ORIGINAL ARTICLE

Effectiveness of 5% *temulawak* extract (*Curcuma xanthorrhiza*) on post-extraction fibroblast cells in Wistar rats (*Rattus norvegicus*)

Nyoman Ayu Anggayanti[1*]
Putu Lestari Sudirman[1]
Made Indira Paramita[1]

[1]Department of Dentistry, Udayana University, Denpasar, Indonesia

*Correspondence:
ayu.anggayanti@unud.ac.id

Received: 24 May 2023
Revised: 25 July 2023
Accepted: 31 July 2023
Published: 31 July 2023
DOI: 10.24198/pjd.vol35no2.47023

p-ISSN 1979-0201
e-ISSN 2549-6212

Citation:
Anggayanti NA, Sudirman PL, paramita MI. Effectivity of 5% *Temulawak* Extract (*Curcuma xanthorrhiza*) on Post-Extraction Fibroblast Cells in Wistar Rats (*Rattus norvegicus*). Padj J Dent, July. 2023;35(2):151-155.

ABSTRACT

Introduction: Fibroblast is a key element in the wound healing process. During the proliferation phase, fibroblast cells are important for producing collagen and elastic cells. *Curcuma xanthorriza* is a medicinal herb that contains active compounds such as curcumin and flavonoid, both of which have the ability to increase fibroblast migration and accelerate wound healing. This study aims to observe the effectiveness of 5% *temulawak* extract (*Curcuma xanthorrhiza*) on post-extraction fibroblast cells in Wistar rats (*Rattus norvegicus*). **Methods:** The experimental study was conducted on 30 rats, divided into a control group and a treatment group. The subjects received intramuscular anesthesia prior to extraction of mandibular incisor. The treatment group was injected with 5% curcuma extract into their wound socket, while the control group was given placebo gel injection. The mandibular samples were obtained and analyzed on the 3rd, 5th, and 7th day. The number of fibroblast cells was observed using a light microscope with three different fields of view at 400x magnification. **Results:** The mean number of fibroblasts in the experiment group was higher than that in the control group. On the 7th day, the treatment group showed 342.50 fibroblast cells, while the control group only showed 298.25 cells. The number of fibroblast cells in the 3rd day treatment group was comparable to that in the 5th day of control group. **Conclusion:** It can be concluded that 5% *temulawak* extract (C*urcuma xanthorrhiza*) has a significant effect increasing post-extraction fibroblast cells in Wistar rats (*Rattus norvegicus*).

KEYWORDS
temulawak, curcuma xanthorrhiza, post-extraction, fibroblast.

INTRODUCTION

Based on the 2018 health survey (RISKESDAS) conducted by the Indonesian Health Ministry, as many as 57.6% of Indonesian citizens suffered from oral health diseases, which often lead to dental extraction.[1] Dental extraction is a procedure in dentistry when a tooth is removed from its socket. This procedure would induce trauma inside the socket.[2]

Wound healing is achieved in three phases: 1) the inflammatory phase, 2) the proliferation phase, and 3) the remodeling phase.[3] The proliferation phase occurs from day 2 to 14 postoperatively. During this process, fibroblast migration takes place. Fibroplasia plays a role in producing an extracellular matrix to dominate the wound cavity and provides a base for keratinocytes.[4] Therefore, fibroblast is a principal cell in wound healing process.

Dentists often prescribe analgesics and anti-inflammatory medications to reduce post-extraction pain. However, it is believed that traditional medicine is a safer alternative with a lower risk of side effects.[5,6] *Temulawak* (*Curcuma xanthorrhiza*) is an herbal plant commonly used as traditional medicine in Indonesia.[7] *Curcuma xanthorrhiza* contains alkaloid, flavonoid, phenolic, and curcumin, as well as other substances with promising abilities in increasing fibroblast cell count.[7-9]

