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Research Article

EXPERIMENTAL STUDY ON THE HEALING EFFECT OF PINE RESIN ON SKIN WOUNDS IN RATS

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ABSTRACT. The skin is the largest organ in the body and is constantly exposed to trauma. Many agents and methods have been tried from past to present to accelerate healing in skin injuries. Today, the use of herbal origin agents is gaining popularity. Resins are known to have anti-inflammatory, antibacterial, antifungal and antioxidant properties. In our study, we examined the effect of resins obtained from pine trees on wound healing. In the study, 3 groups were formed as pine resin group, control group and antibiotic group, with 7 animals in each group (21 animals in total). All animals were photographed once a day for 15 days in terms of wound dimensions and wound area measurements were performed digitally with ImageJ program. At the end of the 15th day, the animals were euthanized and histopathological examination was performed. As a result of the study, local pine resin application showed a greater improvement in terms of wound area in the first three days compared to the antibiotic and control group, while the antibiotic and pine resin group showed similar improvement on days 3-15 and showed more improvement compared to the control group. When examined histopathologically, pine resin and antibiotic groups showed similar effects and increased epithelialization, inflammatory cell infiltration and granulation cell formation.

Keywords: *Epithelialization, experimental, healing, herbal.*

INTRODUCTION

The skin is the largest organ in the body and is constantly exposed to trauma. When skin injuries occur, it is very important to ensure a smooth and rapid healing [1]. A complex series of cellular, physiological, and molecular processes aimed at preserving the structural and functional integrity of the injured tissue comprise the basic reaction known as wound healing. Hemostasis, inflammation, proliferation, and remodeling are the four phases of wound healing. Numerous plant-based products have demonstrated therapeutic potential as agents that promote wound healing [2,3].

Pine resin, also known as rosin, pine gum or pitch, is a sticky substance that oozes from the bark of pine trees. It is the natural resin secreted by Pinaceae family members including *Pinus longifolia* Roxb., *Pinus sylvestris* L., and *Pinus palustris* Mill. It is secreted from injured areas on the trunks of pine trees or during the ripening of cones, providing protection and healing of the tree. Initially secreted as a liquid, the resin solidifies white and yellow upon contact with air and dries on the surface [4]. Pine resin has been used for various purposes throughout history [5].

Studies have shown that pine resin has antimicrobial and anti-inflammatory properties, which make it effective in promoting wound healing. Pine resin contains compounds such as terpenes, flavonoids, and phenols, which have been found to have antibacterial, antifungal, and antioxidant properties. These compounds help to reduce inflammation and pain, promote tissue regeneration, and protect against infection [6].

It's important to note that while pine resin has a history of traditional use for various purposes, scientific research on its specific effects and medical applications is still limited. The aim of this study is to determine the effectiveness of pine resin on experimentally created skin wounds in rats.

MATERIALS AND METHODS

Animals

In all, 21 male Wistar albino rats, two months of age, weighing between 230 and 250 g at birth, were employed in the study, seven rats per group. The rats had unlimited access to food and water and were kept in a 12-hour light/dark cycle at a temperature of 22±2°C. The Muğla Sıtkı Koçman University Animal Experiments Local Ethics Committee approved the study, and it has permit number 24/08/2023-23/23.

The Preparation of Pine Resin

The study's pine resins were gathered from red pine (*Pinus brutia* Ten.) forests located in the Muğla region. The collected resin was dried in the shade, frozen to solidify it, and then ground into a uniform powder using a grain grinder. Red pine resin generally contains terpenoids in its composition, and factors such as elevation do not significantly affect the content [7]. It is well known that benzene and alcohol provide the highest extraction efficiency for terpenoid compounds. Alcohol extraction, which is applied to the skin, was used in this investigation. After weighing the powdered resin at 100 g, 500 ml of an ethyl alcohol-water solvent (consisting of 70% ethanol and 30% ultrapure water) was added. For three days, the mixture was mixed with a vortex mixer for five minutes each day. After that, it was filtered through Whatman No. 1 filter paper, moved into falcon tubes, and kept at +4°C until the experimental phase [8].

