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RESEARCH ARTICLE

DEVELOPMENT AND ESTIMATION OF ANTI-INFLAMMATORY ACTIVITY OF TOPICAL ETORICOXIB EMULGEL BY CARRAGEENAN INDUCED PAW OEDEMA METHOD

Obanewa Opeyemi Adegbenro¹, Oyeniran Taiwo Opeyemi² ¹Department of physiology, University of Ibadan, Nigeria²Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Nigeria.**ABSTRACT**

Emulgel is one of the emerging topical drug delivery system for the delivery of hydrophobic drugs which overcome various disadvantages of ointments and creams such as greasiness and phase inversion.

Etoricoxib is poorly aqueous soluble Non steroidal Anti-Inflammatory Drug (NSAID). It is used in osteoarthritis, rheumatoid arthritis, acute gouty arthritis, ankylosing spondylitis, low back pain, acute postoperative pain, and primary dysmenorrhea. Its oral delivery is associated with greater chance of adverse effects or therapeutic failure and large amount of drug is lost in the vicinity of the target organ. Also oral administration of etoricoxib causes gastro-intestinal irritation. The aim of the present study was to develop an emulgel formulations using the gelling agent like Carbopol 934 and HPMC K4M with emulsifiers like Span 20 and Tween 20. Six emulgel formulations were developed and evaluated on different parameters like physical appearance, pH, viscosity, extrudability, drug content, spreadability, *in-vitro* diffusion studies, and skin irritation test. Best formulation of batch EG1 was further evaluated for stability study and anti-inflammatory activity in carrageenan induced rat paw edema. Anti-inflammatory effect of formulation EG1 was compared with standard market product Indomethacin.

Keywords: Anti-inflammatory effect, carrageenan, emulgel, etoricoxib, gelling agent, hydrophobic.

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INTRODUCTION

Drug delivery via skin has a potential view for a long time because skin is easy to access with large surface area and the route is non invasive. This route of drug delivery has gained popularity due to some advantages like, it avoids first pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration^{1,2}.

Gels are a relatively newer class of drug delivery systems constituting large amounts of aqueous or hydro alcoholic liquid in a complex network of solid particles. Topical gel formulations provide suitable delivery system for drugs because they are less greasy and can be easily removed from the skin³. They are intended to be applied to the skin or certain mucous membranes for protective, prophylactic or therapeutic purposes. They embrace a higher aqueous component that assists in dissolution of drugs, and facilitate easy drug release through the vehicle that is essentially a liquid⁴. Despite many advantages some limitations are associated with gels, major drawback remains, which is

its ability to deliver hydrophobic drugs. To overcome this limitation, emulgel have been emerged as an effective delivery system aiding the incorporation of hydrophobic drugs in gel dosage forms. When gels and emulsions are utilized in combination, the dosage forms are referred as emulgel⁵. Emulgel is emerging field for the topical drug delivery, and till date it has less marketed product, so it is interesting and challenging to focus on emulgel⁶. Emulgel is the approach using the benefits of both emulsion and gels, gaining the dual controlled release effect where the emulsion either oil in water or water in oil is gelled by incorporation in the gel base, simply the emulgel are emulsion in gel. Emulgel possess the properties as thixotropic, grease less, easily spreadable, easily removable, emollient, non staining, water soluble, long shelf life, bio friendly and pleasing appearance that improves the patient acceptability⁷.

Etoricoxib is a Non steroidal Anti-Inflammatory Drug (NSAID) belonging to the class of cyclooxygenase 2 (COX-2) inhibitors used in the treatment of relieving

moderate pain and swelling of joints associated with different forms of arthritis⁸. Clinical trials have established the efficacy of etoricoxib in osteoarthritis, rheumatoid arthritis, acute gouty arthritis, low back pain, acute postoperative pain, and primary dysmenorrhea⁹.

Its very low aqueous solubility and poor dissolution can cause formulation problems and limit its therapeutic application by delaying the rate of absorption and the onset of action by oral route. Moreover, oral treatment is associated with greater chance of adverse effects or therapeutic failure and large amount of drug is lost in the vicinity of the target organ. Also oral administration of etoricoxib causes gastro-intestinal irritation¹⁰.

In the present study, an attempt has been made to develop emulgel formulations of etoricoxib so as to reduce the side effects of oral administration and to enhance percutaneous absorption.

The selected formulations were further characterized for anti-inflammatory activity and compared with standard market product Indomethacin.

