THE REPUBLIC OF TURKEY
FIRAT UNIVERSITY
THE INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF SURGERY

EFFECTS OF OZONATED OILS (SESAME OIL, *NIGELLA SATIVA* OIL AND *HYPERICUM PERFORATUM* OIL) ON WOUND HEALING PROCESS IN RATS

MASTER THESIS

Tamara RIZAOĞLU

ELAZIĞ – 2018

APPROVAL PAGE

Prof.Dr. Mustafa Kaplan

Director of Instutu of Health Sciences

This thesis was found to comply Master Thesis standards

Prof.Dr. Ali Said DURMUŞ

Head of Surgery Department

This thesis had been read by us and accepted as Master Thesis due to in terms and of quality

Prof.Dr. İbrahim CANPOLAT

Supervisor

Master Examination Jury Members

Prof.Dr. Ali HAYAT

Prof.Dr. İbrahim CANPOLAT

Doç.Dr. Enis KARABULUT

ETHICAL DECLARATION

I declare that I have performed this thesis study with my own studies, that it is not contrary to the ethics at all stages from the planning of the works to obtain of the findings and writing phase, that I have obtained all information and data in this thesis under academic and ethical rules, that I have referred to data, information and interpretations that were included in this thesis but which was not obtained in the findings of this thesis.

Tamara RIZAOĞLU

20.04.2018

Supervisor

Prof. Dr. İbrahim CANPOLAT

Department of Surgery

ELAZIĞ

ACKNOWLEDGMENT

I thank all who in one way or another contributed somehow to the completion of this thesis. First, I give thanks to God for protection and ability to do work.

I would like to express my sincere gratitude to my supervisor Prof. Dr. İbrahim CANPOLAT for his patience, kind support, immense knowledge, motivation, directions and thorough guidance during my research work. His guidance helped me in all the time of research. Throughout this project, I benefited from his advice, particularly so when exploring new ideas. His positive outlook and confidence in my research inspired me and gave me confidence. His careful editing contributed enormously to the production of this thesis. My deep thanks also goes to Assoc. Prof. Dr. Enis KARABULUT, Dr. Sema ÇAKIR, Dr. Hawar QADIR and I want to thank all the remaining professors and colleagues for their helps and supports.

I would like to thanks Head of Department of Pathology Prof. Dr. Yesari ERÖKSÜZ, and Dr. Burak KARABULUT for evaluating pathological samples.

I would like to thank all of my friends, who have patiently supported me throughout the entire process, both by keeping me harmonious and helping me putting pieces together. Your friendship makes my life a wonderful experience. I cannot list all of the names here, but you are always in my mind. I will be grateful forever for your kindness.

Last but not the least; I have to thank my parents for their love, encouragement, praying and supports throughout my life. And the greatest thanks to my father as my role model and motivated to achieve my ambitions. God has mercy on him. My special thanks to my fiancé Mohammad JADALLAH for his great love, support and patient.

My brother and sisters (Jafar, Lamis, Hiba, Inas, Sareen) deserve my wholehearted thanks as well.

Sincerely

Tamara RIZAOĞLU

Elazığ, 2018

CONTENTS

Page No.

LIST OF TABLES

Page No.

LIST OF FIGURES

Page No.

ABBREVIATIONS

O. H.: Ozonated *Hypericum perforatum* oil

O. N.: Ozonated *Nigella sativa* oil

O. S.: Ozonated Sesame oil

1. SUMMARY

EFFECTS OF OZONATED OILS (SESAME OIL, *NIGELLA SATIVA* OIL AND *HYPERICUM PERFORATUM* OIL) ON WOUND HEALING PROCESS IN RATS

In this study, the effects of three different ozonated oils (sesame oil, *Nigella sativa* oil and *Hypericum perforatum* oil) on wound closure rate, healing process and possible complications were examined macroscopically and microscopically.

Twenty-one adult Wistar albino female rats were used in the study. Subjects were divided into three groups, i.e. (Early wound healing (7 days), medium wound healing (14 days) and late wound healing (21 days). Four full-thickness skin wounds of equal size (10 mm in diameter) to the back regions of all rats were formed. The wound was left open during healing.

While the first wound (control group) received no treatment the second wound, ozonated sesame oil, in the third wound ozonated *Nigella sativa* oil and lastly ozonated *hypericum perforatum* oil were used. The first, second and third main groups were euthanized on days 7, 14 and 21, respectively.

There was no significant difference in wound healing between groups in the first 7-day. In the 14 day groups, it was found that the healing was better in the group of *Nigella sativa* oil and sesame oil group ($P<0.05$). In 21 day groups all wound healing was improved, but *Nigella sativa* oil group had earlier improvement compared to the others ($P<0.01$).As a result, the best wound healing was achieved with *Nigella sativa* oil and sesame oil.

Keywords: Wound healing, ozonated Sesame oil, ozonated *Nigella sativa* oil, ozonated *Hypericum perforatum* oil

2. ÖZET

RATLARDA OZONLANMIŞ YAĞLAR KULLANILMASI (OZONE EDILMIŞ SUSAM YAĞI, ÇÖREK OTU YAĞI VE KANTORON YAĞI) YARA İYİLEŞTİRME PROSESİNİN ETKİSİ

Bu çalışmada üç farklı ozonlanmış yağın (susam yağı, çörek otu yağı ve kantaron yağı) yara iyileşmesi üzerine etkileri araştırıldı. Farklı ozonize edilmiş yağların yara kapanma hızındaki etkileri, iyileşme süreci ve oluşabilecek komplikasyonlar makroskopik ve mikroskopik olarak incelendi.

Çalışmada, 21 adet erişkin wistar albino dişi rat kullanıldı. Denekler üç ana gruba (erken yara iyileşmesi (7 gün), orta dönem yara iyileşmesi (14 gün) ve geç yara iyileşmesi (21 gün) ayrıldı). Tüm ratların sırt bölgelerine eşit ebatta (10 mm çapında) 4 adet tam katlı deri yarası oluşturuldu. Yara bölgesi olarak ratların kolay ulaşılamayacakları sırt bölgesi seçilerek, Yara iyileşmesi süresince yaralar açık bırakıldı.

İlk yarada herhangi bir tedavi uygulanmadı (kontrol grubu). İkinci yarada ozonlanmış susam yağı, üçüncü yarada ozonlanmış çörek otu yağı ve son yarada ozonlanmış kantaron yağı kullanıldı. İlk ana grup 7 gün, ikinci ana grup 14 gün, üçüncü ana grup 21 gün sonra ötenazi edildi.

Yara iyileşmesinin incelendiği ilk 7 günlük gruplarda anlamlı bir fark oluşmadı. 14 günlük gruplarda çörek otu ve susam yağı gruplarında iyileşmemin daha iyi olduğu saptandı ($P<0.05$). 21 günlük gruplarda tüm yaralar iyileşti ancak çörek otu yağı grubunun diğerlerine göre daha önce iyileştiği belirlendi ($P<0.01$).

Sonuç olarak farklı üç ozonlanmış yağın kullanıldığı bu çalışmada en iyi yara iyileşmesi çörek otu yağı ve susam yağından elde edildi..

Anahtar Kelimeler: Yara iyileşmesi, ozone edilmiş susam yağı, ozone edilmiş çörek otu yağı, ozone edilmiş kantoron yağı.

3. INTRODUCTION

3.1. Skin

Skin; has a wide variety of functions, acts as a barrier between the external environment and the organism and reflects our spiritual reactions (1). The anatomy of the skin is shown in (Figure 1).

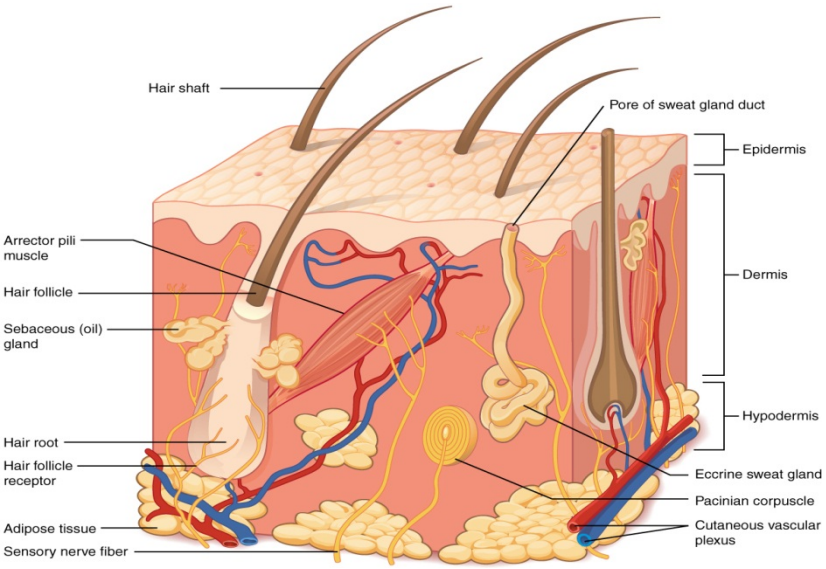

Figure 1. The anatomy of the skin

3.1.1. Skin Functions

- It acts as a physical guard against the future danger,
- Provide thermoregulation,
- Synthesize Vitamin D3,
- Syntheses and deposits lipid reserves ,
- It perceives the senses,
- Coordinates immune response (2)

3.1.2. Histology of Skin

The skin histologically consists of three layers:

- *Epidermis*
- *Dermis*
- *Hypodermis*

Epidermis

The outermost layer of the skin, mechanical, chemical and bacterial against external influences, it makes the task of protecting the skin (1).It allows the permeability of the skin and prevents water loss. Sensor receptors sense touch, pressure, and pain and heat senses (2). It can be divided into 5 cell layers different from each other.

It is listed as follows from bottom to top:

- *Stratum basale*
- *Stratum spinosum*
- *Stratum granulosum*
- *Stratum lusidum*
- *Stratum corneum (*2).

Dermis

This layer provides the skin elasticity and strength, and consists of three components;

1. Cells (Fibroblast, histiocyte, lymphocyte, plasma cells and mast cells)
2. Fibers (collagen, elastic and reticulum)
3. Basic substance (Hyaluronic acid, chondroitin sulfate and dermatan sulfate)

The dermis consists of two layers, the papillae and the reticular layer:

Papillary layer (just below the epidermis): Extension by the epidermis makes a tight connection with indentations. Terminal capillaries and nerve endings are found here. Collagen fibers in the direction perpendicular to the skin surface and as loose bundles extend to the papillae.

Reticular layer (located on subcutaneous): Collagen fibers are seen parallel to the skin surface and in more tightly bundles. It is more intense (1). It combines skin with deep tissue. Thermoregulation helps with veins. Deposits lipid reserves (2).

Hypodermis

Lipocytes, called fat cells, cluster to form lobules. This layer prevents heat loss, protection against trauma and serves as a substitute food store.

3.1.3. Skin Supplements

Skin supplements include sweat and sebaceous glands, hairs, nails, blood and lymphatic vessels and nereves. Sweat glands are divided into two: eccrine and apocrine sweat glands. Sebaceous glands except soles and palms, can be found in all parts of the skin. Skin muscles inclue the arrector pili muscles, a smooth muscle type, the only muscle found in the skin. They originate form the bottom of the hair follicle, cross upwards in the dermis and end up in the epidermis. Blood vessels course deep or superficially in the skin and establish plexuses where they met. Deep plexus are commonly located in border of subcutis-cutis and superficial plexus in subpapillary of the dermis. Epidermis lacks of vessels. Lymphatic capillaries underlie blood capillaries and lymph vessels accompany skin and venules. The dermis consists of encapsulated special free nerve endings transmitting sensory stimulation. In addition,

in the skin autonomic nerves that innervate sweat glands, blood vessels and arrector pili muscle are available (1).

3.2. Definition of Wound

Wound is interruption of ordinary congruity of structures typically limited to those caused by physical means and called likewise damage and injury (3).Wounds result from pathologic procedures starting inside or outwardly to the included organ (s). Acute wounds ordinarily continue through an efficient and timely reparative process that outcomes in uphold rebuilding of anatomic and functional probity. Chronic wounds have neglected to continue through an organized and timely procedure to deliver anatomic and functional probity, or continued through the repair process without setting up a maintained anatomic and functional outcome (4).

3.2.1 Wound Types

The Wounds are divided into two major classes, open and closed:

Open Wound

- Cut wounds (incision wounds)
- Laser and lacerations
- Puncture wound
- Avulsions
- Abrasion

Closed Wound

- Contusions
- Hematomas
- Crush injuries (5)

3.3. Acute Wounds

It is wound types that are performed in a manner compatible with anatomical and functional repair within normal healing period. Under appropriate conditions, healing occurs within about 20 to 30 days. These types of injuries are usually short-lived tissue injuries that are not very invasive surgical procedures, and healing is short-term and occurs in the four main distinct stages (11, 12).

3.4. Chronic Wounds

Chronic wounds are more likely to heal in longer time than expected due to a disruption in the normal healing period (11).Factors such as infection, decreased blood flow, tissue hypoxia, necrosis, exudate and excessive increase of inflammatory cytokines may cause delay in recovery. Burn injuries and vascular-induced ulcers tend to be chronic (10).

Healing does not occur when tissue oxygen level is below 30 mmHg. The chronic wound environment is hypoxic, acidic, hypoglycemic, hyperglycemic, hyper lactic and hypercarbia (10).

In chronic wounds, the stage of inflammation usually lasts longer and no healing occurs until this stage is suppressed. The disruption of the inflammatory stage manifests itself by the cleavage of the connective tissue epithelium of MMP secreted by the neutrophil and the degradation of PDGF (Platelet-derived Growth Factor) and TGF-β (transforming growth factor-β) by means of the neutrophil-releasing elastase enzyme (12).

In chronic wounds there is an increase in MMP and a decrease in PDGF, TGF-β and TIMP levels. Fibronectin destruction is observed. There is a delay in

epithelialization due to the disorder in the migration of epithelial tissue cells. Infection has a significant negative impact on wound healing. Severe bacterial densities occur within 48 hours of open wounds. If the infection cannot be cured, healing is delayed (14).

