

# Aceclofenac Organogels: *In Vitro* and *In Vivo* Characterization

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**Abstract: Purpose:** To develop and evaluate the suitability of lecithin organogels containing aceclofenac for topical application and compare its *In vitro* and *In vivo* effects with conventionally used hydrogels.

**Methods:** The components and their concentration necessary for organogels formation were evaluated using phase diagram. Solubility of aceclofenac was determined. The *In vitro* skin permeation ability of aceclofenac from ethyl oleate based lecithin organogels [EO/lecithin organogel] and hydrogel was investigated. The *In vivo* characterization of ethyl oleate based organogel study was compared with that of hydrogel. The alterations in microstructure of organogels during diffusion study were elucidated. Viscosity and micellar size of the organogel sample were estimated. The safety of optimized organogel was determined using histopathological investigation.

**Results:** The flux calculated for skin permeation ability of aceclofenac was in the order EO/lecithin organogel > hydrogel. The *In vivo* results also demonstrated that organogels are more effective in faster drug release as compared to hydrogels. It was observed that viscosity of gels decreased with increasing stress. The size of micellar aggregation increased with water added and has been revealed in dynamic light scattering (DLS) study. The histopathological data showed that EO/lecithin organogel were safe enough for topical purpose.

**Keywords:** Aceclofenac, topical, lecithin organogel, ethyl oleate.

## 1. INTRODUCTION

The skin is an exceptionally effective barrier to most drugs for therapeutic treatment. Very few drugs in therapeutic amount are permeated through skin such as nitroglycerine, scopolamine, nicotine, clonidine, fentanyl, estradiol, testosterone, Lidocaine, and oxybutinil [1]. Therefore, the systems that make the skin more permeable and thereby enhance transdermal delivery are of great formulation interest. The strategies to deliver the medicament into the skin and for systemic circulation have been evolved. The extensive research has been reported on lipids as skin penetration enhancers [2-5]. Lipids in the form of vesicles such as liposomes, niosomes [6-8], ethosomes [9] and transfersomes [10] have been evaluated. The lipid-based formulations have been in use since decades. The importance of lipids has especially increased after realizing the utility of natural phospholipids. Lecithin, the natural bio-friendly molecules are ubiquitous phospholipids that accounts for more than 50 % of the lipid matrix of biological membranes. Soybean lecithin in an apolar organic solvent, on addition of water, forms an entangled dynamic network of long and flexible worm-like multi-molecular aggregates termed as 'organogels' [11]. These are characterized by high viscosity and complete optical transparency. Lecithin organogels are emerging as carriers for drug molecules with diverse physicochemical properties including macromolecules [12]. Transdermal transport rates of scopolamine and broxaterol from lecithin organogels

were faster than commercial patches [12]. Similarly, improved skin penetration of indomethacin and diclofenac has been observed with lecithin-based organogels in isopropyl palmitate [13]. Piroxicam has been successfully incorporated in lecithin organogels [14]. Recently, results have shown that ketorolac tromethamine could be incorporated at high concentrations into lecithin organogels [15]. Aceclofenac, a phenylacetic acid derivative, is a drug of choice in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Researchers have attempted development of oral drug delivery systems for aceclofenac [16]. The chronic oral administration of aceclofenac tends to cause severe gastric irritation [17]. Topical administration of aceclofenac offers the advantage of enhanced drug delivery to the affected areas, by passing gastric irritation. Luigi et al [17] have evaluated clinically efficiency of topical aceclofenac cream (1.5%w/w) in patients. The formulation showed improved therapeutic efficacy. Yang *et al.* [18] has formulated micro-emulsion containing aceclofenac (3%w/w) for topical delivery. Micro emulsions were prepared using different oil phase viz oleic acid, linoleic acid, triacetin and labrafac. Labrasol was used as surfactant. Transcutol was mixed as a cosurfactant for enhancing skin permeability of aceclofenac. Micro emulsion containing linoleic acid as oil phase showed highest flux ( $J_{ss}=32.05 \pm 9.17 \mu\text{g}/\text{cm}^2/\text{hr}$ ) as compared to the formulations prepared using other oil phase. However, micro emulsions suffers from the disadvantage that it requires large amounts of surfactant and cosurfactant necessary for stabilizing the nanodroplets [19]. Microemulsions exhibits poor viscosity and spreadibility and hence are difficult to administer. On the other hand lecithin organogels does not requires use of surfactants or any additional pene-

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tration enhancer as 'lecithin' present already in the system serves both as stabilizer and penetration enhancer. Organogels compared to microemulsions has high viscosity and spreadability and hence can be administered to the skin with much ease.

