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Evaluation of Skin Permeation and Pharmacological Effects of Tenoxicam Nanoemulsion in Topical Formulations

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ABSTRACT

This article was aimed to investigate the skin permeation, the anti-inflammatory and analgesic effect of topical formulations containing Tenoxicam in a nanoemulsion form in drug concentration 0.5% and 1%. Skin permeation study was carried out using rat abdominal skin. Two tests were employed to evaluate the pharmacological effects included the Paw Oedema induced by Carrageenan injection and Hot plate test for anti-inflammatory and analgesic effect respectively. Finally, skin irritation test was performed to confirm the safety of both formulae. The obtained results suggested that Tenoxicam in lower concentration (0.5%) gives insignificantly greater cumulative % of in-vitro rat skin permeation (41.5%) than higher concentration (1% Tenoxicam releases 40.1%) in 24 hr, this confirm that Tenoxicam may possibly permeate through the human skin. The study also revealed that group 2 (animals treated with Tenoxicam nanoemulsion 0.5% in MC gel), group 3 (animals treated with 1% Tenoxicam nanoemulsion prepared in HPMC) and group 4 (animals treated with commercial analogue (Feldene® Gel) produced maximum percent oedema inhibition after 1 hr (70.95%, 78.74% and 72.94%) respectively and then continued significantly for 3 hrs. Also the same previous animal groups showed maximum increase in reaction time [analgesic effect] after 1.5 hr (18.7, 27.6 and 21 seconds) respectively. Skin irritation test suggested that both topical formulae were non-sensitizing and safe for human topical use. Finally it was concluded that formulae containing (oleic acid 10%; Tween 20 43.33%; PG 21.66% and water 25%) nanoemulsion equivalent to 0.5% Tenoxicam in MC gel base and 1% Tenoxicam in HPMC gel base possessed a good and an acceptable in-vitro skin permeation and pharmacological effects.

INTRODUCTION

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) comprise a large group of chemically different compounds having mainly three pharmacological properties in common: analgesic, anti-inflammatory and antipyretic. Tenoxicam is well absorbed after oral doses; peak plasma concentrations occur within about 2 hours in fasting subjects; this may be delayed to about 6 hours when tenoxicam is given with food but the extent of absorption is not affected. Tenoxicam is over 98.5% protein bound and penetrates synovial fluid [1]. The plasma elimination half-life is about 60 to 75 hours; with daily administration, steady-state concentrations are reached within 10 to 15 days. Tenoxicam is completely metabolised to inactive metabolites which are excreted mainly in the urine; there is some biliary excretion of glucuronide conjugates of the metabolites [2].

It is used in the symptomatic management of musculoskeletal and joint disorders such as osteoarthritis and rheumatoid arthritis, and also in the short-term management of soft-tissue injury. Tenoxicam is given by mouth as a single daily dose usually of 20 mg. Doses similar to those given by mouth have been given by intramuscular or intravenous injection for initial treatment for 1 to 2 days. Tenoxicam has also been given by rectal suppository [3].

A number of possible mechanisms may contribute in whole or in part to the mechanism of action of Tenoxicam. NSAIDs inhibit prostaglandin synthesis by inhibiting cyclo-oxygenase, which catalyses the formation of cyclic endoperoxidases from arachidonic acid. Tenoxicam prevented arachidonic acid induced death in mice with a potency of about one-quarter of that of indomethacin [4].

Tenoxicam possessed potent anti-inflammatory activity in experimental models of acute and chronic inflammation. In acute rat models of kaolin-induced inflammatory oedema, Tenoxicam was more potent than naproxen, diclofenac sodium or indomethacin when 10 mg/kg of each drug was given [5].

Pretreatment with Tenoxicam in the rat model of acute kaolin-induced inflammation produced a rapid and long-lasting analgesic effect with a 3-fold increase in pain threshold. In other studies with rats, the analgesic activity of Tenoxicam as assessed by scald-induced hind paw hyperalgesia was equivalent to that of peroxicam, diclofenac sodium, indomethacin and naproxen but more potent than that of mefenamic acid and aspirin [6].

Tenoxicam produced a potent antipyretic effect in rats treated with subcutaneous injection of yeast. In comparison to some reference non-steroidal anti-inflammatory drugs, Tenoxicam was equivalent to indomethacin in its antipyretic effect, weaker than piroxicam, diclofenac and naproxen, and more potent than aspirin. In children, Tenoxicam was not a suitable alternative to paracetamol in the treatment of fever because of its weak antipyretic activity [7]. Results of data collected from patient using Tenoxicam confirm that its efficacy and safety compare favorably with that shown by other non-steroidal anti-inflammatory drugs in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, gout and soft tissue injury [8].

