

Cyclic Monoterpene Extract from Cardamom Oil as a Skin Permeation Enhancer for Indomethacin: *In Vitro* and *In Vivo* Studies

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The *in vitro* and *in vivo* effect of pretreatment by cardamom oil, a crude drug extract, in ethanol/water vehicles on the transdermal delivery of indomethacin was investigated. The cyclic monoterpene components in cardamom oil were also determined and quantified in this study. The permeation of indomethacin was significantly enhanced after pretreatment of cardamom oil both in the *in vitro* and *in vivo* studies. The result of various pretreatment periods showed that the indomethacin flux decreased as the length of the pretreatment increased. Both natural cardamom oil and a cyclic monoterpene mixture composed of the components of the oil showed similar enhancement on indomethacin permeation, indicating cyclic monoterpenes are the predominant components altering the barrier property of stratum corneum. The results also showed that three minor components in cardamom oil (α -pinene, 6.5%; β -pinene, 4.8%; α -terpineol, 0.4%) had a synergistic effect with 1,8-cineole (59.3%) and *d*-limonene (29.0%) to enhance the permeation of indomethacin.

Key words indomethacin; cardamom oil; cyclic monoterpene; transdermal delivery

Many investigations have examined the possibility of the percutaneous delivery of drugs, but a major difficulty is the impermeability of stratum corneum. An effective method employed to reduce this diffusional barrier is to use permeation enhancers. Considerable research is now in progress on the use of crude drug extracts as permeation enhancers to improve drug permeation. These enhancers include: fatty acid extract from cod-liver oil and volatile oils from anise, ylang-ylang, chenopodium, eucalyptus and cardamom.^{1–5)}

Natural volatile oils are commonly of low cutaneous irritancy and are therefore good candidates for skin permeation enhancement.⁶⁾ To date, most investigations have focused on the monoterpene constituents of volatile oils. We have developed cardamom oil from the seed of *Amomum cardamomum* (Zingiberaceae) as a potential permeation enhancer for a number of drugs including piroxicam, diclofenac sodium and indomethacin.^{4,5,7)} Cardamom oil is commonly used as a flavor and it possesses pharmacological activities of antispasmodic, anti-inflammatory, analgesic and antimicrobial actions.^{8,9)}

The aim of this study is to further investigate the effect of cardamom oil on the *in vitro* and *in vivo* transdermal permeation of indomethacin gels using rabbit as an animal model. For liquid permeation enhancers, incorporating a large quantity into the original formulation would influence the viscosity, solubility and other physicochemical properties of the dosage form. Pretreating the skin with permeation enhancers before application of a drug would be a good method to promote its permeation.^{10,11)} Accordingly, the duration of pretreatment period, the concentration of cardamom oil and the pretreatment vehicles were evaluated to optimize and maximize the permeability of indomethacin. The cyclic monoterpene components in cardamom oil were also determined and quantified in this study, and the enhancing effect of the simulated cardamom oil made in our laboratory was compared with that of natural cardamom oil.

MATERIALS AND METHODS

Materials Indomethacin, *p*-phenylphenol, 1,8-cineole and *d*-limonene were purchased from Sigma Chemical Co. (U.S.A.). α -Pinene was supplied by Aldrich Chemical Co. (U.S.A.). β -Pinene was purchased from Tokyo Chemical Industries, Ltd. (Japan). α -Terpineol was obtained from Janssen Chemical Co. (Belgium). Indomethacin gel (Inteban[®]) was a gift from Sumitomo Chemical Co. (Japan). Commercially available formulation 1 and 2 were gifts from two pharmaceutical corporations of Taiwan. The extraction method of cardamom oil was reported previously.¹²⁾ All other chemicals and solvents were of analytical grade.

