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Research Article

Wound healing activities of eucalyptol on full thickness excisional skin wound model in rats

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ABSTRACT

A plant-derived monoterpene, eucalyptol (1,8-cineole), has been suggested to have antioxidant, anti-inflammatory, and antimicrobial properties. The wound-healing effects of eucalyptol remain unknown. This study aimed to evaluate the topical use of eucalyptol at two concentrations on a full-thickness excisional skin wound model in terms of wound healing, gene expression, biochemical changes, and histopathological changes. Sixty Wistar rats were randomly divided into 5 groups (n = 12 per group). The control group (C) was untreated. Full-thickness excisional skin wounds were created in the following groups: the vehicle group (V), which was treated with polysorbate 80 solution (negative control). The Dexpanthenol group (D) was treated with dexpanthenol ointment (positive control). Eucalyptol 5% (E5) and Eucalyptol 10% (E10) groups were treated with 5% and 10% eucalyptol, respectively. Wound areas were measured on days 1, 4, 7, 11, 14, 18, and 21 days after wound creation. Wound tissues were collected on days 7 and 21. Histopathological, gene expression (TNFα, IL10, TGFβ1, VEGF), and biochemical (MDA, rGSH, GPx, CAT) analyses were performed on the wound tissues. TNFα gene expression levels were upregulated in Groups V and E5 ($P<0.05$). IL10 levels were upregulated in the V, E5, and E10 groups ($P<0.05$). TGFβ1 was upregulated in all groups compared with the control. Significant differences in rGSH, GPx, CAT, and MDA levels were found among all groups ($P<0.05$). Topical administration of 10% eucalyptol accelerates wound healing. With respect to epidermal thickness, 5% eucalyptol resulted in superior wound healing. Further studies should include different wound models, tissue analyses, and combinations of eucalyptol with other agents.

Keywords: Cineole, Cutaneous, Dermatology, Experimental, Topical

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Introduction

Wound healing is a complex physiological process involving diverse cell types and cellular phenomena, including reactions. When including three intricate stages—inflammation, tissue regeneration, and remodeling (maturation) of newly formed tissue— it may occur over varying timescales, from several days to months or years. Chronic wounds may lead to severe morbidity and mortality, especially in the elderly and particularly when concurrent diseases such as diabetes mellitus and vascular diseases are present. Surgery is also a potential cause of wound complications. Nonhealing wounds incur high treatment costs and significantly affect quality of life (51). Nearly 234 million surgical procedures are performed worldwide each year. To improve wound healing, promising therapeutics based on active components have been used (7). The treatment aims to support wound healing and prevent failure leading to nonhealing wounds (14, 44, 51).

Eucalyptol (1,8-cineole), a plant-derived monoterpene, is a natural constituent of a number of plant species, such as bay leaves, camphor laurel, rosemary, tea tree, mugwort, wormwood, sage, sweet basil, and their essential oils (17, 25, 28). Eucalyptol has been approved

by the United States Food and Drug Administration (FDA) for human use in cosmetics, aromatherapy, dentistry, and as a flavoring agent (8, 17, 28). Anti-inflammatory, antioxidant, and antimicrobial effects of eucalyptol have been reported in various diseases, including pancreatitis, colon damage, respiratory diseases, cardiovascular and neurodegenerative diseases (4, 5, 28, 49, 50). The wound-healing effects of essential oils containing eucalyptol have been partly investigated (8, 17, 29, 48), but the effects of eucalyptol alone on wound healing remain unknown. Consequently, a significant number of people depend on herbal medicine for effective wound healing. Numerous in vitro and in vivo parameters are employed to evaluate the activity of medicinal plants, using extracts, fractions, and isolated compounds (3, 33).

The aim of this study was to evaluate the wound-healing activity of eucalyptol (1,8-Cineole) in a full-thickness excisional skin wound model in Wistar rats, by assessing wound healing and contraction, gene expression, biochemical and histopathological changes.

Materials and Methods

Materials and Animals: Eucalyptol was purchased from Sigma-Aldrich (US). Sixty healthy adult male Wistar rats (six to eight weeks old, weighing 200–300 g) were purchased from Hatay Mustafa Kemal University Experimental Research and Application Center. The animals were placed in the laboratory to undergo routine health checks and were given a one-week adaptation period prior to the study. All rats were maintained individually in polycarbonate cages. The water and food were provided ad libitum. The light-dark cycle was 12:12 h; the temperature (23 ± 2 °C) and humidity (55%) were constant in a climate-controlled room.

