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Research Article

FORMULATIONAND EVALUATION OF TRANSDERMAL GEL OF LORNOXICAM IN COMBINATION WITH CHEMICAL ENHANCERS

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ABSTRACT

Transdermal delivery of drugs through the skin to systemic circulation provides aconvenient route of administration for a variety of clinical indications. The purpose of present investigation was to develop Lornoxicam transdermal gel and its iontophoretic delivery to enhance its permeation for systemic effect and to avoid side effects and minimize frequency of administration. Lornoxicam (NSAIDs) is a COX-1 and COX-2 inhibitor used in the treatment of inflammation, pain and edema, rheumatoid arthritis and so on. Transdermal gel of Lornoxicam was formulated using triethanolamine (5%) as solvent, carbopol 934p as gelling polymer and various penetration enhancers and enhancement in its permeation by using chemical enhancers and iontophoresis was investigated. Formulated gel was evaluated with respect to different physiochemical parameters such as pH, viscosity, spreadability, gel strength. Permeation study was carried out using cellophane membrane and phosphate buffer pH 6.8 for 6 hours. Anti- inflammatory activity of Lornoxicam gel was studied in albino rats by carrageenan induced paw edema method in which Lornoxicam gel was delivered through rat's skin by passive diffusion and diffusion with chemical enhancers. Optimized formulation (F3) with chemical penetration enhancers (tween 80, 2%) showed higher anti-inflammatory activity (40 %) as compared to F1 (22%) in 1 to 4 hours. So, diffusion of Lornoxicam is affected by presence of chemical enhancers.Stability studies carried out at different temperatures and humidity did not show any significant change in drug content, % CDR, viscosities and other parameters at the end of 12 weeks indicating that all the formulations were stable. Physiochemiclly stable and non-irritant Lornoxicam gel was formulated which could deliver significant amount of active substances across the skin *in-vitro* and *in-vivo* which elicit the anti-inflammatory activity.

Keywords: Lornoxicam, penetration enhancers, NSAID's, tween 80, anti-inflammatory activity.

INTRODUCTION

There are several techniques of conventional drug delivery system where tablets, capsules, pills, liquids, aerosols, ointments were used as drug carrier. Among them, novel drug delivery system employs uniqueness in controlling rate of drug delivery and targeting the delivery of drug to specific tissue. These systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation[1].

Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic actionand optimization of drug delivery through human skin is important in modern therapy[2,3]. The outer most layer of the human skin, stratum corneum (SC) having multilayered structure, forms a strong barrier to most of the substances including drugs[4]. Number of approaches has been developed to enhance and control transport of drugs across the skin which expand the range of drugs delivered. Among them chemical method is one which is based on strategies like increasing skin permeability[5]. The treatment of skin disease as well as musculoskeletal disorder might be advantageous from topical administration, obtaining a considerable reduction of oral side effect with improved patient compliance.[4]

Lornoxicam is a potent non-steroidal anti-inflammatory drug, used for the variety of inflammatory conditions. The mechanism of action Lornoxicam is primarily due to inhibition of prostaglandin synthesis through the inhibition of cyclooxygenase (COX) enzymes. Like other NSAIDs, common side effect of Lornoxicam is gastrointestinal irritation. Thus the delivery of the Lornoxicam through the skin for inflammation is desireable[6]. In order to increase therapeutic efficacy of topically applied drug, it is necessary to employ chemical enhancers. An attempt has been made, to enhance the permeation of Lornoxicam by using chemical enhancers in gels made using carbopol934p to study the topical delivery of Lornoxicam through the rat's skin. Tween and Span were used as synthetic penetration enhancers[4].

MATERIALS AND METHODS

Materials and reagents

Lornoxicam was provided by Naprod Life Science P. LTD (India) as a gift sample, Carbopol 934p, Triethanolamine, Tween, Span, Ethanol (S.D fine chemicals Pvt. Ltd, Mumbai). Reverse osmosis (RO) water was used for preparing all the solutions and samples.

