DERMAL PENETRATION ENHANCEMENT BY CRUDE DRUGS: IN VITRO SKIN PERMEATION OF PREDNISOLONE ENHANCED BY ACTIVE CONSTITUENTS IN CARDAMON SEED

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Several extracts of crude drugs were prepared and tested as penetration enhancers for the diffusion of prednisolone through mouse skin in vitro. The acetone extract of cardamon seed (Eelettaria cardamomum) was selected for further study to identify the active principles. The result showed that terpineol and acetyl terpineol are the active components in cardamon seed.

KEYWORDS cardamon seed; terpineol; acetyl terpineol; penetration enhancer; skin transport; mouse skin

Several organic solvents, alkyl sulfoxide, phosphine oxides, sugar esters and surfactants are known to enhance drug transport through skin. The screening test was carried out to identify new enhancers from extracts of herbal medicine and other plants. The acetone extract of cardamon seed was active in in vitro permeation experiments using diffusion cells. Further, acetyl terpineol (d-α-terpinyl acetate) and terpineol (d-α-terpineol) were determined to be active principles.

MATERIALS AND METHODS

Fractionation of the Acetone Extract of Cardamon Seed Cardamon seed was obtained from local markets in Osaka. It was coasely cut and processed using 5 times the volume of acetone at room temperature. The extraction procedure was repeated 3 times. The acetone extract was obtained by concentrating the filtered solution under reduced pressure below 40°C. Cardamon seed acetone extract (40 g) was fractionated into two fractions by silica gel column chromatography (Merck, Silica Gel 60, elution solvent:hexane-EtOAc).[1] The amount of each fraction was as follows: fraction 1, 21.4 g; fraction 2, 14.4 g. Fraction 1 was further separated and a compound was identified as acetyl terpineol and fraction 2 as terpineol, based on MS, NMR and IR spectra. The recovery for acetyl terpineol from fraction 1 was 13.3 g (62.1%) and that for terpineol from fraction 2 was 8.7 g (6.0%).

Animals The excised skin was obtained from the abdominal surface of a male ddY mice (Oriental Bio-Service), after the removal of the abdominal hair with an electric clipper and a hair remover (Kobayashi Kocei).

Diffusion Cell Diffusion cells were supplied by Crown Glass CO., N.J., USA. The experimental system was as follows: effective area of permeation; 1.77 cm^2; receiver temparature, 37°C; receiver volume, 8 ml. Samples of 100 μl were collected at 12 and 24 h post application from the receiver compartment. The samples were assayed by HPLC (Zorbax-ODS (150 x 4.5 mm), H_2O:acetonitrile = 60:40, 1.0 ml/min, 254 nm). The results are presented as the means of 4 experiments.

Permeation Procedure The receiver compartment of the cell was filled with 8 ml of saline (0.9% NaCl), stirred with a magnetic stirring bar at 150 rpm, and the donor compartment with 2 ml of emulsion consisting of 20%(w/v) enhancer, 10%(w/v) propylene glycol, 69%(w/v) isopropylalcohol and 1%(w/v) prednisolone. Emulsion without enhancer was also used for comparison.

Statistical Analysis Effects of the test drugs were compared to that of the control group by using

Student's t test.

RESULTS AND DISCUSSION

The effect of the acetone extract in enhancing the dermal penetration of prednisolone was greater than that of Azone® (1-dodecylazacycloheptan-2-one), which has been reported to be a penetration enhancer for a number of drugs[2] (Table I).

Fractions 1 and 2 of the acetone extract increased the penetration of prednisolone from the emulsion (Table I). Taking into account the molecular weight of acetyl terpineol, terpineol and Azone, these results indicated that, in the solvent system tested, terpineol and acetyl terpineol had higher penetration enhancing power than Azone (Table I).

Table I. Estimated Amount of Penetrated Prednisolone (*$p < 0.05$)

Preparation	Concentration of enhancer (%(w/v))	Amount penetrated (μg/ml) After 10 h	After 20 h
Control	-	0.4±0.3	1.1±0.3
Azone	5	63.2±0.1*	71.5±2.8*
	20	80.2±4.9*	91.4±6.1*
Acetone extract	20	243.8±13.2*	335.8±1.0*
Control	-	1.7±0.4	2.3±0.4
Azone	20	62.2±0.6*	93.9±1.9*
Fr.1	20	133.0±4.4*	184.0±3.8*
Fr.2	20	0	73.6±8.9*
Control	-	1.1±0.1	1.6±0.1
Azone	20	82.5±1.2*	93.3±1.5*
Acetyl terpineol	5	118.0±6.2*	150.2±11.7*
(d-α-terpinyl acetate)	10	135.6±1.7*	211.5±2.6*
	20	187.5±4.7*	279.9±3.6*
Terpineol	5	94.2±11.7*	145.4±10.4*
(d-α-terpineol)	10	106.1±4.7*	151.9±7.9*
	20	105.3±2.6*	151.3±2.9*

There are relatively large amounts of volatile oils belonging to the terpenoids such as acetyl terpineol and terpineol in the acetone extract. Although the active ingredients of cardamon seed are not yet entirely identified, the terpenoids may be regarded as important.

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