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Research Article

EVALUATION OF THE ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF PIROXICAM-LOADED MICROEMULSION IN TOPICAL FORMULATIONS

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ABSTRACT

Piroxicam is a nonsteroidal anti-inflammatory drug. It has anti-inflammatory, analgesic and antipyretic activity through inhibition of prostaglandin synthetase, via inhibition of cyclooxygenase enzymes. This study was designed to compare the anti-inflammatory and analgesic effects of piroxicam in microemulsion formulation incorporated in different pharmaceutical gel bases to the commercial product (Feldene Gel®). The anti-inflammatory activity of the tested piroxicam formulations was evaluated using right hind paw oedema size of rats induced by carrageenan injection, while the analgesic effect was evaluated using Hot Plate method applied on mice. Our results concerning the anti-inflammatory activity revealed that the tested piroxicam-microemulsion formulae (piroxicam- microemulsion formula incorporated in different gel bases namely 3% HPMC and 3% MC) produced a maximum percent oedema inhibition after 1 hr (75.7% and 76.90%), respectively, while the analgesic effect of the same previous formulae produce a maximum increase in reaction time (analgesic effect) but after 1.5 hr (62.8% and 65.8% seconds) respectively, and then this analgesic effect was continued significantly for 3 hrs. indicating that the tested piroxicam formulations exhibited good and acceptable pharmacological effects (i.e. anti-inflammatory and analgesic effects) in comparison to the commercial product (Feldene Gel®).

Keywords: Microemulsion, piroxicam, Topical, Formulations, Anti-inflammatory effect, Analgesic effect.

INTRODUCTION

Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, rheumatoid arthritis [1], analgesic [2], and antipyretic activities in animal models. The mechanism of action of piroxicam, like that of other NSAIDS, is not completely understood but may be related to prostaglandin synthetase inhibition.

Piroxicam is insoluble in water and cyclohexane, sparingly soluble in di isopropyl ether and toluene, only slightly more soluble in lower aliphatic alcohols methanol, ethanol and isopropanol and soluble in some polar organic solvents such as: Dimethyl form amide 1 gm/10 ml, Dimethyl sulphoxide 1 gm/10 ml and Chloroform 1 gm/10 ml. [3]

Piroxicam is well absorbed from the gastrointestinal tract; peak plasma concentrations are reached 3 to 5 hours after an oral dose. Piroxicam is also absorbed to some degree after topical application. [4] Piroxicam is 99% bound to plasma proteins and it has been detected in breast milk. [4] It is metabolized in the liver by hydroxylation and conjugation with glucuronic acid. [4] and is excreted mainly in the urine with smaller amounts in the feces. Enterohepatic recycling occurs. Less than 5% of the dose is excreted unchanged in the urine and feces. [4] Piroxicam was used in musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, rheumatoid arthritis including juvenile idiopathic arthritis, in soft-tissue disorders, in acute gout, and in postoperative pain. [4]

Inflammation plays essential roles in combating the pathogen and saving the integrity of the organism. First: it activates cells as macrophages to kill the pathogen and initiates the specific immune response. Second: it provides a physical barrier to prevent the spread of the pathogen. Third: it initiates the repair of the injured tissue. Inflammation could be initiated by the presence of antigen, but also by tissue damage, or release of peptides from the sensory nerve terminals. [5]

Inflammation is a complex process also on the molecular level, involving the production of various humoral mediators including kinins (bradykinin), prostaglandins, leukotrienes and a number of cytokines (tumor necrosis factor). In many cases of inflammation, membrane phospholipids are converted to arachidonic acid by phospholipase A_2. Cyclooxygenase acts to convert arachidonic acid to prostaglandins and thromboxanes (cyclooxygenase pathway), whereas lipoxygenase acts to convert arachidonic acid to leukotrienes (lipooxygenase pathway). The concentration of corticosteroids increases in chronic inflammations. It inhibits the conversion of membrane phospholipids to arachidonic acid, thereby inhibiting the synthesis of both the cyclic endoperoxides and leukotrienes [6] and decreasing inflammation.

Pain can be experienced after any tissue injury, but strong stimuli could be also painful even without tissue damage. Pain sensitivity is dynamically changing with the actual physiological status: pain is sometimes absent when tissue damage is obvious and ongoing. [7] On the other hand, inflamed tissues can be more sensitive to painful stimuli (hyperalgesia), moreover even non-noxious stimuli could be "misperceived" as pain (allodynia).