Animals

Approximately 150-200g of male albino rats was used. All the animals were properly fed and housed as per guidelines of Institutional Animal Ethics Committee (IAEC). All the experimental procedure and protocol used in this study were reviewed and approved (SACCP/IAEC/24/2013-14) by IAECof Sri AdiChunchanagiri collegeof pharmacy, B.G Nagara, Karnataka constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical guidelines were strictly followed during all the experiments.

Solubility studies

The solubility of Lornoxicam was determined in distilled water, pH 6.8 phosphate buffer (PB), chloroform and 5% triethanolamine solution in water by shake flask method. Briefly, an excess amount of Lornoxicam is added to each vial containing 10 ml of selected solubilizer. The mixtures were subjected to the mechanical agitation for 72 hrs.in isothermal shaker at 25^{o}C ±1^{o}C followed by the filtration through watmann's filter paper prior to UV.[4]

FTIR interaction studies

Infrared spectroscopy was conducted using a Thermo Nicolet FTIR and the spectrum was recorded in the region of 4000 to 400 cm-1. FTIR studies were carried on pure drug, physical mixture of drug and polymer to confirm the compatibility of the drug with other excipient used for the preparation of gel.[7]

DSC study of physical mixture of drug and polymer

Thermal properties of the pure Lornoxicam and the physical mixture of drug and excipients were analyzed by Shimadzu DSC-60, Shimadzu Limited Japan. The samples were heated in a thermetically sealed aluminum pans. Heat runs for each sample were set from 25 to 350^{0}C at a heating rate of 10^{0}C/min, using nitrogen as blanket gas. The 3gm of sample was used for the analysis.[7]

Preparation of gels

Lornoxicam of 0.2% gel formulation were prepared by dispersion methods[8]. Carbopol 934p as gelling base slowly dispersed into distilled water and allowed to swell for 12 hours. Lornoxicam solution was prepared by dissolving in 5% triethanolamine and the solution was slowly dispersed into gel base with continuous mixing. Penetration enhancers were added to the gel base by gentle stirring, chemical enhancers used were 1% and 2% tween and span. Finally a preservative was added by dissolving it in ethanol. Composition of Lornoxicam gel formulations are shown in table 1.

Evaluation of Lornoxicam gel

pH

The pH of Lornoxicam gels were determined by using a calibrated pH meter(equiptronics). The readings were taken for average of three samples.The pH meter was calibrated before each use with standard 4, 7 and 9.2 buffer solutions[8].

Drug content

Drug content analysis was determined by dissolving 1g of gel in 100ml of phosphate buffer pH 6.8: methanol (50:50). Then 1ml of this solution was transfer to the 10ml volumetric flask and final volume was made by same solutions. Finally absorbance of prepared solution was measured at 380nm using UV visible spectrophotometer. The percentage drug content is calculated[9].

Viscosity and rheological studies

Brookfield digital viscometer (Model LVDV–E, USA) was used for the determination of viscosity and rheological properties of Lornoxicam gel using spindle no T-96. The viscosity of gel was measured at different angular velocities at a temperature of 25°C. A

typical run comprised changing of the angular velocity from 0.5 to 2.5 rpm. The averages of two readings were used to calculate the viscosity.[8]

Spreadability

For the determination of spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 min. Weight (50 gm) was added to the pan. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (S).

Spreadability (g.cm/s) (S) =M×L/T

Where M = weight tide to upper slide, L = length moved on the glass slide, T= time taken[9].

Determination of gel strength

A TA-XT2i (Stable microsystems, Ltd. UK) Texture analyser is used. The experiment was done by placing the gels in standard beaker below the probe. In this an analytical probe is then immersed into the sample. The Texture Analyser was set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 gm were selected. An aluminium probe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of gm[8].

