1), 2015, 1712-1722.

Research Article

"Development and Evaluation of Novel Topical Formulation by Minimizing Side Effect of Dithranol for Skin Diseases''

Dinesh D. Ghube*,[1], Swati S. Rawat[2]

[1]Bhagwan Baba College of pharmacy Aurangabad, Maharashtra, India, [2]Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India,

Received 26 Nov. 2015; received in revised form 12 Dec. 2015; accepted 15 Dec. 2015
Available online 27 December 2015.

Abstract

Skin diseases are the common infectious diseases in human. Skin diseases like Eczema/dermatitis, ringworm infection/dermatophytes and psoriasis are most common skin infectious diseases. Eczematous diseases are very common with an estimated prevalence of more than 10% in the general population. According to statistics 15-25% of all dermatological patients suffer from eczema. Surveys have shown that eczema prevalence is increasing; Psoriasis is a disease with a prevalence of 2-3%in Western Europe and USA. The prevalence of psoriasis in Caucasians has been reported to be between 1.5 and 3 % The prevalence of psoriasis appears to be significantly higher among whites compared with Africans-Americans. Asians or Pacific Islanders Psoriasis is a psychosocially, and at time medically, debilitating disorder that affects 1% to 3% of the population worldwide. The Dithranol is most frequently used with and without prescription for eczema, ringworm, and psoriasis. During dithranol therapy, dithranol irritation and dithranol staining may occur. Dithranol irritation is caused by an inflammatory reaction of the skin to the formation of superoxide radical due to the loss of hydrogen atom from the methylene group at position C-10. The brown staining of the skin and clothing is due to oxidation product of dithranol (anthraquinone dimmers & polymers) which are brown and black respectively .Besides Staining also post inflammatory hyperpigmentation can add to the brownish colour of the skin. Above several side effects cause patient incompliance about using of dithranol. The purpose of present study is to develop the formulation by suitable methods. And then evaluate it with chemical , physical and pharmacologically.

Keywords: Dithranol, psoriasis.

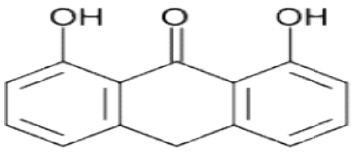
1. Introduction

Psoriasis is common chronic skin disease, which is unpredictable. A large area of the body can be converted with psoriatic lesions. The extent of the lesions may vary considerably between patients, and in time within one patient. Typically lesions are sharply demarcated, thickened, red and scaly. Additionally, the lesions can be painful and may itch and occasionally bleed. Many patients with psoriasis indicate that their social and relational life is influenced substantially by the extent of their skin disease (Do kortej, et.al 1999) Psoriasis is an erythematosquamous skin disorder (Van de kerof pcm 1999).

Corresponding author
E-mail address: ghubedinesh13@gmail.com
(Dinesh D. Ghube)

Drug Profile

Dithranol



Dithranol

Chemical structure: 1,8-dihydroxy-9,10-dihydroanthracen-9-one

Formula C14H10O3 Mol. Mass-226.227 g/mol

Pharmacology

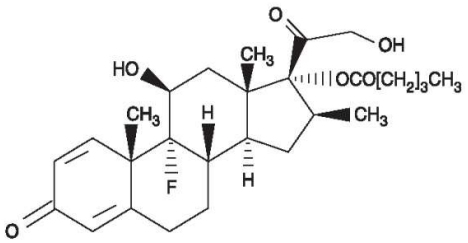
Dithranol accumulates in mitochondrin where it interfaces with the supply of energy to the cell, probably by the oxidation of dithranol releasing free radicals. This impedes DNA replication and so slows the excessive cell division that occurs in psoriatic plaques. In addition Dithranol may act by reducing the elevated levels of cGMP that occurs in psoriasis. Anthralin is a synthetic compound whose precise mechanism of anti-psoriatic action is not yet fully understood. However, numerous studies have demonstrated antiproliferative and anti-inflammatory effects of anthralin on psoriatic and normal skin.

