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FORMULATION AND CHARACTERISATION OF KETOPROFEN TOPICAL GEL

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ABSTRACT

Topical gel of NSAIDs is very useful as palliative product for treating pain and inflammation associated with arthritis. Various synthetic, semi synthetic and natural gelling agents are used with literature suggesting their safety & suitability for topical use. For the present study the gel which are prepared by using different type of polymer carbopol 940(CP), Hydroxy Propyl Methyl Cellulose (HPMC) K4M and xanthan gum were chosen with cyclodextrin (CD)as a penetration enhancers. The prepared gel was evaluated with different parameters such as pH, Homogeneity, Viscosity, Spreadability, drug content, pharmacological evaluation like carrageenan induced rat paw edema, skin irritation study, *In-vitro* diffusion study and Stability studies. The results of the present study showed that the permeation rate of formulation C3 and C4 was enhanced without any significant change in the pH, Viscosity, Spreadability and drug content. The formulations C4 have good significant level during anti-inflammatory study.

Key words: Ketoprofen**,** Rat paw oedema, gel, Carbopol 940, HPMC K4M, Xanthan.

INTRODUCTION

The skin cross a total surface area of approximately 1.8 m^2 and provide the constant between the human body and its external environment. Decrease the systemic drug level and decrease systemic side effects. Most of topical preparations are meant to be applied to the skin. The pH of the skin varies from 4 to 5.6. It is suggested that acidity of the skin helps in limiting or preventing the growth of pathogens and other organisms [1]. Inflammation is fundamental protective response the ultimate goal of which is to rid the organism of the initial cause of cell injury (e.g. microbes and toxins) as well as the consequence of such injury i.e. the necrotic cells and tissues. Inflammation is an extremely complex process; it may be initiated by tissue damage, immunologic reaction, microorganism or other phenomenon leading to the infiltration of inflammatory cells, such as leukocytes [2]. The topical drug products are designed to deliver the drugs into the skin for treating various dermal disorders, and here skin is the target organ. But penetration of drugs through the stratum corneum is essential for both types of deliveries and hence the rate limiting step is percutaneous absorption [3,1, 4]. Gel formulations with suitable rheological and mucoadhesive properties increased the contact time at the absorption site. Discharge of drug from the gel formulation must be sustained for the benefits by prolonged contact time [5]. Gels are advantageous as compared to other topical semisolid due to ease of application, good spreadability, greaseless (non-staining), generally provide faster drug release than creams and ointments, superior optical clarity of synthetic polymer gels e.g. carbomer and poloxamer [6,7].

MATERIALS AND METHODS

The pure API Ketoprofen was procuring from Emcure Pharma Ltd. Pune as a gift sample. Carbopol 940 from Vishal - Chem, Mumbai, Disodium hydrogen

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phosphate and Potassium dihydrogen phosphate, Ethanol (95%), Propylene glycol, Xanthan gum procured from Loba Chemie Pvt. Ltd. Mumbai, Methyl paraben, Propyle paraben procured from S.D Fine Chem Pvt. Ltd., Mumbai. β – Cyclodextrin obtained from Curex Pharma Pvt. Ltd., Jalgaon. Triethanolamine from Merck Specialties Pvt. Ltd., Mumbai, HPMC K4M, Lab fine chem Industries, Mumbai all the chemicals and solvents either purchase or gifted by the manufacturer and use as it is.

Preliminary Studies

The samples of Ketoprofen were checked for colour, odour, stickiness, consistency, appearance, grittiness was observed by visual inspection.

Melting Point Determination

Melting point of Ketoprofen was determined by capillary method. The capillary filled with drug powder was placed in Thiels tube filled with liquid paraffin. The tube was heated and the melting point of drug powder was noted [8].

Spectroscopic Studies

Preparation of Phosphate Buffer pH 7.4

Potassium dihydrogen phosphate 0.2 M was prepared and 250 ml of this solution was uniformly mixed with 0.2 M NaOH 195.5 ml volume was prepared up to 1000 ml with distilled water. The pH of the buffer was adjusted to pH 7.4 [9].

Selection of Spectral Analysis

Ketoprofen was dissolved separately in around 50 ml Phosphate Buffer Saline (PBS) pH 7.4 in a 100 ml volumetric flask with vigorous shaking followed by ultrasonication for about 5 minutes. The standard solutions were then scanned in the spectrum mode of the instrument from 400 nm to 200 nm. [9].

