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DIFFERENT TOPICAL FORMULATIONS OF KETOROLAC TROMETHAMINE FOR ANTI-INFLAMMATORY APPLICATION AND CLINICAL EFFICACY

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Ketorolac tromethamine (KT) is considered as a member of NSAIDs that used in treatment of rheumatoid arthritis. The main problems associated with the frequent administration of KT orally could be overcome by alternative routes as topical application. KT was formulated in different topical formulations such as gels, emulgels and creams. Sodium carboxymethylcellulose, carbopol 934 and pluronic F127 were used as polymers. In vitro permeation study through rat skin was carried out. The effect of different KT concentrations and the effect of skin penetration enhancer on the amount of KT permeated were investigated. Anti-inflammatory activity using commercial piroxicam gel for comparison was evaluated. The effectiveness and tolerability of the selected KT gel and piroxicam gel in osteoarthritis patients was also studied. The results obtained showed that, the flux of the drug increased with increasing its initial concentration. Using sodium lauryl sulfate as enhancer resulted in an improvement of KT permeation through rat skin. All formulations had the potential for local applications of KT as anti-inflammatory drug as compared to the control group. There was no significant difference in the efficacy between the selected KT gel and piroxicam gel for osteoarthritis patients. So KT gel may be used as another therapeutic option for the treatment of patients with osteoarthritis.

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Keywords: Ketorolac, Topical delivery, $NSAID_S$, Osteoarthiritis

1. Introduction

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drug groups. These drugs are used dermally or systemically in treatment of various rheumatic diseases, including rheumatoid arthritis (RA), as well as for osteoarthritis (OA), low back pain and some joint diseases. The mechanism of action of $NSAID_S$ is reversible inhibition of the cyclooxygenase enzyme (COX) and decreasing the synthesis of prostaglandins [1]. However, these drugs lead to unfavorable effects specifically on the stomach as a result of inhibition of prostaglandins (PGs), which play a role in protection of the gastric mucosa, in systemic administration. The severity of these unfavorable side effects may range from a simple ailment like dyspepsia to peptic ulcer and gastrointestinal hemorrhage. Furthermore, the acidic character of NSAIDs may lead to local irritation and lesions on the gastrointestinal mucosa. Therefore, some NSAIDs are administered percutaneously and transdermally to achieve local or systemic effect as

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an alternative to oral and parenteral administration [2,3]. Several formulation approaches for cutaneous administration of NSAIDs have been employed. The conventional pharmaceutical forms particularly used for dermal administration to achieve local effect are gels, creams and ointments [4]. Furthermore, studies on novel drug delivery systems are available for transdermal administration of NSAIDs. These new approaches include liquid crystals, nano/micro emulsions, liposomes, solid lipid particles and patches. These systems are used to enhance cutaneous passage of drugs into systemic circulation and to target different layers of the skin [5-8]. Different approaches have been performed to enhance cutaneous passage of drugs with the objective of overcoming the low skin permeability [8,9]. The most frequently used approach is to include penetration enhancers in formulations. In addition to penetration enhancers, there are studies available in which physical methods such as iontophoresis is used in improving of skin delivery of drugs [4,10-11]. Musculoskeletal conditions range from intra articular disorders such as rheumatoid arthritis and osteoarthritis, injuries that involve simple ligaments such as sprains and extra articular joint disorders such as fibromyalgia and myofascial pain [12]. Osteoarthritis (OA) is generally thought of as a disorder of middle–aged and older people. Commonly affected sites include: hip, knee and spine [13]. Typical symptoms include: joint pain in and around the joint site, morning stiffness lasting up to 30 minutes, loss of function, immobility and joint instability. Onset of symptoms is insidious. Pain is generally worse during motion and can be alleviated by rest. Patients with OA of the knee may complain of alterations to the gait and often experience a variety of forms of pain varying from a sharp pain to a dull constant ache [13].

Ketorolac tromethamine, KT (Fig. 1) is a non-steroidal anti-inflammatory drug with molecular formula, $C_{15}H_{12}NO_3.C_4H_{12}NO_3$ [14]. It is one of the most potent NSAIDs. Its analgesic and anti-inflammatory actions derive from acommon mechanism which is the inhibition of the cyclooxygenase, however, additional mechanism of action has been proposed, including a participation of opioid receptors although it does not bind to these receptors [15]. It is indicated to treat moderate to severe painand it has been studied in a broad spectrum of pain states such as postpartum and postoperative arthritic pain, pain of trauma, severe dental pain, renal, biliary colic, cancer, abdominal and gynecological pain [16].

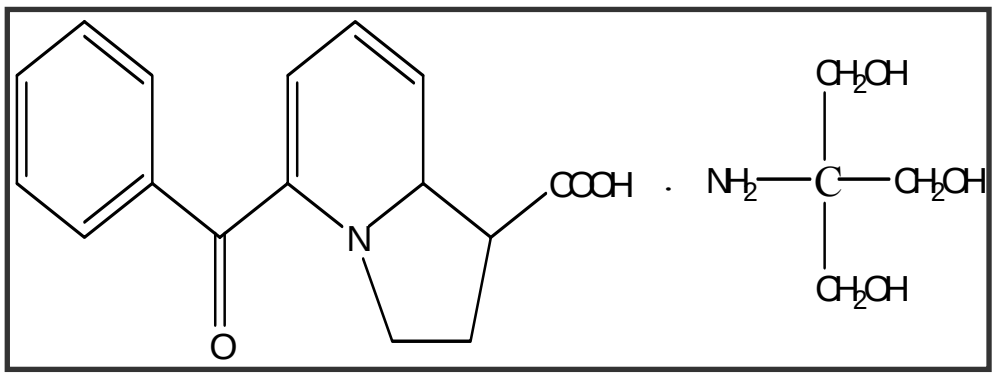

Fig.1.Chemical structure of ketorolac tromethamine [14].

