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Development and *in vitro* Evaluation of Diclofenac Hydroxyethyl Pyrrolidine Formulations Containing Penetration Enhancers

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Diclofenac hydroxyethyl pyrrolidine (DHEP), a novel salt of a known non-steroidal antiinflammatory drug was formulated (1.32% w/w DHEP equivalent to 1% w/w diclofenac) in a hydrogel base using Carbopol of 940 and 941 grade separately and also in emulsion base. Formulations of DHEP with different penetration enhancers in the three bases mentioned above were prepared and evaluated for their physicochemical properties. *In vitro* drug permeation studies were carried out in Keshary-Chien glass diffusion cells using freshly isolated guinea pig skin for all the formulations in comparison with gels containing no penetration enhancer. The experimental study involved colorimetric estimation of the amount of diclofenac diffusing from a formulation across the skin. Our results indicated that the lag time for the drug diffusion was not altered after the addition of an enhancer to any type of formulation but addition of a penetration enhancer increased the rate of drug diffusion when compared to formulations containing drug alone. Cardamom oil (1% w/w) showed maximum enhancement of flux of diclofenac *in vitro* in comparison with other enhancers used in the formulations.

The ability of a drug in a topical formulation to penetrate the skin and to exert its effect is dependent on two consecutive events[1]. The drug must first diffuse out of the vehicle to the skin surface and then it must penetrate this natural barrier en route to the site of action. The process of drug diffusion and its penetration to the site of action depends on physical properties of the drug, vehicle, barrier and penetration enhancers present in the formulation. Topical formulations of non-steroidal anti-inflammatory drugs are becoming increasingly popular due to minimized adverse effects during their use.

Diclofenac hydroxyethyl pyrrolidine (DHEP) also known as diclofenac epolamine is a novel salt of diclofenac with promising transdermal potential[2]. The physicochemical properties of DHEP are suitable for dermal absorption of the active principle diclofenac from a topical form such as gel[3]. The higher water solubility of DHEP (1.89% w/w) allows the active principle to be obtained at the desired concentration, completely dissolved and therefore readily available for rapid absorption through the skin, from a gel or gel like structure. The weak detergency of DHEP may be important in improving the penetration through the external layer of the skin. Due to its surfactant behaviour and ability to interact with membrane components such as lecithins, DHEP can modify membrane permeability and improve its own absorption.

The following study has been designed to investigate *in vitro* permeation of diclofenac from gels and an emulsion base containing DHEP and the effect of various penetration enhancers on its flux using Keshary-Chien Diffusion Cell model.

MATERIALS AND METHODS

Reagents and chemicals

Diclofenac hydroxyethyl pyrrolidine was a generous gift from Amoli Organics Ltd., Mumbai. Carbopol 940 and 941

manufactured by BF Goodrich, USA were received as a gift from a leading multinational pharmaceutical company. D (+)-limonene and phosphate buffered saline (PBS) were purchased from Sigma Chemical Co., USA. Cardamom oil was purchased from M/s Shah International Aromatics, Mumbai. Deet (N,N-diethyl-3-methyl benzamide) and isopropyl myristate (IPM) were of Pharmacopoeial grade and were available in-house.

Preparation of standard calibration curve of DHEP in PBS

Series of dilutions of DHEP in PBS (pH 7.4) ranging from 1 µg to 150 µg were made. To each 1 ml of dilution of DHEP, 0.5 ml potassium ferricyanide followed by 0.2 ml of 6% w/w sodium hydroxide was added. The mixtures were shaken and diluted to 10 ml with distilled water after one minute. Absorbance of reaction mixture was measured at 453 nm against a reagent blank on a Perkin-Elmer spectrophotometer (n=12)Graph of absorbance Vs amount of DHEP was plotted (mean of n=12) and linear regression of Y on X was calculated using a computerized BASICA programme.

Preparation of DHEP formulations

Diclofenac epolamine (1.32% w/w) was incorporated in six different semi-solid formulae to be studied for *in vitro* diffusion across the freshly isolated guinea pig skin. Drug content and content uniformity were determined for all these preparations using colorimetric method of analysis[4].

Preparation of skin membrane

A full thickness abdominal skin was obtained from freshly sacrificed guinea pigs (male, weight 250-300 g) and it was washed thoroughly with distilled water to remove any subcutaneous matter and fatty tissues. It was then immersed in phosphate buffered saline (pH 7.4) at 37° for 30 min for equilibration before using for permeation studies.