IR Spectrum Interpretation

The dry sample of Ketoprofen was mixed with IR grade KBr in the ratio of 1:1. This mixture were scanned over a wave number range of 4000 to 400 cm^{-1} in FTIR instrument and spectral analysis was carried out [10].

Formulation of Topical Gel

In the preliminary study various gelling agents like Carbopol 940, Carbopol 974p, Sodium alginate, Xanthan gum, Locust bean gum, Gaur gum, Sodium CMC, HPMC K4M were tried on the basis of availability and compatibility with drugs. They were tried in different concentrations on the basis of their viscosity and literature review. The formulation batch was showed in Table No. 1.

Evaluation of Topical Preparation

Organoleptic properties

Colour, odour and appearance may get changed on storage. The change in properties indicates decomposition [11].

pH

The apparent pH of product may get altered on storage. Changes in product pH also indicate chemical decomposition. The stratum corneum is remarkably resistant to alterations in pH, tolerating a wide range of 3 to 9 [11].

Spreadability

Two glass slides of 20×20 cm were selected. The gel formulation (2.5g) who's Spreadability has to be determined were placed over one of the slides. The other slide was placed upon the top of the gel such that the gel was sandwiched between the two slides in an area occupied by a distance of 60 cm along. The two slides in position were fixed to a stand without least disturbance and in such a way that only the upper slide would slip off freely by the applied force (weight). Carefully 20 g weight was attached with thread to upper slide. Time taken for the upper slide to pass through the distance of 13 cm was recorded. From above values Spreadability was determined by following equation [12].

$$S = M \times L / T$$

Where: S, is the spreadability of gel formulations M, is the weight (g) tied on the upper plate, L, is the length (cm) of the glass plates, T, is the time taken for plates to slide the entire length.

Drug Content

The quantity of 0.2 g gel was dissolved in methanol in 100 ml volumetric flask sonicated for 1 hour and volume was making up to the mark with methanol. From this solution, 1 ml sample was withdrawn and diluted up to 10 ml with methanol. The sample was analyzed (simultaneous equation method) UV spectrophotometrically at wavelengths of 254 nm. From absorptivity value drug content was determined. Similarly drug content was determined using standard calibration curve of Ketoprofen [13].

Viscosity

Time dependent rheological behavior of semisolids may also signal physical or chemical change. Brookfield viscometers with spindle helipath attachments, which measure the force it takes to drive a spindle helically through a semisolid. Substantial irreversible rheological changes are a sign of poor physical stability[12, 14].

Extrudability

It is a useful empirical test to the measure the force required to extrude the material from a tube. Since the packing of gels have gained a considerable importance in delivery of desired quantity of extrusion of gel from

collapsible tube or jar and therefore the measurement of extrudability [12].

Elegance

Ideally, the application should be undetectable to eye and neither tacky nor greasy. Semisolids should be evaluated for tackiness, greasiness, Spreadability and stiffness [11]. 2010).

***In-vitro* Permeation Study**

In-vitro studies can help in investigating mechanisms of skin permeation of the drug. Diffusion cells generally comprise two compartments, one containing the active component (donor vehicle) and the other containing receptor solution, separated by a piece of excised skin or other membrane. Although many variations of diffusion cells exist, there are two basic designs; the static or non flowing cell, and the flow through cell [14].

Animal Study

Male albino wistar rats, weighing 150-200 gm were used. They were housed in the standard environmental condition and fed with diet and water. All procedure was followed in accordance with the approved protocol [15].

Carrageenan-Induced Rat Paw Edema

Animals are divided into three groups (n=6) starved overnight with water add libitum prior to the day of experiment. The control group receives vehicle topically, while other group receives test drug and standard drug respectively. Left paw was marked with ink at the level of lateral malleolus; basal paw volume was measured plethysmographically by volume displacement method using Plethysmometer (UGO Basile 7140) by immersing the paw till the level of tangential malleolus. The animals are treated with drug. After one hour dosing, animals are treated with 0.1ml of 1% solution of Carrageenan into the sub-plantar side of the left hind paw subcutaneously. The increase in paw volume was calculated as percentage compared with the basal volume [15, 16]. The difference of average values between treated animals and control group was calculated for each time interval and evaluated statistically [2].

