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Research Article

FORMULATION AND EVALUATION OF GEL AND EMULGEL OF CHILI EXTRACT (*CAPSICUM FRUTESCENS* L.) AS TOPICAL DOSAGE FORMS

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ABSTRACT

Capsaicinoid is used in topical treatment and high-dose dermal patch, to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia. It may be used as cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis. The purpose of the present study was to measure penetration ability of capsaicinoid through Spraque-Dawley rat abdomen skin as membrane diffusion. Capsaicinoid had been extracted from chili fructus by reflux method and its content was determined. Capsaicinoid was used as an active ingredient in emulgel and gel formulation. The penetration of capsaicinoid from each dosage forms were evaluated using Franz diffusion cell with Spraque-Dawley rat abdomen skin as membrane. Physical stability of the products were investigated, including influence of temperatures, organoleptic test, pH, globule diameter, viscosity, and consistency. The result of the research showed, chili fructus extract is containing 1.93 ± 0.2 % capsaicinoid. Sum of cumulative penetration of capsaicinoid from gel and emulgel dosage forms were 153.11 ± 2.42 µg cm-^{2}, and 321.22 ± 4.67 µg cm-2, respectively. The percentage of penetrated capsaicinoid from gel and emulgel dosage forms were 19.39 ± 0.31 % and 40.69 ± 0.59 %, respectively. Flux of capsaicinoid from gel and emulgel dosage forms were 11.26 ± 0.20 µg cm-2 hour-1 and 24,28 ± 0,52 µg cm-2 hour-1, respectively. The results revealed that penetration ability of emulgel dosage form is higher than gel, and both of the dosage form is physically stable.

Keywords: Capsaicinoid, Gel, Emulgel, Penetration, Franz diffusion cell

INTRODUCTION

The nutraceutical chemical components are derived from food crops sources, and provide medicinal benefits for long-term health. Examples of these nutraceutical chemicals include capsaicin, antioxidants and phytochemicals. Nutraceutical products were considered as alternative medicine for many years. Nutraceuticals have become mainstream supplement for the diet. Nowadays, research has begun to show evidence that these chemicals found in food are as effective as chemical active pharmaceutical ingredient. Capsaicin, the main ingredient responsible for the hot taste of chili peppers, is an alkaloid (capsaicinoid) which found in the capsicum family, including Capsicum frutescens L (1). Between the capsaisinoids, capsaicin and dihydrocapsaicin together account for about 90% of pungency. Capsaicin has been used as a topical analgesic against rheumatoid arthritis pain and inflammation (2).

Capsaicin is insoluble in water, however it is soluble in organic solvents such as dichloromethane, chloroform, ether. Capsaicinoid had been extracted from chili fructus (*Capsicum frutescens* L.) with dichloromethane as solvent extraction by using reflux extraction method. Capsaicinoid content was determined from the extract by using TLC Densytometry. It is well known that vesicle used in the topical formulation have the great influence in the rate of drug permeation across the skin. Base on properties of the capsaicinoid, in this present study the chili extract was formulated in emulgel dosage form. To obtain the optimal analgesic effects of capsaisinoid in chili extract, it should be able to penetrate through the skin. Topical dosage form was chosen to formulate the extract since capsaisinoid can be well absorbed through the skin and as an alternative to overcome first fase metabolic (3,4). Chosing the suitable pharmaceutical dosage form is one way to increase the absorption of active pharmaceutical inggredient (API) through the skin (4,5,6).

Emulgel is emulsion whether oil in water (o/w) or water in oil (w/o) type, which mixed with the gel base. Emulgel can be used as a hydrophobic drug carrier (6), like capcaisinoid, and comfortable to use and easy to wash. The purpose of the present study was to measure penetration ability of capsaicinoid that contain in extract chili as active compound from emulgel through *Spraque-Dawley* rat abdomen skin as membrane diffusion.

MATERIALS AND METHODS

Material

Capsaicinoid standard (56.7 % Capsaicin and 43.3 % Dihydrocapsaicin) (Sigma Aldrich, Singapore), dichloromethane (Merck, Germany), ethanol 96% (Merck, Germany), Carbopol-940 (Lubrizol, Hongkong), olive oil (Valdoro, Italy), Tween 60 (Merck, Germany) , Span 20 (Tokyo Chemical Industry, Japan), propylene glycol (Dow, Chemical Co.), n-buthanol (Merck, Germany), menthol (Shanghai, Xinhua), butylated hydroxy toluene (SPP Chemical), sodium hydroxide (Merck, Germany), potassium dihydrogen phosphate (Merck, Germany: Chili fruit powder was from BALITTRO Bogor, West Java.

Animals

White female rats, *Spraque Dawley* strain, 2-3 months old, with weight about 150-200 gram (Botanical Agricultural Institute, Indonesia).

