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The effects of Genistein on osseointegration of Titanium implants in experimental ovariectomized model

Efectos de la Genisteína en la oseointegración de implantes de Titanio en un modelo experimental ovariectomizado

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ABSTRACT

Osseointegration is a challenge in the dental implant treatment of individuals with osteoporosis. Genistein is a phytoestrogen with beneficial effects in the prevention of osteoporosis. This study aims to evaluate the protective effects of genistein supplementation on the osseointegration level of titanium implants in ovariectomized rats. The rats in this study were randomly divided into 5 groups with 8 rats in each group: Control, Implant, Ovariectomy–Implant, Ovariectomy–Implant–Genistein, Implant–Genistein. The implants were surgically integrated tibial bones of rats. Genistein was administered at 2 mg·kg^{-1} by oral gavage three times a week. All rats were sacrificed at the end of 3 months. Biochemical analyses were made from the blood serum of the rats, histomorphometric analyses from the implant and surrounding tissues placed in the tibia, and bone mineral density analyses from the mandibles. The bone implant connection (BIC) ratio of the Control–Implant group was higher than the other groups (P<0.05). The BIC ratio of the Ovariectomy–Implant group was lower than the Ovariectomy–Implant–Genistein and Implant–Genistein groups (P<0.05). In terms of thread filling, no statistically significant difference was found between the groups (P>0.05). The jaw bone mineral density (BMD) of the control group was higher than the Ovariectomy–Implant and Implant–Genistein groups (P<0.05). In ovariectomy–Implant–Genistein group jaw BMD was higher than the Ovariectomy–Implant and Implant–Genistein groups (P<0.05). In conclusion, it can be stated that genistein can improve the negative effects of ovariectomy on the bone and increase implant osseointegration. Genistein consumption may increase implant osseointegration in osteoporotic cases.

Key words: Osseointegration; ovariectomy; osteoporosis; phytoestrogen; genistein

RESUMEN

La osteointegración es un desafío en el tratamiento con implantes dentales de individuos con osteoporosis. La genisteína es un fitoestrógeno con efectos beneficiosos en la prevención de la osteoporosis. Este estudio tiene como objetivo evaluar los efectos protectores de la suplementación con genisteína en el nivel de osteointegración de implantes de titanio en ratas ovariectomizadas. Las ratas en este estudio se dividieron aleatoriamente en 5 grupos con 8 ratas en cada grupo: Control, Implante, Ovariectomía–Implante, Ovariectomía–Implante–Genisteína, Implante–Genisteína. Los implantes fueron huesos tibiales de ratas integrados quirúrgicamente. La genisteína se administró a 2 mg·kg^{-1} por sonda oral tres veces por semana. Todas las ratas fueron sacrificadas al final de los 3 meses. Se realizaron análisis bioquímicos del suero sanguíneo de las ratas, análisis histomorfométricos del implante y los tejidos circundantes colocados en la tibia y análisis de densidad mineral ósea de las mandíbulas. La relación de conexión ósea del implante (BIC) del grupo Control–Implante fue mayor que los otros grupos (P<0,05). La relación BIC del grupo Ovariectomía–Implante fue menor que los grupos Ovariectomía–Implante–Genisteína e Implante–Genisteína (P<0,05). En cuanto al relleno de ranura, no se encontró diferencia estadísticamente significativa entre los grupos (P>0,05). La densidad mineral ósea de la mandíbula (BMD) del grupo control fue mayor que los grupos Ovariectomía–Implante e Implante–Genisteína (P<0,05). Al igual que los grupos Ovariectomía–Implante e Implante–Genisteína (P<0,05). En conclusión, se puede afirmar que la genisteína puede mejorar los efectos negativos de la ovariectomía sobre el hueso y aumentar la implante osteointegración. El consumo de genisteína puede aumentar la osteointegración del implante en casos osteoporóticos.

Palabras clave: Osteointegración; ovariectomía; osteoporosis; fitoestrógenos; genisteína

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INTRODUCTION

In treating partial and complete edentulism, osseointegrated dental implant–supported prosthetic treatment is a scientifically accepted method. Osseointegration is the healing process in which clinically asymptomatic rigid fixation of alloplastic materials are achieved and maintained during functional loading [1, 2]. There are several factors that affect the osseointegration of dental implants, including surgical considerations, bone quality and quantity and host–related factors such as the patients' nutritional status. Many micronutrients play an important role in dental implant osseointegration by affecting a number of alveolar bone parameters such as bone healing after tooth extraction [3].

Osteoporosis has been defined as a systemic skeletal disease characterized by low bone mass and deterioration of the microarchitecture of bone tissue, resulting in increased bone fragility and susceptibility to fracture. Osteoporosis, which causes massive bone loss in trabecular and cortical bones, affects the jaw bones as well as other body bones. Studies have shown that osteoporosis adversely affects bone–implant connection, especially in the trabecular bone. Therefore, osteoporosis is considered a relative contraindication for dental implants [4].

Estrogen deficiency decreases osteoblast activity and increases osteoclast activity, thus reducing bone mass. In addition, estrogen deficiency causes an increase in the release of proinflammatory cytokines such as the tumor necrosis factor–alpha (TNF–a), interleukin (IL-6) and (IL-1) from osteoblasts, monocytes and macrophages [5]. These cytokines stimulate stromal cells and preosteoblasts and secrete factors such as macrophage colony–stimulating factor, receptor activator of nuclear factor κ–β ligand (RANKL), IL-6, IL-1, which stimulate the proliferation of osteoclast precursors or osteoclastogenesis [6, 7]. RANKL also plays an important role in cases of malignancy or in postmenopausal osteoporosis, where pathological bone loss is seen [8]. In estrogen deficiency, RANKL production increases and osteoprotegerin (OPG) decreases in osteoblasts. Osteoprotegerin plays a role in reducing the production and activity of RANKL. IL-1α, TGF–β, IL-1β, TNF–α, estrogen and 1,25(OH)2 vitamin D3 stimulate the secretion of osteoprotegerin from osteoblasts [1]. This event shortens the life span of osteocytes and osteoblasts and plays a role in the pathogenesis of senile osteoporosis by causing a decrease in osteoblastogenesis [9].

