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RESEARCH ARTICLE

The effect of astaxanthin gel and zeaxanthin combination on wound healing in diabetic rats

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Abstract

Background: Diabetes mellitus is a metabolic disorder characterised by uncontrolled increases in glucose levels in the blood due to a decrease in insulin function. Diabetic ulcers are complications that can arise from diabetes mellitus because of peripheral artery disease or complications in neuropathy. Astaxanthin and zeaxanthin are natural carotenoids and contain tremendously powerful antioxidants, they were combined with the goal of generating greater synergistic effects as natural antioxidants. **Objectives**: To determine the effect of the combination of astaxanthin and zeaxanthin (2%:5% b/v) on the gel characteristics and to identify the effectiveness of gel preparations in healing diabetic ulcers in rats. **Methods**: The effectiveness of the gel preparation combination was determined by using male rats administered with variations in concentrations of astaxanthin and zeaxanthin. **Results**: The test results of the effectiveness of gels in healing diabetic ulcers presented that all formulas were significantly effective with average scores of 62.67% for formula 1, 50% for formula 2 and 57.25% for formula 3. The formula that possesses the best average value is formula 1, with a wound healing length of nine days, followed by formula 2 and 3 with wound healing lengths of 11 days. **Conclusion**: The gel formulation of the astaxanthin and zeaxanthin combination was successfully developed and resulted in an effective preparation evaluation and the potential for diabetic ulcer healing in a test model of male Wistar rats.

Introduction

Diabetes mellitus is a metabolic illness characterised by high blood glucose levels caused by insulin shortage or dysfunction (Tarigan, 2015). Diabetes mellitus is a condition that affects a large number of people. Diabetic ulcers are one of the consequences of diabetes (IDF, 2017).

Diabetic ulcers are a type of diabetic complication that occurs as a result of neuropathy or peripheral artery dysfunction (ADA, 2017). Diabetes mellitus neuropathy affects about 20 - 40% of diabetic patients, and about 50% of them develop the peripheral vascular disease, which leads to amputation (Candra *et al.,* 2019).

Astaxanthin is a powerful antioxidant that is able to protect cells and tissues from free radical damage by decreasing oxidative/nitrative stress (Al-amin *et al.,* 2019). Astaxanthin is a powerful fat-soluble antioxidant that was discovered in aquatic organisms like shrimp, salmon, and crabs. Astaxanthin has a pKa of 13.07 and possesses singlet oxygen free radical neutralising action, causing it to be 40 times more effective than beta carotene and 1000 times stronger than vitamin E in terms of lipid peroxidation protection (Singh *et al.,* 2020).

Zeaxanthin is a nutritious carotenoid with a pKa value of 18.91. It was discovered primarily in dark green leafy vegetables and egg yolks and belonged to the xanthophyll pigment family. Another xanthophyll, lutein, is structurally associated with zeaxanthin (Edwards, JA., 2016). It is an anti-inflammatory and has antioxidant qualities, as well as anti-angiogenic properties, which assist in protecting against phototoxic harm and decreases oxidative stress (Xue *et al.,* 2015).

Gels are semisolid systems in which a three-dimensional network of particles or macromolecules dissolved in the dispersing phase restricts the dispersing medium mobility. Gels are not sticky; hence, they require less energy to formulate. They are also stable and possess good aesthetics, so they offer more potential as a means of providing topical medications than ointments. Furthermore, gel effectively transports medicinal substances. Gel preparations also possess the advantage of being easy to evenly spread on the skin, providing a cooling sensation, and not leaving blemishes on the skin. Gel preparation forms are selected at a good level by patients, especially in the treatment of diabetic mellitus wounds (Afianti & Murrukmihadi, 2015).