The assessment of percutaneous absorption of molecules is a very important step in the evaluation of any dermal or transdermal drug delivery system. A key goal in the design and optimization of dermal or transdermal dosage forms lies in understanding the factors that determine a good in vivo performance. Certainly, the most reliable skin absorption data are collected in human studies; however, such studies are generally not feasible during the initial development of a novel pharmaceutical dosage form or consideration of a new drug candidate. Animals or isolated animal skin models, generally more available than human skin, are of prime importance in basic research to improve our understanding of the processes, pathways and driving forces of various agents across the skin barrier. However, in vitro experiments with human skin are difficult to conduct due to the scarcity of this material and the fact that gender, race, site, age and skin condition of the donor cannot be controlled satisfactorily [9]. Therefore, various animal skin alternatives have been used to predict the percutaneous absorption through human skin.

The extent of skin permeation of a compound may depend on the route of absorption. There are several pathways which can be involved in the transdermal permeation of chemicals [10-13].

As the majority of molecules applied onto the skin permeate along the SC lipid domain, the organization of these regions is very important for the barrier function of the skin. The SC lipid composition and organization differ from that of other biological membranes, with long chain ceramides, free fatty acids, cholesterol and cholesteryl esters being the main lipid classes [14-16].

Mechanism by which Nanoemulsions penetrate skin

A dermally applied nanoemulsion is expected to penetrate the stratum corneum and to exist intact in the whole horney layer, alter both lipid and polar pathways [17]. The drug dissolved in the lipid domain of the nanoemulsions can directly penetrate the lipid of the stratum corneum, thereby destabilizing its bilayer structure. These interactions will increase the lipid pathway permeability to drugs. On the other hand, the hydrophilic domain of nanoemulsions can hydrate the stratum corneum to a greater extent and play an important role in percutaneous uptake of drugs.

When the aqueous fluid of nanoemulsions enters the polar pathway, it increases the interlamellar volume of the stratum corneum lipid bilayer, resulting in disruption of its interfacial structure. A lipophilic drug like Tenoxicam can then permeate more easily through the lipid pathway of stratum corneum. Moreover, droplet size of the nanoemulsion may also affect its efficiency, where the small droplet size of the nanoemulsion make it an excellent carrier for enhancing percutaneous uptake of Tenoxicam and more easily through the lipid pathway of stratum corneum [18].

Inflammation is the body's main basic response to injury. The analgesic and anti-inflammatory activity of NSAIDs can be evaluated pharmacologically by numerous method, all of these method depend primarily on inducing an inflammation or pain, then investigating the anti-inflammatory effects of the drug. There are numerous methods can be applied for investigation of anti-inflammatory or analgesic effect of NSAIDs. Among these methods; hot plate method [19-22], formalin induced edema test [23-26] and edema size of rat hind paw [27].

Skin irritation is defined as a locally arising, non-immunogenic inflammatory reaction, which appears shortly after stimulation and usually disappears during a few days [28]. The presence of erythema, oedema, dryness of the skin, fissures, desquamation, itching and pain characterizes both irritant contact dermatitis and allergic contact dermatitis. Some of these characteristics are signs of an inflammatory reaction and an altered homeostasis. All together, these symptoms are the ultimate physiological manifestation of a complex chain of biochemical, neural, vascular and cellular responses following the initial irritation signal [29,30]. Skin irritation test can be also carried out to study the safety of topical preparation when applied to the skin.

MATERIALS AND METHODS

Carrageenan, type I, Sigma chemical Co. [USA]. Tenoxicam nanoemulsion formulae incorporated in different cellulose derivatives gel bases coded as F1 and F2 were formulated in our laboratory. Feldene Gel®, Pfizer International Pharmaceutical Industries Co., Egypt.

Preparation of topical formulations

Topical formulations of Tenoxicam nanoemulsion were prepared firstly by formulation of Tenoxicam in a nanoemulsion form by selecting the oily phase, surfactant and cosurfactant in the specified Smix ratio [31-34]. The prepared nanemulsion was incorporated into the cellulose derivatives gel bases to formulate the topical preparation of Tenoxicam. Two formulae were used for this study as they were the most stable among variant formulae had tested before in a previous study [35-38]. The two used formulae were coded as F1 and F2. The formulation characteristics of prepared Tenoxicam tested formulae were represented in table No.1.