Previous studies have stated that *Curcuma xanthorrhiza* extract is effective in accelerating the healing progress of a second-degree burn wound.[8,10-12] In their research, 5% *Curcuma xanthorrhiza* extract showed a significant result with a 40% wound closure rate on the 9th day, as proved by the formation of granulation tissue.[7] The granulation tissue is a result of fibroblast proliferation and angiogenesis, which aid in faster wound closure. Another study on 5% *Curcuma xanthorrhiza* extract also showed that the application of the extract increased the number of fibroblasts, tissue granulation, blood vessel density, and wound contraction in male diabetic Wistar rats.[8,12-15] These findings indicate the excellent potential of 5% *Curcuma xanthorrhiza* extract as a wound-healing agent. However, research on its use to accelerate healing in post-extraction dental sockets is still minimal. Therefore,

This study aims to observe the effectiveness of 5% *temulawak* extract (C*urcuma xanthorrhiza*) on post-extraction fibroblast cells in Wistar rats (*Rattus norvegicus*).

METHODS

The *Wistar* Rats were used as experiment subjects and study had been approved by the Faculty of Veterinary Medicine at Udayana University. The experiment was performed in accordance with the guidelines of the Health Research Ethics Commission of the Faculty of Medicine, Udayana University. The rats were kept in the Faculty of Veterinary Medicine, Udayana University, given an initial examination for systemic health conditions and stored in boxes with sawdust. All animals were kept at room temperature with controlled humidity.

Extract of *temulawak* (*Curcuma rhizome*) was mashed and macerated with 96% ethanol. Every 10 grams of the extract was mixed with 10 ml of 2% CMC-Na for dilution to produce a 5% *Curcuma xanthorrhiza* extract gel.[6] The animals were divided into two groups of 15 subjects each. Subjects in the treatment group (Group 1) were given 5% *Curcuma xanthorrhiza* extract gel, injected into the post-extraction socket twice a day. Subjects in the control group (Group 2) were given placebo gel. Prior to the extraction of the mandibular right incisors, the subjects in both groups were given intramuscular anesthesia (ketamine and xylazine). The extraction was performed with a sterile excavator. The post-extraction socket was then injected with 0.1 ml of 5% *Curcuma xanthorrhiza* extract gel using a small cannula. The subjects in the control group were given 0,1 ml of placebo gel. The animals were euthanized on the 3rd, 5th, and 7th day. The mandibular samples were obtained to retrieve mucosa specimens. The specimens were then processed with haematoxylin and eosin (HE) staining.[7] The number of fibroblast cells in the specimen was counted using a binocular microscope at 400 times magnification. Three different fields of view were examined. The total number of fibroblasts in each field of view was summed and then divided by three to calculate the average. The counted fibroblast cells were considered active fibroblasts, characterized by smooth chromatin, large cytoplasm, round nucleus, and distinct appearance.

Data obtained was processed using SPSS software. The normality test was performed with the Shapiro-Wilk test. Next, the Levene test was performed to test the homogeneity of the sample. The tests showed that the data was normally distributed and homogenous, so it was further tested with the parametric One Way ANOVA with a confidence level of 95% or $p<0.05$. Lastly, a Least Significant Difference (LSD) post hoc test was performed to find out the differences between both groups in detail.

RESULTS

The results of the average number of fibroblasts that have been observed are shown in Table 1. Based on Table 1, the highest mean number of fibroblast cells was in the treatment group on 7th day with 342.50 cells. Based on Table 2 and Table 3, it was found that the data used in this study was normally distributed and homogeneous. Thus, a One-Way ANOVA test was carried out. The findings, as presented in Table 4, displayed a significant effect on the number of post-extraction fibroblast cells by giving 5% *Curcuma xanthorriza* extract gel in Wistar rats. Thus, an LSD post hoc test was carried out.