In vitro permeation study

Keshary-Chien glass diffusion cell was used for this part of study[5]. Phosphate buffered saline, used as a receptor medium, was filled in the lower part of the diffusion cell (10 ml). It was placed on a magnetic stirrer with a small magnetic needle placed inside for uniform distribution of diffusant. The temperature of the cell was maintained at 37±0.5° by a thermostatically controlled waterbath which circulated water through the outer jacket of the cell. Isolated guinea pig skin was carefully placed over the 1 cm^2 orifice of the receptor chamber and around 0.3 g of precisely weighed formulations was applied on the epidermal side of the mounted skin. As open glass cap was tightened on isolated skin with a clamp to hold it in place for an entire duration of the study. Samples (1 ml) were removed from the receptor compartment at hourly intervals and equal volume of fresh PBS was added. Samples were assayed using a colorimetric method of analysis after subsequent dilution.

Preparation of DHEP formulations containing penetration enhancers

Diclofenac epolamine (1.32% w/w) along with 1% w/ w penetration enhancers was incorporated in three formulae selected for further *in vitro* diffusion in an exactly similar way as described above. Drug content and content uniformity were determined for all these preparations using colorimetric method of analysis.

Analysis of *in vitro* diffusion samples

One ml of PBS sample removed from receptor chamber of Keshary-Chien glass diffusion cell was analyzed for diclofenac using linear regression. Appropriate reagent blank was used for each set of this study.

Measurement of viscosity and pH of DHEP formulations

Viscosity of DHEP formulations was measured at 25° and 720 r.p.m. speed using a Plate and Cone viscometer (Sheen 490). The pH of 10% suspension of gel/emulsion base in distilled water was determined using a Metler digital pH meter.

Stability studies

Based on the results of *in vitro* permeation studies, a total of 6 formulations of DHEP were chosen for stability testing in final packing, at four different temperature conditions 4°, 20°, 37° and 45° and two relative humidities (56% and 75%) for a period of 3 months. The parameters of stability assessment used were; assay of drug content,

pH, viscosity, weight gain of packed formulation and visual change if any. The time intervals for testing were 0, 1, 2, 4, 6, 8 and 12 weeks. For assessment of drug content, ten mg (equivalent to 100 µg of drug) of carbopol gel or emulsion base containing 1% w/w diclofenac was accurately weighed and 1 ml PBS was added, vortexed and filtered to obtain a clear solution. It was then subjected to colorimetric analysis at 453 nm against a suitable reagent blank on a Perkin-Elmer spectrophotometer (n=6).

RESULTS AND DISCUSSION

Standard calibration curve of DHEP in PBS yielded the following equation for linear regression of Y on X calculated using the computerized BASICA programme :

For 10-150µg range Y = (0.008)X + 0.011 n=6, r=1.00

Precise and uniform distribution on diclofenac in every formulation was verified by drug content and content uniformity evaluations, which were found to be satisfactory.

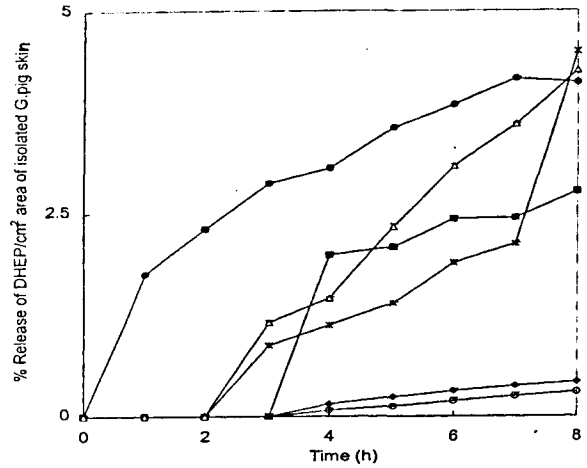

Fig -1 *In Vitro* diffusion of DHEP from semi - solid formulations using Keshary - Chien diffusion cell. Diclofenac epolamine was formulated in six different compositions and *in vitro* drug diffusion across the isolated guinea pig skin was studied for 8h, using Keshary-Chien diffusion cell. The release of DHEP from each formula is shown above as: formula1(◆), formula 2(□), formula 3(O), formula 4(O), formula 5(★) and formula 6 (▲)

In vitro permeation study

Figure 1 gives % release of DHEP from the formulation in the receptor medium across the skin with time. This study was carried out for 8 h and % release is calculated based on the amount of drug originally present in the formulation that was applied on the skin. Formulae 4, 5 and 6 released reasonably good amount of DHEP, whereas, formulae 1, 2 and 3 failed to show similar or better pattern of drug diffusion. We, therefore selected formulae 4, 5 and 6 for further drug diffusion studies using penetration enhancers. Formula 4 and formulae 6 were hydrogels of carbopol 940 and 941 grade while formula 5 was an emulsion base. New batches of DHEP formulations were made based on the above results and four different penetration enhancers, d(+)-limonene, cardamom oil, deet and isopropyl myristate (IPM) were incorporated at 1% w/w concentration, to obtain a total of 12 formulations.