In-vitro skin permeation study

Skin membrane preparation

The abdominal hair of male rabbits, weighing 1.5 ± 0.003 Kg, was shaved using razors 24 h before treatment. After anesthetizing the rabbit with ether, the rabbit was sacrificed and the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was wiped with isopropyl alcohol to remove adhering fat and the skin was allowed to dry at room temperature. The dried

Table No.1: Formulations of Topical Tenoxicam Nanoemulsion

Formula Code	Nanoemulsion Composition			Drug Conc.	Gel Base
	water	Oil [oleic acid]	*S mix		
F1	25	10	65	0.5 %	MC [3%]
F2	25	10	65	1%	HPMC [3%]

*S mix = Surfactant: Co-surfactant [Tween 20: propylene glycol].
*S mix in ratio is 2:1.

samples were wrapped in aluminum foil and stored at 4 °C until use [39]. Previous research works demonstrated the maintenance of SC barrier characteristics after storage in the reported conditions [40].

In-Vitro Permeation Studies

The in vitro skin permeation of Tenoxicam nanoemulsion from MC and HPMC gel bases was investigated through rabbit skin using a modified USP 17 dissolution apparatus I. A glass cylindrical tube [2.5 cm in diameter and 6 cm in length] was attached instead of the basket and was tightly covered with a rabbit skin [with a diffusional area of 4.91 cm2] with the stratum corneum facing to inside the tube and the dermis facing to outside the tube (receptor solution). Tenoxicam loaded nanoemulsion gel bases were placed in the cylindrical tube at the stratum corneum surface. The cylindrical tube was dipped in 200 ml methanolic phosphate buffer (30%:70%) at pH 7.4 to allow the establishment of the sink conditions and to sustain permanent solubilization [41]. The release study was carried out for 24 hours at 32°C ± 0.5°C. The stirring shaft was rotated at a speed of 100 r.p.m.

At predetermined time intervals [1, 2, 3, 4, 5, 6, 8, 12, 24 hours], aliquots of one milliliter of the release medium were withdrawn and diluted then filtered for analysis and replaced with equal volume of the buffer solution to maintain a constant volume [42]. The absorbance of the collected samples was measured by UV at max of 370 nm using the same buffer solution as a control formula. All experiments were run in triplicates. At the end of 24 h, the amount of drug remaining on the skin was determined by extraction into methanol followed by UV analysis [29]. While the amount of drug retained in the skin was calculated by the formula [43].

% Tenoxicam retained in the skin = Total amount of Tenoxicam in the gel – (Amount of the Tenoxicam permeated at the end of 24 hr] + Amount of Tenoxicam on the skin at the end of 24 hr).

Animals

White hairless male albino rats weighting between (170 and 200 gm) were selected for evaluation of the anti-inflammatory activity by measurement of oedema size resulting from carrageenan injection in the right hind paw region of the body. While white male albino mice weighting between (25 and 30 gm) were selected for analgesic activity study by the hot plate method. Animals were housed 5 per cage in the animal facility of the Faculty of Pharmacy, Al-Azhar University. Animals were kept under constant temperature (23 ± 1°) and a 12-hr light/dark cycle (lights on at 7 am). Each animal was allowed free access to standard food pellets and water. All the animals were acclimatized in the animal facility for at least 2 weeks prior the experiments.

Treatment

The animals were divided into four groups, each consisting of six animals.

Group 1 was treated with plain gel base without drug to account for the effect of vehicle.

Group 2 was treated with F1.

Group 3 was treated with F2.

Group 4 was treated with Feldene® Gel (commercial product analogue).

Edema size induced by Carrageenan injection

Certain amount of gel (100mg) was applied topically to the right hind paw of the rats [44,45]. The area of application was occluded with bandages and it was left in place for two hours. The dressing was then removed and the gel remaining on the surface of the skin was wiped off with a piece of cotton. The animals were then injected with 0.1 ml of 1% freshly prepared carrageenan solution in saline in plantar region of right hind paw [46]. The right hind paw thickness was measured from ventral to dorsal surfaces, with a dial caliper immediately before and 0.5, 1, 1.5, 2, 2.5 and 3 hrs after the sub- plantar injection. The size of oedema was expressed as the increase in paw thickness (in mm) after carrageenan injection

% inhibition of edema = [(V control – V treated) / V control] X 100

Where, V control = mean oedema volume of rats in controlled group and V treated = edema volume of each rat in test group.

Hot Plate Test

The hot plate method of was applied to evaluate the analgesic activity of different formulations [47]. Certain amount of gel [100mg] was applied topically to the hind paw of the mice. Thirty minutes after the drug application, the gel remaining on the surface of the skin was wiped off with piece of cotton. Each mouse was placed in two liters beaker placed over a hot plate thermostatically controlled at 55 ± 0.5˚ with a cut of time 30 seconds to avoid tissues damage. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to react in this manner was obtained using a stopwatch and was taken as a reaction time. Recording were taken at 0.5, 1, 1.5, 2, 2.5 and 3 hours after administration.

