

Preparation and Evaluation of Aceclofenac Microemulsion for Transdermal Delivery System

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To develop novel transdermal formulation for aceclofenac, microemulsion was prepared for increasing its skin permeability. Based on solubility and phase studies, oil and surfactant was selected and composition was determined. Microemulsion was spontaneously prepared by mixing ingredients and the physicochemical properties such as investigated. The mean diameters of microemulsion were approximately 90 nm and the system was physically stable at room temperature at least for 3 months. In addition, the *in vitro* and *in vivo* performance of microemulsion formulation was evaluated. Aceclofenac was released from microemulsion in acidic aqueous medium, and dissolved amounts of aceclofenac was approximately 30% after 240 min. Skin permeation of aceclofenac from microemulsion formulation was higher than that of cream. Following transdermal application of aceclofenac preparation to delayed onset muscle soreness, serum creatine phosphokinase and lactate dehydrogenase activity was significantly reduced by aceclofenac. Aceclofenac in microemulsion was more potent than cream in the alleviation of muscle pain. Therefore, the microemulsion formulation of aceclofenac appear to be a reasonable transdermal delivery system of the drug with enhanced skin permeability and efficacy for the treatment of muscle damage.

Key words: Aceclofenac, Microemulsion, Skin permeation, Delayed onset muscle soreness

INTRODUCTION

Aceclofenac (2-[2,6-dichlorophenyl] aminobenzeneacetic acid carboxymethyl ester) is a nonsteroidal anti-inflammatory drug (NSAID), which has anti-inflammatory, antipyretic, and analgesic actions. Pharmacological studies have shown that the effect of aceclofenac on skeletal arthritis and rheumatic arthritis may be mediated by selective inhibition of prostaglandin E₂. In general, the most widely cited side-effect of NSAID, including aceclofenac, is the gastrointestinal ulcer. The side effect is also accompanied by anemia due to the bleeding. Since these side effects are typically pronounced after the oral administration of NSAIDs, formulations of NSAIDs, such as aceclofenac, that are applicable *via* non-oral routes of administration may be necessary to eliminate these side-

effect. Indeed, transdermal drug delivery of NSAIDs is feasible in recent years (Guy *et al.*, 1987). As expected, the system appeared to minimize gastrointestinal side effects and hepatic first-pass effect. Furthermore, the controlled or sustained release of the active ingredients may be achieved with an enhanced patient compliance (Cullander *et al.*, 1992). However, feasibility of transdermal delivery for aceclofenac has not been evaluated in the literature.

Microemulsion is generally defined as isotropic, transparent, thermodynamically stable mixtures of water, oil, and a surfactant; usually in combination with a cosurfactant, such as short chain alcohol derivatives. Microemulsions can be used as pharmaceutical formulation; oil-in-water microemulsions may be used as a carrier for water-insoluble drugs (Jeppson *et al.*, 1975; Mizushima *et al.*, 1982; Kronevi *et al.*, 1983), while water-in-oil microemulsions may also be applicable in sustained release of drugs as intramuscular preparations (Gasco *et al.*, 1990). In previous studies, flurbiprofen have been successfully formulated with microemulsion system aimed

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Table I. Composition of microemulsion containing aceclofenac with various oil phase

Preparations	Oil phase	Surfactant/Cosurfactant		Drug (g)	EtOH (g)	Water (g)
		Labrasol(g)/Transcutol(g)				
A	Oleic acid (9.5g)	42.75/42.75		3.0	1.0	1.0
B	Linoleic acid (9.5g)	42.75/42.75		3.0	1.0	1.0
C	Triacetin (9.5g)	42.75/42.75		3.0	1.0	1.0
D	Labrafac hydro (9.5g)	42.75/42.75		3.0	1.0	1.0
Cream	Oleic acid (14.0)	20.0/1.0		3.0	1.0	61.0

for transdermal delivery (Lee *et al.*, 1998).