The % Inhibition was proposed by using following formula.

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

Where, Vt - Mean edema volume of test

Vc - Mean edema volume of control

Skin Irritation Test

The method of Draize was selected to observe the presence of erythema and edema on the test sites. Grading of the severity of erythema and edema formation was also investigated after 24 and 72 hours [17].

Experimental Animals

This study employed eighteen male rabbits (1.2-2.5 kg) to test for the skin irritation. They were kept carefully following an acclimation period of 7 days to ensure their fitness in investigation. Test animals were kept in restricted entrance of other rodent. Facility with environmental conditions set to a temperature of 25 ± 2 ºC and day / 12-h night cycle. Animals were provided full of food and drinking water to their easy access. The back region of each rabbit was shaved prior to the testing. The site of application was occluded with gauze and covered with a non sensitizing microporous adhesive tape [16]. After 24 h, the gel was removed and the score of erythema was determined by the Drazie test as follows:

0 - negative erythema; 1 - mild erythema; 2 - fair erythema; 3 - chronic erythema

Accelerated Stability Study of Gel Formulation

Stability of medicinal products may be defined as the capability of a particular formulation in a specific container to remain within its physic-chemical, growth of microbes, efficacy and toxicological condition, i.e. resists deterioration means ability to stabilize the drug to 90% of labeled potency is generally recognized as the minimum acceptable potency level. So Stability study was carried out for 3 months at 40 ^{0}C/75% RH[18,19].

Results and Discussion

Characterization of Drug

Drug powder is crystalline white or almost white in colour, having neutral or almost odourless.

Melting Point

Melting point range obtained for Ketoprofen was 94ºC-97ºC. The reported melting point range of Ketoprofen was 95^{o}C-97ºC.

Characterization of Drug

In present study, the spectrophotometric method was adopted for the evaluation of Ketoprofen using U.V. spectrophotometer labutima.

UV Spectroscopy

λ max Determination

The UV spectrum of Ketoprofen in 7.4 pH phosphate buffer solution scanned in the range of 400-200 nm. The spectrum indicated that the observed λ_{max} of Ketoprofen was 254 nm which was matched with pharmacopoeial value.

Percentage Purity of Ketoprofen

The supplier claims that the provided Ketoprofen drug was 99.95% pure.

Infrared Spectroscopy

The IR spectrum did not show presence of any additional peaks for new functional groups indicating no

chemical interaction between Ketoprofen & the used polymers. After 30 days of storage at room temperature, samples were observed for physical changes but there were no physical changes observed in the mixture of drugs and polymer combination. Ketoprofen with different polymers (Carbopol 940, HPMC K4M, Xanthan gum) indicated that there were no interaction between drugs, polymers and other excipients when compared with infrared spectrum of pure drug as all functional group frequencies were present.

Evaluation and Characterization of Gel

Physical Evaluation of Gel Formulation

The Gel formulations were observed for their visual appearance, odour, colour, texture and feel upon application such as grittiness, uniformity, and stickiness of formulation the observations was showed in Table No.2..

Spreadability

All the gel formulation was also evaluated for spreadability it was found that the range was in between 52 to 72 mm. The C3 gel formulation showed maximum and superior spreadability ie.72 mm. and lowest Spreadability value was noted for H4 formulation was 52 mm. The spreadability of marketed gel formulation was 82mm. The observations were showed in Table No.3.

Homogeneity

All formulation of Ketoprofen topical gel posses the homogeneous in nature. Results of homogeneity are shown in Table No.3.

pH

The pH of formulation C1, C2, C3, C4, H1, H2, H3, H4, X1, X2, X3, X4. Lies in between 6.3 to 7.2 in normal range of skin and did not produce any skin irritation.

Drug Content

Drug content was determined by UV analysis which was found within the limits. Results are shown in Table No.3.

Viscosity

The viscosity of prepared gels was evaluated. The viscosity evaluation were carried out on Brook-field viscometer (DV 2 PRO^{+}). Results and graphs are showed in Fig. 1-3.

***In-vitro* Diffusion Study**

The *In-vitro* diffusion study were taken by using Franz diffusion cell which showed Cumulative % drug release of ketoprofen gel formulation was showed in Figure No. 4-6.