Phytoestrogens are a large group of heterocyclic phenols with a chemical structure similar to estrogen. Phytoestrogens which are abundant in plants, have received increasing attention as a dietary component that can affect various aspects of human health. It has been recently demonstrated that phytoestrogens can prevent osteoporosis. The positive effect of synthetic phytoestrogen application on bone tissue has been shown in the bones of postmenopausal women and osteoporotic experimental animals. Genistein is a typical soybean isoflavone that acts as a phytoestrogen. Genistein is the major phytoestrogen in soybeans. In 1946 the discovery that the cause of infertility in sheep living in Australia was due to genistein in ground clover eaten by sheep supported the estrogenic effect of genistein. In 1987, genistein was found to be a potent and specific repressor of protein kinase. Since most cancer gene codes are tyrosine kinase–dependent, this discovery offers hope for cancer treatment. Genistein competes with estrogen due to defect that it resembles estrogen in structure [5, 6, 7]. The bone–retaining effects of genistein are due to the low doses of the agonistic effect of this phytoestrogen on estrogen receptors in osteoblasts. Osteoblasts or their precursors can secrete cytokines with inhibitory or stimulating effects on osteoblasts in response to genistein or parathyroid hormone (PTH). Genistein stimulates the transformation of osteoblast progenitor cells into osteoblasts while suppressing osteoclast progenitor cells. In addition, genistein interacts with estrogen receptors in osteoblasts and creates a suppressive effect on osteoclasts. Therefore, genistein can indirectly prevent or suppress bone resorption [5, 6, 7, 8].

Genistein offers an alternative treatment option to estrogen supplementation for perimenopausal and postmenopausal women. It acts as a selective estrogen receptor modulator (SERM) that provides an alternative treatment for menopausal signs and symptoms. In addition to treating osteoporosis, its properties such as improving cardiovascular health, bone density and skin quality make genistein a good treatment option for perimenopausal and postmenopausal women [7, 8].

The aim of this study was to examine the effect of genistein, a phytoestrogen, on the level of bone–implant connection in titanium implants applied to the tibia of rats that underwent an ovariectomy.

MATERIAL AND METHODS

Animals and study design

All experimental and surgical procedures in this study were performed in Firat University Experimental Research Center in Elazig, Turkiye. The study was approved by the Firat University Animal Experimental Ethics Committee (Protocol Number: 2018/43). The recommendations of the Helsinki Declaration regarding the protection of laboratory research animals were followed.

In this study, 40 healthy adult female Sprague Dawley rats (*Rattus norvegicus*) of 240–260 g; (Balance Shimadzu, Japan) aged 3–3.5 months were used.

All rats included in the study were obtained from Firat University Experimental Research Center, Elazig, Turkiye. The rats were kept in plastic containers and their temperatures were checked daily. During the experiment, the rats were not limited in terms of food and water, and the light cycle in the lab was adjusted to rotate through 12 hours (h) of darkness and 12 h of light. All rats were selected in the same estrus period to ensure standardization throughout the experimental protocols. The rats were divided into 5 groups with 8 rats in each group.

Control group (n=8): No additional procedures were applied to the rats in this group during the 3–month experimental setup. At the end of the 3–month experimental period, the rats were sacrificed.

Control–implant group (n=8): Under general anesthesia, $TiAl_6V_4$ implants with a 2.5 mm diameter and a 4 mm length were placed in the corticocancellous bone in the metaphyseal parts of the right tibia bones of the rats, and no additional application was done until the end of the study. At the end of the 3–month experimental period, following the surgical placement of the implants, the rats were sacrificed.

Ovariectomy–implant group (n=8): Ovariectomy was performed on the subjects in this group under general anesthesia; $TiAl_6V_4$ implants with a 2.5 mm diameter and a 4 mm length were placed into the corticocancellous bone in the metaphyseal parts of the right tibia bones of the rats in the same session. At the end of the 3–month experimental period, following the surgical placement of the implants, the rats were sacrificed.

Ovariectomy–implant–genistein group (n=8): Ovariectomy was performed on the rats under general anesthesia; $TiAl_6V_4$ implants with a 2.5 mm diameter and a 4 mm length were placed into the corticocancellous bone in the metaphyseal parts of the right tibia bones of the rats in the same session. Following this procedure, 2 $mg \cdot kg^{-1}$ genistein was given to the rats by oral gavage 3 times a week for 3 months. At the end of the 3–month experimental period, following the surgical placement of the implants, the rats were sacrificed [8].

Implant–genistein group (n=8): $TiAl_6V_4$ implants with a 2.5 mm diameter and a 4 mm length were placed into the corticocancellous bone in the metaphyseal parts of the right tibia bones of the rats. Following this procedure, 2 $mg \cdot kg^{-1}$ genistein was given to the rats by oral gavage 3 times a week for 3 months. At the end of the 3–month experimental period, following the surgical placement of the implants, the rats were sacrificed [8].

Surgical procedures

Surgical procedures applied to the rats in the study groups were performed under general anesthesia by intramuscular injection of anesthetics (10 $mg \cdot kg^{-1}$ xylazine, 40 $mg \cdot kg^{-1}$ ketamine). After shaving the surgical areas of the subjects that were administered general anesthesia, antisepsis of the surgical areas was provided with povidone–iodine. In the rats that underwent an ovariectomy, the abdominal cavity was opened with a 2 cm transverse incision in the midline of the abdomen (FIGS. 1 A,B). The ovaries were dissected and excised bilaterally; the abdominal wall, subcutaneous tissues and skin were sutured separately with 5/0 vicryl; the wound was closed primarily (FIGS. 1 C,D) [9].

