Effect of Fenugreek Seed Extract on Osteoblasts, Osteoclasts, and Neocapillaries After Extraction in Hypoestrogenic Rats

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ABSTRACT

Background: *Hypoestrogen is characterized with lower level of estrogen hormone that affects wound healing following tooth extraction. In a hypoestrogenic condition, the post-extraction healing prosses is marked by the osteoblasts count, increased activation of osteoclasts, and suppressed the neocapillaries count. Fenugreek seeds with compounds such as diosgenin, quercetin, galactomannan, trigonellin and lupeol can potentially stimulate osteoblasts formation, suppress the number of osteoclasts cells, and increase the neocapillaries count. Purpose: To evaluate the influence of Fenugreek seed extract (FSE) on administration on osteoblasts, osteoclasts, and neocapillaries count in the post-extraction socket of hypoestrogenic rat teeth.*
Methods: *True experimental study and the design that was used is Post Test Only Control Group Design. Female Wistar rats were used as experimental subjects and assigned to two untreated control groups and three treatment groups receiving fenugreek seed extract at doses of 100, 200, and 400 mg/kgBW. The right maxillae of rats euthanized on days 3, 7, and 30 were processed into histological sections, stained with hematoxylin–eosin (H&E), and examined under a microscope at 400× magnification across ten fields of view.*
Results*: This study was analyzed using the Tukey post-hoc test demonstrated statistically significant differences in osteoblast numbers, osteoclast counts, and neocapillary density ($p < 0.05$).*
Conclusion: *Fenugreek seed extract affects increasing the osteoblasts, decreasing the osteoclasts, and enhance neocapillary development following tooth extraction in hypoestrogenic White Wistar rats.*

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INTRODUCTION

Tooth extraction is a common dental procedure that causes injury to both soft and hard tissues, initiating a wound healing process consisting of hemostasis, inflammation, proliferation, and remodeling phases[1,2]. Successful healing is essential to prevent complications, yet it can be significantly affected by systemic factors, particularly estrogen levels.

Postmenopausal women represent a significant proportion of dental patients requiring tooth extraction, with estimates suggesting that approximately 50% of women over the age of 51 experience estrogen deficiency, and studies have reported that postmenopausal status is associated with a higher risk of impaired post-extraction healing and complications such as delayed bone regeneration and dry socket[3]. In hypoestrogenic conditions, such as postmenopause, wound healing is impaired due to disrupted balance between pro-inflammatory and anti-inflammatory mediators, leading to delayed bone formation and increased bone resorption[4]. Estrogen deficiency also negatively impacts angiogenesis, reducing neocapillary formation necessary for oxygen and nutrient supply during healing[5]. Bone remodeling depends on the balance between osteoblasts, which form new bone, and osteoclasts, which resorb bone[6,7]. Hypoestrogen conditions enhance osteoclast activity through dysregulation of the RANK/RANKL pathway and increased proinflammatory cytokines, resulting in excessive bone resorption[8,9].

During the healing process, osteoblasts contribute to new bone formation, while osteoclasts remove damaged bone tissue[10]. Meanwhile, angiogenesis in the proliferation phase, driven by mediators such as VEGF, is critical for forming neocapillaries that support tissue repair[11-14]. However, all these processes are compromised under low estrogen conditions, leading to delayed healing outcomes[5].

To address this issue, natural therapies such as Fenugreek (Trigonella foenum-graecum L.) have been explored. Fenugreek seeds contain bioactive compounds, including diosgenin and quercetin, which exhibit phytoestrogenic activity by binding to estrogen receptors and mimicking estradiol effects[15,16]. Additionally, Fenugreek demonstrates anti-inflammatory, pro-angiogenic, and wound healing properties, including stimulation of macrophages, fibroblast proliferation, and cytokine expression[16,17].

Therefore, this study focuses on evaluating the effect of Fenugreek seed extract on osteoblast numbers, osteoclast counts, and neocapillary formation following tooth extraction in hypoestrogenic Wistar rats, aiming to improve wound healing outcomes under estrogen-deficient conditions.

RESEARCH METHOD

Fenugreek seeds were obtained from local purchase in Malang, East Java, Indonesia. Seeds were selected based on the following criteria: uniform yellowish-brown color, free from mold or visible contamination, fully mature and dried, and confirmed to be of the species Trigonella foenum-graecum L. based on morphological characteristics. Processing into extracts was carried out at UPT Laboratorium Materia Medica, Malang. Fenugreek seeds were initially cleansed and oven-dried at 50 °C for 24 hours, then ground using an ultra centrifugal device to become 25 g of powder. The powdered material was subsequently subjected to maceration extraction — a method chosen for its ability to preserve thermolabile bioactive compounds — with 96% ethanol at a solvent-to-material ratio of 5 mL/g under room temperature conditions for a minimum duration of three days, with periodic agitation, following the standard maceration protocol described by Departemen Kesehatan Republik Indonesia[18].

The filtrate was dried with a rotary evaporator until a thick extract was obtained, then stored for 72 hours at 37°C and sterilized. According to the in-silico study of this extract, the agent that possible found in FSEE as the estrogen agonist agent are naringenin, quercetin, luteolin, dan kaempferol. These results were obtained through computational screening based on Lipinski's Rule of Five, suggesting favorable predicted oral bioavailability of the compounds, as this rule is commonly applied to assess drug-likeness according to molecular characteristics such as molecular weight[19].