MATERIALS AND METHODS

Etoricoxib was obtained from Abumec Pharmaceuticals Ltd, Kaduna. Carbopol 934, HPMC K4M and Light Liquid paraffin Adler Products Limited, Lagos. Tween 20, Span 20 and Propylene glycol were obtained from Agary Pharmaceutical Limited, Lagos. Methyl paraben was obtained from Bentos Pharmaceutical Products Limited, Oyo state, Nigeria.

All other ingredients used were of analytical grade.

Preparation of Etoricoxib emulgel

The methods used to prepare etoricoxib emulgel was followed as used by Yadav *et al.* in a previous study¹¹. By dispersing carbopol and HPMCK4M in purified water with constant stirring at a moderate speed gels in formulations were prepared. By the means of tri-ethanol amine pH are adjusted to around 6. Aqueous phase was prepared by dissolving Tween 20 in purified water while the oil phase of the emulsion was prepared by dissolving Span 20 in light liquid paraffin. Methyl paraben was dissolved in propylene glycol whereas drug was dissolved in ethanol and both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70° to 80°C; then the oily phase were added to the aqueous phase with continuous stirring until cooled to room temperature. Finally the emulgel was prepared by mixing the both gel and emulsion in 1:1 ratio. The composition of different formulations is shown in Table 1.

EVALUATION OF EMULGEL FORMULATIONS

Physical Examination

The prepared etoricoxib emulgel formulations were inspected visually for their colour, homogeneity, consistency, grittiness and phase separation. The evaluation of the developed formulations for clarity observed visually with naked eye. Smears of gels were prepared on glass slides and observed under a

compound microscope for the presence of any insoluble particles or grittiness¹².

pH

The pH of the sample was measured by a digital pH meter (New Heights Pharmaceuticals Ltd, Nigeria) at room temperature. The electrode was dipped in gel for 10 seconds, and the value was read on the digital interface¹³.

Extrudability

It is an empirical test to measure the force required to extrude the material from tube. In the current study, the method adopted for extrudability is based upon the quantity of etoricoxib emulgel and quantity extruded from lacquered aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of emulgel in 10 seconds. More the quantity extruded, better is extrudability^{14,15}.

Viscosity

The viscosity of formulated etoricoxib emulgel was determined using Brookfield viscometer (Cintex Pharmaceutical Limited, Nigeria) with spindle no. 18 at 100 rpm at 25±0.5°C¹⁶.

Drug content

The drug content of etoricoxib emulgel was measured by dissolving a known weight of the emulgel formulation (one gram) in 100 ml methanol, appropriate dilutions were made and the resulting solution was then filtering using millipore filter (0.45 µm). Absorbance was measured at 275 nm using UVspectrophotometer (Shimadzu UV 1800). Drug content was calculated using the slope and the intercept obtained by linear regression analysis of standard calibration curve¹⁷.

Skin irritation test

A set of 4 rats was used in the study. A 0.5 gm of etoricoxib emulgel formulation was applied on the properly shaven area of skin approximately 2.54x2.54 cm². When the undesirable changes like skin color; change in skin morphology was checked for a period of 24 hr¹⁸.

In-vitro diffusion study

The *in-vitro* diffusion study of prepared gel was carried out in Keshary Chein diffusion cell apparatus. In Keshary-Chein diffusion cell, 500 mg of etoricoxib emulgel was spread uniformly on the cellophane membrane which was previously soaked in phosphate buffer pH 5.5 for 24 h and was sandwiched between donor and receptor compartment. 6 ml of phosphate buffer was used as receptor compartment. The temperature was maintained at 37±0.5°C. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly stirred using magnetic bead at 450 rpm. Sample of 1 ml was withdrawn and replacement was done with 1 ml of fresh buffer. The drug concentration on the receptor fluid was determined spectrophotometrically against blank at 275 nm. The cumulative amount of drug released expressed in % was plotted for formulation^{19,20}.

Anti-inflammatory study

Healthy albino rats of either sex (Wistar strain) weighing 150-200 g were used in current study. The animals were kept in plastic cages with soft bedding, 6

per cage with free access to food and water and were maintained under controlled temperature ($27\pm 2^{\circ}\text{C}$) and 12 hrs: 12 hrs light and dark cycle. Prior to the experiment, food was withdrawn overnight but adequate water was provided to the rats²¹.