Muscle damage can be developed by various reasons such as direct physical shock to muscle (Javinen, 1975; Sorvari *et al.*, 1975), constant hypoxia state due to the block of blood supply (Hanzlikova *et al.*, 1979), long-term continuous severe exercise (Greenberg *et al.*, 1967), and injection of harmless medicines (Gray, 1967). Injured muscle goes through the normal restoration and regeneration processes. Delayed onset muscle soreness (DOMS) is symptom which displeasure or pain occurring in skeletal muscle due to the use of unaccustomed muscle. After exercise it happens in between 8~12 hours, reaches to the maximum in 48 hours, and prolongs for several days. The change of muscle cell membrane permeability and the damage of muscle cell are the biochemical responses after severe exercise. However, changes after DOMS has not been described in detail, LDH and CPK are being analyzed most commonly in order to monitor the DOMS.

In this study, we have studied the feasibility of new formulation for aceclofenac that reduces the side-effect of the drug while maintaining sustained actions and improving patient compliance. We have prepared microemulsion containing aceclofenac and examined the dissolution and skin penetration of aceclofenac from microemulsion *in vitro*. The selected formulation was applied to human volunteers with experimental DOMS, and the efficacy of the new formulation was tested.

MATERIALS AND METHODS

Aceclofenac was supplied by Kyung-Dong Pharm Ind. (Seoul, Korea). Diethylene glycol monoethyl ether (Transcutol®), polyoxyethylene (4) lauryl ether (Brij® 30)(2:1) oleoyl macroglycerides EP (Labrafil®), Linoleic acid and oleic acid, caprylic/capric triglyceride polyethylene glycol-4 complex (Labrafac hydro®) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade and used without further purification.

Based on the results of solubility and phase studies, adequate ratio of each component in microemulsion was determined. Microemulsions were prepared by mixing major ingredients (i.e., oils, surfactant and cosurfactant). Oleic acid, linoleic acid, Triacetin and Labrafac were used as oil phase and Labrasol was used as surfactant. Transcutol was mixed with cosurfactant for enhancement skin permeability of aceclofenac. The cream, a control formulation for aceclofenac, was prepared containing oleic acid, surfactant and large amount of water (Table I). Determination of aceclofenac

Based on the results of solubility and phase studies, adequate ratio of each component in microemulsion was determined. Microemulsions were prepared by mixing major ingredients (i.e., oils, surfactant and cosurfactant). Oleic acid, linoleic acid, Triacetin and Labrafac were used as oil phase and Labrasol was used as surfactant. Transcutol was mixed with cosurfactant for enhancement skin permeability of aceclofenac. The cream, a control formulation for aceclofenac, was prepared containing oleic acid, surfactant and large amount of water (Table I).

The amount of aceclofenac was quantified using a HPLC system consisting of solvent delivery system (M 930, Young Lin, Korea), C₁₈ reverse phase column (WAT 027324, Waters Co., Milford, Massachusetts, USA), multi-wavelength detector (M 720, Young Lin, Korea) and integrator (D520B, Young Lin, Korea).

Dissolution of aceclofenac from microemulsion

The dissolution test was performed in dissolution apparatus according to KP dissolution procedure. Three milliliters of prepared microemulsion were matted into the closed dialysis sac (molecular weight cut off=12,000). Then the sac was loaded in the 500 mL of the dissolution media at 37 ± 0.5°C with stirring at 100 rpm. At designated times, an aliquot of the dissolution media was withdrawn and an equal volume of temperature equilibrated media was replaced. The concentration of aceclofenac was determined by HPLC.

Skin permeability of microemulsions

The skin was removed from the back of male Sprague-Dawely rats weighing 220 ± 10 g (Dae-Han animal Co. Dae-Jeon, Korea). After removal of subcutaneous fat and blood vessel, skin of 2 inch \times 2 inch in size was fixed to Franz diffusion cell. Aceclofenac preparation was applied in donner phase, while receptor phase was filled with each physiological salt solution, and maintained the tem-perature of 37°C. In this study, the effective expansion area contacting with receptor phase was 2.54 cm². At every designated time, 0.5 mL of solution was collected from receptor phase and assayed for aceclofenac concentration.

The accumulated amount of aceclofenac administered per unit area of the skin was represented with function corresponding time. Lag-time method was used to investigate dynamic state of drugs through the skin. Permeation rate and lag-time was calculated by using following formulars.

$$J_s = \frac{1}{A} \left(\frac{dQ}{dt} \right)_{ss} = \frac{DKC}{h}$$

$$D = \frac{h^2}{6T_L}$$

J_s = permeation rate at the equilibrium state,
 A = permeation skin area,
 $(dQ/dt)_{ss}$ = the amount of drugs passing the membrane per unit time at the equilibrium state,
 C = concentration of drugs in donner compartment,
 K = distribution coefficient of drugs,
 h = thickness of membrane,
 D = diffusion integer of drugs through skin,
 T_L = lag-time

Delayed onset muscle soreness (DOMS)

Physically healthy male volunteers were selected. The human subjects were not allowed to drink liquors or take medicines tha may influence the result of the experiment. Their average age was 24.5 years old.