The finished extract can be applied to the treatment group at a dosages of 100, 200, and 400mg /kgBW with the dilution formula % = V / V (volume / volume)[2,20]. We use this dosage because according to the previous study that describe about the dosage of FSEE of the bone mechanical properties of the rats showed that 50mg/kg is the minimal dosage and higher doses usually used in pharmacological studies respectively. To facilitate the administration of the extract, it was done using a gastric sonde and diluted by dilution technique.

This research uses a true experimental Post Test Only Control Group Design which is done in the laboratory in-vivo by comparing the experimental group with the control group at the Oral Biology Laboratory of the Faculty of Dentistry, Brawijaya University. The study samples consisted of female Wistar rats (Rattus norvegicus) aged 4–6 months with body weights ranging from 250 to 300 g. In this design there are 5 large groups determined randomly, namely 3 treatment groups and 2 control groups. Each group consisted of 5 rats per observation time point (days 3, 7, and 30), resulting in 15 rats per group and a total of 75 rats used in this study. Sample size determination was based on Federer's formula for experimental animal studies, which requires a minimum of $(t-1)(n-1) \geq 15$, where t is the number of treatment groups and n is the number of animals per group. The treatment group consisted of rats that had been ovariectomized and performed tooth extraction and given Fenugreek seed extract at dose 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW respectively. Meanwhile, the control group consisted of rats that underwent tooth extraction in the post-ovariectomy state and with or without ovariectomy, the rat's tooth extraction wound was left without sutures.

This study was previously received prior ethical clearance from the Health Research Ethic Committee of the Faculty of Medicine, Universitas Brawijaya on number 173/EC/KEPK/06/2022. Subsequently, the rats were maintained for one week before the research in a plastic box with the use of wood husks replaced every two days and the need for food and drink that must be met. After one week of maintenance, rats in the K2 control group and the experimental groups were ovariectomized to obtain hypoestrogen conditions by freeing the oviduct and ovaries from fatty tissue and surrounding connective tissue. The rats were then maintained for four weeks post ovariectomy while still meeting the needs of food and water. The 4-week post-ovariectomy maintenance period was chosen based on previous studies confirming that significant estrogen depletion and its systemic effects on bone metabolism are established within 4 weeks following ovariectomy in rats. After this period, rats in all groups had their maxillary right molar teeth carefully extracted under general anesthesia using a combination of ketamine (50 mg/kgBW) and xylazine (5 mg/kgBW) administered intraperitoneally, following the tooth extraction model in experimental animals as previously described[20]. The rat's tooth extraction wound was left without sutures. Rats that had undergone tooth extraction were given post-extraction care by administering Sagestam (gentamicin) antibiotics at a dose of 0.3 mg/kgBW to prevent post-operative infection, as gentamicin provides broad-spectrum coverage against gram-negative bacteria commonly found in the oral cavity, and Santagesik (metamizole) analgesics at a dose of 3.5 mg/kgBW to manage post-operative pain[4]. These drugs were selected due to their established safety profile and common use in rodent post-surgical care

in Indonesian experimental studies[20]. Rats in the treatment group were given Fenugreek seed extract that had been made from local Fenugreek seeds and diluted beforehand. Each treatment group received a different dosage, group P1 was administered 100 mg/kgBW, group P2 received 200 mg/kgBW, and group P3 was given 400 mg/kgBW, all delivered via gastric sonde.

All rats in the control and treatment groups were then sacrificed on days 3, 7, and 30 after extraction. These time points were selected to represent the three main phases of post-extraction wound healing: day 3 represents the early inflammatory phase characterized by blood clot formation and early granulation tissue; day 7 represents the proliferative phase in which osteoblast activation and neocapillary formation are prominent; and day 30 represents the remodeling phase characterized by mature bone formation and a decrease in osteoclast and neocapillary activityM[5]. The preparations were then viewed and histologically counted using a light microscope (Nikon Eclipse type Ei) with a magnification of 400x and 10 fields of view with the zig-zag method. Osteoblasts were identified as large, mononuclear cells with abundant basophilic cytoplasm, prominent nucleoli, and a cuboidal-to-columnar shape, typically arranged in a single layer along the bone surface. Osteoclasts were identified as large, multinucleated giant cells with eosinophilic cytoplasm located in Howship's lacunae on the bone surface. Neocapillaries were identified as newly formed small blood vessel structures lined by a single layer of endothelial cells with visible lumens containing red blood cells, located within the granulation tissue of the extraction socket. The osteoblasts, osteoclasts, and neocapillaries count that have been obtained from histological observations is then subjected to data analysis testing. Data analysis was carried out using the One-way ANOVA test followed by Tukey's Post-Hoc test to identify significant differences between groups, as recommended for parametric comparison of multiple independent groups with normal data distribution[6].

RESULTS

In this study, the number of osteoblasts, osteoclasts, and neocapillaries were quantified during wound healing following tooth extraction of Wistar strain rats in hypoestrogen state. This study showed that the highest mean number osteoblasts count was observed in treatment group 3, which received a dose of 400 mg/kgBW, reaching 13.76 ± 3.16 on day 7. Conversely, the lowest mean osteoclast count was also found in treatment group 3 at the same dose, with a value of 7.28 ± 2.08 on day 3. Additionally, the greatest mean number of neocapillaries occurred in treatment group 3 at 400 mg/kg body weight, amounting to 11.70 ± 1.31 on day 3.