The animals were divided into two groups of 6 animals each. Standard group was treated with indomethacin given by I.P., test group receive 2 g etoricoxib emulgel formulations of batch EG1 were applied over 9 cm^2 as transdermal patch on the dorsal skin after removing the hair with a clipper. The area of application was occluded with bandages and it was left in place for 2 hrs. The dressing was then removed and the emulgel remaining on the surface was wiped off with cotton. The animals were then injected with 0.1 ml of 1% carrageenan solution in saline in plantar region of left hind paw and the paw volume was measured after 1, 2, 4, 6, 8 hr using water plethysmometer. The right hind paw served as a reference non inflamed paw for comparison.

Paw volume: Initial Rat paw volume was measured using plethysmometer. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark in the plethysmometer²². Mean changes in paw volume were calculated and % inhibition of paw edema was calculated using formula:

$$\% \text{ Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c is mean changes in paw volume of control group and V_t is mean changes in paw volume treated group.

Paw thickness: Paw thickness was measured by compressing the joint by rotating the screw of micrometer screw gauge till the pain elicited as indicated by squeaking or leg withdrawal²³. The distance moved by the screw gauge was recorded and % inhibition of paw thickness was calculated using formula:

$$\% \text{ Inhibition} = \frac{T_c - T_t}{T_c} \times 100$$

Where, T_c is mean change in paw thickness of arthritis control group and T_t is mean changes in paw thickness of treated group.

Statistical analysis: Data was expressed as mean \pm SD and analyzed for statistical significance using Student's t test, one-way analysis of variance (ANOVA) followed by Dunnett's test or two-way ANOVA followed by Bonferroni test. $P < 0.05$, $p < 0.01$ and $p < 0.001$ was considered to be significant.

RESULTS AND DISCUSSION

The objective of the present study was to design and characterize an effective topical drug delivery of etoricoxib. Due to its low aqueous solubility and poor dissolution it is a poor candidate for oral route. Thus a unique approach of formulating hydrophobic drugs, emulgel, where emulsions could be easily incorporated into gels was attempted. The solubility of hydrophobic drugs can be improved by incorporating them into emulsion form, which further helps in enhancing their skin permeability.

In current study 6 emulgel formulations of etoricoxib were successfully formulated using the gelling

agent like Carbopol 934 and HPMCK4M with emulsifiers like Span 20 and Tween 20 developed and evaluated on different parameters.

The results of physicochemical parameters i.e pH, viscosity, extrudability and drug content of all batches are shown in Table 2. All trials of emulgel were found to be homogeneous and smears were transparent without grittiness or presence of any particles. The pH values of emulgel batches were found in the range of 6.8-7.1 which is similar to skin pH. Viscosity is an important parameter for characterizing the emulgels as it affects the extrudability and release of drug. The viscosity range observed for all formulation was 12208-12800 cps.

Extrusion of the emulgel from the tube is an important during application and for the patient compliance. Emulgels with high consistency may not extrude from the tube easily, where as low viscous gel may show quickly extrudability of emulgels. The extrudability of all formulations was found to be satisfactory. The percentage drug content of all formulations was found to be satisfactory and in the range of 93.54 ± 0.52 - $98.74\pm 0.08\%$.

The allergic symptoms like inflammation, redness, irritation, erythema and edema are not appeared on rats up to 24hr. The *in vitro* drug release profiles of etoricoxib emulgel formulations are shown in Figure 1. Maximum drug release was observed was 80.82% for emulgel formulations of batch EG1 at the end of 210 min and minimum release 54.62% was shown by batch EG3. Drug release kinetics data of the emulgels is given in Table 3.

Local injection of carrageenan into rat hind paw induces acute inflammatory responses such as edema. The development of the edema induced by carrageenan has been described as a biphasic event²². A rapid early phase (up to 2 h) is triggered by the concerted release of histamine, bradykinin, 5-hydroxytryptamine or cyclooxygenase products. And a more sustained late phase (2 to 5 h) is regulated by neutrophil infiltration and sustained production of arachidonic metabolites (prostanoids) (primarily by cyclooxygenase) or nitric oxide from inducible nitric oxide synthase²³. Standard indomethacin shows 54.861% mean paw volume percentage inhibition, while emulgel formulation of batch EG1 shows 18.59% (Table 4).

In a previous study Prakash *et al.* has evaluated topical etoricoxib gel for anti-inflammatory study for 6 hrs. Current study checked this study for 8 hrs to observe the changes for a longer duration²⁴.

