RESEARCH ARTICLE

FORMULATION, EVALUATION AND ANTI-INFLAMMATORY ACTIVITY OF TOPICAL ETORICOXIB GEL

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Abstract: The present research has been undertaken with the aim to develop a topical gel formulation of etoricoxib, which would attenuate the gastrointestinal relater toxicities associated with oral administration. Etoricoxib is a highly selective cyclooxygenase-2 (cox-2) inhibitor. In the present study gels with carbopol, HPMC K4M, MC, HPC were prepared with different permeation enhancers like, DMSO, lemongrass oil, menthe oil, oleic acid. They were evaluated for physicochemical properties, drug release. After *in vitro* evaluation of gel formulations, *ex vivo* permeation of etoricoxib was evaluated across rat epidermis and human cadaver skin. The best formulation was then evaluated for the anti-inflammatory and skin irritation study. And were kept for stability studies for period of three months and found that they were stable and finally it was concluded that the formulation F25 was the best and comparable with that of marketed product.

Keywords: Etoricoxib, carbopol, hydroxyl propyl methyl cellulose, methyl cellulose, hydroxyl propyl cellulose, anti-inflammatory activity.

INTRODUCTION

The use of non-steroidal anti-inflammatory drug is well recognized for regional inflammatory disorders such as muscle pain, osteoarhtritis and rheumatoid arthritis [1, 2]. Etoricoxib is a non-steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities. It is potent, highly selective Etoricoxib, which is described chemically as 5-chloro-6'-methyl-3-[4-(methylsulfonyl) phenyl]-2, 3'-bipyridine. However its use has been associated with a number of gastrointestinal disorders. These potential side effects may be over come by the topical administration of the drug [3, 4].

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, cream, gel, ointments, liquids, aerosols and injectables, as drug carriers. Delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders. This route of drug delivery has gained popularity because it avoids first pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration. Due to the first past effect only 25-45% of the orally administered dose reaches the blood circulation. In order to bypass these disadvantages the gel formulations have been proposed as topical application. The present research has been undertaken with the aim to develop a topical gel formulation of etoricoxib, which would attenuate the gastrointestinal relater toxicities associated with oral administration. Also, etoricoxib having molecular weight of 358.84 and melting point in the range of 134-135°C can be considered ideal to permeate through the skin.

In the present study, topical gel formulations of etoricoxib were prepared using HPMC K4M and carbopol 934 as gel forming polymers. After *in vitro* evaluation of gel formulations, *ex vivo* permeation of etoricoxib was evaluated across rat epidermis and human cadaver skin.

MATERIALS AND METHODS

Etoricoxib was a gift sample from M/S Hetero Drugs Ltd., Hyderabad. HPMC K4M, carbopol 934, methylcellulose, HPC, DMSO, oleic acid, lemongrass oil, mentha oil, all others from S.D fine chemicals, Mumbai. The Institutional Ethical Committee of NET Pharmacy College, Raichur, Karnataka, approved all experiments with animals. The department of anatomy, Navodaya Medical College, Raichur, provided cadaver skin.

Preparation of etoricoxib gel

Specified amount of drug was dissolved in appropriate and preweighed amount of ethanol to which preweighed amount of permeation enhancer was added. To the resulting solution appropriate quantity of polymer was added slowly and kept under constant stirring until clear gel was formed. Gel formulations of etoricoxib were prepared using different concentrations of carbopol, HPMC K4M, MC, HPC were prepared with different concentrations of permeation enhancers like, DMSO, lemongrass oil, menthe oil, oleic acid. Optimized formulae were given in Table 1.

