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Carbopol and Sodium Carboxymethylcellulose Based Methylsulfonylmethane Gels for Treatment of Osteoarthritis: *In-vitro* and *In-vivo* Evaluation

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ABSTRACT

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The objective of present study was to prepare transdermal gels of methylsulfonylmethane (MSM) alone or in combination with aloe vera and sesame oil using polymers like carbopol 940 (CP) and sodium carboxymethylcellulose (NaCMC). The physicochemical compatibility of drug and the polymers was confirmed by infrared spectroscopy and differential scanning calorimetry. The gels were evaluated for various physicochemical parameters such as pH, homogeneity, spreadability, viscosity measurement and drug content. *In-vitro* drug release study across cellophane membrane was performed. CP based gels (F1- F4) showed higher drug release ranging from 89.7% to 96.4% than NaCMC based gels (F5- F8) with drug release ranging from 79.5% to 89.1% at the end of study. Also, CP based gels showed more prominent anti-inflammatory activity (69.25 to 81.5% inhibition) than NaCMC based gels (54.4 to 72.3 % inhibition). CP based F4 formulation was considered to be optimized formulation based on its in vitro drug release and anti-inflammatory activity studies. The F4 formulation was further subjected to skin irritation study, short term stability study and *In-vivo* study on osteoarthritic patients. It was observed that Lequesne's score was significantly reduced ($p<0.05$) as compared to placebo, demonstrating that patient's symptoms can be improved with MSM gel containing aloe vera and sesame oil. However, further clinical studies on larger number of patients are required. From the results obtained, it can be concluded that the MSM gel containing aloe vera and sesame oil can be better alternative for treating the patients with osteoarthritis.

Keywords: Methylsulfonylmethane, Transdermal gel, Osteoarthritis, Aloe vera, Sesame oil.

INTRODUCTION

Osteoarthritis (OA) is characterized by progressive loss of articular cartilage and bony overgrowth seen mostly in elderly individuals. The initial bland progression of OA may become clinically relevant as an inflammation brought about by the increasing deposition of cartilaginous debris[1]. For the patient, the most important aspect of the condition is pain and associated impairment of movement[2]. As cartilage is not innervated, the pain arises from secondary effects, such as synovial inflammation and fluid accumulation leading to joint capsule distension and stretching of the periosteal nerve endings[3].

Although paracetamol and Nonsteroidal anti-inflammatory drug (NSAIDs) are widely used in the management of osteoarthritis, there is limited evidence that they actually improve the underlying pathology of the disease. Adverse drug reactions are common with NSAIDs, and this factor limits their long term use[4]. Drugs that may prevent or retard the progression of articular cartilage breakdown in osteoarthritis are now receiving attention. Chondroprotection is a new field in the management of osteoarthritis that is designed to improve cartilage repair as well as enhance joint remodeling[5]. Due to this fact, there has been a search for topical medication that will work to reduce patient's symptoms, that will regenerate cartilage and act as anti-inflammatory without causing any side effects.

Methylsulfonylmethane (MSM) is a naturally occurring nutrient found in normal diet[6]. It is considered to be one of the least toxic substances in nature. There is a need for supplementation of MSM since it is lost in the process of cooking. MSM is an effective natural analgesic. It blocks the inflammatory process and enhances the activity of cortisol, a natural anti-inflammatory hormone produced in the body[4]. MSM can restore the flexibility and permeability of cell walls. This helps to equalize the pressure and reduce or eliminate the cause of pain.

MSM, glucosamine and chondroitin combination has been used in many studies in osteoarthritis all over the world as nutritional supplements aiding cartilage repair. They are found to be uniformly safe in all studies compared to NSAIDs[7-9]. Also, natural agents such as aloe vera and sesame oil have shown potential anti-inflammatory activities which may give synergistic effect when combined with MSM[10,11].

The transdermal route of administration has been recognized as one of the potential routes for local and systemic delivery of drugs. This route offers many advantages over the oral dosage form such as improving patient compliance in long term therapy, avoiding first-pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizing inter and intra-patient variability and

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making it possible to interrupt or terminate treatment when necessary[12,13]. Out of various semisolid dosage forms, gels are becoming more popular due to ease of application and better percutaneous absorption. Gel tends to be smooth, elegant, non-greasy, producing cooling effects and utilize better drug release as compared to other semisolid formulations[14,15]. Till date, there is no any reported clinical evidence regarding the efficiency of transdermal MSM gel in osteoarthritis. Few Aloe vera-MSM gels are available in the market but none contains sesame oil as one of their ingredients. Also, the marketed Aloe vera-MSM gels contain aloe vera as the major component due to which the efficacy of MSM against osteoarthritis remains hidden.

Hence, an attempt was made to prepare MSM gel formulations containing MSM as a major component along with sesame oil and aloe vera, using two gelling agents, namely carbopol 940 (CP) and sodium carboxymethylcellulose (NaCMC) and evaluate their anti-inflammatory activity in osteoarthritis. In addition, the effect of presence of aloe vera and sesame oil in the MSM gel formulations on the anti-inflammatory activity was studied.