C1- 86.814±0.978%, C2- 86.526±0.964%, C3- 87.124±0.965%, C4- 85.846±0.636%,

H1- 79.307±1.759%, H2- 83.254±2.725%, H3- 75.473±0.513%, H4- 84.012±1.255%,

X1- 75.635±0.490%, X2- 74.804±0.795%, X3- 76.958±1.766%, X4- 75.676±0.516%.

And Cumulative % drug release of marketed gel formulation was– 93.423±1.601

The formulation C3 and C4 showed good released pattern.

Anti-Inflammatory Activity

In Carrageenan induced rat paw edema test C3 shows 71.30 % inhibition after 7 hr as compared to Standard (Marketed) 73.627 at 3 hr. which showed that it was potent anti-inflammatory activity and its effectiveness. H4 And X4 formulation does not show any significant level upto 7 hrs. $P<0.05$, $**P<0.01$ as compared to control, as per one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Values are presented as mean ± SEM of n = 6 animal in each group. The results was showed in [Fig- No. 7-9].

Skin Irritation Study

The skin irritation study done on Wister rats skin showed no irritation to the skin. The results were showed in [Table No. 4].

Accelerated Stability Testing

The formulations were kept for stability studies there was no significant change observed in physical parameter i.e.(appearance color change and grittiness) at 40^{0}C±2 /75%±5 RH. The result of drug content was given in [Table No.5].

There was negligible difference in the drug content observed after stability study suggested that all the formulations are stable under the given conditions for 90 days.

Table 1. Formulation Batches of Ketoprofen Gel

Ingredients (%)	Formulation Code											
	C1	C2	C3	C4	H1	H2	H3	H4	X1	X2	X3	X4
Ketoprofen (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Carbopol 940	0.5	1	1.5	2	-	-	-	-	-	-	-	-
HPMC K4M	-	-	-	-	2	3	4	5	-	-	-	-
Xanthan gum	-	-	-	-	-	-	-	-	0.5	1	1.5	2
Triethanolamine	q.s.	q.s.	q.s.	q.s.	-	-	-	-	-	-	-	-
Propylene glycol	10	10	10	10	10	10	10	10	10	10	10	10

Ethanol 95%	30	30	30	30	30	30	30	30	30	30	30	30
β cyclodextrin	0	3	4	5	0	3	4	5	0	3	4	5
Metaben/C_8-H_8-O_3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total (%)	100	100	100	100	100	100	100	100	100	100	100	100

Table 2. Physical Evaluation of Gel Formulation

Formulation/ Properties	Colour	Odour	Consistency	Appearance	Grittiness	Stickiness
C1	Transparent	Slightly alcoholic	Good	Smooth	No	None
C2	Transparent	Slightly alcoholic	Good	Smooth	No	None
C3	Transparent	Slightly alcoholic	Good	Smooth	No	None
C4	Transparent	Slightly alcoholic	Good	Smooth	No	None
H1	White milky	Slightly alcoholic	Good	Smooth	No	None
H2	White milky	Slightly alcoholic	Good	Smooth	No	None
H3	White yellowish	Slightly alcoholic	Good	Smooth	No	None
H4	White yellowish	Slightly alcoholic	Good	Smooth	No	None
X1	Transparent	Slightly alcoholic	Good	Smooth	No	None
X2	Transparent	Slightly alcoholic	Good	Smooth	No	None
X3	Transparent	Slightly alcoholic	Good	Smooth	No	None
X4	Transparent	Slightly alcoholic	Good	Smooth	No	None
Marketed Formulation	Transparent	Slightly alcoholic	Good	Smooth	No	None

Table 3. Spreadability, pH, Homogeneity, Drug Content of Gel Formulation

Formulation	Spreadability (mm)	Homogeneity	pH	Drug Content (%)
C1	64.01±0.85	Homogeneous	6.41±0.73	96.05±1.65
C2	67.02±0.69	Homogeneous	6.7±0.49	94.64±0.86
C3	72.01±0.65	Homogeneous	7.1±0.70	96.24±0.38
C4	68.02±0.95	Homogeneous	6.39±0.71	95.15±0.75
H1	54.00±0.62	Homogeneous	7.2±0.69	86.71±1.98
H2	57.01±0.78	Homogeneous	6.41±0.53	88.92±0.99
H3	58.02±0.89	Homogeneous	6.3±0.62	90.78±0.81
H4	52.0±0.91	Homogeneous	7.0±0.71	89.26±0.81
X1	64.01±0.77	Homogeneous	6.5±0.76	91.81±1.49
X2	68.03±0.69	Homogeneous	6.8±0. 87	90.72±1.13
X3	69.03±0.57	Homogeneous	6.6±0.55	89.88±1.30
X4	68.02±0.62	Homogeneous	6.3±0.64	86.21±0.77
Marketed	82.4±0.59	Homogeneous	7.1±0.36	98.45±0.67