Evaluation of etoricoxib gel

The prepared gels were evaluated for physical appearance, spreadability, pH, drug content, and *in vitro* release study. The physical appearance and homogenesity of the prepared gels were tested by visual observations. The spreadability [5] of the gel formulations was determined. The pH of the gel formulations was determined using a pH meter. The measurement was performed at 1st, 15th and 30th day after preparation to detect any ph fluctuations with time. For assay of the drug in gels, etoricoxib was extracted from 1gmof each gel formulations with ethanol, and diluted with phosphate buffer of pH 6.8. The resultant mixture was filtered through membrane filter (pore size 0.45 mm). The absorbance of the sample was determined spectrometrically at 238 nm (Shimadzu UV-VIS spectrometer) after appropriate dilution with prepared buffer. The viscosity of the gel formulations was determined using Brookfield viscometer (Model DV III) with spindle no.CP52 at rpm at the temperature of 37° C. The *in vitro* drug release from gel formulations was studied across cellulose membranes using modified Keshery Chien diffusion cell. The receptor compartment was filled with the solution of 75ml phosphate buffer of pH 6.8 and maintained at 37 ± 0.5° C with constant magnetic stirring. 100mg of gel was placed on the donor compartment. The samples (5ml) were collected from the receptor compartment at predetermined time interval and replaced by equal volume of fresh receptor solution to maintain constant volume allowing sink condition throughout the experiment. The amounts of etoricoxib in the sample were assayed spectrometrically at 238nm against appropriate blank.

Ex vivo evaluation

Sample of whole adult human skin (45 age) was obtained from breast reduction operation (provided by Navodaya Medical College, Raichur). Subcutaneous fat was carefully trimmed and then rinsed with normal saline. Skin was sealed in aluminium foil and a plastic bag and stored at –20 °C until used [6].

Permeation studies were performed for different formulation across cadaver skin in modified diffusion cell at 32 ± 0.5 °C. Phosphate buffer pH 6.8 was used as an *ex vivo* fluid in receptor compartment to ensure sink conditions and stability of the drug [7]. This whole assembly was kept on magnetic stirrer and the solution was stirred continuously using a magnetic bead. The sample was withdrawn at different time interval and replaced with equal volume of diffusion media. Samples were analyzed in UV spectrophotometer at 238nm and the cumulative amount of drug permeated per square cm of transdermal system was calculated.

Anti-inflammatory activity study

Anti-inflammatory [8] study was conducted using 30 albino rats

Table 1. Optimized formulae of etoricoxib gels

Formulations	Drug	Carbopol	HPMC	HPC	MC	Lemon grass oil	Menthol oil	Oleic acid	DMSO	PG	Hydro alc. vehicle	Triethanola-mine
F9	1	2	--	--	--	2	--	--	--	5	90	Q.S
F11	1	2	--	--	--	--	2	--	--	5	90	Q.S
F15	1	2	--	--	--	--	--	--	2	5	90	Q.S
F25	1	--	2	--	--	2	--	--	--	5	90	Q.S
F27	1	--	2	--	--	--	2	--	--	5	90	Q.S
F31	1	--	2	--	--	--	--	--	2	5	90	Q.S
F65	1	--	--	--	2	--	--	--	2	5	90	Q.S

Table 2. Physicochemical properties and drug release

Formulations	Viscosity CPs	Spreadability (gm^2cm/sec)	Drug content (%) ±SD, n=3	pH ± SD, n=3	% Drug released in 6 hrs
F9	3465.84	32.05	99.86 ± 0.843	6.3 ± 0.6	92.88
F11	2962.73	22.66	98.62 ± 0.744	6.4 ± 0.67	86.16
F15	3409.93	26.22	97.54 ± 0.413	6.7 ± 0.471	76.32
F25	2180.12	16.66	97.98 ± 0.362	6.8 ± 0.351	99.28
F27	2291.92	19.05	96.58 ± 0.578	6.5 ± 1.456	92.83
F31	2720.49	21.22	96.98 ± 0.486	6.7 ± 0.471	89.42
F65	2098.92	15.92	97.92 ± 0.315	6.8 ± 0.351	99.89

(approved by Institutional Animal Ethical Committee, N. E. T. Pharmacy College Raichur, Karnataka,) of either sex weighing (100-150 g) and divided into 5 groups. In all groups, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of carrageenan in normal saline in left hind paw of the rats. The medicated formulation (0.25g) or base was applied topically with gentle rubbing to the paw of each rat of respective group one hour before and one hour after the carrageenan challenge. The paw edema volume was measured using plethysmometer at 1, 2, 3 and 4 hour after injection of carrageenan. The average paw edema volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula.

