JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

A Preventive herb against bone loss in diabetic rats: *Zingiber officinale*

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Research Article

Volume: 6, Issue: 2
August 2022
Pages: 76-83

ABSTRACT

The study aims to determine and compare bone mechanical and material properties in experimentally diabetic rats treated with ginger extract. Forty female, healthy Wistar albino rats were used in the study. Rats were divided into five groups; Control (C), Sham (S), Ginger (G), Diabetic (D), and Diabetic rats treated with Ginger (DG). Diabetes mellitus was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin. Ginger-treated rats received 200 mg/kg ginger extract by oral gavage in a 30-day-trial. At the end of the study, tibiae were harvested and subjected to a three-point bending test. Plasma samples were also analyzed for calcium and phosphorus concentrations. It was observed that the bending strength significantly decreased in the groups Ginger (234.78 ± 16.79; P = 0.019) and the Diabetic (223.90 ± 29.90; P = 0.028) compared to group Control (275.75 ± 33.47). In addition, the bending strength of the diabetic rats treated with ginger (DG group; 251.92 ± 15.90) was also significantly higher than the rats in the Ginger and Diabetic groups (P = 0.032 and P = 0.037, respectively). Although the plasma calcium concentrations showed no differences among any of the groups, the plasma phosphorus levels decreased significantly in group Diabetic (3.47 ± 0.28; P = 0.05) compared to Control (5.11 ± 0.21). However, there was a significant increase in plasma phosphorus in group DG (4.32 ± 0.12; P = 0.05) compared to Diabetic. In conclusion, ginger extract treatment of diabetic rats improves bone material properties. The adverse effects of diabetes on the mechanical properties of the bone were prevented by using ginger extract in diabetic rats.

Keywords: biomechanics, diabetes mellitus, ginger, *Zingiber officinale,* rats.

Article History

Received: 26.05.2022
Accepted: 21.07.2022
Available online: 11.08.2022

DOI: https://doi.org/10.30704/http-www-jivs-net.1120974

To cite this article: Suzer, B., Seyidoglu, N., Tufekci, K., Deniz Karakci, D., Bakir, B. (2022). A Preventive herb against bone loss in diabetic rats: Zingiber officinale. ***Journal of Istanbul Veterinary Sciences, 6*(2),** 76-83. **Abbreviated Title*: J. İstanbul vet. sci.***

Introduction

Diabetes is caused by deficiencies in insulin production or insufficient insulin production along with insufficient insulin resistance. Diabetes mellitus (DM) is a chronic metabolic disease with high blood glucose levels (Gong, 2012; Moseley, 2012; Yan and Li, 2013). DM frequently results in crucial complications affecting the heart, blood vessels, eyes, nerves, and kidneys. In addition, adverse effects of diabetes on bone health have been progressively recognized and reduced and delayed bone formation were shown in diabetic animals (Follak et al., 2005).

Bone homeostasis is balanced between bone resorption by osteoclasts and bone formation by osteoblasts (Ckarke, 2008). The failure in the balance

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between these two important cell functions leads to bone loss (Karsenty and Wagner, 2002; William et al., 2003). Insulin stimulates anabolic effects on bone by binding receptors on osteoblasts (Thomas et al., 1996). Thus, decreased insulin levels or reduced insulin signaling in osteoblasts may cause inhibition of bone formation in diabetes (Thrailkill et al., 2005; Gandhi et al., 2006). Diabetes also leads to imbalance in osteoclast/osteoblast function, and thereby decreases bone quality (William et al., 2003; Nyman et al., 2011).

Minerals are important for structural components of body as well as for regulation of chemical reactions of body processes. The alteration of bone structure, bone metabolism rate, endocrine system and calcium-phosphorus are balanced by nutrients and natural supplements. Researchers observed that especially herbal foods can affect bone resorption and have protective roles against bone loss (Hwang et al., 2018; Zammel et al., 2018). It is possible to treat bone loss pharmacologically by inhibiting osteoclastogenesis and/or by inducing osteoblast activity. Several drugs such as bisphosphonates, are commonly used for the treatment of bone loss (Rogers et al., 2011; Das and Crockett, 2013). However, the effects of plants on human health have been utilized for thousands of years (Koehn and Carter, 2005; Jones et al., 2006; Newman and Cragg, 2014). Especially the traditional herbal medicines have been preferred by clinicians because of their fewer side effects. These medicines are also more appropriate for long term treatments compared to chemically synthesized ones (Hidaka et al., 1999).

