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The Effect "Ox-Water" on Wound Healing of Diabetic Rats *(Rattus norvegicus)* Induced by Multiple Low Dose of Strptozotocin (MLD-STZ)

Aulanni'am Aulanni'am[1,2,*], DyahKinasih Wuragil[2,3], Anita Herawati[2], Januar Gondosapoetra[4]

[1]Department of Chemistry, Faculty of Sciences, Brawijaya University, Indonesia
[2]Biosains Institute, Brawijaya University, Indonesia
[3]Faculty of Veterinary Medicine, Brawijaya University, Indonesia
[4]Moor Sukses International, Co. Ltd, East Java, Indonesia

Abstract: Diabetes mellitus (DM) is one of glucose metabolic disorders characterized by pancreatic β cell destruction that followed the conditions of hyperglycemia and healing of wounds that are difficult to cure. Oxygenated water is believed able to suppress the growth of bacteria in the wound. Many therapies both with chemicals or herbal substances have been reported. The aimed of this research was to investigate the activity of 100 ppm Ox-water to treat wounds on the state of diabetic conditions through the inhibition of anaerobic bacteria, through the blood glucose levels and histopathology of skin, expression of GLUT 2 expression by immunohistochemistry on diabetis rats. Diabetic rats induced by 20 mg/kg BW of MLD STZ for 5 days. This study used male rats *(Rattus norvegicus* Wistar strain*)* with 10-12 weeks of age and 200 g of body weight. Rats were divided into five groups; (A) control group (healthy rats); (B) diabetic rats with brisket wound due to incisi; and (C), (D) and (E) were diabetic rats with brisket wound due to incisiand received therapies with 100 ppm Ox-water spray on skin wound and 2 mL force feeding in ones, twice and third times a day for 14 days, respectively. MLD-STZ resulted in diabetic rats and had bear up for 14 days after last injection without any therapies. This findings showed a decreased of brisket wound length on rats on groups with 100 ppm Ox-water therapies .There was alsoan improvement of skin on brisket wound after100 ppm Ox-water therapies. This finding suggests the efficacy of using 100 ppm Of-water for wound therapy on the condition of diabetic patients with spray method directly on the wound.
Key words : diabetes mellitus, 100 ppm Ox-water, GLUT-2, blood glucose levels, wound length.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose levels and are usually followed by the injuries that aggravate the patient's condition. Base on the latest data compiled by the World Health Organization (WHO), the prevalence of DM in developing countries is potential to increase. It had been reported that currently about 150 million people suffer from DM and is estimated to grow in twice by 2025. The increase is due to population growth, aging, unhealthy diet and obesity. Indonesia has a high probability in the increasing number of diabetic patients in which most of the population is age 45-65years old thus affecting productivity in the country [1].

Hyperglicemia condition because glucose metabolism disorder caused by a deficiency of insulin and β cell pancreas destruction because some complications also worsened the infection. There have been reported a lot of diabetic patients with food infection [2]. Wounds in diabetics is usually difficult to treat due to high blood glucose levels are disrupt immune function to attact viruses or bacteria that trigger the production of Reactive Oxygen Species (ROS) excess.

Basically, the goal of diabetes treatment is to lower blood glucose levels to normal, but to prevent hypoglycemia. Oral treatment with chemical drugs has side effects when consumed prolonged time. The Effects often complaining aregastrointestinal and renal disorders as well ashypoglycemia [3]. Oxygen is a substance which dissolved in water. Water containing a high oxygen concentration can help circulation on metabolism. High oxygen on circulation will lead ephitelialization of cells also to reduce and kill bacterial which cause an infection. The purpose of this study was to investigate the activity of 100 ppm Ox-water to treat wounds on the state of diabetic conditions through the inhibition of anaerobic bacteria, through the expression of GLUT 2 expression by immunohistochemistry.