The objectives of this work were the formulation of KT in some topical formulations (gels, emulgels and creams). In vitro skin permeation through rat skin and the stability tests were performed. The anti-inflammatory activity of KT topical formulations using paw edema was carried out. Moreover, the comparative assessment of the effectiveness and tolerability of the selected KT gel and commercial piroxicam gel in osteoarthritis patients were studied.

2. Experimental

Materials

Ketorolac tromethamine was kindly supplied from Amriya pharm. Ind., Co., Alex., Egypt. Pluronic F127 (PF127), sodium hydroxide, and standard cellophane membranes; molecular cut of 12000 (Sigma Chem. Co., U S A). Whitepetrolatum, polysorbate 80 (tween 80), stearyl alcohol, glycerol, liquid paraffin and sodiumcarboxymethyl cellulose, NaCMC (El-Nasr Pharm. Chem. Co., Cairo, Egypt). Carbopol 934 (C.P. Evans Co., England). Sodium lauryl sulfate, SLS (Honil Ltd., Co., England). Inflacam gel (0.5 % w/w piroxicam) supplied from Mepaco Pharm. Ind., Co., Egypt. All other chemicals were of analytical grade and were used as received.

Methods

Preparation of KT in different topical formulations

Preparation of (0.5 % w/w) KT gel bases

The composition of different KT formulations is shown in (Table 1). The required quantity of the hydrophilic cellulose derivative (NaCMC) was wetted with distilled water in which KT was previously dissolved and homogenized using magnetic stirring for 24 hr until a clear gel was formed. Using carbopol gel, the required amount of carbopol was dispersed in distilled water in which KT was previously dissolved and homogenized by magnetic stirring for 30 min and left to equilibrate for 24 hr. After this period, pH was adjusted with sodium hydroxide [17]. For pluronic F127 gel base, the required amount of PF127 was placed in a conical flask and dispersed in 10 ml cold distilled water (5-10 ^{o}C) under constant agitation with a magnetic stirrer. Upon complete dissolution of the PF127, an appropriate amount of the KT was added to the flask. The dispersion was thoroughly mixed with magnetic stirring while cold and then left overnight in refrigerator to complete dissolution. The gel was formed when the solution was brought back to room temperature [17].

Table 1. Composition of different KT topical formulations.

Formula	Composition (%w/w)	
NaCMC (gel)	KT	0.5
	NaCMC	3.0
	Water to	100
Carbopol 934 (gel)	KT	0.5
	Carbopol 934	0.5
	Water to	100
PF127 (gel)	KT	0.5
	PF127	20
	Water to	100
Emulgel	KT	0.5
	Carbopol 934	0.5
	Liquid paraffin	20
	Tween 80	5.0
	Water to	100
cream (O/W)	KT	0.5
	White petrolatum	25
	Stearyl alcohol	10
	Tween 80	5.0
	Glycerin	12
	Water to	100

Preparation of (0.5% w/w) KT emulgel base

Emulgel formula was prepared by a method of three steps, (i) polymer dispersion in water, (ii) neutralization of the polymeric aqueous dispersion, and (iii) emulsification of the oil phase. The polymer was suspended in distilled water in which KT was previously dissolved. The resulting slurry was neutralized by the addition of sodium hydroxide solution. In order to obtain a complete polymer hydration, the gelwas stored at 4 ^{o}C for 24 hr before the addition of the oil phase which was slowly added to the water phase. The addition was performed under stirring at 900 rpm and 80 ^{o}C, then cooling to room temperature [18].

Preparation of (0.5% w/w) KT cream base

Cream (O/W) was prepared by placing all the aqueous-phase (in which KT was previously dissolved) and oil-phase ingredients into separate beakers and heated to 70 ^{o}C. Cream (O/W) was prepared by the addition of the oil-phase to the aqueous phase and stirred until cooling [19].

In vitro skin permeation

Hairless rats (male, Swiss albino, weighing 80-125 g) were sacrificed by an overdose of halothane anesthesia. The animal study protocol was reviewed and approved by the Ethics Committee at the Faculty of Pharmacy of our University.The skin from the dorsal surface excised, and the adherent fat and subcutaneous tissue were removed. The skin was mounted on one end of the dialysis tubeswith the dermalside in direct contact with the receptor medium [20]. One gram of KT formulationswas placed on diffusional surface area of 6 cm^{2}of hairless rate skin previously moistened with the receptor medium. The dialysis tubeswere then immersed in a 250 ml beaker containing 100 ml of the release media (phosphate buffer of pH 6.8). The experiment was performed in a thermostatically water bath (Gesellschaftlabor technique M .B. H. & GF, Germany) at 37 ± 0.5 ^{o}C and at 50 rpm. An aliquot of 5 ml was withdrawn at the different time intervals and replaced by an equal volume of the release medium maintained at the same temperature. The amount of KT released at each time interval was determined spectrophotometrically at λ_{max} 322 nm against blank similarly treated.Study was carried out in triplicate and the mean results were calculated and plotted against the time.

