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Anti-inflammatory and Analgesic Effects of Ketoprofen in Palm Oil Esters Nanoemulsion

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Abstract: Ketoprofen is a potent non-steroidal anti-inflammatory drug has been used in the treatment of various kinds of pains, inflammation and arthritis. However, oral administration of ketoprofen produces serious gastrointestinal adverse effects. One of the promising methods to overcome these adverse effects is to administer the drug through the skin. The aim of the present work is to evaluate the anti-inflammatory and analgesic effects from topically applied ketoprofen entrapped palm oil esters (POEs) based nanoemulsion and to compare with market ketoprofen product, Fastum® gel. The novelty of this study is, use of POEs for the oil phase of nanoemulsion. The anti-inflammatory and analgesic studies were performed on rats by carrageenan-induced rat hind paw edema test and carrageenan-induced hyperalgesia pain threshold test to compare the ketoprofen entrapped POEs based nanoemulsion formulation and market formulation. Results indicated that there are no significant different between ketoprofen entrapped POEs nanoemulsion and market formulation in carrageenan-induced rat hind paw edema study and carrageenan-induced hyperalgesia pain threshold study. However, it shows a significant different between POEs nanoemulsion formulation and control group in these studies at $p<0.05$. From these results it was concluded that the developed nanoemulsion have great potential for topical application of ketoprofen.

Key words: nanoemulsion, ketoprofen, palm oil esters

1 INTRODUCTION

The treatment of pain requires analgesics including inflammatory products. Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) and has been widely used for the treatment of rheumatoid arthritis and osteoarthritis[1)]. Although ketoprofen administered orally is rapidly absorbed, metabolized and excreted, it causes some gastrointestinal complaints such as nausea, dyspepsia and renal side effects like other NSAIDs. Therefore there is a great interest in developing topical dosage forms of ketoprofen to avoid the oral side effects.

Nanoemulsion was chosen as the topical dosage form because of the suitability for efficient delivery of active ingredients through the skin. The large surface area of the emulsion system allows rapid penetration of actives[2)]. In this study, palm oil esters (POEs) was selected as the oil phase of nanoemulsion. Palm oil is derived from the fruit of the palm tree *Elaesis guineenis* and consists of triglycerides, a combination of glycerol and different fatty acids[3)]. Alcoholysis of triglycerides from palm oil produces POEs that uses lipase as a catalyst in a relatively simple process[4, 5)]. POEs is new oil which is cheap but rich in esters and is a suitable candidate for jojoba oil, which is very expensive. This modified or synthesised oil (such as POEs) is a new ingredient for pharmaceutical industry[6-10)]. The oil is non irritant on human skin, increases the skin hydration due to its moisturizing properties, shows high thermal stability[3)] and could be used in cosmetic or medicinal formulations to deliver poorly water-soluble lipophilic actives or drugs[8, 9)].

Formulation of ketoprofen in POEs nanoemulsion and *in-vitro* evaluation through cellulose membrane were already reported in our earlier article[6)]. We have also reported the *in-vitro* evaluation through the rat skin[7)]. Hence, in this study we report, the anti-inflammatory and analgesic effects for ketoprofen from nanoemulsions produced by formulating the POEs as the oil phase and compared to a

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product available in market.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Carrageenan type IV (lambda), Tween 80® were purchased from Sigma (St. Louis, MO) Marketed formulation is Fastum® gel (A.Menarini Industrie Farmaceutiche Riunite, Italy) was bought from a retail pharmacy in Penang, Malaysia. Ketoprofen was purchased from Eurochem Asia limited, China. Palm oil esters (POEs) were provided by co-researchers in Universiti Putra Malaysia. Limonene was purchased from Sigma-Aldrich, Germany. Water used in this study was distilled water and all other solvents and chemicals were of analytical reagent grades.

2.2 Animals

Healthy Male Sprague-Dawley (SD) rats weighing 220-240 g were obtained from animal house facility, Universiti Sains Malaysia. The animals were housed in standard environmental conditions in solid bottom cage with top-ventilated stainless steel cover. The animals were allowed free access to water and standard rat chow (Gold Coin Sdn. Bhd., Selangor). The water bottles were cleaned and fresh water was replaced daily. The animals were allowed to move freely in their cages throughout the study period, to minimize stress in the animals. The cages were housed in animal transit room of school of pharmaceutical sciences at room temperature (22-26℃).