Zingiber officinale (ginger) is a flowering plant whose rhizome, ginger root, or ginger, has been commonly used throughout the world as a cooking spice and has also been used as a natural medicine due to its anti-inflammatory and pain relief agent for musculoskeletal diseases in traditional medicines (Ali et al., 2008; Baliga et al., 2011). There are more than 50 types of antioxidants have been extracted from ginger. The principal pharmacological activity of ginger is related to its active compounds such as 2- and 6-Gingerol (Shukla and Singh, 2007), especially anti-inflammatory effects of ginger have come from 6-gingerol (Semwal et al., 2015). The 6-gingerol has anti-oxidant, anti-tumoral, anti-obesity and anti-diabetic activities besides its anti-inflammatory properties (Semwal et al., 2015). In DM patients, ginger consumption improves glycemic status (Bhandari et al., 2005; Thomson et al., 2007; Shanmugam et al., 2011; Mahluji et al., 2013), insulin sensitivity, lipid profiles (Shanmugam et al., 2011; Huang et al., 2011) and other metabolic disorders by reducing inflammatory factors. Moreover, ginger may inhibit bone loss and contribute bone formation. Zammel et al., (2018) indicated that ginger extract could depress osteoclast activation and decrease their number throughout inhibiting the osteoclastogenesis. Also, ginger extracts caused improvements in the vertebral microarchitecture. These researchers also suggested that the positive effects of ginger may be related to increasing osteoprotegerin (OPG) and/or decreasing the receptor activator of nuclear factor-κB ligand (RANKL) expression by osteoblast. Another study (Hwang et al., 2018) suggested that 6-gingerol inhibited IL-1-induced osteoclast differentiation, and 6 -gingerol might be useful for inflammatory bone loss treatments.

According to the knowledge from the previous studies, ginger seems to be useful to decrease fracture risk, to inhibit bone loss and enhance bone strength. The aim of the study is to determine and compare bone mechanical and material properties in experimentally diabetic rats treated with ginger extract.

Materials and Methods

Animals: The experimental protocols were approved by the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Animal Care and Use Committee of Tekirdag Namik Kemal University, Turkey (Approval No: T2019-232).

In this study, forty female, healthy Wistar albino rats weighing 150-250 g and aged 4 months old were used. The animals were housed under standard laboratory conditions (22±1 oC; 55±10% humidity) in clear, plastic cages, with stainless steel feed hoppers. Wood shavings were used as bedding material. Rats were given tap water and ad libitum access to commercial rodent diet.

Plant supplementation: Fresh ginger rhizomes were purchased from a local store and authenticated at department of Botany in University. The ethanolic extract of ginger and the experimental design was prepared using the method described by Shanmugam et al., (2011). The rhizomes were washed and air-dried. Then the air-dried rhizomes were transformed into powder mechanically and prepared extract 95% ethanol for 24 hours. After that, the extract was filtered and 95% ethanol was added. The process was repeated three times. The three extracts were pooled together then filtered and evaporated to dry. As a result, a dark brown and gelatinous extract was obtained. Before the onset of the experiment, 200 mg/kg gelatinous extract was dissolved in 2% Tween 80 solution.

Experimental design and treatment process (Protocol): The rats were divided into five groups with eight animals in each group based on previous mechanobiological studies (Main and Biewener, 2004; Main et al., 2010; Lynch et al., 2010). The total experiment protocol was maintained for thirty days. The experimental groups as follows: Control (C); Sham (S) (2% Tween 80 was applied); Ginger (G) (Oral gavage 200 mg/kg Ginger extract); Diabetic (D) (50 mg/kg STZ i.p.); Diabetic + Ginger (DG) (Oral gavage 200 mg/kg Ginger extract).

Diabetes mellitus was induced by a single intraperitoneal injection (i.p.) of 50 mg/kg streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in 50 ml citric acid + 40 ml of disodium hydrogen phosphate buffer (pH 4.5) was administered after an overnight fasting. Three days after STZ administration, fasting blood glucose levels of tail vein blood of rats were measured by glucometer (Accu-Chek Instant, Roche, Switzerland). The animals were labeled diabetic with fasting blood glucose of 250 mg/dl and above.

