ORIGINAL ARTICLE

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Demonstration of Effect of Oak Root Extract on Burn Wounds in Rats

ABSTRACT

Objective: The aim of this study was to determine whether oak root extract has protective role on burn injuries by using quantitative and histopathological approaches.

Methods: The study was conducted on 16 adult Sprague-Dawley rats aged 8 weeks (weighing 350 g). The rats were assigned into 2 groups including: group 1 consisting of rats employed as controls and group 2 in which rats received oak root extract. After shaving dorsum of rats, second degree burn injury was created at the right and left sides of the line representing vertebral column in both groups. Wound care was provided by using normal saline in group 1, while it was provided by using oat root extract in group 2. The right side was used for macroscopic evaluations, while left side was used for histopathological examination through biopsy samples obtained on the days 1, 3, 7 and 17. On the day 17, the rats were sacrificed under ketamine anesthesia. Cutaneous tissue samples were taken and macroscopic measurements were made by using a grid paper. Edema, neovascularization, inflammatory cell infiltration and re-epithelization were assessed on the histopathological examination.

Results: When compared to the control group, it was seen that the extent of burn area was reduced and that degree of neovascularization, inflammatory cell infiltration and re-epithelization was increased with decreased edema was ($p<0.001$).

Conclusions: It was seen that oak root extract was effective in the healing of burns.

Keywords: Burn, Oak Root Extract, Healing, Rat

Ratlarda Yanık Yarası Üzerine Meşe Kökü Ekstresinin Etkisinin Gösterilmesi

ÖZET

Amaç: Bu araştırmanın amacı ratlarda yanık yarası üzerine kantitatif, ve histopatolojik yaklaşımlarla meşe kökü ekstresinin koruyucu rolü olup olmadığını belirlemektir.

Gereç ve Yöntem: Çalışmaya 16 adet, 8 haftalık erişkin ve 350 gram ağırlığında Spraque-Dawley rat alındı ve ratlar iki gruba ayrıldı; grup 1: kontrol grubu, grup 2; meşe ekstresi uygulanan grup. Ratların sırt bölgesi traşlandıktan sonra columna vertebralisin olduğu hat işaretlenip sağ ve solunda kalan bölgeye 2. derece temas yanığı oluşturuldu. Grup 1 yanık alanı serum fizyolojik, Grup 2' de ise meşe ekstresi ile günlük pansuman yapıldı. Her bir ratın sırtındaki sağ bölge makroskopik, sol bölge ise histopatolojik inceleme (1. 3. 7. ve 17. günlerde alınan biyopsi) için kullanıldı. 17. gün de ratlar ketamin anestezi altında iken sakrifiye edildi ve cilt doku örnekleri alındı ve makroskopik olarak milimetrik kağıt ile ölçüm yapıldı. Histopatolojik incelemede ödem, neovaskülarizasyon, enflamatuar hücre infiltrasyonu ve re-epitelizasyon değerlendirildi.

Bulgular: Meşe ekstresi uygulanan grup, kontrol grubu ile karşılaştırıldığında yanık alanın azaldığı, neovaskülarizasyon, enflamatuar hücre ve re-epitelizasyon derecesinin arttığı, ödemin ise azaldığı görüldü ($p<0.001$).

Sonuç: Meşe kökü ekstresinin yanık yarasının iyileşmesinde etkili olduğu görüldü.

Anahtar Kelimeler: Yanık, Meşe Kökü Ekstresi, İyileşme, Rat.

INTRODUCTION

Burn injury or burn is defined as disruption of skin and subcutaneous tissue by the effects of heat, electricity, chemicals and radiation. Burn is an important trauma, leading deaths and disabilities with remarkable costs to community. Annually, 2.2 million people receive medical therapy due to thermal burn. Of these, 60,000 people are hospitalized while 5,500 people die due to burn injury (1).