2.3 Preparation of nanoemulsion

Nanoemulsions were prepared by spontaneous emulsification process as reported by our earlier publication[6]. Palm oil esters and Tween 80® are left for 30 min under magnetic stirring 600 rpm at 25℃ to mix thoroughly. Then weighed amount of ketoprofen was added into the solution and mixed thoroughly, until a clear dispersion was formed, which indicated that all the drug solubilization is completed. To the resulting mixture water was added drop by drop while mixing with the aid of magnetic stirrer at 600 rpm and temperature of 25℃. The composition of ketoprofen entrapped nanoemulsion contains: 23.6% POE, 35.9% Tween 80®, 35% distilled water, 2.5% ketoprofen and 3% limonene.

2.4 Carrageenan induced rat hind paw edema test

The change in edema volume of the SD rat's hind paw was measured as described by Yam *et al.*, 2008[11]. Carrageenan was prepared as 1% (w/v) suspension in sterile 0.9% NaCl 1 h before experiment. The rats were divided into three groups as following: group [1] control group (untreated) [2] application of market formulation and group [3] application of ketoprofen entrapped nanoemulsion. The foot pad thickness was measured (Fig. 1) by placing the animal foot between the anvil and spindle of peacock dial thickness gauze (micrometer-Ozaki Ltd., Japan) (Fig. 1). To induce local inflammation, 50 μL of 1% carrageenan (w/v) was injected in to the plantar surface of the left hind paw of the rats. immediately after the carrageenan injection[12], ketoprofen entrapped POEs nanoemulsion, and market ketoprofen gel were applied to the plantar surface of the left hind paw of rats, by gently rubbing 50 times with index finger[13]. The hind paw thickness was measured before injection and subsequent readings of the same hind paw were carried out for 4 h at 2 h intervals and compared to the initial readings. The animals were maintained without access to food during the experiment period. The percentage in thickness changes of the hind paw was calculated according to the following formula:

$$\text{Percentage of increase}(\%) = (C_t - C_0)/C_0 \times 100$$

where,

C_t = Thickness of hind paw at t hour

C_0 = Thickness of hind paw before Carrageenan was injected.

2.5 Carrageenan-induced rat hind paw hyperalgesia pain threshold test

Carrageenan was prepared as 1% (w/v) suspension in sterile 0.9% NaCl 1 h before experiment. A sub-plantar injection of 50 μl of 1 % carrageenan (w/v) was performed on the SD rat hind paw. SD rats were divided into three groups and formulations were applied to these groups according to the method described in section 2.4. Pain threshold was measured mechanically (Fig. 2) using a potable pain threshold device (YMF-P1) equipped with data acquisition system (fabricated by Department of Pharmacology School of Pharmaceutical Sciences, USM, Malaysia)

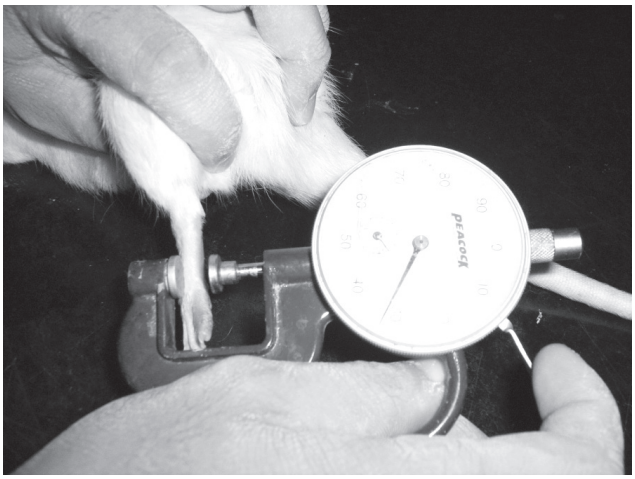

Fig. 1 Measurement of hind paw edema thickness of a SD rat by micrometer.

before the carrageenan was injected, and then at 2 and 4 h after injection of carrageenan. The stimulus force was recorded at which the rat withdrew its hind paw, and vocalization or struggle as an indication of pain threshold[14] (g). The sensor prob applied mechanically with an increasing strenght until the rat withdrew his paw. The maximum paw withdrawal threshold reached and the readings were recorded. From graphs three peaks, which exhibit similar heights were taken into reading and mean results were noted. The percentage change of pain threshold was calculated according to the following formula:

$$\% \text{ of change} = (P_t - P_0)/P_0 \times 100$$

where,

P_t = Pain threshold at t hour.

