



## COMPARATIVE STUDY ON ANTI-INFLAMMATORY SYNERGISTIC ACTIVITY OF LORNOXICAM USING TURMERIC OIL IN TDDS

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### ABSTRACT:

The present research work was an attempt to develop, evaluate matrix-type transdermal therapeutic system containing lornoxicam with hydrophilic polymer (HPMC E-5) and turmeric oil (leaf extract) by the solvent evaporation technique. The turmeric oil has been used for two reasons, one is to see whether it act as natural penetration enhancer (replacing chemical enhancers) and also to compare the anti-inflammatory synergism between the drug and the oil itself. The anti-inflammatory activity of turmeric oil has been well documented. The physicochemical compatibility of the drug, polymers and oil was studied by infrared spectroscopy. The results suggested no physicochemical incompatibility between them. Five transdermal patch formulations (F1 to F5) consists of HPMC E5 (300 mg fixed) and turmeric oil in the concentrations 3%, 4%, 5%, 6% and 7% respectively were prepared. All formulations carried 10% w/v of Polyethylene glycol as plasticizer in dichloromethane and methanol (4:1) as solvent system. The prepared transdermal patches were evaluated for in vitro release, moisture absorption, moisture loss and mechanical properties. The diffusion studies were performed by using modified Franz diffusion cells. The formulation, F3 showed maximum release 97.56% as compared to patch without any penetration enhancer (58.83%). The synergistic activity has been determined by Carrageenan induced rat paw edema test and the results have confirmed the synergistic activity between the drug and turmeric oil. The developed transdermal patches may increase the efficacy of lornoxicam for the therapy of arthritis and other painful muscular conditions.

**Keywords:** Transdermal system, HPMC, Turmeric oil, penetration enhancer, Synergistic activity.

### 1. INTRODUCTION

The idea of delivering drugs through skin is old, as the use is reported back in 16<sup>th</sup> century B.C. The husk of castor oil plant in water was placed on an aching head [1]. Today the transdermal drug delivery is well accepted for delivering drug to systemic circulation. Conventional systems of medication which require multi dose therapy have numerous

problems and complications. The design of conventional dosage form, whether a tablet, an injection or a patch, to deliver the right amount of medicine at the right target site becomes complicated. To address these problems, controlled release drug delivery system, a novel drug delivery approach evolves, which facilitates the drug release into systemic circulation. Controlled drug release can be achieved by transdermal drug delivery

systems (TDDS) which can deliver medicines via the skin portal to systemic circulation at a predetermined rate over a prolonged period of time [2-4]. TDDS has gained a lot of interest during the last decade as it offers many advantages over the conventional dosage forms and oral controlled release delivery systems notably avoidance of hepatic first pass effect, less frequency of administration, reduction in gastrointestinal side effects and improves patient compliance [5]. For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin [6]. Lornoxicam is a newer NSAID of oxicam class. It is a strong analgesic and anti-inflammatory agent. Its analgesic activity is comparable to that of opioids (more effective than 10 mg morphine, when used at doses  $>$  or  $=$  8 mg to control pain after oral surgery). Clinical investigations have established it as a potent analgesic with excellent anti-inflammatory properties in a range of painful and/or inflammatory conditions, including rheumatoid arthritis and postoperative pain [6-8]. Like all NSAIDs, Lornoxicam acts by inhibiting the metabolites of COX branch of arachidonic acid pathway. It inhibits both isoforms in the same proportion; a perfectly balanced inhibition of COX-1 and COX-2 is achieved. As Prostaglandins play an important role in gastrointestinal mucosal protection by strengthening the mucosal barrier for acid and in inhibiting gastric acid secretion. Thus inhibition of prostaglandin synthesis leads to adverse effects. The gastric side effects range from mild dyspepsia and heartburn to ulceration and haemorrhage [9-10]. Lornoxicam is absorbed rapidly and almost completely from the gastro-intestinal tract. Peak plasma concentration is attained within 2.5 hrs. Food reduces the absorption of the drug. The absolute bioavailability of lornoxicam is 90-100%. It has a relatively short