## 2. MATERIALS AND METHODS

### Materials

Soy lecithin [Epikuron200] was a generous gift from Degussa Bioactive (Germany) and it was used without further purification. Aceclofenac was kindly supplied as gift sample by IPCA Lab. Ltd. (Mumbai, India). Ethyl oleate [EO] was purchased from S.D.Fine chemicals (Mumbai, India) and all were of AR grade. HPLC grade methanol, water and glacial acetic acid were purchased from S.D.Fine chemicals (Mumbai, India). Double distilled water was used through out the experiment.

The following notation will be used below for the sake of shortness:

$W_0$  (Molar ratio) - [water]/ [lecithin]; EO- Ethyloleate.

### Methods

#### Solubility Analysis

An excess amount of drug was added in two different test tubes one containing 5 ml of oil [ethyl oleate] and the other containing 5ml solution of oil and lecithin. The tubes were kept on mechanical water bath shaker (Neolab, India) at 37°C for 72 h. The suspension was filtered through membrane filter [0.45 $\mu$ ]. The filtrate was diluted with ethanol and drug concentration was determined. The study was done in triplicate.

#### Preparation of Gel Formulations

##### Organogel Preparation

Accurately weighed quantity [1.14g] of lecithin (i.e. 22.8%w/v) was dissolved in test tube containing 5ml of ethyl oleate. The mixtures were sonicated at a frequency of 15000 KHz for 10 minutes at room temperature to produce 300mM lecithin reverse micellar solution. Aceclofenac [1% w/w] was incorporated in this lecithin solutions. Appropriate amount of distilled water (108 microliter of water for ethyl oleate based organogel i.e.  $W_0=4$  for EO) was added with the help of microlitre syringe to induce organogelation.

##### Hydrogel Preparation

Hydrogel was prepared by using Carbopol 940 as polymer with reported pH adjustment technique [20]. An appropriate amount of Carbopol 940 (1%w/w) was slowly added into beaker containing water under constant stirring, Aceclofenac (1%w/w) was dispersed in propylene glycol in a separate beaker; this mixture was then added to the beaker containing carbopol in water. After the mixture had been kept at ambient temperature for 24 h, a small amount of 0.5% (w/w) triethanolamine was added and mixed well until the gel was formed. A few drops of 2 M sodium hydroxide or 2 M phosphoric acid were added to adjust the pH of the gels between pH 5 and 7.

### Analytical Method for Aceclofenac Estimation

A set of standard solutions of aceclofenac was prepared (5-50)  $\mu$ g/ml in mobile phase (methanol: water: glacial acetic acid). The standard curve was obtained from the area of peak measured using HPLC with a correlation coefficient of 0.999.

HPLC analysis of aceclofenac was performed using a Jasco SERIES 2000 pump set at flow rate of 1 mL/min, a Spectra Jasco SERIES UV 2075 detector set at 276 nm. Samples were injected using rheodyne injector at 20 $\mu$ l capacity per injection was used. The software used was Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Hypersil C-18 (250 mm X 4.6 mm, 5.0 $\mu$ m particle size) and a mobile phase of methanol: water: glacial acetic acid (70:30:0.3).