Side effects

It temporarily stains the skin a yellow-brown and permanently stains clothing fabrics. It may cause a local burning sensation and irritation; this may be minimized by careful attention to the details of treatment and only gradually stepping up through the strengths of dithranol formulations. The surrounding skin can be protected using soft paraffin and the treated area is covered with tube guage.

Uses: anti-psoriatic, anti-inflammatory

Betamethasone Valerate



Betamethasone valerate

Chemical structure

Betamethasone valerate is 9-fluro -11β-methyl-3, 20-dioxopregna-1, 4-dien-17-yl pentanoate.

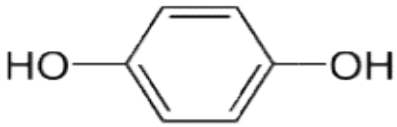
The molecular formula. C27,H37,FO6. MW=476.6

Mechanism of action

Betamethasone Valerate is a glucocorticoid receptor agonist. The anti-inflammatory actions of corticosteroids are thought to involve lipocortins, phospholipase A2 inhibitory proteins which, though inhibition arachidonic acid, control the biosynthesis of prostaglandins and leukotrienes.

Uses: anti-inflammatory

Hyroquinone



Hyroquinone

Chemical structure: Benzene-1,4-diol other names Quinol/1-4 dihydroxy benzene/1-4 hydroxy benzene.

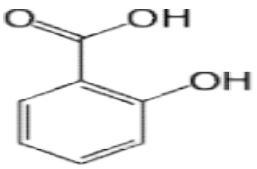
Molecular formula C6H4(OH)2 . Molar mass:110.1 g/mol

Mechanism of action

Hydroquinone is a topical depigmentating agent used in hyperpigmentation conditions by suppressing melanocyte metabolic processes. It also increases melanin excretion from melanocytes and prevents its production.

Uses: Hyperpigmentation skin.

Salicylic acid



Salicylic acid

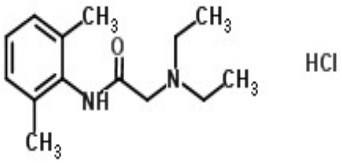
Chemical structure: 2- Hydroxybenzoic acid

Mol. formula C7H6O3. Molar Mass. 138.12 g/mol

Uses: Salicyclic acid has kerotolytic properties and its applied topically in the

treatment of hyperkeratotic and scaling skin conditions such as dandruff and seborrhoeic dermatitis, psoriasis, and acne

Lignocaine Hydrochloride



Lignocaine Hydrochloride

Molecular formula C14H22N20,HCl,H2O Mol. Wt. 288.8

Chemical structure: 2-diethylaminoaceto-2,6-xylidide hydrochloride monohydrate

Mechanism of action

The efficacy profile of lidocaine as a local anesthetic is characterized by a rapid onset of action and intermediate duration of efficacy. Therefore, lidocaine is suitable for infiltration block and surface anesthesia.

Use: local anesthetic

Materials and Methods

Drags and Chemicals

Dithranol, Salicylic acid, Betamethasone Valerate, Hydroquione, Lignociane hydrochloride, soft paraffin, hard paraffin, Cetostearyl alcohol, white bees was Carrageenan, Formalin, etc. All others chemical used were of analytical grade.

Instruments

Brookfield viscometers, pH meter, Plethysmometer were used.

Methods

Physical & Chemical compatibility.

Sampling for DSC (Differential Scanning Colorimetry), & IR (Infra-Red).

The Samples were placed at three different conditions for 21 days.

A) Freeze Temperature. (0 - 4^0C)

B) Room Temperature. (25 - 26^0C)

C) Oven Temperature. (40^0C)

Samples were placed individually and also with or combination.

A) Betamethasone valerate.
B) Dithranol
C) Hydroquinone
D) Salicylic acid
E) Lignocaine hydrochloride.

Above samples were packed individually and also packed with mixed all the same drugs for combinations sample.