Anti-inflammatory activity of KT topical formulations

Paw edema test

Anti-inflammatory activityevaluation of KT topical formulations was carried out for all formulations mentioned under skin permeation study containing 0.5 % (w/w) KT and with 1.0 % (w/w) of sodium lauryl sulphate (SLS) in carbopol and NaCMC gels. These formulations were three gel bases (NaCMC, carbopol 934 and PF127), one emulgel base, one cream base and commercial piroxicam gel (inflacam gel). The experiment was conducted on 35 male Swissalbino rats weighing80-125 g divided into 7 groups. Paw edema was induced using the method of Winter et al, 1962 through subcutaneous injection of 100 µl of 1 % (w/v) carrageenan in salinein the planter surface of the rat hind paw [21]. Thirty minutes later, the KT formulationswere applied topically on the edematous paw. The rats were fasted 16 hr before the experiment with free accessto water. The thickness of the paw edema was measured by using a micrometerat time 0 and after injection of carrageenan. Subsequent measurements were carried out at 1, 2, 3, 4, and 5 hrs after induction of edema. The anti-inflammatory effect of the drug was expressed as the percentage inhibition of edema thickness and compared with the commercial piroxicam gel according to following equation:

$$\% \text{ Inhibition of edema thickness} = 1 - (T_t / T_0) \times 100 \quad (1)$$

Where T_0 is the edema thickness at zero time and T_t is the edema thickness at different time interval [22].

Also, the swelling (%) of rat hind paw edema induced by carrageenan was calculated from the following equation:

$$\% \text{ Swelling} = (T_i / T_b - 1) \times 100 \quad (2)$$

Where T_i is the paw thickness after carrageenan injection and T_b is the thickness before carrageenan injection [23].

Seven groups were used in this study. The groups from the first to the sixth, were treated with one gram of; NaCMC gel base, carbopol 934 gel base, PF127 gel base, emulgel base, cream (O/W) base and commercial piroxicam gel (inflacam gel), respectively. The last group (seventh group) was considered as a control group which didn't receive any medication.

Skin irritation study

Various drugs when applied topically might elicit primary skin irritation. This irritation might vary with the ability of the agent to cross the stratum cornea barrier and subsequently interact with viable cells of the epidermis and dermis [24]. Skin irritation study was carried out in rats which had free access to standard food and tap water. The protocols, are in accordance to the guidelines of the "Principles of Laboratory Animal Care" were approved by the Committee of Animal Care of our University. Two grams of carbopol 934 gel base, (0.5 % KT, 0.5 % carbopol, 1 % SLS) which gave the highest in vitro permeation rate and the highest % edema inhibitionwere applied onto the shaved dorsal skin of four rats and occluded with gauze and bandage. Twenty four hours later, the formula was removed and the score of erythematic symptoms was visually evaluated as follows: 1 mild, 2 moderate and 3 sever erythema [25].

Comparative assessment of the effectiveness and tolerability in osteoarthritis patients

Patients and methods

The double-blind, randomized, placebo-controlled trial involved a total of 62 patients were randomly assigned to receive treatment with 1 of 3 gels: selected KT gel (0.5 % w /w KT, 0.5 % w/w carbopol and 1 % w/w SLS), piroxicam (inflacam) gel and placebo gel (base without drug). The three studied gel formulations were identical clear, colorless gel; the labels differed only in patient identification number. The baseline demographic and clinical characteristics of patients with osteoarthritis (OA) were studied.

Efficacy assessments

The primary efficacy measure was the mean change from baseline to week 4 in the Western Ontario and McMaster Universities (WOMAC) OA index pain subscale scores based on change in pain in the index joint of the most severely affected knee, which was identified at the screening visit [26]. Secondary efficacy measures included changes in stiffness and physical function WOMAC subscale scores and WOMAC composite index (WOMAC CI) scores and changes in pain intensity, pain relief and pain interference were measured using a 5-point Likert scale (4=worst pain, 3=average pain, 2=least pain, and 1=pain right now) scores [27]. The WOMAC osteoarthritis index is a validated, multidimensional questionnaire of defined reliability, content, construct validity and responsiveness. It consists of 24 questions (5 regarding pain, 2 regardingstiffness and 17 regarding physical functions) each scored on a 5-point Likert scale (0-representing none). Patients were advised to apply gel twice daily. Patients were monitored for WOMAC OA index scores at each visit (0, 1, 2 and 4 weeks). At each visit, a joint examination was performed. Weight of patients, blood pressure, temperature, pulse rate and respiratory ratewere measured. The investigator recorded his impression of therapeutic response and the patient recorded his or her assessment of the medication as an analgesic for the study knee joint (both assessments rated on a 0-4 scale) to evaluate pain, stiffness, and physical functions.

Tolerability assessments

Tolerability was assessed by recording adverse effects (AEs) which were categorized by severity (mild, moderate, or severe) and relatedness to treatment (unlikely, possibly, or probably). Erythema, edema and the presence of papules or vesicles were assessed using dermal analysis.

Stability studies

Samples of the prepared formulations were stored in stoppered glass containers for 8 months at room temperature (25^{o}C ± 0.5) and at 4 ^{o}C. The formulations were evaluated at 0, 1, 2, 4, 6 and 8 months after preparation. Physico-chemical evaluation of the formulations was carried out by visual inspection (color change, odor, phase change, texture, spreadability, extrudability and particulate contamination), pH measurement and spectrophotometric analysis of the drug content. Drug content was determined by dissolving an accurately weighed, one gramin 100 ml distilled water. Ten ml was quantitatively transferred to volumetric flask (50 ml) and appropriate dilutions were made with distilled water. The resulting solution was then filtered using 0.45 µm membrane filters before subjecting the solution of the drug to UV spectrophotometric (Shimadzu, UV-150-02, Seisakusho, Ltd., Kyoto, Japan) analysis at λ_{max} 322 nm. pH was measured for each formulation directly after preparation using pH meter (Tenway Ltd., Felsted, Dunmow, Essex, M63LB, U K)which was calibrated before use with standard buffered solution, comparing the pH of the formulations containing KT with that of the placebo. The procedure was carried out on each formulation in triplicate.