Injury of tissue, which is the main inducer of pain, results in local accumulation of chemical mediators that can strongly activate nociceptors. [8] Cell membrane perturbation by injurious agents or forces causes activation of membrane-bound enzymes (particulary phospholipase A_2), which generate arachidonic acid from membrane phospholipids. [5] Arachidonic acid is the substrate for enzymatic cascades that generate within seconds prostaglandins, thromboxanes and leukotriens. These products are all known mediators of pain and inflammation. They induce a self-propagatory process of release of other pain and inflammatory mediators from different cell types. Thus, tissue injury initiates enzymatic cleavage of circulating high-molecular-weight kininogen to produce bradykinin, another potent mediator of pain and inflammation. [8] Further, mast cells in damaged tissue degranulate, releasing histamine and chemotactic agents that promote infiltration of injured tissue with neutrophils and eosinophils. [5] Many tissue injury-induced chemical mediators (such as histamine, bradykinin and prostaglandins) cause dilatation and increase permeability of tissue capillaries. As a result of this localized edema the pH value of the interstitial fluid decreases and activation of the pH sensitive receptor causes further pain. It described that low pH solutions evoke a prolonged activation of sensory nerves and produce a sharp stinging pain. [9] Moreover, the increased pressure in this area activates the low sensitivity mechanoreceptors, which is perceived as painful.

So this work was aimed to compare the anti-inflammatory and analgesic effects of piroxicam in microemulsion formulation incorporated in different pharmaceutical gel bases to the commercial product (Feldene Gel®).

MATERIALS AND METHODS

Chemicals Carrageenan, type I, Sigma-Aldrich Louis, MO, (USA); Feldene Gel®, Pfizer International Pharmaceutical Industries Co.,

(Egypt); Piroxicam-microemulsion in different gel bases, was prepared in our lab. at department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt.

Preparation of microemulsion formulations

Piroxicam is accurately weighed and added simply to the selected premicroemulsion bases from the constructed phase diagrams. Vortexing is required to dissolve piroxicam completely in microemulsion systems. The final piroxicam concentration is adjusted to be 0.5% w/v. [10]

Preparation of cellulosic gel bases containing piroxicam

The weighed amount of MC and HPMC powder was sprinkled gently using magnetic stirrer in 100 ml beaker containing boiling distilled water, and stirred magnetically at a high speed. Stirring was continued until a thin hazy dispersion, without lumps, was formed. Leaving over night in the refrigerator may be necessary for complete gel dispersion. [11, 12] Piroxicam in microemulsion form was added during the stirring process. The release pattern of the drug, from the gel bases, was carried out, by using the cell diffusion model described in the USP 24/NF 19. [13]

Table 1: Shows formulation characteristics of prepared piroxicam tested formulae

Formula	Microemulsion composition					
	Oil OA %	Surfactant T80 %	Cosurfactant PG %	Water %	Piroxicam %	Gel base
H1	10	60	30	50	0.5	3% HPMC
H2	10	60	30	50	0.5	3% MC

OA = Oleic Acid; T80 = Tween 80; PG = Propylene Glycol; % = % w/w; MC = Methyl cellulose ; HPMC = Hydroxy Propyl Methyl Cellulose

The amount of the drug released from the bases was determined spectrophotometerically at λ_{max} 350 nm by measuring the test samples against blank samples. Experiments were triplicated and mean results were reported. [14] The formulation characteristics of prepared piroxicam tested formulae were represented in table (1).

Animals

White 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 standardized conditions at animal facility of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. All animals were acclimatized and kept under constant temperature (25 ± 2°C) and a 12-hr light/dark cycle (lights on at 7 am) for at least 2 weeks prior the experiments. All animal procedures were performed in accordance with the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University - Cairo, Egypt, and followed the recommendations for the proper care and use of laboratory animals (NIH publication No. 85-23, revised 1985). Each animal was allowed free access to standard food pellets and water.

Treatment

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

Group 1:- Control group treated with non medicated gel base.

Group 2:- Treated group with piroxicam- microemulsion incorporated in MC gel.

Group 3:- Treated group with piroxicam- microemulsion incorporated in HPMC gel.