The samples were packed in air tight container.

Evaluations

Samples were sent for DSC and IR. Interpretations of the DSC & IR show physical as well as chemical compatibility and not compatibility.

In the physical compatibility the individual drug DSC result must have to match with Combination drug DSC it means this was necessary to show the individual peak of drug in the combination and at same position of its Melting point. & does not show any changes in melting point. In the chemical compatibility this is necessary that individual drug peak band must have to match with combination IR graphs peak band individually and does not show any new function group peak or the band.

Formulation

Preparation of ointment base.

The base was selected according to the properties of the compounds or contains which are used in the preparation.

Paraffin ointment base were selected.

Procedure

All the inactive ingredient was taken accurately by their weight and it was melted with continued stirring and was cooled with stirring up to make uniform semisolid form. And transfer it in well closed container with label of pharmaceutical aid only.

Preparation of active ointment.

Formula. (100 gm)

Procedure

Melt the base by weight approximately about 50 gm and then the active ingredients were added to it according to descending order of melting points. Firstly base was melted then Betamethasone valerate was added, when it was completely melted then dithranol was added then hydroquinone was added then salicylic acid was added and lastly lignocaine hydrochloride was added. Then it was

transferred to measuring cylinder and remaining amount of melted paraffin ointment base was added to make the volume. Then transferred it in container and stirred it continued to make it uniform and then cooled it at normal temperature.

Physical Evaluations of ointment.

Evaluation of the Formulation (in vitro)

The prepared ointment formulations incorporating active ingredients i.e. Betamethasone valerate, Dithranol, Hydroquinone, Salicylic acid, Lignocaine hydrochloride were subjected for the in vitro evaluation and stability studies by using the various parameters.

Physical evaluation

The colour, appearance and the feel on application of the prepared ointment formulations were noted and the results are shown in table.

Subjective Properties

Subjective properties such as consistency, texture were observed and shown in table.

pH measurement

The pH of the gel was determined by using a digital pH meter (Systronics pH meter type 335) 100 gm ointment was melted at temperature 40^0c and adjusts the temperature knob at 40^0c and pH was determined by dipping the glass electrode completely into ointment solution system so as to cover the electrode. Then

Instrument reading in terms of pH are tabulated in the table. The pH was studied for 30 days.

Rheological properties

Topical formulation i.e. ointment was studied for their rheological properties over a period of one month using Brookfield Viscometer (Brookfield Engg Lab. Inc. Model LVT). In each case, 100 g. of the ointment sample was kept at room temperature. The viscosities of ointment formulation was observed on 0, 2, 7, 14, 22, 30th day. The observations were noted and viscosity was calculated by using the formula

Viscosity = dial reading x factor

The viscosities of the ointment formulation were studied at different speeds using spindle no. IV They are shown in the table.

Determination of Spreadability

Spreadability is a term expressed to denote the extent of area to which the ointment readily spreads on application to the skin or the affected part. The therapeutic efficacy of a formulation depends upon its spreading value. Hence the determination of spreadability is very important in evaluating the formulation characteristics.

Method

Two glass slides of standard dimension (20 cm x 5 cm) were selected. The formulation whose spreadability has to be determined was placed over one of the slides. The other slide was placed on the top of the cream or gel such that the formulation is sandwiched between the two slides in an area occupied by a distance of 6.0 cm alongside 100 gm weight was placed uniformly to form a thin layer. The weight was removed and the excess of ointment adhering to the slides was scrapped off. The two slides in position were fixed to a stand (45^o angle) without slight disturbance and in such a way that only the lower slide was bed firmly by the force of weight tied to it. A 20 gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 5.0 cm and separate away from the lower slide under the direction of weight was noted. The experiment was repeated and the mean time taken for three such determinations was calculated. The results were recorded.