### In Vitro Skin Permeation Studies

Full thickness abdominal skin of albino rats [125-150g] was used. The dermal surface was carefully cleaned to remove sub cutaneous tissues and fats without damaging the epidermal surface. The diffusion of aceclofenac from the different gel samples was investigated across animal skin using Keshary-Chien type diffusion cells. The capacity of diffusion cell was 20ml and effective surface area was 3.14 cm<sup>2</sup>. The receptor compartment was filled with saline phosphate buffer [pH=7.4]. The cells were thermostated at 37 $\pm$ 1 °C and the receptor solution was stirred with a magnetic bar at 200 rpm. A 1.0 g amount of gel samples was loaded on the membrane. The aliquots of 2 ml samples were withdrawn every hour for 6 h from the receptor compartment and replaced with fresh medium. The samples were diluted with mobile phase solvent and then analyzed by HPLC for aceclofenac content. The mean cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot was calculated as flux  $J_{ss}$  ( $\mu$ g/cm<sup>2</sup>/hr) [21] and the permeability coefficient was calculated using Eq. 1:

$$K_p = \frac{J_{ss}}{C_v} \quad (1)$$

Where  $K_p$  is permeability coefficient and  $C_v$  is total amount of drug.

The enhancement of drug penetration due to organogel formulation was noted as enhancement factor (EF), which was calculated using Eq. 2:

$$EF = \frac{K_p(\text{organogel})}{K_p(\text{hydrogel})} \quad (2)$$

The drug fluxes from EO organogel were compared with the flux of hydrogel by applying independent Student's *t*-test. A value of  $P = 0.05$  was considered significant.

### In Vivo Studies

The carrageenan induced rat paw edema method was used as a tool to compare the efficacy of ethyl oleate organogel with that of hydrogel. The male albino rats (weighing 200-250 g) of local strain were used for the anti-inflammatory study by carrageenan-induced rat paw edema method. The animals were kept for 1 week to get acclimatized

in the animal house before the experiment, and they were maintained on unrestricted supplies of food and water.

The animals were divided into four groups, each consisting 6 rats. First two groups were treated with organogel and hydrogel respectively. The other two groups were kept as control and were treated with plain gel base of corresponding organogel and hydrogel without drug to account for the effect of vehicle. Study was conducted by complete cross over design.

Paw edema was induced in unanesthetized rats by subplantar injection of carrageenan (0.1 ml of 1% carrageenan-saline solution) into the right hind paw. One hour later, 1mg of gel was applied topically on the edematous paw. Paw volume was measured using a mercury plethysmometer immediately before the injection of carrageenan and thereafter at 1-hr intervals for 6 hrs. Edema was expressed as the increase in paw volume (milliliters) after carrageenan injection relative to the preinjection value for each animal. The left hind paw served as a reference non inflamed paw for comparison.

The percentage difference between right and left paw edema was taken as % edema produced. The % edema produced with test samples were subtracted from % edema produced in control group to obtain % inhibition produced by respective groups.

### Microstructures of Organogel

Microstructure of EO organogel was observed during *In vitro* diffusion study in order to elucidate the release mechanism of drug from the gel matrix with time. A set of 3 diffusion cells with excised rat skin mounted on the donor chamber was used with experimental condition identical to *in vitro* diffusion study. Weighed amount [1.0 g] of organogel was placed on each of the 3 mounted excised rat skin. Gel samples were removed at 1, 2 and 6 hours of diffusion. The microstructure of the gel was observed and photographed with a digital camera.

### Characterization of Organogel

#### a. Viscosity Studies

Viscosity of both plain and medicated organogel sample was determined. For the viscosity measurements, a Cone and Plate viscometer CAPII H<sup>+</sup> was used. All measurements were made on freshly prepared samples. Each time 0.5 g of sample was used for measurements. The measurements were carried out at different speeds ranging from 100rpm to 900rpm at 37°C.

#### b. Dynamic Light Scattering [DLS] Studies

The dynamic light scattering pattern of plain EO organogels at different *W<sub>o</sub>* ratio was obtained at 25°C (DynaPro-MS800 instrument, Protein Solutions Inc., VA, scattered light monitored at 90°). The gel samples were filtered (20 nm filter, Whatman Anodisc 13, catalog no. 6809-7003) and dust contamination was prevented. At least 20 measurements each of 10 s duration were collected. The high viscosity gel samples were warmed to facilitate transfer in scattering cell [22]. The distributions of hydrodynamic radius of particles in gels were analyzed using *Regularization* software provided by the manufacturer.

