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Effect of Wheat Germ Oil on Wound Healing: An *in Vitro* Study in Fibroblast Cells

Muhammed Gömeç [1]*, Gülnihal İpek [2], Ayşegül Öztürk [3], Zeynep Deniz Şahin İnan [4]

[1]Department of General Surgery, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye
[2]Faculty of Pharmacy Sivas Cumhuriyet University, Sivas, Türkiye
[3]Deparment of Medical Services and Techniques, Sivas Cumhuriyet University, Vocational School of Health Services, Sivas, Türkiye
[4]Department of Histology&Embryology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye

ABSTRACT:
Purpose: Wound healing is a set of mechanisms that are activated to restore structurally damaged tissue. There are many studies aimed at accelerating wound healing. In this context, products obtained from plants come to the fore. In this article, the effects of wheat germ oil (WGO), which is known to have antioxidant and anti-inflammatory properties, on wound healing were investigated by *in vitro* method.
Material and Methods: Wheat germ oil and α-tocopherol were applied to L929, a healthy fibroblast cell line, at different doses for 24, 48 and 72 hours. Cell viability was measured by XTT colorimetric method. *In vitro* wound healing model was applied at the dose where the oil was effective. Obtained results were analyzed statistically.
Results: As a result of the application of α-tocopherol to L929 cells at different doses, it was observed that there was no significant contribution to cell proliferation compared to the control group. However, WGO was observed to significantly increase proliferation at the 100 ng/ml concentration. In the wound healing model, cells treated with WGO at 48 hours were observed to proliferate faster and invade the wound site more rapidly. ($p<0.05$)
Conclusion: The data obtained in our study showed that WGO contributed positively to wound healing. It was determined that this effect was not related to the α-tocopherol in the content of WGO. Although the effect of WGO on wound healing was seen to be positive in our study, our study should be supported by in vivo and clinical studies.
Keywords: *Cell viability, Wheat germ oil, Wound healing, α-tokoferole*

**Corresponding author: Muhammed Gömeç, email: muhammedgomec@cumhuriyet.edu.tr*

INTRODUCTION

A wound is a common condition that occurs as a result of damage to the skin and soft tissue for any reason. These wounds can turn into more complex and chronic wounds as well as being simple (Sendi and McNally, 2016; Van Zanten et al., 2017). The healing of these wounds in the human body has a complex process aimed at restoring the damaged tissue to maintain tissue homeostasis. Complex processes such as cell migration, cell proliferation, collagen synthesis, collagen deposition, angiogenesis and remodeling require well-organized systems to work in harmony. Time is important in wound healing and the length of the healing process is not stable in tissue wounds. This period varies depending on many factors such as the nature of the wound, the general health of the person, its vascular structure, the cause of the wound, its location in the body and whether there is an infection in the wound. Wounds that are delayed in healing and last longer than three months are defined as chronic wounds. Acute wounds tend to heal faster and with fewer complications than chronic wounds. For this reason, it is aimed to heal the wounds as soon as possible. Because the prolongation of the wound healing period can cause problems such as chronic ulcers

and pathological scars (Falanga, 2005). Therefore, it is important to accelerate the wound healing process, for to prevent scar formation and the formation of chronic non-healing wounds.

Wheat Germ Oil contains valuable essential fatty acids. It contains linolenic acid and alpha linoleic acid, which may be beneficial by increasing stamina, lowering cholesterol levels, and aiding muscular dystrophies and other neuromuscular conditions. The tocopherol in WGO can also change the intensity of lipid peroxidation processes by stimulating the redox system (Jacob, 1995; Leenhardt et al., 2008). 20 kg of germ oil can be obtained from one ton of wheat, and approximately 1 kg of germ oil from 20 kg of germ. Wheat Germ Oil has many bioactive ingredients such as vitamin E, thiamine, riboflavin, niacin, tocopherols, phytosterols and policosanols at different rates depending on many variables such as production location and production method (Atwell and Finnie, 2016; Liu, 2007). In addition to these ingredients, it contains peptides that support wound healing and contribute to cell proliferation by having anti-inflammatory activity. In addition, it is known that tocopherols have high antioxidant activity and have various benefits such as delay in aging, regulation of cholesterol levels, and being protective in the progression of degenerative diseases. However, the effect of bioactive molecules other than tocopherols on wound healing is still being investigated (Özcan et al., 2013; Türkoğlu et al., 2021). Wheat germ oil has a strong role in the prevention and treatment of diseases that cause oxidative stress. Many experimental studies have shown that WGO, which is a natural food, can act as an additive in health and cosmetic products (Abdel-Gawad, 2015; Anwar and Mohamed, 2015; Wang and Johnson, 2001).