Spreadibility is calculated by using this formula

$$S = W \times L / t$$

Where S = spreadability
L = length of glass slide
W = Weight tied to upper slide
t = time

In present experiment W = 20 gm, L = 5 cm

Antipsoriatic activity

Mouse tail anodel for psoriasis (H.G. voget et. al, 2002)

Procedure

Male albino mice weighing 25-30 g were used. The tails were treated locally with 0.1 gm ointment applied to the proximal part of the tail.

For the contact time of 2 h a plastic cylinder was slipped over the tail and fixed with adhesive tape. At the end of contact time the cylinders were removed and the tails washed. Animals were treated once daily, 5 times a week, for 3 weeks. Ten animals were used per dosage group. Two hours after the last treatment the animals were sacrificed and the tails prepared histologically (fixation in 4% formalin, paraplastic embedding). Longitudinal sections of about 5 pm thickness were prepared and stained with hematoxylin-cosin. The epidermal thickness was measured as the distance between the demo-epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in each of 10 scales. Out of these 50 measurements the mean for the individual animals was calculated. The sections were examined for the presence of a gramiar layer or isolated gransular cells induced in the previously parakeratotic skin areas (10 sequential scales per animal). The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit. The distances were obtained in pixels (1 pixel = 1.2120 pm). Quantitative values of orthokeratosis were obtained by measuring the lenght of the granular layer per scale (A) and the whole scale lenght (B). The whole scale lenght is defined as the lenght of the scale lying between two adjacent hair follicles, beginning and ending at the turining point between hair follicle and scale. Percent keratosis is calculated by the formula:

(A / B) x 100

Experimental Design

All animals were divided in to five groups, comprises 10 animals in each group.

Evaluations

Ten sequential scales per animal were measured and the results given in % orthokeratosis per scale. Ten animals were taken for one drug concentration or control group. Thus 50-80 individual orthokeratosis values were obtained per test group. Mean and standard error of the mean were calculated per animal and per group. From the individual orthokeratosis values per dosage group (50-80 scales) a frequency distribution is constructed. Therefore the values (ranging from 0 to 100% orthokeratosis) are grouped into classes with a constant class interval of 10% (class 1: 0-10% class 2: 10.1-20%; class 10: 90.1-100% orthokeratosis). The frequency per class was calculated in %:

$$\text{Class frequency} = \frac{\text{No. of scales in the class}}{\text{Total no. of scales}} \times 100$$

For every class the cumulative frequency was constructed by adding the frequencies of all foregoing classes. Due to a non-Gaussian distribution of the orthokeratosis values (100% is the maximal effect). Results were analyzed statistically using one way ANOVAs followed by dunnet's test. The efficacy of test compounds on cpidermal differentiation was calculated from the mean length of orthokeratosis after treatment with the substrate (Oks) and with control (Okc) using the formula:

Histological examination

Ten sequential scales were examined for the presence of a granular layer induced in the previously parakeratotic skin area. The induction of orthokeratosis in those parts of the adult mouse tail. Which have normally a parakeratotic differentiation, was quantified measuring the length of the granular layer (A) and the length of the scale (B). The proportion (A/B) 100 represents the % orthokeratosis was calculated as follows:

$$\text{DA} = \frac{\text{Mean OK of treated group - Mean OK of control group}}{\text{100 - Mean OK of control group}} \times 100$$

The measurements were carried out at the border of the scale with a semiautomatic image evaluation unit.

Measurement of epidermal thickness

It was obtained by measuring the distance between the dermoepidermal borderline and the beginning of the horny layer. Five measurements per animal was made in every 10 scales and the mean of different animals was calculated.

Results and Discussion

This study showed the developed formulation and evaluated by different parameter

Physical compatibility study (DSC).

