WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

The Effect of Turmeric (Curcuma Longa) Ethanol Extract Ointment on Second-Degree Burn Wound Healing in Wistar Rats (Rattus Norvegicus)

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A B S T R A C T

In traditional medicine, turmeric is used as an anti-inflammatory, antiseptic, wound medicine, burn medicine and for liver disorders. Curcumin compounds contained in turmeric have antiseptic properties, prevent bacterial infection in burns, and have anti-inflammatory properties to relieve inflammation in burns and accelerate the healing process. This study aimed to determine the phytochemical content and effectiveness of Turmeric Ethanol Extract Ointment (Curcuma longa Linn) in healing second-degree burns in Wistar rats (Rattus norvegicus). This research is experimental with a pre-and post-test control group design approach, January 2024. The research sample is the turmeric plant (Curcuma longa) obtained from traditional markets in Medan. The experimental animal samples in this study were Wistar strain rats; the number of rats used in this study was 20 male Wistar rats (Rattus norvegicus) divided into 4 treatment groups, so each group consisted of 5 rats. The variables in this study are independent in the form of administration of several topical formulations (ointment base, nebacetin® ointment, turmeric ethanol extract ointment (Curcuma longa) 10% and turmeric ethanol extract ointment (Curcuma longa) 20%, as well as independent variables which include wound contraction and epithelialization period. The statistical analysis used in this study was the One-Way ANOVA test, followed by a post-hoc test. Before the t-test, a descriptive analysis of wound contraction and epithelialization period was performed. If the data in this study is not normally distributed, it will be transformed to make it usually distributed. The results showed that the treatment group with 10% and 20% turmeric ointment had a significantly shorter epithelialization period than the control group. The p-value of 0.015 indicates that this difference has statistical significance. It can be interpreted that using turmeric ointment with a concentration of 10% and 20% significantly accelerates the epithelialization process compared to the control and standard groups in wound healing.

Keywords: Turmeric, Anti-inflammatory, Antiseptic, Wound medicine, Burn medicine

1. Main text

A burn is an injury that occurs due to direct contact or exposure to sources of heat (thermal), electricity, chemicals, or radiation (Tutik Rahayuningsih 2012). Based on depth, burns are divided into four types: superficial (degree 1), deep partial thickness (degree 2), total thickness (level 3), and level 4 (Hakim 2020). Burns can usually be prevented, and different treatments are applied based on the severity of the burn. Ointments, creams, biological and non-biological dressings, and antibiotics are sometimes recommended for levels 2, 3, and 4 burns. However, the misuse of such drugs can increase the risk of antibiotic resistance and fungal infections, even slowing wound healing and increasing the depth of burns (Avni et al., 2010). Every year, the incidence of injuries increases; burns rank sixth as the cause of unintentional injuries after falls, motorcycle accidents, and other factors (Research and Development Agency of the Ministry of Health of the Republic of Indonesia 2013). In 2008, more than 410,000 burn injuries occurred in the United States, with approximately 40,000 cases requiring hospitalization. In India, over 1 million people suffer from burns every year (Fitria, Saputra, and Revilla 2014). Until now, burns remain a concern for clinical nurses as severe burns have led to high post-burn morbidity. In Indonesia, the mortality rate from burns is still high at around 40%, mainly caused by severe burns (Mutia 2015).

Herbal products have been used in the medical field since ancient times, including turmeric root (Curcuma longa). The primary active compound in turmeric is curcumin (Nabofa et al. 2018). Many researchers are interested in studying the benefits of turmeric and its safety level. Some of the benefits of turmeric from research results are its antioxidant properties (Wanninger et al. 2015; Razavi 2021), anti-inflammatory effects (Manarin et al. 2019; Setiadi, Khumaida, and Wahyuning Ardie 2017; Kocaadam and Şanlier 2017), and anti-cancer properties (Hartati 2013; Razavi 2021). This study aims to determine the phytochemical content and the effect of turmeric ethanol extract ointment (Curcuma longa Linn) in healing second-degree burns in Wistar rats (Rattus norvegicus).

2. Methods

This study is experimental with a pre and post-test control group design approach. This research was conducted in January 2024. In this study, a sample of turmeric rhizomes and male Wistar rats (Rattus norvegicus), as many as 20, are divided into four treatment groups. Each group consists of 5 rats (Muthia Milasari 2019). The variables in this study are independent in the form of giving several topical formulations (base ointment, nebacetin ointment®, Turmeric Ethanol Extract Ointment (Curcuma longa) 10% and Turmeric Ethanol Extract Ointment (Curcuma longa) 20%, as well as independent variables that include wound contraction and epithelialization period.

Tool

Maceration vessel, knife, rotary evaporator, water handler, gel container, stirrer rod, a plate measuring 2 x 2 cm.

Material

Turmeric, equates, lanolin, solid paraffin, cetostearyl alcohol, white vaseline, gauze, oil paper, filter paper, 1mm2-sized paper, oil paper, nebacetin ointment®.

Phytochemical Test

Turmeric rhizomes identified several groups of compounds such as flavonoids, tannins, alkaloids, phenols, steroids/triterpenoids, terpenoids, and saponins (Rahmawati, Febrina, and Tjitraresmi 2016).