The One-way Anova test revealed statistically significant variations in the number of osteoblasts, osteoclasts, and neocapillaries in all treatment groups when compared to the positive control group on days 3, 7, and 30 ($p \leq 0.05$). Tukey's Post-Hoc test showed significant differences in the number of osteoblasts, osteoclasts, and neocapillaries between each treatment group and the positive control group both on days 3, 7, and 30 ($p \leq 0.05$), with the exception of neocapillary numbers between the positive control group and treatment group 2 on day 7.

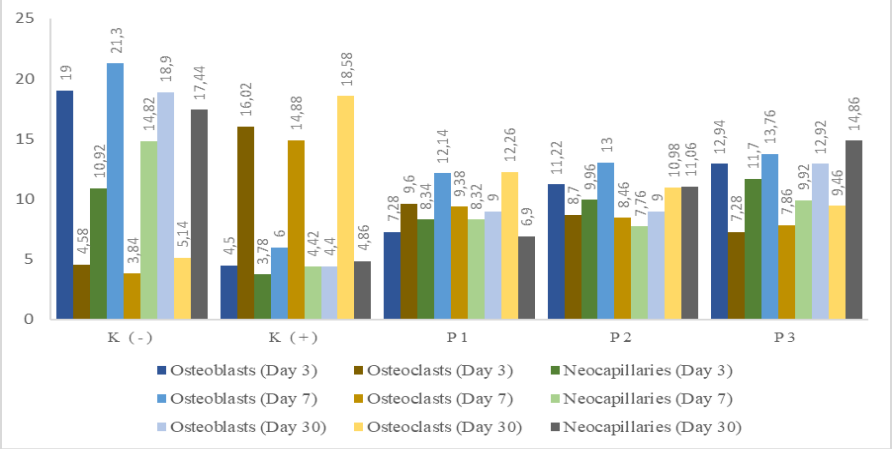

Figure 1. Mean Number of Osteoblasts, Osteoclasts, and Neocapillaries Diagram

Table 1. Results of One-way ANOVA Test of Osteoblasts Number

Observation Time	Group				p-value (One-way ANOVA)
	K(+)	P1	P2	P3	
Day 3	4.46±0.59[a]	8.88±1.26[ab]	11.22±4.09[b]	12.94±8.15[b]	0.021
Day 7	5.98±1.04[a]	12.14±2.63[b]	13.00±4.32[b]	13.76±3.16[b]	0.003
Day 30	4.44±0.92[a]	10.00±1.35[b]	12.22±2.77[b]	13.92±4.73[b]	0.000

Table 2. Results of One-way ANOVA Test of Osteoclasts Number

Observation Time	Group				*p-value (One-way ANOVA)*
	K(+)	P1	P2	P3	
Day 3	16.02±2.24[a]	9.60±1.71[b]	8.70±1.17[b]	7.28±2.08[b]	0.000
Day 7	14.88±2.14[a]	9.38±1.70[b]	8.46±2.17[b]	7.86±1.65[b]	0.000
Day 30	18.58±1.64[a]	12.26±1.31[b]	10.98±2.06[b]	9.46±2.00[b]	0.000

Table 3. Results of One-way ANOVA Test accompanied by Tukey's Post-Hoc Test of Neocapillaries Number

Observation Time	Group				*p-value (One-way ANOVA)*
	K(+)	P1	P2	P3	
Day 3	3.78±0.86[a]	8.34±1.72[b]	9.96±1.17[bc]	11.70±1.31[c]	0.000
Day 7	4.42±0.69[a]	8.32±1.58[b]	7.76±1.22[ab]	9.92±3.43[b]	0.004
Day 30	4.86±1.15[a]	6.90±1.28[a]	11.06±2.38[b]	14.86±1.32[c]	0.000

