

Induction effect combination of *Nigella sativa* extract and bovine bone graft to the area of woven bone on the preservation socket post-tooth extraction

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Abstract

Background: Tooth extraction is a common dentistry procedure followed by alveolar bone resorption. Trauma that occurs in tooth extraction will induce exaggerating inflammatory process, leads to increased alveolar bone resorption. Bone resorption can be minimized by administering a combination of *Nigella sativa* extract and bovine bone graft. The combination material is expected to increase the woven bone area formation and speed up the alveolar bone remodeling process.

Purpose: To determine the effect of induction of a combination of *Nigella sativa* extract and bovine bone graft in the formation of the woven bone area on tooth extraction sockets.

Methods: The lower left incisor of *Cavia cobaya* was extracted and the socket was given materials according to their group, namely, control group, bovine bone graft group, *Nigella sativa* extract group, and a combination of *Nigella sativa* extract and bovine bone graft group. *Cavia cobaya* was sacrificed on day 7 and day 14. Histological observation was carried out with hematoxylin-eosin staining.

Results: The highest formation of the woven bone area resulted from the group that was treated with a combination of *Nigella sativa* extract and bovine bone graft, both on day 7 and day 14.

Conclusion: The combination of *Nigella sativa* extract and bovine bone graft is able to increase the formation of woven bone area in the tooth extraction socket.

Keywords: *Nigella sativa*; Bovine bone grafts; Woven bones; Socket preservation; Medicine

1. Introduction

Trauma that occurs in tooth extraction procedures will amplify the inflammation process, resulting more alveolar bone resorption. Such resorption can be minimized with the preservation socket method. The preservation socket aims to compensate biological resorption of the buccal bone wall, maintain bone volume and bone structure so that it can function optimally and obtain good esthetics. [1,2]. Bone graft is a material that can stimulate bone healing because it has osteogenic, osteoinductive and osteoconductive properties (Nguyen Ngoc Hung, 2012). Bovine bone graft is a xenograft that has a microstructural composition similar to that of humans. In addition, bovine cartilage bone xenograft also contains high levels of calcium and phosphorus [4]. Along with the development of science and technology, several natural ingredients have been extensively researched and used in dentistry to reduce inflammatory responses and accelerate the osteogenic process so as to prevent excessive resorption and stimulate bone formation by combining them with bone grafts (Kresnoadi, et al. 2020; Kresnoadi et al. 2019). Another natural ingredient that has been used in

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various civilizations around the world for centuries to treat various human and animal diseases is *Nigella sativa* (Yimer et al. 2019). Several studies related to *Nigella sativa* stated that the content of thymoquinones has an anti-inflammatory effect that is equivalent to diclofenac. Besides having an anti-inflammatory effect, *Nigella sativa* also has an antioxidant effect, by reducing oxidative stress and increasing the activity of antioxidant enzymes which will reduce free radicals [8].

This study was conducted to determine the effect of induction of the combination of *Nigella sativa* extract and bovine bone graft on woven bone area of tooth socket.

2. Material and methods

This research was approved by the Ethics Committee of the Faculty of Dentistry, Universitas Airlangga with certificate number 566/HRECC.FODM/VIII/2022. This research was a true experimental laboratory study, post-test only control group design. The animal research for this study was *Cavia cobaya* with further details as follows: healthy, active, weighing around 300-350 grams, and 3 - 3.5 months old.

The production of *Nigella sativa* extract was carried out at the Surabaya Industrial Research and Consultation Center. One kilogram of *Nigella sativa* seeds was dried under the heavy sun for 4 hours and crushed with a grinder. The seed fragments were then added 2 litres of 96% ethanol and shaken for 2x24 hours to obtain clear blackish liquid. The process was continued by putting the liquid into the evaporator to separate it from the ethanol, in order to obtain a viscous brownish liquid, at a temperature of 50 – 60 °C.

56 *Cavia cobaya* was grouped into 4 groups; control group was received 25 grams of polyethylene glycol (PEG), *Nigella sativa* group was received 0.5 grams of extract and 24.5 grams of PEG, bovine bone graft group was received 0.5 grams of graft and 24.5 grams of PEG, and combination group was received 0.5 grams of *Nigella sativa* extract, 0.5 grams of bovine bone graft and 24 grams of PEG. *Cavia cobaya* was anesthetized intramuscularly with ketamine at a dose of 20 mg/300 mg body weight. Extraction was performed carefully on the left lower incisor. The socket was then irrigated using sterile distilled water. whose lower left incisor teeth had been extracted were divided into 8 groups, each group consisting of 7 individuals. Each groups received materials according to their group as much as 0.1 ml. Suturing of post-extraction socket was carried out with polyamide monofilament sewing thread. Each group was sacrificed on day 7 and day 14.