MATERIALS AND METHODS

Materials:

Methylsulfonylmethane was obtained as a gift sample from Chaitanya Biologicals Ltd (Mumbai, India). Carbopol 940 was received as gift sample from FDC Ltd. (Mumbai, India). Sodium carboxymethylcellulose (NaCMC), high viscosity, was purchased from Loba Chemie (Mumbai, India). Aloe vera and sesame oil were purchased from Charak Ayurveda (Satara, India) and Rajesh chemicals (Mumbai, India) respectively. All other chemicals used were of analytical grade.

Preparation of MSM Transdermal Gels

a. Carbopol based gel

Different batches of gel coded as F1- F4 were prepared using carbopol 940. For the preparation of each batch of gel formulation, corresponding amount of polymer mentioned in Table 1 was taken in a beaker and sufficient quantity of distilled water was added to it and kept aside for 24 hours for proper soaking. Dispersion of MSM (5g in 20 ml propylene glycol) was mixed with above prepared carbopol gel and stirred well to get uniform dispersion of drug. The required quantities of sesame oil and aloe vera gel were then incorporated as per the given formula. The acidic pH of polymeric solution of carbopol was neutralized by adding of sufficient amount of triethanolamine (Table 1). The amount of triethanolamine required to neutralize the carbopol solution was determined by titrimetric method using phenol red as an indicator.

b. Sodium carboxymethylcellulose based gel

Different batches of gel formulations; F5 - F8 were prepared by taking NaCMC in a glass mortar and few drops of distilled water were added to wet the mass. Immediately after wetting, hot water (60-65^{o}C) was incorporated with continuous stirring. MSM (5g) was then mixed in propylene glycol and triturated with swollen NaCMC (Table 1). The sesame oil and aloe vera gel was then incorporated in sufficient quantity as mentioned in the formula.

Table 1: Formulations data of MSM gels system

		Formulations*							
		CP based				NaCMC based			
S.N	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
1	Methylsulfonylmethane	5	5	5	5	5	5	5	5
2	Carbopol 940	1	1	1	1	-	-	-	-
3	NaCMC	-	-	-	-	5	5	5	5
4	Aloe vera ge	I-	1	-	1	-	1	-	1
5	Sesame oil	-	-	1	1	-	-	1	1
6	Propylene glycol	20	20	20	20	20	20	20	20
7	Ethanol	20	20	20	20	-	-	-	-
8	Triethanolamine	1	1	1	1	-	-	-	-
9	Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
10	Water	100	100	100	100	100	100	100	100

* All quantities are expressed as % w/v

Determination of pH:

2.5 grams of gel was accurately weighed and dispersed in 25 ml of distilled water[15]. The pH of dispersion was measured by using digital pH meter (Systronics µ pH system 362).

Homogeneity:

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate[16].

Spreadability:

It was determined by wooden block and glass slide apparatus**.** For the determination of spreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 minutes[15]. Weight (50 g) was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate till detachment of both plates was taken as measure of spreadability[17]. Spreadability was calculated by using the formula:

$$S = ML/T \quad \text{..... (Eq 1)}$$

Where,

S = Spreadability

M = Weight tied to upper slide

L = Length moved on the glass slide

T = Time taken to separate the slide completely from each other

Viscosity measurement:

Viscosity of the gels was determined using a Brookfield viscometer, (Brookfield DV-II + Pro viscometer) by using small sample adapter having spindle number SC4-18/13R. The gel was subjected to a torque ranging from 10 to 100 %. The viscosity was obtained with the 'Rheocal' software[17].

Investigation of physicochemical compatibility of MSM and excipients:

To study the physicochemical compatibility between MSM, polymers and other excipients used in the gels, infrared (IR) spectra were recorded using an FTIR spectrophotometer (8400 S Shimadzu, Japan) by the KBr pellet method and spectra were recorded in the wavelength region between 3600-450 cm^{-1}. The spectra obtained for MSM and physical mixtures of MSM with polymers and other excipients were compared.

Drug content:

The MSM gel containing 50 mg of MSM per gram of gel was dissolved in 50 ml of methanol. The volumetric flask containing gel solution was shaken for 2 hours on mechanical shaker. This solution was filtered through a 0.45 µm membrane filter and drug content was analyzed using a gas chromatography mass spectroscopy (GCMS-QP 2010, Shimadzu, Japan)[18].