Methods

Preparation of Chili Fruit Extract

The extract was made by reflux method of several amount of Chili fruit powder with dichloromethane as solvent. The extract was then filtered and the residue washed with dichloromethane. The filtrate was collected and evaporated to remove solvent by using rotary vacuum evaporator and vacuum oven until the extract was obtained. The extract obtained was used as an active ingredient in emulgel and gel formulation. Separation method of the content in the extract was conducted by TLC Densytometry (Camag,Switzerland) using n-hexan: dichloromethane:acetic glacial (7: 2.5: 0.5). The identification of the components of the alleged capsaicin was carried out by comparing it with the standard. Dichloromethane is a toxic compound, so to find out the residue of it in extract was measured by Gas Chromatography (7).

Preparation of Chili Fruit Extract Emulgel

Emulgel was made in two steps. The first step was made of oil in water emulsion and base of gel. The second step was mixed of the emulsion and gel base. The emulgel formula can be shown at Table 1.

Table 1: Formulations of Gel and Emulgel Containing Chili Fruit Extract

Material	Consentration (%) (w/w)	
	Emulgel	Gel
Chili extract	Equivalent to capsaicinoid 0.6%	
Carbomer	2.00	2.00
NaOH	0.60	0.60
Olive oil	5.00	-
Tween 60	3.60	-
Span 20	1.40	-
Propilenglycol	5.00	5.00
Ethanol 96%	3.00	3.00
Menthol	1.00	1.00
BHT	0.03	0.03
Destiled water up to	100.0	100.0

The oil phase emulsion was made by dissolving Span 20, Chili fruit extract and BHT in olive oil, while the water phase was made by dissolving Tween 60 in distilled water. Each phase was heated at a temperature of 70-75 °C, and menthol was dissolved in ethanol 96% then blended into propylene glycol. After each phase reaches a temperature of approximately 70-75 °C, the oil phase was added to the water phase followed by the addition of a mixture of menthol-ethanol-propylene glycol. Then, the mixture was stirred using a homogenizer with a speed of 2500 rpm until the room temperature and the emulsion was formed. Then the emulsion was mixed into a base gel which consist of 2% carbomer bit by bit using a homogenizer stirring 3000 rpm for 30 minutes or until a homogeneous mass of emulgel was formed, comparison between the emulsion and base gel was 6: 4.

Making Gel With Extract Chili as Active Ingredient

The gel was made with dispersion of carbomer in distilled water while stirred until completely dispersed. Sodium hydroxide (NaOH) was dissolved in distilled water and then added to the gel base carbomer with 1500 rpm stirred until a thick gel base was formed. Menthol was dissolved in ethanol 96% then blended into propylene glycol. The mixture was added to the gel base, then stirred with a speed of 500 rpm until homogeneous. After gel mass was formed, fruit chilly extract added into the gel by using homogenizer with speed of 3000 rpm for 30 minutes.

Physical Evaluation of Emulgel and Gel

The product was evaluated include organoleptic test, homogenity, pH, viscosity, consistency, the average globule diameter, physical stability at low temperature (4º ± 2 °C), room temperature (28° ± 2 °C), and high temperature (40° ± 2 °C) for 8 weeks with interval 2 weeks of each observation, cycling test, and mechanical tests.

In Vitro Skin Permeation

The in vitro permeation experiments were determined by using Franz diffusion cell. Abdoment skin of female *Spraque-Dawley* rat (2-3 month old) was mounted on the receptor compartment with the side facing upwards into the donor compartment and the dermal side facing downwards into the receptor. Aqueous solutions that consist of ethanol 96%- buffer phosphate pH7.4 pH (EPB) about 13.0 ml,was used as the receptor medium. The donor compartment of the cell was filled with 1-2 gram chili extract. The available diffusion area of cell was 1.52 cm^2 using 0.6 ± 0.1 mm thick of membrane. Franz diffusion cell was used to measure the penetration ability of capsaicinoid. The diffusion cell area was 1.52 cm^2. The receptor phase of cell was sustained at 37°C and stirred by a magnetic stirrer at 300 rpm. At appropriate intervals, 0.5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The sample was analyzed by using TLC densitometer at wavelength of 281.0 nm. Each formulation represents three experiments.

RESULTS AND DISSCUSSION

Extraction

Liquid extract was evaporated using a rotary vacuum evaporator and performed with the temperature not more than 55 °C (8). Evaporation was performed to remove residual solvent from chili fruit extract. Residue of dichloromethan was determined using gas chromatography. The result showed the amount of the dichloromethan was 143 µg ml^{-1}, less than maximum levels of dichloromethane (600 µg mL^{-1})still eligible for pharmaceutical product based on European Medicines Agency ICH (2010). Capsaicinoid content in extract was determined using TLC Densitometer (Camag, Switzerland, and the result showed that capsaicinoid content was 1.92 ± 0.2%. and in emulgel and gel dosage form were 3.12% equivalent to 0.06% capsaicinoid.

