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(RESEARCH ARTICLE)

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Induction effect of Nigella sativa combined with bovine bone graft on osteoblast and osteoclast formation in post extraction tooth socket

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Abstract

Background:. Poor healing of alveolar bone after traumatic tooth extraction procedure increases the difficulty of treatments, especially in the field of prosthodontic. Therefore, adequate socket preservation of alveolar bone is beneficial. *Nigella sativa* (NS) and bovine bone graft (BBG) is expected to reduce osteoclast and increase osteoblast as a marker of alveolar bone healing.

Purpose: This study was aimed to observe the effect of NS combined with BBG on osteoclast and osteoblast count on post-extraction socket of alveolar bone.

Methods: Lower incisors of fifty-six *Cavia cobaya* were extracted and divided into four groups. First group was control group, second group was given BBG, third group with NS, and fourth group with NS and BBG combination. *Cavia cobaya* was terminated on the 7th and 14th day for further analysis of alveolar socket bone. Histological examinations of osteoclast & osteoblast were measured with a 400x magnification light microscope. One-way ANOVA and Tukey HSD tests were performed to analyze the data statistically.

Results: There was a significant difference in osteoclast and osteoblast count between the groups. Both lowest mean of osteoclast and highest mean of osteoblast was found in the NS-BBG combination group on 7th and 14th day.

Conclusion: The combination of NS and BBG can effectively decrease osteoclast and increase osteoblast count on post-extraction socket of the alveolar bone.

Keywords: *Nigella sativa*; Osteoclast; Osteoblast; Socket preservation; Medicine

1. Introduction

Tooth extraction is a common practice in dentistry which indicated when a tooth is not possible to be restored or maintained for a long-term health function and aesthetic. Tooth loss impact is associated with quality-of-life impairment such as ability to masticate, speak, and socialize [1]. It is estimated that post-extraction tooth socket will experience up to 50% reduction of original ridge width [2,3]. Inflammatory phase as a part of wound healing sequence from tooth extraction procedure causes an increase of pro-inflammatory cytokines such as Interleukin (IL)-1β and tumor necrosis factor (TNF)α. The increase of these pro-inflammatory cytokines results in the proliferation of receptor activator of NF-κB (RANK) and receptor activator of NF-κB ligand (RANKL) which leads to increased number of osteoclast so bone resorption ensues [4–6]. Osteoblast itself is a cell that responsible for bone formation, where osteoblast itself is a

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modified fibroblast. Fibroblast received ossification-specific transcription factors, one of them is runt-related transcription factor (RUNX) 2 which will contribute to osteoblast differentiation [7].

Alveolar ridge preservation or socket preservation is a procedure to decrease bone resorption after tooth extraction. Following extraction, socket preservation is a predictable approach to minimize undesired horizontal and vertical ridge reduction [8]. Bone grafting is one of the methods to preserve post extraction socket. Xenograft is a type of bone graft derived from donors of a different species. Bovine bone graft is known to have osteoconductive properties and relatively low cost [9,10]. Bovine bone graft is needed to induce osteogenic activity to accelerate bone formation [6]

Natural ingredient, one of which is *Nigella sativa* is known for its extensive medical from its main active component, thymoquinone. Thymoquinone is known to have remarkable properties, including anti-inflammatory, antioxidant, analgesic, anticancer, and antibacterial [11][12,13]. The properties of *Nigella sativa* and active component can be combined with bovine bone graft in hopes to improving the process of bone formation. This research was conducted to determine the effect of induction combination between *Nigella sativa* and bovine bone graft in post-extraction tooth socket on osteoblast and osteoclast count.

2. Material and methods

This investigation constituted experimental laboratory research which received ethical approval from the Ethics Committee, Faculty of Dental Medicine of our university, number 540/HRECC.FODM/VIII/2022. This research was categorized as a true experimental laboratory research. The study design used was a post-test only control group design using 56 healthy and active males *Cavia Cobaya* weighing around 300-350 grams, and aged around 3-3.5 months old.