Experimental Design: Following induction of general anesthesia (Ketamine HCl 60 mg/kg and Xylazine HCl 10 mg/kg, IP), routine surgical preparation was performed and a square, full-thickness excisional wound (41) measuring 2.25 cm^2 (1.5 × 1.5 cm) was created on the dorsal neck of each rat, except in the control group. The animals were randomly divided into five experimental groups (n=12 each). The control group was left untreated. The Vehicle group (Group V) was treated with a polysorbate 80 solution. Rats in the dexpanthenol group (Group D) were treated with dexpanthenol ointment (Bephantene Plus, Bayer, Istanbul, Turkiye) and served as a positive control. The Eucalyptol 5% group (Group E5) and the Eucalyptol 10% group (Group E10) were treated with 5% and 10% eucalyptol (in polysorbate 80 solution), respectively. All treatments were applied topically to cover the wound area (~1 mL) once daily. Subsequently, all groups were divided into two subgroups (n = 6 each: C-7, C-21, V-7, V-21, D-7, D-21, E5-7, E5-21, E10-7, E10-21) based on the sacrifice date, either day 7 or day 21 after wound creation. All subgroups were physically examined and weighed, and water and food intake were monitored weekly.

On days 7 and 21, the rats were deeply anesthetized (xylazine HCl 10 mg/kg and ketamine HCl 100 mg/kg, IP) and then decapitated. The wound tissue samples were dissected with a surgical blade and scissors. Half of each wound sample from each animal was preserved in 10% formalin for histopathological analysis, and the other half was preserved for biochemical and gene expression analysis.

Wound Area Measurement: Individual photographs of wound areas by a digital camera at 0, 1, 4, 7, 11, 14, 18 and 21 days after wound creation. ImageJ software was used to measure the wound surface areas (cm^2).

RNA isolation and cDNA synthesis: Total RNA was isolated from each scar tissue sample using the Trizol method (43). For this purpose, 50 mg of tissue from each sample was homogenized in 1 ml of Trizol Reagent (Sigma Aldrich, USA), and then the kit protocol was performed. RNA pellets obtained from the samples were diluted with 30–100 µL of nuclease-free water. The purity, concentration, and quality parameters of the samples were evaluated using a nucleic acid meter (Merinton, SMA-1000 UV Spectrophotometer, China) and 1% agarose gel electrophoresis (100 V for 25 min).

To eliminate possible genomic DNA contamination, DNA digestion was performed according to the kit protocol (DNase I, RNase-free, Thermo Scientific, USA). After this process, RNA samples were converted to cDNA in accordance with the kit protocol (High-Capacity cDNA Reverse Transcription Kit, Thermo Fisher Scientific, USA). The reaction protocol on the thermal cycler was as follows: samples were incubated at 25 °C for 10 minutes, then at 37 °C for 120 minutes, and finally at 85 °C for 5 minutes. After the reaction, sample volumes were brought up to 200 µL with nuclease-free water.

RT-qPCR Application: A kit containing SYBR Green dye (Power SYBR® Green PCR Master, ThermoFisher Scientific, USA) was used for RT-qPCR analysis. Expression levels of target genes (IL10, TNFα, TGFβ1, and VEGF) were determined and normalized to the ACTB housekeeping gene. Amplification of the genes was performed using 10 µL of cDNA from each sample. The reaction protocol consisted of an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 60 seconds (Qiagen Rotor Gene Q, USA). Each sample was run in duplicate. Primers for the genes used for amplification are presented in Table 1.

Table 1. Forward and Reverse Sequences of the primers

Gene	Forward and Reverse Primer Sequences	Reference
ACTB	F: 5'-GCAGGAGTACGATGAGTCCG-3' R: 5'- ACGCAGCTCAGTAACAGTCC-3'	(40)
TNFα	F: 5'-ACTGAACTTCGGGGTGATCG-3' R: 5'-GCTTGGTGGTTTGCTACGAC-3'	Designed by the authors
IL10	F: 5'-TTGAACCACCCGGCATCTAC-3' R: 5'-CCAAGGAGTTGCTCCCGTTA-3'	Designed by the authors
VEGF	F: 5'-TCTCCCAGATCGGTGACAGT-3' R: 5'-GGCAGAGCTGAGTGTTAGCA-3'	(35)
TGFβ	F: 5'-TGACGTCACTGGAGTTGTCC-3' R: 5'-CCTCGACGTTTGGGACTGAT-3'	Designed by the authors

Biochemical Analysis: Skin tissues were homogenized in phosphate buffer (pH 7.4) at a 1:10 (w/v) ratio in three 10-second repetitions at 15,000 rpm. Homogenates were centrifuged at 4°C and 5,000 rpm for 30 min. The Obtained supernatants were stored at -80°C for biochemical analysis (28). Tissue analyses of total protein (37), MDA (38), GSH (16), GPx (8), and CAT (2) were performed using previously described methods.

Histopatological Analysis: Skin wound specimens of all the rats were fixed in buffered formaldehyde (10%), dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Longitudinal sections 5–6 µm thick were obtained using a sliding microtome (Leica Microsystems, Germany) and stained with hematoxylin and eosin (H&E).