% Edema inhibition = (1- Vt / Vc) 100

Where, Vt - Mean edema volume of test

Vc - Mean edma volume of control

Statistical significance was calculated by using student's unpaired't' test.

Skin irritation study

In skin irritation [9] study 10 rats (approved by Institutional Animal Ethical Committee, N. E. T. Pharmacy College Raichur, Karnataka,) of either sex weighing between (400-500g) was used. Animals were divided in to 2 groups of 5 animals each. Hairs were depleted from the back of rats with the help of depilatories and area 4 cm2 was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion gel was applied (500mg / rats) once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as.

A - No reaction, B - Slight, patchy erythema, C - Slight but confluent or moderate but patchy erythema, D - Moderate erythema, E - Severe erythema with or without edema.

Stability studies

The stability studies [10] were conducted according to ICH guidelines by storing the TD systems at 40 ± 2°C / 75% RH in stability chamber (Lab-Care, Mumbai) for three months.

RESULTS AND DISCUSSION

The physicochemical properties of the gel formulations are shown in the Table 2. From the results it is clearly evident that all the gel formulations showed the good homogenesity and spreadability. The physical appearance of the gel was translucent in nature. The drug content of the gel formulations was in the range of 97.48 ± 2.456 to 99.86 ± 0.843 % showing content uniformity. The pH of the gel formulations was in the range of 6.3 ± 0.6 to 6.8 ± 0.351, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values (varied from 0.01 to 0.16) as a function of time for all formulations. The physicochemical properties of the prepared gel formulations were in good agreement with those of a marketed product. The viscosity was in the range of 2180.12cps to 3465.84cps.

The in vitro release of etoricoxib from gel formulations through cellulose membrane were carried out to select appropriate composition for gel formulations have suitable consistency for topical application. Release profiles of etoricoxib from various gel formulations across the cellulose membrane deplicated that drug release decrease with increase in concentration of the gelling agent. The steady state flux values were also found lower for the formulations in which polymer concentration was kept high. In addition, viscosity increased as polymer concentration increased. Viscosity is negatively related to the release of active substance from formulations and its penetration through the diffusion barriers. The decrease in the release could be attributed to increased micro viscosity of the gel by increasing polymer concentration [11, 12]. The higher polymer concentration resulting in lower drug release from the vehicles is in agreement with Lauffer's molecular diffusion theory of polymer gels [13], which states that the diffusion coefficient of a solute is inversely proportional to the volume fraction occupied by the gel-forming agent.

The selected gels were evaluated in rat skin and cadaver skin the permeation profiles showed the same pattern Figure 1, 2 as that of the in vitro release profile across the cellulose membrane. A linear relationship [r^2>0.9] was observed between the cumulative amount permeated and time, indicating zero order permeation kinetics and the permeation of etoricoxib was based on diffusion controlled mechanism. An inverse relationship was observed between permeation rate and viscosity of the gel formulation. The cumulative amounts permeated at 6 hrs were 99.28% and marketed product was 99.89%.