Collective cell migration; is an important process for wound repair, cancer invasion, metastasis, immune responses, angiogenesis, and embryonic morphogenesis. Wound healing testing is also a standard *in vitro* technique for investigating bulk cell migration in two dimensions. In this test, a cell-free field is created in the confluent monolayer either by physical exclusion or by removal of cells from the site by mechanical, thermal, or chemical damage. Exposure to the cell-free space causes cells to migrate into the cavity and represents wound closure (Borges et al., 2017; Walter et al., 2010). In the light of this information, the potential effect of wheat germ oil, whose antioxidant activity is known, on the L929 fibroblast cell line, which is frequently used in wound healing studies, was evaluated within the scope of our research. In addition, in this study, it was investigated whether this effect was due to α-tocopherol.

MATERIAL and METHODS

Cell Culture Studies

Healthy fibroblast cell line L929, obtained from ATCC (American Type Culture Collection) by CÜTFAM, was prepared under sterile conditions at 37°C and 5% CO2 in 25 cm2 flasks, in DMEM (1% L-glutamine, % 1 penicillin-streptomycin and 10% fetal bovine serum) were grown in cell culture medium. Cells were passaged when they reached 80% density, and work began after the third passage.

The XTT Cell Viability Test

The effects of WGO and α-tocopherol on cell viability were investigated by XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) test. This method is based on the principle that metabolically active cells reduce XTT, a tetrazolium salt, to orange formazan components. Although the dye formed is water-soluble, the dye density can be read at the given wavelengths with the help of a spectrophotometer. The dye intensity (orange color) is proportional to the number of metabolically active cells.

For cytotoxicity, first all L929 cells, $1x10^4$ cells per well, were taken and inoculated into a sterile 96-well microplate and allowed to adhere overnight. The next day, WGO and α-tocopherol alone were applied to the cells in increasing concentrations and incubated for 24 and 48 hours. At the end of the incubation period, 50 µl of XTT solution was added to each well and incubated for 4 hours in a CO2 oven. Then, the optical density measurement value was read in a microplate reader at 450 nm, the cell viability rate of the control group was accepted as 100% and the calculation was made using the formula % Cell viability = (Concentration O.D./Control O.D.) X 100.

Wound Healing Test

This experiment was performed using a wound healing kit (ab242285; Abcam plc.; Cambridge, UK). The kit consists of 2x24 well plates, each consisting of 12 standard wound-forming plates. The inserts form a standard wound area with a defined gap measuring 0.9x1.8 mm to measure the migration and proliferation rates of cells. Cells that migrate to the wound area can extend their protrusion and eventually invade and seal the wound area. Cell proliferation and migration rates were visualized by staining with a light microscope. The proliferation rate was assessed using the Wound Healing Assay according to the manufacturer's instructions. Briefly, a trans well medial culture plate placed on L929 fibroblast cell line applied with germ oil (Tabia; Pure natural germ oil; MM 01 002, TR) was cultured for 24 hours, 36 hours and 48 hours. A cell suspension containing 0.5 -1.0 x 10^6 cells/mL was formed in medium containing roughly 10% fetal bovine serum (FBS), 250 µL of cells were added to each well for optimum cell distribution. Then, at the end of 24, 36, and 48 hours, the migration distance was measured. Calculation and measurements were made using the following method specified in the kit.

Determination of the surface area of cells migrating to the wound area: Migrated Cell Surface Area = length of cell migration (mm) x 2 x length.

Percent Closure (%) = Migrated Cell Surface Area / Total Surface Area x 100.

Total Surface Area; 0.9x1.8=1.62

Statistical Analysis

Statistical evaluation of the data to be obtained was carried out within the SPSS package program, using the one-way ANOVA Analysis of Variance Test for data with normal distribution, and the Kruskal-Wallis and Mann-Whitney U test, which are non-parametric tests, for data that did not show normal distribution. Values of P<0.05 from the results were evaluated to be considered significant.

RESULTS

Effect of WGO and α-tocopherol on L929 Cell Viability

To evaluate the effects of WGO and α-tocopherol on L929 cell viability, which is a healthy fibroblast cell line, different doses (100, 50, 25 and 12.5 ng/ml and µM, respectively) were applied and measurements were made with the XTT colorimetric method. α-tocopherol, which is also present in WGO, did not cause proliferation in L929 cells at any concentration (p>0.05). However, WGO showed a statistically significant proliferation effect at 100 ng/ml concentration (p<0.05) (Figure 1). In the light of these results, the next stage of the study, the wound healing model, was started.