The H&E-stained sections were examined under light microscopy for epithelialization (complete or incomplete epithelial proliferation in the epidermal layer), degeneration (vacuolation or vacuolar degeneration of epidermal or dermal cells and areas), granulation (presence of necrotic and inflammatory cells), and leucocytic infiltration (scored by a method based on the distribution of polymorphonuclear and mononuclear cells). The captured images were evaluated blindly by two histologists according to a modified method (1, 6). For each parameter, five microscopic fields from all slides per group were scored at 100× magnification on a 0–3 scale, with 0 indicating absence, 1 minimal, 2 moderate, and 3 extensive.

Statistical Analysis: The sample size was determined based on an a priori power analysis using G*Power 3.1 software. Assuming an effect size of 0.8, a significance level (α) of 0.05, and a statistical power (1–β) of 0.80, the minimum required sample size per (sub)group was calculated to be 6 animals. The housekeeping gene was used to normalize target gene expression. Expression results were calculated using 2^(-ΔΔCt) method (33). Results from the experimental groups were compared with those of the control group and presented as fold changes. Statistical analysis of the biochemical and histopathology results was performed using IBM SPSS version 22 (USA). The Shapiro-Wilk test was used to assess the normality of the groups. Analysis of variance (ANOVA) with the post hoc Duncan test was used for group comparisons. Repeated measures analysis of variance followed by the Bonferroni test was applied to compare wound-area changes over time between groups. Significance set as $P<0.05$ in the wound area, histopathology, and genetic expression results. Significance levels for biochemical results were $P<0.01$ and $P<0.001$. All data are presented as mean ± standard error of the mean (SEM).

Results

Clinical Follow Up and Wound Area Results: Feed consumption and body weight were not significantly different between groups and subgroups on weekly assessments ($P>0.05$). Water consumption was significantly lower in the E5 group than in the other groups ($P<0.05$). Significant differences in wound area were observed in time-based, within-group comparisons for all groups ($P<0.001$). Wound areas differed significantly between groups: D - E5 ($P=0.037$) on day 4; D - V ($P=0.019$) and D - E5 ($P=0.02$) on day 7; D - V ($P=0.02$) on day 10; and D - E5 ($P = 0.017$), V - E5 ($P=0.012$), and E5 - E10 ($P=0.041$) on day 21. The group-to-group comparison over time was significant ($P=0.008$). Results of time-group comparisons of wound area are presented in Figure 1. The appearance of the wound area in different groups on different days is presented in Figure 2.

Gene Expression (mRNA) Results: The isolated RNA samples were appropriate for cDNA conversion and gene expression analysis with respect to purity (A260/A280 = 1.86 ± 0.01) and concentration (609.32 ± 33.18 ng/µl). Significant differences were detected in the expression levels of target genes on both day 7 and day 21. Compared to control, TNFα gene expression level was upregulated by more than 5-fold in the V group, while it was upregulated by approximately 3-fold in the E5 group

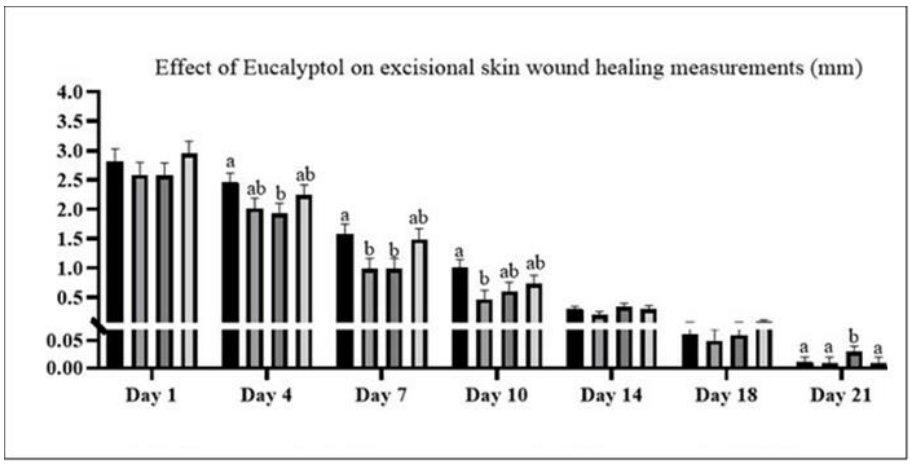

Figure 1. Effect of Eucalyptol on excisional skin wound healing measurements (mm). Wound areas of the rats was measured (mm) on days 1, 4, 7, 10, 14, 18, and 21. Data are presented as mean ± standard error of the mean (SEM). Different letters (a, b, ab) indicate significant differences between groups at the same days (P<0.05).