Statistical Analysis for the results

The statistical analysis for the results was carried out on results of the mid of experiment (1.5hr) by student's "t" test using Excel software and one-way analysis of variance (ANOVA) followed by Tukey [post tests] using Instate software to determine the significance of the obtained results between the prepared medicated gel and the plain one.

Skin irritation test

Various preparations, when applied dermally, might elicit skin irritation. Therefore, to assess the skin-sensitizing potential, Tenoxicam nanoemulsion gel was applied onto the dorsal skin of albino rats. The animals were housed in propylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before experimentation. The dorsal abdominal skin of rats was shaved 24 h before study. The formulations were applied and the site of application was occluded with gauze and covered with non-sensitizing microporous tape. Erythema values for formulations with and without pretreatment with abrading gel were recorded [48]. The patches were removed after 24 hr and the score of erythema was recorded [49].

RESULTS AND DISCUSSIONS

In-vitro skin permeation study:

The in vitro skin permeation of Tenoxicam nanoemulsion gel formulations was investigated through rabbit skin using Modified dissolution cell as shown in Table No.2 and fig.1. The lower drug concentration (0.5% Tenoxicam) in MC gel base gave insignificantly greater cumulative % release (41.5%) higher than (1% Tenoxicam released 40.1%) in HPMC gel base through 24 hr.

This is in agreement with a previous study of release of Ampicillin sodium from different gel bases. It was found that MC had higher release than HPMC [50,51].

In contrast, this is in disagreement with other repots, which revealed that the release of Piroxicam or hydrocortisone acetate from HPMC was insignificant higher release than MC [52,53]. The mechanism by which Tenoxicam permeate through skin is that oleic acid increase permeation through non-polar route, as it increases both diffusivity and partitioning. However, it also increases the partitioning parameter in the polar route by increasing hydration of stratum corneum. Propylene glycol can alter skin structure, thereby modifying the percutaneous absorption. Propylene glycol readily permeates the skin and in so doing may carry the drug molecules across [54]. Besides, particle size plays an important role in percutaneous absorption.

The percent of Tenoxicam retained in the surface of rabbit skin was 36.5% and 35.9% for MC (0.5%) and HPMC (1%) respectively (fig. 2). Release of Tenoxicam from MC gel base showed first order kinetics with t1/2 of 29.48 minutes, while release from HPMC occurred according to diffusion model with t1/2 of 22.14 minutes. The results of drug permeation from all the formulations through the rabbit abdominal skin confirmed that Tenoxicam was released and permeated through the rabbit skin and hence could possibly permeate through the human skin.

Pharmacological Effects

The Anti-inflammatory activity

Paw oedema size induced by carrageenan injection

Treatment of the rats with Tenoxicam nanoemulsion significantly inhibited the edema size induced by carrageenan injection in the intra-plantar area of the right hind paw for each rat. It is observed that the group 2 (animal group treated with 0.5% Tenoxicam nanoemulsion prepared with MC gel), group 3 (animal group treated with 1% Tenoxicam nanoemulsion prepared with HPMC gel) and Group 4 (animal group treated with Feldene® Gel) produced maximum percent oedema inhibition after 1 hr (70.95% , 78.74% and 72.94%) respectively .

Table No.2: In-vitro skin permeation of different Tenoxicam nanoemulsion formulae across rabbit skin.

Time (Hours)	% of Tenoxicam Permeated from Different Formulae (mg/ ml)	
	MC (0.5%) ±SD	HPMC (1%) ±SD
1	4.15±0.15	3.8±0.2
2	4.5±0.15	4±0.25
3	4.9±0.1	4.3±0.3
4	7.1±0.15	6.3±0.5
5	9.6±0.2	9.7±0.5
6	12.5±0.25	11.7±0.7
8	22±0.25	21.4±0.5
12	29.7±0.3	28.6±0.5
24	41.5±0.4	40.1±0.8

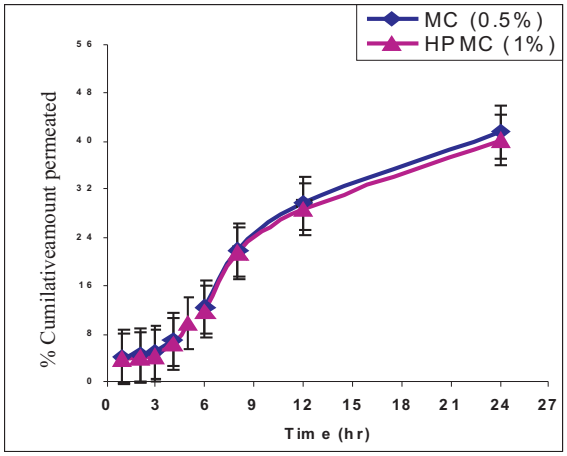

Fig. 1: In-vitro skin permeation of different Tenoxicam gel formulations across Rabbit skin

