Gel edasi: Ethosomal gel using cow’s blood as a supportive of healing diabetic foot ulcer

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Abstract. Diabetes mellitus (DM) is a chronic carbohydrate metabolic disorder. Diabetic foot injury is one of the chronic complications of people with diabetes mellitus because it can cause disability and even cause death. Cow blood waste is a waste that can disturb the environment. Components of the chemical elements contained in cow blood include nitrogen, phosphorus, potassium, organic carbon, erythrocytes and leukocytes which function in the body's defense by fighting infections so that cow blood waste is one alternative solution in the treatment of diabetic feet at a price affordable. Ethosome gel is a gel preparation that has the advantage of penetrating into the skin layer faster and significantly increasing the value of transdermal preparation flux. Based on the results of the study, the desired platelets from cow blood were obtained after sampling from the Makassar city slaughterhouse. The platelets obtained were then weighed as much as 0.5 g, 1 g, and 1.5 g to make a gel formula with a carbopol base of 1%. The gel that has been made is then evaluated like a homogeneous and clear gel physical examination. The pH obtained in formulas 1, 2 and 3 is 6.43; 7.00; and 6.80. The formula 1 has a viscosity of 47,342.6 cps and the formula 2 has a viscosity of 45,678.5 cps while formula 3 has viscosity 35,456.7 cps. In the stability test the results obtained were that the preparation did not experience changes in color and odor.

Keywords: gel, ethosome delivery system, cow’s blood, diabetic ulcer

1. Introduction
Diabetes mellitus (DM) is a disorder of carbohydrate metabolism that is chronic in nature, and can cause chronic complications as well. Diabetes is one of the four priorities of non-communicable diseases, this diabetes has effects such as blindness, heart attack, stroke, kidney failure and amputation. One of the causes of major DM complications is 80% diabetic foot sores from diabetic patients. The population of diabetes mellitus (DM) in Indonesia is ranked 4th in the world with the highest population of people with diabetes mellitus (DM) after the countries of India, China and the United States [1]. Diabetic foot is one of the chronic complications of diabetes mellitus which is most feared by sufferers because it can lead to disability and even death. This is due to the long treatment and the cost of treatment is increasingly expensive. So that Cow Blood Waste can be an alternative solution for this treatment.
Cow blood waste is a waste that can disturb the environment, but it can be seen that cow blood has a high economic value and can also be used as an alternative treatment for diabetes mellitus wounds. The percentage of blood in a cow's body is around 3.5-7% of the total weight. Components of the chemical elements contained in cow blood between nitrogen content 12.18%, phosphorus 5.28%, potassium 0.15% and organic carbon 19.01%[2]. There are two classes of cells in the blood plasma, namely red blood cells (erythrocytes) that play a role in the transport of O2 and white blood cells (leukocytes) that function in the body's defense by fighting infections. Therefore, these components can make cow blood waste as an alternative solution in the treatment of diabetic feet at affordable prices.

Ethosome gel is a lipid vesicle consisting of phospholipids, alcohol (ethanol, or isopropyl alcohol) in relatively high concentrations in water. The gel preparations in the form of ethosomes have the advantage of penetrating into the skin layer faster and significantly increasing the value of the transdermal preparation flux. When compared with liposome gel preparations, liposomes do not penetrate too deeply on the skin[3].

Therefore, gel is made using an ethosome delivery system with active ingredients of cow blood platelets.

2. Methods

2.1. Platelets isolation by PRP method
Cow blood samples taken from slaughterhouses in Makassar. A 2000 ml cow blood sample was stored in a vacutainer tube containing a solution of adenosine-citrate-dextroseacid (ACD-A) and then rotated at a low speed of 4000 rpm for 15 minutes[4].