(n=3)

Table- 4. Skin Irritation Study of Gel Formulation

S. No.	Scores on Respective Days								
	Formulation	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	C1	0	0	0	0	0	0	0	0
2	C2	0	0	0	0	0	0	0	0
3	C3	0	0	0	0	0	0	0	0
4	C4	0	0	0	0	0	0	0	0
5	H1	0	0	0	0	0	0	0	0
6	H2	0	0	0	0	0	0	0	0
7	H3	0	0	0	0	0	0	0	0
8	H4	0	0	0	0	0	0	0	0
9	X1	0	0	0	0	0	0	0	0

10	X2	0	0	0	0	0	0	0	0
11	X3	0	0	0	0	0	0	0	0
12	X4	0	0	0	0	0	0	0	0

Table 5. Results of drug content after stability testing

Formulations	Drug Content (%) 0 Days	Drug Content (%) 90 Days
C1	96.05±0.09	95.78±0.01
C2	94.64±0.01	94.00±0.06
C3	96.24±0.04	96.11±0.05
C4	95.15±0.06	94.86±0.04
H1	86.71±0.05	84.22±0.03
H2	88.92±0.05	86.67±0.06
H3	90.78±0.03	89.45±0.05
H4	89.26±0.05	87.83±0.06
X1	91.81±0.06	89.33±0.03
X2	90.72±0.07	90.23±0.01
X3	89.88±0.05	88.57±0.09
X4	86.21±0.09	85.57±0.04
Marketed	98.45+0.67	98.32±0.45

(n=3)

Figure, 1. Rheogram of C1-C4 with Marketed Formulation

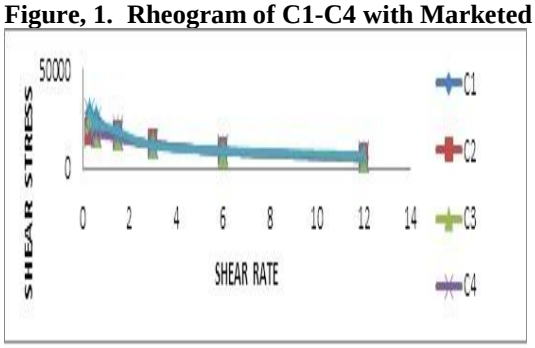
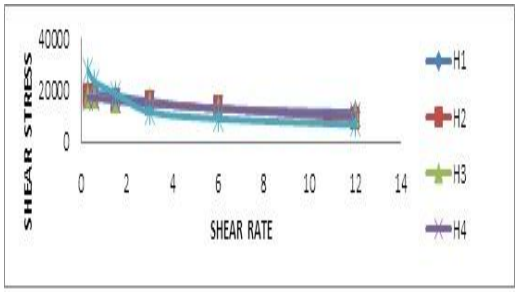
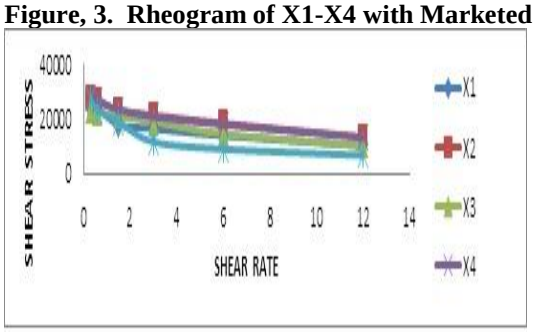