Drug release study:

Drug release study was conducted using Keshary-Chein diffusion cells. The receptor compartment was filled with 50 ml of phosphate buffer having pH 7.4 as diffusion media. Cellophane membrane, previously soaked in phosphate buffer (pH 7.4) for 45 minutes, was mounted on the donor compartment with the help of an adhesive. The MSM gel (1 g containing 50 mg of MSM) was placed atop the semipermeable membrane. Magnetic stirrer was set at 50 rpm and whole assembly was maintained at 37 ± 2 °C. The amount of drug released was determined by withdrawing 0.5 ml of sample at regular time interval for 8 hours. The volume withdrawn was replaced with equal volume of fresh buffer solution[17]. Samples were analyzed for drug content using a GCMS.

Anti-inflammatory activity:

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Seth Govind Raghunath Sable College of Pharmacy, Saswad (Pune, India) (Protocol No. SGRS/IAEC/22/2008-09). The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in the male Wistar strain rats, weighing between 150-200 g. The animals were housed in standard isolation cages (45× 35 × 25 cm) under environmentally controlled conditions with a 12-hours light/12-hours dark cycle. Rats were allowed to access water freely in standard laboratory rat chow (Hindustan Lever Pvt. Ltd, Mumbai).

Animals were divided into 10 groups comprising 6 animals in each group as follows:

1. Group I: For control
2. Group II: For Marketed formulation (MF) (Diclofenac gel)
3. Group III: For Hydrogel containing MSM in carbopol 940 gel base (F1).
4. Group IV: For Hydrogel containing MSM and aloe vera in carbopol 940 gel base (F2).
5. Group V: For Hydrogel containing MSM and sesame oil in carbopol 940 gel base (F3).
6. Group VI: For Hydrogel containing MSM, aloe vera and sesame oil in carbopol 940 gel base (F4).
7. Group VII: For Hydrogel containing MSM in NaCMC gel base (F5).
8. Group VIII: For Hydrogel containing MSM and aloe vera in NaCMC gel base (F6).
9. Group IX: For Hydrogel containing MSM and sesame oil in NaCMC gel base (F7).
10. Group X: For Hydrogel containing MSM, aloe vera and sesame oil in NaCMC gel base (F8).

The change in edema volume of the rat's hind paw was measured as described by Winter et al.[19], using a plethysmometer connected to a pressure transducer with a digital transducer indicator (UGO BASILE 7140). The readings of indicator were calibrated in terms of volume displaced in milliliters using a fiber probe which was marked as 0.5 to 4 g. The MSM gel was applied to the plantar surface of the left hind paw by gently rubbing 50 times with index figure. Rats of control group received only the gel base without drug by the same mode of application[20]. Three hours after the dose, 0.1 ml of a 1% carrageenan solution in normal saline was injected subplantarly into the treated paw and the volume of the paw was immediately measured. The volume of the paw was measured again three hours after the carrageenan injection. The percent swelling of the paw was determined using the following formula:

$$\% \text{ Swelling} = \frac{V - Vi}{Vi} \times 100 \quad \text{..... (Eq 2)}$$

Where V is the paw volume 3 hours after the carregeenan injection and V_i is the initial paw volume. The average paw swelling in the group of the drug treated rats was compared with that of the control rats and the percent inhibition of the edema formation was determined using the following formula[21]:

$$\% \text{ Inhibition} = \frac{1 - VT}{Vc} \times 100 \quad \text{..... (Eq 3)}$$

Where, VT = Mean inflammation of test group.

VC = Mean inflammation of control group.

Skin irritation test:

New Zealand albino rabbits, weighing 2.5-3.0 kg, were given a standard pellet diet and were provided with water *ad libitum*. All the animals were healthy and free from dermal abnormalities. The procedure involving animals were reviewed and approved by the Institutional Animal Ethics Committee of Government College of Pharmacy, Karad (Protocol No: GCOPK/IAEC/2008-09/19).

The hair on the dorsal side of Wistar albino rabbits were removed by clipping one day before this portion of the experiment[22]. The rabbits were divided into 3 groups (n=6). Group I served as the control, group II received transdermal gel (optimized formulation) and group III received a 0.8% v/v aqueous solution of formalin as a standard irritant[23]. A new gel, or new formalin solution, was applied daily for 7 days. Finally, the application sites were graded according to visual scoring scale[24].

The skin irritation test was performed on white rabbits, by applying 1 g gel formulation in 9 cm^2 cotton wool or 1 ml 0.8% formalin soaked in 9 cm^2 cotton wool. The cotton wool was secured firmly in the back of the rabbit with adhesive plaster. The animal was observed for 7 days for any sign of edema and erythema[15].

Stability studies:

The stability of the optimized formulation was investigated as per ICH guidelines by storing the gel at a temperature of 40 ± 2^0C and 75 ± 5% RH for 3 months. The optimized formulation was analyzed for the change in pH, spreadability and drug content by procedure stated earlier[25].