### Evaluation of Optimized Organogel Formulation

#### Histopathological Investigation of Skin Using Organogel Formulation

The rat abdominal skin region measuring approximately 4cm<sup>2</sup> was mounted on two different modified Keshary-Chien diffusion cells. A 3.0 g of organogel was placed on the skin membrane of one diffusion cell, whereas 3.0 g of water [control] was placed on the skin membrane of the other diffusion cell. The skin was fixed in 10% neutral formalin for 24 hours and then cut vertically against the surface at the central region (4mm width). Each section was dehydrated using graded solutions of ethanol and then embedded in paraffin wax. Tissues were divided into small pieces and stained with haematoxylin and eosin. The sections were observed under 100x magnification and photographed.

### 3. RESULTS AND DISCUSSION

#### Solubility Analysis

An attempt was made to determine the solubility of aceclofenac in ethyl oleate oil and its corresponding lecithin reverse micellar solution. Solubility of aceclofenac in ethyl oleate-lecithin reverse micellar system [70.52± 1.02 mg/ml] was found to be almost 13 folds higher than that in ethyl oleate oil [4.90± 0.02 mg/ml]. Lecithin organogels are three-dimensional aggregates of gelator molecules containing reverse micelles. These have solubilising ability for drugs of diverse chemical nature [12]. The solubility enhancement ability of lecithin organogels is attributed to the presence of reverse micelles. Similar enhancement in solubility has been reported previously for broxaterol. It was found that solubility of broxaterol; an antiasthmatic drug in Isopropyl palmitate [IPP] was found to be 11mg/ml whereas in lecithin-IPP reverse micellar system solubility of broxaterol was 75mg/ml [12]. Likewise the solubility enhancement effect in lecithin organogels has been observed with the drugs such as  $\beta$ -estradiol, 17-acetate,  $\beta$ -estradiol cypionate, isosorbide dinitrate and clonidine [12]. The increased solubility of drug results in enhanced permeation of drug from matrix of gels.

#### *In Vitro* Skin Permeation Study

The mean cumulative drug permeation per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot gave the flux (*J<sub>ss</sub>*) (Fig. 1), which was higher for EO organogel containing 1%w/w aceclofenac (95.7 $\mu$ g/cm<sup>2</sup>/hr) than that of hydrogel containing 1%w/w aceclofenac (54.83 $\mu$ g/cm<sup>2</sup>/hr). Calculated values of the Enhancement Factor (EF) for EO organogel with respect to hydrogel formulation are presented in (Table 1). The value of permeability coefficient (*K<sub>p</sub>*) for EO (9.57 x 10<sup>-3</sup> cm<sup>2</sup>/hr) organogel formulation is higher when compared to hydrogel (5.48 x 10<sup>-3</sup> cm<sup>2</sup>/hr). The calculated value of EF for EO organogel formulation is relatively higher (1.746), indicating an enhanced permeation of aceclofenac from this formulation.

Higher permeation profiles of organogel compared to that of hydrogel may be attributed to the presence of lecithin in the organogel. Lecithin enhances skin permeation by affecting the lipids of stratum corneum, altering their arrangement and disordering them transiently [23]. The trans-skin perme-

ability of propranolol hydrochloride, a poorly permeable and water-soluble drug incorporated in lecithin organogel, across human cadaver skin has been investigated and significantly enhanced (approximately 10 times higher) permeability of micellar-borne drug across the human skin was observed employing drug in 200 mM lecithin/iso-octane/water organogel system in comparison to that of pure drug in solution form or emulsified in the petroleum jelly [24].