Wound Healing Assay

The wound healing kit created a standard wound area with a defined gap of 0.9x1.8 mm. Then, 100 µM WGO was applied to L929 cells and allowed to incubate for 24h, 36h, and 48h. Then, at the end of 24, 36 and 48 hours, the migration distance of cells to the wound area was measured. Although there was a positive improvement compared to the control group at 24 and 36 hours, no significant difference was detected *(p>0.05)*. When the measurements at the 48th hour were compared with the control group, it was observed that the cells treated with WGO proliferated faster and invaded and closed the wound area faster *(p<0.05)* (Figure 2).

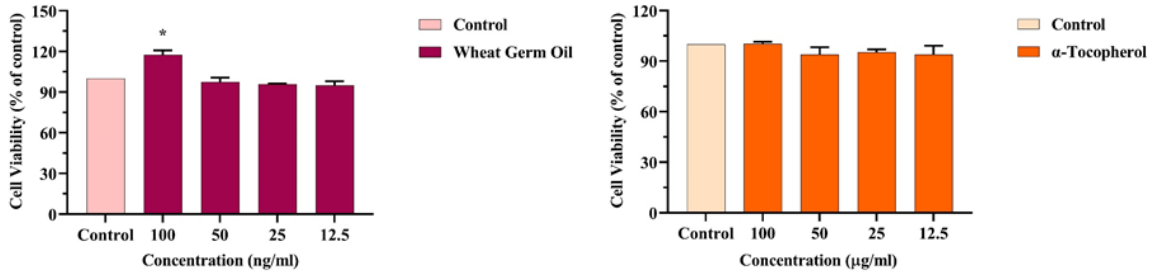

Figure 1. Effect of Wheat Germ Oil and α-tocopherol on cell proliferation in L929 cells. The data are expressed as mean ± standard error mean. *p < 0.05 as compared with control-untreated group.

Table 1. Measurement of migration distances from 5 different places at the end of 24, 36 and 48 hours in the 0.9x1.8 mm wound area when Wheat germ oil at 100 ng/ml concentrations was applied to L929 fibroblast cells.

Five Different Measuring Areas	24 Hour WGO(100 ng/ml)/Control	36 Hour WGO(100 ng/ml)/Control	48 Hour WGO(100 ng/ml)/Control
Length of cell migration	0,9 mm/0.89 mm	0.75 mm/0.79 mm	0.70 mm /0.72 mm
Migrated cell surface area	1.620/1.602	1.35/1.422	1.160/2.01
Percent closure (%)	100/98.89	83.32/87.78	72.78/84.01
Length of cell migration	0.89 mm/0.87 mm	0.78 mm/0.77 mm	0.71 mm/0.76 mm
Migrated cell surface area	1.602/1.566	1.404/1.386	1.238/1.399
Percent closure (%)	98.89/96.67	86.67/85.56	75.89/85.43
Length of cell migration	0.75 mm/0.76 mm	0.75 mm/0.87 mm	0.69 mm/ 0.81 mm
Migrated cell surface area	1.350/1.368	1.350/1.566	1.202/1.458
Percent closure (%)	83.32/84.44	83.32/96.67	73.67/90
Length of cell migration	0.75 mm/0.78 mm	0.83 mm/0.82 mm	0.74 mm /0.80 mm
Migrated cell surface area	1.350/1.44	1.494/1.476	1.321/1.44
Percent closure (%)	82.32/88.9	92.21/91.11	81.21/88.89
Length of cell migration	0.78 mm/0.82 mm	0.75 mm/0.80 mm	0.70 mm/0.79 mm
Migrated cell surface area	1.404/1.476	1.350/1.584	1.242/1.422
Percent closure (%)	86.67/91.11	83.32/97.78	74.78/87.78
Average Value	**90.24/92.002**	**85.984/91.78**	**75.66/87.22**