plasma half-life (3 to 5 hours). It is eliminated following biotransformation to 5'-hydroxy-Lornoxicam, which does not undergo enterohepatic recirculation [11]. Turmeric leaf oil, obtained from the leaves of the herb *Curcuma longa* L. (Zingiberaceae), is used extensively in curries and mustards as a colouring and flavoring agent. In Ayurvedic medicine, turmeric has traditionally been used as a treatment for inflammation, skin wounds and tumors. Extracts have been reported to have effect as antimicrobial, anti-inflammatory, anti-oxidant and anticancer agents. In preclinical animal studies, turmeric has shown anti-inflammatory, cancer-chemopreventive and antineoplastic properties powdered turmeric, or its extract, is found in numerous commercially available botanical supplements. The best characterized of the compounds found in turmeric is Curcumin, which appears to be able to act at multiple sites to reduce inflammation [12-13]. Nonsteroidal antiinflammatory agents may act via single or combination of any of the mechanism involving inhibition of arachidonic acid metabolism, inhibition of cyclo-oxygenase (COX)/ inhibition of the PG synthesis, inhibition of lipoxygenase (LOX), inhibition of cytokines (IL, TNF, etc.), release of steroidal hormones from the adrenals, stabilization of lysosomal membrane and uncoupling of oxidative phosphorylation, etc [14 & 15].

The present research work was an attempt to develop and evaluate matrix-type transdermal therapeutic system containing lornoxicam with hydrophilic polymer (HPMC E-5) and turmeric oil (leaf extract) by the solvent evaporation technique.

## 2. MATERIALS AND METHODS

Lornoxicam was received as a gift sample from Glenmark Pharmaceuticals Pvt. Ltd, Mumbai, India. HPMC E-5 procured from Ozone International, Mumbai, India, and Turmeric leaf oil was as a gift sample from Lala Jagdish Prasad Pvt. Ltd, Kanpur, India. All other

laboratory chemical used in the study was analytical reagents grade.

## 2.1 DRUG, POLYMER AND OIL INTERACTION STUDY

The physicochemical compatibility between Lornoxicam and polymers used in the films was studied by using Fourier transform-infrared (FT-IR- 8300, Shimadzu Co., Kyoto, Japan) spectroscopy. The FT-IR spectra were recorded in the wavelength region between 4000 and 400  $\text{cm}^{-1}$ . The spectra obtained for Lornoxicam and physical mixtures of Lornoxicam with polymers were compared [16-17].

## 2.2 PREPARATION OF TRANSDERMAL PATCH

Transdermal patches of Lornoxicam were prepared by solvent evaporation technique [18-20] and contents of all the formulations

are tabulated in **table 1**. HPMC E-5 and Turmeric oil solution were prepared separately in dichloromethane: methanol (4:1) mixture. The two solutions were mixed together then weighed amount of Lornoxicam was added slowly in this prepared solution. 0.6 ml of PEG 400 was added and mixed to the mixture. Turmeric oil was added for the two purposes as a natural permeation enhancer as well as to impart a synergism to the drug activity. The drug-polymer solution was casted in Teflon plate with area of 3  $\text{cm}^2$  which was wrapped by aluminium foil. The plate was kept aside for drying at room temperature for 24 hrs. Inverted funnel was placed over the Teflon plates to prevent the current of air. After drying, the patches were peeled from Teflon plates, wrapped in aluminium foil, and preserved in desiccators for further studies.

**Table 1: Composition of different formulations containing Lornoxicam**

Formulations code Composition	F-1	F-2	F-3	F-4	F-5
Lornoxicam (mg)	8	8	8	8	8
HPMC (mg)	300	300	300	300	300
Turmeric oil (%w/v)	3	4	5	6	7
Dichloromethane: Methanol (4:1) (ml)	7	7	7	7	7
PEG 400 (ml)	0.6	0.6	0.6	0.6	0.6
Glycerol (ml)	1.6	1.6	1.6	1.6	1.6

## 2.3 EVALUATION OF TRANSDERMAL PATCHES OF LORNOXICAM

### (a) Physical appearance

The prepared patches were physically examined [21] for colour, clarity and surface texture.

### (b) Thickness uniformity

The thickness of patches [22] was measured by using electronic calliper, with a least count of

0.01 mm. Thickness was measured at three different points on the film and average readings were taken.

### (c) Uniformity of weight

The patch of size 1x1  $\text{cm}^2$  was cut and weight of [23] each patch was taken individually, the average weight of the patch was calculated.