Experimental

Animal Model Preparation

This study used male *Rattus norvegicus* Wistar strain obtained from the Animal Model Unit Development (UPHP) UGM Yogyakarta with 10-12 weeks of age, body weight average of 200 g. The use of animal models in this research had been approved by Brawijaya University Research Ethics Committee No. 303-KEP-UB.Rats were divided into five groups; (A) control group (healthy rats) with brisket wound due to incisi; (B) diabetic rats with wound incision in the skin, and (C), (D) and (E) were diabetic rats with brisket wound and received therapies with 100 ppm Ox-water on spray and 2 mL force feeding in ones, twice and third times a day for 14 days, respectively.

Preparation of Diabetic Induced Animal Model

Preparation of diabetic induced animal models was conducted by Multi Low Dose Streptozotocin (MLD-STZ). 100 mg of Streptozotocin was dissolved in 3 mL of pH 4.5.citrate buffer. 20 mg/kg STZ then was injected in five consecutive days, intraperitonially (i.p). After injection for 5 days, then rats were awaited for 14 days to ensure DM condition which characterized to express blood glucose level greater than 200 mg/dL [4].

100 ppm Ox-water Therapies

100 ppm ox-water (Moor Sukses International, Co. Ltd) were administered to C, D, E groups with vary methods as follows: Group C was diabetic with brisket wound with therapies on sparay and force feeding as much as 2 mL and administered ones per day for 14 days, while group B were given with Ox-water therapies for twice a day and the last group of E, rats were theurapized for third times a day.

Blood Glucose levels measurement

Blood glucose levels were assessed using glucometer kit (digital one touch, Lifescan) usingcoccygeal-vein blood sampling. Data was obtained on mg/dL levels

Histopathological of pancreatic β cells observation and analysis

Histological of pancreas was prepared based on Junquiera and Carneiro [5] with slight modification, observation and analysis was conducted to perform

Incision Length Analysis

Wound brisket was prepared by chloroform anaesthesia to prepare unconscious rats, then the brisket were scratched one direction to obtain wound.

Statistical Analysis

All data were recorded individual within the groups. Data shown on mean ±SD. Data comparison were performed using SPSS ver. 16 for Windows and carried out using ANOVA and Tukey Test. A value of $p< 0.05$ was considered significant.

Results and Discussion

Table 1. Blood Glucose Levels of Experimental Animals

Groups	Blood glucose levels (mg/dL)	Percentage of blood glucose level alteration	
		Increased	Decreased
Healthy rats (A)	116.4 ± 14.12^{a}	-	-
Diabetic rats (B)	499.2 ± 41.74^{e}	328	-
Ox-water therapies 1x(C)	417.6 ± 40.23^{d}	-	16.35
Ox-water therapies 2x (D)	331.4 ± 37.73^{c}	-	33.6
Ox-water therapies 3x(E)	187.4 ± 16.01^{b}	-	62.48

Note: superscript showed significantly different between group ($p<0.05$)

Rats induced diabetes was prepare by multi low dose of streptozotocin as previously describes [6]. This technique was able to produce consistency diabetic rats. The previous reports showed the persistence of diabetes mellitus after incubation for 20-30 days. It have been noted that an increasing of blood glucose levels caused by (1) decreasing amount of glucose that enters the cells; (2) reduced use of glucose by various tissues; and (3) increased production of glucose production (gluconeogenesis) in a liver. Our results showed that Ox-water therapies could lower blood glucose levels. The blood glucose lowering mechanism in this research was supported by oxygenation of pancreatic tissue. The presence of ox-water support the cell-oxygen needed to recover their injuries due to free radicals of NO resulted from streptozotocin injection. The pancreatic recovery lead to insulin production to lower glucose levels in a blood and used it as energy for gluconeogenesis.

