

The Effect Of Black Cumin Nanoemulgel (Nigella sativa) On Fibroblasts In Post-Tooth Extraction Wound Healing In Mice

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ABSTRACT

Background: Tooth extraction is said to be successful when accompanied by a good wound healing process. Innovation for post-tooth extraction wound treatment is necessary. Black cumin has active ingredients that function as anti-inflammatories. The formulation used is nanoemulgel because it has the advantages of stability and good adhesion. **Objective:** To evaluate the effect of black cumin nanoemulgel on the number of fibroblasts in the wound healing process after tooth extraction in rats. **Methods:** This study was conducted using post-test only control group design with a total sample of 22 samples divided into 4 groups with each group totaling 5 samples and 2 samples as a drop out. The application is carried out as much as 3 times a day for 5 days. Tissue collection from experimental animals was carried out and then histology preparations were made. Calculations of fibroblast were carried out using hematoxylin eosin (HE) staining and carried out in the apical 1/3 of the 3 visual fields with a V pattern. Statistical tests used are the One-Way Anova test and the Post Hoc LSD test. **Results:** There was significant difference in the number of fibroblasts between groups ($p = 0.000$). Black cumin nanoemulgel concentration of 15% showed highest number of fibroblasts. **Conclusion:** Nanoemulgel black cumin (Nigella sativa) 15% can increase the number of fibroblasts in wound healing post tooth extraction in rats. Nanoemulgel black cumin (Nigella sativa) 15% have the potential to accelerate wound healing process by increasing the number of fibroblasts.

Keywords: Black Cumin, Number of Fibroblasts, Post-Tooth Extraction, Wound Healing

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INTRODUCTION

Tooth extraction, also known as tooth extraction in dentistry, is a dental procedure that removes a tooth from its socket.¹ Tooth extraction causes minimal trauma to the supporting tissue and bone surrounding it.²

A tooth extraction is considered successful if an adequate wound-healing process follows. Dentists must be attentive to the wound-healing process because it can cause pain and discomfort in the patient's oral cavity.² The prolonged healing time of a wound after tooth extraction can result in pain, bleeding, swelling, impaired chewing and speech function, and even infection.³

The wound healing process consists of four stages: hemostasis, inflammation, proliferation, and remodelling.⁴ The proliferation phase begins approximately on the fourth day after the injury and lasts until the 21st day. Fibroblasts migrate with the help of platelet-derived growth factor.⁵ In wound healing, there are several growth factor that can regenerate tissue, one of which is fibroblast growth factor (FGF) that can stimulate proliferation and deposition of fibroblast.⁶

Gengigel is one of the medications available that can be used to quicken the healing of socket wounds following tooth extraction.⁷ Gengigel contains hyaluronic acid, which has anti-inflammatory, antimicrobial, and antioxidant properties that aid in wound healing.⁸

An inventive use of herbal plants to treat wounds following tooth extraction must be considered. In comparison to drugs made from synthetic chemicals, herbal plants are less expensive and have fewer side effects. This is because herbal plants have been shown to cure diseases, and their use is more effective, efficient, safe, and economical.⁹ Black cumin is one example of a herbal plant that can be used.¹⁰

Black cumin (*Nigella sativa*) contains saponins that aid in wound healing (angiogenesis) through Vascular Endothelial

Growth Factor (VEGF). Black cumin (*Nigella sativa*) contains the mineral zinc, which is known to aid in wound healing. This zinc aids in fibroblast proliferation and collagen synthesis.¹¹ Black cumin (*Nigella sativa*) also contains flavonoid compounds that can increase the expression of the Insulin-Like Growth Factor (IGF-1) receptor as a mediator of fibroblast proliferation and collagen synthesis.¹² Flavonoid can increase immunity and reduce inflammation, so flavonoid can stimulate fibroblast growth factor (FGF) that can increase the number of fibroblast.¹³

A type of emulsion with droplet sizes ranging from 1 to 100 nm that is immersed in a gel base is called nanoemulgel, and this formulation will be used in this study.¹⁴ Nanoemulgel formulations have the advantage of good stability and adhesion, providing good delivery of drugs and active substances with low solubility, assisting in drug release control, and extending drug effects.¹⁵ The small droplet size also facilitates nanoemulgel penetration into the skin and mucosa.¹⁶ In addition, nanoemulgel has a lower viscosity than hydrogel preparations, which results in better distribution. This nanoemulgel has the physical characteristics of a semisolid formulation that is thick, has a soft texture, and is easily spread on the skin or mucosa.¹⁷

Previous research by Wulandari et al¹⁸ found that a 10% gel concentration of black cumin (*Nigella sativa*) extract increased the number of fibroblast cells on the eighth day after application to gingival wounds. Another study by Septiana. L¹⁹ found that black cumin oil (*Nigella sativa*) emulgel increased the number of fibroblasts in ulcerative wounds.