Mean percentage inhibition of paw thickness by indomethacin was found to be 29.91%, while EG1 shows 18.986% (Table 5).

CONCLUSION

At present scenario, emulgel is one of the recent technologies used for dual control release of emulsion and gel for topical use. Moreover, they will become a solution for loading hydrophobic drugs in water soluble gel bases for the long term stability. The stability of emulsion is increased, when it is incorporated into gel. Etoricoxib is a hydrophobic drug. In current study Etoricoxib was successfully formulated in an emulgel

drug delivery with gelling agent like Carbopol 934 and HPMCK4M with emulsifiers like Span 20 and Tween 20 developed with simple, commercial feasible manufacturing process.

Etoricoxib emulgel formulation EG1 used in this study showed significant reduction of paw edema thickness and volume at 8 hrs or more after carrageenan injection, demonstrated that the emulgel possess fairly good anti-inflammatory activity.

Based on different evaluation parameters, study concludes that EG1 emulgel formulation was the optimum formulations. However, *in vivo* investigation in relevant animal models may provide further insights into the efficiency of this drug delivery system and its relevance, which inevitably warrants further research.

CONFLICT OF INTEREST

"No conflict of interest associated with this work".

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Table 1: Quantative composition of etoricoxib emulgel formulations

Ingredients (%w/w)	EG1	EG2	EG3	EG4	EG5	EG6
Etoricoxib	1	1	1	1	1	1
Carbopol 934	1	1	1	-	-	-
HPMC K4M	1	1.5	2	-	-	-
Xanthan Gum	-	-	-	1	1	1
Light Liquid paraffin	7.5	5	7.5	5	7.5	5
Tween 20	0.6	1	1	-	-	-
Span 20	-	-	-	0.6	1	1
Propylene glycol	7	7	7	7	7	7
Ethanol	10	10	10	10	10	10
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03
Purified water q.s.	100	100	100	100	100	100

Table 2: Physicochemical characterization of etoricoxib emulgel

Formulation Code	Parameters					
	Nature	pH	Viscosity (cps)	Spreadability gm cm/sec	Drug content (%w/w)	Extrudability
EG1	White, Homogenous	6.8	12208	17.8±0.18	96.25±0.09	++
EG2	White, Homogenous	6.9	12510	25.4±0.09	98.36±0.15	+++
EG3	White, Homogenous	7.1	12415	27.1±0.44	95.23±0.22	++
EG4	White, Homogenous	7.0	12522	29.5±0.21	94.67±0.18	+++
EG5	White, Homogenous	6.8	12800	28.4±0.27	93.54±0.52	++
EG6	White, Homogenous	7.0	12648	32.6±0.15	98.74±0.08	+++

Excellent +++, Good++, Satisfactory+

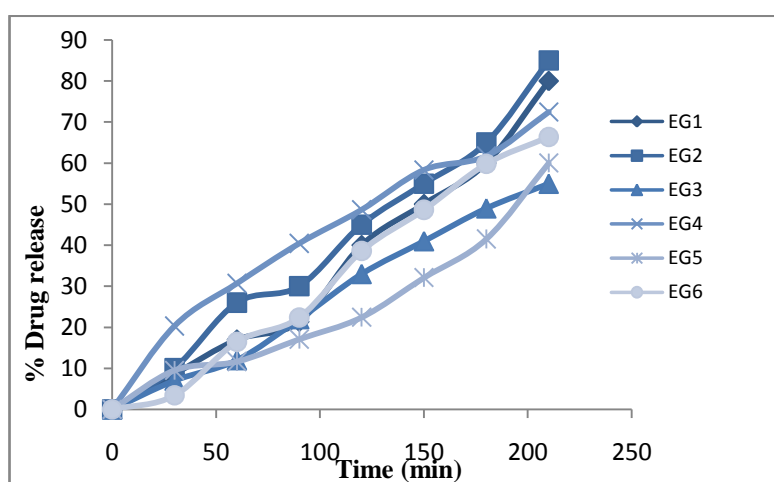


Figure 1: In-vitro diffusion profile of etoricoxib emulgel formulations

Table 3: Drug release kinetic parameters for different etoricoxib emulgel formulations