DOMS was induced by excercise using dumb-bells. Dumb-bell exercise was performed with the arm of non-dominant hand until they feel fatigue. One-repetition maximum was from 8.5 to 11.5 kg and the frequency of exercise was from 35 to 52. Before and after exercise, blood was collected in basilica vein. Activity for creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) was measured by using assay kit (Asan Co.).

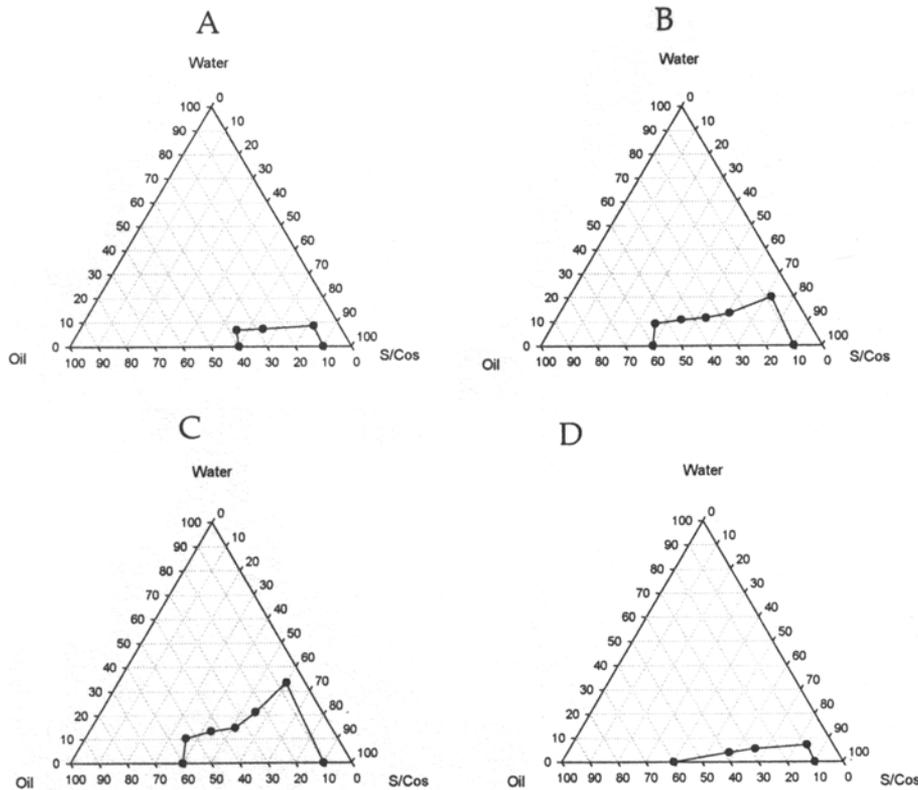


Fig. 1. Phase diagram of mixture of oil, surfactant/cosurfactant and water used in making microemulsion. Sufractant/cosurfactant was Labrasol:Transcutaol=1:1

- A) Oleic acid
- B) Linoleic acid
- C) Triacetin
- D) Labrafac

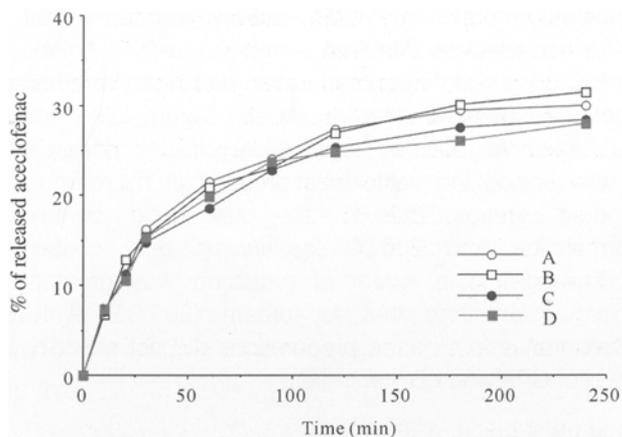


Fig. 2. Dissolution profiles of aceclofenac from micemulsion in pH 5.5 buffer solution
 A : Preparation A B : Preparation B
 C : Preparation C D : Preparation D