Methods

Experimental animals

The animals used in this study were male Wistar rats that measured 180-250 grams, were aged between 2 - 3 months and were obtained from Marmot, Rat, and Mice Rodent Breeders (Kp, Sukaraja Rt.04/Rw.09 Majalaya-Bandung). The rats were healthy and regularly active. This research has been approved by the Health Research Ethics Commission of STIKes Bakti Tunas Husada with No. 039/kepk-bth/V/2021. The research was conducted using *The Posttest Only Control Group Design*. The total number of rats used in this study was 24. These 24 white rats were placed into six test groups, each including four white rats.

Preparation and evaluation of the gel

Gel making began by mixing carbopol in distilled water, which had been preheated until homogeneous. Other excipients were then combined to create a clear, swollen gel. The final mixing was done by adding the deionised water and by using a magnetic 100 rpm for one hour.

Evaluation of gel

Organoleptic test

The shape, odour, and colour of the gel were observed 48 hours after the manufacturing procedure and over the 28-day storage period. Visual observation techniques were employed to monitor the tests (Dewantari & Sugihartini, 2015).

Spreadability test

A gram of gel was weighed and placed on a transparent glass with a diameter of 20 cm and covered with another glass. Then, a 125-gram weight was administered and allowed to stand for about one minute before measuring the constant diameter (Das *et al.,* 2011).

pH test

The sample weighed up to one gram. 10 mL of aqua dest water at pH 7 was administered and agitated. After the mixture was homogenised, pH measurements were obtained by inserting a calibrated pH meter and allowing it to stand for a while to acquire a steady pH.

Homogeneity test

The preparation was administered to two pieces of glass or another suitable clear material in order to examine the homogeneity; the preparation should have had a homogeneous arrangement with no visible coarse grains (Gunawan Didik dan Sri Mulyani, 2004).

Viscosity test

A Brookfield viscometer was used to perform the viscosity test. After 48 hours of gelling, the spindle was adjusted until it was submerged and the speed was set (Septiani *et al*., 2011).

Stability test

The cycling test method was used, and the gel was organised at 4±2 °C for 24 hours before being transferred to a 40±2 °C oven for another 24 hours (one cycle). The test was performed for six cycles or 12 days after the phase separation was detected.

Diabetic rat modelling

The rats were acclimated and fasted for 8 - 12 hours before being injected intraperitoneally with 150 mg/kg BW of alloxan. Depending on the alloxan dose and the resistance of the test animals, three to five days passed between induction and diabetes development. A glucometer was used to determine blood glucose levels (BGL). If a rat's blood glucose level was 200 mg/dL, it was classified as diabetic (ADA, 2017). Following that, the diabetic rats were divided into six groups.

Making diabetic rat wounds

Three to five days after induction with a dose of 150 mg/kg BB that was previously dissolved in 0.9% NaCl isotonis solution, test animals with blood sugar levels above 200 mg/dL were wounded after being anesthetised with an inhaled ether solution (Fröde & Medeiros, 2008). 70% alcohol was applied to the back area that had been shaven, and then the rats were injured using a 2 cm long sterile scalpel. The wound depth was either 0.3 cm or reached the dermis, which was characterised by the discharge of blood. The gel was

administered topically by applying it on the wound with a cotton bud as much as two times a day, every day. To prevent infection in the wound, the cleanliness of the cage was maintained both before and during treatment. The parameters observed in the study included the morphological appearance of the wounds, wound size, and the length of time taken for the wound to heal.

Result

Three formulations were created after the gel was generated in Table I.