3.5. Definition of Wound Healing

Wound healing is one of the most complex biological events after birth. It is a complex process of the replacement of dead tissue by a vital tissue. The response of the body to local injury begins very early in the process of inflammation, and results in repair and regeneration. Healed wounds constitute a range of repair and they should be characterized and indicated (Figure 2). Perfectly healed wound is one that has come back to ordinary anatomic structure, appearance, and function. A minimally healed wound is characterized by the restoration of anatomic continuity, but without a uphold functional result and consequently the wound may repeat. Between these two extremes, an acceptably healed wound is characterized by restoration of maintained functional and anatomic coherence (4, 6, 7).

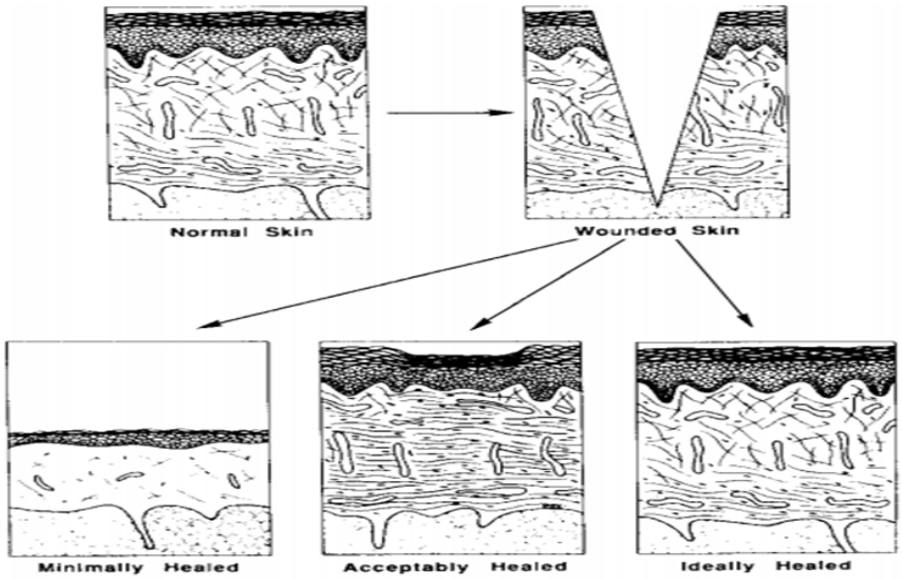

Figure 2. A pictorial portrayal of prototypic types of wound healing (6).

3.5.1. History of Wound Healing

History of wound healing; It goes back to 2200 years B.C “Before Christ”. In the inscriptions written by the Sumerians, the wounds were reported to be first cleaned with water and milk and then be closed with honey (15).

Forty-eight cases in Smith papyrus which were penned in 1700s B.C, The seventh of the report deals with injuries and care. The use of cotton stools and banding techniques have been described. The Hippocratic collections (BC 400) are the definition of primary and secondary wound healing (16).

Celcus (30 BC - 45AD) refers to the use of ligatures to hemorrhage in the third book and Four cardinal signs of inflammation are rubor, tumor, color, and dolor definition (15).

Egyptians, copper stones or copper pigment from Crystals they have dressed and even today are considered to be antiseptic properties of these substances. In the 14th century the increase in injuries by the use of firearms , has opened a new field of wound care. Surgeons have used natural wound healing in wound care methods that do not rely on the process by washing with boiled water and baking with boiling oil but efforts have disastrous consequences. In the mid-16th century, surgeon Ambroise Pare reported that these applications were wrong (15, 17, 18).

In wound care by the presence of iodine and chlorine in the middle of the eighteenth century further went forward. In 1846 Semmelweiss published the benefits of the use of hypochlorite solution in sepsis prophylaxis in puerperium (17).

Joseph Gamgee, 1880, also referred to by its name, very soft cotton and wool wrapped in gauze pad was composed identify. These pads were used as absorbents (17).

The open wounds began to be better able to investigate the results of the healing process when are covered with a suitable material, were Rev 1896 's, began to burn in the homograft applications increased efforts in this direction. Davis used placenta's amniotic and chorionic layers as biological dressing material in 1910 (19).

Many synthetic cover materials have been developed out of natural biological agents. The first of these is sponge ivola in 1961.Then plastic spray agents such polyoix entered into this field. Many chemical, physical and biological agents have been identified that affect wound healing over the topical route (19, 20).

3.5.2. Phases of Wound Healing

Wound healing consists of four phases separated from each other but intermix with each other. These phases can not be completed within the time of the failure should occur, or may occur in any one phase may result in the delay or closure of the wound healing (8).

- The hemostasis phase,
- The inflammatory phase,
- The proliferative phase,
- The maturational or remodeling phase.

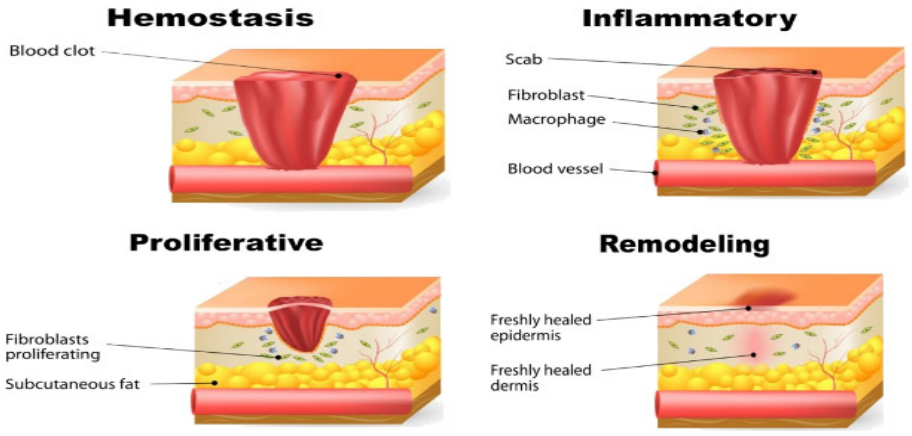

Figure 3. Wound Healing Phases

3.5.2.1. The Hemostasis Phase

Hemostasis is the first phase of wound healing. This phase occurs right after initial injury. At the time of the injury, usually bleeding occurs. Bleeding activates hemostasis. At the site of vascular injury, vasoconstriction is started immediately by neurogenic mechanisms and secretion of endothelia. This vasoconstriction prevents the further loss of blood while the fibrin clot forms a temporarily seal over the injury site and prevent the influx of microorganisms. After an injury, platelets are the first cells appear within injury site. Injury site exposes extracellular matrix proteins, such as febrile collagen, fibronectin and other adhesive proteins which allow platelets to adhere and become activated. During adhesion platelets undergo aggregation and at the same time release many mediators, such as serotonin, adenosine diphosphate, and thromboxane, and also release adhesive proteins, such as fibrinogen, fibronectin, thrombospondin, and von Will brand factor VIII. These mediators and locally generated thrombin induce further platelet aggregation and secretion, and form the platelet plug. During platelet aggregation, thrombin converts soluble circulating fibrinogen to insoluble fibrin which in turn traps to form the physical entity of the hemostasis plug; this is the process of primary hemostasis (9, 10, 11).

The blood coagulation pathways are divided into extrinsic and intrinsic pathways, converging where factor X is activated (11). Platelet aggregation also triggered a specific enzyme in blood known as Hagemen factor XII, to initiate the intrinsic coagulation pathway. In this cascade, by activation of some proenzyme, prothrombin is converted to thrombin. In the meantime, extrinsic coagulation pathway is activated by tissue factor, a cellular lipoprotein exposed at sites of tissue injury. There are interconnections between intrinsic and extrinsic pathway. For

example, a tissue factor-factor VIIa complex also activated factor IX in the intrinsic pathway (9, 11). In addition, platelets also produce cytokines, such as platelet derived growth factors that call in cells to participate in later phases of healing. For example, they are responsible for some processes including the synthesis of collagen, influx of fibroblasts and regulation of cell migration (12).

3.5.2.2. The Inflammatory Phase

The second phase of wound healing is inflammatory phase that starts immediately after hemostasis, and last about 4-6 days. Vasodilation follows the initial vasoconstriction that increases vascular permeability in response to histamine. Vasodilation allows the leakage of vascular fluid from intravascular space to the extravascular compartment (13). By vasodilation, neutrophils, lymphocytes, and monocyte migrate into injury site (10). Neutrophils predominant for the first few days and then disappear if the wound does not become infected. Neutrophil also initiate wound repair by activating local fibroblasts and epithelial cells. Later in inflammation, monocytes differentiate into macrophages and become major phagocytic cell at the injury site (9, 10). Both neutrophils and macrophages have surface receptors that permit them to recognize, bind and engulf foreign materials such as bacteria and tissue debris. After engulfing, bacteria and debris are digested by the inflammatory cells.

Besides the having phagocytic property of macrophages, the cells synthesize some cytokines including growth factors. These are involved in the migration, proliferation and organization for tissue repair (14). Macrophages also produce special enzymes called matrix metalloproteinase (MMPs) such as collagenase and

elastase into the wounded area. Collagenase plays an important role in wound debriment and shaping of connective tissue (10).

3.5.2.3. The Proliferative Phase

This phase is characterized by three critical changes in the injured site namely re-epithelialization, neovascularization and granulation (10). During this phase, fibroblasts stimulate the production of collagen, which gives the tissue its tensile strength and its structure. Wounds in a moist environment demonstrate a faster and more direct course of epithelialization (12).

3.5.2.4. The Maturational Or Remodeling Phase

During this phase, the loose granulation tissue differentiates into stable extracellular matrix. Collagen fibers reorganize, remodel and mature and the wound gaining its final tensile strength. Macrophages and fibroblasts which activate ECM-bound growth factor and MMPs play a vital role in this phase (10).

At the end of the granulation phase, myofibroblasts (differentiated fibroblasts) are activated and the wound starts to contract. With wound closure, Type III collagen undergoes degradation and type I collagen synthesis peak. This process is controlled synthesis of new collagen and lysis of old collagen by the actions of MMPs. The MMPs is controlled by tissue 26 inhibitors of metalloproteinases. There must be balance between tissue inhibitor of metalloproteinase and MMPs in wound remodeling process (9). During new connective tissue formation, fibronectin and hyaluronic are replaced, collagen bundles grow in site and strength neovascularization stops, and metabolic activity within the ECM diminishes. The density of cells such as macrophages, keratinocytes, fibroblasts and myofibroblasts

are reduced by apoptosis. The balance between the synthesis of new collagen and the degradation of old is also important to wound repair and remodeling. At the end of the remodeling phase, the new connective tissue matures and changes from pink-red to white color (10).

3.6. Types Of Wound Healing

3.6.1. Primary Wound Healing

It is a form of healing that is not shaped, cleansed from dead tissues, free of foreign bodies, without tissue loss, and aseptically brought into contact with the wound edges by being brought face to face. Wound healing is the most desirable form of healing (8).

3.6.2. Secondary Wound Healing

It is a form of wound healing in which large tissue loss is shaped, healing is impaired, irritation and consequently the granulation tissue is shaped. Inflammation, localized mortality and susceptibility are observed on wound edges , Removal of dead tissues is by way of irritation. The granulation tissue fills the location of the dumped tissues (8, 11).

3.6.3. Tertiary Wound Healing

Delayed primer is called wound healing. Large tissue is observed in loss and contaminating wounds. Such injuries are left open for a period of time in order to take measures against the risk of infection. When adequate blood is observed, the primary wound is closed as if the wound had healed (8, 10, 11).

3.7. Factors Affecting Wound Healing

3.7.1. Local Factors

- ✓ Foreign bodies
- ✓ Dead tissue
- ✓ Infection
- ✓ Local ischemia
- ✓ Surgical technique
- ✓ Sewing and sewing materials
- ✓ Vascularization of the wound area
- ✓ Moisture content of wound area
- ✓ A distance to one of your wound lips
- ✓ Ambient temperature
- ✓ Growth Factors
- ✓ Mechanical stress
- ✓ Radiotherapy
- ✓ Nitric oxide, Histamine, Oxygen free radicals (10).

3.7.2. Systemic Factors

- ➢ Age and race
- ➢ Anemia
- ➢ Nutrition, Malnutrition
- ➢ Steroids, Antimetabolites
- ➢ Smoking
- ➢ Systemic diseases

- Immune suppression (10).

3.8. Wound Healing Effect of Some Plants and Their Active Ingredients

Since ancient times, people have used plants and their preparations to heal their wounds. Often, their use is simply based on tradition, with no scientific evidence of effectiveness and little knowledge about the default active compounds or their mode of action. As wound healing is a complex biological process, several in vitro and in vivo tests are available. Some of the many potentially beneficial natural products come from plants such as, *Aloe vera*, *Hypericum perforatum*, *Nigella sativa*, *Sesamum Indicum L*. *Momordica charantina* L., *Matricaria recutita* L., *Calendula officinalis*, *Aesculus hippocastanum*, *Symphytum officinale*, *Centella asiatica*, *Liquidambar orientalis* Mill (20).

Hyperbaric oxygen therapy, medical ozonotherapy, electric current, laser beams,

The effects of methods such as ultrasound in the repair of tissue lesions have been demonstrated in experimental studies (24). With technological advances, these methods of treatment will become useful methods to find in important contributions to wound healing. However, studies are still ongoing to ensure optimal efficiency (25).

3.9. Ozone

Ozone is a highly energetic, 3 atomic molecule derived from oxygen. Oxygen in the atmosphere; oxygen atom (O), oxygen molecule (O2) and ozone (O3) and ozone is less stable than normal oxygen (21).

Medical ozone unlike technical ozone, pure medical oxygen is prepared by a silent electrical discharge. An ozone / oxygen mixture is obtained at the desired dose and concentration. The concentration ratios range from 1 to 100 micrograms/ millimeter according to the ozone/oxygen mixture ratios (0.05% O3 to 5% O3) (22).