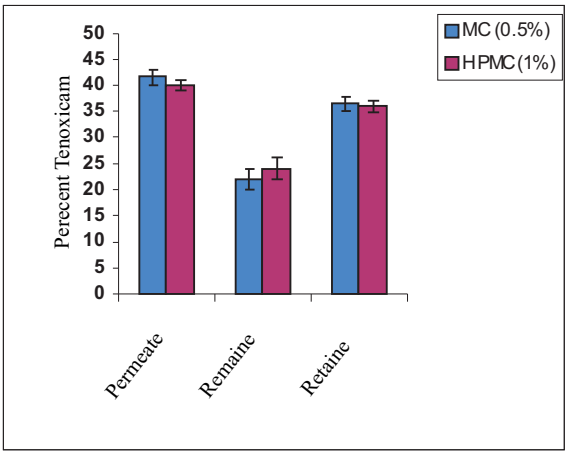

Fig. 2: Comparison of the drug levels from in-Vitro skin penetration Studies: receptor compartment [permeated], remained on the skin, and retained in the skin

Table No.3: Percent oedema inhibition by topical application of Tenoxicam gel and Feldene® gel.

Groups	Percent oedema inhibition					
	0.5 (hr)	1 (hr)	1.5 (hr)	2 (hr)	2.5 (hr)	3 (hr)
Group 1	0	0	0	0	0	0
Group 2	34.18	70.95	66.18	65.48	59.94	52.19
Group3	38.18	78.74	78.43	76.02	71.58	70.43
Group 4	52.26	72.94	71.09	65.89	64.71	52.63

Table No.4: Reaction time after topical application of Tenoxicam gel bases and Feldene® gel.

Groups	Reaction time in seconds after varying time intervals					
	0.5 (hr)	1 (hr)	1.5 (hr)	2 (hr)	2.5 (hr)	3 (hr)
Group 1	7.25	7.2	6.9	6.5	6.3	5.9
Group 2	9.8	14.25	18.7	17.4	13.16	11.45
Group 3	12.5	19	27.6	25.7	23.1	18.46
Group 4	11	15	21	20	15	12

It was observed that, 1% Tenoxicam nanoemulsion in HPMC gel base produces insignificant higher anti-inflammatory effect than 0.5% Tenoxicam Nanoemulsion in MC gel base and Feldene® Gel. These results were recorded in Table No.3 and graphically represented in Fig.3. From the statistical analysis of the data, we noted that all the investigated formulae were significantly inhibiting edema size and p value less than 0.05 were considered as significant.

The analgesic effect evaluation

Hot plate test

The analgesic effect of the Tenoxicam nanoemulsion formulae in different gel bases was studied by Hot Plate method and compared with control groups. As it shown in Table No.4 and Fig.4, it was observed that the reaction time was significantly increased in animal groups pretreated with the Tenoxicam nanoemulsion formulae in comparison with the control group.

Group 2, group 3 and group 4 animals produced maximum increase in reaction time (analgesic effect) after 1.5 hr (18.7, 27.6 and 21 seconds) respectively. Also the statistical analysis by ANOVA the results of hot plat experiment revealed that all the formulae significantly increase the reaction time (analgesic effect) in mice and p value less than 0.05 were considered as significant. After carrying out the experiments for evaluation of both the analgesic and the anti-inflammatory effects of Tenoxicam-nanoemulsion formula in different gel bases and Feldene® Gel, it is clear that chosen Tenoxicam-nanoemulsion gel bases have an acceptable analgesic and anti-inflammatory effect.

Skin irritation Test

The results of skin irritation test based on visual observation score revealed that, both formulations and Feldene® Gel were non-sensitizing and safe for use, as there is no erythema occurred after application of Tenoxicam nanoemulsion formulae on the rat skin.