***In- vitro* diffusion study**

The experiments were conducted in Franz diffusion cells with donor compartment and a receiver compartment. A suitable size of pre-treated cellophane membrane was mounted in between donor and receptor cells of the Franz diffusion cells (locally fabricated). The receiver contains 15 ml phosphate buffer solution (PBS), PBS pH 6.8 was constantly stirred by magnetic stirrer at 100 rpm and was maintained at a temperature of 37 ± 1°C throughout the experiments. A formulation that is drug equivalent to 2 mg Lornoxicam was applied homogenously in the donor compartments; 1ml samples were withdrawn from receiver at predetermined time intervals over 6 hours and immediately replenishedwith an equal volume of fresh PBS. Samples were assayed for drug content spectrophotometrically. Sink condition was maintained throughout the experiments[9].

***In-vivo* anti-inflammatory activity**

Anti-inflammatory effect of topically applied LRN gel was determined in male albino rats (150-200g, 8-10 weeks) by carrageenan induced paw oedema method. For this purpose, rats were divided into three groups (n=3): group 1- normal control receiving 1%(w/v) carrageenan saline, group 2- LRN gel without penetration enhancers (F1), group 3- LRN optimised gel (F3). Briefly, 30 min after formulation application (0.25 g), rats of both treated groups were challenged by a subcutaneous injection of a 1% (w/v) solution of carrageenan in saline (0.1 ml) into plantar site of right hind paw. Then the volume of paw was measured in plethysmograph immediately after injection and considered as zero hour volume. Then after volume was taken at 0.5, 1, 2, 3, 4, 5, 6hrs and the percentage inhibition of edema is calculated by following formula.

Percent reduction of edema = **C-T×100/C**

Where, C= mean volume of edema for control, T= mean volume of edema for treated group[10].

RESULTS AND DISCUSSIONS

Solubility

LRN is poorly soluble in water (0.0385±0.02 mg/ml). Among the different solubilizer screened LRN exhibited the highest solubility in 5% triethanolamine (42.5±0.01 mg/ml). Solubility of LRN in chloroform and PBS pH 6.8 was 0.25±0.2 and 0.15±0.2 mg/ml respectively. Hence 5% triethanolamine is selected for the formulation of LRN gel.

FTIR interaction studies

From the FTIR studies, all the characteristic peaks of Lornoxicam were present in the spectrum of drug and polymer mixture, indicating compatibility between drug and polymer. The spectra were recorded over the wave number range 4000-400 cm^{-1}. The FTIR spectrum of Lornoxicam showed a characteristic peak at 3,065 cm^{-1} corresponding to NH stretching vibration. Intense absorption peak was found at 1,733 cm^{-1}due to the stretching vibration of the C=O group in the primary amide. The stretching vibrations of the S=O group appeared at 1,034 cm^{-1}. C–Cl bending vibration at 948 cm^{-1}which indicates groups is match with structure of drug andconfirms the purity of the drug. There is no shift of peaks or disappearance of principle peaks or modification of the principlepeaks indicating that there is no interaction between the drug and excipients. FT-IR spectrum of pure drugand its physical mixture is represented in figure 1 and 2.

DSC studies

Any possible drug polymer interactions were studied by thermal analysis. The DSC thermogram of Lornoxicam was typical of a crystalline substance, exhibiting a sharp

exothermic peak at 223.66°Ccorresponding to its melting and decomposition. The thermogram of Lornoxicam with excipients showed the existence of the drug exothermic peak at239.07^{0}C. This showed not much shift in the exothermic peak of pure drug which indicates absence of interactions between Lornoxicam and other excipients. The DSC thermogram of pure drug, its physical mixture and formulation are shown in the figure 3 & 4.

Evaluation of Lornoxicam gel

1%- 1.5 % w/w of carbopol was used for the preparation of gel on the basis of optimum viscosity and spreadability because at 0.5% w/w it produced gel with fluid consistency and above1.5% it produced a gel with high viscosity and low spreadability.

pH of gel

The pH of Lornoxicam gels were determined by using a calibrated pH meter (equiptronics) and pH of the gel were found to be 6.8±0.4 (n=3) and tabulated in table 2.