2.2. Making Ethosomes
Ethosome is prepared using the cold method. The organic phase was made from cow blood platelets mixed according to comparison, then added 2 mL of ethanol and homogenized using a magnetic stirrer at 30 ° C with a centrifugation speed of 700 rpm for 20 minutes. During stirring, propylene glycol is added gradually as much as 1 mL. In another phase, aquadest is heated at 30 ° C. This liquid phase is added to the organic phase. This mixture is stirred for 5 minutes at the same speed[5].

| No | Formula | Platelets (g) | Propylene glycol (ml) | Ethanol (ml) |
|----|---------|---------------|-----------------------|--------------|
| 1  | E1      | 0.5           | 1                     | 2            |
| 2  | E2      | 1             | 1                     | 2            |
| 3  | E3      | 1.5           | 1                     | 2            |

2.3. Gel Making
Carbopol 1% b / v was made by mixing 1 g of carbopol powder in 100 mL of aquadest. The 20 mL ethosome that has been made is added to the expanded carbopol then stirred using a magnetic stirrer at 30 ° C and a constant speed until homogeneous. pH is neutralized using triethanolamine and stirred at low speed until transparent gel is formed[6].

2.4. Physical examination
Prepared blood ethosomal gel of cow platelets was examined visually for color, homogeneity, consistency, dispersion and phase separation[7].

2.5. Viscosity testing
The measurement of the viscosity of the prepared gel was carried out by Brookfield viscometer. The gel is rotated at 50 rpm using spindle number 7[8].
2.6. **pH testing**

pH is measured in each gel, using a pH meter, which is calibrated before each use with a standard buffer solution at pH 4, 7, 9. Electrodes are inserted into the sample for 10 minutes to take readings at room temperature [7].

2.7. **Stability Test**

Stability test was carried out to show the physical change of the gel with the freeze thaw method by placing the gel preparation at 100 °C for 24 hours then the gel was transferred at room temperature around 25-29 °C for 24 hours. After that, viscosity and dispersion tests are carried out [8].

2.8. **Testing on experimental animals**

In vivo testing was conducted at the Faculty of Pharmacy’s Biopharmaceutical Laboratory, University of Hasanuddin. Before the experiment began, all the animals tested were induced with sugar for seven days, in order to increase blood sugar levels in experimental animals. If blood sugar levels don't increase, alloxan is given. The tested animals used are rabbit (*Oryctolagus cuniculus*) which is healthy. First, the rabbit is anesthetized using ether then shaving the hair on the part that will be injured (back), then injured with a soldering iron with a diameter of 3 cm wound. In this experiment 5 rabbits were used with different blood concentration treatments. Rabbit I, wound given 1 gram gel formula 2.5%. Rabbit II, the wound is given 1 gram of gel formula as much as 5%. Rabbit III, wound is given 1 gram gel formula 7.5%. The wound is smeared with the test preparation every 24 hours and then covered with gauze. The length of the wound is measured and closed again until the wound heals. It was recorded that the day began to decrease the length of the wound, the formation of karopeng and the day of closed wound 100%.

3. **Result**

![Figure1](image-url)

**Figure1.** cow’s blood platelets, (1) Platelet poor plasma, (2) Platelet rich plasma (3) Erythrocytes

Bovine blood platelets obtained from the centrifugation process are made into ethosomes according to formulas E1, E2 and E3, each formula produced 20 mL ethosomes which were mixed on the basis of 1% carbopol gel. Physical examination of the gel preparations was carried out, gel results were
obtained that were good according to the predetermined requirements clear, homogeneous, and no phase separation occurred.

pH testing was carried out with the following results:

**Table 2. pH and viscosity test results**

| Formula | pH   | Viskositas (cps) |
|---------|------|-----------------|
| E1      | 6.43 | 47.342.6        |
| E2      | 7    | 45.678.5        |
| E3      | 6.8  | 35.456.7        |

All three formulas showed a good pH of 6.43; 7.00; and 6.80 respectively for E1, E2 and E3. This pH condition is also in accordance with the pH of the skin which is 5.5-7. After testing the pH, the viscosity test was then carried out using the Ostwald viscometer and obtained results as in table 2. gel formula E1 had a viscosity of 47,342.6 cps and E2 formula had a viscosity of 45,678.5 cps while the E3 formula had a viscosity of 35,456.7 cps. Gel viscosity values are in accordance with the characteristics of a good gel which has a viscosity between 1000-100,000 cps.

Stability testing of the gel has been carried out using the Freeze thaw method by storing the preparation at 100 °C for 24 hours, after which it is stored at room temperature 25-29 °C for 24 hours. After testing the viscosity, it was found that the ethosomal gel formula 1 had a viscosity of 43,232 cps, and the ethosomal formula 2 had a viscosity of 40,552 cps while the formula 3 had a viscosity of 32,854 cps. The viscosity value shows a decrease in the viscosity value before the stability test is carried out even though the change is not very significant and remains within the required gel viscosity limit.