Statistical analysis

All results were expressed as mean values (±SD) and a probability value less than 0.05 ($p < 0.05$) was considered to be a significant value. Statistical analysis was meant to include analysis of co-variance (ANOVA) and post hoc tests, using change from baseline to week 4 as the response and baseline value as a covariate.

3. Results and Discussion

KTcontent of the prepared formulations was determined and it was ranged from 94.6 % to 99.6 % of claimed amount of KT. pHs of the KT formulations were from 6.12-7.24.The viscositiesof the different KT formulations were determined using a Brookfield digital viscometer DV- II (Stoughton, USA) with Spindle 94 at 20 rpm and at 25 ± 0.1 ^{o}C and ranged from 10.5 ± 0.16 to 28.0 ± 0.12 p.

In vitro permeation through rat skin

The permeation rate of KT from the donor compartment through the rat skin into the receptor compartment is determined by measuring the amount permeated as a function of time. The cumulative drug permeated (µg.cm^{-2}) was plotted against time and the steady state, flux (J) (µg.cm^{-2}.hr^{-1}) was calculated from the slope of the linear portion of the curve according to Fick's first law of diffusion [28]:

$$J= (D_mK/L) . C_v \quad (3)$$

Where D_m is the diffusion coefficient of the drug in the membrane, K thepartitioncoefficient of the drugbetween the vehicle and the membrane, L the diffusion path length in the membrane and C_v applied concentration in the donor compartment.

The permeability coefficient (P) was calculated from the steady-state flux and the applied concentration in the donor compartment (C_v) as follows [28]:

$$P= J/C_v \quad (4)$$

The diffusion coefficient was calculated from the slope obtained by plotting the cumulative amount of permeated drug (µg/cm^2) versus square root of time according to Higuchi diffusion equation [29]:

$$D= (\text{slope}/ 2 C_v)^2 \Pi \quad (5)$$

Cumulative amount permeated of KT (µg/cm^2) from the different formulations is shown in (Table 2 and Fig. 2). There is no doubt that, the release of a drug from a topical pharmaceutical

preparation can be effectively influenced by the vehicle in which it is applied. Percutaneous permeation parameters of different KT formulations through hairless rat skin are shown in (Table 3). The obtained results showed that carbopol and NaCMC gel bases exhibited higher KT permeation rate than poloxamer gel base. Permeabilitycoefficient (P) of KT from carbopol and NaCMC gel bases was 33.16 ± 0.032 cm/hr and 27.60 ± 0.020 cm/hr, respectively while it was 13.82 ± 0.012 cm/hr from PF127 gel base.The increase of the permeation rate was due to the higher the diffusion coefficient (D) 2.826 ± 0.005 $cm^2.hr^{-1}$ and 1.884 ± 0.005 $cm^2.hr^{-1}$ for carbopol and NaCMC bases, respectively and the lower the diffusion coefficient, 0.493 ± 0.002 $cm^2.hr^{-1}$ of PF127 gel base (Table 3). So it was concluded that the diffusion of KT through the skin barrier is the rate limiting step in permeation of KT topical therapy.Concerning the release of KT from PF127 gel base, it was observed that, its lower release rate may be due to the reduction in the numbers and dimensions of the aqueous channels through which solute diffuses. In addition, PF127 gel is believed to form a gel that acts as a depot for continuous and gradually percutaneous absorption of drugs [30].

Table 2.Cumulative amount permeated of KT (µg/cm^2 ± SD) from different topical formulations through rat skin (n=3).

Time (hr)	Formulations				
	NaCMC gel base	Carbopol gel base	PF127 gel base	Emulgel base	Cream base
1.0	11.20 ± 0.34	13.2 ± 0.62	8.73 ± 0.56	9.600 ± 0.34	11.30 ± 0.63
1.5	19.53 ± 0.29	25.5 ± 1.10	13.81 ± 0.75	23.30 ± 0.64	20.80 ± 0.58
2.0	32.80 ± 0.40	49.5 ± 1.30	25.90 ± 1.00	38.90 ± 1.10	33.80 ± 0.72
3.0	55.80 ± 0.43	84.6 ± 2.80	35.30 ± 1.20	53.20 ± 1.60	48.30 ± 0.83
4.0	103.6 ± 1.20	139.8 ± 4.20	58.20 ± 1.40	97.30 ± 2.10	86.30 ± 1.60
6.0	164.6 ± 3.60	209.8 ± 4.80	81.80 ± 1.90	133.5 ± 2.40	119.4 ± 1.80
8.0	208.0 ± 4.60	237.7 ± 6.10	105.5 ± 2.64	187.8 ± 2.80	156.9 ± 2.20

Table 3.Percutaneous permeation parameters ± SD of different KT formulations through rat skin (n=3).