In this study, specially produced titanium implants were used ($TiAl_6V_4$) and a specially produced application set was used to apply them. The implants were 2.5 mm in diameter, 4 mm in length and in screw form with an Resorbable Blast Material (RBM) surface structure (Implance Implant Systems, AGS Medical Corporation, Istanbul, Türkiye). An incision was made from the proximal cranial part of the right tibial bone to access the surgical field. The soft tissues and periosteum were dissected with a periosteal elevator and bone tissue was reached. The corticocancellous bone platform was exposed in the metaphyseal region of the right tibias, and the implant sockets were prepared perpendicular to the bone surface. Irrigation was performed with sterile saline in the surgical area to prevent heating while opening the implant sockets [1].

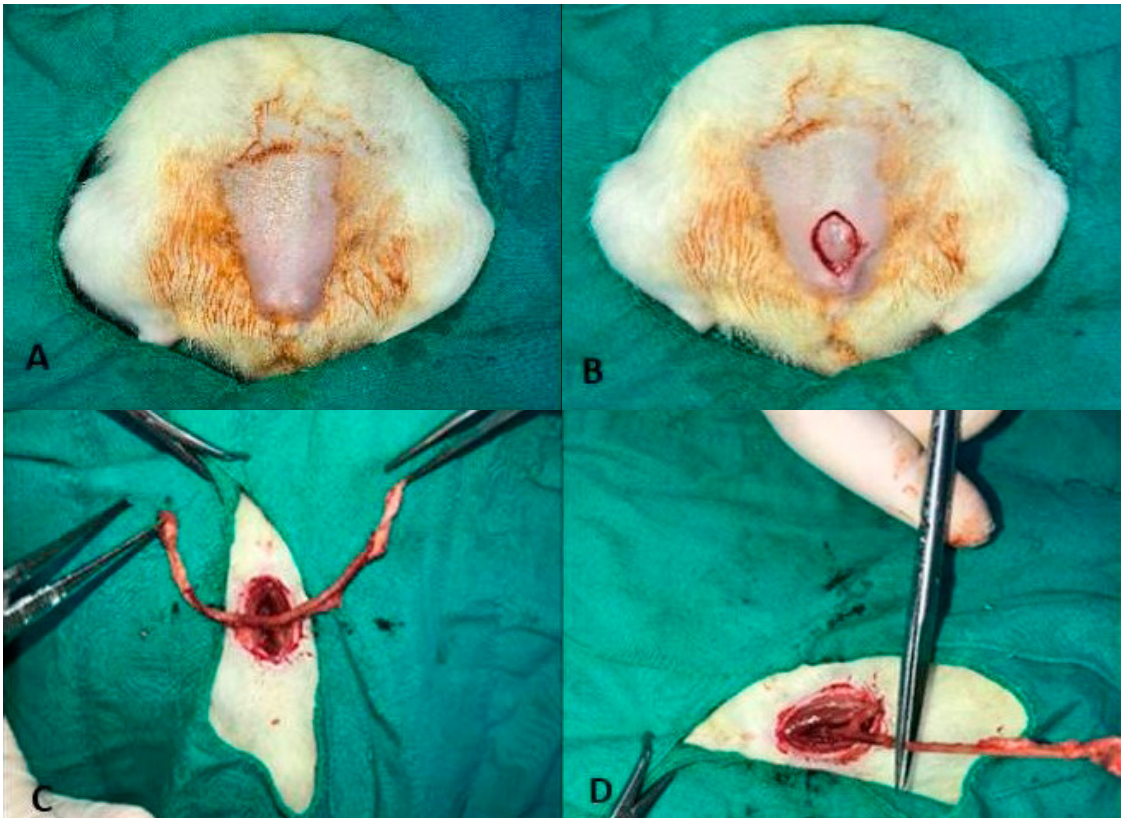

FIGURE 1. Shaving the surgical (A) area and opening the abdominal cavity with a transverse incision (B). Bilateral excision of the ovaries by dissection (C, D)

The implant sockets were prepared with an initial bur (AGS Medical Implance Implants, Istanbul, Türkiye) with a diameter of 1.8 mm at a revolution speed of 500-600 rpm with physiodispenser (WH DENTAL İmplantmed Classic, Bürmoos, Austria), then an intermediate bur (AGS Medical Implance Implants, Istanbul, Türkiye) with a diameter of 2.2 mm and a final bur (AGS Medical Implance Implants, Istanbul, Türkiye) with a diameter of 2.5 mm. The implantation process was completed by placing the implants in the sockets with the special carrying parts. After the implants were placed, the periosteum and soft tissues were repositioned in their original positions with 4-0 silk sutures. Antibiotics (40 $mg \cdot kg^{-1}$ cephalosporin) and analgesics (0.1 $mg \cdot kg^{-1}$ tramadolhydrochloride) were administered intramuscularly for 3 d to prevent infection and pain after surgical procedures [1].

Genistein administration

Genistein, (DSM Nutritional Products Ltd, Research and Development, Switzerland) containing at least 98% genistein, was used as genistein. Genistein was weighed (WL 603 Digital Precision Scale, USA) at a precision of 2 $mg \cdot kg^{-1}$ for each subject and diluted with physiological saline. Rats in the Ovariectomy–Implant–Genistein and Implant–Genistein groups were fed with this prepared solution 3 times a week for 3 months by oral gavage. The genistein dose used in this study was selected based on a previous study [8].

Collecting blood samples and biochemical analysis

Just before the sacrification procedure of the rats, blood was taken from their hearts with 10 mL injectors. The blood taken was placed in 10 mL gel tubes and kept for 10 min, and centrifuged (NF 200, NÜVE, Ankara, Turkiye) 1007 g force. After the blood samples were centrifuged serum samples were obtained; alkaline phosphatase (ALP), calcium (Ca), phosphorus (P), aspartate aminotransferase (AST), alanine aminotransferase (ALT) rates were analyzed with the photometric method (Advia Chemistry XPT, Siemens, Healthineers, Erlangen, Germany) in Firat University Faculty of Medicine Biochemistry Laboratory.

