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# The Effect of Egg Yolk Oil on the Mast Cell Concentration in Excisional Wound Healing of STZ-Diabetic Rats

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**Abstract**: Diabetes mellitus (DM) is serious metabolic disease. Impaired wound healing in DM leads to significant morbidity and mortality due to various clinical and socioeconomic issues. Wound healing is a complex mechanism involving different tissues and cells. Mast cells (MC) are the first group of cells to respond to injury and contribute to three main phases of wound healing: inflammation, proliferation, and scar formation/remodeling. The aim of this study was to evaluate the effect of egg yolk oil (EYO) on MC concentration in excisional wounds of streptozotocin (STZ) diabetic rats. Female Sprague-Dawley rats were allocated in three groups (6 rats per group) as: Group 1 (non-diabetic, topically treated with 2% fusidic acid ointment), Group 2 (STZ-diabetic, topically treated with 2% fusidic acid ointment), and Group 3 (STZ-diabetic, topically treated with EYO). On third day after single intraperitoneal injection of 65 mg/kg STZ, two full-thickness skin excisional wounds were generated on the back of all rats (day 0). On day 7 and day 14, randomly selected three rats per group were sacrificed under deep anesthesia. Skin sections were stained with toluidine blue, and MC numbers were determined. Differences in these numbers among the groups were analyzed statistically. Group 2 and Group 3 had statistically higher MC concentration on day 0 compared to Group 1 ($p$<0.001). Group 3 had statistically lower MC concentration on day 7 compared to Group 1 and Group 2 ($p$<0.01). In addition, increased MC degranulation was observed in Group 3 on the same day. The results of this study suggest that EYO induces MC degranulation, which is related to wound healing process, and decreases MC concentration in the first few days of the wound healing in STZ-diabetic rats. This decrease in MC concentration in DM is likely to be enable the wound to heal earlier than some other cases.

**Keywords:** Wound healing, STZ, Egg yolk oil, Mast cells, Diabetes mellitus

## Introduction

Wound healing is a complex and systematic progression, and consisting three phases: inflammation, proliferation, and remodeling (Falanga, 2005). It has been documented that unresolved inflammation causes chronic wounds (Nguyen et al., 2019). In diabetic patients, chronic foot ulcers caused by impaired wound healing are common, and morbidity, mortality, and excess care costs are quite high (Ramsey et al., 1999).

Mast cells (MC) are found abundantly in skin like barrier organs, and they initiate allergic reactions. It has been shown that MC enhance acute inflammation, stimulate re-epithelialization and angiogenesis, while they promote scarring in normal wound healing; their numbers increase in chronic wounds, hypertrophic scars and keloids (Gniadecki, Gajkowska, Bartosik, Hansen, & Wulf, 1998; Komi, Khomtchouk, & Santa Maria, 2019; Noli & Miolo, 2001). MC granules contain many mediators such as heparin, histamine, tryptase, chymase, vascular endothelial growth factor, and tumor necrosis factor alpha; they release these mediators by degranulation during wounding, accelerate the angiogenesis and fibroblast proliferation and increase collagen synthesis (Artuc, Hermes, Steckelings, Grützkau, & Henz, 1999; Hatamochi, Fujiwara, & Ueki, 1984; Tonnesen, Feng, & Clark, 2000). It has been also reported that MC accumulate at the wound edge and may participate the collagen remodeling (Iba, Shibata, Kato, & Masukawa, 2004). When all these are taken into account, it can be suggested that MC can be involved in three phases of wound healing, which are inflammation, proliferation and remodeling phases.



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In recent years, the importance of MC in chronic diseases and wounds has been recognized, and effects of many natural products on MC concentration have been investigated (Estevão et al., 2015; Farahpour, Hesaraki, Faraji, Zeinalpour, & Aghaei, 2017; Kempuraj, Caraffa, Ronconi, Lessiani, & Conti, 2016; Ozay et al., 2018; Sarandy et al., 2018; Shi & Shi, 2012; Souza Neto Junior et al., 2017). For example, Farahpour, Mirzakhani, Doostmohammadi, & Ebrahimzadeh (2015) have reported that, *Pistacia atlantica* hulls ointment enhances MC distribution and infiltration, which in turn promotes the neovascularization, and it can therefore be considered as an appropriate compound for wound healing medicine. This study aimed to evaluate the effect of egg yolk oil (EYO) on MC concentration in excisional wounds of streptozotocin (STZ) diabetic rats.

## Method

### Preparation of EYO

To obtain EYO, the method which was developed by Herring & Ala (1980) was used. Boiled fifteen organic chicken eggs' shells were removed, egg whites were separated, and only solid egg yolks were put in the cleaned and sterilized pan. After that, the egg yolks were heated up to 190-200 °C for 20 minutes to release EYO. When EYO come out from the egg yolk, it was stirred for another 5 minutes. Then, pure EYO was obtained by throwing redundant egg yolks and filtering it from sterile gauze. EYO was kept in an amber flask at +4 °C until further use in this study.