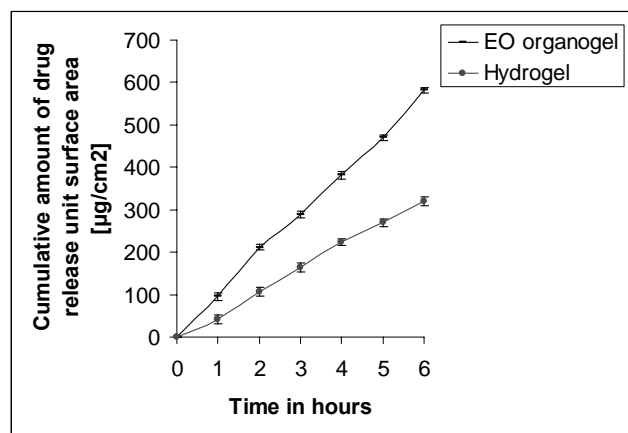


Fig. (1). Comparative *In vitro* skin permeation data of organogel and hydrogel with 1% w/w aceclofenac.

### *In Vivo* Study

The data were compared by ANOVA technique and differences at  $p < 0.005$  were considered significant. Both the formulations [ethyl oleate organogel and hydrogel] showed a significant reduction in paw edema after 3 hrs (Table 2), however percent inhibition observed with organogel was relatively more and was found to be  $94.44 \pm 2.22$ , whereas that with hydrogel was found to be  $38.89 \pm 3.56$ . After 3 hrs

the percent inhibition decreased with time till the end of study for both the formulations. The results of *in vivo* studies were found to be supporting the outcome of *in vitro* study that the drug release is faster from organogel as compared to hydrogel.

### Microstructures of Organogel

The Fig. (2) demonstrates the changes in microstructure of EO organogel during different time periods of *In vitro* diffusion study. The drug in organogel is mainly entrapped in intact 3-dimensional network of gel matrix (Fig. 2A) containing lecithin reverse micelles in nonpolar organic liquid [Ethyl Oleate in this case] along with few microlitres of polar liquid [water in this case]. As the diffusion process continues more and more amount of water penetrates in the gel structure. This greater percolation of water in the gel results in breaking of gel matrix and subsequent release of drug (Fig. 2B). As the time goes on the number of reverse micellar aggregates decreases and the organogel matrix breaks open, more pores are formed and finally it gets converted to a coarse macro emulsion (Fig. 2C).

### Characterization of Organogel

#### a. Viscosity Studies

For any vehicle to be used for topical drug delivery applications, it is essential to study its rheological behavior. The latter is important for its efficacy in delivering the molecules onto or across the skin site and also for its ease of application. Lecithin organogel, prior to gelling, i.e., before the addition of polar phase, exhibit Newtonian behavior but follow Maxwell's rheological (viscoelastic) behavior on addition of the polar phase [25] (Figs. 3a,b). The viscosity data has been summarized in Table 3. It was observed that non-medicated organogel has higher viscosity compared to medi-

Table I. Results of Flux, Permeability Coefficient and Enhancement Factor Organogels and Hydrogel

No.	Gel Formulations	Flux ( $\mu\text{g}/\text{cm}^2/\text{hr}$ )	Permeability Coefficient ( $\text{Kp} \times 10^{-3}$ ) ( $\text{cm}/\text{hr}$ )	Enhancement Factor (EF)
1	Ethyl oleate organogel	$95.7 \mu\text{g}/\text{cm}^2/\text{hr}$	$9.57 \times 10^{-3} \text{ cm}/\text{hr}$	1.746
2	Hydrogel	$54.83 \mu\text{g}/\text{cm}^2/\text{hr}$	$5.48 \times 10^{-3} \text{ cm}/\text{hr}$	-

Table 2. Results of *In Vivo* Study for Organogel and Hydrogel

Time [Hours]	% Inhibition After ( $\pm$ SEM) Ethyl Oleate Organogel	% Inhibition After ( $\pm$ SEM) Hydrogel
1	$33.33 \pm 2.67$	$27.78 \pm 2.70$
2	$77.78 \pm 3.70$	$33.30 \pm 2.74$
3	$94.44 \pm 2.22$	$38.89 \pm 3.56$
4	$77.78 \pm 2.22$	$48.41 \pm 1.03$
5	$61.11 \pm 2.11$	$27.78 \pm 2.78$
6	$61.90 \pm 2.67$	$19.05 \pm 2.05$