Group 4:- Treated group with Feldene Gel®.

Paw oedema size induced by carrageenan injection

Certain amount of the investigated gel base (100 mg) [15] was applied topically to the right hind paw of the rats. [16] The area of application is lightly 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 paw volume was determined immediately before carrageenan injection and considered as zero time. The animals were then injected with 0.1 ml of 1% freshly prepared sterile carrageenan solution in saline into sub-plantar region of right hind paw of rats. [17] The contralateral paw received an equal volume of saline. The right hind paw thickness was measured from ventral to dorsal surfaces, with a dial caliper [18], after 0.5, 1, 1.5, 2, 2.5, and 3 hrs after the sub-plantar injection of carrageenan. The size of oedema which expressed as a percentage change in paw thickness (in mm) from control (pre-drug, zero time) and measured by Dial micrometer (M&W.Ltd, Sheffild, England) after carrageenan injection was recorded.

Hot plate test

The hot plate method described by Gupta et al. [19] was carried out to evaluate the analgesic activity of different formulations of microemulsion containing piroxicam. A hot-plate test was performed using an electronically controlled hotplate (Ugo Basile, Comerio, Italy) heated to 53°C (± 0.1°C). Certain amount of gel (100 mg) was applied topically to the hind paw of the mice. Thirty minutes after the drug administration, the gel remaining on the surface of the skin was wiped off with piece of cotton. Each mouse was placed unrestrained on the hot plate for baseline measurement just prior to saline or drug application and considered as zero time or control. Measurements of pain threshold for the treated animals were taken after 0.5, 1, 1.5, 2, 2.5 and 3 hours after drug application. Latency to lift and licking a hind paw or attempted to jump from the apparatus was recorded for the control and drug-treated groups. The cut-off time was 30 seconds to avoid further tissues damage from exposure to hot plate.

Statistical analysis

It was carried out by Student's t-test using Excel software and one-way analysis of variance (ANOVA) followed by Tukey-Kramer (post tests) using INSTAT software to determine the significance of the obtained results between the prepared Piroxicam Microemulsion Formulae and the conventional commercial Feldene Gel®.

The % of the effect (inhibition) was calculated by the following equation: = [(Control – drug)/ control] x 100.

RESULTS AND DISCUSSION

The Anti-inflammatory activity of piroxicam microemulsion:

Paw Edema Size induced by Carrageenan injection:

Topical treatment of the rats with piroxicam significantly inhibits the edema size induced by carrageenan injection into the sub-plantar area of the right hind paw for each rat.

It is observed from figures (1) and (2) that the groups that treated with piroxicam microemulsion formulae exhibit a maximum percent oedema inhibition after 1 hr (75.7% and 76.90%) respectively, which is lower than that of Feldene Gel® (77.6%) after 1 hr.

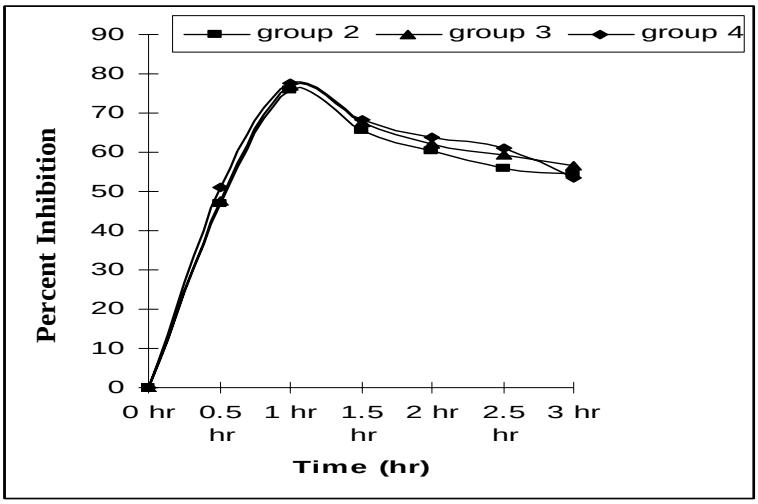

Fig. 1: Shows the Percent Oedema Inhibition by Topical Application of Different Piroxicam Gel bases.

Group 2( ) Treated group with piroxicam-microemulsion incorporated in MC gel.

Group 3( ) Treated group with piroxicam-microemulsion incorporated in HPMC gel.