### Experimental Animals and Study Design

All rats were fed commercial rat feed and water *ad libitum*, and were kept on a 12 h light/12 h dark cycle. Three groups of rats which were consisted randomly selected six female Sprague-Dawley rats (190-230 g) were allocated as: Group 1 (non-diabetic, topically treated with 2% fusidic acid ointment), Group 2 (STZ-diabetic, topically treated with 2% fusidic acid ointment), and Group 3 (STZ-diabetic, topically treated with EYO). On third day after single intraperitoneal injection of 65 mg/kg STZ (dissolved in 0.1 mol/L sodium citrate buffer, pH 4.2), the rats with fasting blood glucose level of 250 mg/dL and above were considered as diabetic. All ethical approvals were taken from the Pamukkale University Animal Experiments Ethics Committee (PAUHDEK 2015/3).

### Excision Wound Model

Excision wounds were created according to Nagappan, Segaran, Wahid, Ramasamy, & Vairappan, 2012. The dorsal thoracic skin of the anesthetized rats was shaved with electric razor, hairs were removed and the region disinfected using 70% alcohol (Figure 1A). Two mutually rounded wounds were formed on the back of each rat (Figure 1B and C) using sterile punch-biopsy apparatus (a diameter of 6 mm). For this purpose, the punch-biopsy apparatus applied to the shaved area with a uniformly pressure single twist until the subcutaneous dermal layer was separated (day 0). The wounds were cleaned using sterile gauze and compressed. All rats were put into cages separately. Before the applications of ointments, all wounds were cleaned with sterile saline solution. After that, all wounds were covered with sufficient ointments (Ozay et al., 2013) (Figure 1D and E). 2% fusidic acid was applied to Group 1 and Group 2, and EYO was applied to Group 3. Applications to the wound areas were continued for 14 days, once a day.