### (d) Drug content uniformity

The patches were tested for the content

uniformity [20-32]. The patches of size 1 cm<sup>2</sup> was cut and placed in a 100 ml volumetric flask. The contents were stirred using a magnetic bead for 24 hrs to dissolve the patches. Subsequent dilutions were made with phosphate buffer (pH 7.4). The absorbance of the solution was measured against the corresponding blank solution at 376 nm using UV-visible spectrophotometer. The experiment was repeated three more time to validate the result.

#### (e) Folding endurance

The folding endurance was measured [21, 25-27] manually for the prepared patches. A strip of patch (2 x 2 cm<sup>2</sup>) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of folding endurance.

#### (f) Tensile strength

Tensile strength of the patches was determined [23-25] with the apparatus fabricated according to the description given in various articles. It consisted of two load cell grips. The lower one was fixed and upper one was movable. The test film of size (4 x 1 cm<sup>2</sup>) was fixed between these cell grips and force was gradually applied till the film broke. The tensile strength of the film was taken directly from the dial reading in kg. Tensile strength is expressed as follows-

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

#### (g) Percentage moisture uptake

The patches were weighed accurately and placed in a desiccators where a humidity condition of 80-90 % RH was maintained by using saturated solution of potassium chloride. The patches were kept until uniform weight is obtained, then taken out and weighed. The percentage of moisture uptake [29] was calculated as the difference between final and

initial weight with respect to initial weight.

$$\% \text{ moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} * 100$$

#### (h) Percentage moisture loss

The patches were weighed individually and kept in a desiccator containing calcium chloride. The final weight was noted when there was no change in the weight of individual patch. The percentage of moisture content was calculated [26-28] as a difference between initial and final weight with respect to final weight.

$$\% \text{ moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} * 100$$

#### (i) In vitro drug permeation studies

The fabricated patch were cut into 1 cm<sup>2</sup> and placed on the commercial semi permeable membrane (regenerated cellulose which was permeable to low molecular weight substances) and attached to the Modified diffusion cell such that the cell's drug releasing surface towards the receptor compartment which was filled with 200 ml of phosphate buffer saline solution of pH 7.4 at 37±1°C. The elution medium was stirred magnetically [32-35]. The aliquots (5 ml) was withdrawn at predetermined time intervals and replaced with same volume of phosphate buffer of pH 7.4. The samples were analysed for drug content using UV spectrophotometer at 376 nm.

#### (j) In Vivo studies

##### Test for Anti-inflammatory synergism By Carrageenan Induced Rat Paw edema test:

##### Animals

Wistar rats (140-190 g) of both sexes were used for the studies. The animals were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. The animals were kept in the

polyacrylic cage (22.5×37.5 cm) and maintained under standard housing conditions (room temperature 24-27 °C and humidity 60-65%) with a 12 h light and dark cycle. Food and water were available ad libitum but the food was not allowed from 1 h prior to the behavioural study. The experimental protocol was approved by the Institutional Animal Ethical Committee (AUUP/AIP/M.Pharm./004/2017), and experiments were conducted in accordance with the CPSCEA guidelines on the use and care of experimental animals.

#### Paw edema induced by carrageenan:

The Rats were divided into four groups (n=5). Acute inflammation was produced [36-37] by sub planter administration of 0.1 ml of 1% w/v carrageenan in normal saline in the left hand paw of the rats. The paw volume was measured at 0-h, 0.5-h, 1-h, 2-h and 3-h carrageenan injection by using plethysmometer. The Animals of group I received normal saline (3 ml/kg b.w., intraperitoneal, i.p) and served as saline control. The groups II received the oil containing transdermal patch containing 5% w/v of turmeric oil extracted pure leaf oil obtained by the leaves of *Curcuma longa*. Group III received drug Lornoxicam (8mg/film) as transdermal patch and the group IV received the combination of turmeric oil and drug Lornoxicam (8mg/film) treatment against carrageenan induced paw edema respectively. Animals of all groups were treated with the extract and reference drug 1 hour before the administration of carrageenan. Instrument used for this activity is Plethysmograph.