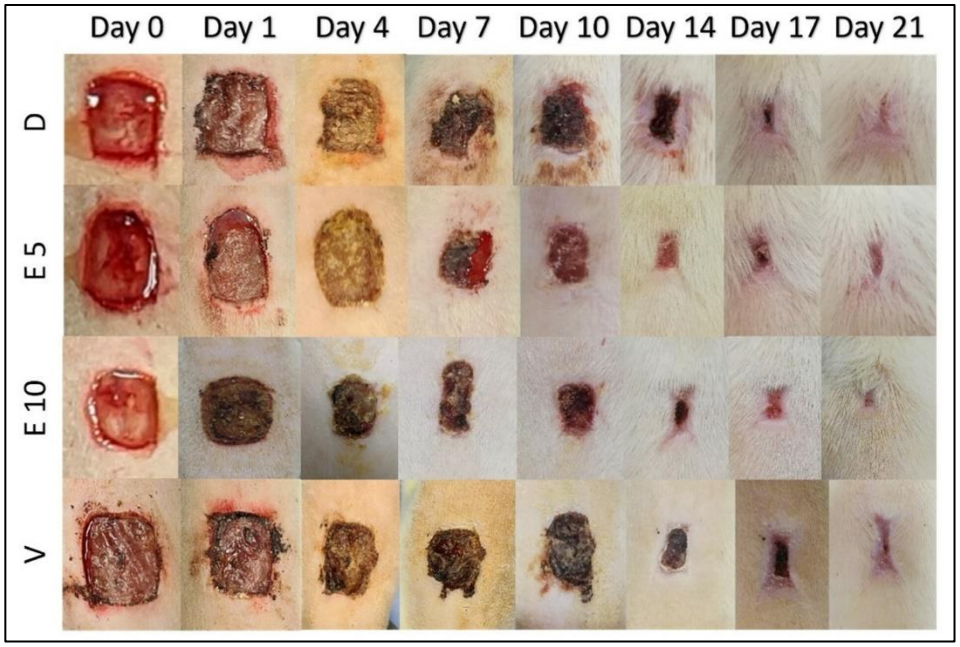

Figure 2. Typical macroscopic results of topical treatments of the groups (D: Dexpanthenol, E5: Eucalyptol 5%, E10: Eucalyptol 10%, V: Vehicle) on the days 0, 1, 4, 7, 10, 14, 17 and 21. Dexpanthenol (D), %5 Eucalyptol and %10 Eucalyptol (E10), resulted in a better closing of the wound area macroscopically.

(P<0.05). On the other hand, IL10 gene expression levels were upregulated more than 10-fold in the V, E5, and E10 groups compared with the control (P<0.01, P<0.001, and P<0.05, respectively). TGFβ1 was also upregulated more than 5-fold in all groups compared with the control group (Figure 3).

TNFα gene expression levels in the E10 group were upregulated approximately threefold compared with the control group (P<0.05). IL10 gene expression levels were upregulated by more than fivefold in all groups except group D. Moreover, TGFβ1 gene expression levels were approximately 5-fold upregulated in the V group compared to control group (P<0.05), and approximately 2- and 5- fold upregulated in the D and E10 groups, respectively (P<0.05) (Figure 4).

Biochemical Results: MDA levels in wound tissues were significantly lower in E5-7 and C-7 than in all other 7-day groups (P<0.001). Significantly higher rGSH and GPx levels were observed in the C-7 group than in the other groups (P<0.001). Significantly lower rGSH levels were observed in the V-7 group (P<0.001). CAT levels were significantly lower in the V-7 and E5-7 groups (P<0.001) and slightly lower in the C-7, D-7, and E10-7 groups.

Wound tissues on day 21 showed significantly higher MDA levels in the V-21 group. Significant differences were found among groups, with levels of rGSH, GPx, and CAT highest in the C-21 group and lowest in the V-21 group. The results of the biochemical analysis of the 7th- and 21st-day groups are presented in Tables 2 and 3, and a summary is given in Figure 5.

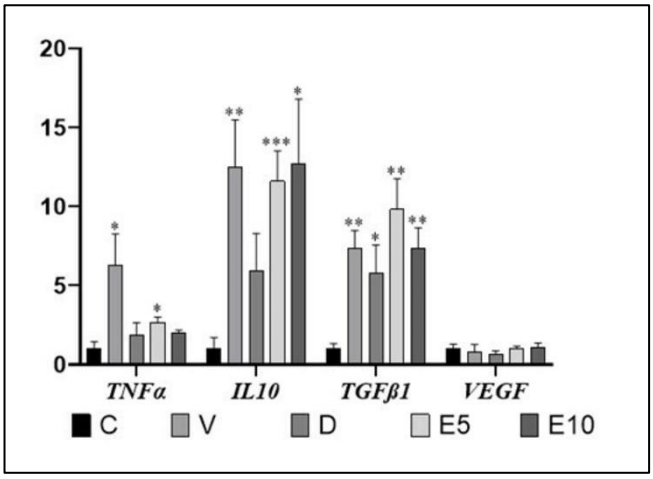

Figure 3. Relative gene expression levels on day 7th of wound healing. Data are presented as mean ± standard error of the mean (SEM). Asterisks: *:P<0.05, **:P<0.01, ***:P <0.001.