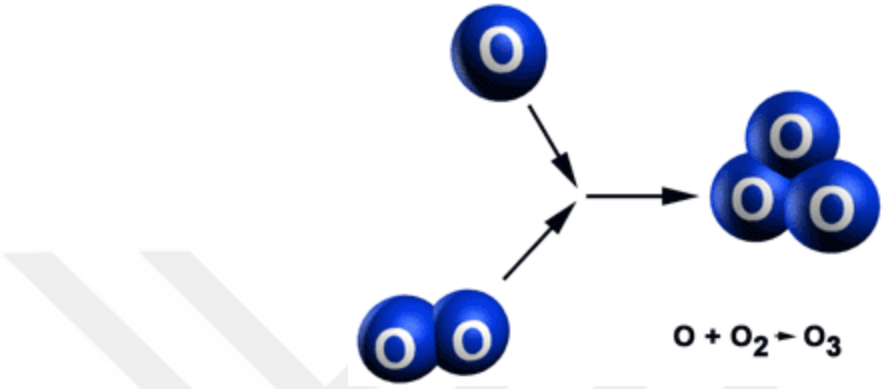

Figure 4. The number of oxygen atoms in ozone molecules

Compared to basic gases such as nitrogen, oxygen and carbon dioxide, which form the atmosphere, ozone, which is at a relatively low temperature, affects the climate plays an important role in the protection of surface life. It is located in the stratosphere layer, which is close to 90% of the ozone in the atmosphere and is between 10 and 50 km from the surface. The maximum ozone concentration is 19-23 with 10 ppm. kms. The remaining 10% ozone amount is 10-15. Km of the Troposphere. Ozone (O3) results from the bonding of oxygen atoms (O) to other oxygen molecules (O2), which are generated by high energy solar radiation by affecting normal oxygen molecules (O2). Ultraviolet-B (UV-B), which poses a major threat to surface organisms, nearly all of the rays are absorbed by ozone in the layer of stratosphere (22).

3.9.1. History Of Ozone Use

In 1840, Ozone or oxygenated water was first used in Switzerland was discovered by the chemist Christian Fredrick Schönbein.

In 1856, it began to be used in disinfection of operating room.

In 1860, the waters began to be cleaned with ozone in Monaco. Ozone, besides killing bacteria and the viruses, it was also seen to have removed the smell of the water and the bad taste.

In 1900, Nikola Tesla patented the first ozone generator.

In 1902 H.J. Clarke used ozone in anemia, cancer, diabetes, influenza and morphine poisoning.

In 1915, Dr. Albert Wolf, treated gangrene and wounds during the First World War with ozone.

In 1926, Dr. Otto Warburg reported that in Berlin ,the cancer had come from a cell- low oxygen level, and with this determination, the 1931 and 1944 Nobel prizes was given.

In 1935 E.Payr created the beginning of ozone application in the meaning of today with the ozone therapy study in surgery.

In 1977, Renate Viebahn has technically explained the effects of ozone on his body (20).

Strong antibacterial, antiviral and antifungal effect, immunomodulatory effect, Due to its positive effect on the transport and release of oxygen in the tissues, as well as it’s quick and efficient healing properties, medical ozone can be used in a wide range of indications. Ozone therapy alone or in combination with other treatment

methods is currently being implemented in approximately 350 diseases to increase their effectiveness (22).

Benefits (23);

1. No side effects or undesirable effects
2. Simple application methods,
3. Patients are well tolerated,
4. Having low cost
5. Being a practical, safe and effective preventive treatment.

Ozone Used Areas (24, 25, 26).

1. Food industry
2. Cleaning of wastewater
3. Drinking water cleaning (in water disinfection)
4. In cold storage
5. Medical treatment

3.9.2. Ozonated Oils

Unsaturated lipid substrates react with insufflated gaseous O2/O3 mixture leading to therapeutically active ozonated derivatives

Briefly, the postulated mechanism known as Criegee reaction provides that ozone combines with an unsaturated binding to form a primary, unstable primary ozonized which breaks down easily to form a zwitterion {There's the -ion part at the end, which is a chemical species with a charge. The first part of the name comes from the German word 'zwitter', meaning hermaphrodite or hybrid. In other words, this term means half anion and half cation. Zwitterions are sometimes called dipolar ions, because they have a negative end (the anion) and a positive end (the cation).}

and a carbonyl fragment. In an anhydrous environment, these substrates combine to give the typical cyclic trioxolane derivative. However, the word "ozonated" is itself without a scientist which means that if it is not associated with "how many" peroxides are present in the oil. In fact, from a therapeutic point of view, the ozonized compositions have the ability to deliver O2 active and / or other useful species deep in the lesion without causing primary irritation of the skin. The few studies on the therapeutic effects of ozonated oils on acute skin scarring in animal models does not analyze the dose / behavior response, expressed as the amount of peroxide present in the ozonated derivative used (27). Recently, a quantitative evaluation of the therapeutic effect of locally applied ozonized sesame oil on acute skin scarring in mice as an animal model has been developed (28). The results indicate that low (<1000) and high (> 3000) doses, expressed in terms of peroxide value (see the corresponding section in this article), delay skin healing. Such evidence is reinforced by a number of results between groups where the "average" concentration (about 1500) has the most beneficial effect in accelerating the rate of wound closure (27).

From an industrial application point of view, the overall quality of ozonated derivatives depends on several parameters, such as: (i) the type and quality of ozone generators; (ii) the ozonation conditions, in terms of reactors and time, type and quantity of material, presence of water and / or catalysts; (iii) the efficiency of the ozonize, in terms of production of O3 concentration, gas flow, carrier gas (28).

3.10. Nigella sativa (Black seed)

3.10.1. History of *Nigella sativa*

Nigella sativa is a cultivar plant which has been known for a long time and in our country bread is widely used in some cheese varieties. *Nigella sativa* was also used by ancient Egyptians for the purpose of treatment. The pharaohs' private doctors always have a bowl of noodles ready to serve as medicines for colds, headache, toothache and inflammation to facilitate digestion after a sensible meal feast. In the works of Hippocrates and Discords, they mentioned the name "Melanthion" from coconut. “Use Black seed regularly, since it is a cure for every disease except death” (Prophet Muhammad), and its many uses have earned Black Cumin the Arabic approbation “The Blessed Seed“.

At the beginning of the middle Ages, cattlemen have gained importance in European countries, and Karl and Ludwig der Fromme from the German kings gave the natives the opportunity to cultivate cattlemen in the 9^{th} century. After explaining the therapeutic effects of the great Turkish medical knowledge and philosopher Ibn-i Sina in 1031, the cavy was discovered as an important medicinal plant. Up to the 18th century, black cats have been used for many purposes in the treatment of rabies and snake bites and tumors, anti-inflammatory (anti-inflammatory) and milk boosters. However, after being forgotten and neglected for 200 years in western countries, a coincidence has been rediscovered at the end of the 20th century (29, 30).

Today, in Europe and America, veterinary drugs that are starting to be produced herbal medicines are regarded as preferred side effects in animals especially in terms of protection and development. It has been found that broad

spectrum medicines applied to diseases seen in animals affect people who consume them by accumulating in the animals' structures. This effect has been shown to impair people's immune system (31).

3.10.2. General features of *Nigella sativa*

Family: Ranunculaceae

Sub-family: Ranunculoideae

Species: Nigelleae

Species: *Nigella sativa* L. (32)

Black seed (Nigella sativa L.) is a medicinal plant annual belonging to Ranunculaceae family which grown naturally in Southwest Asia and the Mediterranean Region.12 species of black seed are naturally grown in Turkey’s flora. The seeds of Nigella sativa, Nigella damascene and Nigella arvensis are used in folk medicine and as a spice. The black seed is cultivated commonly in Afyon, Isparta, Burdur and Konya regions in Turkey. Black seed is intensively used in as uncrushed in bakery products (bread, muffins, biscuits, etc.) and in some cheese (brynza, cottage cheese, etc.) in Turkey the seeds are black and cornered (33).

Figure 5. Nigella sativa

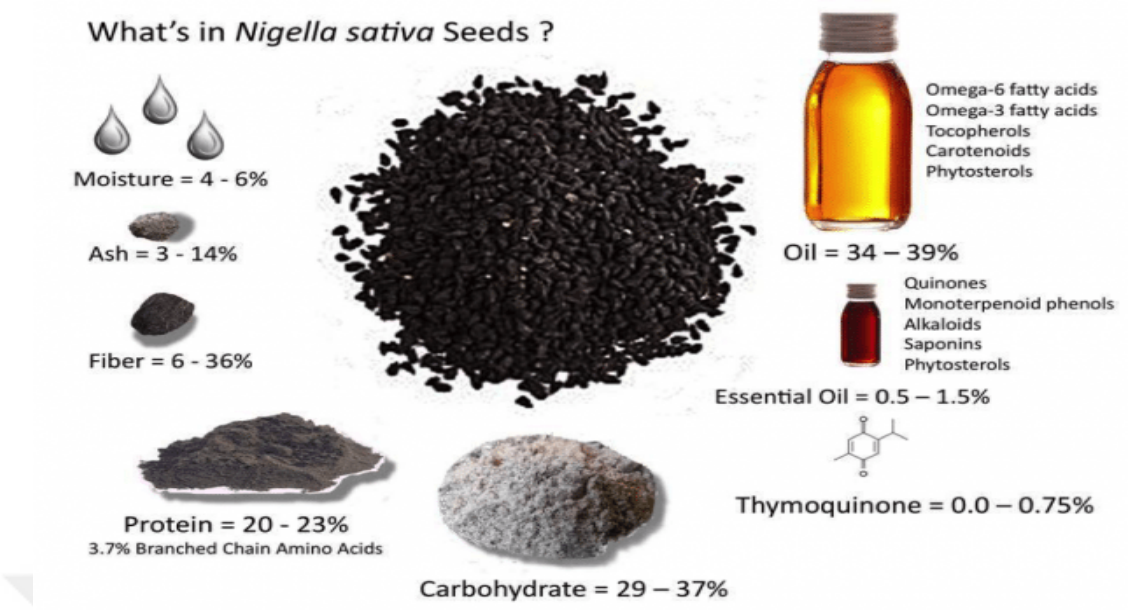

Figure 6. Whats in *nigella sativa* seeds.

3.1.0.3. Chemical Content of Nigella sativa

Nigella sativa consists of essential oil (0, 38-0, 49%), fixed fat (30-40%), protein (20-30), saponin, melantin, nigellin and tannin. The chemical content of the *Nigella sativa* varies according to the harvest season of the plant, the seasoning of the plantain and the cultivation. It has been determined that the volatile oil obtained from cattail seeds grown in the vicinity of Cairo contains 67 compounds and the most significant of these components are: p-cymene, TQ, a-pinene and ß-pinene. Also in the seeds of *Nigella sativa* s are small amounts of vitamins B1, B2 and B6, amino acids which are the building blocks of proteins; minerals such as iron, calcium, magnesium, zinc and selenium, which are known as trace elements and play a role in many important metabolic activities in the organism, which must be taken from outside with food and water. The active substance in the seeds of nigella was isolated only in nigella in 1959 (34).

It has been determined that the volatile oil of *Nigella sativa* has taken part in various reactions (35), these tasks are:

- It has antihistaminic, anti-inflammatory, anti-infective properties and has bronchodilator and vasodilator characteristics,
- It is known that crystallized nigella inhibits histamine release protein kinase C, which is known as a triggering agent,
- Essential oils balance the immune system,
- It regulates allergic reactions,
- It supports metabolism, reduces cholesterol and sugar,
- Increases interferon production by stimulating bone marrow,
- It has been determined that tracer elements contain unnatural cofactors for enzyme reactions.
- seeds have shown to stabilize mood, decrease anxiety

3.11. Hypericum Perforatum L. (St.John's wort)

The genus Hypericum is spreading especially in areas where temperate climates such as the Mediterranean Basin are dominant. It grows naturally in Europe, Asia, North Africa and North America. *Hypericum perforatum* L. belongs to the family Hypericaceae and is known as a variety of names such as yellow stamens, canes, chestnuts, sheepskin, lambs, and swordfish: There are 70 different species of Hypericum in Turkey and 350-400 different species in the world (36). The climate of the Earth is common throughout temperate and tropical regions, mostly on grassy river banks, on roadsides, in arid regions in summer, in wet winter areas, and on unsavory fields. Slightly acidic so it’s best in neutral soil (37).

H. perforatum L leaves are single, mutually or spiral (Fig.8). It is located in 5 sepal buds. Five petals each other it settled in a wrinkled form in the independent bud. Stamens clustered or many. The ovary is in top condition. Axial or parietal placental branching. Seeds do not carry endosperm (38).

Figure 7. St. john’s wort flower

The term Greek hyper hypericum (above) and eikon (picture) revenue from the combination of the words. Because in ancient Greece and Rome Hypericum's branches are the mystical powers of this plant and put them on their paintings or sculptures in their homes, believing that they would protect them from evil forces (39). Perforatum word is given in exocrine gland because it is similar to the holes in the plant's leaves (Fig.8). The name stems from the fact that it blooms around 24 June, which is traditionally celebrated as the birthday of John the Baptist. Wort is an old English word for plant (40).

Figure 8. Leaf images of H. perforatum L. plant

H. perforatum extracts century's trauma, burn, rheumatism, pain, enuresis, depression (41), bruising, swelling, inflammation, for the treatment of bacterial and viral infections have traditionally been used anxiety medication (42).

The healing effect of the wounds of this plant is well known. Human health and well characterized from the wound in Anatolia are used in many more people because of the positive effect. *H. perforatum* wound-healing effect, as well as sedative, antiseptic, antioxidant, anti-depression, spasm, antiviral and antimicrobial, hepatoprotective, diuretic effect and is referred to by the presence of antibiotic (36,43,44).

H. perforatum L. oily preparations, topical minor burns, wounds, skin infections and are used for various pains. Plant preparations are used in anxiety and depressive problems (41).

Wound-healing effect of *H. perforatum* prepared from St. John's Wort oil has been known for a long time. The color of the oil and the effect are due to a hypoxanthine which is a diantronate pigment. This drug leads to photosensitization

when used extensively and exposed to sunlight, inflammation and dermatitis occur in the mucosa and skin (45, 46).

Wound healing in H. perforatum shorten the period of inflammation (anti-inflammatory effect), resistance to infection. Besides, increase in the percentage consisting fibroblasts has been shown to increase collagen synthesis in fibroblasts (47).