Nondecalcified histomorphometric analysis

Histological examination of the titanium implants were analyzed according to the undecalcified preparation method after they were removed together with the surrounding bone. After the implants and the surrounding bone tissue were removed as a block from the soft tissue, they were kept in a 4% buffered formalin solution for at least 24 h. The samples were dehydrated and embedded in photopolymerized methylmethacrylate. The implants were cut with a precision cutting (EXACT Technologies Inc., USA) device from the middle with the surrounding bone tissue. After this process, the samples were abraded with a precision sander and sections with a 50 µm thickness were obtained from each sample. These sections were stained with toulidine blue for histomorphometric analysis and the samples surfaces were covered with a coverslip using methyl methacrylate. Digital images of all samples at 4×, 10× optical magnifications were taken and recorded with a digital camera connected to a light microscope (Nikon, Japan). Histological bone–implant connection (BIC) (%) and thread filling ratios (%) measurements were made for each sample using the Image Analysis Program (Nikon, Japan). The ratio of the total surface length in contact with the bone to the circumference of the implant was evaluated as the percentage (%) of bone–implant connection (BIC) for each implant. The thread filling (TF) ratio for each implant; was calculated as the percentage (%) of bone–filled thread area to the total thread area [10, 11].

Evaluation of bone mineral density

Densitometric evaluation was made using the software in Dual Energy X–Ray Absorptiometry (DEXA) (Hologic® QDR 4500C Acclaim Series Elite/N:49458, USA) device of Firat University Faculty of Medicine, Department of Nuclear Medicine, Version 12.3 package. In the evaluation of the mineral density (BMD), bones of the jaws and right femurs of the rats were recorded.

Statistical analysis

The IBM SPSS Statistics 22 (IBM SPSS, Turkiye) program was used for statistical analysis. While evaluating the study data, the conformity of the parameters to normal distribution was evaluated with the Shapiro Wilks test. While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, frequency), in comparison of quantitative data, parameters that did not show normal distribution were found between groups. The Kruskal Wallis test was used in comparisons and the Mann Whitney U test was used to determine the group that caused the difference. Significance was evaluated at the $P<0.05$ level.

RESULTS AND DISCUSSION

Biochemical parameters as can be seen in TABLE I. ALP levels in the implant–genistein group were lower compared to all other groups ($P<0.05$). While serum Ca levels in the control group were higher than the Ovariectomy implant and Ovariectomy implant genistein groups ($P<0.05$), there was no difference in Ca levels between the other groups ($P>0.05$). P levels in the control group were detected to be higher than the Ovariectomy implant and Ovariectomy implant genistein groups (P:0.001; $P<0.05$). P levels of the Ovariectomy implant group were lower than those of the Ovariectomy implant genistein and Implant genistein groups ($P<0.05$). On the other hand, there was no difference in AST levels between any groups ($P>0.05$). However, ALT levels in the implant genistein group was lower compared to Control, Control implant, Ovariectomy implant and Ovariectomy implant genistein groups ($P<0.05$). There was no difference between the other groups in terms of ALT levels ($P>0.05$).

As seen in TABLE II; there was a difference between the groups in BIC levels ($P<0.05$). The highest BIC level was detected in the controls (FIG. 2) ($P<0.05$).

The BIC levels in the ovariectomy implant group (FIG. 3) were lower than the ovariectomy implant genistein (FIG.4) and implant genistein group (FIG. 5) ($P<0.05$).

When the groups were evaluated in terms of thread filling, no statistically significant difference was found between the groups ($P>0.05$). As seen in the TABLE III, there was a difference in mandibular bone mineral density (BMD) between the groups ($P<0.05$), while the highest BMD was found in the control group ($P<0.05$).

TABLE I
Statistical data of ALP, Ca, Phosphorus, AST and ALT parameters of the groups

Groups	ALP (U·L^{-1})	Ca (mg·dl^{-1})	Phosphorus (mg·dl^{-1})	AST (U·L^{-1})	ALT (U·L^{-1})
	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)	Mean ± SD (median)
Control (N=8)	78.88 ± 14.97 (74)	10.91 ± 0.28 (10.8)	5.85 ± 0.32 (5.8)	206.75 ± 32.01 (199.5)	73.88 ± 7.53 (73)
Control Implant (N=8)	99.25 ± 50.39 (87.5)	10.92 ± 1.04 (10.6)	5.56 ± 0.53 (5.6)	210.5 ± 28.14 (203)	74.5 ± 13.31 (68.5)
Ovariectomy Implant (N=8)	109 ± 52.39 (98)	10.42 ± 0.39 (10.4)	5.05 ± 0.22 (5.1)	216.25 ± 41.17 (230.5)	79.75 ± 13.53 (78)
Ovariectomy Implant Genistein (N=8)	109.5 ± 28.07 (120.5)	10.26 ± 0.26 (10.3)	5.35 ± 0.14 (5.4)	239.88 ± 39.23 (247.5)	86.13 ± 14.59 (88)
Implant Genistein (N=8)	53.88 ± 12.9 (52)	10.57 ± 0.52 (10.5)	5.56 ± 0.28 (5.6)	194.5 ± 45.85 (185.5)	64.5 ± 22.7 (57.5)
P–value	0.009*	0.020*	0.001*	0.163	0.015*

Kruskal Wallis Test *P<0.05

TABLE II
Bone implant connection (BIC) and Thread Filling (TF) parameters of the groups

Groups	BIC (%)	TF (%)
	Mean ± SD (median)	Mean ± SD (median)
Control Implant (N=8)	67.6 ± 9.44 (68)	48.25 ± 8.83 (48.8)
Ovariectomy Implant (N=8)	43.26 ± 6.09 (44)	35.73 ± 15.46 (29.4)
Ovariectomy Implant Genistein (N=8)	53.99 ± 9.02 (52.8)	49.57 ± 19.79 (44.5)
Implant Genistein (N=8)	54.57 ± 10.95 (54.6)	43.3 ± 9.24 (41.9)
P–value	0.002*	0.214