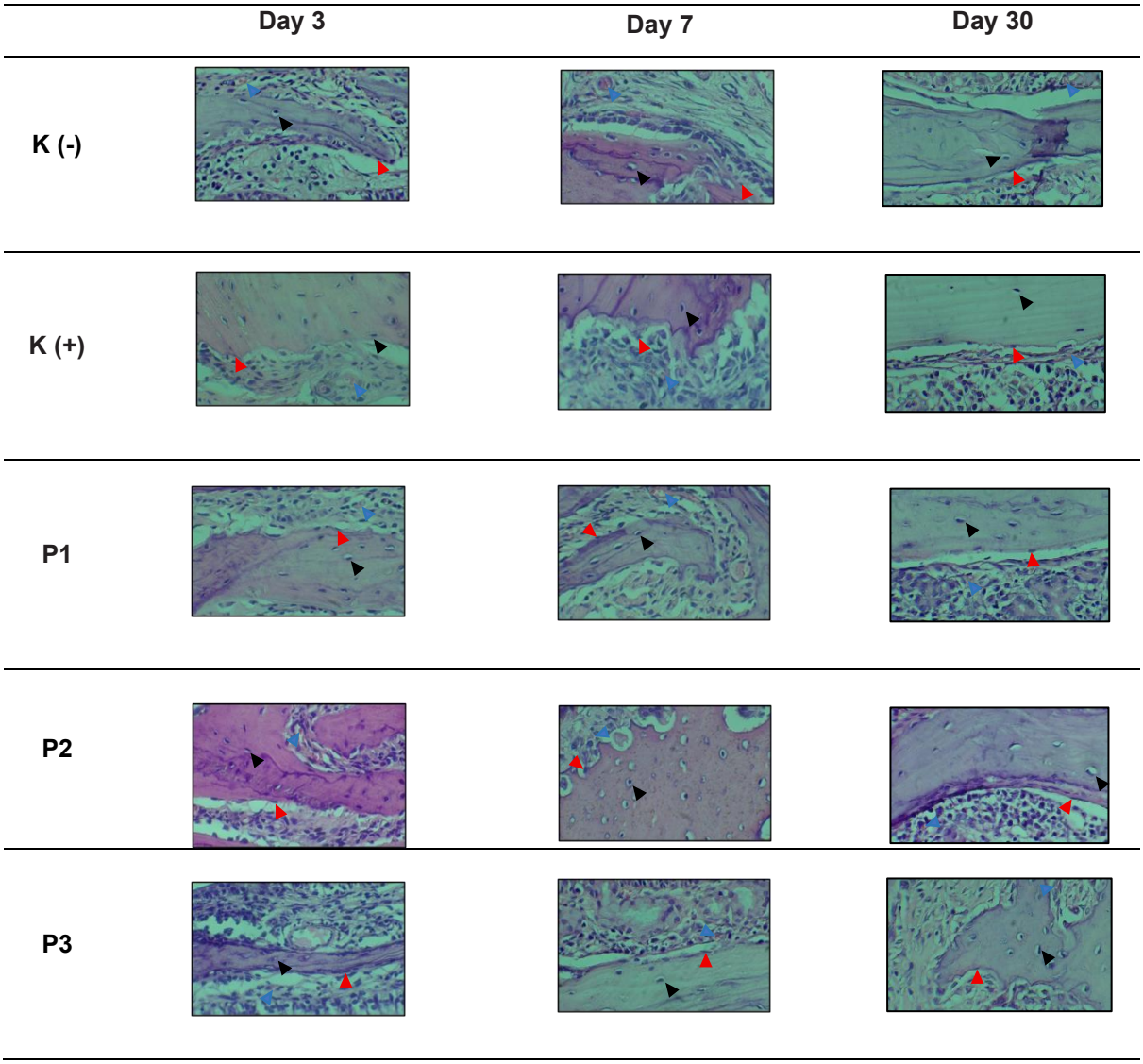

Figure 2. Results of Histological Examination of Osteoblasts, Osteoclasts, and Neocapillaries with Hematoxylin-Eosin Staining on days 3, 7, and 30 (magnification 400×). Osteoblasts (indicated by black arrows) appear as large mononuclear cells with basophilic cytoplasm and round nuclei, arranged along the bone surface. Osteoclasts (indicated by red arrows) appear as large multinucleated cells with eosinophilic cytoplasm located in resorption lacunae. Neocapillaries (indicated by blue arrows) are identified as small vascular channels lined by endothelial cells with visible erythrocytes within the lumen. K(-): negative control (without ovariectomy); K(+): positive control (ovariectomized, without FSE); P1: 100 mg/kgBW FSE; P2: 200 mg/kgBW FSE; P3: 400 mg/kgBW FSE.

DISCUSSION

The outcomes of the study of the negative control group and the positive control group at all observation times (p-value <0.05) showed a significant difference in the mean numbers of osteoblasts, osteoclasts, and neocapillaries on days 3, 7, and 30 due to the effects of estrogen. This finding is consistent with previous studies showing that the application of bioactive materials to post-extraction sockets can significantly increase osteoblast numbers during bone regeneration[21,22]. The positive control group (K2) exhibited a pronounced increase in osteoclast numbers, reflecting the hypoestrogenic state of the rats. This study also showed an increase in the number of osteoblast and neocapillaries cells, as well as a significant decrease in the number of osteoclast cells in all treatment groups compared to the positive control group. This study aims to reveal the effect of Fenugreek Seeds Extract on hypoestrogen condition by contains several active compounds such as

steroidal sapogenins (diosgenin), polysaccharides (galactomannan), alkaloids (trigonelline), amino acids (4-hydroxyisoleucine), and flavonoids (quercetin). Biological activity analyses indicated that the metabolites quercetin, naringenin, luteolin, and kaempferol are predicted to function as estrogen agonists based on their high predicted activity scores. Additionally, these metabolites exhibited elevated Pa scores, suggesting potential anti-inflammatory effects and inhibition of TNF expression[16,22], so in post-menopausal patients where the amount of estrogen decreases, fenugreek phytoestrogens can act as estrogen mimicking because the active ingredients in Fenugreek have the ability to bind to ERα which resembles estradiol or has an estrogen-like structure and function[10,15,24-26]. In addition, Fenugreek phytoestrogens are also able to work like estrogen replacement therapy (ERT) as evidenced by previous studies indicating that administration of fenugreek extract markedly elevates estradiol levels, so in other words, Fenugreek extract has an effect in restoring the hormonal balance of individuals during menopause[15].