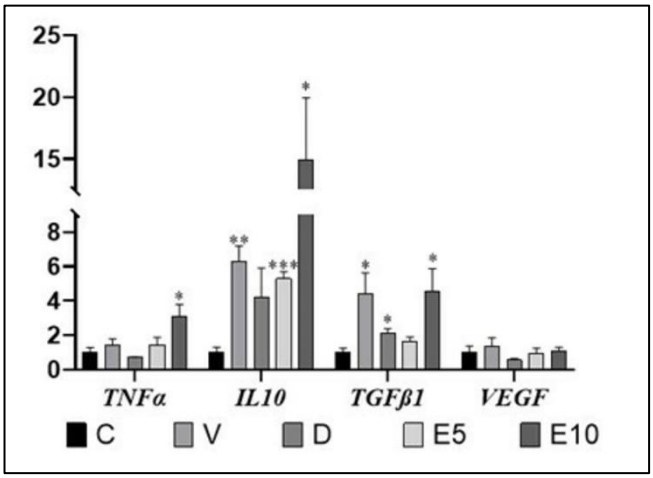

Figure 4. Relative gene expression levels on day 21st of wound healing. Data are presented as mean ± standard error of the mean (SEM). Asterisks: *:P<0.05, **:P<0.01, ***:P<0.001.

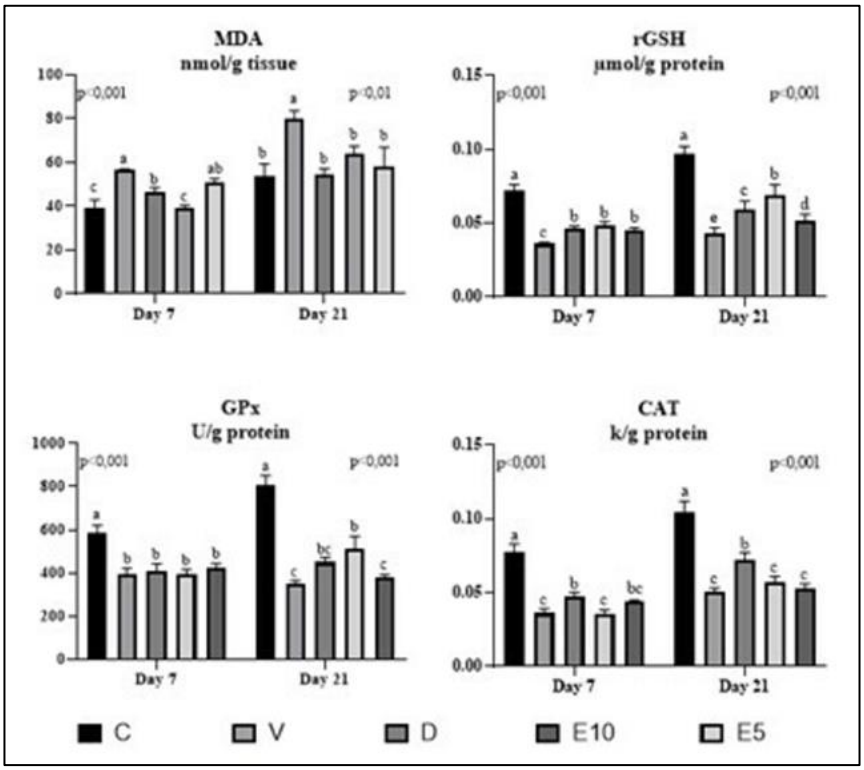

Figure 5. Relative biochemical results on 7th and 21st days of wound healing. Data are presented as mean ± standard error of the mean (SEM). Different letters (a, b, c, ab, bc) indicate significant differences between groups at the same days (P<0.05 and P<0.001).

Table 2. Results of biochemical analyses on day 7 of wound healing.

Groups	MDA nmol/g tissue	rGSH µmol/g protein	GPx U/g protein	CAT k/g protein
C	39.44±3.49^{c}	0.072±0.004^{a}	587.17±34.01^{a}	0.078±0.005^{a}
V	56.37±0.48^{a}	0.036±0.001^{c}	396.58±26.75^{b}	0.036±0.003^{c}
D	46.69±1.79^{b}	0.046±0.002^{b}	409.43±32.24^{b}	0.047±0.003^{b}
E5	39.44±1.28^{c}	0.048±0.003^{b}	394.94±21.86^{b}	0.035±0.003^{c}
E10	50.73±1.79^{ab}	0.045±0.002^{b}	425.10±20.09^{b}	0.044±0.001^{bc}
P	***	***	***	***

$^{a-c}$ Differences in the same column are statistically significant. Data are presented as mean ± standard error of the mean. ***; P<0.001.

Table 3. Results of biochemical analyses on day 21 of wound healing.