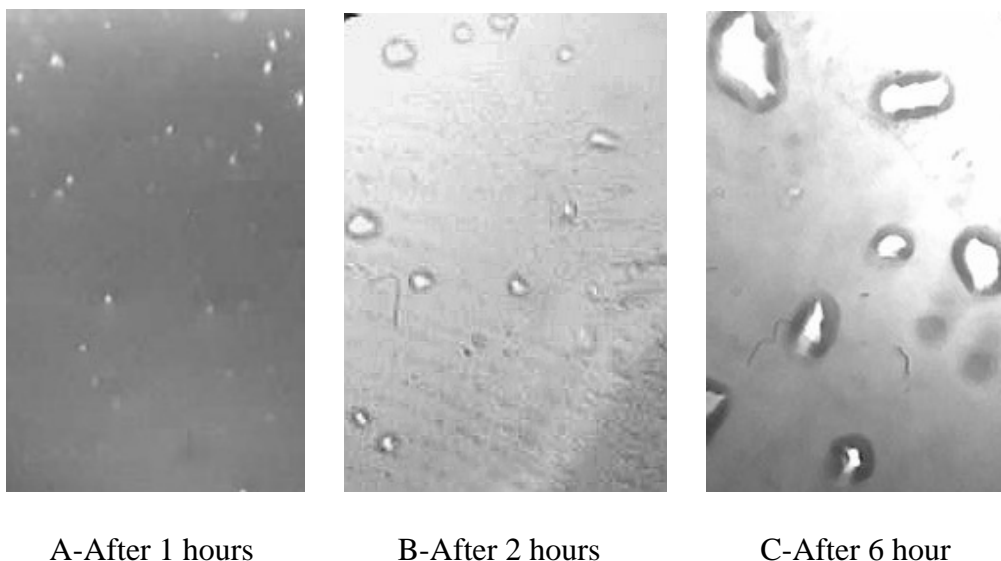


Fig. (2). Microstructure organogel during *In vitro* diffusion studies.



Fig. (3). a) Inverted vial contains Ethyl oleate/lecithin mixture, before addition of water and is less viscous and hence falling towards the closure of the vial.

b) Inverted vial contains Ethyl oleate/lecithin/water mixture, after addition of water becomes gel of high viscosity and hence remaining at the top of the inverted vial.

cated organogel containing aceclofenac. This was observed because aceclofenac being hydrophobic in nature gets partitioned predominantly in hydrophobic region of micellar aggregate. This results in decreased hydrophobic interaction among lecithin molecules present in oil. This results in weak interaction among lecithin molecules and hence low viscosity. The amount of drug added is only 1%w/w and hence the observed decrease in viscosity is of lower magnitude. The viscosity of gel altered in presence of stress. As the stress

increases the viscosity of gel decreases. Kumar *et al.* [26] have reported similar type of decrease in viscosity with increasing stress for lecithin-cyclohexane-water system.

#### b. Dynamic Light Scattering [DLS] Studies

Dynamic Light scattering studies was carried out for EO organogel in order to determine the hydrodynamic radius (Rh) of lecithin reverse micelles at different  $W_o$  ratios. Addition of water was restricted till  $W_o=4$ . On continuing the water addition above  $W_o=4$  the 3-dimensional network collapses and separation of the homogenous organogel takes place in a two-phase system consisting of low viscous liquid and a compact organogel or jelly-like phase. At still higher concentrations of the polar phase, the transformation of the separated compact organogel into a solid, nontransparent precipitate is observed.

It was found that hydrodynamic radius (Rh) of the micelles increases with increase in  $W_o$  ratio (Table 4). These longer cylindrical micelles entangle to form a network like structure giving high viscosity [27].

#### Evaluation of Optimized Organogel

Based on the superior *In vitro* skin permeation data and *In vivo* data, ethyl oleate/lecithin organogel was selected as the best optimized formulation and hence it was subjected to histopathological study.

