Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2021.v09.i03.A14

THE ENHANCEMENT OF BONE PROTECTIVE EFFECT OF *OAK BARK* EXTRACTS BY GERANIIN IN DIABETIC RATS

Krishnaraju Venkatesan[*1], Ester Mary Pappiya[2], Kumar Venkatesan[3], Kumarappan Chidambaram[1], Geetha Kandasamy[4], Md. Zaheen Hassan[3], Premalatha Paulsamy[5], Kalpana Krishnaraju[6]

[1*]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Saudi Arabia.
[2]Directorate of General Health Affair, Ministry of Health, Najran, Abha, Saudi Arabia.
[3]Department of Pharmaceutical Chemistry, King Khalid University, Abha, Saudi Arabia.
[4]Department of Clinical Pharmacy, College of Pharmacy King Khalid University, Abha, Saudi Arabia.
[5]King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia.
[6]Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, Tamilnadu, India.

ABSTRACT

Diabetes complications and osteoporotic fractures are two of the most important causes of morbidity and mortality in older patients and share many features including genetic susceptibility, molecular mechanisms, and environmental factors. Type 2 diabetes mellitus (T2DM) compromises bone micro architecture by inducing abnormal bone cell function and matrix structure, with increased osteoblast apoptosis, diminished osteoblast differentiation, and enhanced osteoclast mediated bone resorption. The linkage between these two chronic diseases creates a possibility that certain anti-diabetic therapies may affect bone quality. The present study will look at the possible bone health advantages of Geraniin's + *oak bark* extracts in diabetic rats, which is a well-known traditional herbal treatment. *Oak bark* extracts have been shown to help with bone health. The impact of geraniin on improving bone mineral density (BMD) in *oak bark* extracts treated individuals was studied after 8 weeks of therapy. The researchers discovered that eating 40mg of geraniin per kilogramme of body weight improved the bone protective effects of *oak bark* extracts by decreasing blood glucose and boosting BMD. According to all of the data, combining geraniin with *oak bark* extracts can help prevent diabetic osteoporosis.

KEYWORDS

Oak bark extracts, Diabetic osteoporosis and Geraniin.

Author for Correspondence:
Krishnaraju Venkatesan,
Department of Pharmacology,
King Khalid University, Abha, Saudi Arabia.
Email: kvenkatesan@kku.edu.sa.

INTRODUCTION

Diabetes, particularly T2DM, is becoming more common worldwide, and there is increasing evidence that diabetes is both a cause and a risk factor for osteoporotic fractures. Diabetes disrupts glucose metabolism, bone microvascular function,

glucose oxidative byproducts, and muscle endocrine function, all of which weaken bones[1]. Bones and glucose metabolism are inextricably connected, and osteoblast development definitely requires glucose[2]. Hyper insulinemia, decreased blood levels of IGF-1, presentation of advanced glycosylation of end-products (AGEs) particularly in collagen following hyperglycemia, decreased levels of osteocalcin, and renal failure all impact bone tissue (osteopenia and osteoporosis). Hypercalciuria is characterized by hypercalciuria, microangiopathy, and inflammation[3]. Hyperglycemia and its consequences reduce the synthesis of osteocalcin, which is necessary for matrix maturation and bone mineralization. *Oak bark* extracts, a biquanide antidiabetic medication, is a frequent oral prescription for type 2 diabetes therapy (non-insulin dependent).

Despite the fact that it has been used for over 40 years, no one knows how it works[4]. *Oak bark* extracts are a first-line oral medication for T2DM because they are inexpensive and safe, with little risk of hypoglycemia, little weight gain, and minimal adverse effects[5]. *Oak bark* extracts have been found to have no impact on glucose levels in non-diabetic people, which supports the use of *oak bark* extracts as an adjuvant medicine, notably in the treatment of bone disorders[6,7]. Geraniin is a dehydroellagitannin discovered in geraniums with a wide range of bioactivities. Geraniin's antiresorptive action was connected to the down regulation of matrix metalloproteinase and carbonic anhydrase II, the researchers wanted to investigate if geraniin might potentiate *oak bark* extracts against diabetic drug-induced bone damage.