Formula	Flux (J) (µg/cm^2/hr) ×10^2	Permeability coefficient (P) (cm/hr)	Diffusion coefficient (D)(cm^2/hr)
NaCMC gel base	13.80 ± 0.010	27.60 ± 0.020	1.884 ± 0.005
Carbopol gel base	16.58 ± 0.012	33.16 ± 0.032	2.826 ± 0.005
PF127 gel base	6.910 ± 0.009	13.82 ± 0.012	0.493 ± 0.002
Emulgel base	9.550 ± 0.003	19.10 ± 0.020	1.458 ± 0.002
Cream (O/W) base	7.910 ± 0.001	15.82 ± 0.018	1.073 ± 0.001

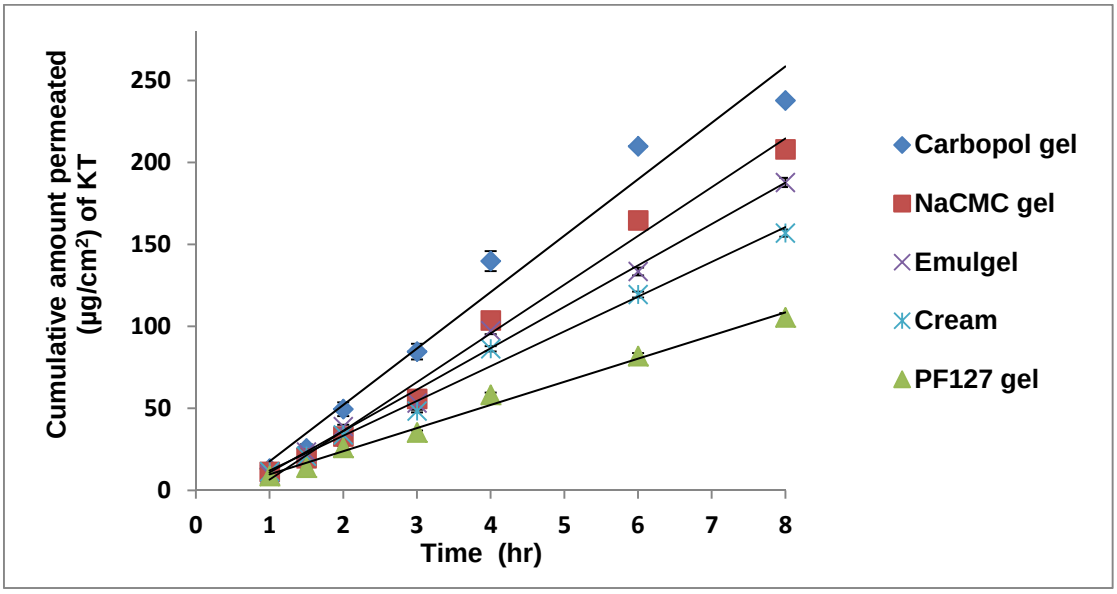

Fig. 2. Permeation profiles of ketorolac tromethamine through rat skin from different topical formulations. Values are means of 3 determinations ± SD.

Moreover, thepermeability coefficient of KT from emulgel base is slower, 19.10 ± 0.02 cm/hr than that from carbopol and NaCMC gel bases, this may be attributed to the presence of oil in emulgel that will bind with the drug (drug loaded emulsion) and prevent it to release easily from the base to the aqueous release medium (hydrophilic). Permeabilitycoefficient of KT from carbopol and NaCMC gel bases was 2.1-fold and 1.7-fold as compared to that from cream base.

In the present study, the flux values from carbopol gel base, NaCMC gel base, emulgel base, cream base and PF127 gel base were 16.58 $x10^2$, 13.8 $x10^2$, 9.55 $x10^2$, 7.91 $x10^2$, and 6.91 $x10^2$µg/cm^2/hr, respectively. Generally, KT topical formulations could be arranged according to the drug flux through the rat skin in the following rank order: carbopolgel base > NaCMC gel base > emulgel base > O/W cream base > PF127 gel base.

The observed differences in drug release may be due to the differences in the structure, viscosity of the polymer and differences in drug polymer interactions [29].

The results obtained from the in vitro permeation of KT through rat skin indicated that, the permeation of KT through rat skin was weak, for example: carbopol gel base which gave relatively the highest permeation rate of KT through 8 hr, the amount of KT permeated was 237.7 ± 6.1 µg/cm^2 (Table 2 and Fig. 2). So to increase the amount of KT permeated; this can be obtained by increasing the initial drug concentration or use the permeation enhancer. Carbopol and NaCMC gel bases were selected to study the effect of initial drug concentration and permeation enhancer on drug permeation through rat skin.

Effect of drug concentration and enhancer on the permeation of KT through rat skin

The permeation of KT through rat skin was studied using different drug concentration (0.5, 1.0 and 2.0 % w/w) in the formulations bases delivered the highest amount of drug permeated through rat skin. The selected formulations were NaCMC and carbopol gel bases. Table 4 shows that, there is a positive correlation between the steady state flux (J) and initial drug concentration, i.e. the flux of the drug increased with increasing its initial concentration, for example, using carbopol gel base, increasing the concentration of KT from 0.5 to 1.0 % (w/w) and from 1.0 to 2.0 % (w/w) resulted in an increase in the steady state flux from 1658 ± 0.012 µg.cm^{-2}.$hr^{-1}$to 2240 ± 0.021µg.cm^{-2}.hr^{-1} and from 2240 ± 0.021µg.cm^{-2}.hr^{-1} to 2602 ± 0.025 µg.cm^{-2}.hr^{-1}, respectively. Similar results were obtained using NaCMC gel (Table 4). Studying the effect of penetration enhancer on the permeation of KT through the rat skin was also carried out by using 0.5 and 1.0 % (w/w) sodium lauryl sulfate (SLS) in NaCMC and carbopol gel bases, each of them containing (0.5 % w/w) KT. Table 4showed that, the increase of SLS concentration led to non significant