The manufacture of *Nigella sativa* extract was carried out at the Surabaya Industrial Research and Consultation Centre, East Java, Indonesia. A kilogram of *Nigella sativa* seeds was dried for 4 hours under the sunlight and crushed with a grinder to turn them into smaller pieces. The *Nigella sativa* seed fragments were added with 2 liter of 96% ethanol and shaken for 2x24 hours. The results were filtered and a clear black liquid was obtained. The *Nigella sativa* filtrate was put into the evaporator to separate it from the ethanol, in order to acquire viscous brownish liquid at a temperature of 50 – 60 °C.

Cavia cobaya was anesthetized intramuscularly with ketamine at a dose of 20 mg/300 mg body weight. Tooth extraction was performed on the left lower incisor with the same motion, direction and force. After tooth was successfully pulled out completely, the socket was irrigated using sterile distilled water. The material added to the socket was according to the *Cavia cobaya* group. Control group was given only 25 gram of polyethylene glycol (PEG), BBG group was given a combination of 0.5 gram of bovine bone graft and 24.5 gram of PEG, NS group was given 0.5 gram of NS extract and 24.5 gram of PEG, and NS-BBG group was given combination of 0.5 gram of NS extract, 0.5 gram of BBG, and 24 grams of PEG. All material was injected as much as ± 0.1 ml using a blunt-tip syringe. The post extraction socket wound area was sutured with polyamide monofilament sewing thread. All groups were terminated on the 7th day and the 14th day.

The subject jaws were removed for decalcification using ethylenediaminetetraacetic acid (EDTA) for 52 days. The tissue was then prepared with paraffin block preparations for histology examination (Haematoxylin-Eosin (HE) staining). Samples were observed under light microscope to count osteoblast and osteoclast cells with 400x magnification and 9 fields of view. The observed area was counted manually with average results per field of view. The result data obtained were analyzed with the Shapiro-Wilk test, Levene test, One-way ANOVA test and Tukey high significant difference (HSD) test.

3. Results

3.1. Day 7

The histological examination identified the presence of osteoblast and osteoclast cells on 7th day. Osteoblast cells had a cuboidal or polygonal cell appearance that was found along the surface or edges of bone (Figure 1). The osteoclast cells appeared as a large dome-shaped multinuclear cell composed of fused mononuclear osteoclast progenitor cells (Figure 2).