Diabetes mellitus also related with many complication such as the severe of infection due to anaerobic bacteria. The high glucose levels in a blood play as an energy source to bacterial growth especially on skin wound. Many researchers had been reported for the difficulty of wound healing on DM patient and cause damage and lead to amputation [7]. This study also observed for the progression of administering ox-water on DM treatment by force drinking and spray directly to rat's brisket wound based on collagen formation on rat's injuries due to incisi (Fig. 1).

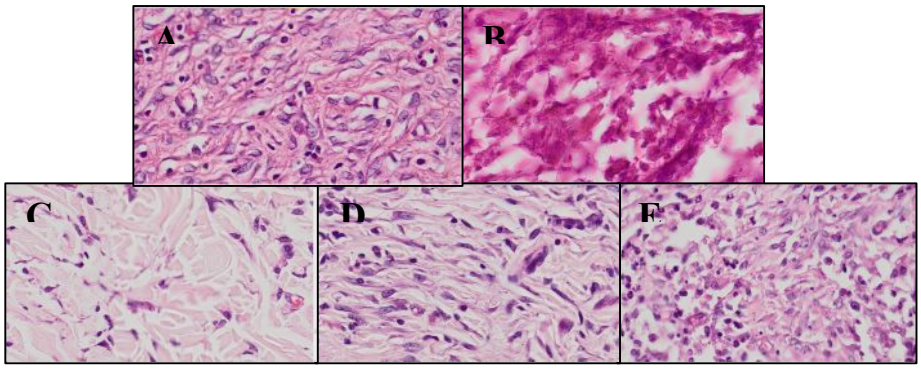

Figure 1.*Brisket skin woundtissue section of treatment rats (400x)*Healthy rats; (B) Diabetics Rats; (C) Diabetic Rats with one ox-water therapies/day;(D) Diabetic Rats with twice ox-water therapies/day, and (E) Diabetic Rats with three times ox-water therapies/day

The control group who suffered wounds without therapy showed wound closure but still looks the Scar, and indicate the presence of new epithelial formation process. While in diabetic rat's wound, showed the inflammation. Wound healing ability is depend on differences in receiving stimulus, growth factors and

cytokines as well as inhibiting factors in each individual [8]. Non diabetic rats showed the density of the collagen tissue, and epithelial formation occurs on days 3 to 14 after the incision. The formation of new epithelial wound repair is the phase consists of several stages of migration, mitosis and differentiation of epithelial cells to restore the integrity of the cell. With collagen synthesis by fibroblasts, formation on dermis will be completed by formation of granulation and dermis [9].

Besides, it also reported for macroscopically appearance of wound healing progression of rat's incisi base on wound length. The ox-water showed potential ability to reduce wound length on diabetic rats with incisi (Fig. 2 and Table 2).