***In-vivo* study:**

a. Study Design:

The present study was conducted at Gosavi Hospital, Koregoan, Satara, India. *The selection of patients was based on following inclusion and exclusion criteria.*

b. Inclusion Criteria:

1. Patients of either sex aged more than 45-50 years.
2. Patients diagnosed with primary osteoarthritis with Lequesne's score in range of 10-18.
3. Patients who can understand the study procedure so that they can come for the regular follow up[3].

c. Exclusion Criteria:

1. Patients with arthritis due to other cause like rheumatoid arthritis and gout.
2. Patients who had any other serious medical illness.
3. Patients who had received the study medication in the past month and participated in any of the clinical trials in past month[3].

Twelve patients were enrolled in the study based on the inclusion and exclusion criteria. The procedure involving patients were reviewed and approved by the Institutional Ethics Committee, Satara College of Pharmacy Satara, India (Protocol No. SCOP/IEC/2008-09/8). Patients were divided into two groups comprising six patients in each group. Group A received optimized formulation where as Group B received placebo. The optimized gel and placebo gel were prepared and packed in appropriate container and given to the patients for application. Patients were instructed to apply approximately 1.5 g of gel to the knee every 8h, and massage it firmly and rapidly into the skin until it disappeared rapidly. All the patients were instructed not to take any analgesics except paracetamol (only if needed) during the study period[3]. Patients were provided with 500 mg paracetamol as rescue medication in poorly controlled pain.

Lequesne's index is computed as described previously[26]. Lequesne's index at 4^{th}, 8^{th} and 12^{th} week was compared with baseline values, in order to study pain score in osteoarthritic patients.

Statistical treatment:

The statistical analysis was performed using 'Graphpad Instat 3.01' software. Values were expressed as mean ± SD. Statistical analysis of skin irritation test was performed using one-way ANOVA followed by Dunnett's multiple comparison tests. Lequesne's index taken at 4^{th}, 8^{th} and 12^{th} week was compared with baseline value by paired *t*-test. The *p* value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

pH and homogeneity:

The pH value of all developed formulations (F1-F8) was in the range of 6.2-7.0, which is compatible to normal pH range of the skin (shown in Table 2). All developed gels (F1-F8) showed good homogeneity with absence of lumps (Table 2).

Table 2: Values of evaluation parameters of developed gel

Formulations	pH	Homogeneity	Spreadability (g.cm/sec)	Viscosity (cps)	Drug Content (%) (mean± SD)
F1	6.9	Good	22.06	9826	98.92 ± 3.32
F2	6.2	Good	23.08	9632	98.53 ± 3.50
F3	6.5	Good	25.86	9142	101.30 ± 3.29
F4	7	Good	27.27	8951	99.95 ± 2.86
F5	6.5	Good	18.07	10132	98.82 ± 3.43
F6	6.6	Good	18.75	10032	99.02 ± 3.54
F7	6.5	Good	20.55	9989	98.92 ± 5.44
F8	6.3	Good	21.39	9821	101.46 ± 3.12

Spreadability:

The value of spreadability indicates that the gel is easily spreadable by small amount of shear. Spreadability of NaCMC based gels was less than that of CP based gels, indicating that spreadability of gel containing CP was good as compared to gel containing NaCMC (Table 2). This may be attributed to the soft and less viscous nature of CP gels. The presence of ethanol also adds to the spreadability due to dehydrating effect on polymer[27]. CP based F4 formulation showed the highest spreadability.

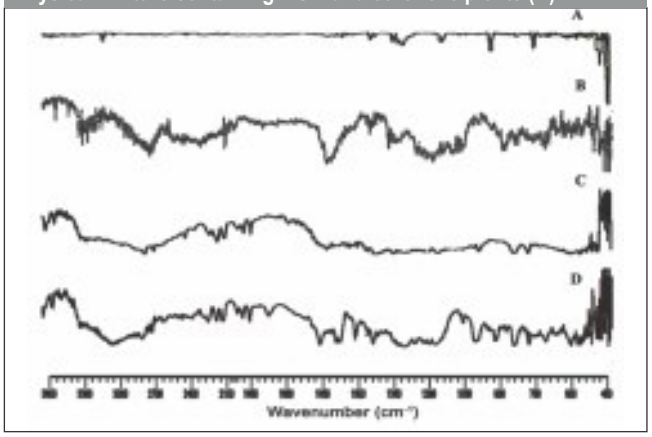
Viscosity measurement:

The viscosity of various MSM gels was measured using a Brookfield viscometer and are given in Table 3. The rheological behaviour of all formulated gels systems was studied. Viscosity of various formulated MSM gels were found to be in the range of 8000 to 11000 centipoises (shown in Table 2). NaCMC gels showed high viscosity than the CP gels due to their high concentration and slightly rigid nature.

Investigation of Physicochemical Compatibility of Drug and Polymer

The IR spectral analysis of MSM alone showed that the principle peaks were observed at wave-number 930 cm^{-1} (S=O st), 1170 cm^{-1} (S=O st, sy) and 1320 cm^{-1} (S=O st, as), confirming the purity of drug (Fig. 1). In the IR spectra of physical mixtures of MSM, polymers and excipients, the characteristic peaks of MSM were observed indicating the compatibility of MSM with polymer and other excipients**.** However, some additional peaks were observed with the physical mixtures, possibly because of presence of polymers.