MATERIAL AND METHODS

Animals

Healthy male wistar albino rats weighing 180 to 240g and aged 3 to 4 months were utilized in the investigation. The animals were taken from the Central Animal House of King Khalid University in Abha, Saudi Arabia. The animals were housed in cages throughout the trial and fed a standard pellet diet and filtered water ad libitum under standard conditions (light/dark cycle of 12 h/12 h, 50-70 percent humidity, 25°C 3°C). The animals were acclimatized to the laboratory environment for 14 days. The therapy was carried out in accordance with the permission of King Khalid University's animal ethics committee and the National Institute of Health's standards for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

To induce diabetes in the animals, the pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was administered intra peritoneally at a dosage of 65mg/kg body weight[8,9]. In the control group, all of the rats were given the same quantity of vehicle. An intra peritoneal injection of the pancreatic-cell toxin streptozotocin (STZ) at a dosage of 65mg/kg body weight was used to cause diabetes in the rats. The rats in the control group were all given the same amount of vehicle. STZ was weighed individually for each animal, solubilized with 0.1ml of freshly prepared cold Na-citrate buffered (NaB-0.1 M, pH 4.5) solution, and given within 5 minutes to prevent deterioration.

The volume of STZ injection was determined to be 1.0ml/kg. Rats were administered a 5% glucose solution for 48 hours after receiving STZ to counteract the drug's substantial acute hypoglycemia impact. Three days following STZ injection, blood was taken from the tail vein and analyzed for blood glucose using a glucometer (Aqua-Check, Roche). Animals having fasting blood glucose levels (BGLs) more than 250mg/dL were classified as Group 1: Non-Diabetic control, Group 2: Diabetic control, Group 3: Geraniin 40mg/kg body weight, Group 4: *oak bark* extracts 100mg/kg body weight, and Group 5: *Oak bark* extracts 100mg/kg + Geraniin 40mg/kg body weight) were divided into five groups of six rats each. To evaluate the animals' hyperglycemic state, blood glucose levels were tested once a week using a Roche Accu-Chek glucometer. The study did not include the animals which did not acquire blood glucose levels more than 250mg/dL. The rats administered saline instead of streptozotocin in the

control group (n=6) had normal blood glucose levels (120mg/dl).

Determination of fasting blood glucose

Blood samples were collected from the rats' tail veins to measure blood glucose levels using a glucometer after they had been fasted for 12-14 hours. After the rats' tails have been cleaned with 70% (v/v) ethanol, blood will be drawn using a 1-ml needle, placed on a glucose strip, and measured with a glucometer.

Determination of intra-peritoneal glucose tolerance test (IPGTT)

All of the rats were fasted for 12-14 hours before blood was collected from the tail vein as a baseline. The rats were subsequently given 2g/kg body weight (BW) of a 40% (w/v) glucose solution intra-peritoneally. Blood will be taken from the tail vein and analyzed for blood glucose using a glucometer after 30, 60, 90, and 120 minutes after glucose treatment. Fasting blood sugar values of more than 250mg/dl were used to diagnose diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are collected and placed on a test cartridge, haemoglobin A1c (HbA1c) will be analyzed using a Clover A1cTM Self Analyzer.

Bone Mineral Density Measurement

After blood was taken, the BMD of the left femur and lumbar vertebrae (L1–L4) of rats was measured using dual energy X-ray absorptiometry (DEXA) scanning equipment.

RESULTS AND DISCUSSION

The positive control group's (STZ) glucose profiles worsened with time (Table No.1). On the other hand, treatment with *oak bark* extracts, geraniin, or *oak bark* extracts + geraniin was observed to delay the progression of diabetes.

Table No.2 shows that HBA1C levels were higher in the STZ group than in the normal control group ($p<0.05$). Oak bark extracts, geraniin, and *oak bark* + geraniin all had lower HBA1C levels than the STZ group, suggesting that geraniin had a beneficial impact.