Cavia cobaya jaws were cut for decalcification process using ethylenediaminetetraacetic acid (EDTA) for 52 days. Histology examination was done using Hematoxylin-Eosin (HE) staining. Samples were observed under a light microscope to measure the trabecular surface area of the alveolar bone with 400x magnification equipped with a Leica stereo microscope camera and Image Raster 4.1 software. The data were analyzed with the Shapiro-Wilk test, Levene Test, Kruskal-Wallis Test and Mann-Whitney Test (Social Sciences software (SPSS) 24.0 edition (SPSS™, Chicago, United States).

3. Results

Mean value and standard deviation of woven bone area on day 7 and day 14 can be seen on Figure 1. The highest average value of woven bone area was found in the *Nigella sativa* + bovine bone extract group on day 7, while the lowest value was found in the control group on day 7. If the results was compared between day 7 and day 14 treatment group, the average woven bone area in the treatment group tended to increase compared to the control group.

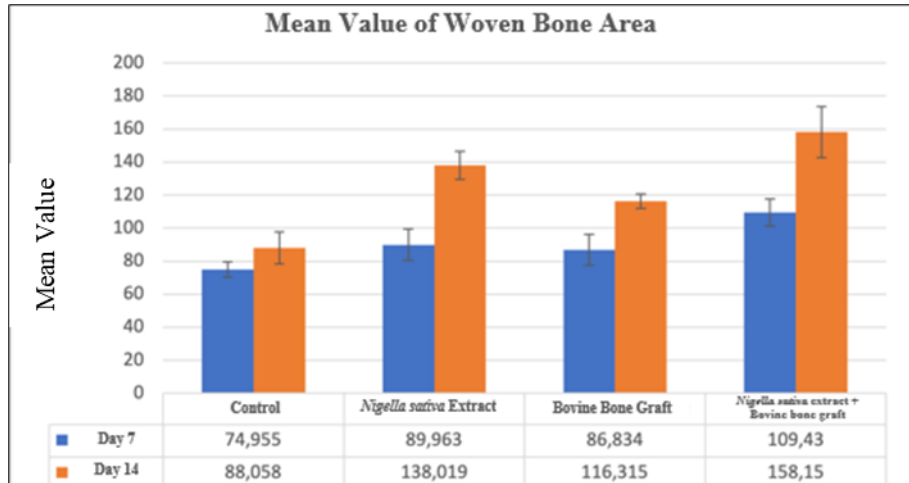


Figure 1 Chart of mean value and standard deviation of woven bone area on day 7 and day 14