![Image of dispersive power curve of gel formula E1](image1)

![Image of dispersive power curve of gel formula E2](image2)
In the dispersion test, it was found that the gel condition was still good because the gel area was spread not so widely that the viscosity did not decrease significantly because, if the gel area spread wide enough, the viscosity decreased. The results obtained indicate that conditions are not much different from those before testing. Therefore, the gel can be said to be stable with changes in temperature. The following is the spread power curve of each formula.

Experimental animals have been adapted using a 40% sugar solution for 7 days but the rabbit's blood glucose level has not matched the desired blood sugar level. Therefore, alloxan induction is carried out intraperitoneally at a dose of 155 mg / 2 kg BW to make rabbit pancreas damaged because if the pancreas is damaged, the beta pancreatic cell does not produce insulin which plays a role in the conversion of glucose to glucogen so that glucose in the rabbit's blood is not controlled and cause blood sugar levels to increase.

4. Conclusion
Based on physical testing, the ethosome gel from cow blood platelets that have been made fulfills the characteristics of the gel in general. however, invivo testing that has not been done yet, making this gel cannot be claimed to be effective in healing ulcer wounds (diabetic ulcer).

References
[1] International Diabetes Federation. 2015. IDF Diabetes Atlas Seventh Edition 2015: Dunia : IDF
[2] Ernawati H, Chotimah NC, Kresmatita S, Ichriani GI. 2015. Pemanfaatan Limbah Darah Sapi dan Kambing sebagai Pupuk Ramah Lingkungan untuk Mendukung Pertanian Lahan Gambut yang Berkelanjutan.Palangkaraya: Udayana Mengabdi. Vol 14(1); 13-17
[3] Akib NI, Suryani, Halimahtussaddiyah R, Prawesti N. 2014. Preparasi Fenilbutazon dalam Pembawa Vesikular Etosom dengan Berbagai Variasi Konsentrasi Fosfatidilkolin dan Etanol. Kendari: Universitas Halu Oleo. Vol 2(1); 112-118
[4] Choi HM, Kim SH, kim CK, Choi HG, Shin DH, Uhm KI, Jo D. 2015. The cheapest and Easiest Way to make Platelet-rich Plasma Preparation. Vol 21(1): 12-17.
[5] Supraja R. Sailaja AK. 2017. Formulation of Mefenamic Acid Loaded Ethosomal Gel by Hot and Cold Methods. Hyderabad : Nano Biomed Eng. Vol 9(1): 27-35
[6] Acharya /A, Ahmed MG, Rao BD, Vinay CH. 2016. Development and evaluation of Ethosomal Gel of Lornoxicam for Transdermal Delivery: In-Vitro and In-Vivo Evaluation. Karmataka: Manipal Journal of Pharmaceutical Sciences. Vol 2(1): 13-20
[7] Singh MP, Nagori BP, Shaw NR, Tiwari M, Jhanwar B. 2013. Formulation Development & Evaluation of Topical Gel Formulation Using Different Agents and Its Comparison with Marketed Gel Formulation. Rajasthan: International Journal of Pharmaceutical Erudition. Vol 3(3); 1-10.
[8] Elmitra. 2017. Dasar-dasar Farmasetika dan Sediaan Semi Solid. Yogyakarta: Deepublish
[9] Alfianika, Ninit. 2018. *Buku Ajar Metode Penelitian Pengajaran Bahasa Indonesia*. Yogyakarta: Deepublish.

[10] Barupal Ak, Vandana G, Ramteke S. 2012. Preparation and characterization of ethosomes for Topical delivery of Aceclofenac. Bhopal: Indian Journal of Pharmaceutical Sciences.

[11] Hamdi, Asep Saipul. 2014. *Metode Penelitian Kuantitatif Aplikasi dalam Pendidikan*. Yogyakarta: Deepublish

[12] Parmar P, Mishra A, Pathak A. 2016. Preparation of Ethosomal Gel of Clotrimazole for fungal Infection by Mechanical Dispersion Method. Bhopal: Current Research in Pharmaceutical Sciences. Vol 6(2): 45-49