Gruplar	MDA nmol/g tissue	rGSH µmol/g protein	GPx U/g protein	CAT k/g protein
C	53.95±5.34^{b}	0,097±0.005^{a}	803.70±48.74^{a}	0.105±0.007^{a}
V	80.16±3.56^{a}	0.043±0.004^{e}	352.05±13.19^{c}	0.051±0.002^{c}
D	54.35±2.73^{b}	0.059±0.006^{c}	449.50±22.57^{bc}	0.072±0.005^{b}
E5	63.63±3.89^{b}	0.069±0.007^{b}	515.71±53.70^{b}	0.057±0.004^{c}
E10	57.98±8.91^{b}	0.052±0.004^{d}	377.83±16.32^{c}	0.053±0.003^{c}
P	**	***	***	***

$^{a-c}$ Differences in the same column are statistically significant. Data are presented as mean ± standard error of the mean. **: P<0.01; ***: P<0.001.

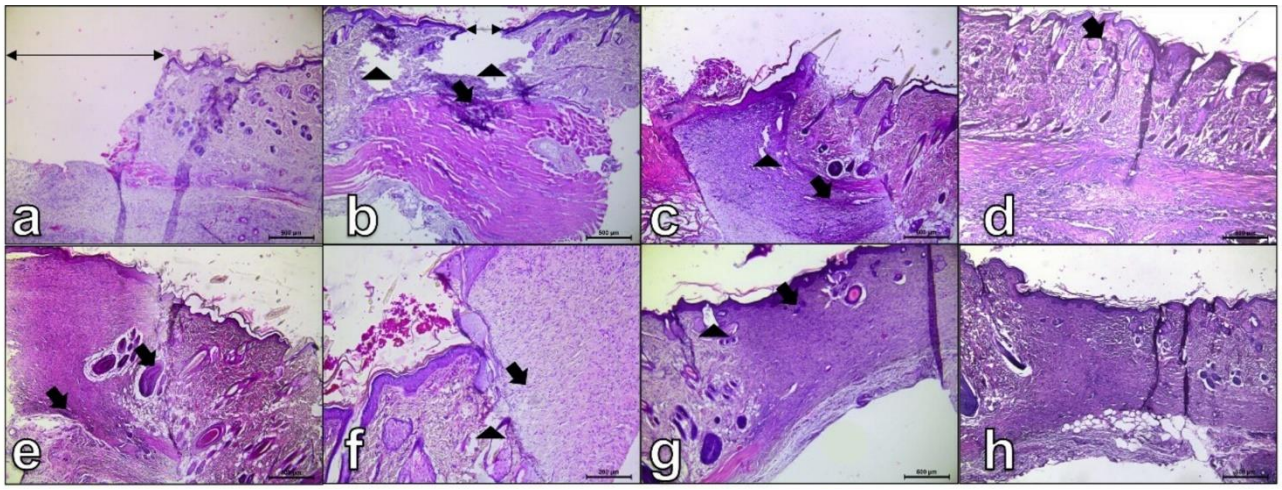

Figure 6. Histopathological microscopic images (40x) of wound tissue sections from the groups of days 7th and 21th (a: Group V day 7, b: Group E5 day 7, c: Group E10 day 7, d: Group D day 7, e: Group V day 21, f: Group E5 day 21, g: Group E10 day 21, h: Group D day 21). Double-pointed arrow: incomplete epithelialization area, black triangle: area of degeneration, single-ended arrow: area of granulation.

Histopathological Results: Areas of incomplete epithelialization were observed more frequently in the V-7 group than in the E5-7 group. epithelialization was completed in the E10-7 and D-7 groups. Epithelialization was complete in all 21-day groups. The increase in epidermal thickness was highest in the E5-7 group and lowest in the E5-21 group. Areas of vacuolization-like degeneration in the dermis were less extensive in the treatment groups than in the V group, except in the E5-7 group. Granulation areas were found to be similar among groups during the same time period. In the treatment groups, the white blood cell density was similar in the 7-day groups and lower in the 21-day groups than in the V-21 group. In the treatment groups, leucocyte density was similar among the 7-day groups and lower in the 21-day groups than in the V-21 group. Cross-sections from the wound areas are presented in Figure 6, and a summary of the histopathological results is presented in Figure 7.