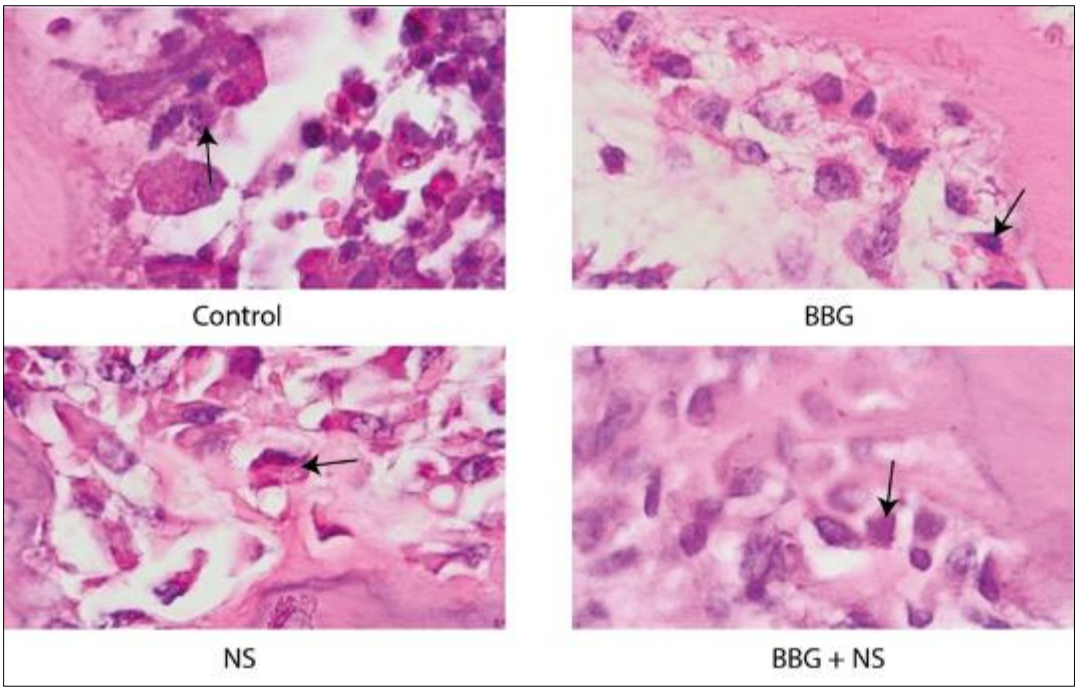

Figure 1 Black arrows identifying osteoblast on 7th day

HE staining observed through a light microscope at a magnification of 400x.

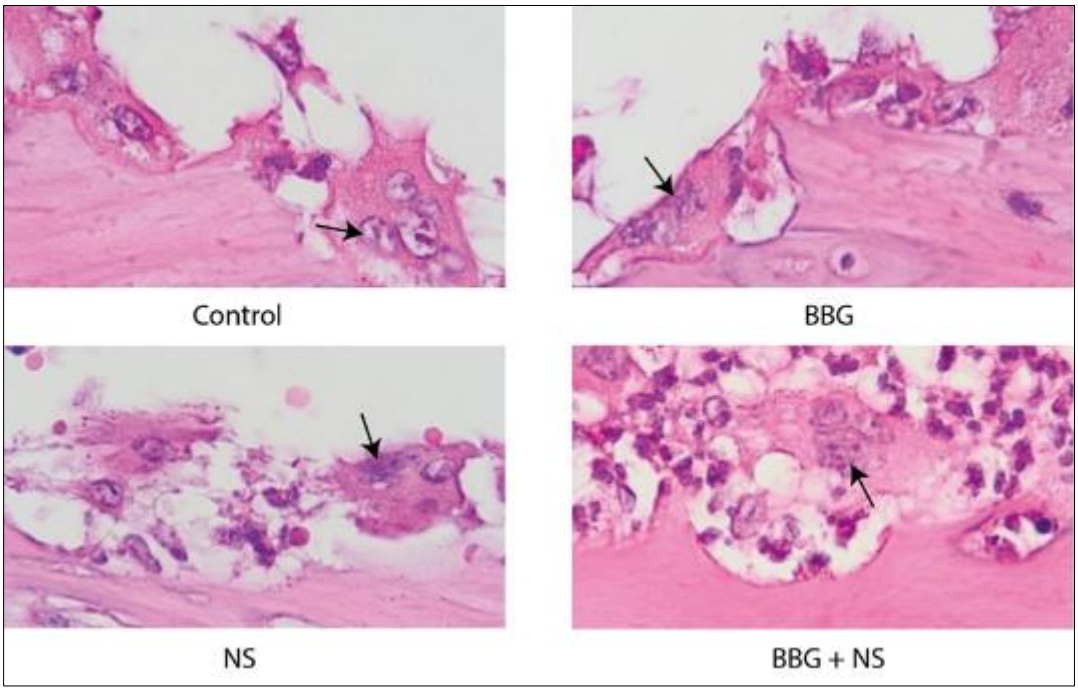

Figure 2 Black arrows identifying osteoclast on 7th day

HE staining observed through a light microscope at a magnification of 400x.

The result of osteoblast and osteoclast cells mean value and standard deviation in histological observation on 7th day of examination were shown in Figure 3 and Figure 4. The highest average value of osteoblast on the 7th day was found on NS-BBG combination group. While the lowest value of osteoblast was found on control group. On the other hand, the lowest average for osteoclast on the 7th day was found on NS-BBG combination group, and the highest average was found on control group. Statistical analysis was done prior to the test results for each group. One-way ANOVA test

showed significancy value of 0.000. Tukey HSD test showed that the mean value of osteoblast and osteoclast cells of NS-BBG combination group was differed significantly compared to control group and BBG group, but not to NS group (Table 1).