Blood analysis: The blood samples were collected into Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes by heart puncture of the overnight-fasted rats under isoflurane anesthesia at the end of the trial. The samples were centrifuged in the same day at 3000 rpm for 10 minutes to separate the plasma, transferred the plasma into microtubes and samples were stored at -80 °C until the analysis day. The changes of plasma calcium and phosphorus levels were determined with commercial kits (Calcium Ref No: 90004, and phosphorus Ref No: 80015, Biolabo, France) using the spectrophotometric method, and the parameters were measured by microplate reader (Biotek, Epoch, USA).

Bone preparation and mechanical testing: Tibiae were harvested and wrapped with Phosphate-Buffered Saline (PBS) soaked gauze and frozen at-20°C until further analyses were conducted (Van Haaren et al., 2008). Prior to tests, bones were thawed at room temperature. Then, tibiae were subjected to mechanical compression test. For three-point bending tests a custom-made, low strength material testing machine was used designed and manufactured by Tufekci et al. (2014). To measure force and corresponding displacement, the loading machine had a load-cell (100 N, Tedea Huntleigh Malvern, USA) and a Linear Variable Differential Transformer (LVDT) (10-mm stroke, Novotechnik Tr10, Germany). Force and displacement measurements were recorded by an oscilloscope (Nicholet-Oddysey XE, USA) at the rate of 50 data/sec. The span length was set to 21mm and the load was applied at the mid-shaft with a constant loading speed of 10mm/min (Jast, 2011). Loading was applied until the bones were broken. Maximum bone breaking force (Fmax) was obtained from the load-displacement curve which was the highest value of the force. After biomechanical test, 2mm sections of mid-shaft of tibia were taken from the fracture site and the section were photographed under a stereomicroscope (Motic, Model: SMZ-168, Hong Kong). Pictures were transferred to Solidworks R17 3D CAD software (Dassault Systèmes, Waltham, MA; USA) and cortical area (Acort) and the minimum principal moment of inertia (Imin) was calculated. Furthermore, flexural (bending) strength (σf), and bending modulus of elasticity (E) were calculated by using the equations below respectively:

$$\sigma_f = \frac{My_{max}}{I_{min}} \qquad E = \frac{FL^3}{48\delta I_{min}}$$

Where M is the ultimate moment at the middle of the specimen, Y_{max} is the maximum vertical distance between neutral axis and the outer edge of the specimens, I_{min} is the minimum moment of inertia related the neutral axis, F is the applied force, L is the length of the support span, δ is the deflection due to corresponding force.

Statistical analysis: Data for the measured parameters were checked for normally distribution and assumptions for homogeneity of variance (Shapiro-Wilk test). If data normally distributed, One-way ANOVA test was applied, and the differences between groups were analyzed by the post hoc Tukey test. If data did not provide normality and homogeneity assumptions, data were subjected to Kruskall-Wallis test followed by post-hoc Mann-Whitney U multiple comparison test of significance using SPSS (IBM SPSS, Version 23.0, Chicago, IL). The differences were considered significant at $P < 0.05$.

Results

Mechanical test measurements for the tibiae of the rats were presented in the Table I. According to these results, F_{max}, I_{min}, A_{cort}, and elastic modulus showed no significant differences among experimental groups (P>0.05). However, changes were observed between experimental groups in terms of bending strength of the tibia. It was seen that the bending strength value significantly decreased in the Ginger and the Diabetic groups compared to the Control (P=0.019 and P=0.028, respectively group G and D). In addition, the bending strength value of the DG (Diabetic+Ginger) group was also significantly higher than the rats in the

Table I. Mechanical test measurements for the tibiae of the rats in experimental groups

Groups	F_{max} (N)	σ_f (MPa)	I_{min} (mm^4)	A_{cort} (mm^2)	*E* (GPa)
Control (C)	62.6 ± 8.73	275.7 ± 33.47^{ab}	1.30 ± 0. 25	3.30 ± 0.32	14.9 ± 2.12
Sham (S)	60.1 ± 16.84	272.3 ± 50.80	1.12 ± 0.26	3.43 ± 0.50	13.9 ± 3.01
Ginger (G)	65.7 ± 5.01	234.8 ± 16.79^{ac}	1.54 ± 0.23	3.76 ± 0.20	14.7 ± 4.75
Diabetic (D)	64.5 ± 15.80	223.9 ± 29.90^{bd}	1.57 ± 0. 53	3.68 ± 0.44	11.3 ± 3.36
Diabetic+Ginger (DG)	67.2 ± 9.03	251.9 ± 15.90^{cd}	1.56 ± 0.30	3.61 ± 0.37	16.3 ± 4.26