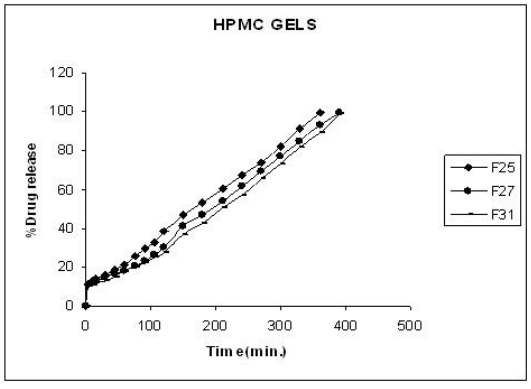

Figure1. Comparison of release of HPMC gels from human cadaver skin

The results reveal that there is an increase in permeation flux ($P < 0.05$) with increasing concentration of permeation enhancer. Formulation (F25) was found to be comparable with marketed product depicted in Figure 3 with respect to physico chemical properties and drug permeation characters. Rammler and Zaffaroni have reviewed the chemical properties of DMSO and suggested that the rapid movement of this molecule through the skin, a protein barrier, depends on a reversible

configurational change of the protein occurring when DMSO substitutes for water. Terpenes (menthe oil and lemon grass oil) have been extensiv-

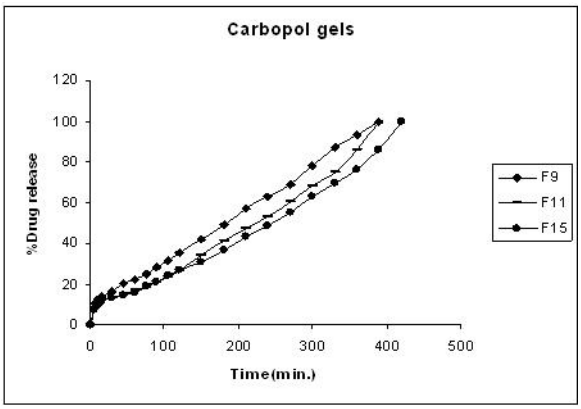

Figure 2. Release of carbopol gels from human cadaver skin

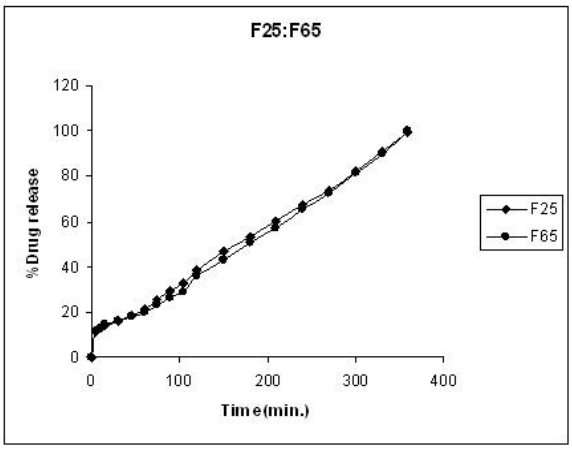

Figure3. Comparison of release of formulation F25 with marketed from human cadaver skin

ely investigated as skin permeation enhancers [14 - 16]. A proposed mechanism for terpenes to improve the skin permeation of drugs is mainly the increase in drug diffusivity in the skin [17] by modifying the intercellular packing, disrupting highly ordered structure of lipids [18]. It has been suggested that the molecular mechanism is attributed to the preferential hydrogen bonding of oxygen containing monoterpenes with ceramide head groups there by breaking the lateral/ traverse hydrogen bond network of lipid bilayer [19].

Anti-inflammatory activity: Percentage increase in paw volume (inflammation) and percentage inhibition of inflammation in control group and groups treated with test and marketed products and the results are given in the Tables 3 – 4, and Figure 4, 5.