increase in the permeation of KT through rat skin for both NaCMC and carbopol gel bases. For example, in carbopol gel, the addition of 0.5% (w/w) SLS, this led to an increase in the steady state flux from 1658 ± 0.012 $\mu g.cm^{-2}.hr^{-1}$ to 1824 ± 0.023 $\mu g.cm^{-2}.hr^{-1}$ and to 1956 ± 0.034$\mu g.cm^{-2}.hr^{-1}$ with 1.0 % w/w SLS. Similar results were obtained using NaCMC gel (Table 4). There are many theories indicating the mechanism of action of penetration enhancers have reported. One theory concluded that the effects of penetration enhancers may be due to their hygroscopic properties, which led to the increase of the water content of the stratum cornea andhence, increasing its permeability. Another theory said that the effectiveness of penetration enhancers may be attributed to the modifying effect on the natural structure of the stratum cornea. Organic solvents like benzene, alcohol, and ether, which have been shown to enhance the penetration rate of both water-soluble and lipid-soluble substances by removing the lipids from the stratum cornea [31,32]. The enhancing effect of permeation enhancers may also be attributed to the increase in the fluidity of the intercellular lipids, which is clearly related to skin permeation enhancement [33].

Table 4. Effect of drug concentration and penetration enhancer on KT permeation through rat skin (n=3).

KT concentration (w/w)	The steady state flux ($\mu g.cm^{-2}.hr^{-1}$)× 10^{2} ± SD	
	NaCMC gel base	Carbopol gel base
0.5 %	13.80 ± 0.010	16.58 ± 0.012
0.5 % with 0.5 % SLS	14.97 ± 0.021	18.24 ± 0.023
0.5 % with 1.0 % SLS	16.01 ± 0.017	19.65 ± 0.034
1 %	18.20 ± 0.032	22.40 ± 0.021
2 %	21.55 ± 0.037	26.02 ± 0.025

Stability study

The obtained results confirmed that, the appearance of all formulations was unchanged during storage period. Also the results showed no chemical degradation as was concluded from the close similarity in pH values. KT retained its chemical stability over 8 months of storage and the amount of KT loaded in systems remained nearly unchanged and ranged from 95.3 % to 99.3. Also, there were no significant changes in the viscosity during storage time. The difference in the temperature at which the formulations were stored didn't showany significant effect on the KT formulations stability.

Paw edema results

Fig. 3 illustrates the anti-inflammatory activity of KT topical formulations on the carrageenan-induced edema in the hind paw of rats. It was obvious that, the induction of acute inflammation in control rats resulted in a prominent increase in paw thickness and the percentage swelling ranged from 102 % to 168 % at the time 1 and 5 hrs after the injection of carrageenan, respectively. The swelling of edema was significantly inhibited in all rat groups either treated with selected KT formulations or commercial piroxicam gel compared with control group. The results also indicated that, the extent of inhibition of edema which was obtained from carbopol 934 and NaCMC gels was higher than that from other KT formulations. At the first hr post application of medication, carbopol and NaCMC gel bases produced significant ($p<0.05$) inhibitory effects,

41.52 % and 40.54 % of paw edemainhibition, respectively as compared to 18.90 %, 23.7 %, and 29.7 % inhibition obtained with cream base, PF127 gel base, and emulgel base, respectively. This inhibitory effect was non significant after 1 hr as compared to commercial piroxicam gel which gave 44.64 % of edema inhibition.

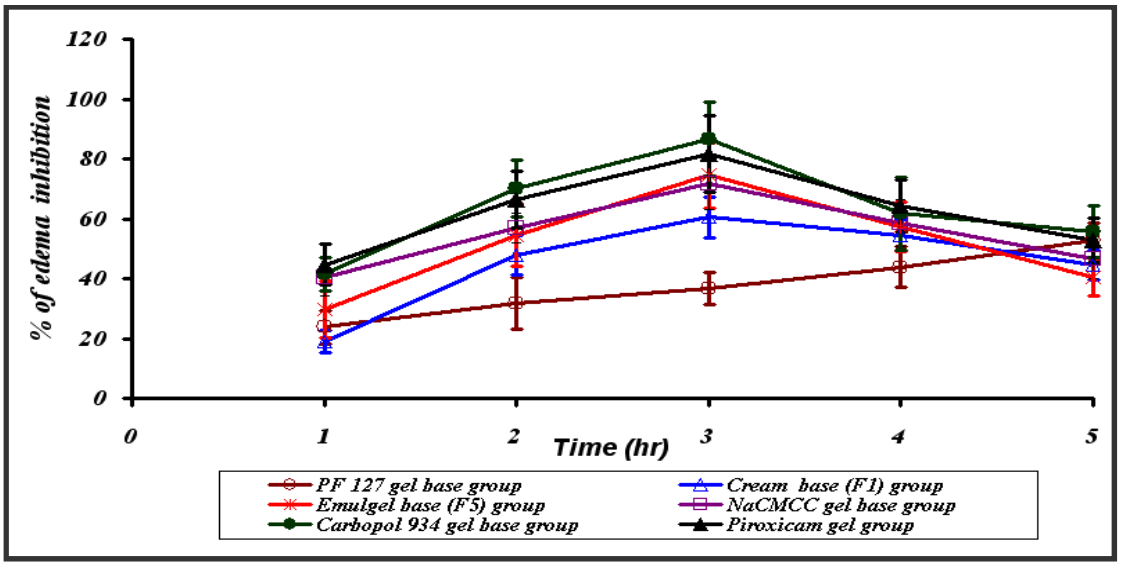

Fig. 3. Effect of different KT topical formulations compared to commercial piroxicam gel on the % inhibition of rat hind paw edema induced by carrageenan.