Determination and Quantification of Cyclic Monoterpene Components from Cardamom Oil The relative percentage of cardamom oil components was calculated based on their peak areas obtained from integration of the GC chromatograms. GC was performed on a Varian STAR 3400 CX GC with a Varian STAR Chromatography Workstation 3.0 computer software for integration and a flame ionization detector (FID). Nitrogen was used as carrier gas at a flow rate of 0.9 ml/min. DB-Petro 100 column was used in the GC analysis. Programmed column conditions were an initial temperature of 68 °C which was then raised 20 °C/min to 160 °C. Injector and detector were set at 250 °C.

***In Vitro* Permeation Study** The diffusion cell used in this *in vitro* study was Franz vertical diffusion assembly. The abdominal skin of male Wistar rat (6—8 weeks old; 200—250 g), male New Zealand rabbit (12—14 weeks old; 2.5—3.0 kg) and the whole adult human skin (41—45 years old) obtained from breast reduction operations were used as the barrier membranes. One milliliter of cardamom oil in different vehicles was applied to the skin surface for a specific time by the occlusion dressing technique. The applied area was then gently swabbed clean with cotton to remove the residual solution. Two grams of indomethacin gel (1%, w/w) was then applied to the treated skin membrane. The area of the skin available for permeation was 2.54 cm². The donor compartment was covered with parafilm. The receptor com-

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partment contained 20 ml pH 7.4 phosphate buffer with 10% (v/v) PEG 400 as a solubilizing agent. The temperature of the receptor cell was maintained at 37 °C. Samples (0.5 ml) were withdrawn from the receptor at regular intervals and an equal volume of fresh receptor solution was added. Samples were assayed using HPLC system.

The cumulative amount of the drug permeated through the skin was plotted as a function of time and a linear regression analysis was used to determine the flux (J) of indomethacin. The effectiveness of cardamom oil pretreatment was determined by comparing the flux of pretreated skin (J_p) to that for untreated skin or control group (J_0). This was defined as the enhancer index (EI):

$$EI = J_p / J_0$$

In Vivo Permeation Study The hair of rabbit was removed from the skin of the abdominal region. A piece of cotton cloth (6×10 cm²) was moistened with 10 ml of cardamom oil vehicle, and the cloth was then applied to the shaved surface for a specific time by the occlusive dressing. Subsequently the wet dressing was peeled off and the applied area was swabbed clean to remove the residual solution. An accurately weighed 6 g of indomethacin gel was spread uniformly over a sheet of cloth (6×10 cm²) and applied to the treated skin area. Arterial blood samples were withdrawn from the central ear artery.

Plasma Analytical Procedure of Indomethacin A 1-ml aliquot of plasma was pipetted into a glass-stoppered centrifuge tube, along with 1 ml of McIlvaine buffer (pH 4) and 50 μl of internal standard solution. The mixture was shaken by vortex and extracted with 7 ml of ether-cyclohexane (8:2) by mechanical shaking for 20 min. After centrifugation for 10 min at 3000 rpm, 5 ml of the mixed solution was transferred to another tube and evaporated to dryness on a water bath at 40 °C. The residue was redissolved with the mobile phase of HPLC system. The HPLC analysis of indomethacin has been described previously.⁴⁾

RESULTS AND DISCUSSION

Quantification of Cyclic Monoterpene Components from Cardamom Oil The volatile oils obtained by steam distillation from *Amomum cardamomum* are well known to contain high amounts of cyclic monoterpenes.³⁾ Table 1 lists the constituents in the volatile oil of cardamom seeds. 1,8-Cineole was the main component of the cyclic monoterpenes, followed by *d*-limonene, α -pinene, β -pinene and α -terpineol. This profile was somewhat different from that of the previous studies which showed a trend of 1,8-cineole > *d*-limonene > α -terpineol > α -pinene in the components of cardamom oil extracted from the seeds of *Elettaria cardamomum*.^{13,14)} It may be due to the difference of plant origin and extraction method of these cardamom oils.