3.11.1. Active Compounds of *Hypericum Perforatum L.*

H. perforatum L. contains at least 10 classes of bioactive compounds; Hypericin pseudohypericin, hyperforin, adhyperforin ,tannins, essential oils, amino acids, phenylpropanals, xanthene's and other water-soluble compounds (organic acids, peptides and polysaccharides), naphthodiacline derivatives, flavonoids, fluoroglucinol derivatives, procyanidins and hypericin and hyperforin are likely to be psychoactive components (41). These components are present at different concentrations in each plant. Genetic variations in species and / or development, ecological growth conditions, harvest time, for example the method of preparation, exposure to light and storage conditions are the reasons for this difference. Among these, it has been shown that hyper hormones are most effective in wound healing but other substances are helpful (41, 48, 49).

3.12. Sesam Seed (Sesamum indicum L.)

Sesame (*Sesamum indicum L.*) was first described by Linnaeus in 1751. *Sesamum indicum,* belongs to Pedaliaceae family on Tub florae team (50).

Figure 9. Sesame Indicum (57)

The word "sesamum" was taken from the Arabic by Hippocrates. Sesame is also known as 'gingerly, beniseed, sim-sim and till'. In the words of sesame Sanskrit language, it appeared to be oil and carries the same meaning as in this case has led to the recognition that the culture of sesame oil plant is the first (51). There are numerous stories about how sesame initially turned out and utilized. In a memorable book called the "Thebes Medicinal Papyrus", found in Egypt, sesame was indicated as the potential origin of therapeutic vitality and impact (53).

India is believed to be the origin of Sesame seeds and sesame seeds are mentioned in the Hindu legend, these legends and tales passes sesame as a symbol of immortality (52).

It is grown in Africa about 6,000 years ago, where it is believed to have spread to Egypt, India, the Middle East, China and other regions. In an ancient text in Egypt (Thebes Medical Papyrus, 1552 BC) the medical effects of sesame seeds are explained. Hippocrates emphasizes high nutritional value. In an ancient Chinese book (300 BC) describing the medical effects of plants, sesame describes it as a plant with many good physiological effects, high energy content, internal peace and long-

term use and anti-aging effects. Sesame oil in Ayurveda, the traditional medicine of India. BC It is used as a massage oil since 700 years (53).

Sesame seeds were brought from Africa to America in the 17th century and began to be used in oil industry in Europe in 1840's years (54). It is known that the existence of sesame in Anatolia is based on very ancient histories. The first document related to sesame belonging to the Ottoman Empire is dated 1850.

Due to the antioxidant component comprises an oilseed in addition to being important in the pharmaceutical and cosmetic industry has found a wide application area. Because Sesame oil is an edible oil, its use as a vegetable oil has been limited in our country because it is not economical to use it. Sesame seeds, on the other hand, constitute the raw material in the production of tahini and tahin helva in our country, it is also used as a spice and bakery products (55).

3.12.1. Seed Composition and Quality

Sesamumindicum L. (Sesame) as an herbaceous annual plant, it is cultivated due to its edible seed, oil and flavorsome value (50). Various bioactive components of the seed were reported including vital minerals, vitamins, phytosterols, polyunsaturated fatty acids, tocopherols and unique class of lignans such as sesamin and sesamolin. A group of phenylpropanoids named lignans present along with tocopherols and phytosterols and provide defense against reactive oxygen species through antioxidant characteristics (55). Subsequently, sesame seeds as rich source of antioxidants and bioactive compounds have also been used to treat burns indicating different effects on wound healing (56). In addition, sesame contains substances that can inhibit lipid peroxidation process, improve the supply of blood to collagen tissues, increase the fibril collagen longevity and reduce cell damages

Sesame seed is a decent vitality source. It is rich in fat, protein, starches, fiber and a few minerals, Average composition of dried and whole sesame seeds are exhibited in Table1.

Table 1. Average composition of dried and whole sesame seeds (56).

Nutrient (proximates)	**Value per 100 g**
Water	4.69 g
Energy	573 kcal
Protein	17.73 g
Total lipid (fat)	49.67 g
Ash	4.45 g
Carbohydrate	23.45 g
Fiber	11.8 g
Sugars, total	0.30 g

3.12.2. Sesame Oil

Sesame varieties have four different colors, white, yellow, brown and black. Varieties contain high fat, protein and essential amino acids. Sesame seeds are especially rich in amino acids such as lysine; methionine and cysteine Sesame seeds contain 40 - 60% fat. The most common fatty acids in sesame oil are; 35.9% to 42.3% oleic acid , 41.5% - 47.9 linoleic acid, 7.9% - 10.2 palmitic acid, 4.8 - 6.1% stearic acid and arachidonic acid (0.3 - 0.6%) with linoleic acid in low proportion (0.3 - 0.4%) (60).

The most important characteristic of Sesame Oil is resistance to oxidative degradation. Sesame Oil high stability; in the composition of sesamol, sesaminol located just tocopherols from compounds acting on other edible oil from the oil-specific and potent antioxidant than these, hydrocarbons and some is due to the antioxidant effect of certain sterol (58).

Sesame oil has many physiological functions such as lowering estrogenic activity, blood lipids and arachidonic acid level. The key step in determining the color, composition and quality of sesame oil is roasting. Antioxidant factors that provide stability are affected by roasting parameters. It was stated that the antioxidant compound and total phenolic content were obtained by roasting at 200 ° C for 20 minutes (59).

Purpose

Considering these advantages of ozonated oils, in this study, it was aimed at investigating which of the following results would be most effective in healing wounds in a shorter time using *nigella sativa*, sesame seeds and *hypericum perforatum* in treatment of an experimental wound induced in rats.

4. MATERIALS AND METHODS

4.1. Chemicals

Ozonated Oils (*Nigella sativa* oil, *Hypericum perforatum* oil and Sesame oil) were used. (Figure 10, 11)

4.2. Animals

Twenty -one 2-month-old female Wistar albino rats weighing 220–250 g were used and the study was carried out in the Fırat University Experimental Research Center. Animals were divided into groups before being taken to the experiment, rats were maintained and fed under standard conditions, and they were left free in the cages. The approval for the study was received from the Local Ethics Experimental Animal Committee of Fırat University, Turkey (19.12.2016-219).

4.3. Method

General anesthesia of rats was performed i.m. injection of 10 mg/kg Xylazine HCI (Rompun, Bayer) and 80 mg / kg Ketamine Hydrochloride (Ketalar, Parke-Davis). Following adequate anesthesia depth, i.e. loss of the pedal and eyelid reflex, the rats were placed on the operation table in the abdominal position and their back regions were shaved, scrubbed and painted using povidone iodine, covered with sterile surgical drapes and prepared for aseptic surgery (Figures 12, 13). All surgical instruments and materials were sterilized in a autoclave . All operation procedures were performed following strict aseptic and a traumatic surgical guidelines.

In the back region of the rats four full-thickness skin wounds of 1 cm in diameter were created with punch biopsy tool (Figures 14, 15) on the back of each rat. The wound sites were divided into four groups according to agents used. While

the left cranial wound site was served as a control group, the right cranial which marked with “S” was allocated to group OS, left caudal marked with “Kt” to group OH and right caudal marked with “Ç” to group ON (Figures 16-18). While control group wound site received no agent throughout the study, group OS, OH and ON wound sites were applied ozonated sesame oil (Figure16), ozonated *Hypericum perforatum* oil (Figure 17) and ozonated *Nigella sativa* oil (Figure 18) twice a day (morning and evening), respectively.

All wounds were left uncovered throughout the study. The status of wound healing was evaluated macroscopically, the size of individual wounds in each rats were measured by a caliper (Figure 19) and photographed digitally every day, beginning on the day of wounding.

The rats were also divided randomly into three groups, days 7 (group 1), 14 (group 2) and 21 (group 3) of animals with equal number (no: 7) according to the day of euthanasia in order to evaluate the early, middle and late findings of wound healing.

Group 1 (early wound healing) subjects were euthanized on postop day 7, group 2 subjects (middle-term wound healing) on postop day 14, group 3 subjects (late wound healing) on postop day 21 by carbon dioxide inhalation (Figure 20). The wound sites were harvested with a scalpel and placed into %10 formalin liquid and presented to pathology department for histopathological evaluation.

4.4. Histological examination

Tissue samples were left in 10% buffered neutral formaldehyde solution for 24 hours, were processed following routine histological procedures, blocked in paraffin which then were sectioned on a microtome 5 µm in thickness and stained with

hematoxylin-eosin (H&E) and toluidine blue and evaluated under light microscope with respect to: ulceration, necrosis, reepithelialization, acute and chronic inflammation, inflammatory cell density, myofibroblasts, fibroblastic activity, vascular proliferation, mastocytes and collagen fiber bundle organization, surface closure.

Histopathological evaluation was performed according to the wound healing assessment criteria shown in Table 2. Inflamation, ulseratiom and vascular proliferation was scored non (-), mild (+), moderate (++), and severe (+++). Closure of the wound surface, epithelization and fibroblast activity increase were graded as present (+) or absent (-). The mean scores of the histopathological findings were calculated scoring system, i.e. non (-), mild (+), moderate (++) and severe (+++) with 1, 2, 3 and 4 points, respectively.

Table 2. Wound healing score evaluation criteria

İnflammation	**Ulceration**	**Vessel Proliferation**	**Surface Closure**	**Epithelisation**	**Fibroblast Activity Increase**
No	No	No	No	No	No
Mild	Mild	mild (less than 5 vessels)	Yes	Yes	Yes
Moderate	Moderate	Moderate (6-10 vessels)	-	-	-
Severe	Severe	Severe (More than 10 vessels)	-	-	-

4.5. Statistical Analysis

All data was presented as mean ± SD. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunne's post hoc test where multiple comparisons were made (SPSS22.0, USA). Differences set to $P<0.001$ and $P<0.05$ were considered statistically significant.

Figure 10. Appearance of used Ozonated Oils(*Nigella sativa* oil" Çörekotu Yağı"; *Hypericum Perforatum* oil" Kantaron Yağı" and Sesame oil"Susam Yağı").

Figure 11: Ozonated Oils(*Nigella sativa* oil" Ç"; Sesame oil"S" and *Hypericum Perforatum* oil" Kt") ") ready for use.

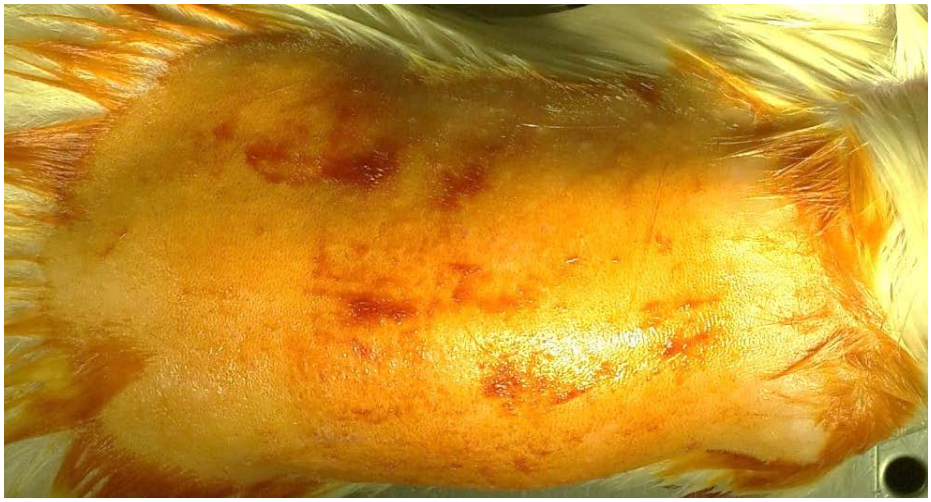

Figure 12. The operation site is scrubbed with povidone iodine solution.

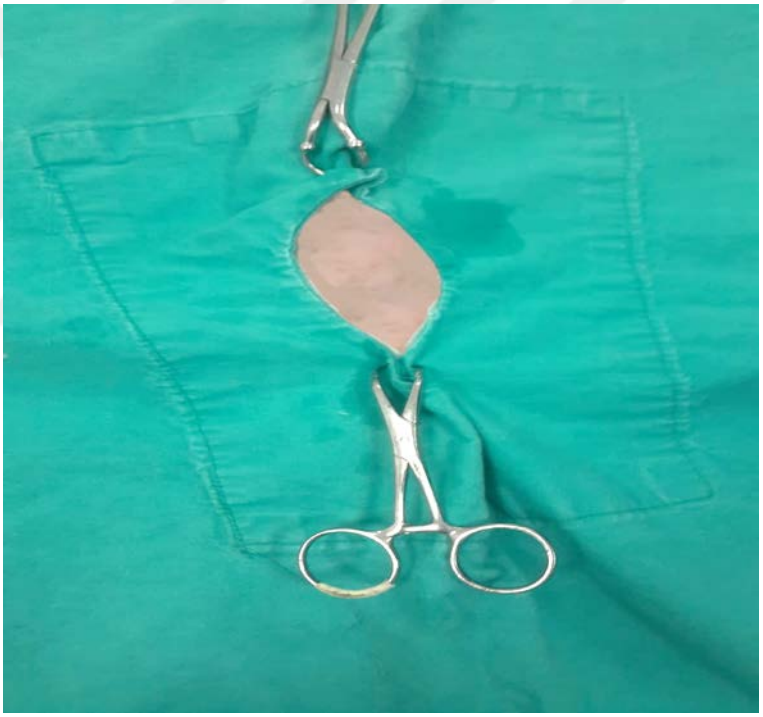

Figure 13. Shaved operation area is visible.