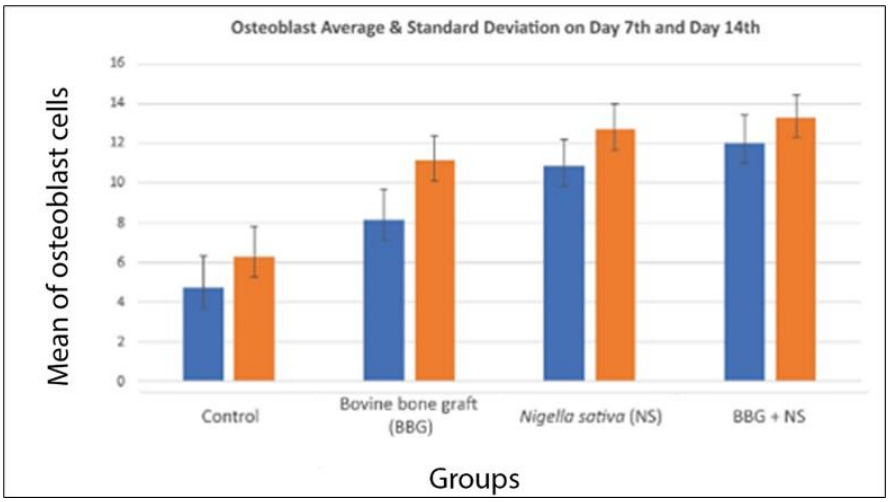

Figure 3 Bar chart of osteoblast count average and standard deviation on 7th and 14th day for control, BBG group, NS group, and NS-BBG combination group

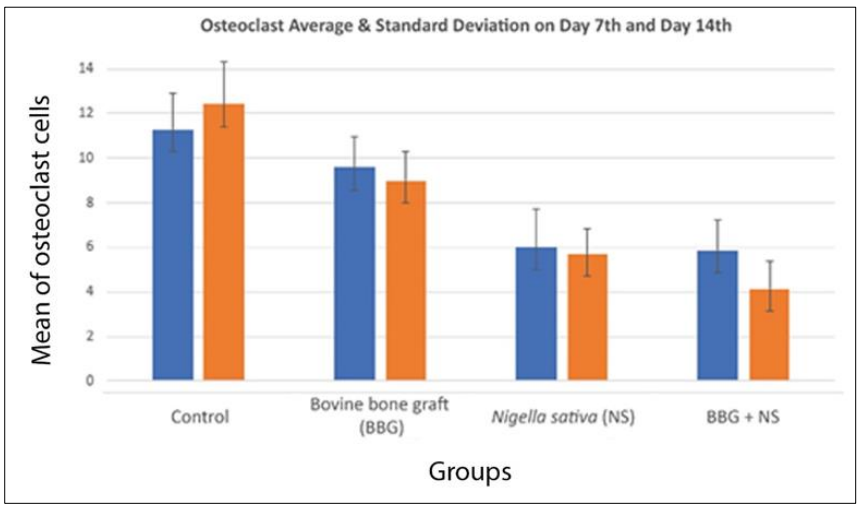

Figure 4 Bar chart of osteoclast count average and standard deviation on 7th and 14th day for control, BBG group, NS group, and NS-BBG combination group

Table 1 Statistical analysis data on the quantity of osteoblasts and osteoclasts in each treatment group on 7th day

	Group	**Mean ± SD**	**Normality Test**	**Homogeneity Test**	**One-way ANOVA Test**	**Tukey HSD Test**			
						1	2	3	4
Osteoblast	1	4.71 ± 1.604	0.224	0.891	0.000	-	*	*	*
	2	8.14 ± 1.574	0.110			*	-	*	*
	3	10.86 ± 1.345	0.873			*	*	-	-
	4	12.00 ± 1.414	0.752			*	*	-	-

Osteoclast	1	11.29 ± 1.604	0.224	0.371	0.000	-	-	*	*
	2	9.57 ± 1.397	0.064			-	-	*	*
	3	6.00 ± 1.732	0.240			*	*	-	-
	4	5.86 ± 1.345	0.873			*	*	-	-

* = significant difference ($p<0.05$)

Note: Group 1: control group on 7th day; group 2: BBG group on 7th day; group 3: NS group on 7th day; group 4: NS-BBG group on 7th day.