Several active ingredients of Fenugreek, diosgenin and lupeol compounds with phytoestrogen properties contained therein are known to have chemical structures similar to estrogen, these component also have an anti-inflammatory effect, which is in high doses can significantly reduce RANKL expression in the bones of ovariectomized rats when compared with the control group, potentially contributing to the prevention of excessive osteoclastogenesis by decreasing the RANKL/OPG ratio. RANKL stimulates osteoclast formation, while OPG counteracts the effects of RANKL. The reduction of the ratio may indicate that Fenugreek has the potential to reduce excessive osteoclast activity[27]. RANK itself is expressed on osteoclasts in response to CSF-1/CSF-1R signaling. Compounds derived from fenugreek have been reported to mitigate excessive bone resorption by inhibiting CSF-1R phosphorylation, thereby disrupting the signaling cascade that regulates osteoclast activation and proliferation. Consequently, inhibition of CSF-1R reduces RANK expression in osteoclasts[4]. Therefore, the lack of RANK/RANKL-induced signaling pathway will limit osteoclast differentiation and will prevent excessive bone resorption.

Fenugreek extract also increases bone turnover and bone strength supported by increased ALP and mineralization in osteoblast cells[28]. During this process, TGF-β stimulates osteoblast proliferation and migration. Enhanced proliferation and activation of osteoblasts subsequently elevates osteoprotegerin (OPG) expression. The increase in OPG significantly impacts osteoclast activity, as OPG functions as a decoy receptor for RANKL, preventing its interaction with RANK on the membranes of osteoclast precursors. This inhibition of RANKL-RANK signaling suppresses osteoclastogenesis, promotes osteoclast apoptosis, reduces bone resorption, and ultimately enhances bone formation[27,29]. Hypoestrogen inhibits angiogenesis or neocapillary formation by disrupting the polarization activity of M1/M2 macrophages. This can cause VEGF secreted by M2 macrophages to decrease so that the induction of the angiogenesis process is disrupted, thereby impairing the development of new capillary blood vessels, which results in a lower neocapillary count compared to normal conditions[30–32]. The neocapillaries count in post-tooth extraction sockets under hypoestrogen conditions increased due to the effects of compounds contained in Fenugreek seeds. Phytoestrogens can be said to be estrogen mimics so that they have a sufficient tendency to bind to ERα and Erβ so that hormonal activity is formed which is very influential in the process of neocapillary formation[31,33]. The hormonal activity formed can make the polarization activity of M1 macrophages and M2 macrophages back in balance or M2 macrophages increase again which previously decreased. This condition causes VEGF secreted by M2 macrophages to increase so that the

induction of the angiogenesis process or neocapillary formation is back on track, which was previously disrupted, and the neocapillaries count increases.

On day 3, osteoblast and osteoclast decreased and increased the mean number of cells counts within each group based on the phase of bone healing. The average results on day 3 are appropriate, where on that day it is still in the early phase of inflammation characterized by the formation of blood clots and granulation tissue that fills the socket. On day 3, the mesenchymal cells had begun differentiating into osteoprogenitor cells, which subsequently proliferate into osteoblasts along the margins of the extraction socket[20,34]. Osteoclasts have not yet reached their peak because in this phase, The socket remained largely filled with blood clots, while clusters of multinucleated giant cells began to appear on the surface of the retraction wall and in areas of the bone cavitycavity in contact with these cells[5,35].

The number of neocapillaries on day 3 in the treatment group showed a rise in the number of neocapillaries from the control group K2, The number of neocapillaries in post-tooth extraction sockets under hypoestrogen conditions increased due to the effects of compounds contained in Fenugreek seeds. Diosgenin and lupeol compounds with phytoestrogen properties contained therein are known to have chemical structures similar to estrogen. Therefore, phytoestrogens can be said to be estrogen mimics characterized by phenolic rings and two hydroxyl groups so that they have a sufficient tendency to bind to ERα and Erβ so that hormonal activity is formed which is very influential in the process of neocapillary formation[16,36]. The resulting hormonal activity can restore the balance between M1 and M2 macrophage polarization, or lead to a resurgence in M2 macrophage numbers that had previously declined. This condition causes VEGF secreted by M2 macrophages to increase so that the induction of the angiogenesis process or neocapillary formation is back on track, which was previously disrupted, and the number of neocapillaries increases[14,15,36].

The results of osteoblasts by day 7 indicated that osteoblast cells numbers after tooth extraction has increased in all control and treatment groups where it should be on day 7 osteoblast activity is still in the early stages of proliferation and osteoblasts help mineralize soft callus which is dominantly carried out by osteoclasts by secreting matrix (collagen type I) which will later become woven bone. Furthermore, in the results of the mean number of osteoclasts on day 7 in groups K2, P1, and P2 there was a discrepancy, which should be on day 7 tends to show the highest osteoclast activity, At this stage, simultaneous bone resorption by osteoclasts cells is seen, while woven bone formation begins at the base of the socket[34,37]. The discrepancy was not statistically significant or it can be said that there was no notable change in the mean between day 3 and day 7. However, the increase in osteoblasts numbers on day 7 of the study could be due to the expression of BMP-2 protein which is dominated by osteoblasts. The main function of BMP-2 is to accelerate the production of alveolar bone[18]. So that in this study it can occur presumably due to the accelerated production of alveolar bone after tooth extraction followed by faster activation of osteoclast cells along with the effectiveness of the work of Fenugreek Seed extract which has a faster and more effective effect. Another case with the suitability of data on the average number of neocapillaries on day 7, all treatment groups showed an increase in the number of neocapillaries from the K2 control group with the same mechanism of role and chemical compounds that occurred on day 3[38].