Table I: Astaxanthin, zeaxanthin and combination gel formula

Materials	Concentration (% b/v)		
	F1	F2	F3
Astaxanthin	2	-	2
Zeaxanthin	-	5	5
Carbopol	0.75	0.75	0.75
Triethanolamine	qs	qs	qs
Propylene Glycol	10	10	10
Glycerin	2	2	2
DMDM Hydantoin	0.1	0.1	0.1
Aquades ad	100	100	100

The pH value of the gel was steady, with an average pH of six from days 0 - 21. Based on the results, the pH of the gel preparation in the skin ranged from 4.5 to 7.0. The spreadability test was carried out to determine the gel's ability to spread on the skin's surface (Figure 1), with the intention that the gel would spread freely. Figure 2 shows the viscosity test results. Results between 5 - 7 cm signified good gel dispersion. Figure 3 demonstrates that the percentage of wound healing increased more in the test gel and positive control group from day 0 to day 14 than in the normal and negative groups. The Shapiro-Wilk method was performed to conduct a normality and homogeneity test ($p > 0.05$). All data groups were substantially different ($p < 0.05$), and the *post hoc* test was administered to determine which groups were significantly different. According to the results of the *post hoc* LSD test for the gel effect on a diabetic rat model, the Astaxanthin formula with a concentration of 2% was tremendously effective with an average value of 63.67%, followed by F3, which was the combination of Astaxanthin and Zeaxanthin(2%:5%) with an average value of 57.25%.

