

Determination of Effect on Cutaneous Wound Healing of Ozonated Hazelnut Oil[†]

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Abstract: In this study, the wound healing effect of ozonated hazelnut oil was investigated on 54 male Sprague-Dawley rats. 7 experimental groups were designed. A circular area with a diameter of approximately 2 cm on dorsal surface was drawn using a coin and full thickness of the marked area was cut approximately 3 cm away from the ears by sterile scissors and forceps. All oils were daily applied on wound surface of experimental animals in volume of 0.2 mL during 15 days. In this study, statistical analysis of the data was performed using the SPSS 22.0 package program. Wounds of all experimental animals were photographed by a digital camera on days 0, 3, 6, 12 and 15 after wounding. Ozonated oil treated wounds had significantly higher than the other groups on the day 3 after wounding. There is a quantitatively healing is determined on 3 day on rats. It has been observed in the stability period that ozonated oils must be stored at a temperature below 4 °C. Vitamin E should be added as an additive in the medical use of ozonated hazelnut oil in wound healing.

Keywords: ozonated hazelnut oil; Sprague-Dawley; wound healing

1. Introduction

In recent years, ozone has been used as for medical purposes as well as a disinfectant. Ozone therapy is the application of the ozone/oxygen mixture to the circulatory system or body cavities. Ozone Therapy is widely used clinically in many diseases. (Infections, Arterial Circulatory Disorders, Age-Related Macular Degeneration, Pulmonary Diseases, Dental and Gum Diseases, Joint Diseases, Intestinal Diseases, Skin Diseases) [1]. Ozonated vegetable oils are one of the most applied methods of ozone treatment because they are easy to apply and obtain. The most commonly used oil at this point is ozonated olive oil [2,3].

Ozonated olive leaves active oxygen as a penetrating the tissues, thereby increasing blood flow in this region, activating metabolic processes, accelerating granulation and epithelization in pathological regions. Ozone (O₃) is the most effective substance in the environment that destroys microbes and toxins, providing pure oxygen that tissues and cells need [4]. The ozonated olive oil and cream helps to soothe the waist and knee aches by rapidly being absorbed into the area of

application. There are a lot of studies in the literature about the use of ozonated olive oil and different vegetable oils in the medical field and studies on ozonated hazelnut oil are limited [5,6]. For this reason, the effect of ozonated hazelnut oil and experimental wound healing was evaluated in this study. In this study, first of all, it is aimed to be determined which kind of mechanism can help the effect of ozonated hazelnut oil on wound healing on Sprague-Dawley rat models.

2. Materials and Methods

Six groups for test (healthy inbred male Sprague Dawley rats weighing 200–250 g grams between 3–4 months old) and one of the control group were included 7 male animals at each group samples selected for comparisons are as follows : Group I: Control, Group II: Refined hazelnut oil, Group III: Non-refined hazelnut oil, Group IV: Ozonated Refined Hazelnut Oil, Group V: Ozonated Non-refined Hazelnut Oil, Group VI: Ozonated Olive oil and Group VII: Ozonated Non-refined Hazelnut oil with hexane, these seven groups of oils were tested on male rats. Control group animals were not treated as agent of oil. All the oils were applied daily for 15 days to the wounded area of 0.2 mL volume one day after the wound was created in the test animals. The entire study protocol was reviewed and approved by Abant İzzet Baysal University Animal Experiments local ethics committee (Protocol no: 2014/33). For ozonation process, refined hazelnut oil (brand name is Çotanak) and ozonated olive oil (brand name is Arifoğlu) as commercially from sold the market, unrefined cold-pressed hazelnut oil which was called non-refined hazelnut oil was obtained at laboratory through 50–60 °C in press. In this study, statistical analysis of the data was performed using the SPSS 22.0 package program. Differences among groups and between groups were analyzed using mean value and standard error by ANOVA-Tukey post hoc test. Values of $p < 0.05$ were considered significant.

3. Results

The results of the study was shown that after 3 days of wound formation, the percentage of wound healing was significantly higher in ozonated refined hazelnut oil application group compared to the group treated with ozonated non-refined hazelnut oil as shown (Figure 1) ($p < 0.05$). The oil applied on the created wound area on 6, 12 and 15 days after wound formation did not show any significant difference as shown (Figure 2). However, this significant difference was not observed on days 6, 12 and 15.