*n = 40, Data was presented as mean±standard deviation of the mean. Fmax: Maximum breaking force, σf: Bending strength, Imin: Minimum principal moment of inertia, Acort: Cortical area, E: Elastic modulus. The groups: Control, Sham, rats supplied with ginger (Ginger), diabetic rats (Diabetic) and diabetic rats treated with ginger (Diabetic+Ginger). a, b, c, d Superscripts show that there is a difference between the groups indicated with the same letters.

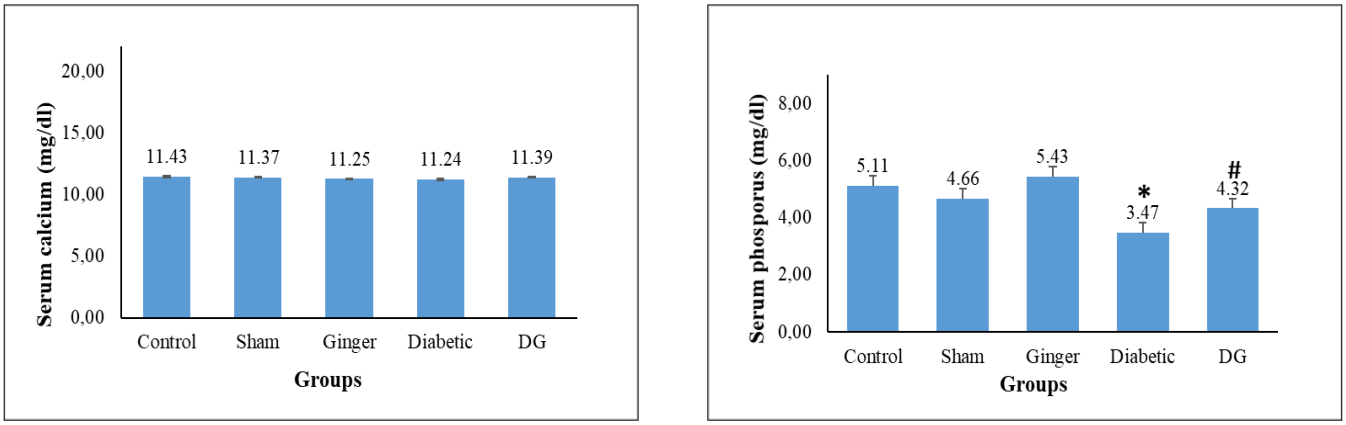

Figure I. The serum indicators of the rats in the control and experimental groups.
(A), The serum calcium concentration of the rats. (B), The serum phosphorus concentration of the rats. The groups: Control, Sham, rats supplied with ginger (Ginger, G), diabetic rats (Diabetic, D) and diabetic rats treated with ginger (Diabetic+Ginger, DG). * $P < 0.05$; Diabetic rats versus Control group. # $P < 0.05$; Diabetic rats treated with ginger versus Diabetic group.

higher than the rats in the G and the D groups (P=0.032 and P=0.037, respectively). However, no significant difference was observed between the Control and the Sham groups (P>0.05).

The plasma calcium and phosphorus for all the groups are shown in Figure I. There were no differences in plasma calcium among any of the groups (P > 0.05). However, the plasma phosphorus value in group D was lower than Control (P = 0.05; 5.11 ± 0.21 and 3.47 ± 0.28, respectively group Control and D), and a significant increase in group DG group than group D (P = 0.05; 3.47 ± 0.28 and 4.32 ± 0.12, respectively group D and DG).

Discussion

Diabetes causes changes and deterioration in bone metabolism and microarchitecture through various mechanisms at the bone's structural and molecular levels. These changes cause detrimental effects on bone biomechanical properties and increase the risk of fractures. Furthermore, these changes also cause impaired healing of the bone tissue (Funk et al., 2000; Janghorbani et al., 2006; Mashadi et al., 2013).