Thermal injury may involve epidermis, whole or part of dermis or even subcutaneous tissue. Increased depth of burn affects wound healing negatively. Estimation of burn depth is essential in management and eventual decision-making process regarding surgical intervention. Burns are evaluated in three degrees: superficial burns involving epidermis (first degree); superficial or superficial-profound burns involving epidermis and dermis in part (second degree); and full-thickness burns involving epidermis and whole dermis (third degree). The management of burns include supportive care and wound care with antibiotic ointments. However, full-thickness excision or grafting should have to be performed in third degree burns (2). In recent years, several natural and traditional products such as aloe vera, olive oil, centaury oil and honey without any side effects have been increasingly used in the management of burns. Thus, investigators are seeking natural products with proven biological effects in the management of burn (3-8). It is thought that extract derived from oak root is one of these products, which is commonly used in Maraş province (5). Based on the information gathered from local people, roots of oak are boiled following mincing, which then applied over burn wound for treatment (5). Oak root extract is a traditional cure used by local people of Maraş province. There are several oak species in Maraş province. Particularly, Quercus coccifera (kermes oak) is used in the management of burns. It is an evergreen, shrub reaching 2-3 meters tall and rarely a small tree reaching up to 10 meters tall with dense branching. Young buds are covered by fine hairs; are dark-skinned; and these hairs are thrown away. Leaves are hard as leather with elliptical or large egg-like shape. Kermes oak is native to Mediterranean region (5). Oak root extract is used as a popular treatment in folk medicine due to its anti-oxidant, anti-bacterial, anti-carcinogenic, anti-viral properties as well as biological activities such as immunostimulant, analgesic and anti-allergic effects (9-12). The aim of present study was to demonstrate effect of oak root extract on second degree burns in macroscopic and histological manner.

MATERIAL AND METHODS

This study was approved by Ethics Committee on Experimental Animal Studies of KSU, Medicine School. The study was conducted at Experimental Animals Research Center of Erciyes University.

Animals and Experimental Groups: The study was conducted on 16 adult, male Sprague-Dawley rats aged 8 weeks (weighing 350 g). The animals were housed in separate Plexiglas cages placed in a room without window under standard conditions with temperature of 21±2°C by maintaining 14-to 10-hours dark-light cycle. All rats were fed by standard rat pellet before and throughout experiments. The rats were assigned into 2 groups as follows: Group 1: control rats; Group 2: rats receiving oak root extract

Burn Injury Model: After shaving dorsum of rats, the comb model was applied to the right and left side of the line representing vertebral column. Four comb-like brass plates (weighing: 200 g; 2x1 cm in size with 0.5 cm intervals) were placed in boiling water for 15 minutes, which were then placed on the dorsum of rats for 20 s (13). In the pilot study, it was seen that second degree burn injury was induced with this model as determined by biopsy on the day 1 (Figure 1). The area on the right side was used for macroscopic monitoring while the area on the left side was used for histopathological examination.

Figure 1. Second degree burn injury.

Collecting Plant, Aqueous Extract and Application of Oak Root Extract: Wound care was performed on daily basis by using normal saline (NS) in control group while it was performed on daily basis by using oak root extract prepared using traditional method (Roots of Kermes oak were washed and minced after peeling. Then, roots were re-washed and dried under sunlight. Distilled water (200 ml) was added to ten grams of oak root, placed into boiling water for 20 minutes. Then, the mixture obtained was filtrated and centrifuged at 3500 rpm over 15 minutes in a sterile manner. The mixture was placed into dark-colored bottles and stored at 4°C.) (5).

Examination of Animals and Findings of Burn: All animals were assessed on the days 1, 3, 7

and 17 by macroscopic evaluation. Necrosis was measured on the day 17 by using a grid paper. Recovery time of burn injury was defined as time to complete healing with removal of crusts.

Histopathological Examination: For histopathological examination, incisional biopsy samples (5x25 mm in size) were obtained from the area on the left side of vertebral column on the days 1, 3, 7 and 17. Incision sites were closed by using 2/0 silk sutures. Tissue samples obtained were placed in 10% formalin, which were then processed for histopathological examination. The tissue samples were embedded to paraffin blocks and 5 µm thickness sections were stained by hematoxylin-eosin. Histopathological examination was performed by using a Leica microscope (x200). Inflammation, edema, fibroblast level and neovascularization were assessed as follows: grade 0, normal; grade 1, focal infiltration; grade 2, moderate infiltration; grade 3, severe infiltration.

