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GERANIIN PREVENTS DIABETIC INDUCED BONE LOSS IN EXENATIDE TREATED DIABETIC RATS BY REDUCING BLOOD GLUCOSE AND SUPPRESSING BONE TURNOVER

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is linked to skeletal problems, such as a higher risk of fractures. This skeletal fragility could be caused by a lack of blood supply and bone strength. Exenatide, a glucagon-like peptide-1 receptor agonist, was demonstrated to increase bone morphology and strength in T2DM mice over time by increasing blood supply to the bone and thereby encouraging bone production. The goal of this study is to see if geraniin has any preventive or therapeutic effects in albino rats that have been given exenatide and have been given diabetes. Streptozotocin was used to induce diabetes. Diabetic rats were given either Exenatide (3g/kg/day) or geraniin (40mg/kg) alone or in combination for eight weeks. BMD of the femur and lumbar vertebrae was assessed by dual-energy X-ray absorptiometry at the end of the trial (DXA). Serum glucose and glycosylated haemoglobin serum were also tested. Exenatide and geraniin, alone and in combination, reduced elevated blood glucose levels substantially. Exenatide medication significantly lowered HBA1C levels as compared to the positive control. The combination of geraniin and Exenatide significantly decreased blood glucose and HBA1C levels. In the femur and lumbar vertebrae, exenatide had little effect on BMD, whereas geraniin treatment greatly improved these results. This research implies that combining geraniin with exenatide may have a preventive and therapeutic role in the treatment of diabetic osteoporosis.

KEYWORDS

Exenatide, Geraniin and Blood glucose.

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INTRODUCTION

Despite having a normal or high bone mineral density (BMD), people with type 2 diabetes mellitus (T2DM) experience bone fragility, reflecting changes in bone quality[1,2]. The cellular and molecular mechanisms that lead to decreased bone strength and quality in T2DM patients are poorly

understood; however, accumulation of advanced glycation end products as a result of hyperglycemia and oxidative stress, changes in collagen cross-linking, and suppression of bone turnover are all thought to play a role in the aetiology of diabetic fractures[3,4]. Glycemic management has been shown to be a crucial intervention in reducing fractures in T2DM[5]. implying that early lifestyle management and anti-diabetic medication delivery are essential to minimise hyperglycemia.

However, selecting anti-diabetic drugs is critical since some anti-diabetic drugs might increase fracture risk by increasing the risk of hypoglycemia and falls or affecting bone turnover and quality. Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are peptides produced in the gastrointestinal tract that increase insulin secretion in a glucose-dependent way in response to meal absorption, thereby preventing hypoglycemia[6].

GLP-1 resistance does not appear to occur in T2DM, in contrast to GIP, and as a result, GLP-1 receptor agonists (GLP-1RAs), such as Exenatide, have been developed and sold for T2DM treatment[6,7]. Recent research has shown that geraniin can help with bone development, resorption, and microstructure alterations[8]. In light of these findings, clinical evaluation of Exenatide's effects on bone strength and quality in a diabetic mouse model is critical. The goal of this study was to see if geraniin could help diabetic rats given Exenatide enhance their bone mineral density.

MATERIAL AND METHODS[9,10]

Animals

The animals were acclimatised to the laboratory environment for 14 days. The treatment was carried out in accordance with the consent of King Khalid University's animal ethics committee and the National Institute of Health's guidelines 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 mice, the pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was given intraperitoneally at a dose of 65mg/kg body weight. In the control group, all of the rats were given the same amount of vehicle. STZ was weighed separately for each animal, solubilized with 0.1ml of freshly prepared cold Na-citrate buffered (NaB-0.1 M, pH 4.5), and administered within 5 minutes to minimise deterioration. The volume of STZ injection was calculated to be 1.0ml/kg.

Rats were administered a 5% glucose solution for 48 hours after receiving STZ to counteract the drug's strong acute hypoglycemia effect. Three days following STZ injection, blood was taken from the tail vein and analysed for blood glucose using a glucometer (Aqua-Check, Roche). Animals having fasting blood glucose levels (BGLs) more than 250mg/dL were classified as diabetic. Group 1 (Non-Diabetic control), Group 2 (Diabetic control), and Group 3 (Geraniin 40mg/kg body weight), Group 4 (Exenatide 3µg/kg/day) and Group 5 (Exenatide 3µg/kg/day + Geraniin 40mg/kg body weight) were each divided into five groups of six rats.