Cardamom oil[6] has recently been reported to possess penetration enhancement capability, especially on NSAIDs and therefore was included in this part of the study. Cardamom oil, a volatile oil distilled from the seeds of Amomum cardamomum (Zingiberaceae) has been evaluated for its potent penetration enhancement effect on piroxicam, indomethacin and diclofenac when used at 0.1-1% concentration range[6]. Due to its fragrance it would impart a high degree of acceptability to the formulation in addition to the desired enhancing effect.

All these preparations released greater amounts of DHEP with time when compared to those containing no enhancer. However, cardamom oil brought about maximum enhancement of drug diffusion in comparison to d(+)-limonene, deet and IPM in all three formulations studied. Figure 2, 3 and 4 show the data obtained for formula 4, 5 and 6, respectively.

We selected two DHEP formulations from each class i.e. carbopol 940, carbopol 941 and an emulsion base which showed best drug release among all from the same class. Therefore, a total of six formulae; gel 4 with cardamom oil and IPM, emulsion base with cardamom oil and deet, gel 6 with cardamom oil and IPM were chosen to carry out stability studies. All these six formulations were found to be stable at different temperatures and relative humidities during the 3 months period of study. There was no change in viscosity, weight, drug content and final pH of formulations under stability analysis.

Colorimetric method of analysis for diclofenac in the gel or emulsion base was standardized by obtaining a linear

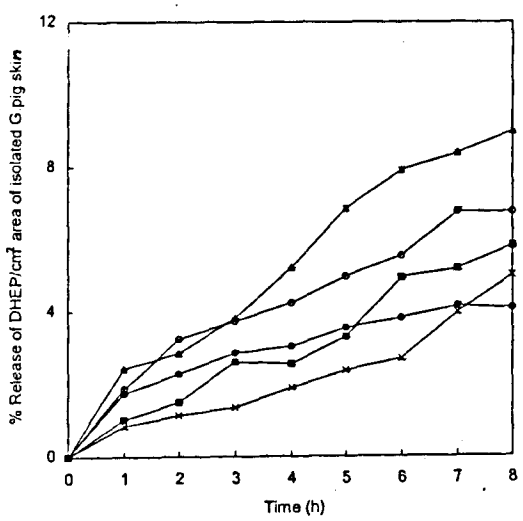

Fig.2 Effect of penetration enhancers on *In vitro* DHEP diffusion from carbapol 940 hydrogel. Donor side of Keshary-Chien diffusion cell contained 0.3 g of DHEP gel with or without penetration enhancer applied on epidermal surface of mounted skin and receptor compartment was filled with fresh phosphate buffered saline. At hourly intervals, 1ml sample was withdrawn from receptor chamber and analysed for DHEP diffused across 1 cm² of isolated guinea pig skin. Enhancers used were cardamom oil(▲), IPM (o), Limonene(□) and deet (x). Gel 4, without enhancer(O).

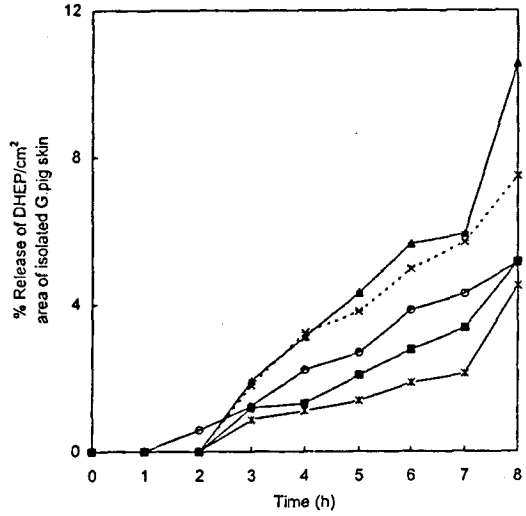

Fig. 3 Effect of penetration enhancers on *in vitro* DHEP diffusion from emulsion base. *In vitro* diffusion of DHEP from an emulsion base was carried out using Keshary-Chien diffusion cell. The experimental procedure remained same as given in Fig.2 and enhancers used were cardamom oil(▲), IPM (o), limonene (□) and deet (x). Emulsion base, without enhancer (-✱-). Each data series plotted above represents the mean of six determinations ± standard error

relationship between drug concentration and absorbance. λ_{max} for epolamine salt of diclofenac in PBS was at 453 nm (n=6) and none of the enhancers or excipients from base shifted it on the either side. We further validated this analytical method by using bases containing other NSAIDs such as nimesulide or ibuprofen in place of our formulations, which did not show any absorbance at 453 nm (n=6).