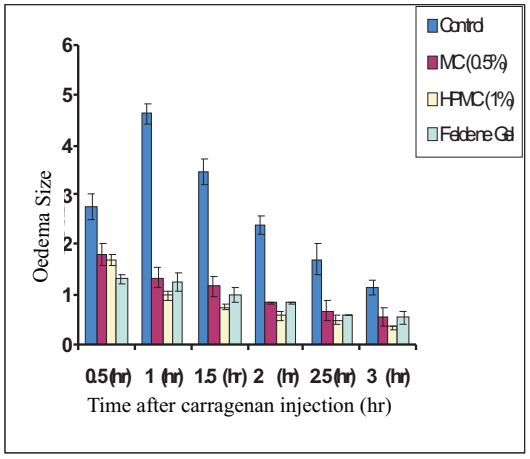

Fig. 3: Anti-inflammatory effect of Tenoxicam gel bases and Feldene® gel

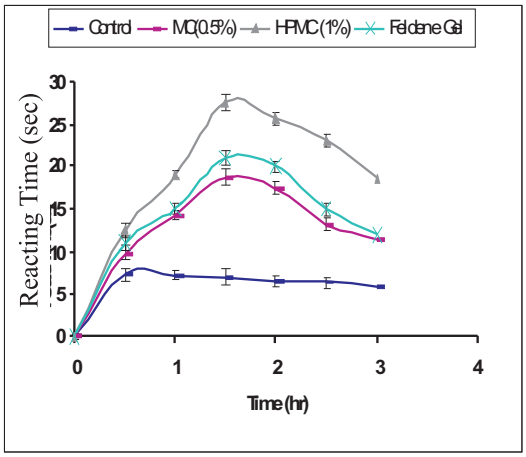

Fig. 4: Mean reaction time versus time profiles in mice after topical application of Tenoxicam gel bases and Feldene® gel

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CONCLUSION

After carrying out the experiments for evaluation of the skin permeation test, the analgesic and the anti-inflammatory effects of Tenoxicam nanoemulsion formulae in different gel bases, it is clear that all the studied medicated gel bases have an acceptable permeation, analgesic and anti-inflammatory effect. The study also revealed that group 2 (animals treated with Tenoxicam nanoemulsion 0.5% in MC gel), group 3 (animals treated with 1% Tenoxicam nanoemulsion prepared in HPMC) and group 4 (animals treated with commercial analogue [Feldene® Gel) produced maximum percent oedema inhibition after 1 hr (70.95%, 78.74% and 72.94%) respectively and then continued significantly for 3 hrs.

Also the same previous animal groups showed maximum increase in reaction time (analgesic effect) after 1.5 hr (18.7, 27.6 and 21 seconds) respectively. Skin irritation test suggested that both topical formulae were non-sensitizing and safe for human topical use. It was clear that HPMC formulae showed maximum percent oedema inhibition and maximum increase in reaction time higher than that obtained by MC or commercial formulae. Finally it was concluded that formulae containing (oleic acid 10%; Tween 20 43.33%; PG 21.66% and water 25%) nanoemulsion equivalent to 0.5% Tenoxicam in MC gel base and 1% Tenoxicam in HPMC gel base possessed a good and an acceptable in-vitro skin permeation and pharmacological effects.

REFERENCES

1. Nilsen OG. Clinical pharmacokinetics of tenoxicam. Clin Pharmacokinet., 1994; 26, 16–43.

2. Guentert TW. Relative bioavailability of oral dosage forms of Tenoxicam, Arzneimittelforschung, 1994; 44: 1051–1059.

3. Todd PA, Clissold SP. Tenoxicam: an update of its pharmacology and therapeutic efficacy in rheumatic diseases. Drugs, 1991; 41, 625–46.

4. Strub KM, Aeppli L, Daum A, Muller RKM, A New Non-Steroida Antiinflammatory Agent with Analgesic Propeties, Presented at the 15 th International Congress of Rheumatology, Paris;1981, June; 21-27.

5. Strub KM, Aeppli L, Daum A. Antiinflammatory and Analgesic Effects of Tenoxicam in Rats, Presented at the 3 rd International Congress of Inflammation, Paris, 1984; 3-7 Sep.

6. Strub KM, Aeppli L, Daum A. Antiinflammatory and Analgesic Effects of Tenoxicam in Rats, In Russo-Marie, Advances in Inflammation, Ravan Press, New York, 1986; Vol. 10, pp. 387-390.

7. Simila S, Kylmanna T. Antipyretic Effect of Tenoxicam and Paracetamol in Febrile Children, Drugs under Experimental and Clinical Research, 1985; 11,731.

8. Fenner H., Drug Invest., 1990; 2,[1], Suppl.3.

9. Behl CR, Kumar S, Malick AW, Patel SB, Char H, Piemontese D. in " Methods for Skin Absorption", Choice of membranes for in vitro skin uptake studies and general experimental techniques, Kemppainen, B.W. and Reifenrath, W.G. [eds.], CRC Press, FL, 1990; pp. 1–21.

10. Trommer H, Neubert RHH. Overcoming the stratum corneum: the modulation of skin penetration, Skin Pharmacol. Appl. Skin Physiol., 2006; 19, 106-121.

11. Netzlaff F, Schaefer UF, Lehr CM, Meiers P, Stahl J, Kietzmann M, Niedorf F. Comparison of bovine udder skin with human and porcine skin in percutaneous permeation experiments, Altern. Lab. Anim., 2006; 34, 499-513.