Physical compatibility of Betamethasone valerate, Dithranol, Hydroquinone, Salicylic acid and lignocaine HCL drugs combination was determined by Differential Scanning Calorimetry (DSC). In the individual DSC studies of the drugs, Betamethasone valerate peak was obtained at 207.36-210.51 ^{o}C, Salicylic acid peak at 157.58-158.69 ^{o}C, Lignocaine peak at 67.00-68.01 oC and combination peaks at 67.00 – 68.01 ^{o}C, 157.58-158.69 ^{o}C, 170.78-172.36 oC, 176.37-181.35 ^{o}C, 207.78-210.57 ^{o}C. These peaks match the peaks outcomes from pure drugs. The characteristic endothermic peaks of individual drug are due to melting of drug.

The DSC thermogram of mixture showed five different peaks at,

67.00-68.01 ^{o}C (endothermic),
157.58-158.69 ^{o}C (endothermic),
170.78-172.36 ^{o}C (endothermic),
176.37-181.35 ^{o}C (endothermic),
207.78-210.57 ^{o}C (endothermic).

The DSC thermogram of drug combination showed drug compatible with each other, Drug showed physical compatibility.

Chemical compatibility study (FT-IR).

FT-IR spectra of Betamethasone valerate alone, Dithranol alone, Hydroquinone alone, Salicylic acid alone, Lignocaine HCL and their mixture were measured. FT-IR spectrum of all alone dedicated its characteristic peaks. Characteristic peaks of any of this should not affected by presence of any drugs in its mixture. These results revealed that there is no molecular interaction between all this five drugs in the solid state. For more confirmation of no molecular interaction between the five drugs, conclusively, the chosen five drugs are compatible when mixed together in the solid state. Drugs showed the chemical compatibility

Physical evaluation.

The results of various physical parameters for evaluation of the prepared topical ointment formulations are reported below.

All results of different parameters of evaluation were recorded. The physical parameters such as color, appearance and feel on application were observed and shown in table.

Table 4 - Physical Evaluation.

Sr. No	Physical Parameter	Formulation
1	Colour	Yellow
2	Appearance	Greasy
3	Feel on application	Smooth

The subjective properties such as consistency, texture and irritation were observed and shown in table.

Table 5- Subjective Properties.

Sr. No	Parameter	Ointment
1	Consistency	Good
2	Texture	Smooth

The pH values of topical ointment formulation were studied at 40 ^{O}C temperature by adjusting temperature knob and pH was observed and shown In table.

pH of ointment.

pH of ointment was determined using digital pH meter. 100 gm. Of ointment was melted at 40 ^{O}C and pH determined. Studied for 30 days.

Rheological Properties

For stability study of prepared herbal gel formulation the rheological studies are carried out viscosity is observed at different speeds at room temperature or 25 ^{O}C, and compared the observation and shown in table. For speed 0.3,0.6,1.5,3.0 R.P.M. using spindle no. IV

Spindle No. IV

Formula Dialed Reading x Factor = Viscosity (Cp.)

Spreadibility

All formulations were studied for their spreadability. Spreadibility was calculated by using formula

S = W x L / T

W= wt of sample
L= length of slide
T= time taken
The values are shown in table

Pharmacological evaluation
Antipsoriatic activity
This study showed the percent of orthokeratosis produced on mice tail. Control group reading mean percent of orthokeratosis ± SEM was calculated (p value ***p <0.0001) and the results were analyzed statically by using one way ANOVAs followed by Dunnet's test.

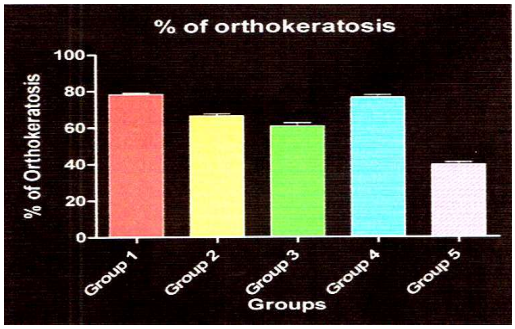


Fig. 3. Shows percentage of orthokeratosis.