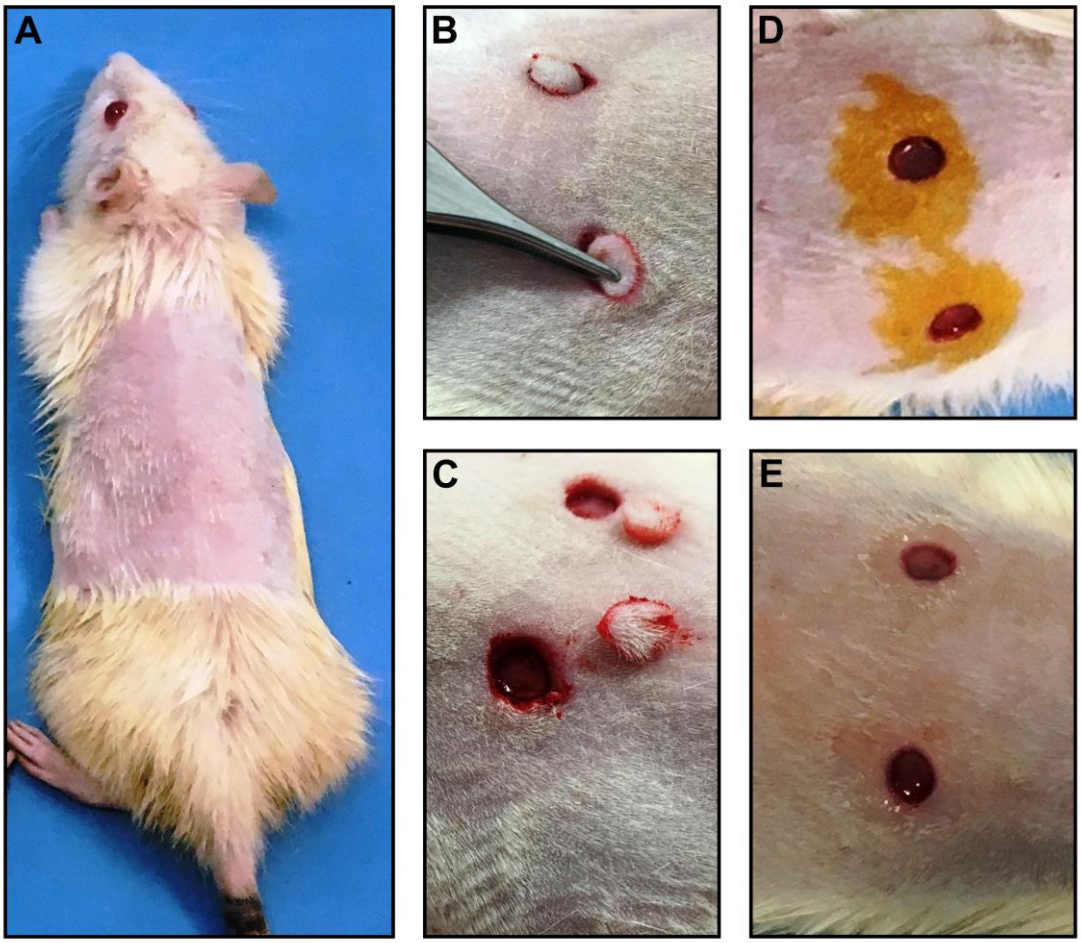


Figure 1. Excision wound model in the study

On day 7 and day 14, randomly selected three rats from each group were sacrificed under deep anesthesia. Wound skin samples were fixed in 10% formalin and embedded in paraffin after routine tissue processing. 5 µm skin sections were stained with toluidine blue (TB) (Toluidine Blue Staining Protocol for Mast Cells, n.d.) and analyzed with light microscope. MC numbers were determined by counting in the randomly selected five 400X fields by two observers.

**Statistical Analyses**

All values were expressed as means ± standard deviation (SD). The differences in the numbers of the MC among the groups were analyzed using the Kruskal-Wallis test and significant differences among groups were evaluated by the Mann-Whitney U test using Minitab 16 (Minitab Inc., State College, PA). All $p$ values $<0.05$ were considered to be statistically significant.

## Results and Discussion

MC were examined in the randomly selected microscopic fields (Figure 2) and counted for each sampled rat per examined day.

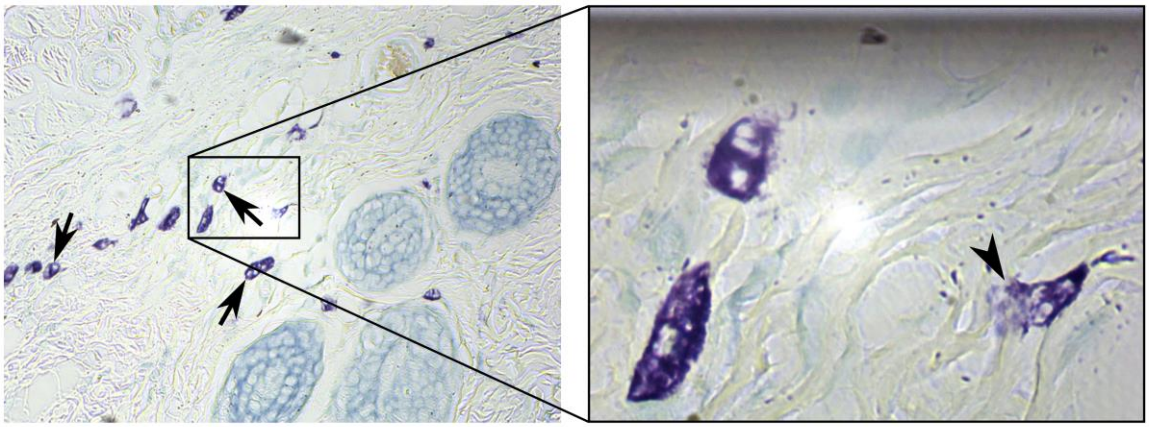

Figure 2. Photomicrographs of excisional skin wounds in rats. Note numerous MC (arrows) in the skin and the degranulation of MC in inset (arrowhead). Toluidine blue. ×200. Inset ×1000.

There were no significant differences among the groups in terms of MC concentration regardless of the days of lesion ($p>0.05$), but there were among the days of lesion regardless of the groups ($p<0.001$). Descriptive data on topical applications in the rats are presented in Table 1. In Group 1, MC concentration tended to decrease consistently, which was lower on day 7 compared with day 0 and lower on day 14 compared with day 7. However, in Group 2 and Group 3, MC concentrations decreased on day 7 compared with day 0, and then they showed an increase compared with day 7, but not more than the concentrations on day 0. Group 2 and Group 3 had statistically higher MC concentration (17.20 and 17.72, respectively) on day 0 compared to Group 1 (12.48) (Figure 3) ($p<0.001$). Group 3 had statistically lower MC concentration (4.87) on day 7 compared to Group 1 (10.93) and Group 2 (8.90) (Figure 3) ($p<0.01$). In addition, increased MC degranulation was observed in Group 3 compared with Group 1 and Group 2 on day 7. On day 14, there were no significant differences among the numbers of MC of the groups.

Table 1. Effects of EYO topical application on MC concentration in STZ-diabetic rats

| **Groups** | **Day 0** | | **Day 7** | | **Day 14** | |
|---|---|---|---|---|---|---|
| | **Mean** | **±SD** | **Mean** | **±SD** | **Mean** | **±SD** |
| Group 1 | 12.48[a] | 0.86 | 10.93[a] | 1.46 | 7.80[a] | 0.66 |
| Group 2 | 17.20[b] | 2.19 | 8.90[a] | 1.47 | 11.37[a] | 2.11 |
| Group 3 | 17.72[b] | 1.65 | 4.87[b] | 0.58 | 11.83[a] | 2.26 |

Means that do not share a superscript letter within same column are significantly different from each other ($p<0.05$).