Ginger (*Zingiber officinale*) is a perennial tropical plant belonging to the Zingiberaceae family. Ginger has been widely used as a food flavoring and herbal medicine for many years (Mashadi et al., 2013). The clinical usage of ginger as a medicinal or alternative therapy is of great interest due to its diverse influences such as antibacterial, anti-inflammatory, antihyperglycemic, antitumor, renal protection, and cardiotonic effect in different systems (Danwilai et al., 2017; Marx et al., 2017). However, few studies have assessed on bone strength and bone fracture risk. So, further research is needed to identify the efficiencies of ginger intakes for bone health. Therefore, in the

present study, we focused on some biomechanical properties of the tibia in diabetic rats treated with ginger to investigate the potential effects of ginger to prevent fracture risk.

The bone tissue is a model that has a lifelong cycle of construction and destruction. Calcium and phosphorus are the most important factors for the cycles. Xiao et al., (2015) showed that in diabetic rats, plasma calcium and phosphorus levels were found lower than the control ones. It was reported that decreasing in plasma calcium and phosphorus could be due to impaired renal reabsorption and osmotic diuresis by hyperglycemia in diabetic rats. Also, it was suggested that alteration of calcium may correlate with abnormality of fasting serum glucose and insulin levels (Sun et al., 2005). In the present study, a significant decrease in plasma phosphorus in D group compared to Control (Control: 5.11±0.21 and D: 3.47±0.28, P=0.05). Ginger treatment improved the phosphorus levels in group DG as to D significantly (D: 3.47±0.28 and DG: 4.32±0.12, P=0.05). However, there was a slight decrease in plasma calcium in D group compared to Control, and an increase in DG group compared to D group. These results may be due to ginger ingredients which have roles on enhance of bone phosphorus mobilization. It was reported that Ginger contains calcium, phosphorus and magnesium which have important roles on bio-functions, especially bone formation and curbing muscle spasms (Kikuzaki and Nakatani, 1993). Also El-Mottaleb et al., (2016) reported that aqueous extract of raw ginger has potential hypoglycemic properties that reflected on bone formation in diabetic rats treated with ginger.

A_{cort} and I_{min} values, which are the geometric properties of the bone and calculated from the broken bone section, were not statistically significant. However, when the mean maximum bone breaking force (F_{max}) values are examined, a higher F_{max} was observed in the G, D and DG groups than in the Control group. A similar increase was also observed in I_{min} and A_{cort} values. Therefore, it can be said that the high strength value in these groups (G, D and DG groups) was an increase not due to the superiority in the microstructure of the bone, but rather due to the geometry. To better evaluate the situation in the microstructure, the elastic modulus and bending strength values should be considered. In this study, the mentioned values were determined by calculating the relevant values (A_{cort} and I_{min}) of the fracture section of the bone in the computer environment. When the sham group was compared with the Control group, it was observed that there was no significant difference in mechanical and geometric properties. These findings suggested that operational stress had no adverse effects on the mechanical and geometrical properties of the bone in the present study.

The mineral and collagen structure of the bone is effective on bending strength. Even if the collagen totally normal, alterations in collagen features may change the amount and arrangement of the mineral, which would affect the bone mechanics (Currey, 2003). The modulus of elasticity is mostly influenced by the mineral structure and depends largely on the degree of mineralization. Because, the elastic modulus of collagen is so low (Hamed et al., 2010). Changes in mineralization have an intense effect on the elastic modulus of the bone. High mineralization of the bone cause high elastic modulus values and this causes low work to fracture (Currey, 1984). In the comparison of the Control and G groups, it was observed that the elasticity modulus was barely unchanged and the bending strength values were lower in the G group. This result suggested that ginger has an adverse effect on collagen tissue of the bone in healthy individuals. To our knowledge, there is only one study mentioned the detrimental effects of ginger on trabecular bone. Therefore, our result can be explained with the research by Khan et al., (2012), that 6-gingerol has harmful effects on cancellous bone and consequently has a negative effect on the mechanical properties of the bone.