Group 4 ( ) Treated group with Feldene Gel®

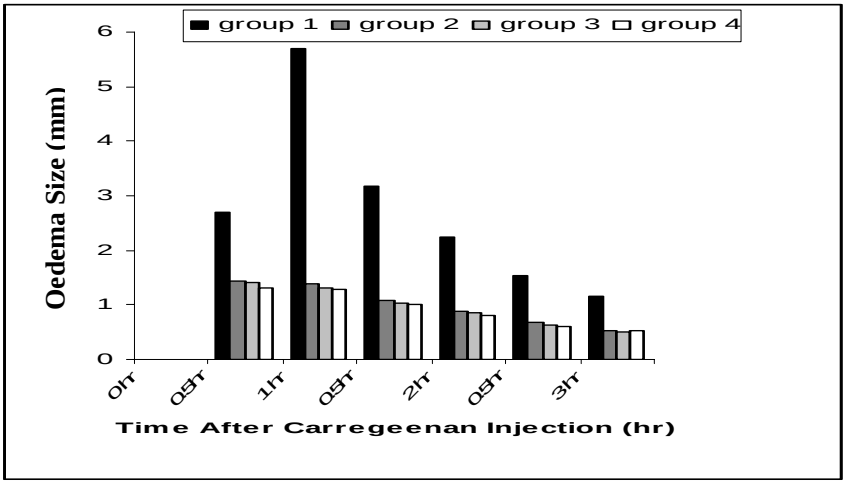

Fig. 2: Shows the anti-inflammatory effect of different piroxicam gel bases using paw oedema of rats induced by carrageenan injection

Group 1( ) Control group treated with non medicated gel base.

Group 2( ) Treated group with piroxicam-microemulsion incorporated in MC gel.

Group 3( ) Treated group with piroxicam-microemulsion incorporated in HPMC gel.

Group 4( ) Treated group with Feldene Gel®.

From the statistical analysis of the data using student's t. test using Excel software and one-way analysis of variance (ANOVA) followed by Tukey-Kramer (post tests) using Instate software we noted that all the investigated formulae were significantly inhibiting edema size and p value less than 0.05 were considered as significant as shown in table (2).

Table 2: Shows The Anova Analysis Of Anti-Inflammatory Effect Of Different Piroxicam Gel Bases.

Groups	Count	Sum	Average	Variance
Column 1	6	5.568	0.928	0.1112
Column 2	6	5.744	0.9573	0.1362
Column 3	6	6.011	1.0018	0.1367
Column 4	6	16.51	2.7531	2.6249

Source of Variance	SS	df	MS	F	P-value	F crit
Between Groups	13.65	3	4.5514	5.913	0.0046	3.0983
Within Groups	15.394	20	0.7697			
Total	29.049	23				

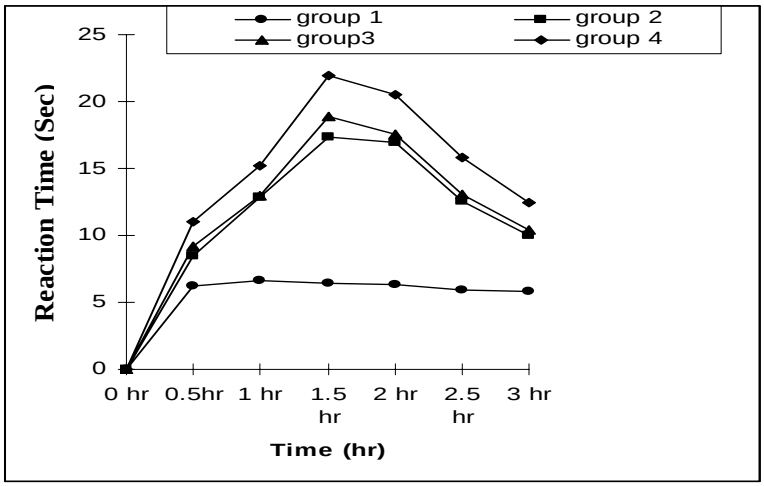

Fig. 3: Shows the mean reaction time in seconds versus time profiles in mice after topical application of different piroxicam gel bases.

Group 1(●) Control group treated with non medicated gel base.

Group 2(■) Treated group with piroxicam-microemulsion incorporated in MC gel.