Table 3. Mean paw edema volume of the albino rats

Time (hrs)	Control	Test (F25)	Standard (F65)
0	0.224	0.204	0.224
1	0.258	0.163	0.192
2	0.279	0.103	0.112
3	0.332	0.097	0.106
4	0.364	0.096	0.099
5	0.392	0.099	0.103
6	0.428	0.105	0.109

Table 4. Percentage inhibition of the edema in albino rats

Time(hrs)	Control	Test (F25)	Standard (F65)
0	-	24.36	21.38
1	-	37.59	25.58
2	-	63.08	59.85
3	-	70.72	68.07
4	-	73.41	72.66
5	-	74.54	73.72
6	-	75.56	74.53

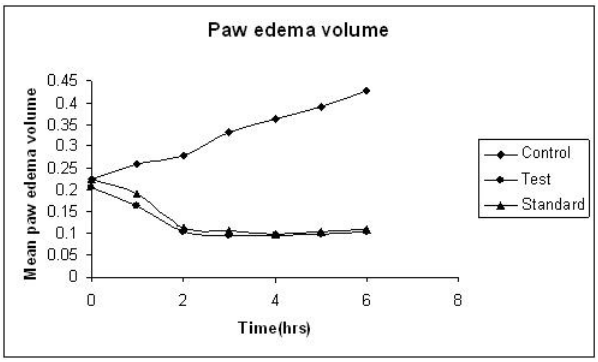

Figure 4. Comparison of rat paws edema volume of control, test and marketed (standard)

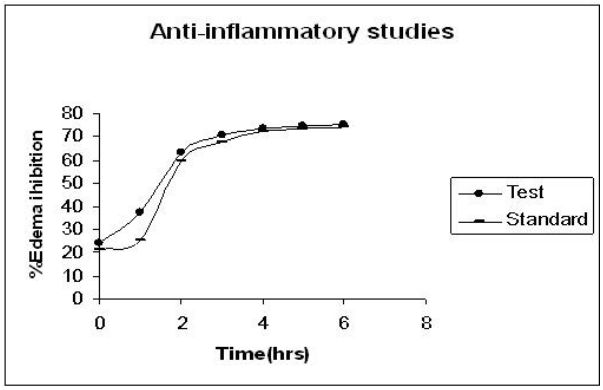

Figure 5. Comparison of % inhibition of edema volume of control, test and marketed (standard)

In control group which received carrageenan alone, a rapid and continuous increase in paw volume (i.e. inflammation) was observed and the inflammation was sustained during the entire period of 6 hrs of study.

In the groups which received test products, the percentage increase in paw volume was low when compared to the control group, indicating that the test and marketed product possess good anti-inflammatory activity. The inflammation due to carrageenan was markedly inhibited by the test and marketed products. The percentage inhibition was much higher in the initial time points. A comparison of the percentage inhibition of inflammation (i.e., anti-inflammatory activity) of test and marketed products is made in Table 4. Comparable anti-inflammatory activity was found with the test product with that of marketed product these results indicated the rapid onset of action and higher activity during the initial periods due to their enhanced dissolution and absorption rates.

Skin irritation studies: Following seven days application of the gel, the results of skin irritation test were tabulated in Table 5. The results indicated that the control preparation (which did not contain any drug), test gel (F 25) and marketed products did not cause any skin reaction. It can be assured that etoricoxib and the excipients did not cause any skin irritation and can be used in the gel formulation.

Stability studies: These formulations were stored at 40 ± 2˚C / 75 % RH in stability chamber (Lab-Care, Mumbai) for three months. After three months. Results did not show any significant variations (p> 0.05). These results indicate that drug remain stable after stability studies.

CONCLUSION

Etoricoxib is a non steroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities. It is potent, highly selective cyclooxygenase-2 (cox-2) inhibitor. To overcome the side effects associated with oral etoricoxib therapy and to have the benefits associated with topical therapy; etoricoxib topical gels are prepared in this study.

From the above results it can be concluded that the etoricoxib gel formulation F25 containing 2% HPMC with 2% lemongrass oil was suitable for topical application and it shows comparable results with that of marketed product.

Table 5. Skin irritation study results

S. No.	Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control	A	A	A	A	A	A	A
2	Test (F25)	A	A	A	A	A	A	A
3	Marketed (F65)	A	A	A	A	A	A	A

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