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Research Article

FORMULATION AND PENETRATION TESTING OF ETHOSOME AZELAIC ACID ON ABDOMINAL SKIN WHITE MALE RATS (*RATTUS NORVEGICUS*) WITH FRANZ DIFFUSION CELL

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ABSTRACT

Objective: Development of transdermal drug delivery systems has several advantages, especially drugs to have a poor penetration of stratum corneum in the skin. Azelaic acid has been proven bactericidal and bacteriostatic to acnes bacteria (*Propionibacterium acnes*). Azelaic acid products in market as cream and gel can only penetration in stratum corneum about 4% of the dosage used. Thus, it is necessary to increase the penetration of azelaic acid to formulate into a carrier system such as ethosome.

Methods: Manufacture of suspension ethosom azelaic acid using thin layer hydration method or classical method. Suspension ethosom of azelaic acid to obtained subsequent freeze dried before formulated in cream preparation. After that, the penetration test for ethosom cream and non ethosom cream of azelaic acid with Franz Diffusion Cell.

Results: Optimization formulation ethosom of azelaic acid with variations concentration of ethanol 30%, 35% and 40%. Ethosome with 35% ethanol had entrapment efficiency higher than 30% and 40% ethanol as 94.48±0.14% and had smaller particle size 179.3±2.23 nm. Penetration test for ethosome cream and non-ethosome cream of azelaic acid showed that cumulative amount was 1334.074±27.086 μg/cm^2 h and 491.032±3.935 μg/cm^2 h.

Conclusion: Ethosome cream of azelaic acid has better penetration capabilities than non-ethosom cream of azelaic acid.

Keywords: Azelaic acid, Penetration enhancer with vesicles ethosome, Franz diffusion cell.

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INTRODUCTION

Drug that can be used to inhibite the growth of *Propionibacterium acne* is azelaic acid. The mechanism of action azelaic acid is primarily through inhibition of protein synthesis, but RNA and DNA synthesis is also decreased [1]. Azelaic acid is bacteriostatic at low concentrations and bactericidal at higher levels. Due to differential uptake of azelaic acid at different pH levels and in nutrient depletion, the minimal inhibitory concentrations for *P. acnes* vary from 0.1 to 250 mmol/L [2-4]. Azelaic acid not only as antiacnes but also has other activities as antihiperpigmentasi skin [4-6], anticeratinitation, cytotoxic cell and antipoliferation in keratinocytes [3].

Based on the Food and Drug Administration, *in vitro* penetration test of Azelex® cream, in which azelaic acid can only penetrate in stratum corneum by 4% of the dosage used [7-10]. Thus, it is necessary to increase the penetration of azelaic acid percutaneously with azelaic acid formulated into a carrier such as ethosome system [11].

Ethosomes are soft, malleable lipid vesicles composed mainly phospholipids, alcohol (ethanol or isopropyl alcohol) in relatively high concentration (20–45%), and water. High concentration of alcohol (20–45%) in the formulation provides soft, flexible characteristics, vesicles stability, and it also disrupts lipid bilayer structure, so it can be increased membrane permeability [12,13].

MATERIALS AND METHODS

Materials

Azelaic acid (Sigma, US), Phospolipon 90 G (90% hydrogenated soy phosphatidylcholine, US), ethanol 96 % (Merck, German), propylene glycol (Dow Chemical Co), potassium dihydrogen phosphate (Merck, German), hydrochloric acid (Brataco, Indonesia), sodium hydroxide (Brataco, Indonesia) methanol (Merck, German), dichloromethane (Merck, German), and cream base dan aqua demineralization (Brataco, Indonesia) were used.

Preparation ethosome of azelaic acid

Preparation ethosome of azelaic acid was thin-layer hydration method. The thin layer was using rotary evaporator by dissolving phospolipon 90 G with a ratio of 1:1 to azelaic acid in dichloromethane and methanol (2: 1). Then, the thin layer formed is stored in the refrigerator for 24 h than in hydration with the water phase. The water phase used consisted of ethanol (with concentration variations of 30%, 35%, and 40%), azelaic acid, and phosphate buffer pH 7.4.

Characterization ethosome of azelaic acid

After obtaining, the suspension ethosome of azelaic acid then proceed with the characterization to obtain the most optimum in formulation. The parameters used are entrapment efficiency, particle size distribution, potential zeta, and polydispersity index.

Entrapment efficiency test

Azelaic acid in made variations concentration were 200, 250, 300, 350, 400, and 450 ppm using phosphate buffer pH 6.8. The solution was then measured uptake by ultraviolet (UV)-visible spectrophotometer at a wavelength of 204 nm and made a calibration curve [14,15].

Entrapment efficiency test was done by the indirect method. Determination of total ethosome suspension was done measuring the absorbance of ethosome suspension dissolved in methanol. Than, concentration of azelaic acid in the supernatant with Cellulose Acetate filter 0.22 μm [14,16,17].