1. Group 1 received ointment of novel combination produced 78.33 % of orthokeratosis and showed 64.08 % of drug activity.
2. Group 2 received ointment of Dithranol (0.5 % w/w) + salicylic acid (10.00 % w/w) produced 66.50 % of orthokeratosis and showed 44.48 % of drug activity.
3. Group 3 received ointment of betamethasone valerate (0.1 % w/w) + salicylic acid (10.00 % w/w) produced 60.83 % of orthokeratosis and showed 33.70% of drug activity.
4. Group 4 received ointment of dithranol (0.5 % w/w) + betamethasone valcrate (0.1 % w/w) + salicylic acid (10.00 % w/w) produced 76.50 % of orthokeratosis and showed 61.05 % of drug activity.
5. Group 5 received paraffin ointment base, produced 39.66 % of orthokeratosis.

Conclusion

DSC of combination showed endothermic peaks of individuals drugs and it showed peak at same position which got in alone DSC of same drug. There were no minute changes of drugs physical constant in thermogram of combination. The drugs showed the physical compatibility.IR of combination showed the band at same position which got in alone IR pf same drugs. There is no molcular a change was observed in IR of combination. The drugs showed the chemical compatibility .From the results of physical evaluation parameters the colour - yellow, appearance - greasy feel on application - smooth. This shows desirable physical parameters. The results of subjective properties of prepared formulation, having good consistency and smooth texture.
The pH of novel ointment studied and showed a minute change in pH after 2 days, 7 days, 14 days and 30 days. The range of pH was 5.59-5.62. pH the normal range of the pH (5.5-6.50) of the skin therefore making the formulation less sensitive and ensures better acceptability to the skin. Rheological study was made. The formulations were subjected to accept stability testing. The data collected for viscosities of the prepared ointment over a period of one month. Firstly viscosity was studied at speed 0.3 rpm by using spindle no. IV and readings were taken for prepared formulation, and then the speed was increased to 0.6 rpm again spindle no. IV was used, and then the speed was increased to 1.5 rpm again spindle no. IV was used, and then last speed 3.0 rpm at spindle no. IV. The viscosities of the prepared ointment formulation at different speeds showed no significant change. So it was concluded that ointment was stable. The spreadability for prepared ointment formulation was studied. The prepared ointment formulation showed desirable spreadability values.
From the stability studies it is clear that the formulated ointment showed no significant changes in pH, spread ability, viscosity and phase consistency keeping at different temperatures for 30 days at 0^{o}c and 30^{o}c. It was concluded that ointment was stable. According to results of antipsoriatic activity it was concluded that, the effect of novel combination showed equivalent effect to that of the standard group and could not produced

any changes in existing dithranol preparations effect. According to all result it was concluded that novel combination was stable and more effective than existing preparation including to overcome the existing side effects of dithranol preparations.

References

1. www.demis.com/
2. Kool J., Population base epidemiologic study of psoriasis with emphasis on quality of life assessment. Dermatol clin, 14 (1916) 485-96.
3. www.cdcinfo@cdc.gov/
4. Tarek afifi, Gillian de Gannes, Changzheng huang, Youwen Zhou. Topical therapies for psoriasis. Evidence. Based review CME, (1976) 519-525.
5. Do korte j. Members Fimc Onder zoek naar kwaliteit van leven bij psoniasis, Ned Tijdschr Dermatol & venereal, (1999) 59-61.
6. Whitefield M., Leeder G.M., Mosedale A. R. Inhibition of dithransol inflammation by free radical. Scavenges (Letter) Lancet; (1985) 1:173.
7. Whitefield M. Pharmaceutical formulations of Anthralin. Br. J. Dermatol; 105 suppl20 (1985).
8. Ashton R E., Andre P. Lowe N J., Whitefield M. Anthralin; historical and current perspectives J a, A cad Dematol; 9: (1983) 173-92
9. Wiegrebe W. Plimier e, Mayer K K, Runne U, Schultz Amling W, Rosmarinowski J. Experimental contribution to the dithranol – Brown problem. Arch Dematol Res; 277: (1985) 153-5.
10. Vogel H.G. Mouse tail model for psoriasis, Drug Discovery and Evaluation Pharmacological Assays; second edition; (2002) 1323-1324

Table 1. Formula of paraffin ointment base.