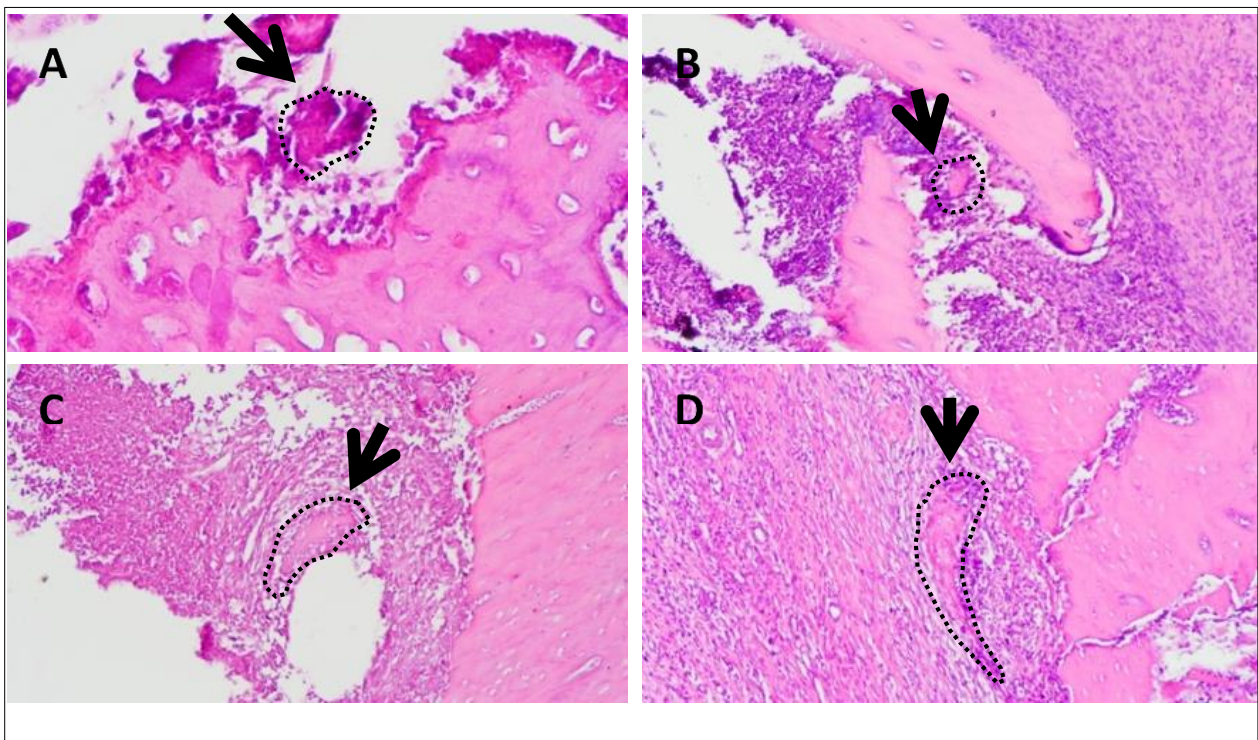


Figure 2 Histological view on the tooth sockets of *Cavia cobaya* in each group on day 7. (A) Control group; (B) Bovine bone graft group, (C) *Nigella sativa* extract group; (D) Combination of bovine bone graft + *Nigella sativa* extract. The arrows indicate the woven bone area

Based on the Kruskal-Wallis test, the p value was 0.000 ($p < 0.05$). Further details was explained by Mann-Whitney test, which there was a significant difference on day 7 and day 14 (p value = 0.002) between the control group and the combination group.