Permeation of Indomethacin Gel through Various Skin Types In order to select a suitable animal model for indomethacin in the present study, the skin of Wistar rat and New Zealand rabbit, two animal models commonly used in the *in vivo* experiments, was used as the membrane in this *in vitro* permeation study to compare the permeabilities of indomethacin with that through human skin. The cumulative amount-time profiles of indomethacin through various types

Table 1. Percentage of Cyclic Monoterpene in Cardamom Oil Derived from *Amomum cardamomum*

Component	Percentage (%)
α -Pinene	6.5
β -Pinene	4.8
<i>d</i> -Limonene	29.0
1,8-Cineole	59.3
α -Terpineol	0.4

Each value represents the mean.

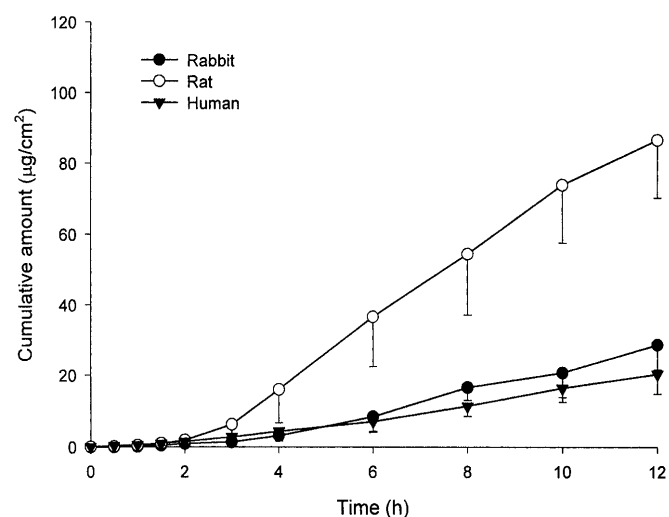


Fig. 1. Cumulative Amount-Time Profiles of Indomethacin from Inteban® Gel across Various Skin Types

Each value represents the mean ± S.D. ($n=3$).

of skin from Inteban® gel ointment are shown in Fig. 1. The permeability increased in the order of human \leq rabbit < rat. Rodent skins are known to be generally more permeable than is human skin.¹⁵⁾ In addition, human breast skin showed a lower permeation rate than the other anatomic sites.¹⁶⁾ Since the permeability of indomethacin through rabbit skin was close to that through human skin (Fig. 1), the rabbit was used as the animal model for the following *in vitro* and *in vivo* experiments.

In Vitro Permeation Study The ethanol/water mixtures incorporated with cardamom oil were used as pretreatment vehicles for the *in vitro* permeation study. Firstly, the influence of pretreatment of ethanol proportions on transport behavior of indomethacin was investigated over the 30–70% ranges. The permeation of indomethacin from Inteban® gel ointment after 30 min pretreatment of ethanol/water vehicles decreased in the order of 50% (ethanol proportion, w/w) > 30% > 70% (Fig. 2, blank column). There was no significant difference (t -test, $p > 0.05$) between the indomethacin flux of the non-pretreatment group and the 30% ethanol/water pretreatment group, indicating 30% ethanol pretreated for 30 min is not enough to enhance the drug's permeability. The highest flux of indomethacin at a concentration of 50% ethanol pretreatment may be related to the increased reduction of diffusion barrier by extracting stratum corneum lipids and proteins.¹⁷⁾ At larger ethanol proportions (70%), the decrease in skin permeation due to dehydration is the dominant effect.¹⁸⁾ The outer proportion of the stratum corneum is dehydrated after treatment of higher ethanol proportion, and