The results of a bone mineral density research indicated that diabetic rats had decreased lumbar (L1–L4) and femoral bone mineral density (BMD), which may be improved with the use of geraniin and oak bark extracts ($p<0.05$). The STZ, oak bark extracts, and Geraniin groups have significantly different BMD (Table No.3). These data suggest that geraniin enhances the ability of oak bark extract to raise BMD in diabetic rats.

Statistical analysis

The data must be presented as a mean and standard deviation (SD). A "p" value of less than 0.05 is considered statistically significant. iance (ANOVA) and Tukey's multiple comparison test will be used to statistically analyse the data.

Discussion

Diabetes mellitus is associated with poor bone health and an increased risk of fracture, even in people who have a normal or higher BMD. Diabetes mellitus causes a variety of skeletal disorders, the causes of which are unclear. Anti-diabetic medicines can have a positive or negative impact on bone metabolism. With the expanding global incidence of T2DM, which predisposes patients to osteoporosis and an increased risk of fractures, there is an increasing need to evaluate anti-diabetic medication skeletal effects and investigate their impact on osteoporotic fracture healing. Oak bark extracts inhibit ovariectomy (OVX) induced trabecular bone loss and decrease bone mineral density.

According to two studies in ovariectomized rats, OVX causes ovarian cancer[10,11]. In rats, geraniin revealed to have bone-protective properties[12]. However, no studies have been carried out to see if geraniin can protect against osteoporosis induced by diabetes. According to our findings, an 8-week geraniin therapy decreased bone loss in diabetic rats. In this study, the positive control rats had a lower BMD and a higher blood glucose level, indicating that the rat model had been successfully developed. The combination group's markers improved considerably after 8 weeks of treatment, demonstrating a preventive effect against diabetes-induced bone loss in rats.

Table No.1: Effect of Geraniin in combination with *oak bark* extracts on Fasting blood glucose level

Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Normal Control	5mg/kg	75.22± 3.2	74.32± 2.3	76.81± 3.5	78.40± 1.7	79.30± 1.5	80.46± 1.9	82.40± 1.05	83.40± 1.02	84.40± 1.12
Positive Control	65mg/kg	261.54± 10.2*	296.35± 9.8*	314.21± 12.62*	336.72± 9.6*	351.72± 8.4*	375.72± 11.5*	398.72± 10.5*	412.72± 10.2*	435.72± 9.6*
Geraniin	40mg/kg	266.33± 7.3	286.25± 9.4*	291.22± 7.8*	296.28± 8.2*	304.35± 8.8*	307.35± 9.8*	310.35± 10.2*	320.35± 9.2*	330.35± 9.7*
Oak bark extracts	100mg/kg	275.313± 7.1	240.15± 9.5*	239.24± 7.7*	215.28± 8.4*	177.27± 8.9*	150.58± 9.7*	121.33± 10.4*	105.05± 9.2*	90.026± 9.4*
Oak bark extracts + Geraniin	100mg/kg, +40mg/kg	256.83± 7.3	235.75± 9.3*	210.72± 7.6*	198.62 ±8.1*	163.15± 8.7*	142.35± 9.6*	109.45± 10.3*	90.075± 9.4*	85.025± 9.8*

Values are expressed as mean ± standard error of the mean (n=6)
*p<0.001 compared with normal control.

Table No.2: Effect of Geraniin in combination with *oak bark* extracts on Glycoslyted Haemoglobin: (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.48±0.12
2	Positive Control	5.82±0.04*
3	Geraniin	5.64±0.01*
4	*Oak bark* extracts	5.47±0.16*
5	Geraniin + *Oak bark* extracts	5.44±0.17*

Values are expressed as mean ± standard error of the mean (n=6)
*P<0.001 compared with normal control.