Wound Model and Measurements

An intramuscular injection of 10 mg/kg Xylazine hydrochloride (Rompun, Bayer, 23.32 mg/mL, Germany) and 70 mg/kg Ketamin hydrochloride (Ketalar, Parke-Davis, 50 mg/mL, USA) was used to anesthetize the rats to create a skin wound model. The dorsal area's interscapular region was shaved in a 3-cm line. After the shaving area was cleaned with povidone-iodine, a full-thickness skin wound model was created using a 5mm punch trephine. Following the procedure, the wound site was measured in millimeters for both length and width, photographed (iPhone 12 mini 26mm F 1.6, Apple, USA), and recorded until the day of sacrifice. To ensure uniformity in the photographs, the photographs were taken with a ruler next to the wound, this ruler was taken as a standard for measurements. The photos were uploaded to the computer-based Image J (Wayne Rasband National Institutes of Health, USA) program to calculate the wound surface areas.

Experimental Design

There were 21 Wistar albino rats utilized in total, divided into three groups of seven animals each. These are the groups and applications that have been created: Group control: from the time the wound formed, the rats in this group did not receive any medication or treatment. Pine resin group: 0.1 ml of pine resin was applied to the surface of the wound once a day until the day of sacrifice. Antibiotic group: received 0.1ml a nitrofurazone antibiotic pomade (Furacin, Sanofi-Aventis, %0.2, France) was applied to the surface of the wound once daily until the day of the sacrifice. On the 15th day of the study, the rats were first anaesthetised and then euthanised with high dose anaesthetic (Ketalar, Parke-Davis, 50 mg/mL, USA).

Histopathologic Analyses

For histopathological analysis, the panniculus layer and the surrounding full-thickness skin were also taken from the body. The skin samples were preserved in a 10% formaldehyde solution for histological examination. After fixation, the tissues were processed through an alcohol and xylene series before being embedded in paraffin blocks. Sections 3 mm thick, transferred from the paraffin blocks onto slides, were stained with hematoxylin-eosin. After a microscopic analysis was done on these sections. The tissues were evaluated under microscope for epithelialization, inflammatory cell infiltration and granulation cell formation. The findings were scored as 0; none, 1; mild, 2; moderate, 3; severe. The mean values of the groups were then evaluated nonparametrically [9].

Statistical Analysis

The statistical package SPSS 26 (Inc., Chicago, USA) was used to evaluate the data statistically. Using analytical techniques, the variables' conformance to a normal distribution was assessed (Kolmogorov-Smirnov and Shapiro-Wilk tests). The wound area was compared between groups using a one-way ANOVA test. When variables between groups differed significantly, pairwise post-hoc comparisons were performed using the Tukey test.

For histopathologic examination, the Kruskal-Wallis-Walli's assay was employed. Bonferroni correction was used to assess the results of paired comparisons of variables, which were carried out using the Mann-Whitney U test. Results were deemed statistically significant if the P-value was less than 0.05.

RESULTS AND DISCUSSION

Wound Size and Healing

Fig. 1. *Shows a photo of daily wound areas.*

The experimentally induced wounds were checked and photographed at the same time every day for 15 days. In the controls, it was observed that the wounds were regularly shrinking and healing was macroscopically realized on the day 11 except for the control group. In macroscopic examination, scab formation was observed in all three groups, but the least scab formation was observed in the pine resin group (figure 1).