3.2. DAY 14

On the 14th day, osteoblast and osteoclast cells were also found. along the surface or margins of bones. Osteoblast cells, which have a cuboidal or polygonal cell morphology, could be easily observed (Figure 5). The multinuclear osteoclast cells, which were made up of fused mononuclear osteoclast progenitor cells, had the appearance of big dome-shaped cells (Figure 6).

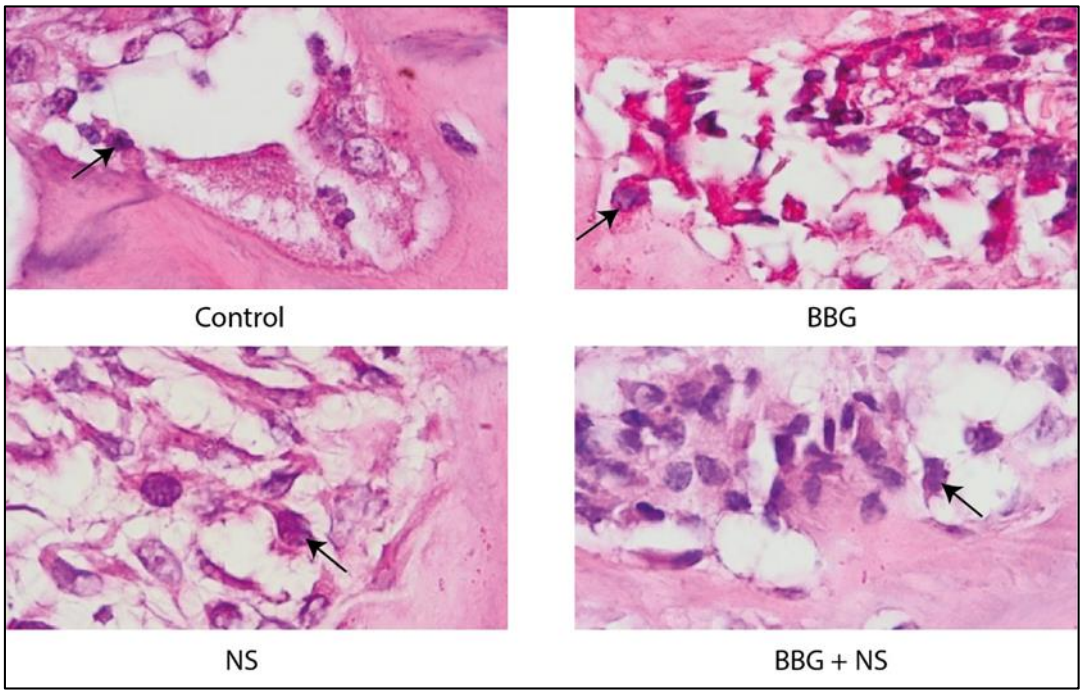

Figure 5 Black arrows identifying osteoblast on day 14

HE staining observed through a light microscope at a magnification of 400x.