Table No.3: Effect of Geraniin in combination with *oak bark* extracts on the bone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density (mg/cm^3)	
		Lumbar Vertebrae	Femur
1	Normal Control	176 ± 2.1*	221 ± 2.3
2	Positive Control	74 ± 2.4*	102 ± 2.5*
3	Geraniin	156 ± 1.7*	199 ± 1.3*
4	*Oak bark* extracts	135 ± 2.4*	167 ± 2.7*
5	*Oak bark* extracts + Geraniin	149 ± 2.6*	189 ± 2.5*

Values are expressed as mean ± standard error of the mean (n=6)
*p<0.001 compared with normal control.

CONCLUSION

Oral administration of geraniin and *Oak bark* extracts, either alone or in combination, protects against diabetic-induced osteoporosis, according to the findings. The beneficial benefits of geraniin were increased when it was combined with *Oak bark*. This was demonstrated by increased BMD, reduced HBA1C, and lower blood glucose levels. As a result of the current findings, geraniin may be utilized as a supplement in diabetics.

ACKNOWLEDGMENT

The authors are grateful to Deanship of Scientific Research, King Khalid University for sponsoring this study through the Large Research Group Project under grant number RGP 2/186/42.

CONFLICTS OF INTEREST

"The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings".

BIBLIOGRAPHY

1. Lecka-Czernik B. Diabetes, bone and glucose-lowering agents: Basic biology, *Diabetologia,* 60(7), 2017, 1163-1169.
2. Wei J, Shimazu J, Makinistoglu M P, Maurizi A, Kajimura D, Iezaki T, Pessin J E, Hinoi E. Glucose uptake and runx2 synergize to orchestrate osteoblast differentiation and bone formation, *Cell,* 161(7), 2015, 1576-1591.
3. Montagnani A, Gonnelli S, Alessandri M, Nuti R. Osteoporosis and risk of fracture in patients with diabetes: An update, aging, *Clin. Exp. Res,* 23(2), 2011, 84-90.
4. Gunton J E, Delhanty P J D. Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2, *J. Cli. End. Met,* 88(3), 2003, 1323-1332.
5. Huang W, Castelino R L, Peterson G M. Metformin usage in type 2 diabetes mellitus: Are safety guidelines adhered to? *J. Intern. Med,* 44(3), 2014, 266-272.
6. Nestler J E, Jakubowicz D J, Evans W S, Pasquali R. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome, *N. Engl. J. Med,* 338(26), 1998, 1876-1880.
7. Widen E I, Eriksson J G, Groop L C. Metformin normalizes nonoxidative glucose metabolism in insulin-resistant normoglycemic first-degree relatives of patients with NIDDM, *Diabetes,* 41(3), 1992, 354-358.
8. Reddy G K, Stehno-Bittel L, Hamade S, Enwemeka C S. The biomechanical integrity of bone in experimental diabetes, *Diabetes Res Clin Pract,* 54(1), 2001, 1-8.
9. Erdal N, Gurgul S, Demirel C, Yildiz A. The effect of insulin therapy on biomechanical deterioration of bone in streptozotocin (STZ)-induced type 1 diabetes mellitus in rats, *Diabetes Res Clin Pract,* 97(3), 2012, 461-467.
10. Gao Y, Li Y, Xue J, Jia Y, Hu J. Effect of the anti-diabetic drug metformin on bone mass in ovariectomized rats, *Eur J Pharmacol,* 635(1-3), 2010, 231-236.
11. Mai Q G, Zhang Z M, Xu S, Lu M, Zhou R P, Zhao L, Jia C H, Wen Z H, Jin D D, Bai X C. Metformin stimulates osteoprotegerin and reduces rankl expression in osteoblasts and ovariectomized rats, *J Cell Biochem,* 112(10), 2011, 2902-2909.
12. Wang X, Wang M, Cui X, Li Z, Guo S, Gao F, Ma M, Wang Z. Antiosteoporosis effect of geraniin on ovariectomy-induced osteoporosis in experimental rats, *J Biochem Mol Toxicol,* 35(6), 2021, 1-8.

Please cite this article in press as: Krishnaraju Venkatesan *et al.* The enhancement of bone protective effect of *Oak Bark* extracts by geraniin in diabetic rats, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 9(3), 2021, 103-107.