Viscosity and rheological studies

The viscosity of LRN gels were shown in table 3 and rheological property were shown in figure 5. Rheological studies showed that the viscosity of the formulations decreases with increase in share rate, which indicates the characteristics of pseudo plastic flow or non-Newtonian flow (shear thinning).

Drug content

The drug content of Lornoxicam gel was found to be 94.04±0.2% to97.07±0.1% and tabulated in table 2.

Spreadability

The spreadability of the LRN gels were found to be 5.16 to7.00 gm.cm/sec (table 2)which is indicative of good spreadability.

Gel strength

The gel strength of the Lornoxicam gels formulations were determined by using texture analyser (TA.XT2i, Stable micro systems, Ltd. UK) and gel strength of gels were tabulated in table 2. From the results it indicates that increases in the concentrations of the polymer increase the gel strength and vice versa.

***In- vitro* diffusion studies**

From the *iv-vitro* studies it's found that the percentage of LRN release after 6 hours was 42% to 74% from all formulations. Formulation F3 releases highest percentage of drug (74%) in 6 hours and F1 released lowest percentage (42%) in 6 hours. This clearly indicates that tween 80 (2%) showed higher permeation among all permeation enhancers (fig. 6).

***In-vivo* anti-inflammatory activity**

In anti-inflammatory activity test using carrageenan induced paw oedema method optimized Lornoxicam gel (F3) and non-optimised gel (F1) (without enhancers) exhibited anti-inflammatory activity up to 6 hours (fig. 7) and peak activity was observed between 1- 6hrs for both formulations. Percentage oedema inhibition produced by the application of gel F1 was 9 – 22% between 1-6 hrs and for F3 percentage oedema inhibition was 18 - 40% after 1- 6 hrs. The observed increase in activity of F3 was around 2 fold as compared to gel without enhancers (F1). The result confirmed the fact that significant amount of Lornoxicam was delivered from the gel to induce the anti-inflammatory effect (fig. 7).Tween 80 enhances the topical penetration of the drug by increasing the partitioning and solubility within stratum corneum.

CONCLUSION

Above investigation presents physiochemically stable topical gel of Lornoxicam which would minimise oral side effects of Lornoxicam and deliver significant amount of Lornoxicam across skin to prevent inflammation. From anti-inflammatory activity studies it's concluded that the diffusion of Lornoxicam with chemical enhancers is more effective than passive delivery and this may be due to the modification of stratum corneum or partitioning of drug by chemical enhancer. Formulation with Tween 80 (2%) showed better inhibition of edema in between 1-6 hours can provide anti-inflammatory effect similar to other marketed formulation

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Table: 1 Composition of LRN gel formulations

S.no	Ingredients	F1	F2	F3	F4	F5
1.	LRN(mg)	200	200	200	200	200
2.	Carbopol 934(%)	1.5	1.2	1	1.5	1.2
3.	Triethanolamine (5%) (ml)	10	10	10	10	10
4.	Ethanol (ml)	1	1	1	1	1
5.	Methylparaben(mg)	40	40	40	40	40
6.	Tween 80 (%)	-	1	2	-	-
7.	Span 60 (%)	-	-	-	1	2
8.	Dis.Waterq.s	100 ml	100 ml	100 ml	100 ml	100 ml

Table 2: Characteristics of various gel formulations

Formulation code	pH	Spredability g.cm/s	Gel strength g/sec	Drug content (%v/w)
F1	6.5	5.16	203.33	93.81± 0.058
F2	6.8	6.16	183.33	95.44± 0.029
F3	6.8	7.00	170.00	97.07± 0.014
F4	6.4	5.10	206.66	94.90± 0.034
F5	6.5	6.10	180.00	96.52± 0.018