Figure 14. Biopsy Punch

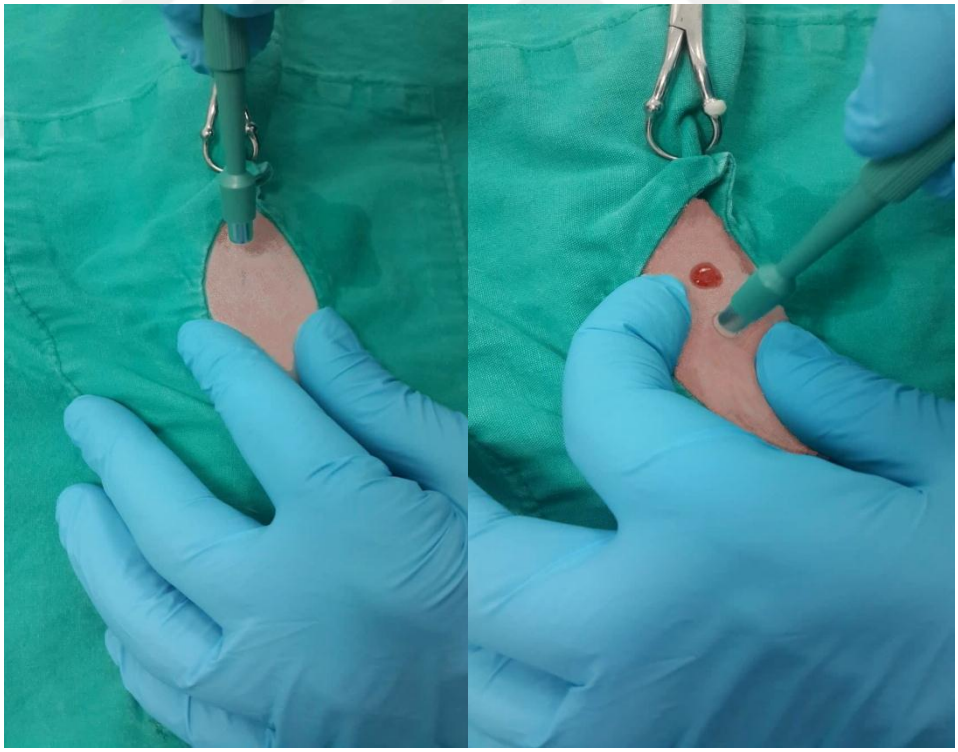

Figure 15. Creation of full-thickness skin wound with biopsy punch

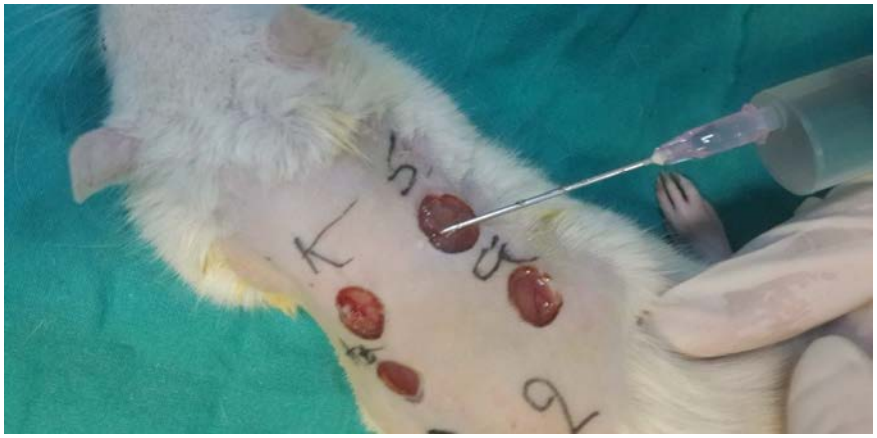

Figure 16. Appearance of ozonated sesame oil administration to the wound site "S"

Figure 17. Appearance of ozonated *Hypericum Perforatum* oil administration to the wound site "Kt"

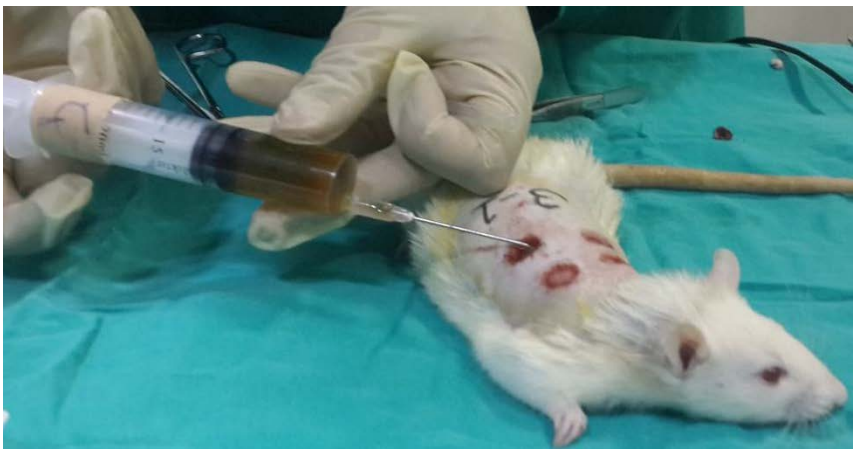

Figure 18. Showing ozonated Nigella sativa oil administration to the wound site "Ç"

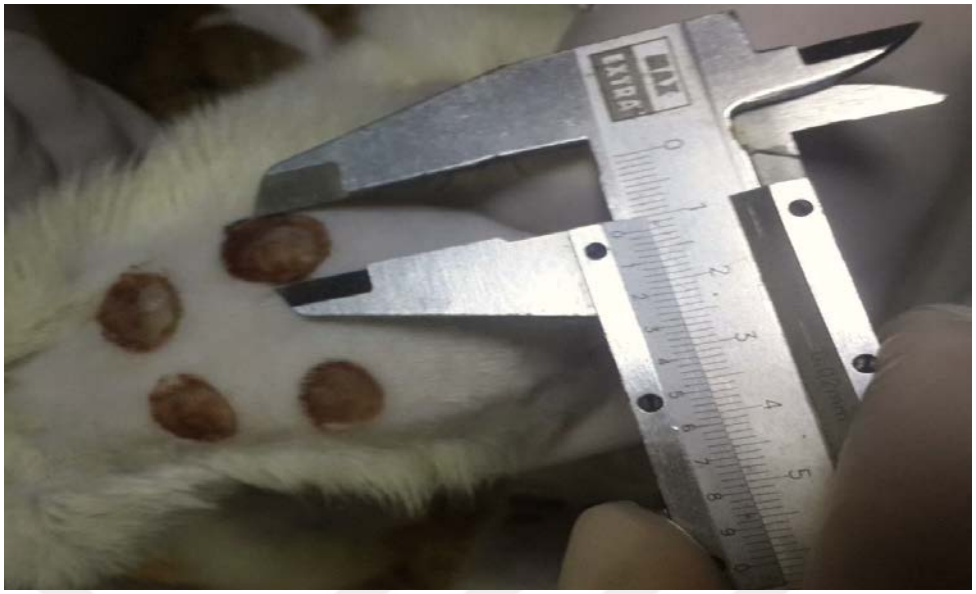

Figure 19. Measurement wound size with vernier caliper.

Figure 20. Carbon dioxide inhalation unit used for euthanasia.

5. RESULT

5.1. Clinical Results

During the experimental study, no postoperative complications including wound infection and abnormal health status were recorded.

Data obtained from the measurements of four wound sites in groups 1-3 are presented in Tables 3-5, respectively.

Table 3. Data of four wound sites measured by vernier caliper in cm in group 1.

According to euthanasia day	**According to the wound sites**			
Group 1	**Control**	**Group O.S.**	**Group O.N**	**Group O.H.**
Day 1	1.06±0.08	1.04±0.08	1.04±0.08	1.07±0.09
Day 2	1.06±0.08	1.04±0.08	1.04±0.08	1.07±0.09
Day 3	1.06±0.08	1.04±0.08	1.02±0.09	1.07±0.09
Day 4	0.98±0.10	1.00±0.10	0.98±0.12	1.04±0.10
Day 5	0.96±0.12	1.00±0.08	0.97±0.12	1.04±0.10
Day 6	0.95±0.11	1.00±0.10	0.97±0.11	1.02±0.09
Day 7	0.95±0.09	0.98±0.10	0.95±0.78	1.01±0.08

Table 4. Data of four wound sites measured by vernier caliper in cm in group 2.

According to euthanasia day	According to the wound sites			
Group 2	**Control**	**Group O.S.**	**Group O.N**	**Group O.H.**
Day 1	1.01±0.03	1.07±0.09	1.01±0.03	1.05±0.11
Day 2	1.01±0.03	1.07±0.09	1.01±0.03	1.05±0.11
Day 3	0.97±0.07	1.07±0.09	0.98±0.06	1.04±0.12
Day 4	0.95±0.05	1.01±0.11	0.97±0.07	1.04±0.12
Day 5	0.95±0.05	0.97±0.09	0.90±0.1	1.00±0.11
Day 6	0.97±0.05	0.94±0.09	0.90±0.1	0.98±0.08
Day 7	0.97±0.04	0.94±0.09	0.90±0.08	0.97±0.11
Day 8	0.950.05	0.90±0.05	0.88±0.08	0.95±0.11
Day 9	0.90±0.05	0.84±0.07	0.80±0.15	0.94±0.11
Day 10	0.72±0.13	0.74±0.17	0.71±0.13	0.75±0.24
Day 11	0,70±0.20	0.64±0.17	0.54±0.19	0.58±0.15
Day 12	0.61±0.15	0.47±0.11	0.37±0.11	0.50±0.18
Day 13	0.51±0.08	0.41±0.17	0.20±0.15	0.35±0.12
Day 14	0.41±0.11	0.20±0.06	0.18±0.07	0.30±0.1

Table 5. Data of four wound sites measured by vernier caliper in cm in group 3.

According to euthanasia day	According to the wound sites			
Group 3	**Control**	**Group O.S.**	**Group O.N**	**Group O.H.**
Day 1	1.13±0.13	1.13±0.10	1.1±0.10	1.03±0.10
Day 2	1.13±0.13	1.12±0.11	1.1±0.10	1.03±0.10
Day 3	1.11±0.14	1.12±0.11	1.08±0.12	1.03±0.10
Day 4	1.08±0.12	1.11±0.12	1.01±0.15	1.02±0.12
Day 5	1.02±0.07	1.00±0.12	0.92±0.11	1.01±0.11
Day 6	1.02±0.01	1.00±0.12	0.90±0.08	0.98±0.12
Day 7	1.01±0.05	0.91±0.06	0.89±0.07	0.94±0.09
Day 8	0.98±0.06	0.90±0.05	0.88±0.08	0.91±0.10
Day 9	0.90±0.05	0.81±0.08	0.78±0.08	0.80±0.08
Day 10	0.74±0.15	0.55±0.07	0.58±0.89	0.58±0.16
Day 11	0.60±0.06	0.40±0.82	0.48±0.16	0.50±0.14
Day 12	0.51±0.08	0.30±0.08	0.44±0.13	041±0.14
Day 13	0.41±0.08	0.19±0.04	0.20±0.08	0.28±0.12
Day 14	0.34±0.10	0.14±0.05	0.18±0.06	0.25±0.09
Day 15	0.22±0.07	0.11±0.03	0.15±0.09	0.20±0.14
Day 16	0.20±0.07	0.10±0.01	0.12±0.05	0.17±0.09
Day 17	0.14±0.05	0.10±00	0.10±00	0.12±0.07
Day 18	0.12±0.04	0.00	0.00	0.00
Day 19	0.00	0.00	0.00	0.00
Day 20	0.00	0.00	0.00	0.00
Day 21	0.00	0.00	0.00	0.00

Based on the statistical results, no significant difference was found between control as well as groups O.S, O.N and O.H in postop day 7 (group 1). However, when the data of group 2 (postop 14) were statistically evaluated the healing rate of wound sites treated with ozonated *Nigella sativa* oil (group O.N) was significantly higher (P<0,01), which is followed successively by the ozonated sesame oil (group O.S, P<0,01) and ozonated *Hypericum perforatum* oil (group O.H, P<0,05) compared to the control group (Tables 6, 7).

On postop day 18, while all wound sites treated with ozonated oils (groups O.S, O.N, O.H) reached full recovery, healing in control wound site were still continuing, which were also observed to reach fully recovered on postop day 19 (Table 5). As a result, healing processes in all wound sites (control, group O.S, O.N, and O.H) of all subjects completed before postop day 21 (Table 5, Figures 21-24).

Table 6. Mean and standard deviation of data considering group variables.

Groups (euthanasia day)	Groups (wound site)	N	Mean	Std. Deviation
1 (postop day 7)	Control	7	1,05714	0,078680
	O. S	7	1,04286	0,078680
	O. N	7	1,04286	0,078680
	O. H	7	1,07143	0,095119
2 (postop day 14)	Control	7	0,97143	0,095119
	O. S	7	0,94286	0,053452
	O. N	7	0,92857	0,095119
	O. H	7	0,95714	0,053452
3 (postop day 21)	Control	7	0,45714	0,09759
	O. S	7	0,22857	0,13801
	O. N	7	0,18571	0,06901
	O. H	7	0,31429	0,08997

Table 7. P value of wound site groups (groups OS, ON, OH) compared to the control group considering three groups

Dependent Variable	Groups (wound site)		P value
Day 1	O.S	Control	,977
	O.N	Control	,977
	O.H	Control	,977
Day 7	O.S	Control	,829
	O.N	Control	,605
	O.H	Control	,972
Day 14	O.S	Control	,001
	O.N	Control	,000
	O.H	Control	,039