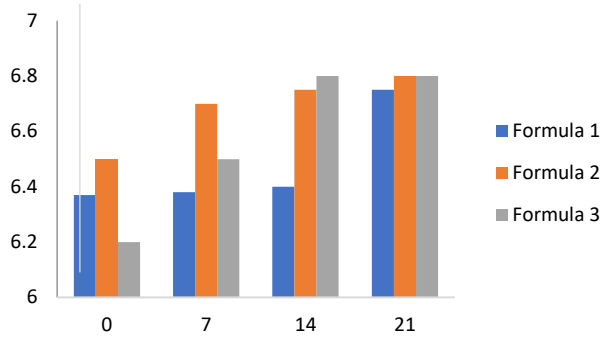

Figure 1: Graph of spreadability test results

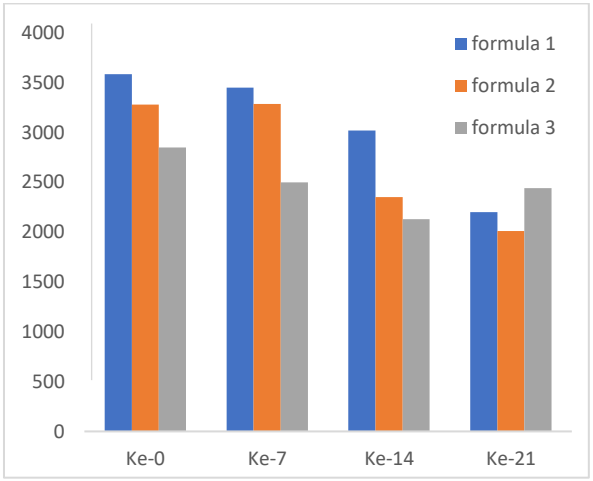

Figure 2: Graph of viscosity test results

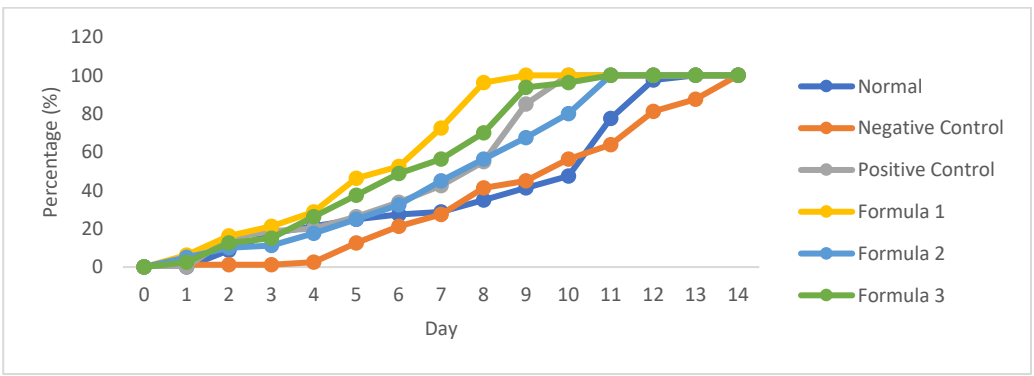

Figure 3: Graph of wound healing

Discussion

These three gel preparation formulas produced gels that were organoleptically stable at 27° - 28°C for 0 - 21 days. The gel had good spreadability, according to the results of the spreadability test (Ainar & Elvira Putri, 2015; Lukman *et al.,* 2017). If the pH of the preparation is too acidic, the skin will shrink and get injured; if the pH is too alkaline, the skin will peel and become dry (Ansari & Tranchant, 2009). The homogeneity test was administered to determine how effective the gel preparation materials were when mixed together (Juvita *et al.,* 2013). When the gel preparations were observed under a microscope with a magnification of 100x, it was revealed that none of the preparations possessed any coarse grains, indicating that the concentration of the extract had no effect on gel homogeneity (Syamsumi, 2006).

In accordance with SNI 16-4399-1996, acceptable topical formulations ranged from 2000-50000 Cps (BSN, 1996). This number is connected to the properties of topical medicines that are easy to remove from the tube and apply to the skin in order to meet packaging standards. The viscosity of a substance determines its resistance and the force required for it to flow (Ansari &Tranchant, 2009). The test results revealed good viscosity values. Organoleptic evaluation of the gel using the cycling test method revealed that it was good organoleptic preparation because there were no transparent semisolid preparations or coarse grains (Syamsumi, 2006).

On the ninth day, blood glucose levels were checked again to identify if the rats were still hyperglycemic (Table II). The rats possessed hyperglycemia; however, only the F2 group's glucose levels did not decrease compared to the pre-induction glucose levels. This was due to the fact that alloxan affects pancreatic β cells in variable degrees. Therefore, not all pancreatic β cells are harmed, and β cells have the ability to regenerate. As a result, β cells continue to produce insulin, lowering blood glucose (Brem Harold and Tomic Canic M, 2007). Compared to the other treatment groups, the administration of 2% astaxanthin gel had the highest average percentage of wound healing, with the percentage reaching 100% on the ninth day. This was due to the fact that astaxanthin acts as an antioxidant in wound healing by stimulating the expression of type 1 procollagens, such as *Fibroblast Growth Factor Basic* and *Endothelial Growth Factor*. FGF assists with granulation tissue development, epithelialisation, and tissue remodelling (Mizuta *et al.,* 2012).

Table II: Average blood glucose levels

Group	Blood glucose level (mg/dL) (Average ± standard deviation)		
	1	2	3
Normal control	108.25 ± 11.00	108.75 ± 18.19	106.75 ± 23.29
Negative control	118.50 ± 30.82	393.75 ± 173.36	323.00 ± 49.25
Positive control	103.75 ± 10.59	477.50 ± 98.08	296.75 ± 95.28
Formula 1	110.50 ± 25.51	408.75 ± 162.12	366.75 ± 168.29
Formula 2	129.00 ± 6.63	350.25 ± 142.85	376.00 ± 181.14
Formula 3	119.50 ± 17.33	400.25 ± 156.51	399.50 ± 162.59

The data were statistically evaluated using the *One Way ANOVA* method. From day 0 - 14, the percentage of wound healing was examined. Astaxanthin and zeaxanthin have good anti-inflammatory activity, beginning at the initiation stage of angiogenesis, in which growth factors were stimulated. Astaxanthin and zeaxanthin played a critical role in increasing granulation tissue. Astaxanthin's anti-inflammatory effects allowed diabetic wounds to heal faster (Xue *et al.,* 2015).

Conclusion

The evaluation of the combination of astaxanthin with zeaxanthin demonstrated that a gel preparation with a good concentration is effective in speeding up wound healing in diabetic mouse models, with a percentage of 57.25% over 14 days.

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