This is the formula used to calculate % paw edema inflammation-

Percentage inhibition (%)

$$= \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

### 3. RESULTS & DISCUSSION:

#### Physicochemical compatibility studies of drug and polymer-

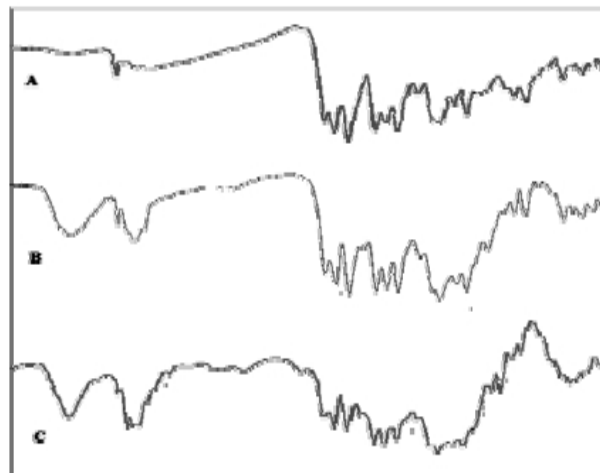


Figure 1: FTIR Spectra of, A= pure drug Lornoxicam, B= Physical mixture of drug and HPMC E5, C= Physical mixture of Lornoxicam, HPMC E-5 and Turmeric oil.

#### Drug-excipient compatibility studies

As described in the methodology section the Fourier transform infrared spectroscopy studies were carried out for pure drug alone and along with polymers and turmeric oil. FT-IR spectra of Lornoxicam alone (**Figure 1: A**), and their physical mixture with HPMC E 5 are shown in figure (**Figure 1: B**) respectively. From the results it was observed that, the characteristic peaks of Lornoxicam were not affected and prominently observed in FT-IR Spectra of physical mixture of Lornoxicam and polymers. This indicated that there is no interaction between Lornoxicam and polymers thus, they are compatible with each other. The interpretation obtained from FT-I.R peaks between the drug Lornoxicam and the Polymer (H.P.M.C E5) and the Turmeric oil showed in **figure1: C** and from that it can be concluded that there is no significant interaction between them.

**Evaluation of prepared Transdermal patches:****Table 2: Evaluation of various parameters of transdermal patches:**

Formulation code	Thickness (mm) $\pm$ S.D.	Weight variation (g) $\pm$ S.D.	% Drug content $\pm$ S.D.	Folding endurance $\pm$ S.D.	Tensile strength (kg) dyne/cm <sup>2</sup>	% Elongation	% Moisture uptake
<b>F-1</b>	0.48 $\pm$ 0.12	0.072 $\pm$ 0.004	93.24 $\pm$ 0.2	98 $\pm$ 2.6	0.62	11.34%	0.30%
<b>F-2</b>	0.39 $\pm$ 0.011	0.074 $\pm$ 0.002	95.35 $\pm$ 0.5	60 $\pm$ 14.2	0.27	14.02%	0.30%
<b>F-3</b>	0.44 $\pm$ 0.079	0.072 $\pm$ 0.004	94.34 $\pm$ 0.1	97 $\pm$ 12	0.60	16.56%	0.39%
<b>F-4</b>	0.43 $\pm$ 0.062	0.073 $\pm$ 0.004	92.68 $\pm$ 0.5	91 $\pm$ 2.6	0.58	12.35%	0.39%
<b>F-5</b>	0.41 $\pm$ 0.080	0.075 $\pm$ 0.003	96.59 $\pm$ 0.3	75 $\pm$ 7.5	0.33	15.43%	0.25%

**Physical appearance**

The patches formed were smooth and transparent/translucent in appearance.

**Thickness**

With the help of Digital callipers, the thickness of patches was measured and the average thicknesses were noted. The thickness results are given in **Table 2**. The result indicates that there was no much difference in the thickness within the formulations. The order of the thickness of patches is F-1 > F-3 > F-4 > F-5 > F-2.

**Weight variation**

Drug loaded patches (1 x 1 cm<sup>2</sup>) were tested for uniformity of weight and the results of weight uniformity are given in **Table 2**. Lesser S.D. values indicate that the patches were uniform. This is in agreement with the uniformity of the thickness. The order of weight uniformity is F-5 > F-2 > F-4 > F-3 = F-1.