The next healing phase is day 21 to day 30, the socket became filled with new mature bone with dense cancellous bone, so osteoclast activity has decreased, while there will be more osteoblast activity[34,37]. Tooth extraction initiates a cascade of biological events within the alveolar bone, resulting in coordinated

osteoclastic resorption and subsequent osteoblastic bone formation throughout the healing period[7,39]. On day 21, osteoblasts had started to be trapped within the bone trabeculae and subsequently form osteoid. As a result, osteoblast activity has begun to decline and slowly turn into osteocytes[40]. The thing that affects the decrease that occurs on day 30 is the possibility that all control and treatment groups experience the process of resorbing the residual ridge on day 30 which is in line with Wolff's Law developed by German anatomist and surgeon Julius Wolff in the 19th century which states that increased stress as a stimulus for new bone formation, while decreased stress tends to cause bone loss. So that on day 30 there will be more osteoclast cells than osteoblast cells because in post-extraction cases it is expected that there will be a decrease in mechanical load that causes bone loss at the extraction site because there is no antagonistic contact between the teeth[41].

In conclusion, fenugreek seed extract is capable of increasing osteoblast and neocapillary counts, also reducing osteoclast numbers in post-tooth extraction sockets of hypoestrogenic rats. The most optimal results were observed at a dose of 400 mg/kgBW, with the highest osteoblast count recorded on day 7 (13.76 ± 3.16), the lowest osteoclast count on day 3 (7.28 ± 2.08), and the greatest neocapillary count on day 3 (11.70 ± 1.31). Regarding the control groups, the negative control group K(-) demonstrated normal wound healing progression with balanced osteoblast and osteoclast activity, confirming that the hypoestrogenic state in the positive control group K(+) was successfully established through ovariectomy, as evidenced by significantly elevated osteoclast counts and reduced osteoblast and neocapillary numbers compared to K(-). Post-operative care using Sagestam (gentamicin) was selected for its broad-spectrum antibacterial coverage against oral cavity microorganisms to prevent surgical site infection, while Santagesik (metamizole) was chosen as an analgesic due to its efficacy in managing acute post-surgical pain with a well-established safety profile in rodent experimental models[20]. This study has several limitations that should be acknowledged: (1) the study was conducted exclusively on an animal model (Wistar rats), and direct extrapolation of findings to human clinical settings requires further investigation; (2) serum estrogen levels were not measured to objectively confirm the hypoestrogenic state following ovariectomy; (3) the individual bioactive compounds responsible for the observed effects were not isolated or tested separately; (4) the percentage composition of each active compound in the fenugreek seed extract was not quantified; and (5) long-term observations beyond day 30 were not performed, leaving the durability of the healing effects unknown.

CONCLUSION

Fenugreek seed extract (Trigonella foenum-graecum) significantly enhances post-extraction bone wound healing in hypoestrogenic Wistar rats by increasing osteoblast proliferation, reducing osteoclast activity, and promoting neocapillary formation in a dose-dependent manner. The most pronounced effects were observed at 400 mg/kgBW, with the highest osteoblast count (13.76 ± 3.16) on day 7, the lowest osteoclast count (7.28 ± 2.08) on day 3, and the greatest neocapillary count (11.70 ± 1.31) on day 3. These findings indicate its potential as a complementary natural therapy for postmenopausal patients, particularly when hormone replacement therapy is contraindicated. However, limitations include the use of an animal model, absence of direct estrogen measurement, lack of isolation of specific bioactive compounds, and no long-term evaluation beyond day 30.

The observed effects are likely mediated by phytoestrogenic compounds that mimic estrogen activity and regulate bone remodeling and angiogenesis. Among the identified compounds—diosgenin, quercetin, galactomannan, trigonelline, lupeol, naringenin, luteolin, and kaempferol—diosgenin and lupeol are considered

key contributors due to their steroid-like structure and roles in suppressing RANKL expression and promoting angiogenesis. Nevertheless, the exact composition and contribution of each compound were not quantified. Future studies should include hormonal assessment, phytochemical analysis, and clinical trials to confirm efficacy and determine the most active components.