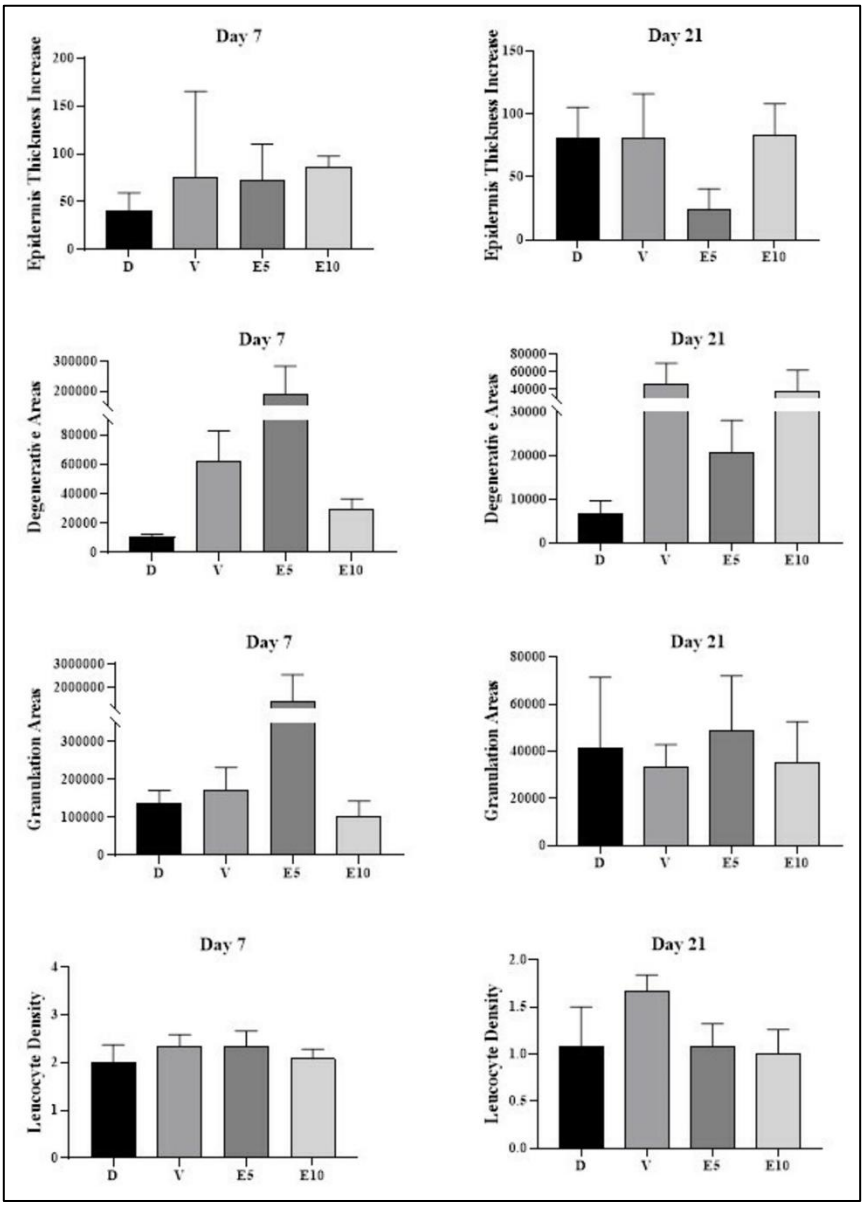

Figure 7. Histopathological examination results of 7th and 21st days according to groups (Epidermis thickness increase: percentage - %, Degenerative and granulation areas: square micrometers - µm2, Leucocyte Density: Leucocyte count/5 µm2)

Discussion and Conclusion

Wound repair occurs as a response to injury. Ideal successful wound closure achieved in the shortest possible time without adverse effects (10, 53). Wound healing processes are continuous, active, and highly complex, and they may be affected by many factors (24, 53). Any undesired effect on the natural sequence of healing may lead to disruption of subsequent stages, resulting in delayed or abnormal healing, formation of scar tissue, or chronic wounds (48). Topical use of anti-inflammatory and free radical-scavenging products protects tissues from oxidative damage and supports wound healing (23, 32, 52). Many studies have attempted to develop an optimal treatment, particularly for open wounds (16, 34, 48). The purpose of our study was to evaluate the topical use of eucalyptol (1,8-Cineole) at two concentrations in an excisional, full-thickness skin wound model. Our results provided key data on wound area measurements and on histopathological, biochemical, and gene expression changes to inform further studies using different wound models.

Food and water consumption are indicators of welfare and stress in experimental animals (7). There was no difference in feed consumption, but the decrease in water consumption in the E5 group may be related to reduced animal movement or a negative effect on overall clinical condition. However, since there was no significant difference in water consumption between group V and the other groups, water consumption was not considered a significant indicator. Wound area measurements are the

most important clinical measure for evaluating wound healing in studies. In wound-model studies, a more rapid decrease in wound area is expected within- or between-group comparisons (16, 48). Based on differences in wound area measurements in our study, within-group comparisons demonstrate consistent wound healing and correct application of working standards. Time-group comparisons of wound areas show that eucalyptol produces more favorable results at 10% concentration but is insufficient at 5% concentration when applied topically.

In terms of gene expression, an increase in TNF-α levels may indicate a prolonged inflammatory phase of wound-healing process. An increase in IL-10 may enhance macrophage activity in the wound area (21, 30, 45). TGF-β1 is mostly related to fibroblast activation initiation of the proliferative phase, angiogenesis, granulation tissue formation, and re-epithelialization (30, 31, 36, 39, 46). The results for TNFα, IL10, and TGFβ1 gene expression levels in the 7-day treatment groups in our study support the conclusion that the D group had the greatest effect. Since group D is the positive control group, it cannot be concluded that 7-day treatments have a significant effect on gene expression. The results revealed that topical administration of Eucalyptol, especially at a 5% concentration, could increase the expression of TNFα, IL-10, and TGFβ1. These results may indicate that eucalyptol treatment at a 5% concentration predisposes to proinflammatory cytokine production, to initiation of the proliferative phase with fibroblast activation, and to anti-inflammatory and immunosuppressive events during the late phase of wound healing. Growth factors influence the last phase of wound healing. Vascular endothelial growth factor (VEGF) stimulates cell migration, proliferation, and extracellular matrix protein synthesis (30, 47). VEGF gene expression levels in the present study may indicate similar cell migration, proliferation, and extracellular matrix protein synthesis because levels were similar in the 7-day and 21-day treatment groups. Thus, topical use of Eucalyptol may be considered to have had no effect on the final phase of wound healing in terms of gene expression.