Kruskal Wallis Test *P<0.05

TABLE III
Mandibular and femur bone mineral density (BMD) of the groups

Group	Mandibular (BMD)	Femur (BMD)
	Mean ± SD (median)	Mean ± SD (median)
Control (N=8)	0.36 ± 0.03 (0.4)	0.24 ± 0.01 (0.2)
Control Implant (N=8)	0.35 ± 0.03 (0.4)	0.25 ± 0.03 (0.3)
Ovariectomy Implant (N=8)	0.33 ± 0.01 (0.3)	0.23 ± 0.03 (0.2)
Ovariectomy Implant Genistein (N=8)	0.37 ± 0.02 (0.4)	0.23 ± 0.01 (0.2)
Implant Genistein (N=8)	0.33 ± 0.03 (0.3)	0.25 ± 0.02 (0.2)
P–value	0.008*	0.322

Kruskal Wallis Test *P<0.05

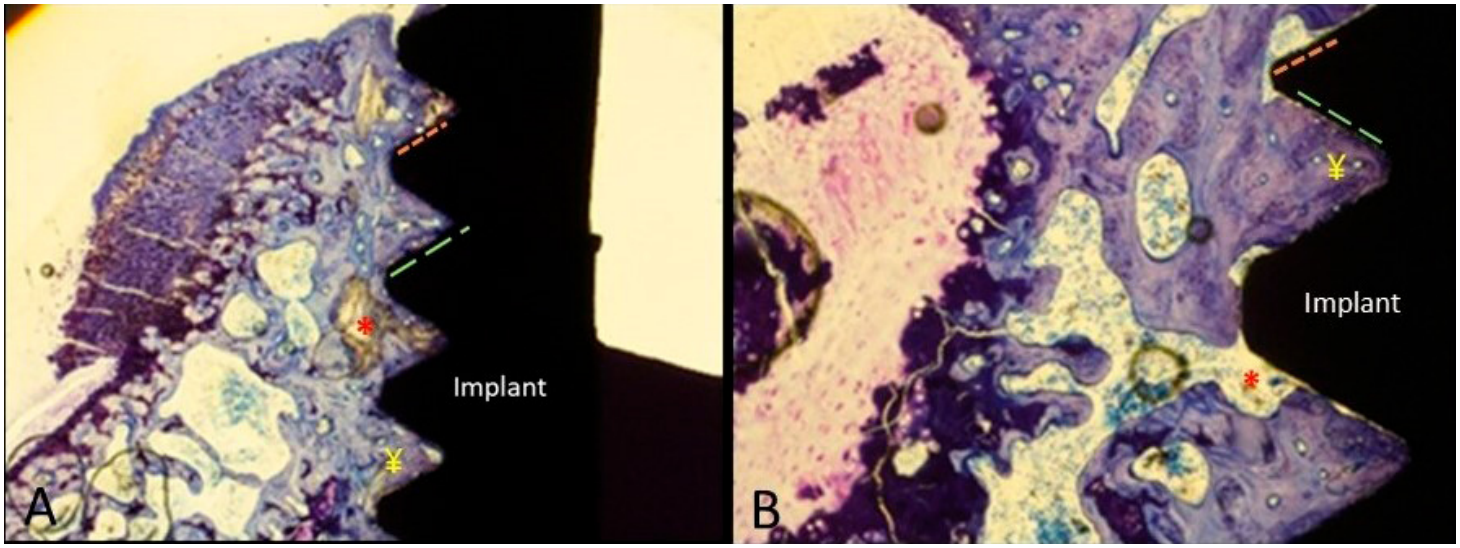

FIGURE 2. Non–Decalcified histologic images of the Control–Implant Group; (A:4×, B:10× magnification, Methylene blue). Implant surface not contacting bone (brown line), Implant surface contacting with bone (green line).*: Area without bone filling, ¥: Area with bone filling. Total implant surface: £, Bone Implant Contact Ratio (%): £–α(β)/£. Thread filling detected by measuring the ratio of the bone filled areas in total thread areas. Thread filling areas (à), non–bone areas (ĕ), total area (Ă). Thread filling Ratios (%):Ă–ĕ/Ă (à/Ă)