Re-epithelization was assessed at the junction of mature and immature epithelium while collagen skin attachments were assessed at dermis. The area involving uninjured collagen and skin attachments was considered as the area without burn, while the area with dense fibroblast and de novo collagen production was considered as burn healing area. The margin between these areas was defined as origin of re-epithelization: grade 0, no epithelization; grade 1, epithelization<15 micron; grade 2: epithelization of 15-25 micron; and grade 3, epithelization>15 micron.

Statistical Analysis: All analyses were performed by using SPSS for Windows (Statistical Package for Social Sciences) and SigmaStat 3.5 software. Shapiro Wilk test was used to test normal distribution. Kruskal-Wallis test and Student-Newman-Keuls method were used for comparison between quantitative variables. Quantitative variables were expressed as mean and median (25-75%). $p<0.05$ was considered as statistically significant.

RESULTS

Table 1 presents histopathological assessment of inflammation, edema, fibroblast level, neovascularization and re-epithelization in groups 1 and 2. When groups were assessed for inflammatory cell infiltration, it was found that there was no significant difference between groups on the days 3 and 17 ($p>0.05$) while group 2 significantly differed from group 1 on the day 7 ($p=0.022$). When compared regarding edema, it was found that there was a significant difference on the day 7 between groups ($p<0.001$). It was also seen that there was no significant difference in neovascularization on the day 3 while there were significant differences on the days 7 and 17 ($p<0.001$). When groups were compared regarding fibroblast infiltration and re-epithelization, it was seen that there was no significant difference between groups on the days 3 and 7 ($p>0.05$) while there was significant difference on the day 17 ($p<0.001$).

In the macroscopic evaluation of burn area, significant difference was detected on the day 17 ($p<0.05$). It was seen that healing time was significantly shorter in group 2 (oak root extract) than controls ($p<0.05$) (Figure 2a, Figure 2b).

Table 1. Histopathological assessment of inflammation, edema, fibroblast level, neovascularization and re-epithelization in groups 1 and 2.

Parameter	Group 1	Group 2	*P*
Neovascularization day 3	0.37± 0.51	0.50± 0.53	0.642
Neovascularization day 7	0.75±0.46	1.5±0.53	0.010
Neovascularization day 17	1.37±0.51	2.75±0.46	<0.001
Fibroblast day 3	0.12±0.35	0.00±0.00	0.334
Fibroblast day 7	1.0±0.00	1.0±0.00	1.000
Fibroblast day 17	1.5±0.53	2.5±0.53	0.002
Inflammation day 3	1.12±0.35	1.25±0.46	0.554
Inflammation day 3	1.37±0.51	2.12±0.64	0.022
Inflammation day 3	1.37± 0.51	1.75± 0.70	0.246
Edema day 3	1.0±0.53	1.37±0.74	0.266
Edema day 7	0.75±0.46	1.75±0.46	<0.001
Edema day 17	0.5±0.53	0.37±0.51	0.642
Re-epithelization day 3	0.37±0.62	0.62±0.51	0.350
Re-epithelization day 7	1.0±0.00	1.37±0.51	0.060
Re-epithelization day 17	1.25±0.46	2.37±0.51	<0.001
Burn area day 1 [cm^2]	7.88±0.21	7.76±0.22	0.271
Burn area day 17 [cm^2]	4.82±0.87	3.68±0.88	0.021
Recovery time [day]	26.25±3.01	21.87±2.99	0.011

Values are expressed as mean ± std. deviation.