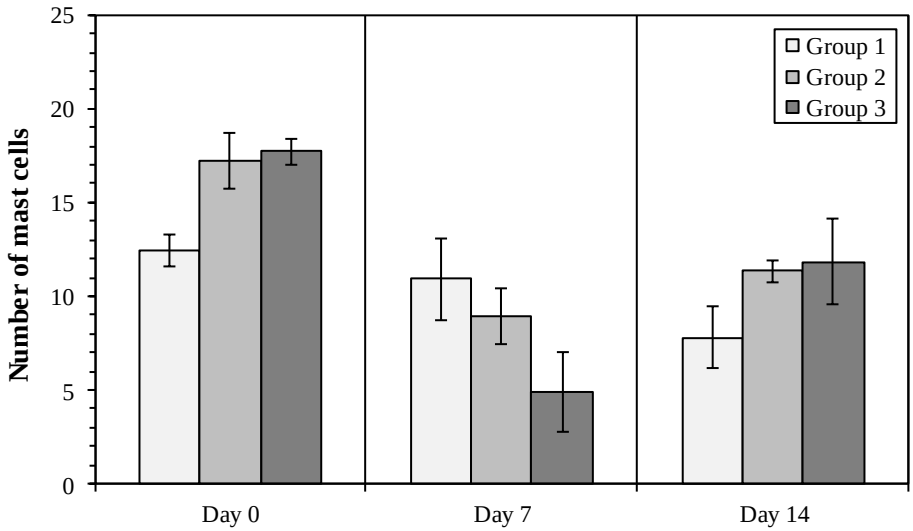


Figure 3. MC concentration of the groups in the study on days 0, 7, and 14.

In diabetic patients' skin, high numbers of MC have been observed in some studies (El Safoury, Fawzy, El Maadawa, & Mohamed, 2009; Tellechea et al., 2016). Similar to these studies, it was found in the present study that the DM groups (Group 2 and Group 3) had higher MC concentrations than Group 1 on day 0. This result may have been due to the chronic proinflammatory state on the skin caused by diabetes (Leal et al., 2015; Tellechea et al., 2013).

It has been reported that the number of MC significantly decreases in adelmidrol + trans-traumatic acid treated diabetic wounds (Siracusa et al., 2018). It was detected in the present study that EYO significantly reduced MC concentration in Group 3 on day 7 compared to Group 1 and Group 2. This result is in agreement with the results of the study carried out by Babaei et al. (2017) in which the effects of omega-3 fatty acids on diabetic wounds were investigated. It has been also suggested that the eggs of the poultry are one of the source of the omega-3 fatty acid (Shinn, Proctor, & Baum, 2018). Taking this knowledge into account, it can be stated that the omega-3 fatty acid content of the EYO may cause this reducing effect on MC concentration.

Activation of MC and release of histamine are necessary processes for normal wound healing (Weller, Foitzik, Paus, Syska, & Maurer, 2006). MC, by means of degranulation, also release many mediators, and these mediators are triggered and modulated inflammatory stage, connective cellular elements proliferation, and remodeling of connective tissue matrix (Noli & Miolo, 2001), which are the phases of wound healing process. It has been shown that MC degranulation increases in diabetic human and mice skin (Nishikori, Shiota, & Okunishi, 2014). In the present study, increased MC degranulation was mostly observed in Group 3 on day 7 compared to Group 1 and Group 2. In light of this finding, it can be assumed that EYO triggers the MC degranulation, and hence causes a decrease in MC concentration and consequently accelerates the wound healing by affecting epithelial cells and fibroblasts. These cells are involved in matrix formation and remodeling (Noli & Miolo, 2001). Similarly, it has been found by Souza Neto Junior et al. (2017) that 10% *Ximenia americana* containing ointment decreases MC concentration and accelerates the early wound contraction in rat skins. It has been also assumed by the same researchers that degranulated MC rapidly disappears and therefore MC cannot be identified in histological sections easily (Souza Neto Junior et al., 2017).

## Conclusion

The results of this study suggest that EYO induces MC degranulation, which is related to wound healing process, and decreases MC concentration in the first few days of the wound healing in rats with DM. This decrease in MC concentration in DM is likely to be enable the wound to heal earlier than some other cases.

## Recommendations

It can be recommended from the results of this study that EYO may be a promising therapeutic agent for the DM-caused wound healing, but further molecular analyses are needed to investigate the active compounds and the mechanisms of their actions.

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