Table 3: Viscosity of formulations

Shear Rate (RPM)	Viscosity of the formulations (cps)				
	F1	F2	F3	F4	F5
0.5	33000	30000	25500	32500	31500
1	15200	13200	12200	15000	13100
1.5	10800	10100	9200	10100	10000
2	8500	7900	7300	8400	7800
2.5	7300	7100	6100	7100	7100

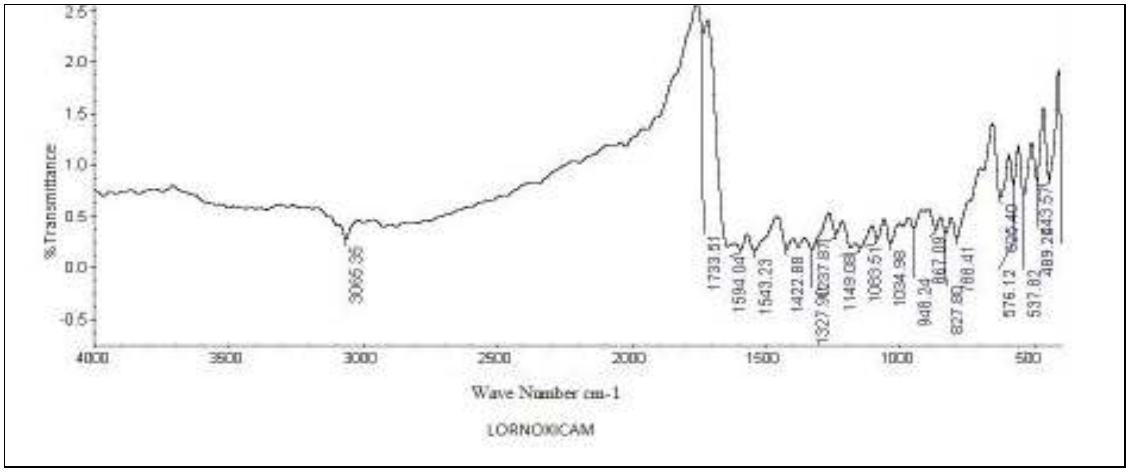