In our study, we have tested penetration enhancement effect of four enhancers from different chemical classes of compounds, d(+)-limonene, cardamom oil, deet, IPM at 1% w/w concentration in combination with ethyl or isopropyl alcohol.

Our findings indicate that in three different compositions of DHEP formulations, hydrogel containing carbopol 940 or 941 and emulsion base containing high concentration of fatty acid, only 1% w/w cardamom oil brought about the maximum enhancement of DHEP permeation across the isolated guinea pig skin. When

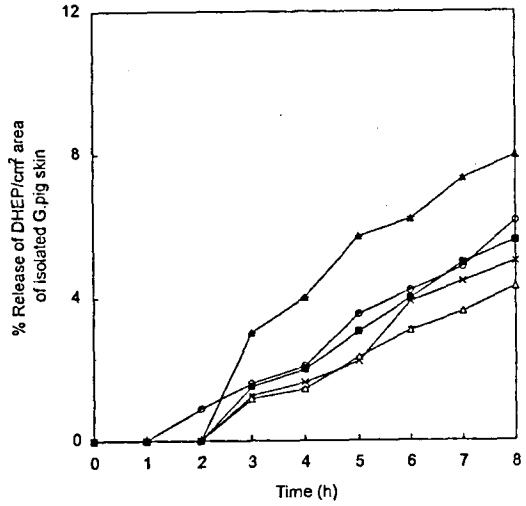

Fig.4 Effect of penetration enhancers on *in vitro* DHEP diffusion from Carbopol 941 hydrogel. *In vitro* diffusion of DHEP from a carbopol 941 based hydrogel was carried out using Keshary-Chien diffusion cell. The experimental procedure remained same as given in Fig.2 and enhancers used were cardamom oil(▲), IPM (o), limonene (□) and deet (x). Gel 6, without enhancer (Δ). Each data series plotted above represents the mean of six determinations ± standard error.

1.32% w/w DHEP (equivalent to 1% diclofenac) was incorporated in a base containing carbopol 940 (gel 4), d-limonene, deet and IPM did not enhance drug diffusion substantially, whereas, cardamom oil doubled it resulting in nearly 10% of drug release during 8 h of study (figure 2). Carbopol 941 based gel 6 exhibited diffusion profile similar to that of carbopol 940 based gel 4 (figure 2, 4).

Table -1: pH values and viscosities of gel 4 (carbopol 940 base) with and without penetration enhancers.

Formulation	pH	Viscosity (cps)
Gel 4	6.74	3900
Gel 4 + Limonene	7.03	3150
Gel 4 + cardamom oil	6.94	3150
Gel 4 + Deet	7.14	3300
Gel 4 + IPM	6.91	3250

Table - 2: pH values and viscosities of formula 5 (emulsion base) with and without penetration enhancers.

Formulation	pH	Viscosity (cps)
Formula 5	7.78	1400
Formula 5 + Limonene	7.89	1100
Formula 5 + Cardamom oil	7.87	1500
Formula 5 + Deet	7.85	1500
Formula 5 + IPM	7.60	1200

Table - 3: pH values and viscosities of gel 6 (carbopol 941 base) with and without penetration enhancers.

Formulation	pH	Viscosity(cps)
Gel 6	6.63	2200
Gel 6 + Limonene	6.71	1700
Gel 6 + Cardamom oil	6.96	1500
Gel 6 + Deet	6.91	1600
Gel 6 + IPM	6.77	1500

DHEP release from emulsion base (formula 5),was also increased to a maximum of 10%, by cardamom oil, however, even combination of deet and IPA enhanced % release of drug considerably well (figure 3). Also, alkaline pH (7.87) of the emulsion base, favoured the enhancing effect of cardamom oil when compared to acidic pH of carbopol gels. This finding is in agreement with earlier published studies[6], which have indicated that the penetration index of diclofenac with 1% cardamom oil was higher when pH increased from acidic (pH 5.8) to alkaline range (pH 7.4). The increase in % release of the drug brought about by different enhancers in formula 4, 5 and 6 has been statistically significant at different time intervals ($p \leq 0.05, t \geq 1.73$). The viscosity of 940 and 941 carbopol gels as well as emulsion base was considerably reduced (15-30%) by the addition of penetration enhancers (Table 1, 2 and 3).

In conclusion, our results indicate that addition of penetration enhancer to a gel or an emulsion base increased rate of drug diffusion without reducing the lag time for diffusion. Among the various enhancers tested, cardamom oil was found to exert maximum enhancement of diclofenac flux. Therefore, incorporation of cardamom oil in DHEP formulation may result in release of DHEP in appreciable amount at the site of application.

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