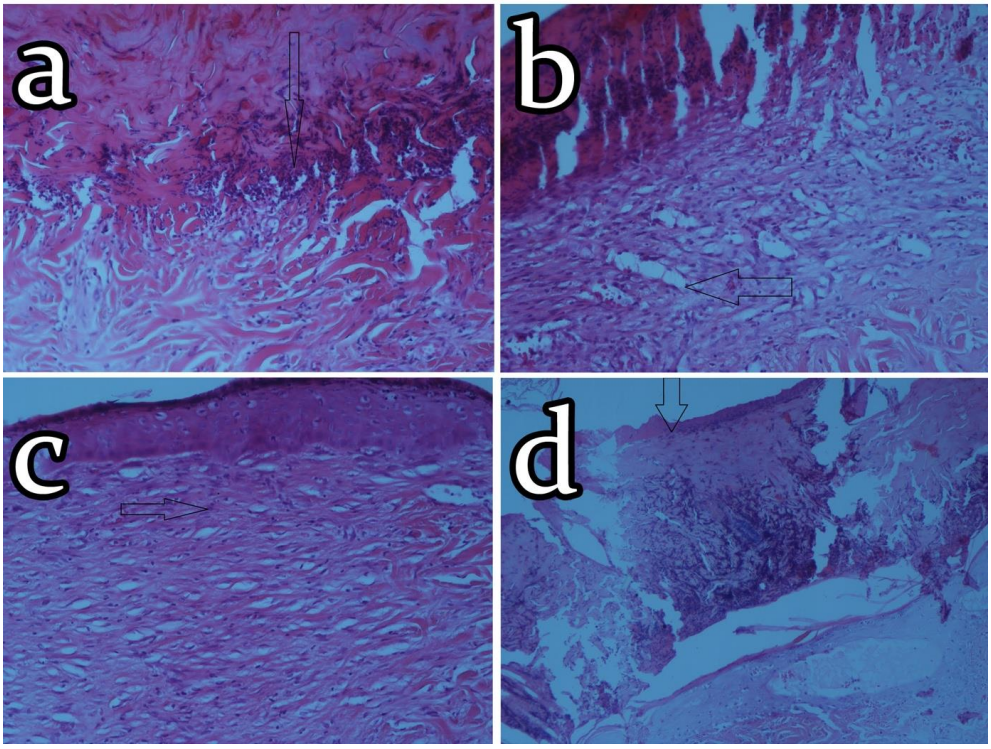

Figure 2. Burn injury increased inflammation, fibroblast level, neovascularization and re-epithelization. Oak root extract reduced the inflemasyon, increased fibroblast level, neovascularization and re-epithelization, reaching normal tissue (H&E, x 200). **2a:** Control group skin tissue; **2b:** Oak root extract group in skin tissue; **2c:** Group2 fibroblast; **2d:** Group2 epithelization.

Table 2 presents histopathological assessment including inflammation, edema, fibroblast level, neovascularization and re-epithelization on the days 3, 7 and 17 within groups. Regarding re-epithelization, significant differences were observed between days 3 and 7 as well as between days 3 and 17. Regarding neovascularization and inflammation, significant differences were observed between days 3 and 7 as well as between days 3 and 17 ($p<0.001$). In group 2, it was seen that there was a gradual increase in neovascularization, fibroblast infiltration and re-epithelization from day 3 to 17 ($p<0.001$). In addition, there was markedly decreased edema on the day 17, indicating statistical significance.

Table 2. Histopathological assessment including inflammation, edema, fibroblast level, neovascularization and re-epithelization on the days 3, 7 and 17 within groups.

Table 2a. Control group

Parameter	3rd day	7th day.	17th day.	*p*
Neovascularization	0.37 ± 0.51^{bc}	0.75 ± 0.46^{a}	1.37 ± 0.51^{a}	<0.001
Fibroblast	0.12 ± 0.35^{bc}	$1.00\pm0.00b^{ac}$	1.5 ± 0.53^{ab}	<0.001
Inflammation	1.12 ± 0.35^{b}	1.37 ± 0.51^{a}	1.37±0.51	N.S.
Edema	1.0 ± 0.53^{c}	0.75±0.46	0.5 ± 0.53^{a}	0.033
Re-epithelization	1.0 ± 0.0^{bc}	1.25 ± 0.46^{a}	1.25 ± 0.46^{a}	<0.011
Burn area [cm^2]	7.88 ± 0.21^{bc}	5.62 ± 0.83^{a}	4.82 ± 0.87^{a}	<0.001