The course of wound healing is largely associated with the antioxidant activity of the therapeutic agent. Maintaining balanced oxidant production is desirable for optimal wound healing. Antioxidants accelerate wound healing by removing reactive oxygen species and increasing collagen synthesis (22). MDA level is known to be one of the markers directly proportional to oxidative stress (12, 19). In our study, the significantly lower MDA levels in wound tissues at seven days in the group treated with Eucalyptol 5% may indicate an antioxidant effect during the early phase of wound healing. Wound healing processes impair the oxidant–antioxidant balance and induce overproduction of oxidants. Antioxidants such as rGSH, GPx, and CAT (20, 27, 28) would mediate a response in healing tissue that protects oxidative balance. The role of GSH in the early phases of wound healing and a lack of GSH in delayed wound healing have been reported (42). The fact that the rGSH level of the control group was significantly higher than that of the other groups in 7th-day wound tissues in our study, but significantly lower than that in the V group, shows the effect of Eucalyptol on early wound healing when applied topically at both 5% and 10% concentrations. GPx activity changes were higher in the group administered Eucalyptol at 10%, but the difference was not statistically significant and therefore not meaningful. CAT is a common antioxidant enzyme that utilizes oxygen, and a significant decrease in CAT was expected in the early phase of wound healing (42). Similarly, our study revealed a decrease in CAT activity following wound creation. These findings suggest that eucalyptol does not have a significant antioxidant effect via CAT activity but may have a significant antioxidant effect via rGSH and GPx. Notably, CAT activity was higher in the dexpanthenol groups. Significantly lower levels of MDA in wound tissues on day 21 may indicate that Eucalyptol is effective as an antioxidant at concentrations of 5% and 10% during the late stage of wound healing. Significant increases in rGSH and GPx levels also suggest that eucalyptol at concentrations of 5% and 10% is relatively more effective as an antioxidant during the early phase of wound healing. Higher antioxidant levels and lower MDA in the E5-21 treatment group indicate that it is more effective at a 5% concentration.

Histopathologically, completion of epithelialization is a known indicator of wound healing (26). Increases in epidermal thickness, leucocyte density, areas of degeneration and granulation are used histopathologically to determine that wound healing is beyond ideal (53). That epithelialization was not completed in groups V-7 and E5-7 in our study, but was completed in groups D and E10-7, indicates that eucalyptol modulates epithelialization in wound tissue in a dose-dependent manner, particularly in the short term. Granulation areas, increased epidermal thickness, vacuolization-like degenerative changes in the dermis, and leukocyte density, which occur as a result of an inflammatory response secondary to trauma, have insufficient beneficial effects on wound healing in the E5 group, especially on day 7, thereby supporting better wound healing in the E10 group. We consider that the 5% concentration of eucalyptol may have provided optimal antioxidant balance and supported epithelial regeneration, while the higher concentration (10%) may have induced a stronger contractile response through enhanced fibroblast activity and collagen deposition.

In summary, this study demonstrated the potential effects of topical eucalyptol administered at two doses, based on clinical, histopathological, biochemical, and gene expression assessments of excisional full-thickness skin wounds in rats. According to our results, topical

administration of eucalyptol at a 10% concentration accelerates wound healing. Wound healing is found to be slower when eucalyptol is applied topically at a 5% concentration. Considering the increase in epidermal thickness, a 5% concentration of eucalyptol resulted in improved wound healing. Biochemical and gene expression changes induced by topical eucalyptol during skin wound healing were assessed. Further studies shall include different wound models, tissue analyses or combining eucalyptol with other agents.

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Ethical Statement

The ethical approval of the study was obtained from the Local Ethics Board of Animal Experiments of Hatay Mustafa Kemal University (Decision no: 2020/04-33).

Conflict of Interest

The authors declared that they have no conflicts of interest.

Author Contributions

MZYD: Design, Experiment, Scientific Writing. ÖK: Experiment, Data analysis. HÖ: Laboratory work, Scientific Writing. CTİ: Laboratory work, Scientific Writing, Review. FK: Laboratory work, Scientific Writing. AY: Laboratory work, Scientific Writing. ZY: Experiment, Data analysis.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that the experimental work was conducted in accordance with the ARRIVE guidelines for the protection of animals used for scientific purposes and with European Community guidelines (EEC Directive of 1986; 86/609/EEC) for the care and use of laboratory animals.

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