Fig. 1: IR spectra of MSM (A), MSM + CP (B), MSM + NaCMC (C), Physical mixture containing MSM and other excipients (D).



Drug content:

The percentage drug content of all prepared transdermal formulations were found to be in the range of 98-102%. The percentage drug content of formulations confirmed that the drug contents were in specified limits (shown in Table 2).

In vitro drug release studies:

Fig. 3 shows the cumulative amount of MSM released versus time profile for all eight formulations. In all the formulations the drug was released immediately with about 19-25% of the drug release in first hour of the study and thereafter the drug release continued gradually. All the formulations showed sustained release effect. CP based formulations (F1, F2, F3 and F4) containing 1% w/v of CP showed the drug release of 89.7%, 95.17%, 97.57% and 98.4% after eight hours. However, NaCMC based formulations (F5, F6, F7 and F8) showed more sustained release which might be due to higher

Fig. 2: DSC thermograms of MSM (A), Physical mixture containing MSM and other excipients (B).

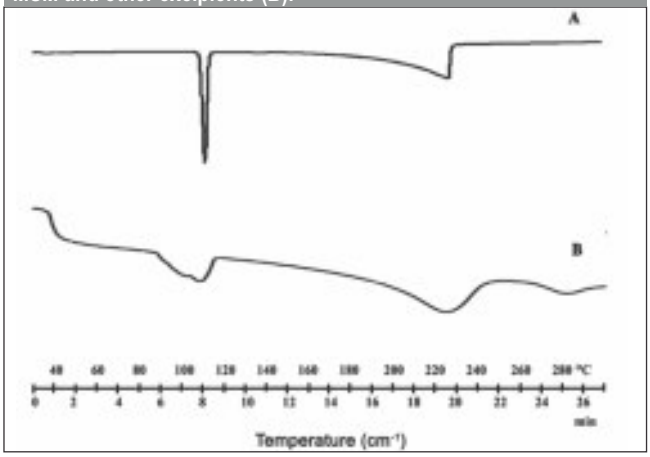
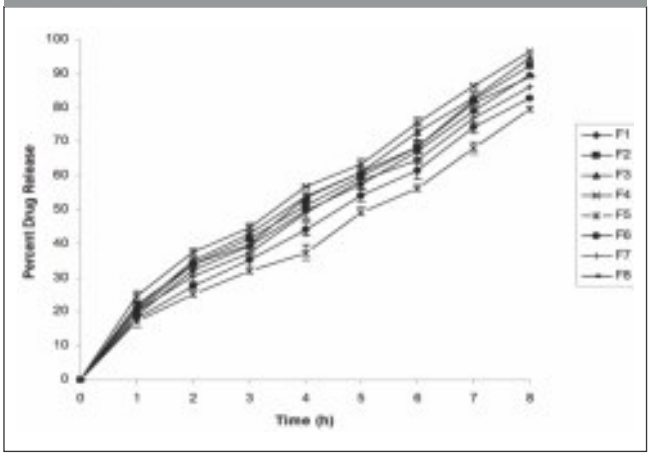


Fig. 3: Drug release profile of formulation F1-F8.



concentration of NaCMC (5% w/v) in these formulations increasing their viscosity. F5 showed the least drug release (79.59%), while F8 showed 89.17% drug release. An increase in the release rate was observed in the CP based gels and the NaCMC based gels in the order F1<F2<F3<F4 and F5<F6<F7<F8 respectively. The incorporation of aloe vera and sesame oil decreased the viscosity of the hydrogels which may be responsible for increase in the release rate of the respective hydrogels. Also aloe vera and sesame oil have been reported as penetration enhancing agents[28, 29]. The initial fast release of MSM from the prepared system could be explained by the fact that these systems were formulated in aqueous vehicle. The matrix formed was already hydrated and therefore hydration and water permeation would no longer limit the drug release. As the polymer concentration increases, it leads to decrease in drug release. The release of MSM from all the formulations (F1-F8) followed zero order kinetics ($r^2 > 0.988$)

Anti-inflammatory activity:

Hydrogels prepared from MSM were quite stable. Due to the presence of MSM, aloe vera, and sesame oil, anti-inflammatory effect of hydrogels have been observed just in three hours after carrageenan injection. CP based formulations showed more prominent anti-inflammatory activity than NaCMC based formulations. This may be because of presence of ethanol as penetration enhancer. This can also be attributed to the presence of 1% carbopol as polymer in formulation F1-F4, which was less viscous as compared to the NaCMC. The percent inhibitions of carrageenan induced edema formation by 5% MSM gels in carbopol 940 and in NaCMC along with the marketed formulation are shown in Table 3.