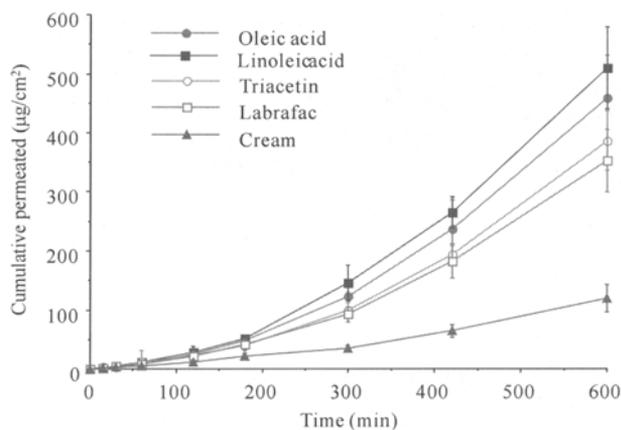


Fig. 3. Cumulative skin permeation of aceclofenac through rat skin. Each bar represents the mean \pm SE from 3 experiments

RESULTS

Preparation and stability of microemulsion

The solubility of aceclofenac was determined in each component of microemulsion systems such as surfactant, cosurfactant and oil and water. Aceclofenac was insoluble in water and sparingly soluble in oil. It was soluble in surfactants, Labrasol was the best solvent for aceclofenac among the surfactants tested for solubility. Transcutol, an enhancer for skin permeability, was the good cosolvent for aceclofenac. Therefore, Labrasol/Transcutol was chosen as surfactant/cosurfactant. The solubility of aceclofenac in Labrasol and Transcutol was 395.76 ± 2.71 mg/mL, 288.15 ± 8.15 mg/mL respectively.

Prior to the preparation of microemulsion with aceclofenac, phase diagram of oil-water-surfactant was constructed. As indicated in the previous section,

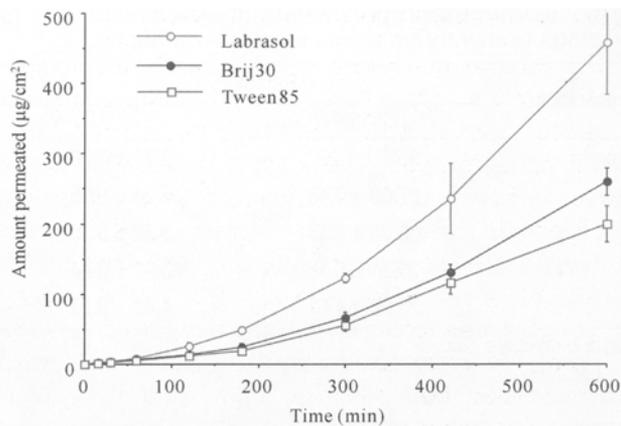


Fig. 4. Effects of surfactant in cumulative skin permeation of aceclofenac through rat skin

Labrasol/ Transcutol was used as surfactant mixture. The influence of weight ratio of surfactant to cosurfactant on the area of o/w microemulsion region was investigated on the pseudo-ternary phase diagram. Fig. 1 illustrates phase equilibrium diagram for four mixtures with different oil phases. There was no difference among phase-diagrams of those mixtures, indicating that oil did not affect the phase of oil-water-Labrasol/transcutol mixture.

Microemulsions with four types of oil were prepared according to the prescription of Table I. Average diameter of microemulsion was found to be approximately 90 nm; In this study, the minimum diameter was found for Triacetin (82.1 nm) while the maximum diameter was for oleic acid (97.5 nm). However, no statistical difference was found for the diameter.

When the physical stability was observed, all microemulsions showed no separation of the phase for three months, indicating that the preparation has a reasonable physical stability.

Dissolution of aceclofenac from microemulsion

The dissolution of aceclofenac from microemulsion was studied at pH 5.5 medium by using dialysis membrane method (Fig. 2). Dissolution of aceclofenac from four types of microemulsion was similar temporal profile and there were no appreciable differences in amount of released. In all microemulsion formulations, amount of aceclofenac dissolution was approximately 30% at 240 min.