To establish the animals' hyperglycemic status, blood glucose levels were monitored once a week for the duration of the trial using a Roche Accu-Chek advantage glucometer. The study did not include the animals who did not develop blood glucose levels greater 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 obtained from the rats' tail veins to test blood glucose levels using a glucometer after they had been fasted for 12-14 hours. After the rats' tails have been washed with 70% (v/v) ethanol, blood will be drawn with a 1-ml needle, placed on a glucose strip, and quantified with a glucometer.

Determination of intra-peritoneal glucose tolerance test

All of the rats were fasted for 12-14 hours before blood was taken from the tail vein as a baseline. The rats were subsequently given 2g/kg body weight (BW) of a 40% (w/v) glucose solution intraperitoneally. Blood will be collected from the tail vein and analysed for blood glucose using a glucometer after 30, 60, 90, and 120 minutes after glucose therapy. Fasting blood sugar readings of less

than 250mg/dl were used to diagnose diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are obtained and dropped on a test cartridge, haemoglobin A1c (HbA1c) will be analysed using a Clover A1cTM Self-Analyzer. The Clover A1c TM Self-Analyzer's LCD screen will show the percentage of HbA1c in the blood sample.

Bone Mineral Density Measurement

The BMD of the left femur and lumbar vertebrae (L1–L4) of rats was assessed using a dual energy X-ray absorptiometry (DEXA) scanning equipment after blood was collected (Lunar, WI, USA).

RESULTS AND DISCUSSION

The positive control group's (STZ) glucose profiles declined over time (Table-1). However, both alone and in combination, exenatide and geraniin have been shown to protect against diabetes progression.

HBA1C levels were higher in the positive control group than in the normal control group (p 0.05), as indicated in Table No.2. In contrast to the positive control group, Exenatide and geraniin, alone and in combination, were shown to lower HBA1C levels, implying that geraniin plays a favourable effect.

The results of a bone mineral density study demonstrated that diabetic rats had reduced lumbar (L1–L4) and femoral bone mineral density (BMD), which could be improved by using Exenatide and geraniin alone or in combination (p 0.05). The positive group's BMD differed significantly from the other treatment groups (Table No.3). These data suggest that geraniin may be able to protect bones against anti-diabetic pharmaceutical side effects.

Statistical analysis

The data must be presented as a mean and standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey's multiple comparison test will be used to analyse the data from different groups statistically. Statistical significance is defined as a p value of less than 0.05.

Discussion

Despite its severe impact on life quality, bone fragility is typically overlooked by diabetologists among all consequences of diabetes mellitus. Bone fragility is a result of chronic hyperglycemia, but it can also be caused by the direct or indirect effects of anti-diabetic medications.

The basic causes of bone fragility in T2DM appear to be connected to the quality rather than the quantity of bone tissue, and it is crucial to determine how marketed anti-diabetic drugs change bone quality, given the inconsistent data on BMD in T2DM. We wanted to see how exenatide, marketed for the treatment of type 2 diabetes, affects bone mass density in diabetic rats in this study. Exenatide has been shown to improve the bone phenotype in diabetic mice by increasing bone production, however the treatment has no impact in non-diabetic settings.

It's still unclear whether the increased bone formation is related to exenatide's direct actions on osteoblasts or to systemic effects. The GLP-1R has been found to be expressed in marrow mesenchymal cells such as adipocytes and osteoblasts[11-13] that exenatide's effects on bone formation could be direct by binding to the GLP-1R expressed on osteoblasts. However, we can't rule out the possibility that exenatide has a direct anabolic effect on bone via enhancing osteoblast proliferation and differentiation rather than influencing their bone-forming activities. Exenatide, on the other hand, reduced the harmful effects of glucose on bone formation in a dose-dependent manner while having no effect under normal glucose circumstances[13]. Geraniin was discovered to have bone-protective effects in rats[8].

However, no research has been done to examine if geraniin can protect against osteoporosis caused by anti-diabetic drugs. An 8-week geraniin treatment reduced bone loss in diabetic rats, according to our data. In previous studies, we discovered that diabetic rats had lower BMD than normal rats. Exenatide had no effect on BMD in the current investigation. The deleterious effects of diabetic-induced bone degeneration were reversed after co-administration with geraniin.