Table 1. Mean results and standard deviation of fibroblast cell count

Groups	Observation	n	Mean	SD
Control	Day-3	4	107.00	6.807
	Day-5	4	194.00	10.132
	Day-7	4	298.25	11.309
Treatment	Day-3	4	190.00	12.275
	Day-5	4	289.75	12.399
	Day-7	4	342.50	5.252

Table 2. Shapiro Wilk test results

Groups	Euthanasia	n	*p - value*
Control group	Day-3	4	0.556
	Day-5	4	0.507
	Day-7	4	0.116
Treatment group	Day-3	4	0.444
	Day-5	4	0.567
	Day-7	4	0.562

Table 3. *Levene's test* results

Groups	*Levene's statistic*	*p - value*
Control group	0.289	0.756
Treatment group	1.574	0.259

Table 4. One Way ANOVA test results

Groups	*p - value*
Control group	0,001
Treatment Group	0,001

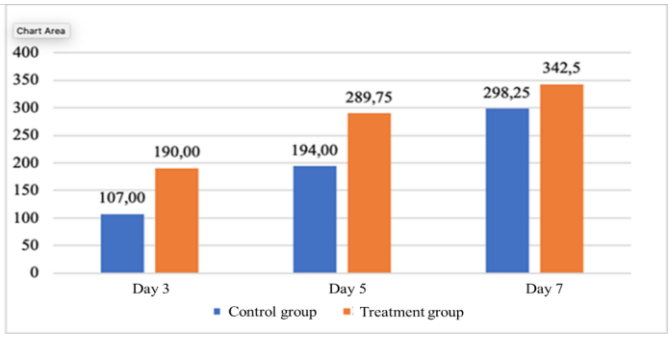

Figure 1. Bar graph of the average number of fibroblast cells

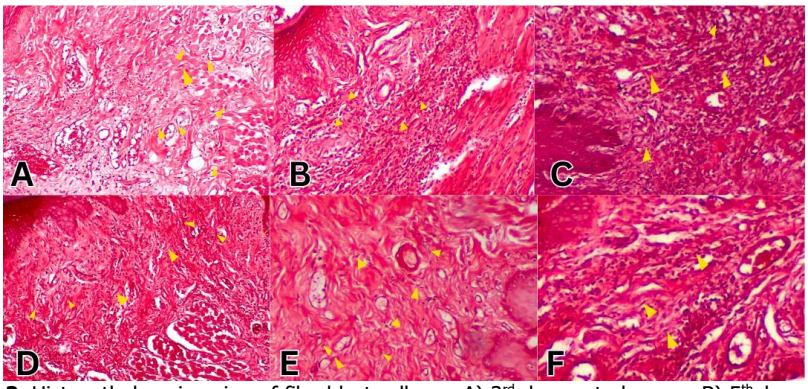

Figure 2. Histopathology imaging of fibroblast cells on: A) 3rd day control group; B) 5th day control group; C) 7th day control group; and D) 3rd day treatment group; E) 5th Day treatment group; F) 7th day treatment group. HE staining, 400x magnification.

Table 5. Least significant difference (LSD) test results

Groups	Euthanasia day	Comparison	Mean difference	p-value
Control Group	Day-3	Day-5 Day-7 Day-3	87.00	0.001
		Day-7	191.25	0.001
	Day-5	Day-3 Day-5	87.00	0.001
			104.25	0.001
	Day-7		191.25	0.001
			104.25	0.001
Treatment Group	Day-3	Day-5 Day-7 Day-3	99.750	0.001
		Day-7 Day-3	152.50	0.001
	Day-5	Day-5	99.750	0.001
			52.750	0.006
	Day-7		152.50	0.001
			52.750	0.006

Table 6. The results of the LSD test on average fibroblast cells between the treatment group and the control group on the 3rd, 5th, and 7th days after tooth extraction in Wistar rats

Control	Treatment	Mean difference	*p - value*
Day-3	Day-3	83.00	0.001
	Day-5	182.25	0.001
	Day-7	235.50	0.001
Day-5	Day-3	4.00	0.782
	Day-5	95.75	0.001
	Day-7	148.50	0.001
Day-7	Day-3	108.25	0.001
	Day-5	8.50	0.558
	Day-7	44.25	0.001

In addition, the data were tested using post hoc LSD to determine in detail the significant differences between the days of decapitation and between groups, and how they affected the number of fibroblast cells. In the 5th day control group and the 3rd treatment group, there was no significant difference; it also occurred in the Day-7 control group and the Day-5 treatment group. This indicates the number of fibroblasts in the Day-3 and Day-5 treatment groups had the same amount as the Day-5 and Day-7 control groups.