Fig. 1: FTIR of pure Lornoxicam

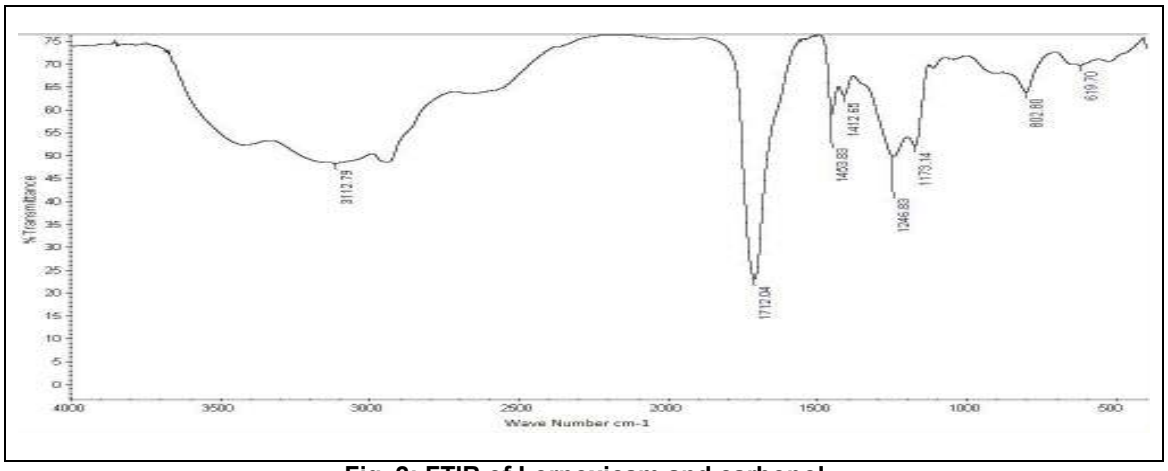

Fig. 2: FTIR of Lornoxicam and carbopol

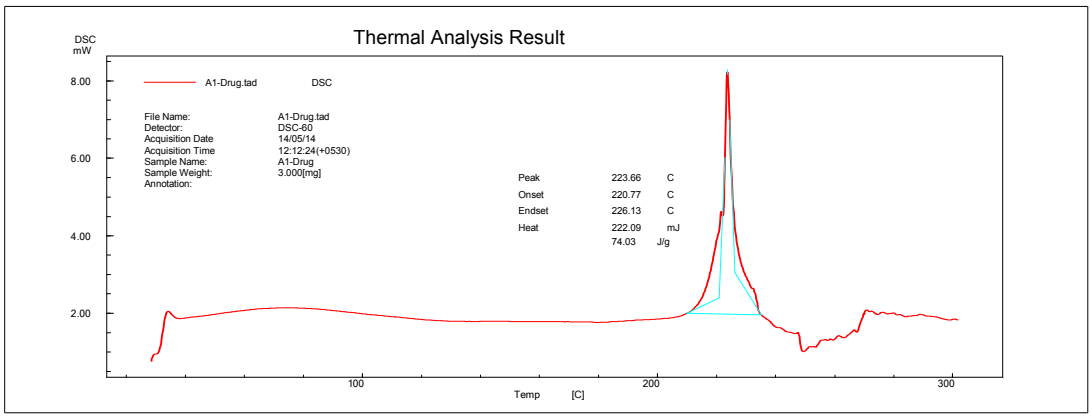

Fig. 3: DSC Thermogram of Lornoxicam

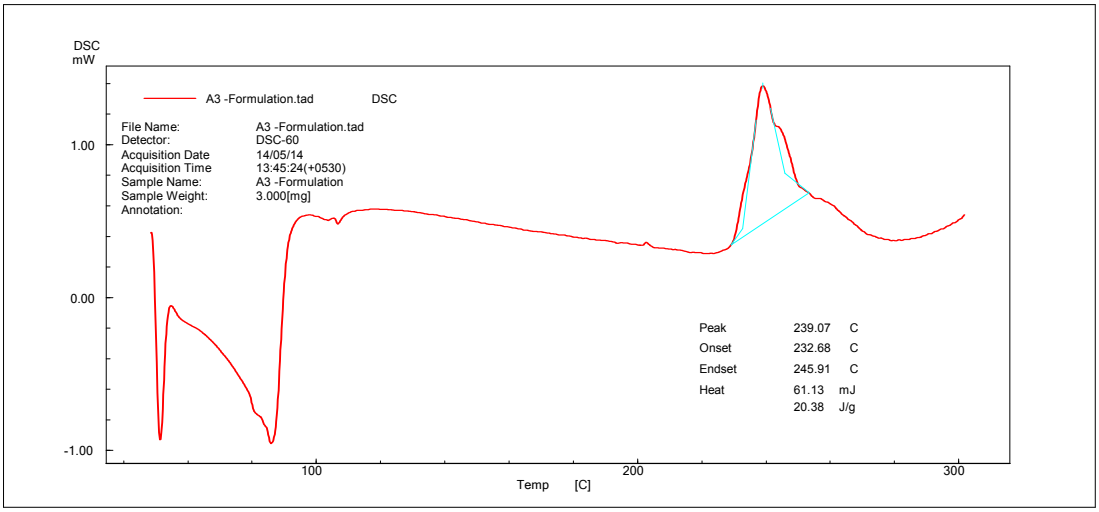

Fig. 4: DSC Thermogram of gel formulation

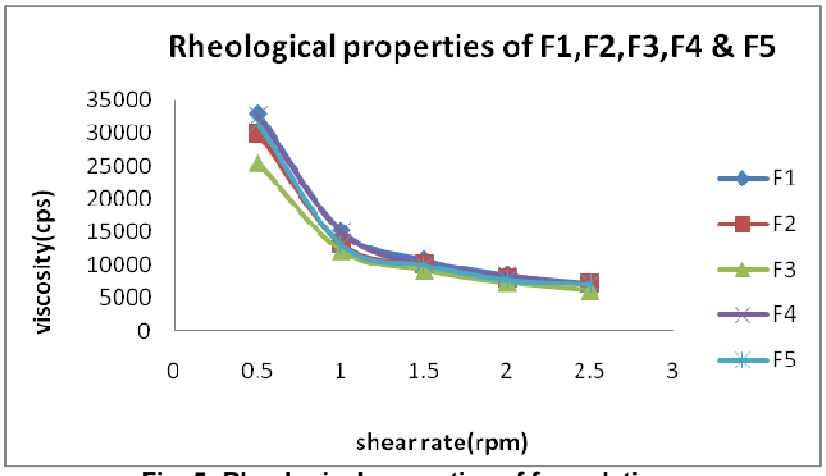