Table No.1: Effect of Geraniin in combination with Exenatide on Fasting blood glucose level

S.No	Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
1	Normal Control	5mL/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
2	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*
3	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*
4	Exenatide	3µg/kg/day	243.32 ±7.3	235.23 ±9.4*	215.22 ±7.8*	210.24 ±8.2*	180.32 ±8.8*	150.35 ±9.8*	126.32 ±10.2*	101.33 ±9.2*	90.35 ±9.7*
5	Exenatide + Geraniin	3µg/kg/day +40 mg/kg	238.33 ±6.3*	217.24 ±8.4*	200.22 ±7.7*	154.26 ±4.2*	158.35 ±8.6*	138.39 ±7.8*	110.2 ±9.2*	80.35 ±8.2*	84.35 ±8.7*

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 Exenatide on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.42±0.14
2	Positive Control	5.80±0.06*
3	Geraniin	5.68±0.03*
4	Exenatide	5.43±0.10*
5	Exenatide +Geraniin	5.41±0.15*

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 Exenatide onthe bone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density (mg/cm3)	
		Lumbar Vertebrae	Femur
1	Normal Control	178 ± 2.2	220 ± 2.5
2	Positive Control	78 ± 2.6*	100 ± 2.3*
3	Geraniin	158 ± 1.5*	200 ± 1.7*
4	Exenatide	90 ± 2.2*	105 ± 2.5*
5	Exenatide +Geraniin	165 ± 2.5*	214 ± 2.6*

Values are expressed as mean ± standard error of the mean (n=6)

*P<0.001 compared with normal control.

CONCLUSION

Geraniin increased bone mass in a diabetes-induced rat model, and co-supplementing geraniin with exenatide reduced diabetic-induced bone loss. As a result, co-administration of geraniin with exenatide as a treatment strategy is likely to decrease bone loss and fracture risk in T2DM patients.

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CONFLICT 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. Yamamoto M, Yamaguchi T, Yamauchi M, Kaji H, Sugimoto T. Diabetic patients have an increased risk of vertebral fractures independent of BMD or diabetic complications, *J Bone Miner Res,* 24(4), 2009, 702-709.
2. Yamamoto M. Insights into bone fragility in diabetes: The crucial role of bone quality on skeletal strength, *Endocr J,* 62(4), 2015, 299-308.
3. Yamamoto M, Sugimoto T. Advanced glycation end products, diabetes, and bone strength, *Curr Osteoporos Rep,* 14(6), 2016, 320-326.
4. Karim L, Bouxsein M L. Effect of type 2 diabetes-related non-enzymatic glycation on bone biomechanical properties, *Bone,* 82, 2016, 21-27.
5. Napoli N, Chandran M, Pierroz D D, Abrahamsen B, Schwartz A V, Ferrari S L, *et al*. Mechanisms of diabetes mellitus-induced bone fragility, *Nat Rev Endocrinol,* 13(4), 2017, 208-219.
6. Baggio L L, Drucker D J. Glucagon-like peptide-1 receptors in the brain: Controlling food intake and body weight, *J Clin Invest,* 124(10), 2014, 4223-4226.
7. Nuche-Berenguer B, Lozano D, Gutierrez-Rojas I, Moreno P, Marinoso M L, Esbrit P, *et al*. GLP-1 and exendin-4 can reverse hyperlipidic-related osteopenia, *J Endocrinol,* 209(2), 2011, 203-210.
8. 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,* 2021, e22774.
9. Gunton J E, Delhanty P J D, Takahashi S I, Baxter R C. Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2, *J. Clin. Endocrinol, Metab,* 88(3), 2003, 1323-1332.
10. 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.
11. Pereira M, Jeyabalan J, Jorgensen C S, Hopkinson M, Al-Jazzar A, Roux J P, e*t al*. Chronic administration of glucagon-like peptide-1 receptor agonists improves trabecular bone mass and architecture in ovariectomised mice, *Bone,* 81, 2015, 459-467.
12. Lee H M, Joo B S, Lee C H, Kim H Y, Ock J H, Lee Y S. Effect of glucagon-like peptide-1 on the differentiation of adipose-derived stem cells into osteoblasts and adipocytes, *J Menopausal Med,* 21(2), 2015, 93-103.
13. Pereira M, Gohin S, Roux J P, *et al*. Exenatide improves bone quality in a murine model of genetically inherited type 2 diabetes mellitus, *Front Endocrinol (Lausanne),* 8, 2017, 327.

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