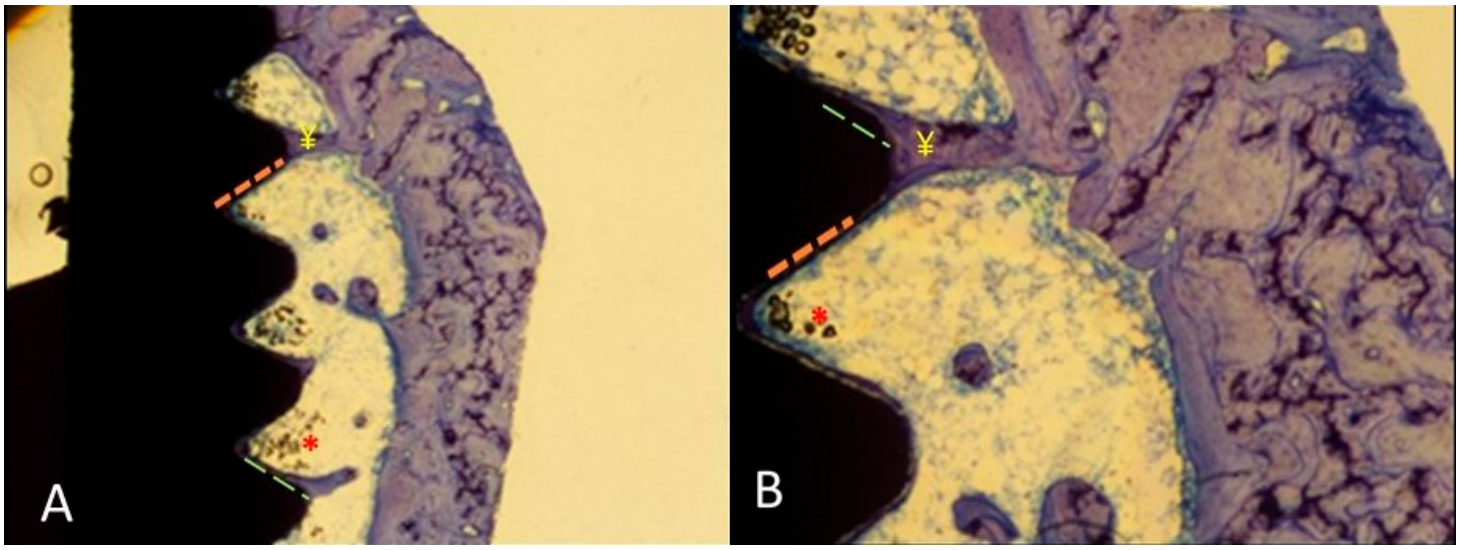

FIGURE 3. Non–Decalcified histologic images of the Ovariectomy–Implant Group; (A:4×, B:10× magnification, Methylene blue). Implant surface not contacting bone (brown line), Implant surface contacting with bone (green line).*: Area without bone filling, ¥: Area with bone filling. Total implant surface: £, Bone Implant Contact Ratio (%): £–α(β)/£. Thread filling detected by measuring the ratio of the bone filled areas in total thread areas. Thread filling areas (à), non–bone areas (ĕ), total area (Ă). Thread filling Ratios (%):Ă–ĕ/Ă (à/Ă)

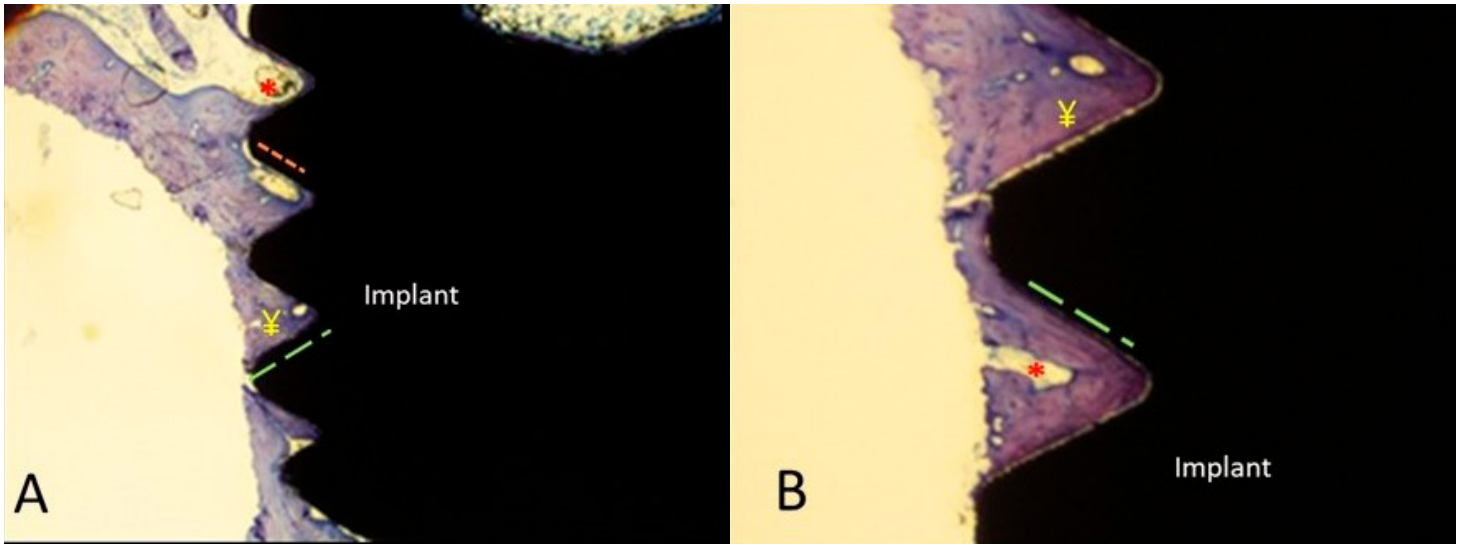

FIGURE 4. Non–Decalcified histologic images of the Ovariectomy–Implant–Genistein Group; (A:4×, B:10× magnification, Methylene blue). Implant surface not contacting bone (brown line), Implant surface contacting with bone (green line).*: Area without bone filling, ¥: Area with bone filling. Total implant surface: £, Bone Implant Contact Ratio (%): £–α(β)/£. Thread filling detected by measuring the ratio of the bone filled areas in total thread areas. Thread filling areas (à), non–bone areas (ĕ), total area (Ă). Thread filling Ratios (%):Ă–ĕ/Ă (à/Ă)

Mandibular and femur bone mineral density of the groups. BMD: Bone mineral density. Mandibular BMD in the ovariectomy implant genistein group was higher than the ovariectomy implant and implant genistein groups ($P<0.05$). There was no statistically difference between the other groups in terms of mandibular BMD ($P>0.05$). There was no statistical difference between the groups in terms of femoral BMD ($P>0.05$).

It has been shown that genistein, a soybean isoflavone that acts as a phytoestrogen, may treat osteoporosis [7, 8]. Genistein improves bone health in bone loss due to estrogen deficiency [7, 8]. Changes in the bone after osteoporosis affects the bone–implant connection negatively; therefore, implant treatment in patients with osteoporosis is approached with suspicion [4]. Compounds used in the treatment of osteoporosis which give positive results may have a positive effect on the bone–implant connection [4]. This study suggests that genistein may positively affect osteointegration in patients with osteoporosis.