P_0 = Pain threshold before carrageenan was injected

2.6 Statistical analysis

The results were expressed as mean value ± S. D. Significance of differences between means was evaluated by one-way analysis of variance (ANOVA), followed by Dunnett post hoc test using SPSS software version 10. $P > 0.05$ were considered to be statistically significant.

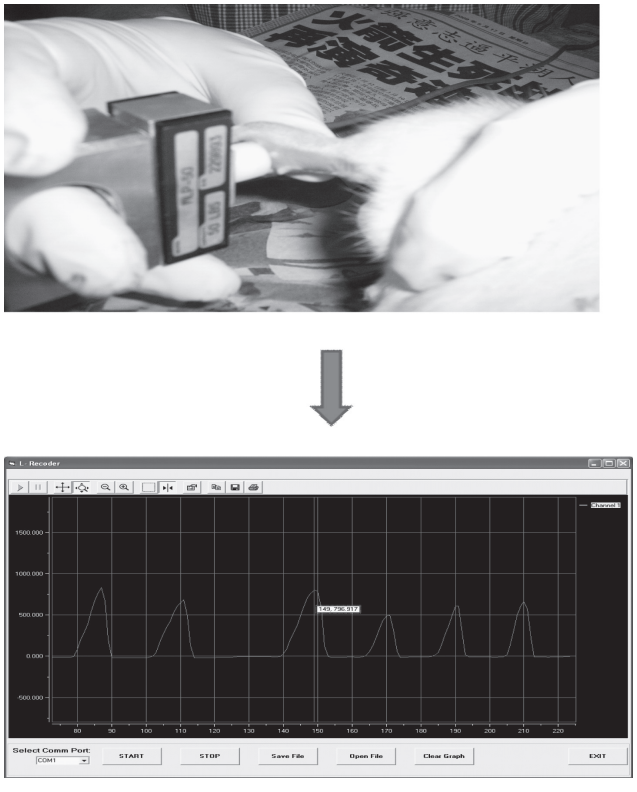

Fig. 2 The threshold pressure measurement taken by potable pain threshold device (YMF-PI)

3 RESULTS

In the results showed (Fig. 3) ketoprofen in both formulations suppressed the edematous response 2 h after carrageenan injection, and this effect persisted up to 4 h. Rats in control group, nanoemulsion and marketed formulations showed the inhibition of the edema at 2 h (70.36 ± 0.02%, 13.98 ± 0.02% and 7.42 ± 0.05% respectively) and 4 h (70.96 ± 0.03, 24.50 ± 0.01% and 33.22 ± 0.02% respectively). The results indicate that the ketoprofen entrapped

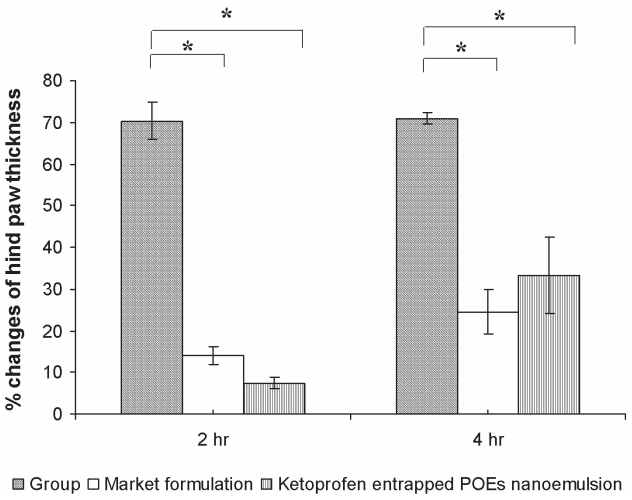

Fig. 3 Anti-inflammatory studies of carrageenan-induced hind paw edema test after topical application of marketed formulation, nanoemulsion and control groups.
* indicates $P<0.05$