Table 2b. Oak root extract group

Parameter	3rd day	7th day.	17th day.	*p*
Neovascularization	0.5± 0.53^{bc}	1.5±0.53^{ac}	2.75± 0.46^{ab}	<0.001
Fibroblast	0.0±0.0^{bc}	1.00±0.00^{ac}	2.50±0.53^{ab}	<0.001
Inflammation	1.25±0.46^{b}	2.12±0.64^{a}	1.75±0.70	0.006
Edema	1.37±0.74^{c}	1.75±0.46^{c}	0.37±0.51^{ab}	<0.001
Re-epithelization	0.62±0.51^{bc}	1.37±0.51^{ac}	2.37±0.51^{ab}	<0.001
Burn area [cm^2]	7.76±0.21^{bc}	5.11±0.75^{ac}	3.68±0.88^{ab}	<0.001

Values are expressed as mean ± std. deviation. **^{abcd}**: The groups in the same column with different letter are statistically significant [p<0.05]

DISCUSSION

Burn is a trauma which can be encountered by majority of population in varying degrees due to several reasons. Burns are traumas that may result in various degrees from functional loss to multi-organ failure and death (1, 2). Moreover, burn injury is also important as it is associated with physical and psychological sequels, temporary or permanent disability as well as most burn injuries are preventable accidents. In these preventable accidents, gathering epidemiological data and interpreting these data to identify risk factors will play an important role in developing strategies for and preventing such accidents. Risk factor can vary between countries and based on educational level, socioeconomic status, habits, physical conditions and environmental conditions (14-16).

Depth of burn is the most important issue in the treatment of burn injuries. Treatment becomes challenging as burn depth increases, leading sequels and complications (1). First degree burns mainly occurs by sunbeam. In general, there is injury in stratum corneum of epidermis. However, injury may extend to stratum lucidum and stratum granulosum or even stratum spinosum and stratum basale. Clinically, it appears as erythema over skin, which is also termed as erythematous burn. There is mild swelling and pain. As first degree burns involve cells in superficial layers, some injured cells without necrosis may recover their original functions. In general, erythema, swelling and pain relieve within few days. Necrotic cells of epidermis are observed but there is no scarring. Mild local pigmentation can be observed in some cases. First degree burns aren't included in the estimation of burn area. It is sufficient to humidify burn area and to provide analgesia in the treatment (1, 2).

All layers of skin are involved in third degree burns and muscle tissue or even bone can be affected by burn injury. Wound can appear as faint, brown or black based on severity of burn and presence or absence of vascular burn and exudation. In the treatment, the main principle is surgical debridement and reconstruction of defect by using a graft or flap (1,2).

In second degree burns, there is damage in whole epidermis and superficial layer of dermis. These burns are characterized by detachment of epidermis from underlying dermis and formation of bullae and vesicles due to accumulation of exudate beneath epidermis. In the second degree burns, burn injury involves more profound layers of dermis, but there are residual skin attachments. It may be readily infected due to substantial necrotic tissue. Bullae or vesicles may be present or not. If necrotic epidermis is peeled, a fainted area with red spots at varying degrees can be observed. These red spots become more prominent 12-24 hours after burn. The wound becomes increasingly dry on the day 1 and 2 while fine, dense thrombi can be seen occasionally (1, 2). In our study, they were shown on the sections obtained on the day 1 after second degree burn.

Topical agents are generally used in the management of second degree burn. These agents include silver sulphadiazin, mafenide acetate, Dakin solution (sodium hypochloride), silver nitrate, bacitracin, neomycine and polymxyin B (1, 2). On the other hand, traditional medicines are also used in the treatment of burn, particularly in first and second degree burns, including aloe vera, olive oil, centaury oil and some plants. Effectiveness has been proven in most of them (3, 4, 6).

It has been reported that honey, olive oil and egg white are most commonly used in wound healing and that yogurt, toothpaste, tomato sauce and sliced potato are used in the treatment of burns in particular (3, 4).