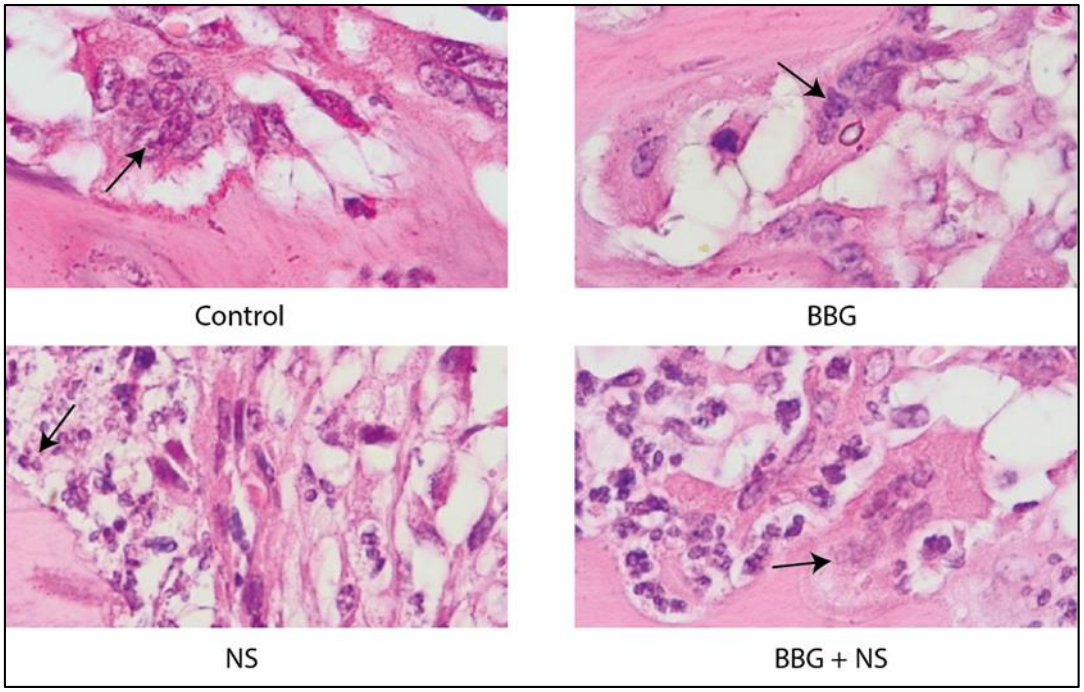

Figure 6 Black arrows identifying osteoclast on day 14

HE staining observed through a light microscope at a magnification of 400x.

Figures 3 and 4 illustrated the average value and standard deviation of osteoblast and osteoclast activity during the 14th day of histological observation. The NS-BBG combination group had the highest average value of osteoblast on day 14, while the control group had the lowest osteoblast level. On the other hand, the control group had the highest average and the NS-BBG combination group had the lowest average for osteoclast on the 14th day.

Prior to each group test results, a statistical analysis was conducted. When comparing the control and treatment group, the one-way ANOVA test showed significance value of 0.000. Tukey HSD test showed that the mean value of osteoblast cells of NS-BBG combination group was differed significantly compared to control group only. Meanwhile, osteoclast cells found on NS-BBG combination group was differed significantly compared to control and BBG group (Table 2).

Table 2 Statistical analysis data on the quantity of osteoblasts and osteoclasts in each treatment group on 14th day

	Group	Mean ± SD	Normality Test	Homogeneity Test	One-way ANOVA Test	Tukey HSD Test			
						5	6	7	8
Osteoblast	5	6.29 ± 1.496	0.591	0.891	0.000	-	*	*	*
	6	11.14 ± 1.215	0.147			*	-	-	-
	7	12.71 ± 1.254	0.062			*	-	-	-
	8	13.29 ± 1.113	0.482			*	-	-	-
Osteoclast	5	12.43 ± 1.902	0.404	0.371	0.000	-	*	*	*
	6	9.00 ± 1.291	0.819			*	-	*	*
	7	5.71 ± 1.113	0.482			*	*	-	-
	8	4.14 ± 1.215	0.147			*	*	-	-

* = There is a significant difference ($p<0.05$)

Note: Group 5: control group on 14th day; group 6: BBG group on 14th day; group 7: NS group on 14th day; group 8: NS-BBG group on 14th day.