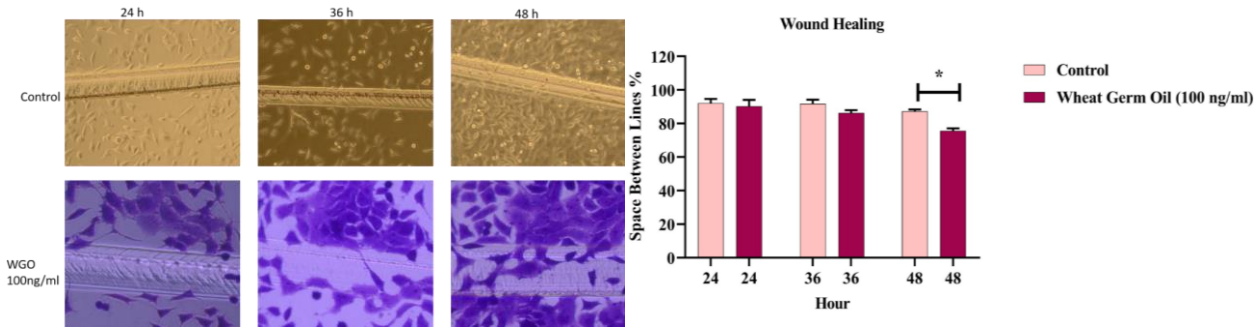

Figure 2. Wound closure image of the cells in the control group of the L929 cell line and the cells applied to WGO at a concentration of 100 ng/ml at 24., 36. and 48. Hours and Comparison graph with the control group. (*=$p<0.05$)

DISCUSSION

Wound healing is a well-coordinated, complex process involving the interaction of different cell types, different chemical and biological reactions, growth factors, and many complex mechanisms, such as cytokines, for the repair of injured tissue from any cause. Poor wound healing, which occurs as a result of a negative situation in this wound healing process, still affects millions of people and continues to be a global health problem (Barrientos et al., 2008; Frykberg and Banks, 2015). For this reason, studies on drugs and products that will contribute to the easier treatment of chronic and complicated wounds continue without slowing down today. In this context, combined treatments come to the fore as well as monotherapeutic agents. Today, natural plant extracts and herbal oils have become the focus of attention as they provide an alternative to these combination treatments and have inspired many studies.

Fibroblasts come to the fore as the first line of defense and first response after dermal injury. Fibroblasts appear locally following injury. The fibroblasts formed to activate the growth factors while forming the granulation tissue, increase the extracellular matrix and provide wound contraction. For all these reasons, fibroblast proliferation is an important step in wound healing (Gurtner et al., 2008).

Wheat Germ Oil, used in traditional medicine, is a natural product obtained from the germ of wheat germ, which is the most important structure of

wheat and contains many bioactive compounds, including α-tocopherol (vitamin E), α-linolenic acid (omega-3 fatty acid), and sterols (Eisenmenger and Dunford, 2008; Yuldasheva, Ul'chenko, and Glushenkova, 2010. It has been reported in many studies that WGO has anti-inflammatory and antioxidant properties, and therefore it has been reported that it will contribute to the prevention, and treatment of diseases, especially in cases of exposure to oxidative stress (Abdel-Gawad, 2015; Anwar, and Mohamed, 2015; Hamdi, 2019; Mahmoud, Mohdaly, and Elneairy, 2015).

The proliferation and migration of fibroblasts, which play an important role in the wound healing process, form the basis of *in vitro* wound healing models and are widely used by the scientific community (Liang, Park, and Guan, 2007; Nicolaus et al., 2017). In this study, an *in vitro* wound healing model was applied to a healthy fibroblast cell line (L929) to demonstrate the possible contributions of WGO to wound healing. In addition, the contribution of α-tocopherol to wound healing was examined. It was evaluated whether the contribution of WGO to wound healing depended on α-tocopherol in its content.

The data obtained in this *in vitro* study revealed that WGO caused a proliferation in fibroblast cells, but α-tocopherol did not contribute to cell proliferation. In the *in vitro* wound healing model, it was observed that WGO contributed to both cell proliferation and cell migration. In a study, (Türkoğlu et al., 2021) showed that the use of multi-layered dressings containing WGO contributed to wound healing. In another study, it was shown that the topical application of WGO had a healing effect on burns in rats (Zakaria et al., 2021). In the light of the results obtained in our study, it was seen that WGO had positive effects on wound healing and this result is compatible with the literature. However, in our study, it was determined that α-tocopherol, which is also included in the content of WGO, does not contribute to wound healing. Although it has been reported in the literature that the antioxidant and anti-inflammatory effects of WGO may be due to α-tocopherol in its content (Hamdi, 2019), in this study, it was observed that α tocopherol in the content of WGO did not have a serious contribution to wound healing, unlike the literature.

CONCLUSION

As a result of our study, the positive effect of wheat germ oil on wound healing was seen in fibroblast cells *in vitro*. Within the scope of our study, it was determined that α-tocopherol in the content of WGO did not cause proliferation in fibroblast cells at similar doses. As a result, it was observed that α-tocopherol alone did not have a significant contribution to wound healing, but WGO made significant contributions to cell proliferation and cell migration, especially in the 48-hour application. More comprehensive in vivo and clinical studies are needed to better understand the role of WGO in healing.

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Conflict of Interest

There is no conflict of interest.

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