12. Hadgraft J. Skin, the final frontier. Int. J. Pharm., 2001; 224 [1-2], 1-18.

13. Guy RH, Hadgraft J. Transdermal drug delivery, New York: Marcel Dekker, Inc., 2003; pp. 1-23.

14. Kanitakis J. Anatomy, histology and immune-histochemistry of normal human skin, Eur. J. Dermatol., 2002; 12 , 390–397.

15. Bouwstra JA, Ponec M. The skin barrier in healthy and diseased state, Biochim. Biophys. Acta., 2006; 1758, 2080-2095.

16. Norlen L. Skin barrier structure and function: the single gel phase model, J. Invest. Dermatol., 2001; 117 ,830–836.

17. Thacrodi D, Panduranga RK. Transdermal absorption of nifedipine from microemulsions of lipophilic skin penetration enhancers, Int. J. Pharm., 1994; 111, 235–240.

18. Sanjula B, Aaiyaz S, Alka A, Javed A, Sheikh S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib, Acta Pharm., 2007; 57 , 315-332.

19. Konig M, Zimmer A M, Steiner H, Holmes PV, Crawley JN, Brownstein MJ, Zimmer A. Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. Nature, 1996; 383, 535-538.

20. Zimmer A, Zimmer AM, Baffi J, Usdin T, Reynolds K, Konig M, Palkovits M, Mezey E. Hyperalgesia in mice with a targeted deletion of the tachyinin I gene Proc. Natl Acad. Sci., USA, 1998; 95, 2630-2635.

21. Zimmer A, Zimmer AM, Hohmann AG, Herknham M, Bonner TI. Increased mortality, hypoactivity and hypoalgesia in cannabinoid CBI receptor, knockout mice. Proc. Natl. Acad. Sci. USA, 1999; 96, 5780-5785.

22. Trafton JA, Abbadie C, Marck K, Basbaum AI. Postsynaptic signaling via the [mu]-opiod receptors responses: of dorsal horn neurons to exogenous to opioids and noxious stimulation, J.Neurosci., 2000; 20, 8578-8584.

23. Hunskaar S, Fasmer OP, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci., 14, 1985; 1064-1070.

24. Kanui TI, Karim F, Towett PK. the formalin test in the naked mole-rat [hetercephalus glaber]: analgesic effects of morphine nefopam and paracetamol, Brain Res., 1993; 600, 123-126.

25. Tjolsen A, Derge OG, Hunskaar S, Rsland JH, Hole K. The formalin test: an evaluation of the method. Pain, 1992; 51, 5-17.

26. Boughton-Smith NK, Deakin AM, Follenfant RL, Whittle BJ, Garland LG. Role of oxygen radicals and arachidonic acid metabolites in the reverse passive Arthus reaction and carrageenan paw oedema in the rat, Br. J. Pharmacol., 1993; 110, 896-902.

27. Mahgoub AA. Grape fruit juice potentiates the anti-infllammatory effects of Diclofenac on the carrageenan-induced

rat's paw edema, Pharmacol. Res., 2002; 45, 1.

28. Harvell JD, Lammintausta K, Maibach HI. in "Irritant contact dermatitis", Guin, J.D. [ed.], Practical Contact Dermatitis. McGraw-Hill, New York, 1995; pp. 7–18.

29. Thomas W, David Basketterb A, Klaus R, Schro D. In vitro skin irritation: facts and future. State of the art review of mechanisms and models Toxicology in vitro, 2004; 18, 231–243.

30. Weltfriend S, Bason M, Lmmintausta K, Maibachh HI. in "Irritant dermatitis", Marzulli, F.N., Maibach, H.I. [eds.], Dermatoxicology, fifth edition. Taylor and Francis, Washington, 1996; pp. 87–111.

31. Mou D, Chen H, Du D, Mao C, Wan J, Xu H, Yang X. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs; Int. J. Pharm., 2008; 353, 270-276.

32. Lianli L, Indranil N, Kwon H K. Development of an Ethyl Laurate-Based Microemulsion for Rapid Onset Intranasal Delivery of Diazepam, Int. J. Pharm., 2002; 237,77-85.

33. Eccleston J. in "Microemulsions", Swarbrick, J. Boylan JC, [eds.] Encyclopedia of Pharmaceutical Technology. vol. 9. New York, NY: Marcel Dekker, [1995]; 375, 421.

34. Azeem A, Mohammad R, Farhan J, Ahmad Zeenat I, Roop K, Khar, 1M, Aqil 1, Sushama T. Nanoemulsion Components Screening and Selection, a Technical Note, AAPS, Pharm. Sci. Tech., 2009; 10[1].