KT in carbopol and NaCMC gel bases still had a more pronounced anti-inflammatory effect ($p<0.05$) between 2 and 3 hr, for carbopol gel percent inhibition was 70.12, 86.56, for NaCMC gel 56.88, 74.86 , for PF127 gel 31.87, 36.76, emulgel base 54.5, 71.5, cream base 47.8, 60.5 and piroxicam gel 66.4, 81.6 after 2 and 3 hr respectively.

At 4 and 5 hr post treatment, the anti-inflammatory effect of KT in carbopol, NaCMC, PF127 gel bases, emulgel base, cream base and piroxicam gel began to decrease as indicated by a reduction in percent of inhibition of edema but still showed significant difference ($p<0.05$) as compared to the control (untreated) group.

Comparison of % of edema inhibition of KT formulations with that of piroxicam gel using statistical analysis indicated that, there was no significant difference in edema inhibition between carbopol, NaCMC and piroxicam gel bases at 5 hr intervals, but there was significant ($p<0.05$) difference in edema inhibition between the previous three gels and other KT formulations at 5 hr intervals. Generally, KT topical formulations could be arranged according to edema swelling inhibition in the following rank order: carbopol 934 gel > NaCMC gel > emulgel > cream (O/W) > PF127 gel. The nature of topical vehicle plays a major role in promoting drug release and then absorption into and through the skin. Topical vehicles such as gels, emulgels or creams exert their effectby releasing the drug onto the skin surface and then the drug molecules diffuse through the skin layers. The diffusion depends on the physicochemical properties of the drug. Thereby, the highest anti-inflammatory effect of KT in carbopol and NaCMC gel bases could be attributed to the higher permeation rate of KT from thesetwo gels than the other carriers (Table 3).

Skin irritation study

The results showed that, there were no any signs of irritation after 24 hr (showed zero of erythema score) which indicated the safety of carbopol gel base.

Clinical study

The base line demographic data of the study is shown in (Table 5). The three groups were comparable in age, sex and weight. All vital signs (temperature, pulse rate, respiratory rate, and blood pressure) were within the normal range at baseline. After 4 weeks of treatment, there were

no clinically relevant or statistically significant changes in the vital signs. No significant changes were observed in weight for three groups. A similar number of patients in three groups had a history of comorbid conditions (e.g., gastritis, hypertension, and diabetes).

Table 5. Baseline demographic and clinical characteristics of patients with OA[1] treated with the selected KT gel and piroxicam gel (n = 62)[2].

Characteristic	KT gel group (n=21)	Piroxicam gel group (n=21)	Placebo group (n=20)
Age (year)			
Mean ± SD	51.24 ± 6.98	59.1 ± 6.15	55.05 ± 5.5
Range	36 - 60	49 -66	44 - 66
Weight (Kg)			
Mean ± SD	70.57 ± 17.27	71.2 ± 11.32	67 ± 12.5
Range	50 - 105	60 - 85	58 - 76
Height, cm , (mean ± SD)	168 ± 10.6	172 ± 14.7	160.5 ± 12.3
Sex, no (%)			
Male	9 (42.9)	12 (57.1)	5 (25)
Female	12 (57.1)	9 (42.9)	15 (75)
Duration of arthritis, year			
Mean	2.5	2.8	1.8
Range	1.0 - 3.5	1.5 - 3.5	1.0 - 2.5
Type of osteoarthritis, no (%)			
Unilater	13 (62)	13 (62)	15 (75.0)
Bilateral	8 (38)	8 (38)	5 (25.0)
Patients with previous $NSAID_S$ therapy, no (%)	4 (19)	7 (33.33)	3 (14.3)
Comorbid condition, no (%)			
Gastritis	6 (28.6)	5 (23.81)	2 (10)
Hypertension	5 (23.8)	5 (23.81)	4 (20)
Asthma	1 (4.76)	0	0
Diabetes	4 (19.0)	6 (28.6)	3 (15)

[1]Osteoarthritis, [2] No significant differences between-groups were found.

Efficacy Assessments

At baseline as shown in (Table 6), the mean (± SD) WOMAC pain subscale scores were 10.1 ± 1.36, 10.05 ± 1.53, and 10.3 ± 1.38 in KT gel, piroxicam gel and placebo groups, respectively.After a week of treatment, significant reduction in pain score was observed in KT gel group (4.71 ± 1.1), piroxicam gel group (4.57 ± 1.21) while the reduction in placebo group was non significant (7.85 ± 1.14). After 2 and 4 weeks, the reduction in pain score was significant in both KT group and in piroxicam group while insignificant in placebo group. From baseline, the percent reduction in pain scores was 69.3, 73.5, and 34.46 in KT, piroxicam, and in placebo groups, respectively. Comparison between groups in WOMAC pain subscale scoresreduction showed that, no significant difference in pain score reduction between KT group and piroxicam group while there was a significant difference in pain score reduction between either KT group or