Result of Physical Test Emulgel and Gel

Physical stability Test

Physical stability test of emulgel and gel were conducted at different temperatures to compare the physical stability. Stability testing was performed by observing at the organoleptic, pH, average globule diameter changes every two weeks for eight weeks. Both, emulgel and gel still showed oganoleptic appearance unchanged during stored eight weeks at low temperatures, room temperature and high temperatures. They were color stable as before, emulgel pale orange color and gel orange color. pH of emulgel and gel unchanged during stored for eight weeks, there were still at pH balance of the skin (4,5-6,5).

The viscosity early of emulgel and gel were 16500 cps and 33900 csp respectively, but after eight weeks slightly changed to emulgel 17250 cps and gel 35000 cps. These viscosity became slightly more viscous both of the dosage form, its posibility caused of ethanol evaporation during storage. The consistency early of emulgel and gel were 357 1/10 mm and 358 1/10 mm respectively, but after eight weeks slightly changed to emulgel 333 1/10 mm and gel 348 1/10mm. Increasing of viscosity of each dosage form straight porportional with decreased of consistency. Diameter globuleof emulgel and on the tree degre temperature were between 0,27-0,29 µm and not showed substantial difference.

Mechanical test was conducted only on the emulgel, because of the composition of the emulgel consisting of two phases. Emulgel received the force of gravitation. By stokes law the force of gravity can affect stability emulsion. Rate of centrifugation at 3800 rpm for five hours comparable by gravitation force received by product for one year. The results showed that there was no phase separation from the mass of emulgel.

The other test was cycling test which conducted to compared the physical condition of the dosage form than before. Both, emulgel and gel stored at different temperatures for eight weeks. The result showed smell of emulgel and gel during eight weeks storage were fragrant menthol unchanged during eight weeks. Both of the product showed characteristics stable and fixed homogeny during eight weeks storage.

In Vitro Penetration Test

This test was performed to determine the amount of capsaicinoid that penetrated through the abdoment skin of female *Spraque-Dawley* rat during a certain time interval. Membrane can be derived from the bark of certain animal body part or artificially (9). The important factor that also considered to penetration testing in vitro was an active substance (capsaicinoid) should dissolve in liquid compartments receptors used. Penetration test in vitro for active ingredient that is hydrophobic like capsaicinoid, it will be difficult to dissolve in the receptor compartment when the medium used is water. Solubility factors may affect the correlation of in vitro-in vivo assay results (10,11). To overcome difference between in vitro and invivo test for a hydrophobic drug, then it is allowed to add solubilizing agent into the receptor (9). Receptor medium used for penetration testing capsaicinoid in this study is a mixture of 96% ethanol and phosphate buffer pH 7.4 with a ratio of 1:1 (EPB). Phosphate buffer pH 7.4 was selected as the receptor medium as simulation of body's biological fluids. The other factors that need attention is when the drug diffusion through the vehicle is a rate limiting step, the viscosity of vehicles may play an important role in controlling the permeation of drug across the skin and should be determined (16).

In these research results indicated that the cumulative of penetrated capsaicinoid from emulgel and gel through the membrane of the abdoment rat skin were 321.22 ± 4.67 and 153.11 ± 2.42 µg cm^{-2} respectively. The capsaicinoid flux from emulgel was significantly higher (*t*-test, $P < 0.05$) than from gel bases with 0.06%. Then flux in a steady state following fick law. Fick Law 1 give the flow of a substance by one unit of cross section in the flow in steady state. When compared with other dosage form such as ointments (9) and hydrogel (4) that only gives the cumulative amount penetrated about 20-60 µg cm^{-2}, emulgel give better results. This is related to the expected therapeutic effect that would be more efficient use of emulgel for better drug release. The percentage of capsaicinoid penetrated from gel and emulgel were 19.39 ± 0.31% and 40.69 ± 0.59%, respectively (Fig.1)

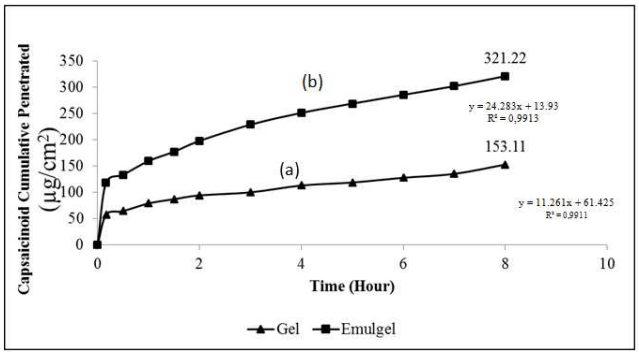

Fig. 1: Penetration profile of capsaicinoid from (a) gel and (b) emulgel.