Sr. No.	Inactive ingredients	Quantity (gm)
1	White bees wax	20
2	Hard paraffin	30
3	Cetostearyl alcohol	50
4	White soft paraffin	900
Total Paraffin ointment base		1000

Table 2. Formula of novel ointment.

Sr. No.	Ingredients	Quantity Given	Quantity Taken
1	Betamethasone Valerate	0.10%	100 mg
2	Dithranol	0.50%	500 mg
3	Salicylic acid	10.00%	10.0 mg
4	Hydroquinone	2.00%	2.00 mg
5	Lignocaine hydrochloride	5	5.00 mg
6	Ointment base (q.s.)	100 % up to	100 gm up to
Prepared ointment of novel combination			100 gm

Table 3. Experimental design of antipsoriatic activity.

Groups	Treatment
Group 1	Ointment of novel Combination
Group 2	Ointment of Dithranol 0.5 % w/w + Salicylic acid 10 % w/w
Group 3	Ointment of Betamethasone valerate 0.1 % w/w + Salicylic acid 10 w/w
Group 4	Ointment of Betamethasone valerate 0.1 % w/w + Dithranol 0.5 % w/w + Salicylic acid 10 % w/w
Group 5	Control (only paraffin ointment base.)

Table 6. pH of Formulation.

Time in days	0	2	4	7	14	22	30
pH	5.60	5.62	5.60	5.61	5.60	5.59	5.60

Table 7. Viscosity (Cp.)

Time in days	Temp 25oC. Spindle No. IV			
Speed	0.3 (RPM)	0.6 (RPM)	1.5 (RPM)	3 (RPM)
0	750000	520000	322000	265000
2	750000	520000	322000	260000
7	740000	520000	324000	262000
14	740000	530000	324000	262000
22	730000	530000	322000	264000
30	740000	530000	324000	264000

Speed 0.3,0.6,1.5,3.0 RPM

Table 8. Spreadibility

Time in days	Spreadability
1	500
7	500
14	500
21	500
30	500

Table 9. Percentage of orthokeratosis produced in mice.

Sr. No.	Animals Marking	Groups				
		Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
1	**Head**	78.33	66.66	55	76.66	43.33
2	**Tail**	80.00	70.00	53.33	70.00	40.00
3	**Back**	80.00	68.33	56.66	68.33	36.66
4	**None**	78.33	63.33	60.00	81.66	40.00
5	**Head + Tail**	73.33	60.00	68.33	78.33	33.33
6	**Head + Back**	81.66	68.33	66.66	80.00	40.00

To be continued

7	**Tail + Back**	78.33	66.66	68.33	78.33	40.00
8	**H. + T. + B.**	76.66	70.00	60.00	78.33	46.66
9	**Fore limbs**	78.33	68.33	60.00	76.66	36.66
10	**Hind limbs**	78.33	63.33	60.00	76.66	40.00
Mean ± SEM		78.33 ± 0.7028***	66.50 ± 1.038***	60.83 ± 1.689***	76.50 ± 1.325***	39.66 ± 1.160

Values ± SEM (standard error mean)***p<0.0001 extremely significant. In regard to non-treated (n = 10 animals/group).

Table 10. Percentage of orthokeratosis & percentage of activity.

Groups	Treatments	Orthokeratosis (%)	Activity (%)
Group 1	**Novel Combination**	78.33 ± 0.7028***	64.08
Group 2	**Dithranol + Salicylic acid**	66.50 ± 1.038***	44.48
Group 3	**Betamethasone valerate + S.A.**	60.83 ± 1.689***	33.70
Group 4	**Dithranol + B.V. + S.A.**	76.50 ± 1.325***	61.05
Group 5	**Control (Paraffin base)**	39.66 ± 1.160	--

Values are mean ± SEM ***p<0.0001 in regard to non-treated (n = 10 animals/group).