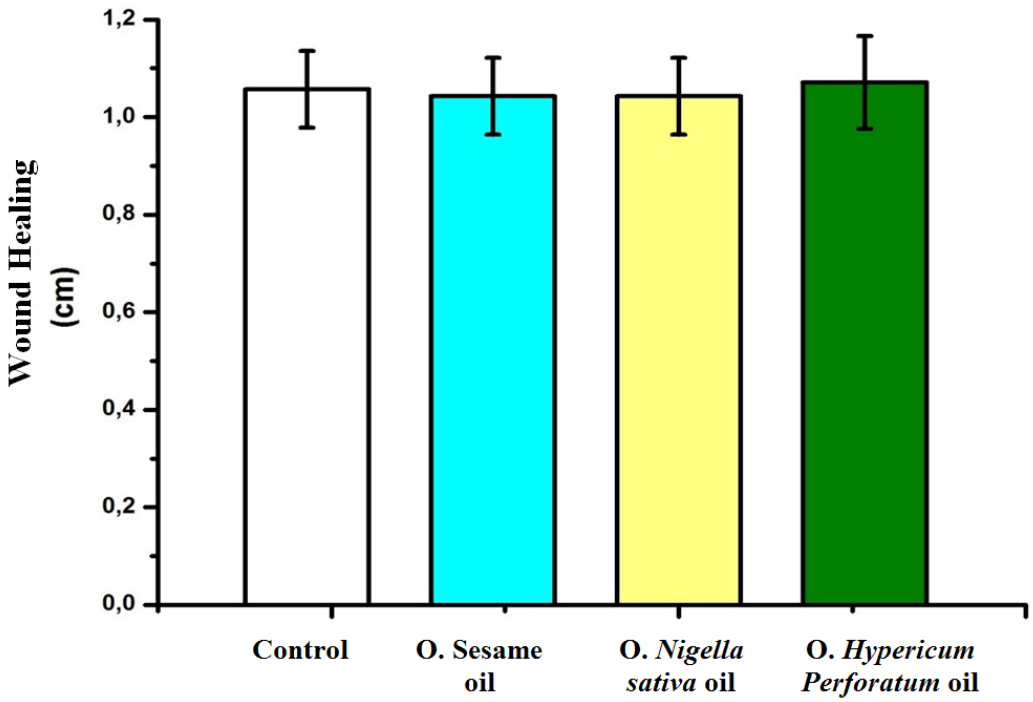

Figure 21. First day on wound healing

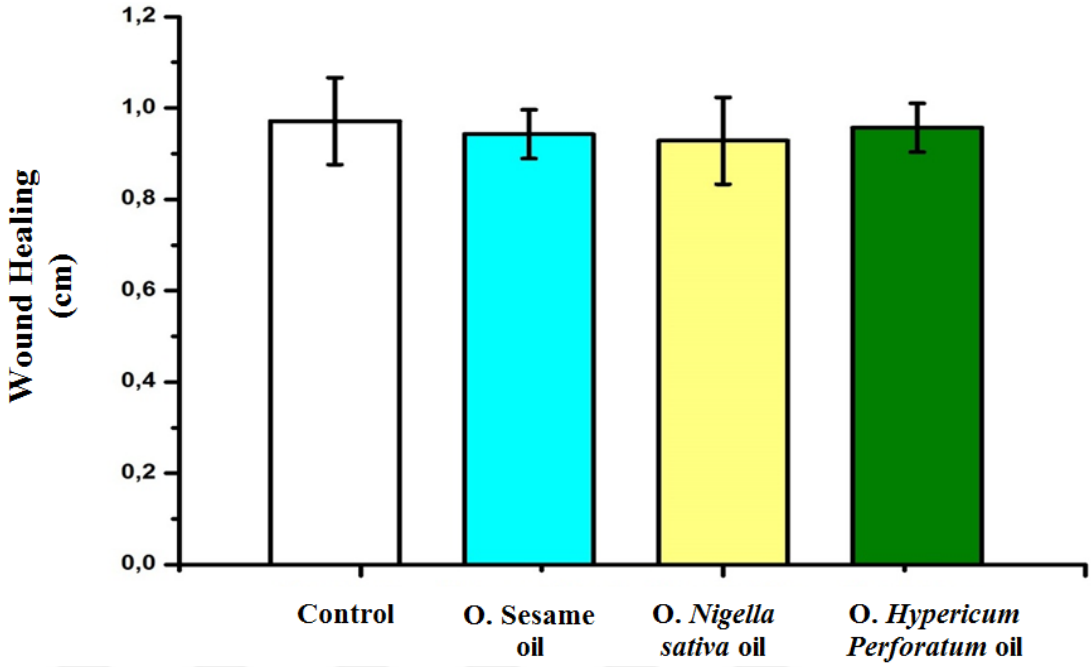

Figure 22. Seventh day on wound healing

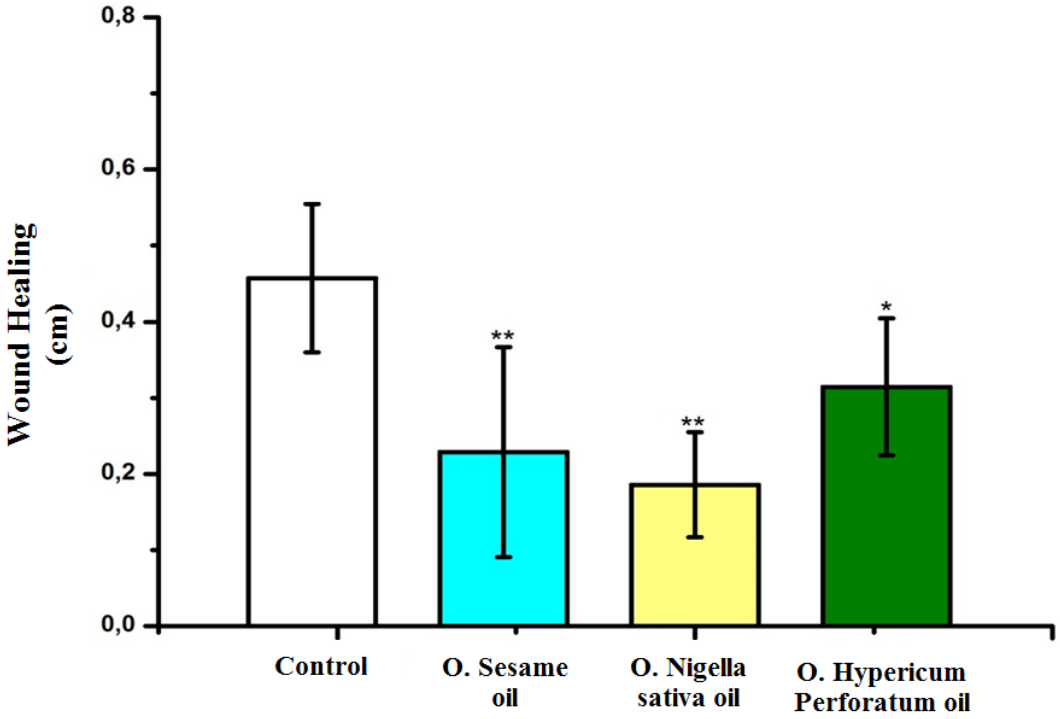

Figure 23. Fourteenth day on wound healing *p<0.05, **p<0.01

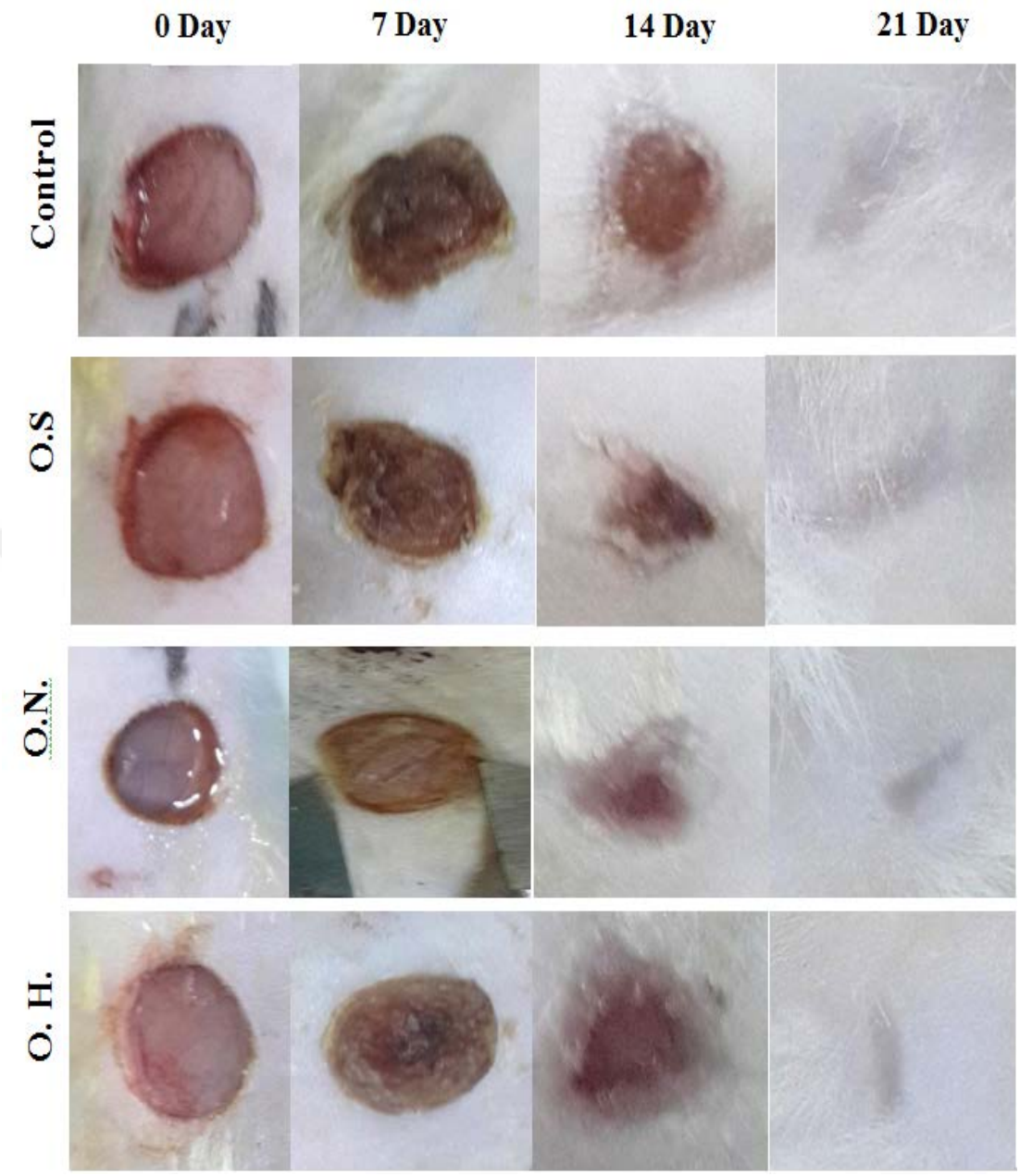

Figure 24: Wound contraction and healing processes throughout the study in four groups (control, groups O.S, O.N, O.H) based on wound sites.

5.2. Histological result

5.2.1. Histopathological Findings in Group I (Day 7):

According to the histopathological scoring data, in control group wound sites conatiend a quite high inflammation and vascular permeability rates, newly formed fibroblast proliferation but no epithelization development. In group O.N, there was a relatively less distinctive inflammation but high vascular permeability and high fibroblast proliferation comapared to control group. In this group unlike control group epithelization process started. In group O.S and O.H moderate inflammation, increased vascular permeability and the edema formation were present. In both groups fibroblast proliferation also inicated (Table 8).

Table 8: Distribution of data according to histopathological scoring in rats euthanized on day 7 (group 1).

Groups	Control	Sesame oil	Nigella sativa oil	Hypericum perforatum oil
inflammation	+ + +	+ +	+	+ +
Ulceration	+ +	+	+	+
Vessel	+ + +	+ +	+	+
Surface Closure	–	–	_	–
Epithelialization	–	–	+	_
Fibroblast	–	+	+	+

No signs (-), mild (+), moderate (++), and severe (+++).

On the seventh day, in all the groups, the lesions were characterized by skin ulcer including inflammatory infiltration, complete epithelial loss and dermatitis. Inflammatory reaction consisted of the neutrophils, macrophages and lymphocytes. There was no difference between the control and experimental groups in terms of

inflammatory response, epithelization and connective tissue formation in all groups (Figure 25: A-D).

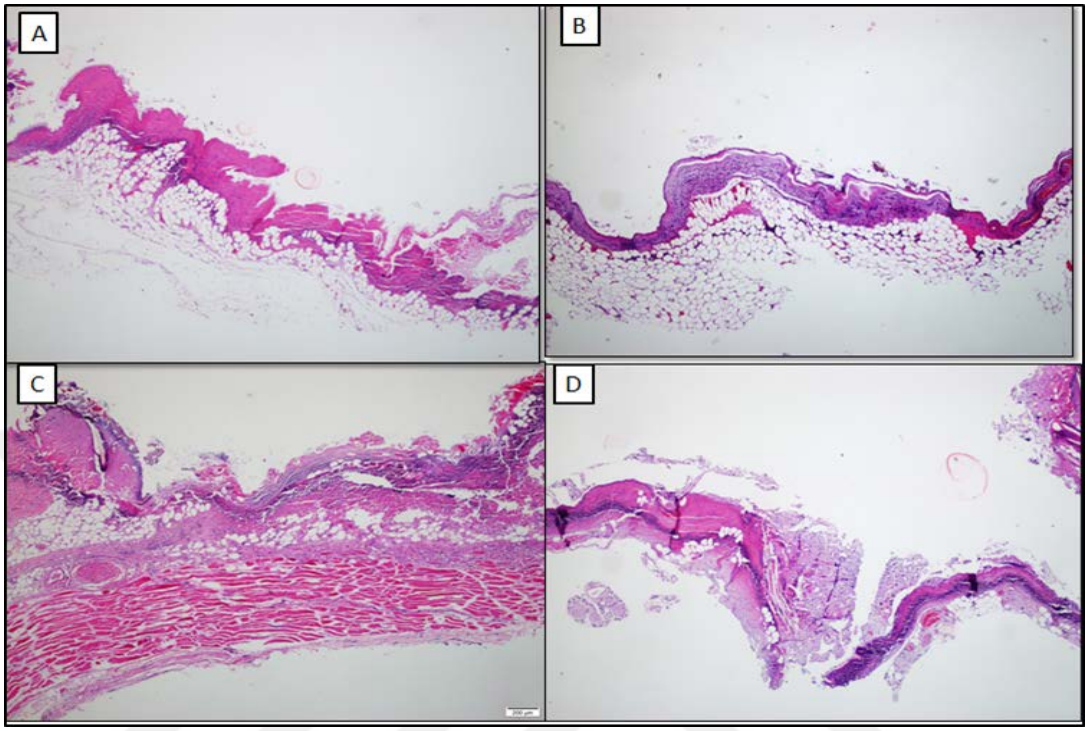

Figure 25. Skin ulcers characterized by necrotic debris and inflammatory infiltration with total absence of epidermis in all groups, including the control group. **A:** Control group, B: O.N. group, C: O.H. group, D: O.S. group, H-E, x4.

5.2.2. Histopathological Findings in Group 2 (Day 14):

According to the histopathological scoring data, **control** and **O.H** groups showed moderate levels of inflammation and increased vascular permeability with newly formed fibroblast proliferation. In these groups epithelization appeared to occur in most parts of the wound area. Unlike the control, in groups **O.S** and **O.N**, no inflamation was observed. In the control group it was determined that there was little edema compared to groups **O.S** and **O.N**. There was less increased vascular permeability in group **O.N** than in group **O.S** (Table 9).