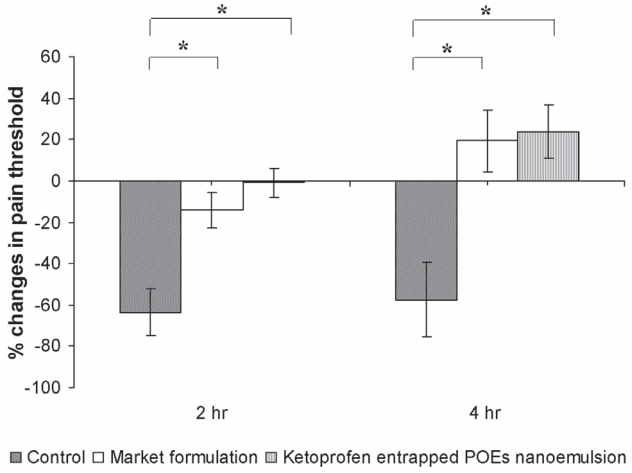

Fig. 4 Analgesic studies of carrageenan-induced threshold pressure test after topical application of marketed formulation, nanoemulsion and control groups.
* indicates $P<0.05$

POEs nanoemulsion and market formulations significantly reduced ($p<0.05$) carrageenan induced paw edema in rats compared to the control group. It is also noted that there is no significant different in paw edema thickness of rats which was treated with nanoemulsion and marketed formulations.

Ketoprofen entrapped POEs nanoemulsion significantly increase in the pain threshold at 2 and 4 h afeter carrageenan injection with blank formulation ($n=6$, $p<0.05$) (Fig. 4). Likewise marketed formulation also showed a significant increase in pain threshold level at 2and 4 h following carrageenan administration in control group (Fig. 4).

4 DISCUSSION

The aim of the present study is to evaluate the anti-inflammatory and analgesic effects of 2.5% ketoprofen entrapped POEs based nanoemulsion and to compare the effects with a marketed formulation. The strength of 2.5% ketoprofen in the POEs based nanoemulsion is selected because all of the topical products available in the market contain 2.5% of ketoprofen. The results of anti-inflammatory and analgesic study showed that ketoprofen entrapped POEs nanoemulsion formulation have the capability to improve the bioavailability of ketoprofen to bio-equalance with marketed formulation in rats. In our previous study[6] we reported that the developed formulation droplets size was found to be in the range of nanoemulsion. Droplets size of ketoprofen unloaded was 84.75nm and droplets size of ketoprofen loaded 229.64 nm. The increase in droplets size could be due to the drug loading in the oil droplets of nanoemulsion. The small size of the nanoemulsion droplets, which lead to the increase in total surface area for releaese, transfer and absorption of the drug. Earlier findings suggests[2] rapid penetration of actives through the skin can be achieved from nanoemulsion system because of the large surface area of the emulsion and small or nano droplets size. Hence nanoemulsions are suitable for efficient delivery of active ingredients through the skin[2].

We reported earlier the results of *in-vitro* release studies of ketoprofen through rat skin showed that the POEs nanoemulsion formulation containing 3% limonene as a skin penetration enhancer produced the effects which are better than the marketed formulation[7]. In the same article we mentioned, the incorporation of limonene as a skin permeation enhancer in the optimized formulation has dramatically increased the release properties of ketoprofen. The use of penetration enhancer is a valuable and important factor for achieving therapeutic plasma levels for many drugs. Terpenes have been used to increase the skin permeation of a large number of compounds and they reported to increase the drug diffusivity in stratum corneum and also drug partitioning into stratum corneum by disrupting the intercellular lipid bi-layers[15]. The intensity of their effects depends mainly on the lipophilicity of the drug and vehicle used[16].

Thus, from the present study it can be suggested that the enhancement of percutaneous absorption of ketoprofen observed in this study might be due to combination of the several mechanisms or factors. Larger total surface area arise from small droplet size and enhancement of drug through stratum corneum by limonene as a percutaneous permeation enhancer appeared to be related mechanisms which have contributed to increased the ketoprofen absorption to achieve similar anti-inflammatory and analgesic properties with marketed formulation.

5 CONCLUSION

The anti-inflammatory and analgesic effects of ketoprofen entrapped POEs nanoemulsion and market ketoprofen formulations were studied. The effects of ketoprofen in rats show the prepared nanoemulsion produced comparable and similar ex *in-vivo* properties with market product. This work reveals the suitability of palm oil esters as a new ingredient for the pharmaceutical industry to deliver ketoprofen by transdermal rout.

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