1. Identify Flavonoids.

As much as 1 ml of test solution is put into 3 test tubes. Tube 1 is a control, and tube 2 is coupled with 1 mL of 10% FeCl3 solution. The flavonoid is positive if there is a dark green/blue discoloration. Tube 3, coupled with a few drops of NaOH 20%, formed yellow if it contains flavonoids.

2. Identification of Tannins

Tannins As much as 2 mL of test solution is inserted into the test tube with a few drops of 1% FeCl3 solution, a positive sign of tannin if the color formed is dark green/ blue.

3. Identification of Alkaloids

For alkaloid tests, as much as 2 mL of test solution is evaporated on a porcelain cup until residue is obtained. The residue is then dissolved with 5 mL HCl 2N. Once cool, the solution is filtered. The solution obtained is divided into 3 test tubes. The first tube serves as a control. In the 2nd tube, three drops of the Dragendroff reagent are added; in the third tube, three drops of Mayer reagent (through the tube wall). The formation of orange deposits in the second tube and yellow deposits in the third tube indicates the presence of alkaloids.

4. Identification of Phenols

Tests are carried out in drip plates; the test solution is added FeCl3 (1% in water/ethanol); if there is a color change, green/red/purple/blue/black shows phenol content.

5. Identification of Terpenoids

The modified Salkowaski test. For 2 mL of water plant extract, add 2 mL of chloroform, followed by a few drops of concentrated sulfuric acid. The solution is well shaken. The formation of a yellow lower layer indicates the presence of terpenoids.

6. Identification of Saponins

Saponins 4 mL test solution is added with 5 mL aquadest, shake, and see the presence of a stable foam. A small extract of 5 mL of water shake in a test tube forms a stable foam (foam as high as 1 cm and stable for 30 minutes). 4 mL of test solution is inserted into the test tube as a control (Muthia Milasari 2019).

Manufacture of Ointment Preparations

Table 1. Topical Preparation Formulations Of Each Ointment

Material Name	**Ointment base**	**Turmeric Ointment *(Curcuma longa)* 10%**	**Turmeric Ointment *(Curcuma longa)* 20%**
Turmeric Ethanol Extract	-	0.5 ml	1 ml
Lanolin	2.5g	2.5g	2.5g
Solid paraffin	2.5g	2.5g	2.5g
Cetostearyl alcochol	2.5g	2.5g	2.5g
White vaseline	42.5g	42.5g	42.5g

Testing on Animal Trials

All animals tried in the form of Wistar rats are done need by using electrical solder that has been motivated with a round-shaped tip then found on the dorsal part of the rat for 10 seconds before doing the need for the rats anesthetized using ketamine (50 mg/kg i.m) that has previously been satisfied. Before continuing with the sampling of extract gels and controls, different tests are carried out to assess the degree and extent of the grade II burn (Thakur et al. 2011). The treatment was given to 24 Wistar rats as tried animals that were divided into groups as in the table below:

Burns evaluation is carried out every 2-4 days, with aspects evaluated from the healing activities of the burn, including wound contraction and epithelialization periods (Thakur et al. 2011). Wound contraction is assessed by measuring the wound diameter using a ruler and then calculating wound contraction using the following formula:

$$\text{Wound Contraction (\%)} = \frac{\text{(size of the initial wound - size of the wound on a specific day)}}{\text{Wound size of a particular day}} X\ 100\%$$

The epithelialization period is measured by calculating the length of time eschar is removed to escape, during which the epithelial period is calculated in the day (Thakur et al. 2011); (Ghazali et al. 2016). The static analysis used in the study was Anova's One-Way test, followed by a post-hoc test. Before another test is done, a descriptive analysis of wound contraction and epithelial period is performed. If the data in this study is distributed abnormally, then there will be a transformation of the data so that the data is distributed normally.

Table 2. Treatment Group in Rats

Group	Treatment
Control	In this group, only the base of ointment
Standard	Nebacetin Ointment is generally used in the treatment of burns in this group.
Turmeric ethanol extract ointment (Curcuma Longa) 10%	This group used Turmeric Ethanol Extract Ointment (Curcuma Longa) 10%
Turmeric ethanol extract ointment (Curcuma Longa) 20%	This group used Turmeric Ethanol Extract Ointment (Curcuma Longa) 20%

3. Results and Discussion

Table 1 presents the results of data normality analysis on burn wound healing parameters. These parameters include the degree of wound contraction on day 3, day 6, day 9, day 12, and day 14 and the epithelialization period. The P value for each parameter reflects the statistical significance level of the data normality test. The analysis showed that on day 3, day 12, and day 14, the data yielded P values less than 0.05, indicating that the data were not normally distributed. While on day 6, day 9, and the epithelialization period, the P value was more significant than 0.05, indicating that the data had a normal distribution. This information is essential to understand the distribution characteristics of the data before conducting further analysis related to burn wound healing.