Table 9. Distribution of data according to histopathological scoring in rats euthanized on day 14 (group 2).

Groups	Control	Sesame oil	Nigella sativa oil	Hypericum perforatum oil
inflammation	+ +	−	−	+
Ulceration	+	−	−	−
Vessel Proliferation	+ +	+	−	+
Surface Closure	−	+	+	+
Epithelialization	−	+	+	+
Fibroblast Activity	+	+	+	+

No signs (-), mild (+), moderate (++), and severe (+++).

In all groups of rats examined on day 14 of the study, fibrous connective tissue regeneration with no sebaceous and sweat glands was evident. Control group wound sites contained no epithelial regeneration but include irregularly laid down fibrous ligament (Fig.26. A). In the experimental groups, i.e. groups O.S, O.H and O.N, had well developed epithelial regeneration. In the experimental groups, epithelization rate can be ordered form the most down to the lowest as group ON, OH and OS (Figure 26. B, C, D).

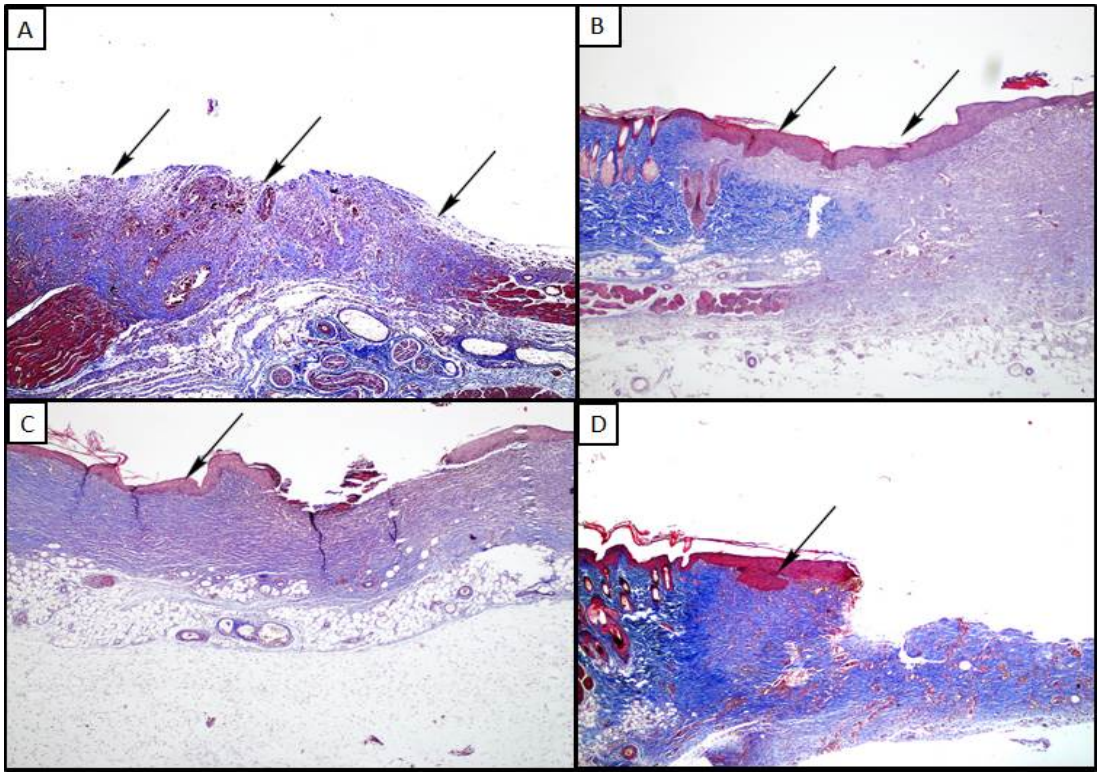

Figure 26: A: Complete removal of epidermis (arrows) and irregular granulation tissue in control group, MT, x10, B.

B: Complete recovery of epidermis (arrows) and development of granulation tissue, MT, x10,

C: Partially epidermal (arrow) and dermal regeneration, MT, x10.

D: Partial epidermal (arrow) and dermal regeneration, MT, x10 in group O.S.

5.2.3. Histopathological Findings in Group 3 (Day 21):

In the third group, lesions in all experimental and control groups

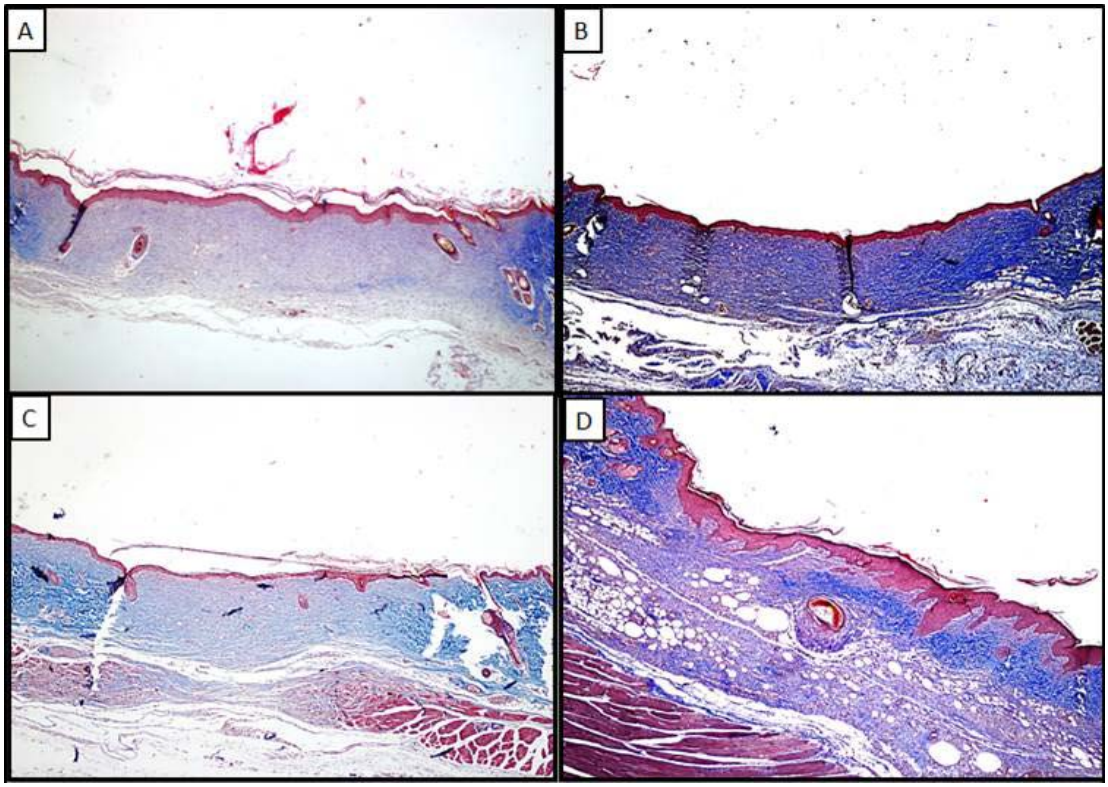

Figure 27. A: Epidermal regeneration and granulation tissue formation in the control group, MT, x10, B. Full recovery of the epidermis (arrows) and granulation tissue development, MT, x10. C. Partially epidermal (arrow) and dermal regeneration, MT, x10. D. Partial epidermal (arrow) and dermis regeneration, MT, x10 in the sesame oil group.

6. DISCUSSION

Wound healing in a defect tissue consists of a number of repair and reorganization processes, during which coagulation systems become active, acute and chronic inflammatory responses arise, new vascular events occur through angiogenesis and vasculogenesis, cells become proliferative, divide, apoptotic, extracellular matrix accumulates (6).

History of wound care and management goes back to very ancient times. In the inscriptions written by the Sumerians before the wound is cleaned with water and milk with to be closed honey. In the mid-eighteenth century, iodine and chlorine were discovered and wound care went forward. Semmelweiss in 1846 published the benefits of hypochlorite solution in prophylaxis of puerperal sepsis, a major cause of women's death in that time (17).

There are many products used under the name of dressing materials for therapeutic purposes in wound healing. Many herbal and fat-containing products have antioxidant activity and these products have been tested for topical wound healing promotion.

Recently, *Hypericum Perforatum*, known as Kantaron oil, has come to the agenda as a topical treatment agent especially in burns wound care (61). Hammer et al. (62) in their study reported that considering its antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effects its anti-inflammatory activities kantaron oil can be uses as active ingredient to induce wound healing. Dunic et al. (63) showed that kantaron oil contains high amounts of quercetin and bibigen (129 mg / ml and 52 mg / ml, respectively). Quercetin and bibigen have been reported to exhibit anti-inflammatory activities similar to indometazine. Lavagne et al. (64)

showed that topical use of H. perforatum-containing oils has positive effects on epithelial regeneration of surgical wounds.

Sesame oil has recently begun to be used in wound healing (65, 66). Suja et al. (65) reported that antioxidant property of sesame oil is due to ingredients such as sesamin, sesamolin and sesamol present in it content. Sesame oil also contains 55% lipid and 20% protein. Kang et al. (66) revealed in vivo and in vitro studies that the sesamol is the most important free radical scavenger agent. In the same studies, these agents, i.e. sesamin, sesamolin and sesamol, were shown to have inhibitory effects in membrane lipid peroxidation.

In a study conducted by Valacchi G. et al (67), was investigated the topical effects of ozonated sesame oil on wound healing, where it revealed that ozonated sesame oil interacts with polyunsaturated fatty acids and therefore has antioxidant effect. This product promotes angiogenesis in wound healing and increases vascular endothelial growth factor as well as cyclin D1 expression.

Studies on the antimicrobial properties of Nigella sativa are available in the literature (68-71). A study (68) determined that antimicrobial properties of Nigella sativa has a potential to accelerate wound healing. In a study by Arici et al. (69), the antibacterial activity of *Nigella sativa* oil was investigated in vitro and a total of 24 antibacterial effects, which were mediated by the combination of thymoquinone, p-cymene and carvacrol components, were observed. Islam et al. (70) tested the antifungal activity of *Nigella sativa* oil was tested against 24 fungal organisms of pathogenic and industrial strains and at the end of the study, they found that *Nigella sativa* oil had significant activity and stronger and wider range against fungi. Bruits

et al. (71) found that *Nigella sativa* oil had especially antioxidants and free radical sweeping features but not pro-oxidant.

Flavanoids and triterpenoids are well-known to enhance wound healing (71). Because flavonoids and triterpenes are also components of *Nigella sativa* we hypothesized that the wound healing in this group would take place in a shorter period.

Ozone, a powerful oxidant, is known as one of the strongest disinfectants because of this property (22). It is also used in the treatment of certain disorders such as dermatitis, alone or in combination with other agents, e.g. ozonated oil (23). Ozone gas reacts with oil by reacting with carbon-carbon double bonds (oleic acid, linoleic, linoleic acid, etc.) found in unsaturated fatty acids of vegetable oils (24). As a result of these reaction different products such as hydrogen peroxide, hydroxyhydroperoxide, aldehyde and ozone are released (26). Ozonated oils are often used as an antibacterial product to treat skin infections. Han CM et al. (72) reported that Nigella sativa exerts a wound-healing effect through its antioxidant property while *Hypericum Perforatum* enhances wound healing via epithelialization and granulation encouraging effects.

In the light of all these studies, we also planned a study showing the effects of ozonated *Hypericum Perforatum* oil (St. John's Wort oil), sesame oil and *Nigella sativa* oil on topical wound healing.

In this study, mean epithelialization days of *Nigella sativa*, sesame oil, *Hypericum Perforatum* oil and control (K) groups were 15,08 ± 0,36, 16,17 ± 0,46, 16,83 ± 0,46 and 16,93 ± 0,46, respectively. These data shows that *Nigella sativa* application has positive effects on wound epithelization rate.

Additionally in our study, wound closure rates were assessed daily throughout the study and it was found that this rate was significantly higher ($p < 0.001$) in the treatment groups compared to the control groups. After 7^{th} day and 14^{th} day in wound treatment, macroscopical findings demonstrated that the granulation tissue was more smooth and alive and histological parameters showed that wound healing developed healthier and vascularization reduced more markedly in experimental groups compared to control.

The wound area is the most obvious symptom of wound healing and can be evaluated measured with the closure rate of the wound surface (74). Contraction, known as the centripetal motion of the wound edges, which speeds up the closing process of open wounds, is governed by the myofibroblast and the extracellular matrix around it. Wound contraction is 80% effective in closing open wounds in where with loose skin texture (74-77). In our study, it was observed that contraction rate was significantly higher in *Hypericum perforatum* group which were proceeded by *Nigella sativa* and sesame oil groups compared to control.

Groups were visualized daily in terms of infections and there was no indication of infection in the wounds.

- It was observed that topical *Nigella sativa* oil improves wound healing by shortening the duration of revitalization compared to the control group ($P<0.001$).
- When the experimental groups (sesame oil, *hypericum perforatum* oil) were compared histologically with the 'control' group, there was no difference between the groups.

- In our study, wound in the wound model created in experimental and control groups and the wound healing rates in the experimental groups of other days were statistically significant for the control groups except the 7th day.
- No evidence of infection was seen in any group when wound healing was observed in rats in the experimental and control groups.

As a result; we investigated the effects of sesame oil, N*igella sativa* oil and *Hypericum perforatum* oil on wound healing considering their antiseptic and antibacterial properties. The wound healing rates were statistically more significant in the experimental groups than in the control groups except the 7th day. Macroscopic evaluation showed that recovery rate was faster in *Nigella sativa* oil and sesame oil group however when the experimental groups (sesame oil, *hypericum perforatum* oil) were compared with respect to histopathologic result no difference between experimental groups in terms of regeneration formation was found. The quantitative data of this study showed that ozonated *Nigella sativa* oil promoted wound healing significantly ($p < 0.001$) as compared to other groups.

These results demonstrate that ozonated oils, especially the ozonated *Nigella sativa* oil may improve acute cutaneous wound repair, mainly via shortening the duration of epitalization. Topical application of specific ozonated oil may be considered as an alternative therapeutic modality to enhance cutaneous wound healing. Therefore we believe that *Nigella sativa* oil can be used as an adjunct or an alternative agent to existing treatments in the future wound healing due to its antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effects.