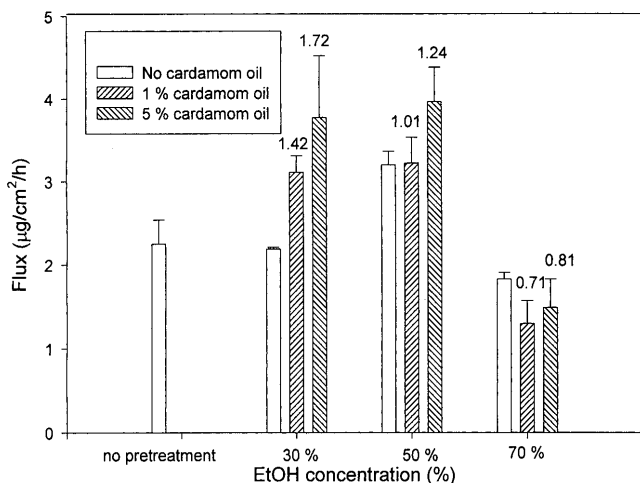


Fig. 2. The *in Vitro* Flux of Indomethacin from Inteban® Gel after Pretreatment of Cardamom Oil in Various EtOH/Water Vehicles for 30 min

Each value represents the mean \pm S.D. ($n=3$).

Table 2. The *in Vitro* Flux ($\mu\text{g}/\text{cm}^2/\text{h}$) of Indomethacin from Inteban® Gel after Pretreatment of Cardamom Oil for Various Periods and Concentrations

Pretreatment period (min)	1%	5%
5	10.84 \pm 2.34	14.19 \pm 4.41
15	5.27 \pm 2.96	7.62 \pm 1.46
30	3.11 \pm 0.20	3.77 \pm 0.75

Each value represents the mean \pm S.D. ($n=3$).

this dehydrated portion would have increased barrier properties of skin.

The flux of indomethacin after pretreatment with ethanol/water vehicles in the presence of 1% and 5% (w/w) cardamom oil is shown in Fig. 2. The number above the column indicates the value of the enhancer index (EI). There is no or a negative effect of cardamom oil pretreatment in 70% ethanol/water vehicle. Moreover, the enhancement of cardamom oil at 5% concentration was higher than that at 1%. Our previous report had shown that the amount of cardamom oil present in the skin is an important factor in the enhancing effect.⁴⁾ In earlier studies with indomethacin as a lipophilic permeant model, drug absorption is markedly enhanced by the addition of *d*-limonene, while hydrophilic terpene enhancers show minor effects.¹⁹⁾ Hence *d*-limonene in cardamom oil may significantly promote the permeation of indomethacin in this study. As a possible mechanism for the enhancement action of *d*-limonene and ethanol, the increase of *d*-limonene concentration is directly proportional to the accumulation of ethanol in the skin.²⁰⁾ So it is considered that *d*-limonene penetrates into the skin when it coexists with ethanol and may change the barrier structure of the stratum corneum.

Cardamom oil (1% and 5% in 30% ethanol/water vehicle) was applied to the donor side as a pretreatment solution for various periods from 5 to 30 min, and the indomethacin flux decreased following the increase of cardamom oil pretreatment period (Table 2). This may indicate that longer duration of the pretreatment period results in a greater extent of barrier property to the stratum corneum. Further investigation is needed and is in progress exploring the mechanism of this unusual result. A 5 min pretreatment period was enough to

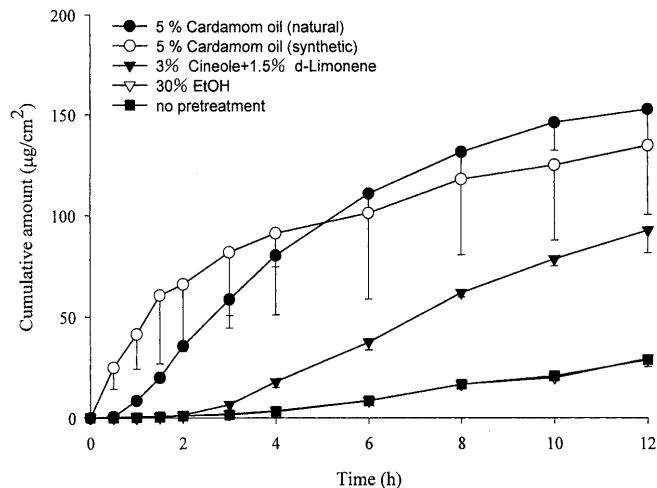


Fig. 3. Cumulative Amount-Time Profiles of Indomethacin from Inteban® Gel after Pretreatment of Various Cardamom Oil Vehicles for 5 min