#### Histopathological Investigation of Skin Using Organogel Formulation

The histology of excised rat skin in control (water) and treated with optimized batch of organogel [i.e. Ethyl oleate based organogel] is shown in Figs. (4a,b). The microscopic observations indicate that the optimized organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultra structure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged. The skin showed

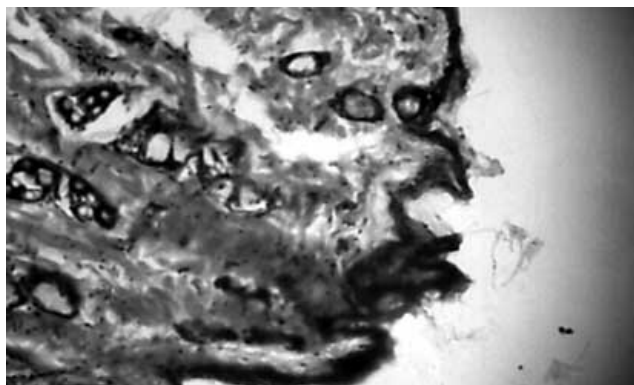
**Table 3. Viscosity of Non-Medicated and Medicated Ethyl Oleate/Lecithin Organogel**

No.	Speed in rpm	Viscosity of Non-Medicated EO Organogel (centipoises)	Viscosity of Medicated EO Organogel (Centipoises)
1	100rpm	288 cp	143 cp
2	500rpm	118 cp	84.46cp
3	900rpm	85 cp	64.86 cp

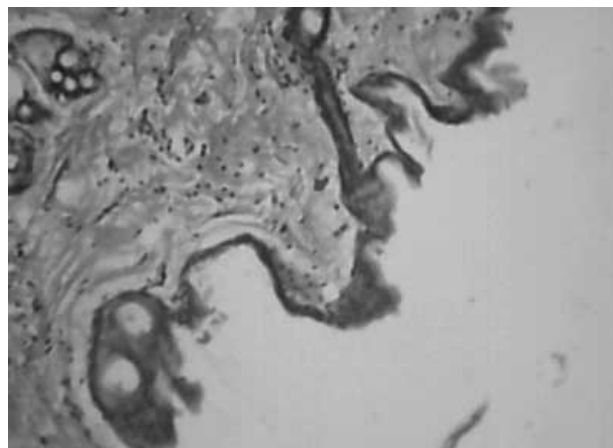
**Table 4. Each Value Represents the Mean  $\pm$  S.D. (n = 3)**

Sr. no.	Wo [hydration ratio]	EO Organogel Micellar Size [nm]
1	1	28.43 $\pm$ 1.15
2	2	33.63 $\pm$ 0.79
3	3	42.41 $\pm$ 3.4
4	4	43.29 $\pm$ 1.2

a)



b)



**Fig. (4). a)** Histopathology of rat skin treated with water (control).

**b)** Histopathology of rat skin treated with optimized ethyl oleate/lecithin organogel (treated).

little hydration effect during diffusion. In the organogels under study, EO used as the oily solvent is biodegradable and has been found nontoxic. EO is primarily used as a vehicle in certain parenteral preparations intended for intramuscular administration [28]. Lecithin used in the formulation has GRAS status and it has been included in the FDA Inactive Ingredients Guide for inhalations; IM and IV injections; otic preparations; oral capsules, suspensions and tablets; rectal, topical, and vaginal preparations. Hence, it can be safely concluded that the EO based lecithin organogels are biocompatible and safe for topical applications.

#### 4. CONCLUSIONS

Findings of this study suggest that ethyl oleate based organogels is able to provide desired anti-inflammatory action. *In vitro* skin permeation study demonstrated that ethyl oleate

based organogel was effective in providing faster drug release. *In vivo* study confirmed the findings of *in vitro* study. The viscosity of medicated organogels was lesser as compared to unmedicated organogels. The size of micellar aggregation increased with water and has been revealed in differential light scattering study. Histopathological study demonstrated that the developed organogel does not have any harmful effects on skin. The results showed that organogel exhibits useful pharmaceutical properties and serves as a better vehicle for topical delivery of aceclofenac than hydrogels. Hence, ethyl oleate based organogels seems to be a promising novel topical formulation for aceclofenac. However, the role of the formulation developed in this study can only be settled with clinical investigations on humans with emphasis on therapeutic index and sideeffects followed by pilot scale studies of manufacturing the product for commercial use.

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