DISCUSSION

This true experimental research used 5% *Curcuma xanthorrhiza* extract gel as the independent variable. A determination test was conducted to determine the type of rhizome used as research material. The results of the determination test have stated that the rhizome used is the rhizome of *temulawak* (*Curcuma xanthorrhiza*). Furthermore, phytochemical tests were carried out to qualitatively determine the active compounds contained in *temulawak*. The active compounds include curcumin, phenols, tannins, steroids, saponins, alkaloids, terpenoids, and flavonoids. This finding is in accordance with previous studies. Specifically, the curcumin and flavonoids have the most impact on wound healing acceleration.[5,6,9,15]

Curcumin's most well-known property is its potent anti-inflammatory effect. Inflammation is a natural response to tissue injury and infection, but excessive or prolonged inflammation can delay the wound healing process. Curcumin helps regulate various proinflammatory molecules like cytokines, chemokines, and enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX). By inhibiting these pro-inflammatory mediators, curcumin helps reduce inflammation at the wound site, which, in turn, allows the healing process to proceed more efficiently. Curcumin is also an excellent antioxidant. It can neutralize free radicals that cause oxidative stress at the wound site. Excessive oxidative stress can impair cell functions and hinder tissue repair. By scavenging these free radicals, curcumin helps protect cells and tissues from damage, enabling better wound healing.

Moreover, curcumin has been shown to influence the activity of various growth factors involved in wound healing, such as increasing the expression of transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). TGF-β plays a crucial role in collagen synthesis and tissue remodeling, while VEGF promotes angiogenesis, which is the formation of new blood vessels. In relation with fibroblast production, curcumin has been found to stimulate fibroblast proliferation and collagen synthesis, promoting the rebuilding of damaged tissue and speeding up the wound healing process.[13,14,16,17]

Similar to curcumin, flavonoids have also been shown to stimulate collagen production. Collagen is the main structural protein in connective tissues, and its increased synthesis is crucial for wound closure and tissue repair. Flavonoids have demonstrated antimicrobial activity against various bacteria and fungi. By preventing or reducing wound infections, flavonoids create a more favorable environment for wound healing to occur without complications. Flavonoids also have anti-inflammatory and angiogenesis-enhancing abilities. Reducing inflammation at the wound site would create a less hostile environment for healing to take place, while improved blood flow to the wound area provides essential nutrients and oxygen, supporting the growth of new tissue and accelerating wound healing.[18,19,20]

The result of this study showed that in the treatment group, the average increase in the number of fibroblasts was highest on Day 7, which was 342.50 cells. While in the control group, the average increase in the number of fibroblast cells was highest on Day 7, which was 298.25 cells (Table 1, Figure 1). The increase in the number of fibroblasts in the treatment group was influenced by the administration of 5% *Curcuma xanthorrhiza* extract gel. The results of the LSD test in the Day-3 treatment group and the Day-5 control group showed no significant difference (Table 5,6,7). This also occurred in the Day-5 treatment group and the Day-7 control group. This indicates that 5% *Curcuma xanthorrhiza* extract gel was effective in increasing the number of fibroblast cells after tooth extraction of Wistar rats.[7,13,14,16,17] Similar to our result, previous studies showed that that 5% *temulawak* extract is effective in accelerating healing of a second-degree burn wound, up to 40% increase in wound closure rate. In their research, 5% *Curcuma xanthorrhiza* extract showed significant result with a 40% wound closure rate on the 9th day, proved by the formation of granulation tissue.[7] The granulation tissue is a result of fibroblast proliferation and angiogenesis, which aid in faster wound closure. Another study on 5% *Curcuma xanthorrhiza* extract also showed that the application of the extract increased the number of fibroblasts, tissue granulation, blood vessel density and wound contraction in male diabetic Wistar rats. [18,19,20]