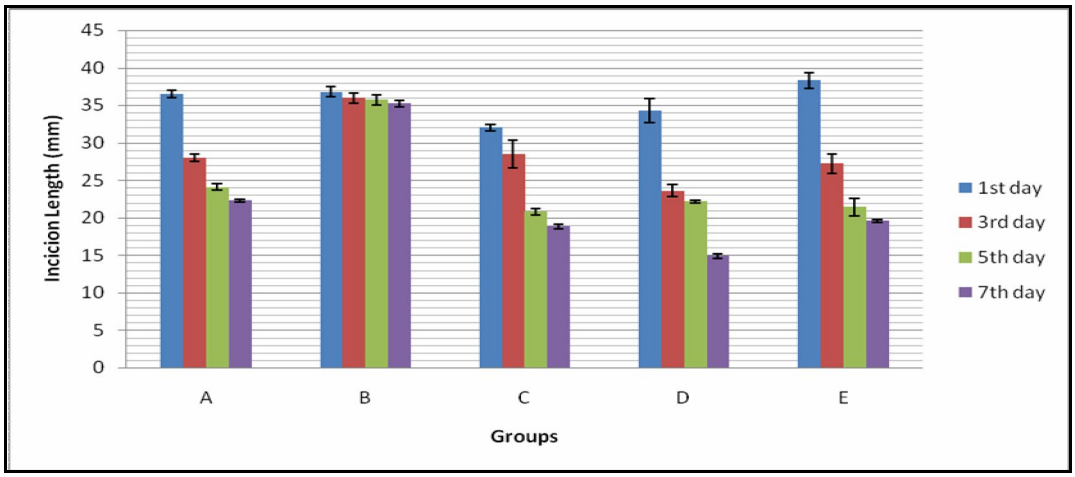

Figure 2. Wound Healing Length of Normal (A) vs Diabetic Rats (B,C,D,E) based on Incision Length. (A)Healthy rats; (B) Diabetics Rats; (C) Diabetic Rats with one ox-water therapies/day; (D) Diabetic Rats with twice ox-water therapies/day, and (E) Diabetic Rats with three times ox-water therapies/day.

Wound healing in diabetic rats begins with the re-establishment of the epithelial tissue supporting s to restore the integrity of the skin. With the synthesis of collagen by fibroblasts which changes its structure into myofibroblast. This myofibroblast performing the function to a contraction in the tissue in the presence of oxygen.Addition of oxygen in wound healing in encouraged the formation of new connective tissue, also encourage the formation of Fibroblast Growth Factor-2 mediated presence of macrophages and platelets [10].

The reduction of length on rat's wound skin by ox-water treatment on diabetic rats showed on this following (Table. 2)

Table 2. Wound healing progression of 100 ppm Ox-water therapies

	1st day	3rd day	5th day	7th day
Healthy Rats (A)				
Diabetic Rats (B)				
Ox-water therapies 1x/day				
Ox-water therapies 2x/day				
Ox-water therapies 3x/day				

Diabetes mellitus condition also related with glucose transporter of GLUT-2 which support insulin secretion and glucose uptake in pancreatic β cells. It also expressed on other tissue affected by DM and showed the severity of DM condition [11]. The enhancing of GLUT-2 lead to severity of DM. Therapeutic effects of 100 ppm ox-water in diabetic rats alter GLUT-2 expression which was reduced after ox-water treatment on diabetic rats. The more ox-water administered in a day, the lower of GLUT-2 expression obtained (Fig. 3 and Fig. 4).