Entrapment efficiency = (T–C)/T ×100%

Information:
T: Total concentration of azelaic acid in ethosome suspension (μg/mL)
C: The concentration of azelaic acid present in the supernatant (μg/mL).

Particle size distribution, polydispersity index, and potential zeta
Measurements of particle size distribution, polydispersity index, and potential zeta ethosome were performed using particle size analyzer.

Determination content of azelaic acid in cream
Ethosome and non-ethosome cream is weighed 1 g, and then, cream is extracted with 10 mL of methanol and then presented ultrasonic to increase the solubility of cream. Thereafter, the preparation was centrifuged at a rate of 3000 ppm for 40 min. The supernatant solution was introduced in flask 25 mL and volume was sufficient with phosphate buffer pH 6.8, calculated of azelaic acid in the preparation using UV-visible spectrophotometer [18].

***In vitro* penetration test**
Penetration test using skin membrane abdominal from male rats with phosphate buffer ph 7.4 as receptor compartment. After that, the abdominal skin of rats was placed between donor and receptor compartment with the dermal side directly related to the medium receptor [4,19]. Sample of 1 g was applied to the skin surface with an area of diffusion was 1.76 cm^2. Then, samples of 4 mL were taken at regular intervals for 12 h. Then, a solution compartment immediately added a volume equal to the volume taken. The solution was then analyzed using a UV-visible spectrophotometry [22].

After concentration of azelaic acid in the sample has been measured, it can be calculated the cumulative amount of azelaic acid penetrated using the following formula [20].

$$Q_t = V_r C_t + \sum_{t=0}^{t-1} Vs C_i$$

Explanation:
Q_t = Cumulative amount of azelaic acid penetrated (μg)
V_r = Volume receptor compartment of Franz diffusion cell (16 mL)
V_s = Sample volume (4 mL)
C_t = Concentration (ng/mL) at minute -n
C_i = Concentration on sampling minute -i

From the analysis will be obtained cumulative amount of azelaic acid per unit area (μg/cm^2) with following formula:

Q = Qt/S

Explanation:
Q = Cumulative amount per unit area (μg/cm^2)
Qt = Cumulative amount penetrated (μg)
S = Area of membrane (1.76 cm^2)

The calculate of flux in steady state through interpolation of linear regression using the following formula:

J = M/(S×t)

Explanation:
J = Flux (μg cm^{-2} jam^{-1})
S = Area of diffusion (cm^{-2})
M = Cumulative amount of azelaic acid to membrane (μg)
T = Time (hours)

Furthermore, from the analysis we get a graph of drug concentration per unit area (ng/cm^2) over time, in order to obtain a straight line, the slope of line is presented rate of drug release [8,20,23].

RESULT AND DISCUSSION

Determination of selected ethosome azelaic acid in formula
The selected formula is a high percentage of entrapment efficiency, particle size distribution (<200 nm), polydispersity index <0.8, and has a potential zeta >/< ± 30 mV (Table 1).

Determination of azelaic acid content in preparations
From data selected was formula 35%. Formula 2 was then formulated in ethosome and non-ethosome cream preparations and the determination of the azelaic acid content can be seen in Table 2. The regression used is y=0.0013×+0.0049, with r=0.9996.

***In vitro* penetration test with Franz diffusion cells**
After the penetration test for 12 h at 10 sampling points showed that the cumulative amount of azelaic acid and penetrated to the preparation ethosomeandnon-ethosomecreamwas16879.269±189.055 μg/$cm^2$and 5759.222±44.779 μg/cm^2 (Table 3). The flux values for the ethosome and non-ethosome cream preparations can be seen in Table 4. In these results, it can be seen that value flux ethosome cream gives a higher than non-ethosome cream preparation. The percentage of the azelaic acid penetrated from each preparation and the results obtained for the preparation penetrated ethosome cream was 74.32%, while for non- ethosome cream, it was 33.49% (Table 5).
Ethosome azelaic acid containing 35% ethanol can affect to structure of stratum corneum (reversible) to increase penetration of azelaic acid through the skin. While azelaic acid in non-ethosome had smaller penetration than ethosome cream. This suggests that possibility hydrophobic of azelaic acid can be retained in the skin layer to longer [13,21].

Table 1: Characterization ethosome of azelaic acid

Formula	D_{mean} volume (nm)	Polydispersity index	Potential zeta (mV)	Entrapment efficiency (%)
Formula 30%	283.5±9.08	0.502±0.06	32.13±7.77	90.08±0.13
Formula 35%	179.3±2.23	0.665±0.02	−34.87±0.35	94.48±0.14
Formula 40%	1377±76.06	0.975±0.04	−22.80±2.25	92.46±0.30

Table 2: Concentration of azelaic acid in cream

Formula	Theoretical concentrations (μg/mL)	Concentrations actually (μg/mL)	Percentage of content	Mean±SD
Ethosome	750	790.077	105.344	106.096±0.720
		800.846	106.779	
		796.231	106.164	
Non-ethosome	500	548.538	109.708	109.605±1.081
		542.385	108.477	
		553.154	110.631	