7. REFERENCES

1. Akkaya, S, Kölemen, F, Kürkçüoğlu, N, Akan T, Atakan N. Dermatoloji Ders Kitabı. S. Medial yayın grubu, Ankara, 1987.
2. Martini FH, Timmons MJ, McKinley MP. Human Anatomy. 3rd edition, New Jersey, USA: Prentice Hall, 2000.
3. Anonymous."WoundHealing"http://www.medicaldictionary.thefreedictionary.com/wound+healing 27.09.2017.
4. Lazarus GS, Cooper DM, Knighton DN, Margolis DJ, Percoraro Re, Rodeheaver G, Robson MC. Definitions and guidelines for assessment of wounds and evaluation of healing. J Wound Repair and Regeneration 1994; 2: 165-170.
5. Marks R, Dykes P, Motley R. Clinical sings and procedures in dermatology. 1st edition, London; Martin Dunitz Ltd,1993.
6. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol 2001; 69: 513-521.
7. Kaariainen M, Kalimo H, Järvinen M. Muscle injuries: biology and treatment. Am J Sports Med 2005; 33: 745-764.
8. Stadler I, Lanzafame RJ, Evans R, Narayan V, Dailey B, Buehner N, Naim JO. 830 nm irradiation increases the wound tensile strength in a diabetic murine model. Lasers Surg Med.2001; 3: 220-226.
9. Witte MB, Barbul A. General principles of wound healing .Surg Clin of North Am 1997; 77 (3), 509-528.
10. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. Clinics in Dermatology 2007; 25: 9–18.
11. Strodtbeck F. Physiology of Wound Healing. Newborn and Infant Nursing Reviews 2001; 1: 43–52.
12. Cotran RS, Kumar V, Collins T. Robbins Pathologic Basis of Disease.2nd edition, Philadelphia: Saunders 1999; 107-109.
13. Hess CT, Kirsner RS. Orchestrating wound healing: assessing and preparing the wound bed. Advances in Skin & Wound Care 2003; 16: 246-257.
14. Phillips SJ. Physiology of Wound Healing and Surgical Wound Care. ASAIO Journal 2000; 46: 2-5.
15. Monaco JL, Lawrence WT. Acute wound healing an overview. Clinics Plastic Surgery 2003; 30: 1-12.
16. Mast BA. Normal wound healing. Plastic and Reconst. Surgery 2000; 1:37-52.
17. Witte MB. Wound healing: general principles of wound healing. Surg. Clin. N. Am. 1997; 77:509- 518.
18. Barutçu A, Aydın OE. Wound Care in Anatolia. EWMA Journal 2006; 6:16-17.
19. Tan SY. History of Most Enduring Medic. Singapore Med J 2002; 43:116-117.
20. Broughton G, Janis JE, Attinger CE. A Brief History of Wound Care .Plast Reconstr Surg 2002; 117-130.

21. Baultan AJ. The Diabetic Foot. Diabetologia 2004; 17:1343-1353.

22. Viebahn –Haensler R. The use of ozone in medicine Karl F.Haug Publishers, Heidelberg 2002, 83-84.

23. Viebahn-Haensler R. Ozone Oksijen Karışımlarının Tıpta Kullanımının Temel Özellik ve Gereklilikleri; Ozonun Tıpta Kullanımı Medikal Ozon Oksijen Derneği 2005, 13-52.

24. Bocci V. Ozone. A new medical drug. Dordrecht, the Netherlands: Springer, 2005; 1–295.

25. Stübinger S, Sader R, Filippi A. The use of ozone in dentistry and maxillofacial surgery: a review. Quintessence Int. 2006; 37:353–9.

26. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marquesm JL. Ozone therapy in medicine and dentistry. J Contemp Dent Pract. 2008; 9:75–84.

27. Bocci V. The case for oxygen-ozone therapy. Br J Biomed Sci. 2007; 64:44–9.

28. Kim HS, Noh SU, Han YW, Kim KM, Kang H, Kim HO and Park YM. Therapeutic effects of topical application of ozone on acute cutaneous wound healing, Journal of Korean Medical Science 2009; 24 (3): 368–374.

29. Valacchi G, Lim Y, Zanardi I, Bocci V and Travagli V. Evaluation of ozonated sesame oil effect in wound healing using the SKH1 mice as a model, in Proceeding of the 7th World Meeting on Pharmaceutics, Bio pharmaceutics and Pharmaceutical Technology. Valletta: Malta 2010.

30. Atta–ur–Rahman MS, Choudhary MI, Rahman H. Nigellimine N–oxide a new isoquinoline alkoloid from the seeds of Nigella sativa. Heterocycles. 1985; 23: 953–955.

31. Grondel JL, Nouws JF, Van Muiswinkel WB. The influence of antibiotics on the immune system: immunopharmakokinetic investigations on the primary anti–SRBC response in carp Cyprinus carpio L., after oxytetracycline injection. Journal Fish Diseases, 1987; 10: 35–43.

32. Arda M, Seçer S, Sarıeyyüpoğlu M. Balık Hastalıkları, Medisan Yayınevi, Ankara, 2002, 1-36.

33. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother, 2000; 14: 323–328.

34. Baytop A. Farmasötik Botanik Ders Kitabı, İstanbul Üniv, 1991, 169.

35. Mahfouz M, El-dakhakhny M. Isolation of a crystaline active principle from Nigella sativa L. Seeds. J. Pharmaco. Sci. U.A.R.1960; 1:1.

36. Ali BH, Blunden G. Pharmacological and toxicological Properties of Nigella sativa. Phytotherapy Research, 2003; 17: 299–306.

37. Baytop T. Türkiye'de bitkilerle tedavi (Geçmişte ve Bugün) .2. Baskı, İstanbul: Nobel Tıp Kitapevi, 1999.

38. Çakmak HE, Bayram E. Muğla orijinli sarı kantaron (Hypericum perforatum L.) populasyonlarının bazı agronomik ve kalite özelliklerinin belirlenmesi, Ege Üniv Ziraat Fak Derg, 2003; 40 (1): 57-64.

39. Meral G, Karabay NÜ. In vitro antibacterial activities of three Hypericum species from west Anatolia, Turkish Electronic J Biotech, Special Issue, 2002, 610.

40. Miller AL. St. John‘s Wort (Hypericum perforatum): clinical effects on depression and other conditions, Alt. Med. Rev., 1998; 3:18–26.

41. Di Carlo G, Borrelli F, Ernst E, Izzo AA. St John‘s wort: Prozac from the plant Kingdom, Trends in Pharmacological Sciences, 2001; 22 (6): 292-297.

42. Greeson JM, Sanford B, Monti DA. St. John's wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature, Psychopharmacology, 2001; 153: 402-414.

43. Hunt EJ, Lester CAE, Lester EA, Tackett RL. Effect of St. John's wort on free radikal production, Life Sciences, 2001; 69: 181-190.

44. Bombardelli E, Morazzoni P. Hypericum perforatum, Fitoterapia, 1995; 66:43-68.

45. Cingi Mİ. Sarı kantaron yağının yara iyileştirmesindeki yeri, Anadolu Tıp Derg, 1991; 13: 35-39.

46. Çırak C. Farklı doku kültürü uygulamalarının iki kantaron türünde (Hypericum perforatum ve H. bupleuroides) mikroçoğaltım yeteneği ve hiperisin ile toplam fenolik birikimi üzerine etkileri, Doktora Tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun, 2006.

47. Radulovic N, Stankov-Jovanovic V, Stojanovic G, Smelcerovic A, Spiteller M, Asakawa Y. Screening of in vitro antimicrobial and antioxidant activity of nine Hypericum species from the Balkans, Food Chem, 2007; 103: 15-21.

48. Öztürk N, Korkmaz S, Öztürk Y. Wound-healing activity of St. John’s wort (Hypericum perforatum L.) on chicken embryonic fibroblasts, J Ethnopharmacol, 2007; 111 (1): 33-9.

49. Nahrstedt A, Butterweck V. Biologically active and other chemical constituents of the herb of Hypericum perforatum L., Pharmacopsychiatry, 1997; 30 (2): 129-134.

50. Draves HA, Walker SE. Determination of hypericin and pseudohypericin in pharmaceutical preparations by liquid chromatography with fluorescence detection, J Chromatogr B, 2000; 749: 57-66.

51. Taşkın K. Susam (Sesamum indicum L.) Bitkisinin in vitro Rejenerasyonu ve Agrobacterium tumefaciens Aracılığı ile Genetik Transformasyonu. Akdeniz Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Antalya, 1997, 58.

52. Uzun B. Susamda Kapalı Kapsüllülük Mutant Karakterine Bağlı Moleküler Merlerin Belirlenmesi. Akdeniz Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, Antalya, 2002, 109.

53. Agosa ET. Physical and Physiological Characteristics’ of Seed of Sesame (Sesamum indicum L.) Varieties Grown in Nigeria. University Of Agriculture, Department Of Plant Breeding And Seed Technology College Of Plant Science And Crop Production, Master Thesis, Abeokuta, 2011, 32.

54. Namiki M. Nutraceutical functions of sesame: A review. Critical Reviews in Food Science and Nutrition, 2007;47: 651-673.

55. Özcan M. Susam Yağı ve Tahinde Fiziksel-Kimyasal Analizler ve Yağ Asitleri Bileşiminin Belirlenmesi. Selçuk Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Konya, 1993, 51.

56. Namiki M. The chemistry and physiological functions of sesame. Food Review International, 1995; 11: 281-329.

57. USDA. USDA National nutrient database for standard reference, release 27. http://www.ars.usda.gov/ba/ bhnrc/ndl 20.05.2016.

58. Anonymous. “Seed” https://www.fairdinkumseeds.com/products-page/brassica-lettuce-and-asian greens/sesame-indicum-white-sesame-seeds/20.05.2017.

59. Mohamed HM . The Use of Sesame Oil Unsaponifiable Matter as a Natural Antioxidant. Food Chemistry, 1998; 62: 269-276.

60. Jannat B, Oveisi MR, Sadeghi N, Hajimahmoodi M, Behzad M, Choopankari E, Behfar AA. Effects of roasting temperature and time on healthy 89 nutraceuticals of antioxidants and total phenolic content in Iranian sesame seeds (Sesamum indicum L.) Iran. Journal of Environmental Health Science and Engineering, 2010; 7: 97-102.

61. Blumenthal M. St John' s Wort. American Botanical Council 1998; 11:470-485

62. Hammer KDP, Hilwing ML, Solco AKS. Inhibition of prostoglandin E2 production by antiinflamatuar Hypericum Perforatum extracts and constituents in RAW264.7 mouse macrophage cells. J Agric Food Chem 2007;55: 7323- 7331.

63. Dunic GZ. Evaluation of Hypericum perforatum Oil Extracts for an Antiinflammatory and Gastroprotective Activity in Rats. Phytoter. Res . 2009;10:1002.

64. Lavagna SM, Secci D. Efficacy of Hypericum and Calendula oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section. Farmaco 2001; 56: 451–453.

65. Suja KP. Antioksidant efficacy of sesame cake extract in vegetable oil protection. Food Chem. 1998; 84: 393–400.

66. Kang MH. Mode of action of sesame ligands in protecting low– density lipoprotein against oxidative dame in vitro. Life Sci. 2000; 66: 161 – 171.

67. Valacchi G, Lim Y, Belmonte G, Miracco C, Zanardi I,Bocci V, Tavagli V. Ozonated sesame oil enhances cutaneous wound healing in SKH1 mice. Wound Repair Regen. 2011 ;19:107-15.

68. Nayak BS, Lexley MPP. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine, 2006;6:41.

69. Arici M, Sagdic O, Gecgel U. Antibacterial effect of Turkish black cumin (Nigella sativa L.) oils. Grasas y Aceites, 2005;56: 259-262.

70. Islam SK, Ahsan M, Hassan CM, Malek MA. Antifungal activities of the oils of Nigella sativa seeds. Pak J Pharm Sci. 1989;2:25-8.

71. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res. 2000;14:323-8.

72. Han MC, Durmuş AS, Sağliyan A, Günay C, Özkaraca M, Kandemir FM, Çomakli S, Öztopalan DF. Effects of Nigella sativa and Hypericum perforatum on wound healing. Turk J Vet Anim Sci 2017; 41: 99-105.

73. Kaya E. Yara iyileşmesi. Genel Cerrahi. Ed: Bilgel H ,İstanbul: Avrupa Tıp Kitapçılık , 2007,169-191.

74. Engin A. Yara iyileşmesi. Temel Cerrahi El Kitabı. (Ed:Sayek İ), Ankara: Güneş Tıp Kitapevleri ,2009, 135-140.

75. Robbins KC. Temel Patoloji (Basic Patology). Fifth edition, İstanbul: Nobel Tıp Kitapevleri,1992, 47-60.

76. Gençcelep M, Aslan L, Yüksel H, Karasu A,Bakır B. Otolog fibrin yapıştırıcının açık yara tedavisinde iyileşme üzerine etkisi: deneysel çalışma. YYÜ. Vet. Fak. Derg. 2001;12 (1-2):101-104 .

8. CURRICULUM VITAE

Personal information:

Name: Tamara Müfit RIZAOĞLU

Date & place of birth: 11th Jan. 1992- Palestine, Jenin

Email address: tamara.assali2015@gmail.com

Education:

- Turkish Center of Istanbul University . (2014-2015)
- Bachelor of Veterinary medicine of An_Najah National University in Palestine (2009-2014)
- (2006-2009) Jenin Secondary Girls School
  Palestine, Jenin

Major Courses:

- Reproduction management and Artificial Insemination in small Ruminant from FAO Organization _ Jerusalem
- First Aid _ Palestine Red Crescent Socitey
- Having ENGLISH proficiency certificate
- Having TURKISH proficiency certificate TÖMER

Skills:

- Excellent computer skills and internet (Microsoft Office).
- Excellent communications Skills.
- Ability to work in team with good interpersonal skills.
- Customer service orientation.
- Able to work to a high degree of detail.
- Able to deal with highly confidential matters professionally & discreetly.
- Photography.

Language:

Arabic: Native Language

English: Good in Reading, Writing & Speaking

Turkish: Good in Reading, Writing & Speaking