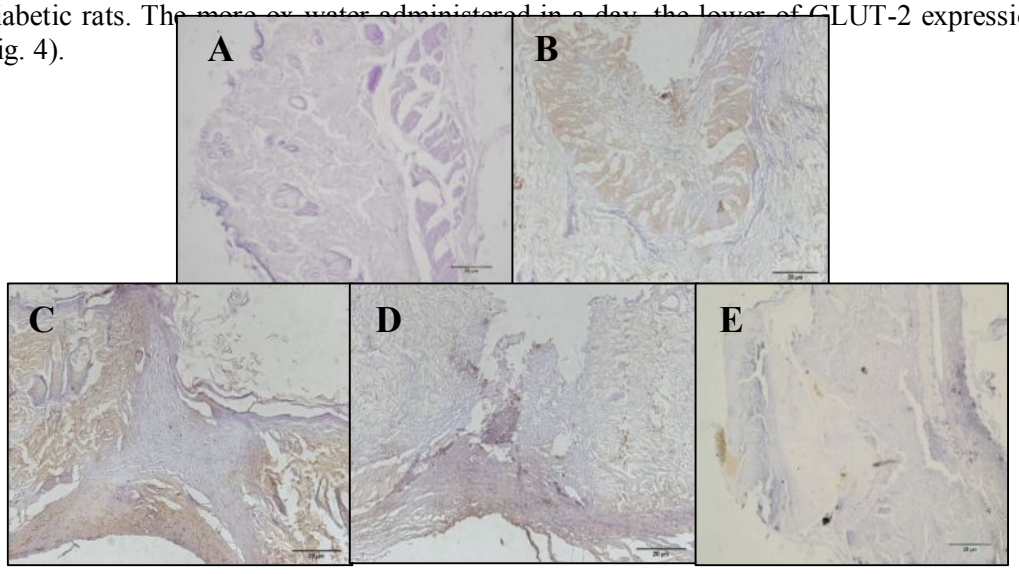

Figure 3. GLUT-2 Expression onrat's BrisketSkin Wound Healthy rats; (B) Diabetics Rats; (C) Diabetic Rats with one ox-water therapies/day;(D) Diabetic Rats with twice ox-water therapies/day, and (E) Diabetic Rats with three times ox-water therapies/day

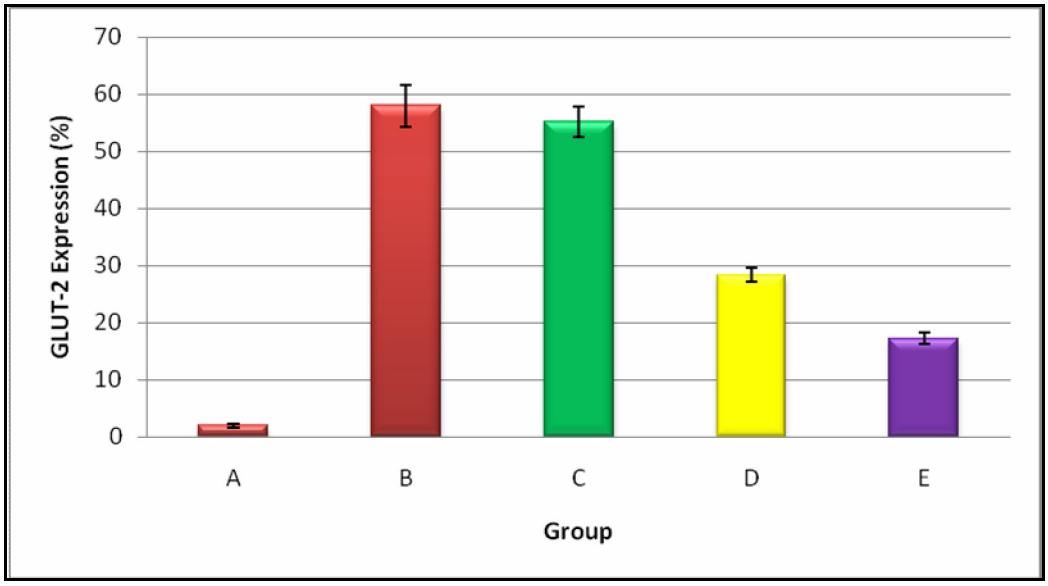

Figure 4. Percentage area of GLUT-2 Expression on rat's brisket skin wound (A) Healthy rats; (B) Diabetics Rats; (C) Diabetic Rats with one ox-water therapies/day; (D) Diabetic Rats with twice ox-water therapies/day, and (E) Diabetic Rats with three times ox-water therapies/day

This finding showed that 100 ppm of ox-water therapy could affect the recovery of DM as well as wound healing as the effect of severity of this disease. This therapy could affect the lowering of blood glucose levels, improvement of collagen formation on brisket skin after incision as well the reduction of wound length and reduction of GLUT-2 expression. The administration of 100 ppm ox-water therapy showed progression for recovering condition. The more frequent of 100 ppm ox-water administration both on spray and drinking on a day, the more recovery parameter showed on this research. Further improvement of therapy and analysis still needed to overcome wound healing problem in diabetic rats to recommend suitable therapy based on providing more oxygenated environment on organism cells.

Conclusions

The conclusion of this worked wasspray and oral therapy of 100 ppm Ox-water could reduce blood glucose levels also reduce degree of wound skin showed by reduction of wound length, formation of collagen network in dermis as well as declining of GLUT-2 expression on brisketskin wound of diabetic rat induced by MLD-STZ.

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