The fact that bending strength was significantly lower in the D group than the Control group, and the average value of the elasticity modulus was lower, although not significant. This result indicates that diabetes has adverse effects on both collagen tissue and mineral tissue. Diabetic fractures occur as a result of decreased bone quality and changes in bone microarchitecture (Jiao et al., 2015). Insulin induces osteoblast proliferation and collagen synthesis (Nyman et al., 2011). The deficiency of insulin hormone due to diabetes causes disruptions in collagen production and may causes a decrease in the bending strength of the bone in diabetic rats. Katayama et al. (1996) also suggested that AGE (Advanced glycation end-products)-modified collagen inhibits osteoblastic differentiation and function in diabetic animals. In addition, diabetes may negatively affect the microvascular environment of the bone and increase bone loss and fracture risk (Shanbhogue et al., 2017). As mentioned above, in the present study, the bending strength was lower in the D group than in the Control group, and the fracture force (F_{max}) values were almost the same as in the Control group. This may occur due to the bone adaptation mechanism; the bone adapted to balance its deteriorated

deteriorated microstructure and lost mechanical properties by increasing volumetrically. However, there was a significant difference in bending strength values between the DG group and the D group. The DG group had higher bending strength and elastic modulus values compared to the diabetic group, although there was no significant difference. It can be concluded that the negative effects of diabetes on the mechanical properties of the bone were prevented by using ginger in diabetic rats. In addition, Zammel et al. (2018) also reported that ginger showed a potential protective effect by reducing changes in vertebral microarchitecture and preserving the mineral composition of the spine.

Conclusion

Despite its mild adverse effects on the collagen and phosphorus mechanism in healthy rats, ginger treatment of diabetic rats indicated that it could be used as supplementary food in diabetic patients due to the possibility of restoring the mechanical and material properties and preventing potential fractures of the bone. Ginger may serve as a potent medicinal agent for the treatment of reducing fracture susceptibility in diabetic patients. Moreover, we hope the current study encourages the researchers to investigate the mechanisms of ginger extract on bone biomechanics.

Acknowledgements

The authors declared that there is no conflict of interest.

References

Ali, B. H., Blunden, G., Tanira, M. O., & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. *Food and Chemical Toxicology, 46*, 409-420.

Baliga, M. S., Haniadka, R., Pereira, M. M., D'Souza, J. J., Pallaty, P. L., Bhat, H. P., & Popuri, S. (2011). Update on the chemopreventive effects of ginger and its phytochemicals. *Critical Reviews in Food Science and Nutrition, 51*, 499-523.

Bhandari, U., Kanojia, R., & Pillai, K. K. (2005). Effect of ethanolic extract of Zingiber officinale on dyslipidaemia in diabetic rats. *Journal of Ethnopharmacology 97*, 227–230.

Clarke, B. (2008). Normal bone anatomy and physiology. *Clinical Journal of the American Society of Nephrology, 3*, 131–139.

Currey, J. D. (1984). Effects of differences in mineralization on the mechanical properties of bone. *Philosophical Transactions of the Royal Society B, 304*, 509–518.

Currey, J. D. (2003). Role of collagen and other organics in the mechanical properties of bone. *Osteoporosis International, 14*, 29–37.

Danwilai, K., Konmun, J., Sripanidkulchai, B. O., & Subongkot, S. (2017). Antioxidant activity of ginger extract as a daily supplement in cancer patients receiving adjuvant chemotherapy: A pilot study. *Cancer Management and Research, 9*, 11–18.

Das, S., & Crockett, J. C. (2013). Osteoporosis - a current view of pharmacological prevention and treatment. *Drug Design, Development and Therapy, 7*, 435–448.

El-Mottaleb, N. A. A. B. D., El-Aziz, E. A. A. B. D., & Mostafa, D. G. (2016). Effect of ginger on bone of streptozotocin induced diabetic rats. *Medical Journal of Cairo University, 84*, 395–403.

Follak, N., Klöting, I., & Merk, H. (2005). Influence of diabetic metabolic state on fracture healing in spontaneously diabetic rats. *Diabetes/Metabolism Research and Review, 21*, 288–296.

Funk, J. R., Hale, J. E., Carmines, D., Gooch, H. L., & Hurwitz, S. R. (2000). Biomechanical evaluation of early fracture healing in normal and diabetic rats. *Journal of Orthopaedic Research, 18*, 126–132.

Gandhi, A., Dumas, C., O'Connor, J. P., Parsons, J. R., & Lin, S. S. (2006). The effects of local platelet rich plasma delivery on diabetic fracture healing. *Bone, 38*, 540–546.

Gong, Z., & Muzumdar, R. H. (2012). Pancreatic function, type 2 diabetes, and metabolism in aging. International *Journal of Endocrinology, 2012*, 320482.