The anti-inflammatory activity of the hydrogels containing 5% MSM in carbopol 940 and NaCMC was compared with that of NSAID topical gel preparation (marketed Diclofenac gel). 5% MSM gel in a NaCMC based F5 formulation showed considerably less inhibition of edema formation (54.40%) whereas CP based F4 formulation showed maximum inhibition (81.55%) which was closer to that of the marketed formulation (87.83%). The lower anti-inflammatory activity of the NaCMC based gels could be attributed to a slower release of drug from the NaCMC due to its high viscosity and high polymer concentration.

Table 3: Percent inhibition of edema by CP based and NaCMC based gel formulations

Sr.No	Formulation	Dose (mg/paw)	No. of Rats	% Swelling	% Inhibition
1	Control	0	6	30.09	-
2	F1	50	6	9.25	69.25
3	F2	50	6	8.49	71.78
4	F3	50	6	6.48	78.46
5	F4	50	6	5.55	81.55
6	F5	50	6	13.72	54.40
7	F6	50	6	12.62	58.05
8	F7	50	6	11.53	61.68
9	F8	50	6	8.33	72.31
10	MF	50	6	3.66	87.83

MF = Marketed formulation

In case of CP and NaCMC based hydrogels, it was observed that the combination of MSM with sesame oil showed more anti-inflammatory activity than its combination with aloe vera gel and its plain formulation. Aloe vera gel and sesame oil were incorporated in to the MSM gel formulations because of their anti-inflammatory properties, however both these agents are also known for their permeation enhancing effect. It has been found that sesame oil, being thick and viscous, can increase the occlusive nature of the gels further leading to increased hydration and permeability. Besides average fraction of proteins and globulins, sesame oil also contains linoleic acid and oleic acid, due to which it causes enhancement by polar and nonpolar pathway[29]. On other hand, aloe vera enhances the permeation by 'pull effect' which is favorable for only those drugs having high molecular weight. MSM has low molecular weight (MW-94.13) and hence aloe vera would not affect the permeability of MSM prominently. The minimal increase observed in the anti-inflammatory activity on combining MSM with aloe vera might be only due to anti-inflammatory action of aloe vera. Thus, formulations containing combination of MSM with sesame oil showed more anti-inflammatory activity due to dual effect of sesame oil i.e. anti-inflammatory and permeation enhancing effect. The percent inhibition observed with the F4 formulation, containing MSM in combination with sesame oil and aloe vera, was nearly the maximum response considered possible for a topical anti-inflammatory drug using this method.

Considering the in vitro drug release profile, anti-inflammatory activity and other evaluation parameters of all formulations (F1- F8), formulation F4 was selected as optimized formulation as it showed 96.4% of drug release, highest anti-inflammatory activity and satisfied the other parameter such as viscosity, spreadability and pH etc.

Skin Irritation Test:

The skin irritation test of optimized formulation F4 showed a skin irritation score (erythema and edema) of less than 2 (shown in Table 4). According to Draize et al[24]., compounds producing scores of 2 or less are considered negative (no skin irritation). Hence the developed transdermal gel formulations were found to be free of skin irritation.

Stability studies:

The stability studies were carried out for optimized formulations. The F4 formulation was analyzed for pH, spreadability and drug content. Three months study revealed that there were no changes observed in homogeneity. All the

formulations showed slight changes in pH, spreadability and drug content but they were in acceptable limits (p<0.05) (shown in Table 5).

In vivo Study:

A total of 12 patients were enrolled - 6 patients were randomized to the placebo group, while 6 patients were randomized to the F4 formulation. The demographic data of the participating patient population is shown in Table 6.

In order to compare the clinical efficacy of F4 formulation and placebo, all patients were clinically evaluated to observe the effect of various efficacy parameters before and at the end of 4, 8 and 12 weeks of therapy. The baseline characteristics of patients in all groups were similar, and there was no difference in the baseline efficacy parameters among all groups. Administration of F4 formulation significantly improved the efficacy parameters compared with placebo.

In present investigation, Lesquesne's index was used to compare the efficacy of F4 formulation with placebo by evaluating the functional and day-to-day activity of the knee joint in osteoarthritis. At baseline both the groups had similar indices suggesting a comparable degree of osteoarthritis. For F4 formulation improvement in functional status based on Lesquesne's index was observed, index being decreased from 13.08 ± 3.21 at baseline to 8.75 ± 2.5 at the end of twelve weeks (p<0.05) (Table 7). However**,** there was no significant decrease found in Lesquesne's index (p>0.05) in case of placebo (Fig. 4).

The study revealed that CP based gel containing MSM in combination with aloe vera and sesame oil has worked very well in osteoarthritic patients. Lequesne's index is a scoring system based on functional mobility of the joint assessing patient's daily activities and hence is a direct evidence of the extent of the disability. For F4 formulation, Lesquesne's score was significantly reduced (p<0.05), demonstrating that patient's symptoms improved very well with this drug. Therefore, the CP based MSM gel containing aloe vera and

Table 4: Skin irritation scores following transdermal gel administration.