4. Discussion

The *Nigella sativa* contained thymoquinone, flavonoid, alkaloid, and saponin. These active constituents played a role in both bone resorption and bone regeneration. Thymoquinone significantly reduce the synthesis of IL-1β, TNF-α, and cyclooxygenase-2 (COX-2) which play a critical role in inflammation [14]. These reduction of pro-inflammatory cytokines results in the reduction of osteoclast formation either directly or indirectly through RANKL, resulting in differentiation and fusion of osteoclast precursors into osteoclasts [15].

Flavonoid in *Nigella sativa* contained high amount of quercetin and kaempferol [16]. Quercetin has the ability to increase osteogenic differentiation by increasing bone morphogenic protein (BMP)-2 and transforming growth factor (TGF)-β1, meanwhile kaempferol has similar osteogenic induction potential with quercetin through increasing cell viability, alkaline phosphatase (ALP) activity, and enhancing calcium mineralization of periodontal ligament stem cells [17]. Yuan et al. (2018) also found that quercetin reduce bone resorption by inhibiting the activation of nuclear factor-kappa B (NF-κB) by TNF-α and the degradation of β-catenin [18]. The enhanced osteogenic activities from flavonoid increased bone regeneration that results in increased amount of osteoblast (Wu et al. 2016). These effects of *Nigella sativa* on wound healing may be the cause of the increased amount of osteoblast and decreased amount of osteoclast on group treated with *Nigella sativa* compared to the control group on both 7th day an 14th day.

Statistical analysis shows that group treated with *Nigella sativa* combined with bovine bone graft has the most reduction on osteoclast and the most increment on osteoblast compared to all other group both in 7th day and 14th day. This occured due to synergistic effect between *Nigella sativa* and bovine bone graft in enhancing bone regeneration and reducing bone resorption. The bone regenerating effects of *Nigella sativa* worked through its active flavonoid content, namely quercetin and kaempferol to promote osteogenic effect which promoted osteoblast amount on alveolar wound healing [16,17]. This bone regenerating effect also enhance by bovine bone graft properties, both were able to increase osteoblast cells count through promoting wound-healing macrophage amount (Shi et al. 2018). The increase of osteoblast leads to increased amount of osteoprotegerin (OPG) which control the differentiation of monocytes by binding to the ligand of the RANKL to compete with RANK [7]. *Nigella sativa* also demonstrate bone resorption reduction by inhibiting osteoclast production through its component, mainly thymoquinone, to reduce inflammation [11,12,14]. Bone resorption also further reduced by BBG through BMP function that reduce osteoclast [21].

The group treated with *Nigella sativa* showed significantly higher number of osteoblast and lower number of osteoclast compared to the group treated with bovine bone graft both on 7th day and 14th day. This significant difference may occur because *Nigella sativa* action on socket wound healing not only through its potential as anti-inflammatory and osteogenic agent but also as antioxidant [22]. Bone defects always experience an instability in its structure and will generate a large amount of reactive oxygen species (ROS). Oxidative stress is caused by ROS activity and impair bone regeneration ability and osteogenesis [23]. Thymoquinone in *Nigella sativa* is able to inhibit oxidation through ROS scavenging effect from its quinine structure [24].

This research showed that post-extraction socket with NS-BBG combination can minimize alveolar bone resorption and promote bone regeneration in the defect site. The choice of using *Nigella sativa* and combine it with bovine bone graft in socket preservation procedure shows an important role in the process of bone remodelling. Bovine bone graft result shows osteoconductive properties which function as a support structure/ scaffold for the regrowth of new bone, which originates from osteoblasts at the base of the socket. *Nigella sativa* extract in the other hand, has shown anti-inflammatory and antioxidant properties, and also accelerates bone regeneration. healing. The research results shown evidence that the induction of *Nigella sativa* extract combined with bovine bone graft significantly increases osteoblast amount and reduces osteoclast amount in post extraction socket.

5. Conclusion

The induction of *Nigella sativa* combined with bovine bone graft on post-extraction tooth socket in *Cavia cobaya* is effective to significantly increase osteoblast count and reduce osteoclast count on 7th and 14th day.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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