Based on this research, it was found that 5% *Curcuma xanthorriza* extract gel has potential as an alternative material that can be used after tooth extraction. It was stated that there was an increase in the number of fibroblast cells that were able to accelerate wound healing, with a significant increase in Day 7 decapitation. However, it is essential to acknowledge certain limitations that may have influenced the findings. Firstly, the sample size was relatively small, limiting the statistical power and generalizability of the results. Secondly, this research only focuses on one aspect (fibroblast cells proliferation) of the wound healing process. Additionally, this research only tested one concentration of the extract. Future research with larger and more diverse samples, encompassing more wound healing aspects such as angiogenesis, level of oxidative stress and/or others, and experiments on other concentrations of the extract would be beneficial in further validating our results and gaining a more comprehensive understanding of the effect of *Curcuma xanthorrhiza* extract on wound healing.

CONCLUSION

Based on this study, it can be concluded that 5% *temulawak* extract (*Curcuma xanthorrhiza*) has significant effect on increasing post-extraction fibroblast cells in Wistar rats *(Rattus norvegicus*).

Author Contributions: Conceptualization, NAA and PLS; methodology, MIP; software, MIP; validation, NAA and PLS; formal analysis, NAA; investigation, MIP; resources, NAA; data curation, PLS; writing original draft preparation, MIP; writing review and editing, NAA and PLS; visualization, PLS; supervision, NAA and PLS; project administration, PLS; funding acquisition, NAA and PLS. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by DIPA PNBP Universitas Udayana TA-2021 grant number B/4969-73/UN14.2.2.VII.1/PT.01.03/202.
Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Faculty of Medicine, Udayana University (No.311/UN114.2.2.VII.14/LT/2021).
Informed Consent Statement: Not applicable.
Data Availability Statement: Research data would be provided under the permission of all authors via corresponding email, paying close attention to applicable ethical rules.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.
Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