Hamed, E., Lee, Y., & Jasiuk, I. (2010). Multiscale modeling of elastic properties of cortical bone. *Acta Mechanica, 213*, 131–154.

Hidaka, S., Okamoto, Y., Yamada, Y., Kon, Y., & Kimura, T. (1999). A Japanese herbal medicine, Chujo-to, has a beneficial effect on osteoporosis in rats. *Phytotherapy Research, 13,* 14–19.

Huang, H. C., Chiu, S. H., & Chang, T. M. (2011). Inhibitory effect of [6]-gingerol on melanogenesis in b16f10 melanoma cells and a possible mechanism of action. *Bioscience, Biotechnology, and Biochemistry, 75*, 1067–1072.

Hwang, Y. H., Kim, T., Kim, R., & Ha, H. (2018). The natural product 6-gingerol inhibits inflammation-associated osteoclast differentiation via reduction of prostaglandin E2 levels. *International Journal of Molecular Sciences, 19*, 2068.

Jast, J. A. (2011). Investigation of ultrastructural and mechanical properties of rat corticol bone using micro-CT, three-point bending testing, and the reference point indentation technique. *Thesis of degree of Master of Science.* Graduate College of the University of Illinois, Urbana, Illinois.

Janghorbani, M., Feskanich, D., Willett, W. C., & Hu, F. (2006). Prospective study of diabetes and risk of hip fracture: The nurses' health study. *Diabetes Care, 29*, 1573–1578.

Jiao, H., Xiao, E., & Graves, D. T. (2015). Diabetes and its effect on bone and fracture healing. *Current Osteoporosis Reports, 13*, 327–335.

Jones, W. P., Chin, Y-W., & Kinghorn, A. D. (2006). The role of pharmacognosy in modern medicine and pharmacy. *Current Drug Targets, 7*, 247–264.

Karsenty, G., & Wagner, E. F. (2002). Reaching a genetic and molecular understanding of skeletal development. *Developmental Cell, 2*, 389–406.

Katayama, Y., Akatsu, T., Yamamoto, M., Kugai, N., & Nagata, N. (1996). Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia. *Journal of Bone and Mineral Research, 11*, 931–937.

Khan, K., Singh, A., Mittal, M., Sharan, K., Singh, N., Preety, D., Sanyal, S., Maurya, R., & Chattopadhyay, N. (2012). [6]-Gingerol induces bone loss in ovary intact adult mice and augments osteoclast function via the transient receptor potential vanilloid 1 channel. *Molecular Nutrition & Food Research, 56*,1860–1873.

Kikuzaki, H., & Nakatani, N. (1993). Antioxidant Effects of Ginger Constituents, *Journal of Food Science, 58*, 1407-1410.

Koehn, F. E., & Carter, G. T. (2005). The evolving role of natural products in drug discovery. *Nature Reviews Drug Discovery, 4,* 206–220.

Lynchi, M. E., Main, R. P., Xu, Q., Walsh, D. J., Schaffleri M. B., Wright, T. M., & van der Meulen, M. C. H. (2010). Cancellous bone adaptation to tibial compression is not sex dependent in growing mice. *Journal of Applied Physiology, 109*, 685–691.

Mahluji, S., Attari, V. E., Mobasseri, M., Payahoo, L., Ostradrahimi, A., & Golzari, S. E. J. (2013). Effects of ginger (Zingiber officinale) on plasma glucose level, HbA1c and insulin sensitivity in type 2 diabetic patients. *International Journal of Food Sciences and Nutrition, 64*, 682–686.

Main, R. P., & Biewener, A. A. (2004). Ontogenetic patterns of limb loading, in vivo bone strains and growth in the goat radius. *Journal of Experimental Biology, 207*, 2577–2588.

Main, R. P., Lynch, M. E., & van der Meulen, M. C. H. (2010). In vivo tibial stiffness is maintained by whole bone morphology and cross-sectional geometry in growing female mice. *Journal of Biomechanics, 43*, 2689–2694.

Marx, W., Ried, K., McCarthy, A. L., Vitetta, L., Sali, A., McKavanagh, D., & Isenring, L. (2017). Ginger-mechanism of action in chemotherapy-induced nausea and vomiting: A review. *Critical Reviews in Food Science and Nutrition, 57*, 141–146.