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REFERENCES

1. Himammi AN, Hartomo BT. Ekstraksi Gigi Posterior dengan Kondisi Periodontitis Kronis Sebagai Persiapan Pembuatan Gigi Tiruan Lengkap pada Pasien Diabetes Mellitus. J Kesehat Gigi [Internet]. 2020;8(1):6–10. Available from: http://ejournal.poltekkes-smg.ac.id/ojs/index.php/jkg/index
2. Lunardhi LC, Kresnoadi U, Agustono B. The effect of a combination of propolis extract and bovine bone graft on the quantity of fibroblasts, osteoblasts and osteoclasts in tooth extraction sockets. Dent J. 2019;52(3):126–32.
3. Chhabra S, Chhabra N, Kaur A, Gupta N. Wound Healing Concepts in Clinical Practice of OMFS. J Maxillofac Oral Surg. 2017;16(4):403–23.
4. Yusharyahya SN, Bramono K, Hestiantoro A, Edwar SQ, Kusuma I. Fenugreek (Trigonella foenum-graceum) increases postmenopausal fibroblast-associated COL1A1 and COL3A1 production dominantly through its binding to estrogen receptor beta. J Appl Pharm Sci. 2020;10(4):22–7.
5. Ma'ruf MT, Dewi PS, Koesoemawati R, Wedagama DM. An Analysis of Noni (Morinda citrifolia) Extract Gel to Increase the Number of Macrophage and Angiogenesis in Mandibles Socket After Tooth Extraction of Guinea Pigs. J Eng Appl Sci. 2019;9306–9.
6. Rusdy H, Pasaribu Saruksuk AS, Dalimunte RS, Dohude GA. Effectiveness of betadine (Jatropha multifida L.) stem sap on the wound healing after tooth extraction in Sprague-Dawley rats. J Kedokt Gigi Univ Padjadjaran. 2021;33(2):145.
7. Kemenkes RI. Profil Kesehatan Indonesia Tahun 2022. Kementerian Kesehatan Republik Indonesia. 2023;
8. Mukai K, Horike SI, Meguro-Horike M, Nakajima Y, Iswara A, Nakatani T. Topical estrogen application promotes cutaneous wound healing in db/db female mice with type 2 diabetes. PLoS One [Internet]. 2022;17(3 March):1–19. Available from: http://dx.doi.org/10.1371/journal.pone.0264572
9. Gushiken LFS, Beserra FP, Bastos JK, Jackson CJ, Pellizzon CH. Cicatrización de heridas cutáneas: Una actualización desde la fisiopatología hasta las terapias actuales. Life. 2021;11(7):1–15.
10. Vígh S, Cziáky Z, Sinka L, Pribac C, Liana Mioara M, Turcuș V, et al. Analysis of Phytoconstituent Profile of Fenugreek – Trigonella Foenuem-Graecum L. – Seed Extracts. Stud Univ Babeș-Bolyai Chem. 2017 Jun 30;62:145–66.
11. Amarasekara DS, Kim S, Rho J. Regulation of osteoblast differentiation by cytokine networks. Int J Mol Sci. 2021;22(6):1–16.
12. Daigo Y, Daigo E, Fukuoka H, Fukuoka N, Ishikawa M, Takahashi K. Wound healing and cell dynamics including mesenchymal and dental pulp stem cells induced by photobiomodulation therapy: An example of socket-preserving effects after tooth extraction in rats and a literature review. Int J Mol Sci. 2020;21(18):1–16.
13. Shen Y, Huang X, Wu J, Lin X, Zhou X, Zhu Z, et al. The Global Burden of Osteoporosis, Low Bone Mass, and Its Related Fracture in 204 Countries and Territories, 1990-2019. Front Endocrinol (Lausanne). 2022;13:882241.
14. Kondo T, Kanayama K, Egusa H, Nishimura I. Current perspectives of residual ridge resorption: Pathological activation of oral barrier osteoclasts. J Prosthodont Res. 2023 Jan;67(1):12–22.
15. Kurniawati A, Cholid Z, Hartanto NI. The Effect of Giving Wungu Leaves Extract (Graptophyllum Pictum L. Griff) on the Decrease in the Number of Osteoclasts in the Post Extraction Socket of Male Wistar Rats. J Int Dent Med Res. 2022;15(4):1511–5.
16. Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling. Genes (United States). 2022;60(8–9):1–18.
17. Soekobagiono S, Alfiandy A, Dahlan A. RANKL expressions in preservation of surgical tooh extraction treated with Moringa (Moringa oleifera) leaf extract and demineralized freeze-dried bovine bone xenograft. Dent J (Majalah Kedokt Gigi). 2018;50(3):149.
18. Aquina M, Permatasari N. The Effectiveness Of Tofu Liquid Waste As A Natural Phytoestrogen For