Group 3(▲) Treated group with piroxicam-microemulsion incorporated in HPMC gel.

Group 4 (◆) Treated group with Feldene Gel®

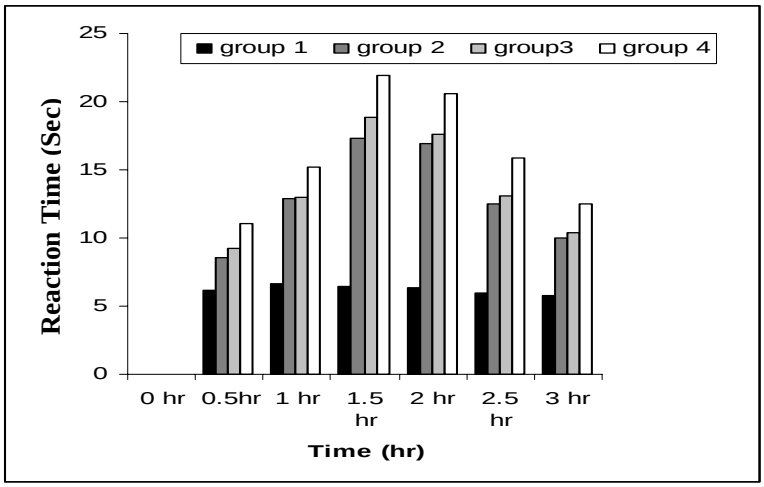

Fig. 4: Shows the mean reaction time in seconds versus time profiles in mice after topical application of different piroxicam gel bases

Group 1(■) Control group treated with non medicated gel base.

Group 2(■) Treated group with piroxicam-microemulsion incorporated in MC gel.

Group 3(□) Treated group with piroxicam-microemulsion incorporated in HPMC gel.

Group 4(□) Treated group with Feldene Gel®.

Table 3: Shows the Anova analysis of the analgesic effect of different piroxicam gel bases.

Groups	Count	Sum	Average	Variance
Column 1	6	97.07	16.178	18.581
Column 2	6	82.14	13.69	14.811
Column 3	6	78.16	13.026	12.648
Column 4	6	37.31	6.22	0.0088

Source of Variance	SS	df	MS	F	P-value	F crit
Between Groups	220.87	3	73.62	7.973	0.001	3.098
Within Groups	184.66	20	9.233			
Total	405.53	23				

The analgesic effect evaluation

Hot plate test

The analgesic effect of the piroxicam-microemulsion formulae in different gel bases were studied by Hot Plate method compared with both control group (group 1) and (animal treated group with Feldene Gel®) group 4.

From the obtained results we noted that the reaction time was significantly increased in animal groups pretreated with the piroxicam-microemulsion formulae in comparison with the group (1) but also it is lower than the reaction time in case of group (4).

It is observed from figures (3) and (4) that the group (2) and group (3) produced a maximum increase in reaction time (analgesic effect) after 1.5 hr (62.8% and 65.8% seconds) respectively, which is lower than that produced by Feldene Gel® (70.5% second after 1.5 hr), and then this analgesic effect was continued significantly for 3 hrs.

Also the statistical analysis the results of hot plat experiment by ANOVA reveals that all the formulae significantly increase the reaction time (analgesic effect) in mice and p value < 0.05 were considered as significant as shown in table (3).

CONCLUSION

After carrying out the experiments for evaluation of both the analgesic and the anti-inflammatory effects of piroxicam-microemulsion formula in different gel bases, it is clear that all the studied medicated gel bases have an acceptable analgesic and anti-inflammatory effect.

From the obtained results in this study, we can conclude that: Group 2 (animal groups treated with piroxicam-microemulsion incorporated in MC gel) and Group 3 (animal groups treated with piroxicam-microemulsion incorporated in HPMC gel) produced maximum percent oedema inhibition after 1 hr (75.7% and 76.90%), respectively, and then continued significantly for 3 hrs. Group 2 and Group 3 give the maximum analgesic effect after 1.5 hr (62.8% and 65.8% second) respectively. HPMC gel base containing piroxicam give the best analgesic and anti-inflammatory effect than MC gel base containing piroxicam respectively. Both HPMC and MC gel bases containing piroxicam showed a pharmacological response less than that obtained by the market product Feldene Gel® which is non significant.

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