Table 1. *Wound sizes (in square millimetres).*

Days		**Control**		**Pine Resin**		**Antibiotic**		
	n	**Mean**	**S. E**	**Mean**	**S. E**	**Mean**	**S. E**	**P-value**
Day 0	7	20.01	0.23	19.97	0.23	19.79	0.18	0.733
Day 1	7	18.37[a]	0.33	16.78[b]	0.36	18.45[a]	0.16	0.001
Day 2	7	15.88[a]	0.26	13.10[b]	0.51	16.34[a]	0.76	0.001
Day 3	7	13.70[a]	0.38	10.51[b]	0.42	12.69[a]	0.84	0.003
Day 4	7	11.90[a]	0.60	8.73[b]	0.61	10.28[ab]	0.49	0.003
Day 5	7	9.94[a]	0.41	7.07[b]	0.42	8.47[ab]	0.51	0.001
Day 6	7	8.26[a]	0.57	5.29[b]	0.35	6.73[ab]	0.57	0.002
Day 7	7	7.47[a]	0.59	3.82[b]	0.45	5.23[b]	0.57	<0.0001
Day 8	7	6.36[a]	0.70	1.91[b]	0.50	3.30[b]	0.57	<0.0001
Day 9	7	5.38[a]	0.70	0.60[b]	0.31	1.55[b]	0.45	<0.0001
Day 10	7	4.67[a]	0.67	0.13[b]	0.13	0.43[b]	0.30	<0.0001
Day 11	7	3.00	0.70	0.00	0.00	0.00	0.00	-
Day 12	7	1.93	0.67	0.00	0.00	0.00	0.00	-
Day 13	7	1.28	0.47	0.00	0.00	0.00	0.00	-
Day 14	7	0.31	0.20	0.00	0.00	0.00	0.00	-
Day 15	7	0.00	0.00	0.00	0.00	0.00	0.00	-

[a-b]: Different letters in the same line are statistically significant (P<0.05).

The images obtained for 15 days were digitized, and the wound area was calculated in square millimeters. According to the statistical data, there was a significant difference between the control and pine resin groups in terms of wound size until the day 11 (P<0.01). This difference was more significant between days 7–10 (P<0.001). While the antibiotic group was significantly different from the pine resin group in terms of wound dimensions in the first 3 days (P<0.01), it was different from the control group on days 7–10 (P<0.001). On days 4–6, the antibiotic group did not show a significant difference in terms of wound size compared to the other groups. On days 11–15, no statistical data could be obtained since healing occurred in the pine resin group and the antibiotic group (Table 1).

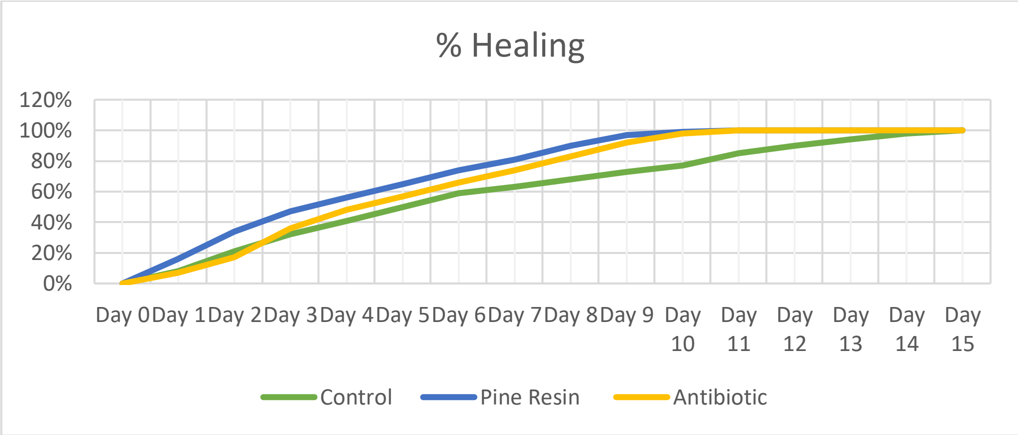

Fig. 2. *Daily healing percentages of wounds.*

The decrease in wound size recorded throughout the study was calculated as the average daily percentage of wound healing in the groups. The following formula was used to calculate the percentage wound (%) size [10]:

$$\textit{Wound healing (\%)} = (\textit{wound area}_{0d} - \textit{wound area}_{1\text{-}15d}) / \textit{wound area}_{0d} \times 100$$

When the amount of healing (%) was analysed, it was observed that the pine resin group gave the highest percentage of healing until the day 11. Although the antibiotic group was behind the control group in terms of the amount of healing (%) on the day 1 and day 2, it showed more healing than the control group in the following days (figure 2).