**Drug content uniformity**

The drug content from the transdermal patches was determined from the calibration curve of Lornoxicam in Phosphate buffer pH 7.4. The Beer's range for Lornoxicam was found to be 5-35  $\mu$ g/ml. Drug content of the

patch was carried out to ascertain that the drug is uniformly distributed into the formulation. The results obtained are represented in the **Table 2**. From the results obtained (i.e., lower S.D. values), it was clear that there was uniform distribution of Lornoxicam in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation.

**Folding endurance**

The recorded folding endurance of the patches was shown in **Table 2**. It depicts all formulations have good film properties. The folding endurance of the patches are in the following order F-1 > F-3 > F-4 > F-5 > F-2. The results indicated as the HPMC concentration increases the folding endurance of the patches increases correspondingly.

**Tensile strength**

Tensile strength was determined by a fabricated apparatus for which design and functioning was prescribed in various articles. The results (average of 3 determinations) are given in the **Table 2**. The order of tensile strength of the patches were F-1 > F-3 > F-4 > F-5 > F-2. With increase in HPMC proportion the tensile strength of patches was increased. It reflects that the soluble polymer develops

cross linking better than insoluble polymer. More the solubility of the polymer higher will be the tensile strength.

**Percentage moisture absorption**

The recorded Percentage moisture absorption of the patches was shown in **Table 2**. The percentage moisture absorption of the prepared patches were in following order F-4 > F-3 > F-2 > F-1 > F-5. The results showed the

moisture absorption of all the patches were within the acceptable limit.

**Percentage moisture loss**

The percentage moisture loss of the prepared patches are in following order F-1 > F-2 > F-3 > F-4 > F-5. The formulation containing HPMC E 5 alone shows significant loss of moisture when compare to other patches.

**In-vitro drug permeation studies**

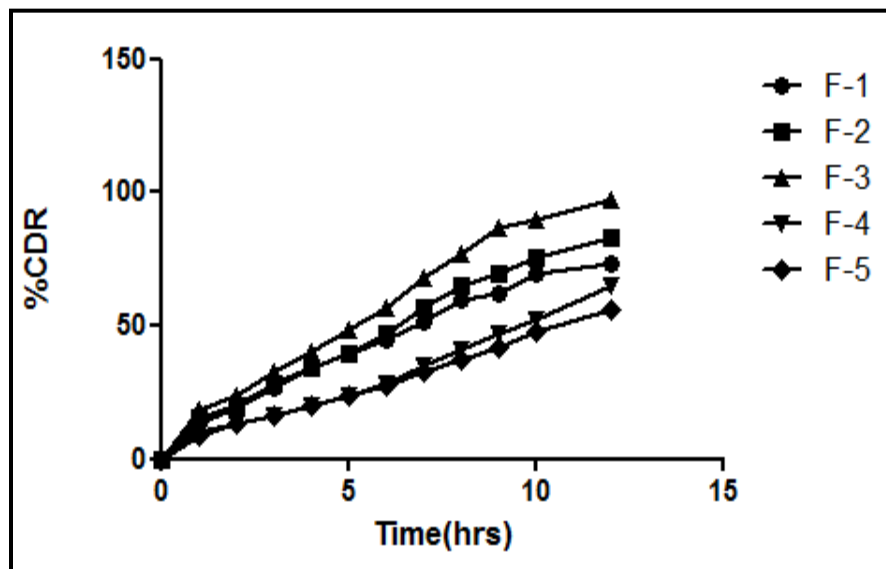


Figure 2: Comparative *In vitro* permeation studies of all prepared patches (F1-F5).

Table 3: Results of model fitting for Lornoxicam TDDS

Formulation code	Zero order	First order	Higuchi / Matrix	Peppas Model	
				R <sup>2</sup> Value	n value
F-1	0.993	0.9865	0.953	0.993	0.868
F-2	0.996	0.952	0.937	0.986	1.14
F-3	0.994	0.991	0.937	0.981	1.081
F-4	0.979	0.859	0.885	0.963	0.964
F-5	0.989	0.957	0.916	0.977	1.06

F-3 formulation (HPMC E-5 with 5% w/v Turmeric Oil) showed the maximum drug permeation (Figure 2), but lasts only for 12 hrs. The % cumulative drug permeated after 12 hrs. was following patterns like F-3> F-2> F-1> F-4> F-5. The formulation F-5 showed lowest drug permeation but it was more than the

formulation prepared without the penetration enhancer with Lornoxicam, but it was shown the permeation for a prolonged time. From the data represented in table 3, it is clearly stated that most of formulations followed mixed order kinetics pattern, rather than single one. But comparatively formulation F4 followed the

zero order pattern rather than others.