From Figure 1 can be seen that the absorption through the skin capsaicinoid happened very quickly. This looked at the 0 to 10 minutes that there is increasing capsaicinoid penetration. Rapid absorption also suspected due to the additional ingredients in the preparation, such as ethanol, propylene glycol, and menthol. The third material that role in a synergistic capsaicinoid absorption through the skin very rapidly on the minutes early. Several other factors that might affect absorption through the bark is medicinal preparation, viscosity and disolusi a remedy in bearer, diffusion of a solute from a bearer to the surface of the skin and penetration drugs through layers of skin especially the stratum corneum.

Capsaicinoid is a compound having good solubility in fats hence perkutan absorption of capsaicinoid also increases. In the formula was used menthol as a cover smell chili, propylene glycol as a humectant, and ethanol as solvent of menthol. In this study the three ingredient were also percutaneous penetration enhancers (12). Ethanol and propylene glycol palyed a role in hydrating the skin. Ethanol worked as percutaneous penetration enhancers by altering the properties of the stratum corneum so capsaicinoid dissolved more into the stratum corneum and make penetration increases (13). Ethanol also could increase the capsaicinoid in donor compartment, because ethanol is a volatile compound that could improve capsaicinoid concentration gradient between the donor and receptor compartments rapidly. While menthol acts as percutaneous penetration enhancers by interfering with the intercellular lipid barrier damage the stratum corneum and enhance the diffusion of the drug in the stratum corneum (12).

The cumulative penetrated capsaicinoid was plotted against time and then made a linear regression equation that could be determined capsaicinoid flux of each preparation (Figure 2). Flux was obtained from the slope of the line in Figure 1, which showed that the flux values taken at steady state following the rules of the law of Fick. Based on Figure 2 could be compared the flux of each preparation. The flux of the preparation gel and emulgel were 11.26 ± 0.20 mg cm^{-2} $hour^{-1}$ and 24.28 ± 0.52 mg cm^{-2} $hour^{-1}$, respectively.

From these results it could be seen that the emulgel provided a higher flux than the gel product. It was proved that the rate of penetration of the preparation emulgel capsaicinoid faster than gel. Other factors that may affect drug absorption through the skin are the viscosity of the preparation, dissolution of a drug in the carrier, the diffusion of dissolved drug from the carrier to the surface of the skin, and the penetration of drugs through the skin, especially the stratum corneum layer (12). The penetration rate is inversely proportional to viscosity grades. The more viscous a preparation it will be more difficult to release the drug from the carrier. Emulgel dosage form has 3-dimensional structure of the gel is more lax. It was indicated that a lower viscosity of emulgel then gel. More amount of water in the formula cause the 3-dimensional structure of the gel was porosity. As a results the capsaicinoid was penetrated easily through the stratum corneum (14).

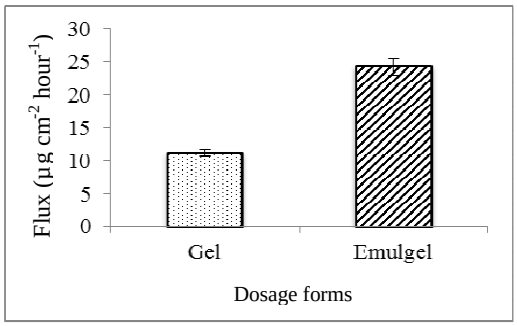

Fig. 2: **Average penetration flux of capsaicinoid from gel and emulgel (average ± SD, n=3)**

The partition coefficient of the drug can also affect the rate of penetration of a drug. Capsaicinoid is not soluble in water. Dissolution of capsaicinoid of the gel dosage form was lower then solubility of emulgel dosage form. In contrast, the solubility capsaicinoid in emulgel higher because in emulgel dosage form consisting of oil and water phases. Oil phase of the emulsion is a good carrier for the drug substance that is not soluble in water.

In addition, in emulgel dosage form contained olive oil, which consists of several types of fatty acids, such as oleic acid, palmitic, linoleic, stearic, and slightly fatty acids myristic acid with the highest oleic acid by 70% (15). Oleic acid in topical preparations could be increased skin permeability by interfering with the composition of the stratum corneum lipid bilayer coating so as to raise their penetration capsaicinoid (13).

Another factor is the diffusion of the drug from the carrier to the surface of the skin. The process of diffusion of a drug is influenced by the solubility of the drug in the carrier. When the drug has low solubility in the carrier, then the process of diffusion of the drug from the carrier will be slower and will eventually be the longer to reach the skin surface.

CONCLUSION

Based on the evaluation physical stability and penetration testing showed that emulgel as topical dosage form in general better than gel.

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