35. Abd-Allah FI, Dawaba HM, Ahmed AMS. Preparation, characterization, and stability studies of piroxicam-loaded microemulsions in topical formulations. Drug Discov Ther., 2010; 4 [4]: 267-75.

36. Badran M, Ahmed AMS, Ibrahim E. Pharmaceutical studies on

percutaneous drug delivery of certain medicaments [M. Sc. Thesis]. Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, 2004.

37. Tarek AA, Hany MI, Fathy IA, Ahmed MS, Ehab F Mohammad TH. In vitro release, rheological, and stability studies of mefenamic acid coprecipitates in topical formulations. Pharmaceutical Development and Technology, 2010; 1–14, Early Online.

38. USP 24/NF 19. Dissolution Apparatus Description. Prepared by the Committee of Revision and Published by the Board of Trustees, 1995; pp. 1791-93.

39. Bhaskar K, Anbu J, Ravichandiran V, Venkateswarlu V, Madhusudan RY. Lipid nanoparticles for transdermal delivery of flurbiprofen: formulation, in vitro, ex vivo and in vivo studies. Lipids in Health and Disease, 2009; 8-6.

40. Swarbrick, J, Lee G, Brom J. Drug permeation through human skin. Effects of storage conditions of skin. J. Invest. Dermatol., 1982; 78, 63-66.

41. Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Ricci M. Lipid nanoparticles for prolonged topical delivery: An in vitro and in vivo investigation. Int. J. Pharm., 2008.

42. Shah KA, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles [SLN] of tretinoin: potential in topical delivery, Int. J. Pharm., 2007; 345, 163–171.

43. Pathak P, Nagarsenker M. Formulation and Evaluation of Lidocaine Lipid Nanosystems for Dermal Delivery. AAPS Pharm. Sci. Tech., 2009; Vol. 10 September, [3].

44. Puri RD, Sanghavi NM. Evaluation of topical non-steroidal drugs of using penetration enhancers, Indian Journal of Pharmacology, 1992; 24: 227-228.

45. Reddy DN, Udupa N. Formulation and evaluation of oral and transdermal preparations of flurbiprofen and piroxicam incorporated with different carries. Drug Dev. Ind. Pharm., 1993; 19[7]: 843-825.

46. Shahiwala A, Misra A. Studies in topical application of niosomally entrapped nimesulide, J. Pharm. Pharmaceut. Sci., 2002; 5[3]: 220-225.

47. Gupta M, Mazumder UK, Kumar RS Kumar TS. studies on Anti-inflammatory, Analgesic and antipyretic Properties of Methanol Extract of Caesalpinia bonducella leaves in Experimental Animal Models, Iranian. J. Pharm. Therap., 2003; 2, 30-34.

48. Drazie J. Appraisal of safety and chemical in foods drug and cosmetics by the staff of the Division of Pharmacology Food and Drug Administration Department of Health Education and Welfare, Topeka, KS: FDA Official of US Business Office1959; P.46.

49. William I, Jordan JR MD, Linda E, Atkinson PhD2, Chinglin L PhD2. Comparison of the Skin Irritation Potential of Two Testosterone Transdermal Systems: An Investigational System and a Marketed Product. CLINICAL l-HERAE'EU'MCSV., 1998; VOL. 20, [1].

50. Janardhanan B, Ramachandra P, Rajappan M, Thengungal K, Ravi I, Probal K. Formulation Development and In Vitro and In Vivo Evaluation of Membrane-Moderated Transdermal Systems of Ampicillin Sodium in Ethanol: pH 4.7 Buffer Solvent System , AAPS Pharm. Sci. Tech., 2007; 8 [1], Article 7.

51. Fang JY, Li-Ren H, Yaw-Bin H, Yi-Hung T. Evaluation of transdermal iontophoresis of enoxacin from polymer formulations, in vitro skin permeation and in vivo microdialysis using Wistar rat as an animal model, Inter. J. Pharm., 1999; 180, 137–149.

52. Attiaa MA, El-Gibaly I, Shaltout SE, Fetih GN. Transbuccal permeation, anti-inflammatory activity and clinical efficacy of piroxicam formulated in different gels, Int. J. Pharm., 2004; 276, 11-28.

53. Raghavan SL, Trividic A, Davis AF, Hadgraft J, Effect of cellulose polymers on supersaturation and in vitro membrane transport of hydrocortisone acetate, Int. J. Pharm., 2000; 193, 231–237.

54. Edurne L, Andriana A, Susana S, Pilar Y. Combined effect of oleic acid and propylene glycol on the percutaneous penetration of Tenoxicam and its retention in the skin, Eur. J. Pharm. Biopharm., 2001; 52, 113-119.