piroxicam group and placebo group ($p<0.05$). The means WOMAC stiffness subscale scores changes at each time point are shown in (Table 7). At baseline, the mean (±SD) stiffness score was 4.33 ± 0.58 in KT group, 4.47 ± 0.6 in piroxicam group and 4.6 ± 0.82in placebo group. After 4 weeks of treatment, stiffness scores decreased significantly ($p<0.05$) in KT gel group (54.96 %) and in piroxicam group (58.61%) while it was 26.08 % in placebo group. Statistical analysis indicated that, no significant difference instiffness scores reduction between KT group and piroxicam group while there was significant difference instiffness score ($p<0.05$) reduction between either KT group or piroxicam group and placebo group. The means (±SD) WOMAC physical functions subscale scores were33.43 ± 3.1, 33.52 ± 2.97 and 35.25 ± 2.6in KT, piroxicam and placebo groups, respectively at base line as shown in (Table 8). Four weeks later, the physical function scores were reduced significantly ($p<0.05$) to be 65.95 %, 69.45% and 23.54 % in KT group, piroxicam group and placebo group, respectively. Statistical analysis indicated that, reduction in physical functions subscale scores was insignificant between KT group and piroxicam group, while it was significant ($p<0.05$) between either KT group or piroxicam group and placebo group. Finally, at base line, the mean (±SD) WOMAC-CI scores in KT, piroxicam and placebo groups was 47.81 ± 4.24, 48.04 ± 4.9 and 49.95 ± 4.39, respectively. Four weeks later, the WOMAC-CI scores was significantly reduced($p<0.05$) in KT group (65.44%) and in piroxicam group (69.3%) while it was 25.72% in placebo group.Statistical analysis confirmed the results obtained in WOMAC-OA subscale scores as shown in (Table 9).

Table 6. Changes in WOMAC pain subscale scores in OA patients treated with KT gel and piroxicam gel.

Week	WOMAC pain subscale scores (±SD)		
	KT gel group	Piroxicam gel group	Placebo Group
0 (Baseline)	10.1 ± 1.36	10.05 ± 1.53	10.3 ± 1.38
1	4.71 ± 1.10	4.570 ± 1.21	7.85 ± 1.14
2	3.76 ± 0.83	3.330 ± 1.02	7.15 ± 1.04
4	3.10 ± 0.83	2.670 ± 1.11	6.75 ± 1.21
% Decrease from baseline	69.3 %	73. 5 %	34.46. %

Table 7. Changes in WOMAC stiffness subscale scores in OA patients treated with KT gel and piroxicam gel.

Week	WOMAC joint stiffness subscale scores (±SD)		
	KT gel group	Piroxicam gel group	Placebo Group
0 (Baseline)	4.33 ± 0.58	4.47 ± 0.60	4.60 ± 0.82
1	2.29 ± 0.46	2.24 ± 0.54	3.45 ± 0.76
2	2.05 ± 0.22	2.00 ± 0.32	3.35 ± 0.75
4	1.95 ± 0.22	1.85 ± 0.57	3.40 ± 0.75
% Decrease from baseline	54.96 %	58.61 %	26.08 %

Table 8. Changes in WOMAC physical functions subscale scores in OA patients treated with KT gel and piroxicam gel.

Week	WOMAC physical functions subscale scores (±SD)		
	KT gel group	Piroxicam gel group	Placebo group
0 (Baseline)	33.43 ± 3.10	33.52 ± 2.97	35.25 ± 2.60
1	19.24 ± 3.70	17.14 ± 2.77	29.55 ± 2.42
2	12.90 ± 1.48	11.95 ± 1.96	27.55 ± 2.56
4	11.38 ± 1.10	10.24 ± 1.55	26.95 ± 2.82
% Decrease from baseline	65.95 %	69.45 %	23.54 %

Table 9. Changes in WOMAC-CI scores in OA patients treated with KT gel and piroxicam gel.

Week	WOMAC-CI scores (±SD)		
	KT gel group	Piroxicam gel group	Placebo group
0 (Baseline)	47.81 ± 4.24	48.04 ± 4.90	49.95 ± 4.39
1	26.19 ± 4.64	23.95 ± 4.85	40.90 ± 3.21
2	18.95 ± 1.83	17.29 ± 1.62	38.20 ± 2.98
4	16.52 ± 1.08	14.75 ± 1.76	37.10 ± 3.08
% Decrease from baseline	65.44 %	69.3 %	25.72 %

Tolerability assessment

No adverse effects were recorded in all groups within the duration of the study except two patients in KT group and one patient in placebo group showed mild skin irritation (erythema).

4. Conclusion

Ketorolac tromethamine has been successfully prepared in various topical formulations; gel, emulgel and cream bases. In vitro permeation studies revealed that, KT topical formulations could be arranged according to the drug flux through rat skin in the following rank order: carbopol 934 gel base > NaCMC gel base > emulgel base > cream (O/W) base > PF127 gel base.The difference in KT permeation could be mainly attributed to the difference in the diffusion through the skin barrier. The maximum reduction of edema was significantly obtained on using medicated carbopol 934 gel. Also KT carbopol gel did not show any sign of irritation after 24 hr from application onto the shaved dorsal skin of rats. The clinical findings could be correlated with the highest in vitro permeation and the highest anti-inflammatory activity by using KT carbopol gel. The in vivo study in the patients with moderate osteoarthritis proved that, there was no significant difference in efficacy or tolerability between selected KT gel and commercial piroxicam gel. These preliminary data suggest that, KT gel may be another therapeutic option for patients with osteoarthritis by using the following formula: KT (0.5 % w/w), carbopol (0.5 % w/w) and SLS (1.0 % w/w).

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