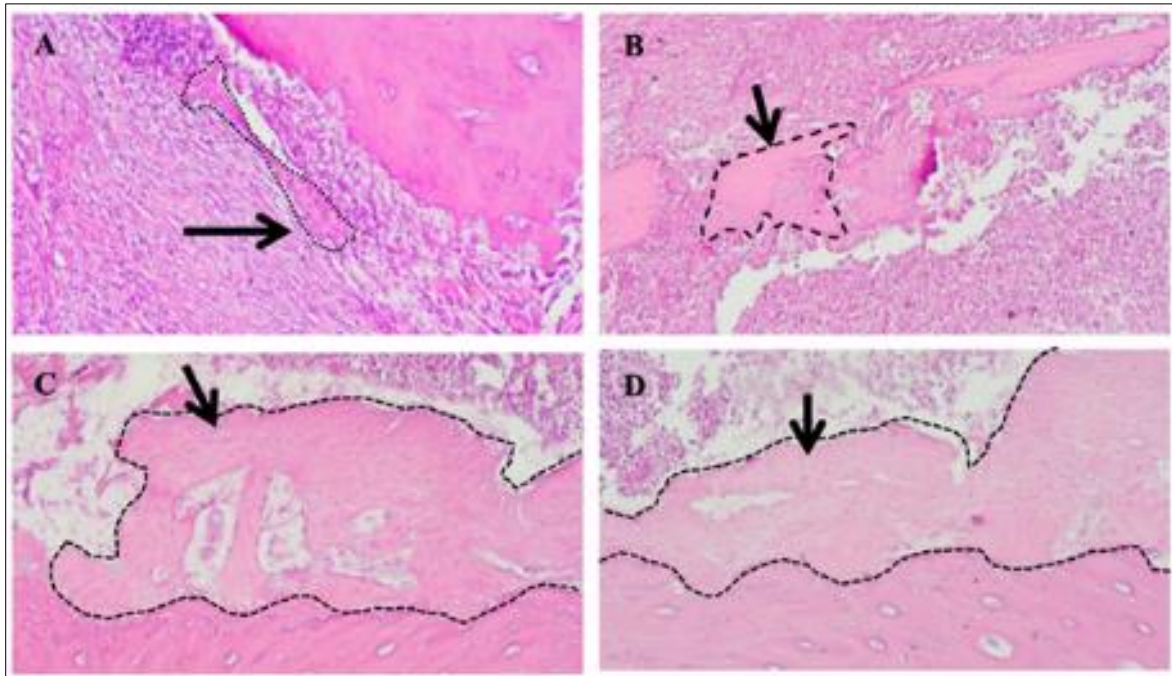


Figure 3 Histological view on the tooth sockets of *Cavia cobaya* in each group on day 14. (A) Control group; (B) Bovine bone graft group, (C) *Nigella sativa* extract group; (D) Combination of bovine bone graft + *Nigella sativa* extract. The arrows indicate the woven bone area

4. Discussion

Alveolar bone is one of the most unstable structures of the periodontium because it undergoes a continuous remodeling process due to its high sensitivity to external mechanical stimuli. The presence or absence of teeth affects the strength of the alveolar bone itself in terms of bone quantity and bone quality. Hence, following tooth extraction, a series of events triggers cellular and morphological changes in the architectural characteristics and dimensions of the alveolar bone. These changes include changes in bone density and bone dimensions which decrease significantly due to bone resorption after tooth extraction [9].

After extraction there is progressive and irreversible atrophy of the alveolar bone, with the greatest changes occurring in the first 3-12 months post extraction, with a loss of nearly 40% in height and 60% in width. [10]. The process of bone resorption starts from the trauma that occurs in tooth extraction which will induce processes of inflammation, proliferation, and remodeling. During the inflammatory phase, inflammatory mediators such as tumor necrosis factor alpha (TNF- α), interleukin-1 β (IL-1 β), and osteoclasts will present, which cause proliferation of nuclear factor kappa-B receptor activators (RANK) and nuclear factor receptors. kappa-B ligand (RANKL).

The results showed that the average woven bone formed on day 14 was higher than day 7. The highest woven bone area on day 14 was the combination group, followed by *Nigella sativa* extract group, while the control group showed the lowest results. This could be due to bovine bone grafts characteristics that allows bone cells to grow around and within the socket. [11]. Bovine bone graft contains bovine bone matrix protein which according to research by Musson et al (2019) has been shown to have an osteoconductive role in the anabolic and catabolic processes of bone formation.

In the group receiving *Nigella sativa* extract, woven bone on day 7 and day 14 was produced the most. The result from combination group was differed significantly compared to the control group, and even had better results when compared to the bovine bone graft group. *Nigella sativa* seeds have a broad spectrum of pharmacological activity including immunopotential and antihistamine, antidiabetic, antihypertensive, anti-inflammatory and antimicrobial activity. It is known as a source of thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene, nigellicimine and nigellicimine-N-oxide, -pinene and thymol and others [12]. Several studies say that thymoquinone has a role in bone healing and prevention of osteoporosis [13].

Apart from thymoquinone, a study by Topcagic et al (2017) also stated that *Nigella sativa* has a high flavonoid content, which contains 8 flavonoids (apigenin, chrysin, galangin, kaempferol, myricetin, naringenin, pinocembrin, and

quercetin). Flavonoids are secondary metabolites that have many biological activities. Many studies reveal that flavonoids can modulate potential self-regeneration and osteogenic differentiation by targeting several signaling pathways such as the Wnt/ β -catenin pathway, the ERK pathway, the PI3K/Akt pathway, and regulate bone-specific markers and transcription factors including ALP, RUNX2, BMP-2, Cbfa1, Osx [14–16].

In the group given the combination of *Nigella sativa* extract and bovine bone graft, they had the highest bone area compared to the other three groups. *Nigella sativa* plays a role in accelerating the inflammatory process due to its content of thymoquinone, flavonoids and alkaloids which are known as anti-inflammatory effects [8,17]. In addition, the content of *Nigella sativa* also has a bone healing effect which can accelerate bone healing and support osteoconductive activity of bovine bone grafts [13,18] so that the combination group of *Nigella sativa* + bovine bone graft was the group with the highest formation of woven bone among the other groups. From the research results, it is evident that the combination of osteoconducting and induction properties of the two materials above significantly increases the formation of woven bone. This proves that the greater the chance of post-revocation socket preservation success.

5. Conclusion

The combination of *Nigella sativa* extract and bovine bone graft was effective in increasing the formation of woven bone in the *Cavia cobaya* tooth socket on day 7 and day 14.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

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