Histopathological Examination

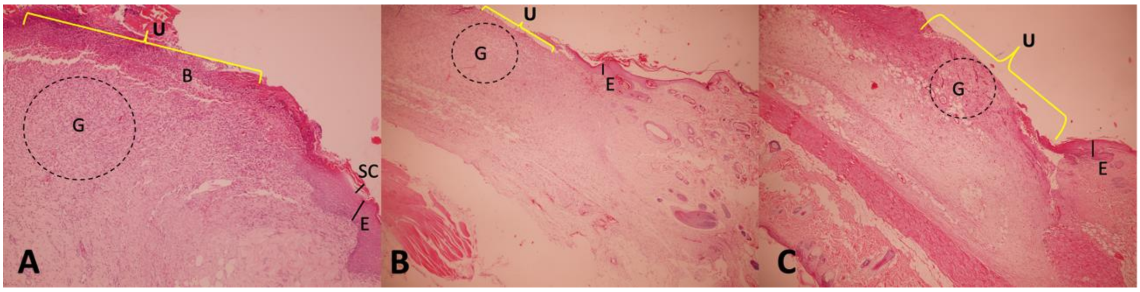

Fig. 3. *Histopathology images under 4x magnification. A) Control group, B) Pine resin group, C) Antibiotic group (G: Granulation tissue, U: Ulceration, B: Bleeding, E: Epidermis, D: Dermis: SC: Stratum corneum)*

At the end of the day 15, tissue samples were stained with hematoxylin-eosin and examined under a microscope. Although ulceration areas were seen in all groups in the microscopic examination, the least ulceration was seen in the pine resin group. The epidermis layer was most uniform in the pine resin group. In the control group, bleeding were observed under the epidermis layer (figure 3).

Table 2. *Histopathologic evaluation. The findings were evaluated as follows: 0; none, 1; mild, 2; moderate, 3; severe.*

Histopathologic Parameters	**n**	**Control**		**Pine Resin**		**Antibiotic**		**P-value**
		Mean	**S.E**	**Mean**	**S.E**	**Mean**	**S.E**	
Epithelization	7	0.88^{b}	0.23	2.75^{a}	0.16	2.38^{a}	0.18	<0.0001
Inflammatory cell infiltration	7	0.88^{b}	0.23	2.13^{a}	0.23	2.13^{a}	0.12	0.001
Granulation tissue formation	7	0.75^{b}	0.25	2.25^{a}	0.16	2.00^{a}	0.27	0.002

$^{a\text{-}b}$: Different letters in the same line are statistically significant (P < 0.05).

There were significant differences between the groups in terms of epithelialization (P<0.0001), inflammation cell infiltration (P<0.01) and granulation cell formation (P<0.01). In all criteria, the control group had higher values compared to the pine resin and antibiotic control group (Table 2).

As the largest organ in the body, the skin is involved in different processes such as protecting the organism from harmful chemicals and pathogens, initiating vitamin D synthesis, excretion and thermal regulation [11]. The skin, which acts as a physical, chemical and bacterial barrier between the internal organs and the external environment, is highly vulnerable to lesions such as wounds because it is constantly exposed to the external atmosphere [12, 13]. When a wound occurs, it is important to treat it quickly to prevent further damage and infection [13]. Wound healing occurs through haemostasis, inflammation, migration, proliferation and remodelling through the interaction of cells, growth factors and cytokines [11, 12].

For a long time, many studies have been conducted to improve wound care. Many organic/inorganic substances and procedures have been tried for this purpose; it is almost impossible to refer to all of them [14]. Recently, there has been a growing interest in the use of traditional agents in the treatment of skin wounds due to their clinical efficacy, simplicity, accessibility and affordability. The World Health Organization (WHO) emphasizes the use of traditional therapies for health protection, prevention, diagnosis or treatment of physical and mental illness [12].