Fig. 5: Rheological properties of formulations

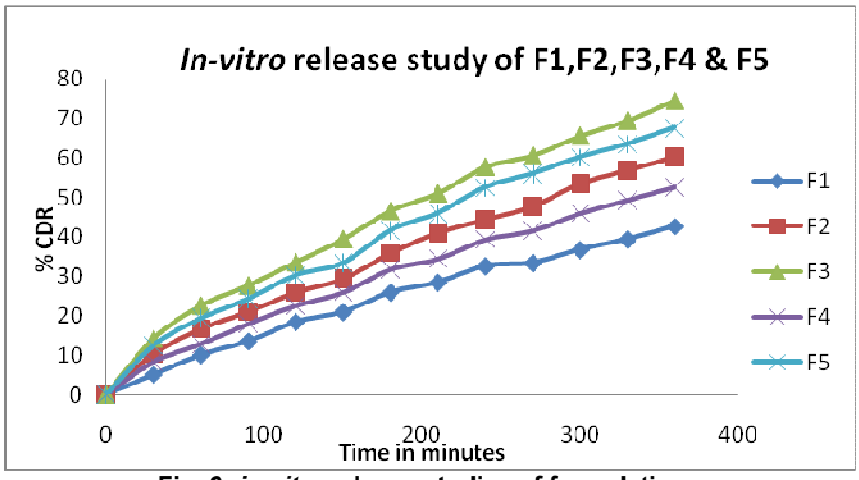

Fig. 6: *in-vitro* release studies of formulations

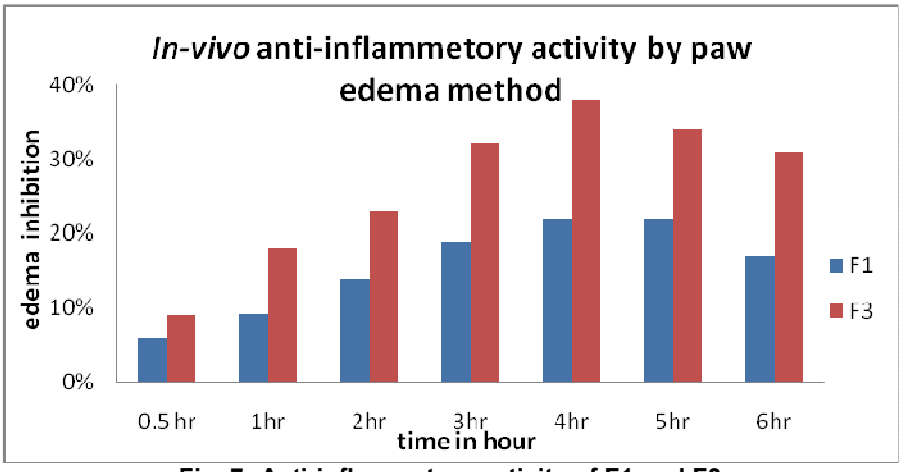

Fig. 7: Anti-inflammatory activity of F1 and F3

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