	Control		F4		Formalin	
Rat No.	E*	E1†	E	E1	E	E1
1	0	0	0	0	2	1
2	0	0	1	1	3	1
3	0	0	0	0	2	2
4	0	0	1	0	3	2
5	0	0	1	0	2	3
6	0	0	0	1	2	3
Mean ±SEM	0	0	0.5 ± 0.5477‡	0.33± 0.5163‡	2.33± 0.5163	2± 0.8944

Where *E = Erythema, †E1 = Edema

Erythema scales: 0 none; 1 slight; 2 well defined; 3 moderate; and 4 scar formation.

Edema scale: 0 none; 1 slight; 2 well defined; 3 moderate; and 4 severe.

‡Significant compared with formalin (p<0.05)

Table 5: Stability study of optimized gel formulation.

Sr. No	Formulation	Months	pH	Spreadability (g.cm/sec)	Drug content % (Mean±S.D)
1	F4	0	7	27.27	99.72 ± 0.1934
		1	7	26.71	99.23 ± 0.0458*
		2	6.9	25.80	99.04 ± 0.0680*
		3	6.8	25.03	98.99 ± 0.0152*

* P<0.05 drug content values for zero months Vs 1st, 2 nd and 3rd months

Table 6: Demographic data of patients

Sr. No.	Parameter	F4 [3F*:3M†]	Placebo [4F:2M]
1	Age (yr)	59.16 ± 10.28	65.66 ± 6.65
2	Height (cm)	167 ± 2	170 ± 3
3	Weight (kg)	69 ± 8	65 ± 2
4	Duration of osteoarthritis (yr)	5.83 ± 3.25	5.66 ± 2.06

Where *F= female; †M= male; n=6; the ratio of male to female patients along with the mean duration of illness is provided. Values are given as mean ± SD.

Fig. 4: The effect of two treatments (Formulation F4 and placebo) on the Lequesne's index.

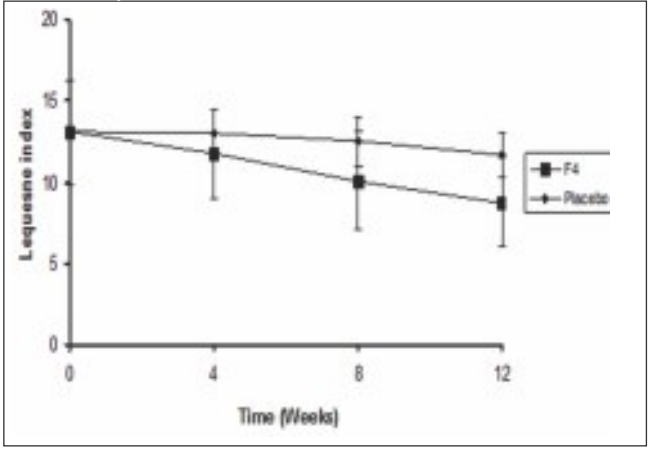


Table 7: Depicting the functional score at different visit.

Sr. no.	Formulations	Baseline (Mean±S.D)	4th week (Mean±S.D)	8th week (Mean±S.D)	12th week (Mean±S.D)
1	F4	13.08±3.21	11.75±2.71[a]	10.08±2.99[a]	8.75±2.75[a]
2	Placebo		13±1.48[b]	12.5±1.48[b]	11.66±1.32[b]

*S.D. = Standard deviation, [a]p<0.05, Baseline Lesquesne's index values for F4 Vs 4th, 8th, and 12th week., [b]p>0.05, Baseline Lesquesne's index values for Placebo Vs 4th, 8th, and 12th week. , (p<0.05 considered as significant; while p>0.05 considered as non significant)

sesame oil can be better alternative for treating the patients with osteoarthritis.

The patient population studied in this study is very limited and further studies are required on large numbers of patients so as to confirm the efficacy of these formulations of MSM.

CONCLUSION

It can be concluded from this study that carbopol based gel formulations containing combination of MSM along with the natural ingredients such as aloe vera and sesame oil can be an efficient natural alternative to the NSAID's, for the treatment of patients with osteoarthritis. However, further studies are required to confirm the results obtained in this study.