Skin permeation of aceclofenac

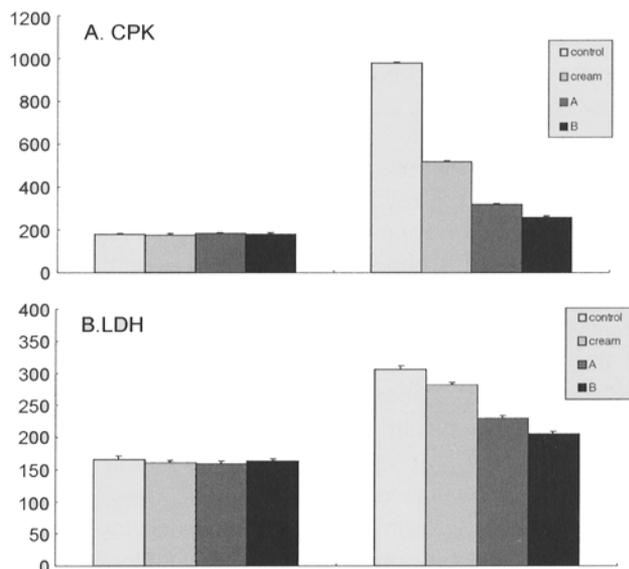
Fig. 3 illustrates the effect of oil affecting drug permeation through rat skin. The permeation of aceclofenac was slow initially, but showed a drastic increase in flux in 5 hours. In linoleic acid- and oleic acid-containing microemulsion, they showed relatively high

Table II. Permeation parameters of aceclofenac through rat skins from various aceclofenac preparations.

Preparation	Parameters	
	J_s ($\mu\text{g}/\text{cm}^2/\text{hr}$)	T_L (hr)
Cream	5.78 ± 1.29	2.71 ± 0.28
A	27.69 ± 0.15	3.24 ± 0.05
B	32.05 ± 9.17	3.57 ± 0.75
C	25.24 ± 5.18	3.91 ± 0.44
D	25.99 ± 4.61	4.42 ± 0.27

J_s ; Steady-state flux

T_L ; Lag-time

**Fig. 5.** Effect of aceclofenac preparation on delayed onset muscle soreness.

A. Serum creatine phosphokinase (CPK) activity (IU/L)

B. Serum lactate dehydrogenase activity (LDH) activity (IU/L)

permeation rate than those with triacetin and Labrafac emulsion. The influence of surfactant on skin permeation rate was the highest in the case with Labrasol (Fig. 4).

Skin permeation parameters are summarized in Table II. Permeation rate of aceclofenac in microemulsion was found to be 4- to 5- fold higher than that in cream.

Effect of aceclofenac microemulsion on DOMS

After the application of different aceclofenac preparations, the change of intergroup serum CPK activity was investigated (Fig. 5A). In control group, CPK activity was 172.23 ± 9.29 (IU/L) before exercise and the activity increased to 979.67 ± 37.69 (IU/L) 24 hours after the exercise. However, in microemulsion treated groups, CPK activity at 24 hr after exercise was 316.67 ± 10.60 (IU/L), 258.33 ± 7.02 (IU/L) in oleic acid- and linoleic acid microemulsions, respectively. Cream formulation also

showed a reduction in CPK activity, but the extent of reduction was less than that of microemulsions. As shown in Fig. 5B, aceclofenac preparation had a similar effect on the LDH increase after exercise. Serum LDH activity increased to 306.00 ± 11.53 (IU/L) in 24 hours after exercise and the value was reduced in microemulsion treated groups (229.33 for oleic acid containing formulation and 206.00 for linoleic acid containing formulation). The extent of reduction was greater for microemulsion than that for cream (281.2 ± 7.9 IU/L). Aceclofenac in all three preparations did not affect basal level of CPK and LDH activities.

DISCUSSION

Microemulsion is a superfine emulsion which consists of particle of less than 100 nm in diameter. Microemulsion as a potential drug delivery systems has been received attention in the recent years. The use of this approach may be application not only for improvement of the therapeutic efficacy of drugs but also for a reduction in the total dose needed and/or minimization toxic side effects. In this study, we report that a microemulsion based topical formulation may be feasible for the transdermal delivery of aceclofenac with enhanced skin permeability and efficacy.

