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RESEARCH ARTICLE

CONSEQUENCE OF CALENDULA OIL ON THE *IN- VITRO* PERCUTANEOUS ABSORPTION OF DICLOFENAC SODIUM

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ABSTRACT:

Terpenes are widely used as penetration enhancers in transdermal drug delivery systems. Calendula oil also contains different terpenes. The enhancing effect of calendula oil on the *in- vitro* percutaneous absorption of diclofenac sodium (DFS) from carbopol gels containing propylene glycol was investigated. Permeation experiments were performed on artificial skin membrane. The permeation effect of calendula oil on DFS was compared with geraniol and menthol (mild accelerants). The enhancing effect of calendula oil was found to be more as compared with geraniol and menthol. However, although the addition of calendula oil increased DFS flux; diffusional lag times were longer than for the control gel.

Keywords: Diclofenac sodium; Calendula oil; Skin penetration enhancer; Percutaneous absorption; Terpenes; Geraniol; Gel formulation

INTRODUCTION:

Diclofenac sodium (DFS) is popular non- steroidal anti-inflammatory drug. Upon oral administration it undergoes first pass metabolism and because of its short biological half- life, the drug has to be administered frequently. The physicochemical, pharmacokinetic and pharmacological properties of this drug make it well suited for transdermal drug development as the biological half life of the DFS is less than 4hrs (1.2to2hrs),molecular weight is less than 400 Dalton (296 Dalton), dose of drug is less 20mg/day for transdermal administration. The DFS is neither highly lipophilic, nor highly hydrophilic and it is suitable for transdermal delivery but shows low permeability and hence not easily absorbed on transdermal application[1,2].

To overcome the less permeability of drugs through the skin, fewer strategies have been used. Most of them use penetration enhancer which is a popular technique. These agents partition into, and interact with, the stratum corneum constituents to induce a temporary, reversible increase in skin permeability. Usually, terpenes enhance drug permeation by any of the following three mechanisms: disruption of the highly ordered lipid structure of stratum corneum; increased drug diffusivity in stratum corneum or increased drug partitioning into stratum corneum[3].

Therefore many compounds, such as terpenes (geraniol and menthol), isopropyl myristate, nicotinic acid esters, hydrogenated soya phospholipid, ethanol, n-octanol and decanol and nonionic surfactants have been reported to enhance the permeation of DFS through the skin [4-9]. Out of them terpenes are widely used as penetration enhancers[10].

The calendula oil is a volatile oil distilled from flowers of *Calendula officinalis* (Asteraceae). After systematic studies, the various constituents of this oil found were α-cadinene, α-cadinol, t-muurolol, menthol, 1,8-cineol, terpene-4-ol,geraniol with p-cymene[11,12].

The enhancing effect of calendula oil on the *in vitro* percutaneous absorption of diclofenac sodium (DFS) from carbopol gels containing propylene glycol was investigated in artificial skin membrane considering the fact that the calendula oil also contains different terpenes which have been identified by GC-MS analysis[12]. The permeation effect of calendula oil on DFS is compared with geraniol and menthol (mild accelerants).

MATERIALS AND METHOD:

Materials:

Diclofenac sodium (a gift sample of Zim laboratory Pvt. Ltd. India), carbopol (Loba chemicals), propylene glycol and triethanolamine (Fine chemical), geraniol and menthol (BASF,Mumbai), calendula oil (extracted from calendula flowers by using Clevenger apparatus) and artificial skin membrane (Dialysis membrane 70 Code-DM003: LA 393-1MT HIMEDIA).

Drug formulations:

The gel were prepared by adding 1% drugs in the formulation shown in Table 1.

Characterization of gel:

pH:

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for 30 minutes. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Table 1: Different formulations of DFS gel

INGREDIENTS (gm)	F1	F2	F3	F4
Diclofenac sodium	1	1	1	1
Carbopol	2	2	2	2
Propylene glycol	15	15	15	15
Geraniol	--	1	--	--
Menthol	--	--	1	--
Calendula oil	--	--	--	1
Triethanolamine	3.50	3.50	3.50	3.50
Water	(ad 100 gm)			

Drug content:

1 g of the prepared gel was mixed with 100ml phosphate buffer. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was recorded using UV spectrophotometer at 276 nm. Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve.

Viscosity:

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 100 rotations per minute and the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookefield Viscometer catalogues.

Spreadability:

One of the criteria for a gel to meet the ideal quantities is that it should possess good spreadability. It is the term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value.

Spreadability (S) is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$S = M \times L / T$

Where M = wt. tied to upper slide

L = length of glass slides

T = time taken to separate the slides

Extrudability study:

The formulations were filled into collapsible metal tubes after the gels were set in the container. The extrudability of formulation was determined. It is a usual empirical test to measure the force required to extrude the material from tube. In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The values of gel formulations obtained are shown in Table 2.

Table 2: Characterization of gel formulations

Test	F1	F2	F3	F4
pH	6.5±0.2	6.8±0.4	6.7±0.5	6.8±0.3
Drug content (%)	98.97±1.2	98.55±0.9	98.75±0.5	99.15±1.5
Viscosity(cps)	6800±50	6800±70	6700±55	6600±65
Spreadability(g.cm/sec)	6.85±0.15	6.45±0.10	7.05±0.35	7.10±0.3
Extrudability	Excellent	Excellent	Excellent	Excellent

Values are the mean ± S.E. of three determinations at 37°C (N=3).