This study was evaluated the effect of black cumin nanoemulgel (*Nigella sativa*) on the number of fibroblasts in healing wounds after tooth extraction in rats.

MATERIALS AND METHODS

This research used a post-test group control design with black cumin (*Nigella sativa*) extract nanoemulgel concentrations at 10% and 15%, gengigel, and distilled water. 1 gram of black cumin nanoemulsion is diluted with 9 ml of gel base to make 10% black cumin nanoemulgel, and an 1,5 grams of black cumin nanoemulsion is diluted with 8,5 ml gel base to make 15% black cumin nanoemulgel. Tooth extraction was conducted on the lower left incisor teeth of male Wistar rats under ketamine-xylazine anaesthesia, using a 1 mL syringe with a short needle measuring 26Gx1/2 inch. Ketamine-xylazine solution was chosen because it provides a short onset of action. Anesthesia is administered intraperitoneally in the left or right lower quadrant of the abdomen with an injection angle of 30-45 degrees. Tooth extraction is performed with an artery clamp and careful luxation movements to prevent the tooth from fracture. Following tooth extraction, the socket is irrigated with distilled water to remove debris or tooth extraction remnants.⁷

In this research, fibroblasts were extracted from male Wistar rats' tooth sockets after tooth extraction. Hematoxylin and eosin staining for fibroblasts. Calculations were performed at 400x magnification in the apical third of the three fields of view with a V pattern, particularly on the left, middle, and right. One Way Anova Test was used as a statistical test.

RESULT

Fibroblasts were observed under a microscope at 400x magnification in three fields of view (shown in Figure 1.). The average number of fibroblasts obtained in the study is shown in Table 1 below :

Table 1. The Average Number of Fibroblast

Treatment Group	N	Mean	Standard Deviation
10% Treatment Group	5	10,9	0,8
5% Treatment Group	5	27,4	1,8
Positive Control Group	5	47,5	2,1
Negative Control Group	5	7,4	1,3

According to Table 1, the negative control group has the lowest number of fibroblasts. The highest number of fibroblasts was found in the positive control group. The black cumin nanoemulgel treatment group at 15% concentration had more fibroblasts than the black cumin nanoemulgel treatment group at 10% concentration.

The histological picture below shows the results of administering black cumin nanoemulgel at 10% and 15% concentrations, positive control, and negative control.

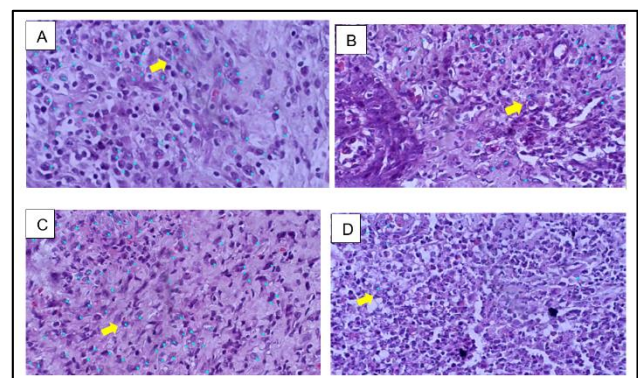


Figure 1. Histological images of the number of fibroblast after tooth extraction. 15% black cumin nanoemulgel treatment control group (A), 10% black cumin nanoemulgel treatment control group (B), Negative control group (aquades) (C), Positive control group (gengigel) (D).

Annotation:

-  : Fibroblast cells with oval or elongated nuclei and spindle shape (contrast).
-  : Fibroblast cells with oval or elongated nuclei and spindle shape.

Table 2. The Result of the Normality Test (Shapiro-Wilk)

Treatment Group	Sig. (P)	Annotation
10% Treatment Group	0,09	Data is Normally Distributed
15% Treatment Group	0,09	Data is Normally Distributed
Positive Control Group	0,29	Data is Normally Distributed
Negative Control Group	0,54	Data is Normally Distributed

Table 2 shows the result of the normality test which demonstrates that each of the treatment group has a p-value greater than 0,05, indicating that each data is normally distributed.

Table 3. The Result of the Homogeneity Test (Levene's Test)

Levene's Test	Sig.	Annotation
	0,220	Data Variance is Homogenous

Table 3 shows the homogeneity test results. The homogeneity test results revealed $p > 0,05$ ($p=0,220$), indicating that the research findings data were homogeneous. Because the data was homogeneous and all groups were normally distributed, hypothesis testing was performed using the One-Way ANOVA parametric test.