Osteoporosis, in rats is confirmed by lower BMD and lower trabecular numbers and thickness, as well as higher trabecular detachment, by changes observed in the proximal tibia, lumbar

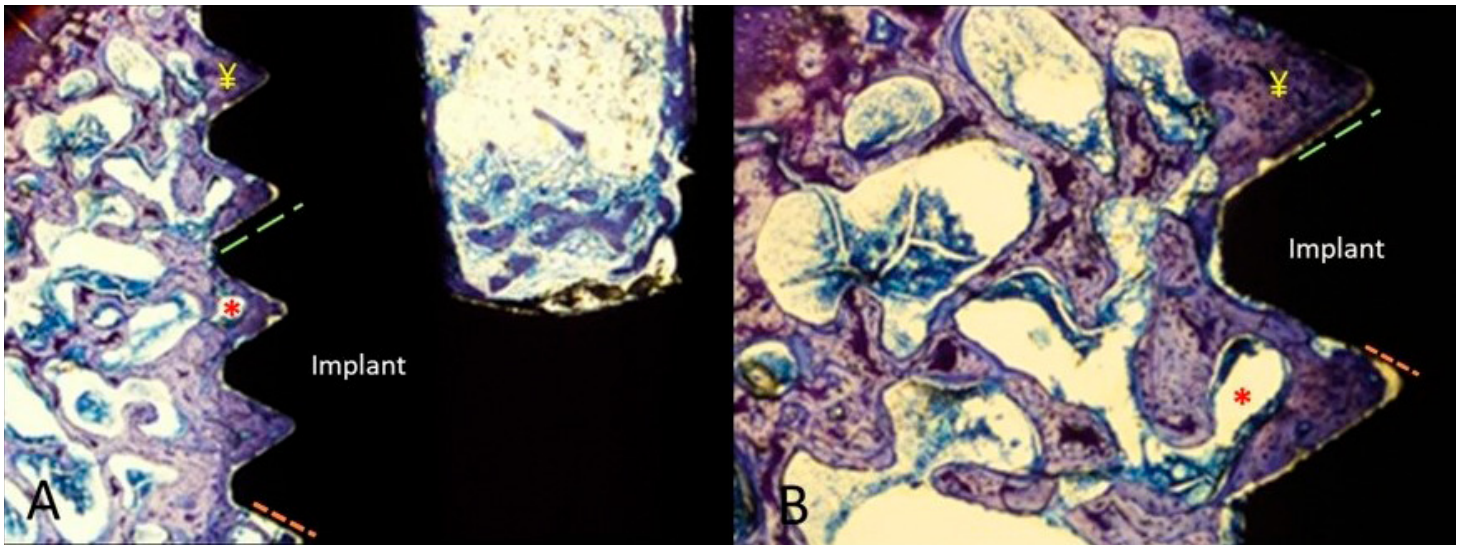

FIGURE 5. Non–Decalcified histologic images of the Implant–Genistein Group; (A:4×, B:10× magnification, Methylene blue). Implant surface not contacting bone (brown line), Implant surface contacting with bone (green line).*: Area without bone filling, ¥: Area with bone filling. Total implant surface: £, Bone Implant Contact Ratio (%): £–α(β)/£. Thread filling detected by measuring the ratio of the bone filled areas in total thread areas. Thread filling areas (à), non–bone areas (ě), total area (Ă). Thread filling Ratios (%):Ă–ě/Ă (à/Ă)

vertebrae, and femur at 14, 30, and 60 days (d) after ovariectomy, respectively. Current data shows that the response of trabecular bones of the proximal tibia, lumbar vertebrae, and femur to ovariectomy is similar in rats to that in humans [12].

In this study, implants were placed in the proximal tibia of rats, and the study was planned as 90 d. Rats are frequently preferred animals in osseointegration studies in the literature. In line with this data, rats were preferred in this study [13, 14, 15]. In a study by Şahin *et al*. [8] in that investigated the suppressive effects of lycopene and genistein on breast cancer, 2 mg·kg^{-1} genistein was administered to rats by oral gavage three times a week [8]. In this study, the rats in genistein groups were administered genistein in powder form containing at least 98% genistein, mixed with physiological saline, at 2 mg·kg^{-1}, by oral gavage, 3 d a week throughout the entire experimental period.

Density and volume decrease in bones seen in osteoporosis occurs in jawbones as well as in other bones. The relationship between osteoporosis and jawbones was first studied in 1960. In osteoporotic patients, the loss of alveolar bone tissue was found to be greater than that of the body of the mandible. Osteoporosis gives its first signs in the alveolar bone due to the fact that the rate of bone remodeling is higher in the alveolar bone than in other bones [16]. It was reported in a rewiev that women have lower mandibular bone mineral content than men, and women over 50 have greater bone loss than men of the same age. In this study, the mandibular BMD of the rats that underwent an ovariectomy was found to be lower than the control group, and this result is consistent with the literature [16, 17, 18].

In the present study, genistein, a phytoestrogen, was used in the rat ovariectomy model. Genistein is a natural compound belonging to the isoflavonoid group [7, 8, 19]. Genistein soy isoflavonoids resemble the molecular structure of estrogen, which is known to stimulate osteogenesis in bone cells, and acts like estrogen [19, 20, 21]. Like estrogen, genistein plays an important protective role against experimentally induced bone resorption in tissue cultures in vitro and stimulates osteoblast–mediated bone formation; that is, it exhibits anabolic effects. Animal model studies with ovariectomized rats (deprived of endogenous estrogens) have proven that genistein is as active as estrogens in maintaining bone health. Studies have shown that phytoestrogens after menopause are more effective than hormone therapy in maintaining bone mineral density in women [20, 21, 22]. Similar studies have shown that phytoestrogens bind to estrogen receptors in the bone and exert an estrogenic effect, reducing bone destruction in menopause [21, 22]. Results of a clinical study involving 136 postmenopausal women which received a soy isoflavone–enriched diet with walking exercise for six months found significant increases in bone mineral content and density in the lumbar spine [23]. In line with this scientific data, this study aimed to investigate the effect of genistein on bone–implant connection in the presence of osteoporosis.