REFERENCES

1. Kemenkes RI. Riset Kesehatan Dasar. Badan Penelitian Dan Pengembangan Kesehatan Kementerian Kesehatan RI. 2018.
2. Danoediningrat CP. Pencabutan Gigi. In: Poedjiastoeti W, Ruslin M, editors. Buku Ajar Bedah Mulut dan Maksilofasial. EGC; 2019. p. 280–8.
3. Purnama H, Sriwidodo, Ratnawulan S. Review Sistematik: Proses Penyembuhan dan Perawatan Luka. Farmaka. 2017;15(2):251–8.DOI: 10.24198/jf.v15i2.13366
4. Primadina N, Basori A, Perdanakusuma DS. Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler. Qanun Medika - Medical J Faculty of Medicine Muhammadiyah Surabaya. 2019 Jan 24; 3(1).DOI: 10.30651/jqm.v3i1.2198
5. Sumayyah S, Salsabila N. Obat Tradisional : Antara Khasiat dan Efek Sampingnya. Farmasetika.com (Online). 2017 Dec 15;2(5).DOI: 10.24198/farmasetika.v2i5.16780
6. Dicky A, Apriliana E. Efek Pemberian Ekstrak *Temulawak (Curcuma Xanthorrhiza* Roxb) terhadap Daya Hambat Pertumbuhan Staphylococcus Aureus dan Escherichia Coli Secara In Vitro. J Ked Univ Lampung. 2016; 1(2).DOI: 10.23960/jkunila12308-312
7. Kesumayadi I, Almas AI, Rambe INH, Hapsari R. Effect of *Curcuma xanthorrhiza* Gel on Methicillin-Resistant Staphylococcus aureus-Infected Second-Degree Burn Wound in Rats. Natural Product Sciences. 2021; 27(1): 1–8.DOI : 10.20307/nps.2021.27.1.1
8. Liberty IA, Rasyid RSP, Subandrate S. Gambaran Histologi Ketebalan Jaringan Granulasi Pada Tikus Wistar Jantan dengan Luka Bakar Setelah Pemberian Ekstrak Kayu Manis *(Cinnamomun burmannii*). J Kedokteran dan Kesehatan : Publikasi Ilmiah Fakultas Kedokteran Universitas Sriwijaya. 2020 Jan 18; 7(1). DOI: 10.32539/JKK.V7I1.7609
9. Tripathi A, Awasthi H, Rokaya DB, Srivastava D, Srivastava V. Antimicrobial and wound healing potential of dietary flavonoid naringenin. Nat Prod J. 2019; 9(1): 61–8.DOI: 10.2174/2210315508666180802104630
10. Sivapriya AS, Kariyil BJ. Therapeutic Potential of Medicinal Plants in Methicillin Resistant *Staphylococcus aureus* Infected Wounds. Challenges and Advances in Pharmaceutical Research Vol. 9. 2022 Nov 10: 99-108. DOI: 10.9734/bpi/capr/v9/3980E
11. Kristianto H. The Effects of Javanese Turmeric (Curcuma xanthorriza) on Fibroblasts, Granulation, Blood Vessel Density, and Contraction in Wound Healing of STZ-Induced Diabetic Rats. Kuwait J of Science. 2021: 50(1): 1-14. DOI: 10.48129/kjs.15261
12. Dompeipen EJ, Kaimudin M. Isolasi Kitin Dan Kitosan Dari Limbah Kulit Udang Isolation. Maj BIAM. 2016; 12(1): 32–9. DOI: 10.29360/mb.v12i1.2326
13. Yunani R, Mudji EH, Apritya D. Perbedaan efektifitasi anestetikum antara zoletil acepromacin dan ketamin acepromacin pada tikus putih,. Kaji Vet. 2015; 3(2): 113–9. DOI:10.35508/jkv.v3i2.1036
14. Hartomo BT, Firdaus FG. Pemanfaatan biomaterial kitosan dalam bidang bedah mulut. Kedok gigi Univ Baiturrahmah. 2018; 6(1): 62–70.DOI: 10.33854/jbd.v6i1.82
15. Panjaitan J, Girsang E, Chiuman L. Effectiveness of *temulawak* (*Curcuma xanthorrhiz*a) ointments as wound healing agents in wistar rats. J Teknol Lab. 2022; 11(2): 95–104. DOI: 10.29238/teknolabjournal.v11i2.376
16. Astuti A, Hendrarti W, Wunas J. Synthesis and characterizations of absorbent dressing turmeric extract curcumin chitosan-alginate hydrogen and ZnO nano for mediate and high exudation. Ann Mechnikov's Inst. 2019; (3): 61–9. DOI: 10.5281/zenodo.3469448
17. Kook KE, Kim C, Kang W, Hwang JK. Inhibitory Effect of Standardized *Curcuma xanthorrhiza* Supercritical Extract on LPS-Induced Periodontitis in Rats. J Microbiol Biotechnol. 2018 Oct 28; 28(10): 1614-25. DOI: 10.4014/jmb.1808.08052.
18. Kumar S, Kumar A, Kumar N, Singh P, Singh TU, Singh BR, et al. In vivo therapeutic efficacy of Curcuma longa extract loaded ethosomes on wound healing. Vet Res Commun. 2022; 46(4): 1033–49. DOI: 10.1007/s11259-022-09952-1
19. Nashi M, Yamamoto S, Maeda K, Taniike N, Takenobu T. A Case of Infective Endocarditis Due to Oral Streptococci After Perioperative Oral Function Management. Cureus. 2021; 13(12): e20446.DOI: 10.7759/cureus.20446
20. de Gea Rico A, Williams JV., Ranjadayalan K, Revington PJ. Infective endocarditis of presumed dental origin and the new NICE guidelines: A case report. Oral Surg. 2021; 5(3): 136–41. DOI: 10.1111/j.1752-248X.2012.01155.x