Since ancient times, humans have been used local plants and trees for several purposes through several ways. First, they used wild type of plants harvested from nature ; followed by domestication of most commonly used plants. Thus, studies investigating folk medicine used have been important so far. Folk medicines have been long tried on humans. Most folk medicine is waiting to be investigated in scientific manner (5).

In our country, some species of oaks have been used for constipation and as a mouthwash in throat diseases in Turkish folk medicine for their ethnobotany. A form of coffee that is prepared by graining acorns of Q. robur, Q. cerris ve Q. ithaburensis subsp. Macrolepis after peeling and roasting has been used for stomach discomfort and

constipation (19). Quercus flakes and tan has astringent and antiseptic effects. It has blood stopping effect. The product prepared from these can be ingested or used as mouthwash in the inflammation of oral mucosa or as a compress in wound care. In addition, it is present in the composition of ointments used in wound healing (18).

In previous studies, several tannins were identified in Kermes oak, including Caffeic acid, Gallocatechin, Fumaric acid, Gallic acid, Catechin, Protocatechuic acid ethyl ester Syringic acid, t-3-hydroxycinnamic acid, 4-hydroxybenzoic acid, Ellagic acid. It has been reported that caffeic acid has antioxidant, anti-carcinogenic, antiviral and immune regulatory effects; that Fumaric acid has antioxidant effects; Gallic acid has analgesic, anti-allergic, antibacterial, antioxidant, antiviral, antiseptic, immunostimulant, protective against cancer, anti-hepatotoxic effects; that Catechin has cholesterol lowering, blood glucose modulating effects; that Protocatechic acid ethyl ester has myoprotoactive, antioxidant, antimicrobial, neuro-enhancing, hypoxia tolerance, collagen arrangement, iron deficiency and cardioprotective effects; that 4-hydroxybenzoic acid has antibacterial, antifungal effects ; and Ellagic acid has antioxidant, anti-mutagenic and cancer protective effects (9-12).

In a study by Stephen C et al., it was shown that oak root extract (Quercus rubras) were effective against S. aureus and hemolytic streptococci and that it improved wound healing (6). Judaki ve ark.ları In the study by Judaki et al., it was shown that jaft's aqueous extract has the strong antimicrobial effect on staphylococcus aureus and pseudomonas aeruginosa (5) In our study, it was seen that wound healing occurred earlier in oak group compared to control group; in addition, no finding of infection was observed.

There are three phases of wound healing: inflammation, proliferation (epithelization, neovascularization, contraction, fibroplasia) and maturation phases. The inflammation phase begins immediately after injury and continues up to 5 days. In our study, significantly higher levels of inflammation on the day 7 in the group 2 indicated abundant macrophages at injury site (17). In addition, significantly higher edema on the day 7 in the group 2 compared to controls indicated ongoing active inflammation at this site.

Neovascularization is increased during proliferation phase. It begins on the day 5 and continues up to day 21. In fact, there are no definitive boundaries between phases in wound healing. The process begins with injury with overlapping phases (2). In our study, neovascularization was found to be significantly higher on the days 7 and 17 in the group 2 when compared to controls, indicating that oak root extract accelerated wound healing by increasing neovascularization. Given less neovascularization on the same days in the control group, it is apparent that oak root extract had increasing effect on neovascularization.

Increased fibroplasia is the final process in proliferation phases. In our study, extent of fibroblast increase was significantly higher on the day 17 in the group 2 compared to controls. This resulted in accelerated wound healing. On the same day, it was found to be lower in the control group, suggesting that oak root extract significantly increased fibroplasia.

When necrotic areas were compared, it was seen that necrosis was significantly lower on the day 17 in oak group compared to controls. This indicated that oak root extract improved wound healing and reduced necrotic areas.

When healing period was assessed, it was seen that complete recovery was achieved in significantly shorter time in oak group when compared to controls. This indicates that oak root extract accelerated wound healing with shorter recovery times.

In conclusion, oak root extract resulted in accelerated wound healing by affecting all phases of wound healing when applied to burn injury in the present study. These results show that oak root extract can be used in burn injury and other injuries. Oak root extract appears as a beneficial mixture. However, further studies are needed about oak root extract.

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