Mandibular Bone Of Ovariectomized Rats. Idj. 2012;1(1):6–13.
19. Torrens-Mas M, Roca P. Phytoestrogens for cancer prevention and treatment. Biology (Basel). 2020;9(12):1–19.
20. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes: Cellular Mechanisms of Wound Repair. Open Biol. 2020;10(9).
21. Gonzalez ACDO, Andrade ZDA, Costa TF, Medrado ARAP. Wound healing-A literature review. An Bras Dermatol. 2016;91(5):614–20.
22. Luthfi M, Juliastuti WS, Risky YA. Angiogenesis of extracted tooth wound on wistar rats after application of okra (Abelmoschus esculentus) gel extract. Pesqui Bras pediatria Clin Integr. 2020;20:1–8.
23. Adiyasa MR, Meiyanti M. Pemanfaatan obat tradisional di Indonesia: distribusi dan faktor demografis yang berpengaruh. J Biomedika dan Kesehat. 2021;4(3):130–8.
24. Beserra FP, Gushiken LFS, Vieira AJ, Bérgamo DA, Bérgamo PL, de Souza MO, et al. From inflammation to cutaneous repair: Topical application of lupeol improves skin wound healing in rats by modulating the cytokine levels, NF-κB, Ki-67, growth factor expression, and distribution of collagen fibers. Int J Mol Sci. 2020;21(14):1–22.
25. Lodra EH, Effendi MC, Permatasari N, Dradjat RS. Potential of fenugreek (Trigonella foenum-graecum L.) metabolites as dental residual ridge resorption inhibitors through estrogen receptor signaling: In silico study. J Pharm Pharmacogn Res. 2023;11(4):683–90.
26. Maryani I, Rochmah YS, Parmana AD. Analisa Gel Kombinasi Platelet Rich Plasma Dan Chitosan Terhadap Peningkatan Jumlah Osteoblas Sebagai Bone Regeneration Pada Luka Pasca Ekstraksi Gigi Tikus Wistar. ODONTO Dent J. 2018;5(2):89.
27. Nursetiani A, Herdiana Y, Farmasi F, Padjadjaran U, Dara T, Manis K, et al. Potensi Biji Klabet (Trigonella foenum-graecum L.) Sebagai Alternatif Pengobatan Herbal : Review Jurnal. Farmaka. 2018;16:475–84.
28. Ceccarelli I, Bioletti L, Peparini S, Solomita E, Ricci C, Casini I, et al. Estrogens and phytoestrogens in body functions. Neurosci Biobehav Rev [Internet]. 2022;132:648–63. Available from: https://www.sciencedirect.com/science/article/pii/S0149763421005558
29. Wani SA, Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci [Internet]. 2018;17(2):97–106. Available from: https://www.sciencedirect.com/science/article/pii/S1658077X15301065
30. Thomas J V, Rao J, John F, Begum S, Maliakel B, IM K, et al. Phytoestrogenic effect of fenugreek seed extract helps in ameliorating the leg pain and vasomotor symptoms in postmenopausal women: A randomized, double-blinded, placebo-controlled study. PharmaNutrition [Internet]. 2020;14:100209. Available from: https://www.sciencedirect.com/science/article/pii/S2213434420300347
31. Zinnia MA, Khademul Islam ABMM. Fenugreek steroidal saponins hinder osteoclastogenic bone resorption by targeting CSF-1R which diminishes the RANKL/OPG ratio. Int J Biol Macromol [Internet]. 2021;186:351–64. Available from: https://www.sciencedirect.com/science/article/pii/S0141813021014264
32. Abo El-Nasr NME, El-Denshary EE, Mahmod SSE, Nofal SM. "Osteo-protective effect of Curcumin; Fenugreek and their combination on ovariectomized female rats". Egypt J Vet Sci. 2018;49(1):1–12.
33. Syarif RD, Kusumaningsih T, Arundina I. Changes in osteoblast and osteoclast cell count after moringa oleifera leaf extract administration during orthodontic tooth movement. J Dentomaxillofacial Sci. 2020;5(2):98–102.
34. Kim H, Wang SY, Kwak G, Yang Y, Kwon IC, Kim SH. Exosome-Guided Phenotypic Switch of M1 to M2 Macrophages for Cutaneous Wound Healing. Adv Sci. 2019;35. Daigo Y, Daigo E, Fukuoka H, Fukuoka N, Ishikawa M, Takahashi K. Wound healing and cell dynamics including mesenchymal and dental pulp stem cells induced by photobiomodulation therapy: An example of socket-preserving effects after tooth extraction in rats and a literature review. Int J Mol Sci. 2020;21(18):1–16.
35. Khoswanto C. Microvascular activity from the wound healing process in wistar rats due to administration of anredera cordifolia (ten.) steenis. J Int Dent Med Res [Internet]. 2021;14(4):1351–6. Available from: https://www.jidmr.com/journal/wp-content/uploads/2021/12/6-D21_1513_Christian_Khoswanto_Indonesia.pdf
36. Li D, Liu Y, Lyu X, Hu C, Yan T, Yan J, et al. Small RNA cargo armed extracellular vesicles alleviate periodontitis in OVX mice via M2 macrophage polarization. Chem Eng J [Internet]. 2022;435:134870. Available from: https://www.sciencedirect.com/science/article/pii/S1385894722003771
37. Chasanah N. Ekspresi TGFB1 Setelah Pemberian Ekstrak Gel Aloe Vera Pada Soket Pencabutan Gigi Tikus Wistar. J Biosains Pascasarj. 2018;20(1):47.
38. Beserra FP, Vieira AJ, Gushiken LFS, de Souza EO, Hussni MF, Hussni CA, et al. Lupeol, a dietary triterpene, enhances wound healing in streptozotocin-induced hyperglycemic rats with modulatory

effects on inflammation, oxidative stress, and angiogenesis. Oxid Med Cell Longev. 2019;2019.
39. Puspita, S., Hanifatunnisa, L. S., Dharmayanti, A. W. S., Mahanani, E. S., & Saleh, E. (2022). Effect of fibroin sponge on alveolar bone remodeling process post-tooth extraction. Odonto : Dental Journal, 9(1), 7–15. https://doi.org/10.30659/odj.9.0.7-15
40. Sa'diyah JS, Septiana DA, Farih NN, Ningsih JR. Pengaruh gel ekstrak daun binahong (Anredera cordifolia) 5% terhadap peningkatan osteoblas pada proses penyembuhan luka pasca pencabutan gigi tikus strain Wistar Effect of 5% binahong (Anredera cordifolia) leaf extract in increasing the osteoblast amount at the tooth extraction wound healing process of Wistar rat . J Kedokt Gigi Univ Padjadjaran. 2020;32(1):9.
41. Kannan KR, Baskaran A, Anjum AD, Roshini A, Kumar VS. Basal Implants in the Mandibular Esthetic Zone: A Case Series. Int J Innov Sci Res Technol [Internet]. 2023;8(3):1338–43. Available from: www.ijisrt.com