Mashadi, N. S., Ghiasvand, R., Askari, G., Hariri, M., Darvashi, L., & Mofid, M. R. (2013). Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. *International Journal of Preventive Medicine, 4*, 36–42.

McCabe, L. R. (2007). Understanding the pathology and mechanisms of type I diabetic bone loss. *Journal of Cellular Biochemistry, 102*, 1343–1357.

Moseley, K. F. (2012). Type 2 diabetes and bone fractures Kendall. *Current Opinion in Endocrinology, Diabetes and Obesity, 19*, 128–135.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products, 79*, 629–661.

Nyman, J. S., Even, J. L., Jo, C. H., Herbert, E. G., Murry, M. R., Cockrell, G. E., Wahl, E. C., Bunn, R. C., Lumpkin, C. K., Fowkles, J. L., & Thrailkill, K. M. (2011). Increasing duration of type 1 diabetes perturbs the strength-structure relationship and increases brittleness of bone. *Bone, 48*, 733–740.

Rogers, M. J., Crockett, J. C., Coxon, F. P., & Mönkkönen, J. (2011). Biochemical and molecular mechanisms of action of bisphosphonates. *Bone, 49*, 34–41.

Semwal, R. B., Semwal, D. K., Combrinck, S., & Viljoen, A. M. (2015). Gingerols and shogaols: Important nutraceutical principles from ginger. *Phytochemistry, 117*, 554–568.

Shanbhogue, V. V., Hansen, S., Frost, M., Brixen, K., & Hermann, A. P. (2017). Bone disease in diabetes: another manifestation of microvascular disease? *Lancet Diabetes Endocrinology, 5*, 827–838.

Shanmugam, K. R., Mallikarjuna, K., Kesireddy, N., & Reddy, K. S. (2011). Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology, 49*, 893–897.

Shukla, Y., & Singh, M. (2007). Cancer preventive properties of ginger: A brief review. *Food and Chemical Toxicology, 45*, 683–690.

Sun, G., Vasdev, S., Martin, G. R., Gadag, V., Zhang, H. (2005). Altered calcium homeostasis is correlated with abnormalities of fasting serum glucose, insulin resistance, and beta-cell function in the Newfoundland population. *Diabetes, 54*, 3336–3339.

Thomas, D. M., Hards, D. K., Rogers, S. D., Ng, K. W., & Best, J. D. (1996). Insulin Receptor Expression in Bone. *Journal of Bone and Mineral Research, 11*, 1312–1320.

Thomson, M., Al-Amin, Z. M., Al-Qattan, K. K., Shaban, L. H., & Ali, M. (2007). Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats. International *Journal of Diabetes and Metabolism, 15*, 3.

Thrailkill, K. M., Lumpkin, C. K., Bunn, R. C., Kemp, S. F., & Fowlkes, J. L. (2005). Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. *American Journal of Physiology-Endocrinology and Metabolism, 289*, 735–745.

Tufekci, K., Kayacan, R., & Kurbanoglu, C. (2014). Effects of gamma radiation sterilization and strain rate on compressive behavior of equine cortical bone. *Journal of the Mechanical Behavior of Biomedical Materials, 34*, 231–242.

Van Haaren, E. H., van der Zwaard, B. C., van der Veen, A. J., Heyligers, I. C., Wuisman, P. I. J. M., & Smit, T. H. (2008). Effect of long-term preservation on the mechanical properties of cortical bone in goats. *Acta Orthopaedica, 79*, 708–716.

William, B. J., Simonet, S. W., & Lacey, D. L. (2003). Osteoclast differentiation and activation. *Nature, 423*, 337–342.

Xiao, L., Wang, X. M., Yang, T., Xiong, Y., Zhang, Z. G., Ding, J., Xu, C., & Xiong, C. Y. (2015). Changes of serum osteocalcin, calcium, and potassium in a rat model of type 2 diabetes. *Cell Biochemistry and Biophysics, 71*, 437–440.

Yan, W., & Li, X. (2013). Impact of diabetes and its treatments on skeletal diseases. *Frontiers in Medicine, 7*, 81–90.

Zammel, N., Amri, N., Chaabane, R., Rebai, T., & Badraoui, R. (2018). Proficiencies of Zingiber officinale against spine curve and vertebral damage induced by corticosteroid therapy associated with gonadal hormone deficiency in a rat model of osteoporosis. *Biomedicine & Pharmacotherapy, 103*, 1429–1435.