Batch Code	Zero order model		First order model		Higuchi model		Korsmeyer-Peppas model		
	R	K	R	K	R	K	Slope(n)	R	K
EG1	0.8976	3.6372	0.9555	-0.0549	0.9846	10.3551	0.6248	0.9981	7.8464
EG2	0.9682	2.6368	0.9562	-0.0358	0.9783	8.8968	0.7077	0.9977	4.7338
EG3	0.9657	2.0245	0.9581	-0.0252	0.9274	10.3922	0.8265	0.9946	2.8082
EG4	0.8561	4.2172	0.9345	-0.0783	0.9758	8.9453	0.5721	0.9984	11.5966
EG5	0.9345	2.6465	0.9548	-0.036	0.9368	10.4181	0.6184	0.9937	7.1578
EG6	0.9621	2.0239	0.9563	-0.0292	0.9682	8.6137	0.7018	0.9954	4.5348

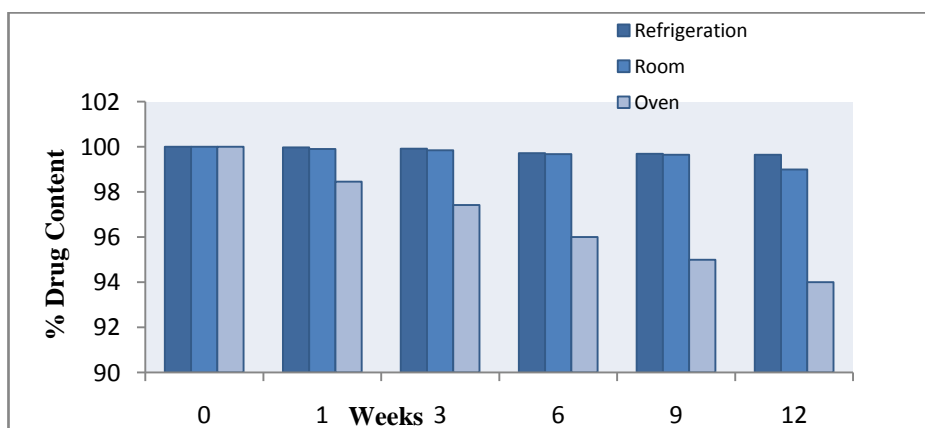


Figure 2: Stability study of etoricoxib emulgel formulation of batch EG1

Table 4: % Inhibition on carrageenan induced paw volume by different treatment

Treatment	Percent inhibition of paw oedema					Mean of % Inhibition
	1 hr	2hr	4hr	6hr	8hr	
Standard (10mg/kg, I.P. Indomethacin)	12.87	21.39	31.79	39.41	47.83	54.86
Etoricoxib emulgel, EG1, 2 gm	4.20	5.12	6.06	11.07	13.51	18.597

N = 6, p < 0.05

Table 5: % Inhibition on carrageenan induced paw thickness by different treatment

Treatment	Percent inhibition of paw thickness					Mean of % Inhibition
	1 hr	2hr	4hr	6hr	8hr	
Standard (10mg/kg, I.P. Indomethacin)	15.08	18.89	22.61	25.23	27.74	29.91
Etoricoxib emulgel, EG1, 2 gm	3.19	5.42	11.37	14.13	16.81	18.98

N = 6, p < 0.05

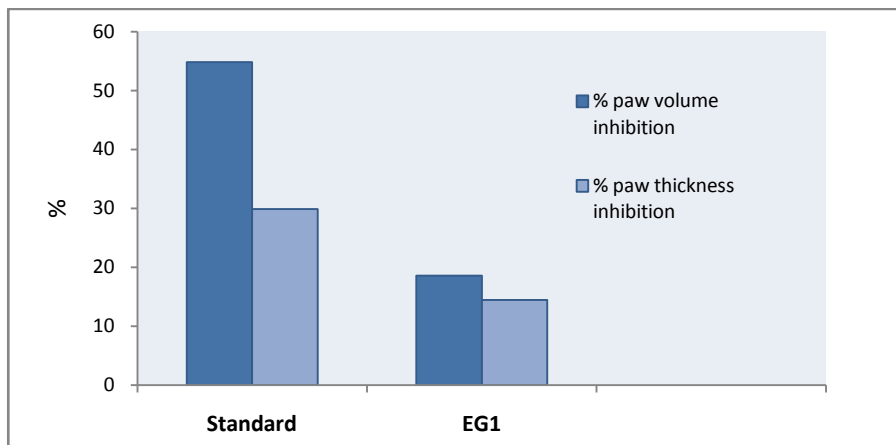


Figure 3: Mean % paw volume and thickness inhibition