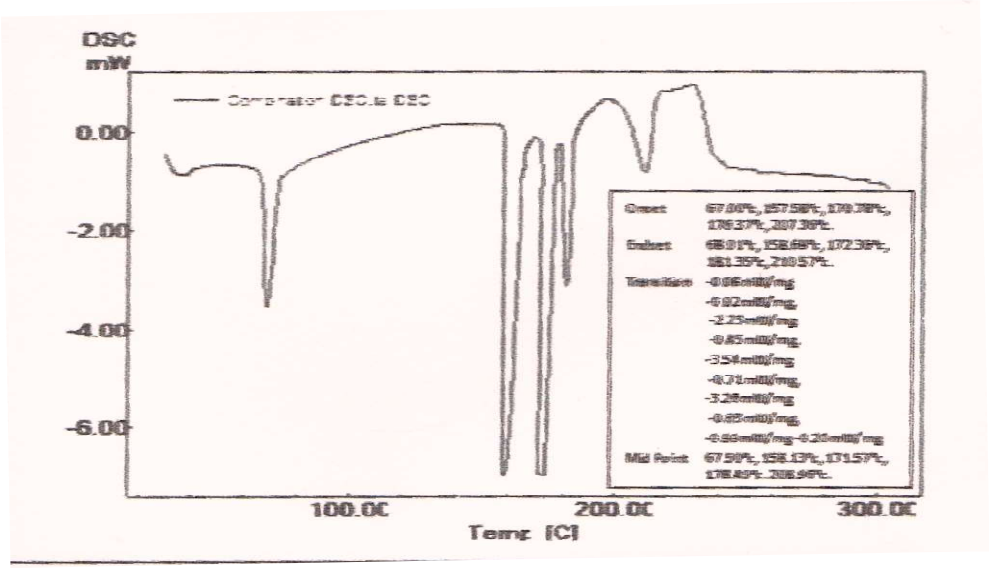


Fig 1. DSC of Combination. Shows five different melting point peaks (Betamethasone valerate, Dithranol, Hydroquinone, Salicylic acid and lignocaine HCL).

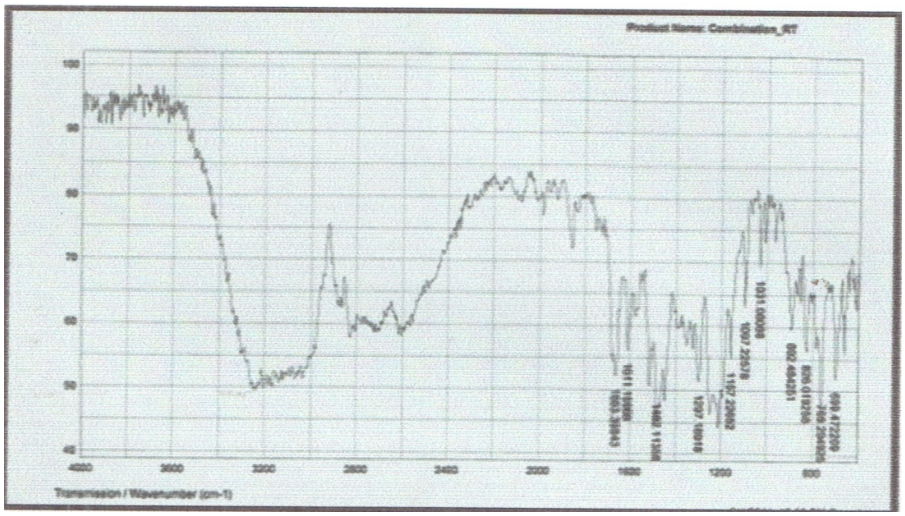


Fig 2. FT-IR of Combination (Betamethasone valerate, Dithranol, Hydroquinone, Salicylic acid and lignocaine HCL).

Source of Support: Nil.

Conflict of Interest: None declared

****