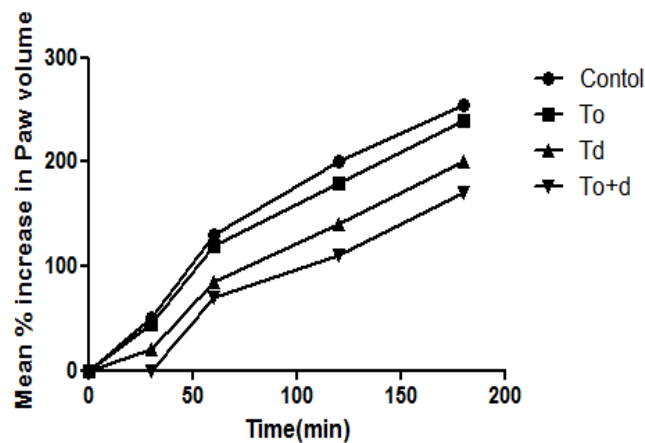
**In Vivo STUDY**

**Carageenan Induced Rat Paw Edema anti-inflammatory response study:**

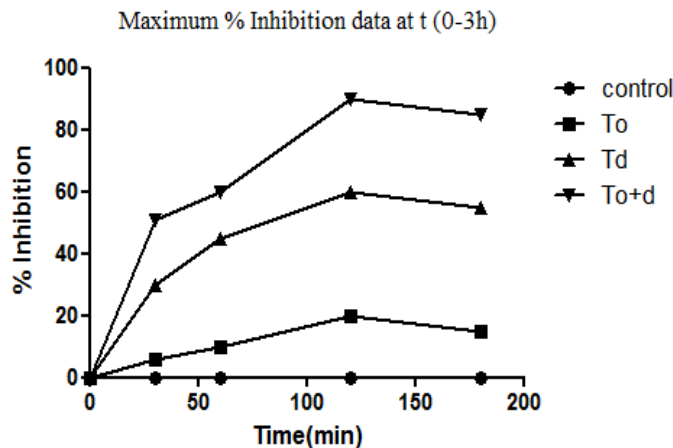
The percentage change of the paw volume of each group has been calculated and then the mean percentage change compared to the each group i.e. Control and Treated. Then the mean difference will also give % Inhibition of paw edema.

**Table 4: Comparative study effect of different formulations on carrageenan induced paw edema in rats (n=5)**

Treatment	Dose (mg/kg)	Paw volume (ml)	% of inhibition	'T' value	*P value
Control	-	1.30±0.030	-		-
Oil	5%(w/v)	1.17±0.025	16.5.0%	2.942	< 0.025
Drug	8mg	1.06±0.038	56.6%	3.521	< 0.025
Oil+Drug	combined	0.92±0.011	85.2.0%	3.559	< 0.025



**Figure 3: Mean %increase in Paw volume at time (0-3h)**



**Figure 4: Maximum % Inhibition at time (0-3h)**



The comparative anti-inflammatory activity of Turmeric oil and the drug against carrageenan induced Paw edema has been successfully investigated and the mean of the % difference of the controlled and turmeric oil, and with drug Lornoxicam and the combined effect against the carrageenan inflammation is compared for one tailed or single tailed paired t test has been evaluated. Results (**Table 4 & figures 3 and 4**) shows significant reduction in inflammation in the group treated with combination of drug and curcumin oil (85.2%, p value 0.025), and curcumin oil treated group as well (16.5%, p value 0.025) as compare to control group.

### CONCLUSION

The synergistic effect of drug Lornoxicam with the combination of Turmeric oil was found to be highly significant for the P value <0.025 as compared to the drug or oil alone.

So in the conclusion on the basis of *in vitro* permeation data and *in vivo* study it can be clearly stated that Turmeric oil was acting for two purposes.

1. It was acted as natural penetration enhancer.
2. The activity of drug alone as anti-inflammatory agent was being significantly enhanced by the Turmeric oil.
3. The synergistic efficacy of drug combined with turmeric oil against the oil itself and drug itself can easily be established.

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