Figure 2. Rheogram of H1-H4 with Marketed Formulation



Figure, 3. Rheogram of X1-X4 with Marketed Formulation



Figure, 4. Cumulative % Release of Carbopol 940 Gel and Marketed Formulation

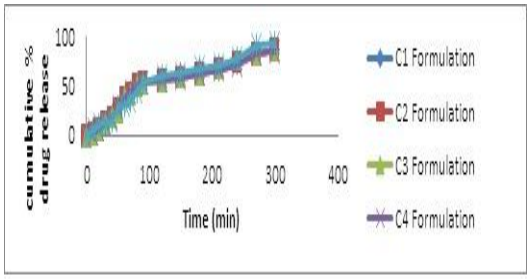


Figure 5. Cumulative % Release of HPMC Gel and Marketed Formulation

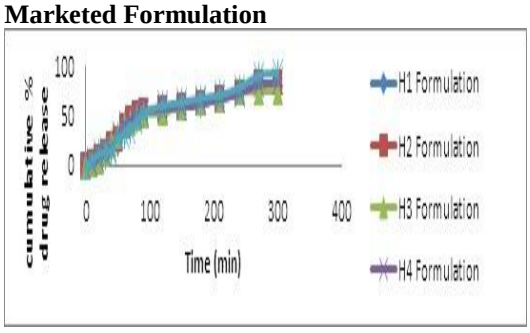


Figure 6. Cumulative % Release of Xanthan Gum Gel and Marketed Formulation

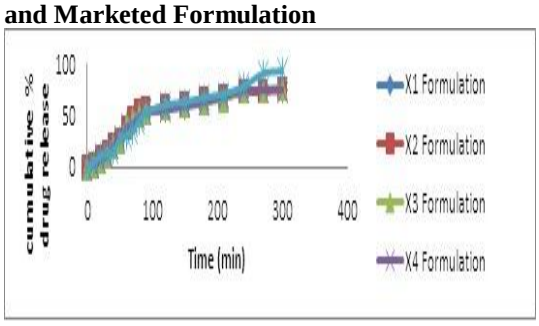


Figure 7. Effect of Ke toprofen on Carrageenan Induced Rat Paw Edema

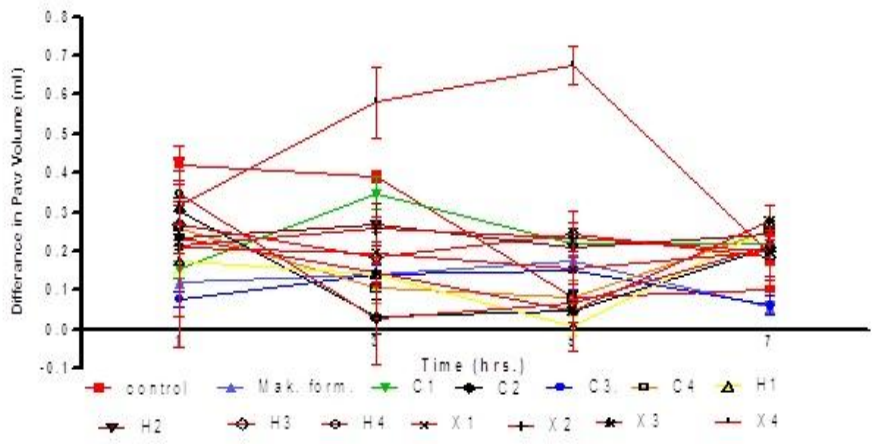


Figure 8. Effect of Ketoprofen on Carrageenan Induced Difference in Rat Paw Edema Volume