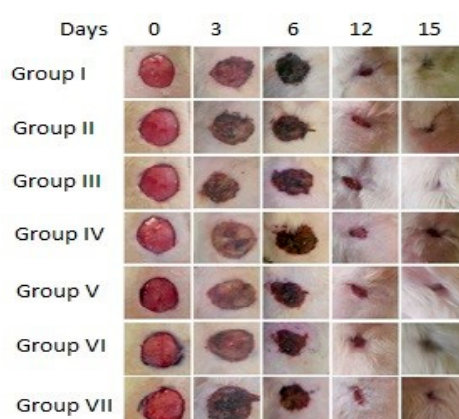


Figure 1. Photography of oil applied on the created wound area on 0, 3, 6, 12 and 15 days after wound formation.

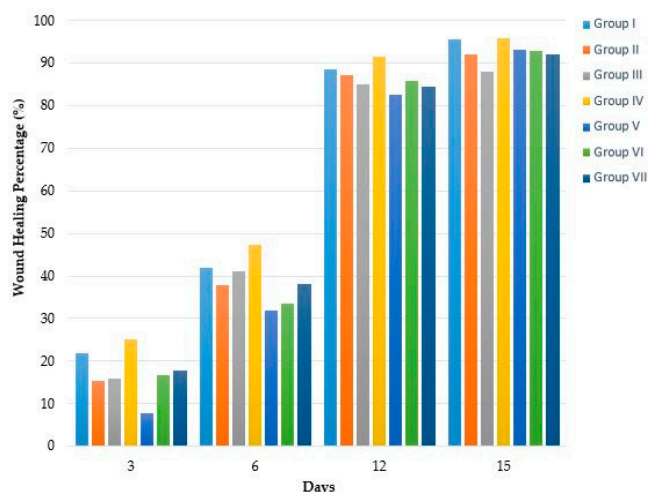


Figure 2. Wound healing percentage (%) of compared groups.

4. Discussion

Ozonated oils are used in many diseases such as joint and skin pathologies especially. The recovery process of the wounds is an extremely important process that is affected by oxidative stress and progression of age causing decrease in the healing process. In this study, a total of 5 hazelnut oil samples were used obtained through refined, non-refined and hexane extraction, an ozonated olive oil application was performed to observe the effect of ozonated hazelnut oil and ozonated olive oil. The best result which experimentally used is a hazelnut oil sample obtained by ozonation process at 7–8 flow rate for 110 min and olive oil with ozone was obtained from commercially sold form. Previous studies have reported the therapeutic potential of ozonated oils in the recovery of cutaneous wounds [7,8]. No studies have been found in the literature on wound healing of ozonated hazelnut oil and the effect of ozonated hazelnut oil on wound healing was investigated for the first time in this study. In addition to this, we have found that wound healing is better in ozonated olive oil compared to ozonated hazelnut oil. According to the results obtained on the comparison between ozonated hazelnut oil and non-ozonated hazelnut oil, the percentage of wound healing after 3 days after the wound was formed was found to be statistically significant in comparison with the hazelnut applied with ozone and unbranched hazelnut oil in the ozonated hazelnut oil application group (Figure 1). It is deduced that the ozonide intermediate, which occurs as a result of ozonation, stimulates fibroblasts and is thus effective on wound healing. However, this significant difference was not observed at 6, 12 and 15 days. At the initial stages of wound healing, there is depolymerization of the type III collagen, which is the main responsibility of the stressor. Depending on the size of the wound, this process varies from 1 to 3 weeks. According to the experimental data which obtained the 7–8 flow rate spread over 110 min must be longer than the ozonation process in this study. The results have found are that the ozonated oil obtained by experimental optimization needs to have a longer ozonation process thus, it may be effective on wound healing for a long time. As a result of the stability analysis of this study that we have obtained ozonated hazelnut oil, the physical and chemical variable rates show that the oils obtained are not stable during the shelf life. Vitamin E, which is also an important agent in wound healing that has also been shown to be completely degraded during the stabilization period. It has been shown in the stability results that vitamin E has to be added extra, which has an important effect on wound healing and prevents radical formation.

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design and helped to draft the manuscript. S.T.K., A.C. and B.K. participated in the animal studies and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no competing interests.

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