Table 1. Results of Data Normality Analysis on Burn Wound Healing Parameters

Wound Healing Parameters	Nilai P
Wound Contraction on Day -3	0.005
Wound Contraction on Day -6	0.341
Wound Contraction on Day -9	0.062
Wound Contraction on Day -12	0.016
Wound Contraction on Day -14	0.009
Epithelialization Period	0.008

Table 2. Results of analysis of One Way ANOVA and Kruskal-Wallis with Wound Contraction as Wound Healing Parameters in The Treatment Group

	Wound Contraction (%)				
Observation Time	**Control**	**Standard**	**Turmeric Ointment (Curcuma Longa Linn) 5 %**	**Turmeric Ointment (Curcuma Longa Linn) 10 %**	**Value**
Day-3	4.22 (8.33)	0.00 (8.66)	22.24 (24.82)	20.52 (25.92)	0.009**
Day-6	8.22 ± 5.22	28.22 ± 20.54	32.28 ± 7.28	34.27 ± 7.58	0.022*
Day-9	7.59 ± 7.92	35.22 ± 8.05	46.25 ± 6.75	52.42 ± 8.42	0.009*

Day- 12	9.22 (29.27)	45.36 (22.22)	64.24 (20.25)	66.57 (8.86)	0.023**
Day- 14	27.27 (37.50)	82.62 (42.22)	76.77 (22.24)	88.29 (3.64)	0.022**

* Data is presented in Median (Range). Different lowercase letters in the same column show significant differences in P values < 0.05

Table 2 displays the results of One Way ANOVA and Kruskal-Wallis analyses evaluating Wound Contraction as the Wound Healing Parameter in the Treatment Group. The table includes observations at different time points (Day 3, Day 6, Day 9, Day 12, and Day 14) for the control group, standard group, and two treatment groups using Turmeric Ointment at concentrations of 10% and 20% (Curcuma Longa Linn). The data is presented as Median (Range), with significant differences in P values (<0.05) denoted by different lowercase letters within the same column. The analysis provides insights into the efficacy of turmeric ointment in promoting wound healing based on the observed levels of wound contraction over time.

Table 3. Results of Epithelial Period Comparison in Each Treatment Group.

Treatment Group	Epithelialization Period *	Value P
Control	21 (2)a	
Standard	18 (2)b	
Turmeric Ointment 10%	20 (2)b	0.015
Turmeric Ointment 20%	20 (2)b	

* Data is presented in Median (Range). Different lowercase letters in the same column show significant differences in P values < 0.05

Table 3 presents the results of the Epithelial Period Comparison in each Treatment Group. The data is presented as Median (Range), with lowercase letters indicating significant differences in P values (<0.05) within the same column. The control group exhibited an Epithelialization Period of 21 (2) days, while the standard group using Turmeric Ointment at a 10% concentration showed an Epithelialization Period of 18 (2) days, and the group using Turmeric Ointment at a 20% concentration had an Epithelialization Period of 20 (2) days. These findings indicate that both concentrations of Turmeric Ointment significantly shortened the Epithelialization Period compared to the control group, with a P value of 0.015 demonstrating statistical significance.

The results indicate that the treatment groups using 10% and 20% Turmeric Ointment exhibited significantly shorter Epithelialization Periods than the control group. The P-value of 0.015 suggests statistical significance in this difference. This implies that using Turmeric Ointment at 10% and 20% concentrations significantly accelerates the epithelialization process compared to the control and standard groups in the context of burn wound healing. Based on these findings, there are significant differences in both the wound contraction parameter and the epithelialization period among the treatment groups.

Research exploring the wound-healing activity of turmeric is still limited, but numerous other studies support these findings. The wound-healing activity of turmeric is attributed to its antioxidant, anti-inflammatory, and antimicrobial properties, creating a favorable microenvironment for wound healing. This is supported by research conducted by Winarti et al. (2017), reporting that the ethyl acetate extract of turmeric has high antioxidant activity with an IC50 value of 66.91 µg/mL, attributed to the presence of compounds like 2-methoxy-4-vinylphenol. Other studies, such as Yanti et al. (2011), have demonstrated the anti-inflammatory activity of turmeric extract, significantly inhibiting the expression of various inflammation biomarkers at both protein synthesis and gene levels in vitro. Additionally, different in vitro antimicrobial studies against pathogenic bacteria have shown significant growth inhibition of bacteria such as Staphylococcus aureus, Escherichia coli, Bacillus cereus, Bacillus stearothermophilus, Pseudomonas aeruginosa, Vibrio cholera, and Salmonella Typhimurium (Adolf J. N. Parhusip, 2006; Asbur, Y., 2017; Muzafri,2019; Sitanggang, Duniaji, & Pratiwi, 2019).

4. Conclusion

The conclusion that can be drawn from this study is that the treatment group with 10% and 20% turmeric ointment experienced a significantly shorter epithelialization period than the control group. The P value of 0.015 confirms the statistical significance of the difference. Using turmeric ointment with 10% and 20% concentration effectively accelerated the burn wound healing process compared to the control group. This finding is consistent with turmeric's antioxidant, anti-inflammatory, and antimicrobial properties that previous studies have supported. Nonetheless, further research is needed to understand the mechanism of burn wound healing in greater detail.

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