Each value represents the mean \pm S.D. ($n=3$).

achieve the maximum level of the enhancing effect, suggesting that cardamom oil distributes in or attacks the surface of skin very quickly. This phenomenon was confirmed by the previous research which shows that *d*-limonene rapidly partitions into the skin after its application.²¹⁾

To verify the enhancing effect of cardamom oil on indomethacin permeation, a mixture was made up in our laboratory simulating the cyclic monoterpene components of cardamom oil (Table 1). The difference of indomethacin permeability between pretreatment of natural and simulated cardamom oil (5%) is compared in Fig. 3. Indomethacin showed lower permeation in the natural cardamom oil pretreatment group during the initial 6 h following application; thereafter the cumulative amount of indomethacin in receptor significantly increased and eventually surpassed that of the simulated cardamom oil pretreatment group. This result may indicate the importance of non-cyclic monoterpene contents in the natural cardamom oil. These contents may be responsible for the difference of *in vitro* indomethacin permeation kinetics between simulated and natural oil treatments. To compute the total amount of indomethacin permeated, the area under the curve (AUC_{0-12h}) from flux (J)-time profiles was calculated. The result showed there was no significant difference (*t*-test, $p < 0.05$) between the AUC_{0-12h} values of natural ($168.21 \pm 32.61 \mu\text{g}/\text{cm}^2$) and simulated ($137.32 \pm 43.42 \mu\text{g}/\text{cm}^2$) cardamom oil pretreatment groups. This indicates that cyclic monoterpenes in cardamom oil are the predominant components enhancing the permeation of indomethacin.

1,8-Cineole and *d*-limonene were mixed in a 2 : 1 ratio as a pretreatment vehicle, based on the composition ratio of these two major components in cardamom oil (Table 1). As shown in Fig. 3, the enhancing effect of indomethacin permeation after pretreatment of this mixture was significantly lower (*t*-test, $p < 0.05$) than that of 5% cardamom oil, suggesting that the enhancing action of the oil results from the synergistic effect of other lower percentage cyclic monoterpenes it contains. A similar result was observed for the enhancing effect of fatty acids in cod-liver oil extract on the buccal permeation of ergotamine tartrate.²²⁾

In Vivo Permeation Study To ascertain the enhancing

Table 3. The *in Vivo* Pharmacokinetic Data of Indomethacin from Various Commercially Available Gels after Pretreatment of 5% Cardamom oil for 5 min

	C_{max} ($\mu\text{g/ml}$)			AUC ($\mu\text{g}\cdot\text{h/ml}$)		
	Non-pretreatment	Pretreatment	E.R. ^{a)}	Non-pretreatment	Pretreatment	E.R.
Inteban®	1.35 ± 0.36	4.33 ± 1.58	3.21	9.95 ± 3.52	39.71 ± 13.89	3.99
Formulation 1	0.71 ± 0.06	1.89 ± 0.85	2.66	5.68 ± 1.14	17.12 ± 8.17	3.01
Formulation 2	4.72 ± 1.22	6.33 ± 1.95	1.34	36.12 ± 7.11	53.64 ± 12.85	1.49

a) E.R. (enhancement ratio) = C_{max} or AUC with cardamom oil pretreatment / C_{max} or AUC without cardamom oil pretreatment. Each value represents the mean ± S.D. ($n=3$).