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REFERENCES

1. Pfeil J, Niethard FU. Lower leg lengthening using the Ilizarov system. Presentation of the various surgical techniques and analysis of lower leg lengthening procedures performed 1986-1989. Orthopade. 1990; 19: 263-72.
2. Mazieres B. Gonoarthroses. Rev Prat. 1996; 46: 2193-200.
3. Vidyasagar S, Mukhyaprana P, Shashikiran U, Sachidananda A, Rao S, Bairy KL. Efficacy and tolerability of glucosamine, chondroithin sulphate and methylsulfonylmethane (MSM) in osteoarthritis of knee in Indian patients. IJPT. 2004; 3: 61- 5.
4. Usha PR, Naidu MUR. Randomised, double-blind, parallel, placebo-controlled study of oral glucosamine, methylsulfonylmethane and their combination in osteoarthritis. Clin Drug Invest. 2004; 24: 353- 63.
5. da Camara CC, Dowless GV. Glucosamine sulphate for osteoarthritis. Ann Pharmacother. 1998; 32: 580-7.
6. Methylsulfonylmethane. Total Health. 1998; 21: 30-1.
7. Ronca F, Palmieri L, Panicucci P, Ronca G. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis Cart. 1998; 6 Suppl A: 14-21.
8. Smalley WE, Ray WA, Daugherty JR, Griffin MR. Nonsteroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons. Am J Epidermiol. 1995; 141: 539-45.
9. McAlindon TE, LaValley MP, Gulin JP, Felson DT. Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta-analysis. JAMA. 2000; 283:1469-75.
10. Vazquez B, Avila G, Segura D, Escalante B. Antiinflammatory activity of extracts from Aloe vera Gel. J Ethnopharmaco 1996; 55: 69-75.
11. Utsunomiya T, Shimada M, Rikimaru T et al. Antioxidant and anti-inflammatory effects of a diet supplemented with sesamin on hepatic ischemia-reperfusion injury in rats. Hepatogastroenterolgy 2003; 50: 1609-13.
12. Chien, YW. Transdermal therapeutic systems. In: Robinson JR Lee VH editors. Controlled Drug Delivery Fundamental and Applications. 2nd ed. New York: Marcel Dekker; 1987. p. 524-52.
13. Kanikkannan N, Andega S, Burton S, Babu RJ, Singh M. Formulation and in vitro evaluation of transdermal patches of melatonin. Drug Dev Ind Pharm. 2004; 30: 205- 12.
14. Gupta M, Verma PRP, Marwaha RK, Faruk A, Singh G. Formulation and evaluation of meloxicam gel. J Pharm Res. 2008; 7: 27-31.
15. Bidkar S, Jain D, Padsalg A, Patel K, Mokale V. Formulation, development and evaluation of fluconazole gel in various polymer bases. Asian J Pharm. 2007; 1: 63-8.
16. Shivhare UD, Jain KB, Mathur VB et al. Formulation development and evaluation of diclofenac sodium gel using water soluble polycrylamide polymer. Digest Journal of Nanomaterials and Biostructures 2009; 4: 285-90.
17. Murthy G, Sai K. Formulation and evaluation of transdermal gels of diltiazem hydrochloride. Indian J Pharm Educ Res. 2008; 42: 272- 6.
18. The official compendia of standard, United States Pharmacopoeia and National Formulary (USP31-NF26), Asian Edition. 2008. p. 978-9.
19. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962; 111: 544-7.
20. Chi SC, Jun HW. Anti-inflammatory activity of ketoprofen gel on carrageenan- induced paw edema in rats. J Pharm Sci. 1990; 79: 974-7.
21. Salunkhe VR, Yadav AV, Shete AS, Kane SR, Kulkarni AS. Anti-inflammatory activity of hydrogel of extract of Pterocarpus Marsupium and Coccinia Indica. Indian Drugs. 2005; 42: 319- 21.
22. Namdeo A, Jain NK. Liquid crystalline pharmacogel based enhanced transdermal delivery of propranolol hydrochloride. J Control Release. 2002; 82: 223- 36.
23. Mutalik S, Udupa N. Glibenclamide transdermal patches: physicochemical, pharmacodynamic, and pharmacokinetic evaluations. J Pharm Sci. 2004; 93: 1577-94.
24. Draize JH, Woodward G, Calvery HO. Methods for study of irritation and toxicity of substances applied topically to the skin and mucous membrane. J Pharmacol Exp Ther. 1994; 82: 377- 90.
25. International Conferences on Harmonization. (2003) Stability Testing of new Drug Substances and Products. Q 1 A (R2). [cited 2009 Sept 10]; Available from: URL http://www.emea.europa.eu.
26. Lequesne MG, Mery C, Samson M, Gerard P. Indexes of severity for osteoarthritis of the hip and knee. Validation-value in comparison with other assessment tests. Scand J Rheumatol. 1987; 65 Suppl 1: 85-9.
27. Contreras MD, Sanchez R. Application of a factorial design to the study of the flow behaviour, spreadability and transparency of a carbopol ETD 2020 gel. Part II. Int J Pharm. 2002; 234: 149-57.
28. Cole L, Heard C. Skin permeation enhancement potential of aloe vera and a proposed mechanism of action based upon size exclusion and pull effect. Int J Pharm. 2007; 333: 10-6.
29. Dinda SC, Vijay Ratna J. Enhancement of skin permeation of ibuprofen from ointments and gels by sesame oil, sunflower oil and oleic acid. Indian J Pharm Sci. 2006; 68: 313-6.