SD: Standard deviation

Table 3: Cumulative penetrated of azelaic acid (n=3)

Time (hours)	Concentration Q (µg/mL)			Mean±SD
	Experiment 1	Experiment 2	Experiment 3	
Etosome cream of azelaic acid				
0.17	682.474	643.138	649.694	658.435±21.075
0.67	1512.107	1495.935	1467.963	1492.002±22.333
2	3360.621	3284.572	3232.124	3292.439±64.609
2.25	3661.495	3574.956	3522.072	3586.174±70.385
2.5	3982.037	3924.344	3879.327	3928.569±51.485
7	9534.572	9889.467	9873.295	9765.778±200.393
8	10897.072	11225.743	11185.533	11102.783±179.282
9	12136.757	12464.991	12437.893	12346.547±182.188
11	14770.411	15244.187	15249.869	15088.156±275.190
12	16684.921	17062.544	16890.341	16879.269±189.055
0.17	26.879	13.767	7.212	15.953±10.014
0.33	40.603	56.774	35.358	44.245±11.163
0.5	82.299	97.159	83.610	87.689±8.227
0.67	152.404	177.316	175.131	168.284±13.796
0.83	236.495	233.872	233.435	234.601±1.655
1	308.348	321.897	323.208	317.818±8.227
9	4319.449	4358.348	4386.757	4354.851±33.790
10	4685.446	4735.708	4769.362	4730.172±42.231
11	5188.243	5259.921	5266.040	5238.068±43.258
12	5710.271	5769.274	5798.121	5759.222±44.779

SD: Standard deviation

Table 4: Flux of ethosome and non-ethosome cream

Formula	Flux (µg/cm².h)	Mean±SD (µg/cm².h)
Ethosome	1302.890	1334.074±27.086
	1351.743	
	1347.589	
Non-ethosome	486.851	491.032±3.935
	491.584	
	494.663	

SD: Standard deviation

Table 5: Percentage penetrated of azelaic acid with Franz diffusion cell

Concentration (µg/mL)	Mean±SD	Percentage penetrated (%)
Azelaic acid in ethosome cream at 12 h		
597.000	591.360±9.769	74.32
597.000		
580.080		
Total content of azelaic acid in ethosome cream before penetration		
790.077	795.718±5.403	
800.846		
796.231		
Azelaic acid in non-ethosome cream at 12 h		
183.145	183.510±0.391	33.49
183.923		
183.462		
Total content of azelaic acid in non-ethosome cream before penetration		
548.538	548.026±5.403	
542.385		
553.154		

SD: Standard deviation

Based on the results of *in vitro* penetration test, flux penetration of ethosome cream higher when compared with non-ethosome cream. That because the reduction of particle size to nano can increase drug penetration in skin. In addition, main component vesicles ethosome is phospholipid and ethanol can increase the penetration of drugs with various mechanisms. In generally the mechanism of drug release from effect ethanol in ethosome and effects elastic form ethosom. When ethosom is applied to the skin, ethanol in ethosome can increase penetration enhancer. The mechanism of ethanol is interacting with fat molecules in stratum corneum which can be decreased rigidity, influence lipid bilayer on stratum corneum, so it can to increase lipid fluidity membrane and permeability of stratum corneum [13,21].

With the disruption of lipid bilayer on the stratum corneum, it will facilitated the ethosome to penetrate into the skin. In this process of ethosome effect. Ethosome is a flexible vesicle that can interact with

lipid bilayer and penetrated in skin by changing the shape of the vesicles to be like the pathway. Ethosome in skin will make it fusion with fat skin and then drug is released in this process resulting in transdermal absorption [13,21].

CONCLUSION

Optimization formulation of ethosome is done with variation concentration ethanol of 30%, 35%, and 40%, indicating the formulation ethosome with 35% ethanol, the highest value of the entrapment efficiency was 94.48 ± 0.14%, and the smallest particle size is 179.3±2.23 nm.

Penetration test for ethosome cream and non-ethosome cream of azelaic acid showed that cumulative amount was 1334.074±27.086 µg/cm^2 h and 491.032±3.935 µg/cm^2 h.

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AUTHOR CONTRIBUTIONS

Novi Nurleni, S. Farm., Apt is a Master Student at the Faculty of Pharmacy, University of Indonesia. Her master research about of "Formulation and Penetration Testing of Ethosom Azelaic Acid on Abdominal Skin White Male Rats (*Rattus Norvegicus*) with Franz Diffusion Cell." Dr. Iskandarsyah, M.S., Apt is Doctor at the Faculty of Pharmacy, University of Indonesia. He is head of Pharmaceutical Laboratory. Has Expertise in Development of pharmaceutical technology. Dr. Ahmad Aulia Jusuf, AHK, PhD is doctor at the Faculty of medicine, University of Indonesia. He is head of the histology department. Has expertise in Stem Cell and Tissue Engineering.

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