Table 4. The Result of One-Way ANOVA Test (Comparative Test in One Group)

Treatment Group	Sig. (P)	Sig. 0,05	Annotation
10% Treatment Group	0,000	$p < 0,05$	Significantly different
15% Treatment Group			
Positive Control Group			
Negative Control Group			

Table 4 shows the results of the One-Way ANOVA test, indicating there is a significant difference in the number of fibroblasts between the research groups with a p-value < 0.05 ($p=0.000$). A Post Hoc LSD test was then performed to determine the differences between the two groups.

Table 5. The Result of Post Hoc LSD Test (Comparative Test between 2 Groups)

Treatment Group	10% Concentration	15% Concentration	Positive Control	Negative Control
10% Concentration		0,000	0,000	0,003
15% Concentration			0,000	0,000
Positive Control				0,000
Negative Control				

Table 5 shows the results of the post hoc LSD test. The test results between treatment groups on the expression value of the number of fibroblasts revealed there was a significant difference in the number of fibroblasts ($p < 0,05$) between groups.

DISCUSSION

According to this research, administering black cumin (*Nigella sativa*) nanoemulgel could affect the number of fibroblasts in post-tooth extraction wounds. The data analysis results in Table 1 shows that the positive control group had the most fibroblasts, and the 15% black cumin (*Nigella sativa*) nanoemulgel treatment group had more fibroblasts than the 10% black cumin (*Nigella sativa*) nanoemulgel treatment group.

Following tooth extraction, a wound-healing process needs to occur. The wound healing process can be accelerated by using a topical medication available in the market, such as gengigel, which contains 0.2% hyaluronic acid. In the early stages of wound healing,

hyaluronic acid forms a temporary structure in the wound tissue that aids in the diffusion of nutrients and cells while also acting as an anti-inflammatory. The hyaluronic acid content of gengigel has been shown to promote fibroblast cell proliferation and hasten wound healing after tooth extraction.⁷

The findings of this study are consistent with previous research by Mastuti et al¹¹ who concluded that black cumin (*Nigella sativa*) ointment could increase the number of fibroblasts in incision wounds in mice. Wulandari et al¹⁸ discovered that a gel formulation containing 10% black cumin extract could increase the number of fibroblasts applied to gingival wounds. According to Septiana. L¹⁹ using black cumin oil nanoemulgel preparations on ulcerative wounds increased the number of fibroblasts. Previous research by Mastuti et al¹¹ demonstrated that the use of 40% and 60% black cumin extract ointment could increase the number of fibroblasts in mouse wounds, with the number of fibroblasts being higher in the 40% black cumin extract ointment treatment group on day 7 because the wound-healing process takes less time and many fibroblasts have been converted into fibrocytes and then into collagen.

Black cumin nanoemulgel (*Nigella sativa*) contains saponins, flavonoids, and thymoquinone, which can increase the number of fibroblasts. The saponin compound found in black cumin aids wound healing by producing Vascular Endothelial Growth Factor (VEGF). The flavonoid content of black cumin is known to increase the amount of collagen and stimulate macrophage activity to trigger the epithelialization process, increase the production of extracellular matrix, growth factors, cytokines, and angiogenesis through the release of growth factors such as Keratinocyte Growth Factor (KGF).²⁰

Thymoquinone, another component of black cumin (*Nigella sativa*), can activate B and T lymphocytes. T lymphocytes will promote the development of cytotoxic and helper T cells, which will phagocytose inflammatory cells and

hasten the healing of wounds, particularly during the inflammatory stage.²¹

The form of the nanoemulgel, which is an emulgel prep in the form of nanoparticles made with a nanotechnology system and small particles²², can also have advantageous effects. The smaller the particle size, the easier it is for the substance to penetrate the skin.¹⁴

The LSD post hoc test results revealed that there were significant differences between the negative control group, the positive control group, and the black cumin (*Nigella sativa*) nanoemulgel treatment group at 10% and 15% concentrations. This research found that black cumin nanoemulgel (*Nigella sativa*) has an effect on the number of fibroblasts in post-tooth extraction wounds. This is proven by a significant difference in the number of fibroblasts in wound healing after tooth extraction between the 10% and 15% black cumin (*Nigella sativa*) nanoemulgel treatment groups versus the negative control group. The positive control group obtained the highest fibroblast results in this study because the amount of gengigel given to each treatment was 0.1 ml, so the active ingredient content of the gengigel was greater than that of the black cumin nanoemulgel, which was administered at 0.02 ml for each treatment.

This study concluded that Black cumin nanoemulgel (*Nigella sativa*) concentration of 15% could increase the number of fibroblasts in wound-healing process following tooth extraction in mice. Black cumin nanoemulgel concentration 15% have the potential to accelerate wound healing process by increasing the number of fibroblasts.

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