Keikhosravi *et al.* [24] investigated the effects of high–intensity interval training and genistein on serum osteocalcin and ALP levels in female elderly rats. It was found that the serum ALP levels were significantly increased in the groups given genistein [24]. In this study, the ALP levels were found to be statistically lower in the implant genistein group than in the other groups, which contradicts this study. On the other hand the results of a study by Qi *et al* [25]. which investigated the effect of genistein and silicon on bone loss due to ovariectomy; genistein and/or silicon was found to reduce serum ALP levels [25]. In this study, the lower ALP levels in the implant genistein group, compared to the ovariectomized groups, supports this study. ALP values may increase in metabolic bone diseases. The low ALP values in the genistein group can be evaluated in this respect, genistein may have suppressed the bone–destructive effect of ovariectomy.

In a study by Park *et al*. [26] on female rats that underwent ovariectomy, it was reported that there was a decrease in serum

Ca levels due to the decline in estrogen levels, and increased Ca reabsorption in the estrogen receptors in the kidneys. Yang *et al.* [27] found a significantly lower serum calcium level in ovariectomized rats in a study using hispidulin, icariin and genistein to compare the effect of estrogen on bone tissue. In addition, serum Ca levels of all groups given hispidulin, icariin and genistein were higher than those of the rats that underwent an ovariectomy. According to the results of the reviewed literature, the results regarding serum Ca levels are controversial [12]. Despite this, the fact that serum Ca levels were found to be significantly lower in the ovariectomy group compared to the control group in this study supports the results of the study of Park *et al.* and Yang *et al.* [26, 27].

Qi *et al.* [25] investigated the effects of genistein and silicon on bone loss due to ovariectomy in rats with a study where the rats were randomly divided into 4 groups after the ovariectomy. As a result of the 10 week treatment of genistein silicone serum P levels were increased in the ovariectomy group [25]. In this study, phosphorus levels of the ovariectomy implant group were found to be statistically significantly lower than those of the ovariectomy implant genistein and implant genistein groups, which supports the current study.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the blood are strong indicators of liver damage [26, 27, 28]. In a study by Huang *et al.* [28] which was investigated the protective effect of genistein on chronic alcohol–induced liver damage and fibrosis in rats, found a significant decrease in AST and ALT enzyme activities in the subjects treated with genistein but showed that genistein did not affect basal plasma AST and ALT activities [26, 27, 28] . In this particular study, the blood AST and ALT levels of the subjects were examined in order to see the effect of genistein on liver function. While there was no statistically significant difference between the AST levels between the groups, the ALT level in the genistein group was found to be significantly lower than the other groups. This result suggests that genistein does not have a negative effect on liver function. In addition, the low ALT values in rats given genistein in this study can be explained by the liver–protective effect of genistein and supports the studies of Huang *et al.* [28].

In a study by Duarte *et al.* [29] evaluating the effect of estrogen deficiency on the bone tissue around the implants integrated into ovariectomized rats, found the bone–implant connection and bone filling values around the implant to be lower in the ovariectomy group. Giro *et al.* [30] reported that estrogen deficiency reduced trabecular bone density in a radiographic study in female rats that investigated the effect of estrogen deficiency treatment with alendronate and estrogen on the bone density around the osseointegrated implant. In a study by Cui *et al.* [31] investigating the osseointegration levels of titanium implants placed in the cancellous and cortical bones of ovariectomized animals, areas without significant bone–implant contact were detected in ovariectomized rats. In this study, when BIC levels were examined, the BIC levels of the ovariectomy–implant group and the ovariectomy–implant–genistein group were found to be significantly lower than those of the control implant group as supported by the literature and revealed that ovariectomy adversely affects BIC [30, 31]. In addition, the fact that the BIC level of the ovariectomy–implant–genistein group was significantly higher than that of the ovariectomy implant group suggests that genistein may positively affect the BIC levels in rats that underwent an ovariectomy. Although there was no statistically significant difference between TF levels in this study, when evaluated numerically, it was seen that the mean TF level of the ovariectomy implant group was lower than the mean TF level of the ovariectomy implant genistein group [29]. This shows that the results of this study are in accordance with the literature.

Lee *et al.* [32] reported that when examining the changes in the microarchitecture of the jaws of ovariectomized rats, the bone mineral density of the ovariectomized rats were lower compared to the control group. These authors have found that there is a decrease consistent with osteoporosis [32] . While there was no significant difference in terms of femoral BMD in this study, when the numerical femoral BMD values were examined, the values of the group that underwent an ovariectomy were controlled and lower than the treatment groups. The fact that the jaw BMD levels were higher in the control group than in the ovariectomy implant group supports the literature.

Qi and Zheng [25], reported in a study investigating the effect of genistein supplementation on markers related to the bone mineral density and bone metabolism in ovariectomized rats, that lumbar spine and femur bone mineral density decreased significantly after ovariectomy in rats, and this decrease was inhibited with genistein supplementation. According to histomorphometric analyses in the same study, investigators stated that genistein supplementation restored bone volume and trabecular thickness of the femur bone in ovariectomized rats [25]. In this study, the mandibular BMD of the ovariectomy–implant–genistein group was significantly higher than the jaw BMD of the implant–genistein group. Again in this study, the fact that the mandibular BMD of the control group was significantly higher than the Implant–Genistein group suggests that genistein does not affect the healthy bone tissue, which supports the literature.

Some limitations were identified while evaluating the findings in this study. The first of these limitations was that only genistein was evaluated in this study. The second was that the effect of genistein at different dosages has not been investigated. Third, this study did not evaluate the survival rate of titanium implants or the long–term success of bone implant connection. Fourth, long bones such as the tibia and femur have different osteogenic properties than the jaw bones (mandible–maxilla) and may therefore respond differently to genistein [33].

CONCLUSION

Within the limits of this study, it has been observed that ovariectomy may adversely affect bone tissue and reduce osseointegration. When the datas of this study are examined, it can be stated that genistein can improve the negative effects of ovariectomy on bone tissue and increase osseointegration. It can be stated that genistein consumption in patients with postmenopausal osteoporosis may contribute to the treatment of the skeletal system, as well as the success of implant treatment in such patients who receive dental implant treatment. Further studies are needed to examine the relationship between genistein and bone tissue–osteoporosis.

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Conflict of interest

The authors declarate there is no conflict of interest.

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