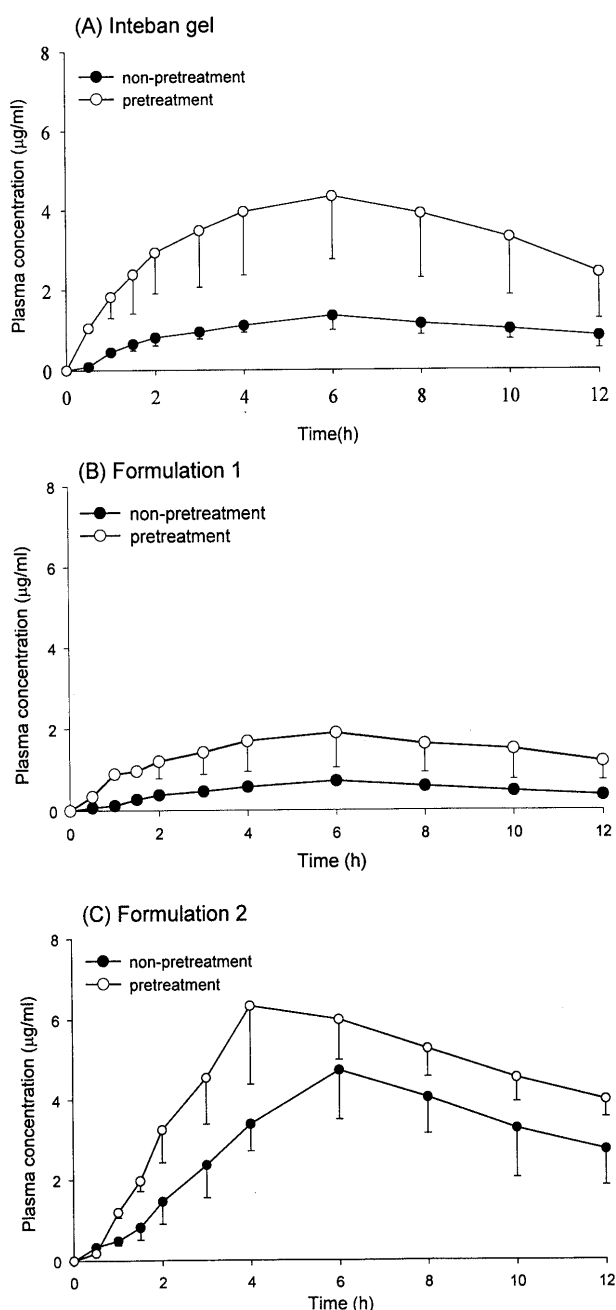


Fig. 4. Plasma Concentration-Time Profiles of Indomethacin after Percutaneous Administration of Three Commercially Available Gels in Rabbits

Each value represents the mean ± S.D. ($n=3$).

effect of cardamom oil in the *in vivo* status, the indomethacin permeation after pretreatment of 5% of the oil in 30% ethanol/water solution for 5 min onto rabbit skin was determined and compared with data from the non-pretreatment group. Previous research illustrates that the rate-determining

step on the absorption of indomethacin is the penetration process from the formulation to the skin rather than the process from the skin to the blood circulation.²¹⁾ This indicates the importance of formulation selection. Accordingly, three commercially available indomethacin formulations were utilized as the donor vehicles for administration to rabbit skin in this study.

The skin was pretreated with 5% cardamom oil in ethanol/water vehicle for 5 min before topical administration of indomethacin, and its plasma concentration-time profiles from 0 to 12 h for each formulation and non-pretreatment group were computed (Fig. 4 (A–C)). Table 3 shows the $AUC_{0-12\text{h}}$ and C_{max} values of the drug with or without pretreatment of cardamom oil. All formulations showed that the plasma concentration of indomethacin significantly increased (*t*-test, $p < 0.05$) after pretreatment of 5% cardamom oil for 5 min. The Inteban® gel showed almost four-fold increase in $AUC_{0-12\text{h}}$ after pretreatment.

Thus, both *in vitro* and *in vivo* permeation studies showed that cardamom oil could effectively promote indomethacin permeability after pretreatment onto the skin for 5 min. It is anticipated that this method will be clinically applicable, since only a short pretreatment period is needed for a patient to benefit from the enhancing effect of indomethacin permeation. Moreover, a shorter pretreatment period will result in less damage of stratum corneum and higher compliance of the patient. Further work is needed and is in progress to elucidate the precise mechanism of the enhancing effect of cardamom oil and the permeation of the oil itself.

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