***In- vitro* permeation study:**

The artificial skin membrane was equilibrated in isotonic phosphate buffer (pH 7.4) for 2 h before being mounted on a Franz-type diffusion cell with an available diffusion area of 1.76 cm^2.The receptor phase consisted of a phosphate buffer solution (pH 7.4, 25 ml), stirred at 600 rpm with the help of magnetic bead and maintained at 37°C. The synthetic semi-permeable membrane was mounted between the donor and receptor compartments. The gel (1 g) formulation was applied to the membrane. Samples were withdrawn through sample port of the Franz diffusion cell at predetermined time interval over 24 hours and analyzed by UV spectrophotometer at 276 nm. The receptor phase was immediately replenished with equal volume of phosphate buffer solution of pH 7.4.

The permeation profiles of DFS through artificial skin membrane from plain carbopol gels (F1), containing calendula oil (F4) and different terpene (F2, F3) as penetration enhancers are given in Fig. 1. The flux, J, was determined from the slopes at steady-state and the lag time from the x-intercept. The permeability coefficient, Kp, was estimated from the flux and the donor drug concentration. The effects of calendula oil and terpene enhancers on DFS permeation parameters are summarized in Table 3. Penetration-enhancing activities are expressed as enhancement ratios(ER) which is the ratio of the Kp value with enhancer to that obtained with plain carbopol gel. The flux values of different gel formulations are shown in Figure 2.

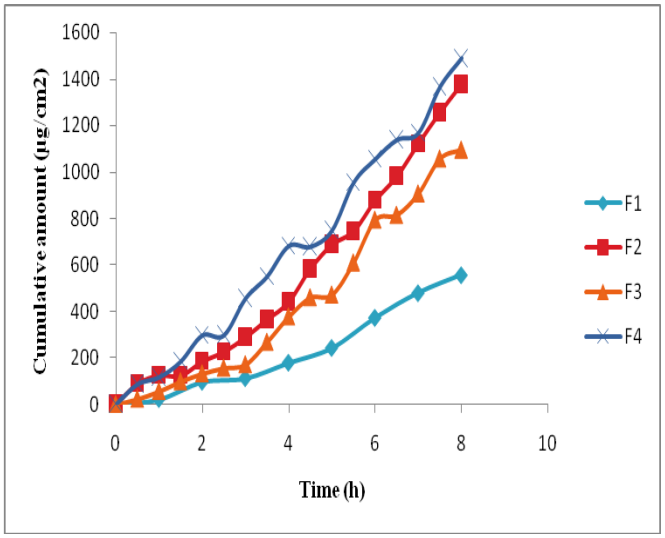

Figure 1: Permeation profile of diclofenac sodium through artificial skin membrane from carbopol gel containing calendula oil and terpenes as penetration enhancers. Each points represents the ± S.E. of two to three experiments.

Enhancement ratio (ER) = Kp value of enhancer gel/ Kp value of plain gel

Permeability coefficient (Kp) = flux value/donor drug concentration

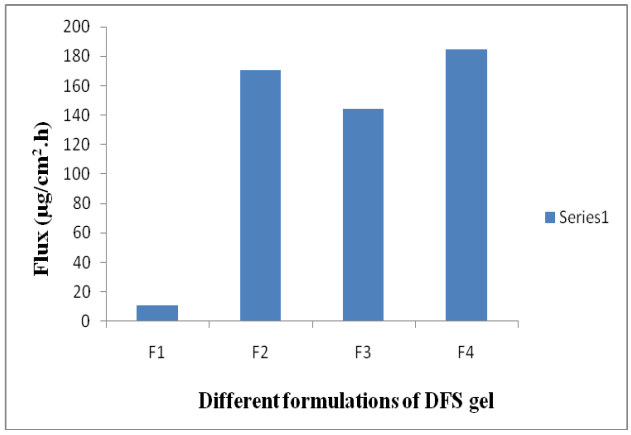

Figure 2: Flux values of different gel formulations

Table 3: Effects of calendula oil and terpenes enhancer on DFS permeation parameters [a]

Enhancer	J(µg/cm^2.h)	Kp ×10^{-3}(cm/hrs)	Lag time(h)	ER
Plain gel	10.61±0.5	1.061±0.006	0.34±0.2	31.20±1.7
Geraniol	170.7±2.5	17.07±0.5	0.73±0.3	233.83±3.5
Menthol	144.4±4.3	14.44±0.8	0.95±0.2	152±3.9
Calendula oil	184.4±3.7	18.44±0.7	0.4±0.08	461±5.7

[a] Values are the mean ± S.E. of three determinations at 37°C (N=3).

RESULTS AND DISCUSSION:

These results demonstrate that the calendula oil is an effective accelerant for DFS drug as compared to geraniol and menthol. The most outstanding penetration enhancer was calendula oil, providing an almost 20-fold increase in DFS permeability coefficient, followed by geraniol, and then menthol. Eventhough penetration enhancing effects of geraniol are closely related with calendula oil, the skin care properties of calendula oil may be an added advantage and it can be used in various transdermal commercial formulations as an effective penetration enhancer.

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CONFLICTS OF INTERESTS: Nil

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