In order to achieve a maximum dissolution aceclofenac in microemulsion, the solubility of aceclofenac to oils and surfactants was tested. Phase studies were carried out to investigate the effect of surfactant/cosurfactant ratio on the extent of stable o/w microemulsion region. The microemulsion in the present study formed spontaneously at ambient temperature when their components were mixed. Based on the solubility and phase studies, Labrasol was found to be a reasonable surfactant for preparation of microemulsion containing aceclofenac. Since the surfactant is non-ionic in nature, the compound has a reasonable safety and is less likely to be affected by pH and ionic strength (Constantinides, 1995). Cosurfactant Transcutol is known to enhance the permeability of drug. Our aceclofenac solubility study indicated that various oils, oils can be divided two groups based on solubilization capacity. In order to investigate the various effects of the solubility of oil on microemulsion, four oils was selected; oleic acid and linoleic acid were found to be a poor solvent while triacetin and labrafac may be categorized as a good solvent. However, there was no difference in phase behavior and particle size of prepared emulsion.

In this study, the dissolution of aceclofenac in microemulsion was investigated. Microemulsion was placed inside the dialysis membrane, and the amount of drug recovered to outer aqueous phase was monitored. Since the skin is acidic, an acidic buffer (i.e., pH 5.5) was used as medium. Drastic increase in dissolution was shown in

60 minutes, and less than 30% of drug was released in 240 min. This observation suggested that the low rate of dissolution may be mediated by the restriction of free dissolution of drug. In the dissolution study, the diffusion rate of drug from oil phase to water phase is an important factor. Thus, we expected to see the difference in dissolution between microemulsions with oils with high and solubilization capacity. Again, there was no difference in dissolution among the microemulsion preparations with different oils. Since the solubility of aceclofenac in surfactant/cosurfactant is sufficiently high, types of oil is not expected to affect the solubility and diffusion of aceclofenac to aqueous phases.

Skin permeation of aceclofenac was high for oils with low solubilization capacity (oleic acid and linoleic acid), and relatively low for oils with high solubilization capacity (triacetin and Labrafac). Oleic acid and linoleic acid are also known to improve the skin permeation of drugs. Although exact mechanism of the improvement is not directly studied, oleic acid or linoleic acid may enhance skin diffusion of the drug by altering fluidity of fat layer. Therefore, it appears reasonable to speculate that increase in fluidity of the stratum corneum rather than the solubility of aceclofenac in the formulation is the primary mechanism of improvement in aceclofenac penetration from the microemulsions across the rat skin. Transcutol is known to facilitate the dissolution of non-soluble drugs and may be used in topical preparations such as ointment, cream and lotion products; In addition, the compound is also known to have skin permeation enhancing action. Labrasol is also a good solvents to non-soluble materials and has a skin permeation enhancing action. These compounds are widely used in the preparation of transdermal formulations.

In general, CPK activity is known to be increased after an acute exercise for a short period of time. The cellular permeability is apparently increased by the muscle damage created by the excessive exercise. The permeability change, in turn, may lead to an increase in the leakage of CPK to the interstitial fluid. As a result, CPK activity increased considerably after 24 hours in case of muscle soreness.

It has been indicated that LDH activity appears to be a good parameter for muscle tissue damage index (Lee Y. E 1997). The activity is also a reasonable index for an exercise ability measurement, since the value represents the activity of energy metabolism. LDH is closely associated in the formation of lactic acid during anaerobic metabolic process. After a severe exercise, as lactic acid is increasing in the tissue, LDH activity is likely to increase with cellular lactic acid level. In this respect, the function of LDH has an important implication because lactic acid can be a primary cause of stress or pain in muscle after a

severe exercise (Dixon and Webb, 1979). In this study, CPK and LDH activity reduced considerably after the application of aceclofenac formulation as compared with the control group. More importantly, aceclofenac in microemulsion showed better efficacy in the restoration of muscle damage as evidence by the reduction in CPK and LDH activities.

In summary, we have demonstrated that skin permeation of aceclofenac from microemulsion formulation was significantly higher than that from conventional cream formulation. In addition, CPK and LDH activity reduced considerably after the application of aceclofenac formulation in exercise models. Therefore, these observations indicate that transdermal delivery of aceclofenac may be feasible with a microemulsion formulation.

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