Compounds of plant origin are the most widely used traditional products for the treatment of skin wounds. Herbal products are prepared as extracts, emulsions, creams and ointments and are commonly applied topically, systemically and orally [12, 15]. Resins are plant-based therapeutic agents and there are studies reporting accelerated wound healing when used topically for the treatment of wounds [16-19].

Resins are known to have anti-inflammatory, antibacterial, antifungal and antioxidant activities [16-18]. However, these activities of resins depend on the metabolites such as flavonoids and terpenes they contain. The content of resins may differ depending on the regions where they are collected [17]. Content determination in resins is important for determining the content of the resin. Although previous studies reported that pine resins contain 90% resin acids and 10% neutral substances, we did not determine the content of pine resins in our study. For this reason, the content of the resin and which metabolites were responsible for the effects obtained at the end of the study could not be determined.

In an article published by Forrest (1982), he stated that pine resin and pine resin products have healing efficacy on wounds [20]. Since then, there have been studies on the wound healing efficacy of pine resin and products derived from pine resin [21]. In

Kazakhstan, "Bialm" ointment containing pine resin was produced and used in experimental studies [21-23]. In Russia, "Biopin" ointment, also produced from pine resin, was used experimentally in the treatment of wounds [24]. Rozbahani et al (2019) experimentally examined the effectiveness of pine resin on wounds with their study [25]. Park et al (2017) examined the effectiveness of abietic acid in pine resin on wound healing. Studies have shown that products obtained from pine resin have antimicrobial activity and accelerate healing [26].

The results obtained from our study are similar to previous studies indicating that pine resin accelerates healing. However, while the efficacy of pine resin on wound healing was examined in our study, its antimicrobial activity was not examined.

Rozbahani et al. (2019) reported that the amount of healing in the pine resin and positive control groups was the same. In our study, the pine resin group showed more healing in the first three days than the positive control group. However, there was no significant difference between the positive control group and the pine resin group in the amount of healing and histopathologic findings in the following days. These findings are consistent with the study by Rozbahani et al [25].

Histopathologically, pine resin significantly increased epithelialization compared to the control group. This finding matches previous studies indicating that pine resin forms a larger epithelial area [21, 25].

Sipponen et al. (2007) clinically examined the effects of spruce resins on chronic wounds in a case report [27]. They found that the use of spruce resins triggered healing in ulcerative wounds that did not tend to heal for a long time. This result shows that resins produced from spruce, a pine species, have started to be used clinically successfully.

In their study, Park et al. (2003) reported that resin increased inflammation and hyperaemia findings in the first days but accelerated healing from the 3-4th days. However, in our study, pine resin accelerated healing from the first day. Sipponen et al. (2007) also reported that resin was effective in chronic wounds. These studies suggest that resin stimulates healing by creating inflammation. In our study, although resins did not show macroscopic signs of inflammation, they increased cell infiltration in the last days of healing at the microscopic level compared to the control group [22, 27].

There are studies in which ointments obtained from pine resin were used, but studies in which the resin was applied pure on the wound are limited [23, 24]. Thanks to the results obtained from our study, pure application of pine resin on the wound accelerated healing and did not cause any macroscopic signs of inflammation. These findings are similar to those of honey, which has been applied directly on the wound since ancient times.

CONCLUSION

The efficacy of pine resin has been experimentally examined in various studies and it has been stated that it accelerates wound healing and creates a more regular epithelial area. Clinically, pine resin types have started to be used in chronic wounds. It has been reported that the resin causes inflammation and triggers healing in chronic wounds, but with our study, it was concluded that resins accelerate healing in acutely formed wounds. In order to obtain more meaningful data, more studies in which pine resins are applied both experimentally and clinically are needed.

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Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: O.B., T.M.D., Design: O.B., A, S., Data Collection or Processing: O.B., A.S., T.M.D., Z.A., Analysis or Interpretation: O.B., Z.A., Literature Search: O.B., A.S., T.M.D., Z.A., Writing: O.B., T.M.D.

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