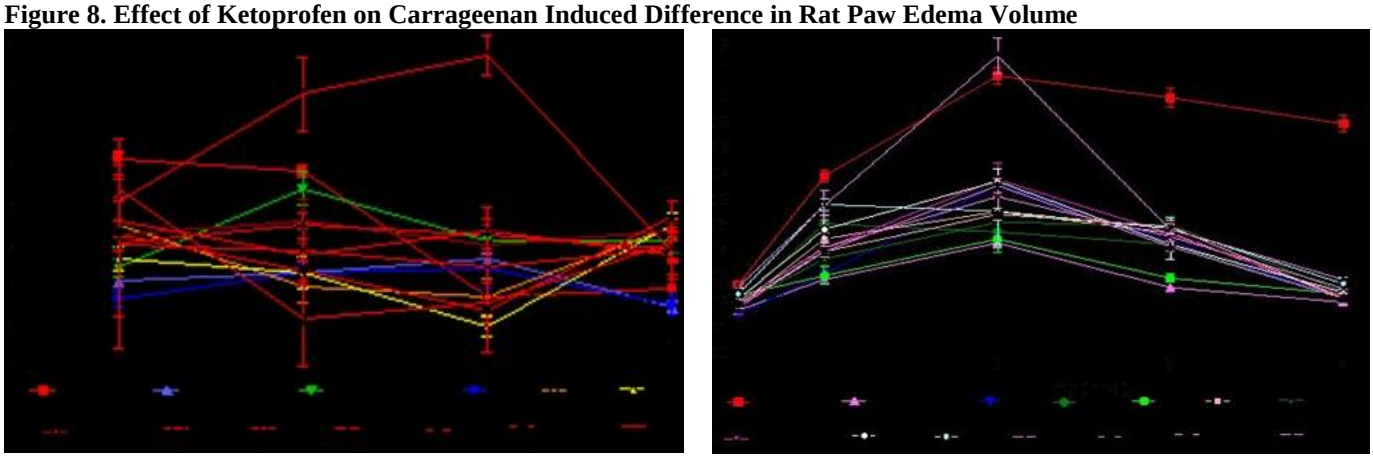
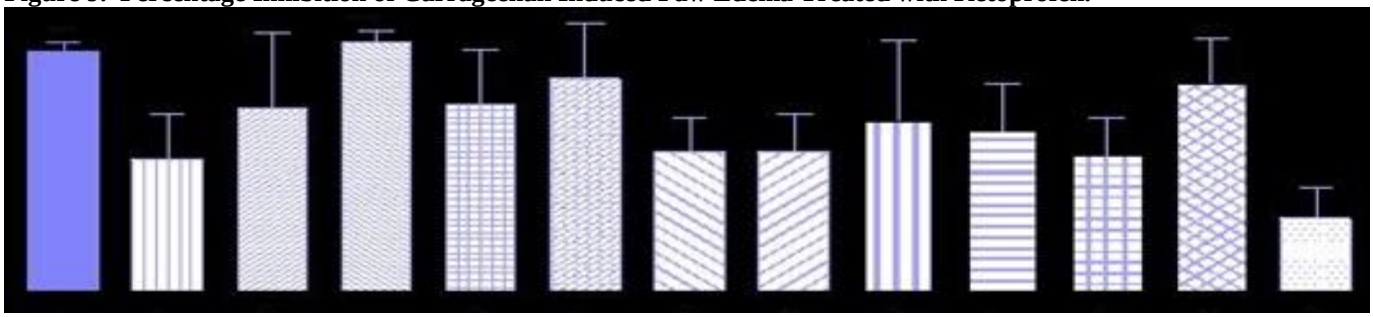


Figure 9. Percentage Inhibition of Carrageenan Induced Paw Edema Treated with Ketoprofen.



CONCLUSION

The present investigation was aimed to formulate topical Ketoprofen anti-inflammatory gel. The study of literature survey reveals that the ketoprofen was more effective as topical as compared to oral. The gel was formulated using different concentration of gelling agent carbopol-940 and HPMC K4M and Xanthan gum polymers with cyclodextrin as a penetration enhancer.

All the gel formulations were evaluated for their pH, viscosity, drug content and spreadability, *In-vivo* animal study includes carrageenan induced paw edema for anti-inflammatory activity and skin irritation study on rabbits. It was establish that all gel preparations were smooth in texture, homogeneous in nature, and elegant with no unpleasant odour and no grittiness was observed which indicated good texture of formulation. Marketed formulation was homogeneous nature, smooth and good in texture. The viscosity of all formulation follows a pseudoplastic flow behavior. The material flows as soon as a shear stress was applied; the slope of the curve gradually decreases with increasing rate of shear. The viscosity was derived from the slope which was found to decrease as the shear rate increased. During stability study both the formulation were found to be stable at room temperature with respect to pH, spreadability and Viscosity but there were some changes in rheological characteristics but that was not prominent The study indicated the overall stability of formulation for three months. Present study showed that

the permeation rate of formulation C3 and C4 was enhanced without any significant change in the pH, Viscosity, Spreadability and drug content. The C4 formulation has posses' significant anti-inflammatory action during the study. The C3 and C4 formulation does not produce any skin irritation after application of ketoprofen topical gel. It was more worthwhile to evaluate the formulation at the clinical level as a further study. Marketed formulation shows better significant in result in case of spreadability, Viscosity, drug content and also cumulative % drug release.

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