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GERANIIN PREVENTS DIABETIC INDUCED BONE LOSS IN EMPAGLIFLOZIN TREATED DIABETIC RATS

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ABSTRACT

Fractures appear to be more common in patients with type 2 diabetic mellitus (T2DM). There is no compelling evidence that empagliflozin, a sodium-glucose co-transporter-2 (SGLT) inhibitor, increases the risk of fracture. The purpose of this trial was to see how geraniin worked in combination with empagliflozin to prevent diabetic and antidiabetic drug-induced bone loss. Streptozotocin was used to induce diabetes. Diabetic rats were administered either empagliflozin (10mg/kg/day) or geraniin (40mg/kg) alone or in combination for eight weeks. BMD (Bone mineral density) of the femur and lumbar vertebrae was assessed by dual-energy X-ray absorptiometry (DXA) at the end of the trial. Serum glucose and glycosylated haemoglobin serum were also tested. Both alone and in combination, empagliflozin and geraniin significantly reduced elevated blood glucose levels. Empagliflozin therapy significantly lowered HBA1C levels when compared to the positive control. The combination of geraniin and empagliflozin significantly decreased blood glucose and HBA1C levels. In the femur and lumbar vertebrae, empagliflozin had little effect on BMD, whereas geraniin treatment considerably improved these results. This research reveals that geraniin supplementation in diabetics taking empagliflozin may be an effective way to reduce diabetic-induced bone loss.

KEYWORDS

Bone mineral density, T2DM and SGLT.

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INTRODUCTION

Patients with type 2 diabetes have a higher risk of bone fractures than those without the disease[1-3] for a variety of reasons, including diabetes' effects on bone macroarchitecture, microarchitecture and turnover, as well as the existence of comorbidities that may enhance the risk of falls[4]. In the

management of patients with type 2 diabetes who are at risk for fractures, the bone safety profiles of glucose-lowering medications may need to be evaluated[4,5] Some sodium-glucose cotransporter 2 (SGLT2) inhibitors raise serum phosphate levels, most likely due to enhanced tubular reabsorption, which can be harmful to bone[6].

Empagliflozin is a type 2 diabetes medication that is a strong and specific SGLT2 inhibitor. Empagliflozin has gotten a lot of interest recently as a medication class with a unique mechanism for treating hyperglycemia. Recent research has shown that geraniin can help with bone development, resorption and microstructure alterations[7]. The goal of this study was to determine how geraniin influenced BMD in empagliflozin-treated rats and to examine how it influenced the effects of empagliflozin.

MATERIAL AND METHODS[8,9]

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 the rats, 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 minimize 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. Blood was drawn from the tail vein three days after STZ injection and samples were tested for blood glucose using a glucometer (Aqua-Check, Roche) (Aqua-Check, Roche). Diabetic animals were defined as those with fasting blood glucose levels (BGLs) more than 250mg/dL. The rats were divided into three groups of six animals each Group-1 (Non-Diabetic control), Group-2 (Diabetic control) and Group 3 (Geraniin 40mg/kg body weight), Group 4 (Empagliflozin 200mg/kg body weight) and Group 5 (Empagliflozin 200mg/kg + Geraniin 40mg/kg body weight).

Blood glucose levels were tested once a week for the course of the trial using a Roche Accu-Chek advantage glucometer to determine the animals' hyperglycemic condition. 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

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. 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 No.1). However, both alone and in combination, empagliflozin and geraniin have been shown to protect against diabetes development.

Table No.2 shows that HBA1C levels were greater in the positive control group than in the normal control group (p 0.05). Empagliflozin and geraniin, alone and in combination, were observed to lower HBA1C levels, in contrast to the positive control group, showing that geraniin had a beneficial effect.

The findings of bone mineral density study revealed that diabetic rats had lower lumbar (L1-L4) and femoral bone mineral density (BMD), which was recovered by Empagliflozin and geraniin alone and in combination treatment (p 0.05). The BMD of the positive group and the other treatment groups differed significantly (Table No.3). These findings imply that geraniin may be able to protect bones from the effects of anti-diabetic medications.

Statistical analysis

The results must be expressed in terms of mean and standard deviation (SD). The data from different groups will be analysed statistically using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test. A p value of less than 0.05 is considered statistically significant.

Discussion

The newest family of oral drugs for the treatment of type 2 diabetic mellitus (T2DM) is sodium-glucose co-transporter 2 (SGLT2) inhibitors[1]. SGLT2 inhibitors can be used alone or in combination to treat type 2 diabetic mellitus (T2DM). The SGLT2 inhibitors can be used alone or in combination with other diabetes medications. The regulation of renal calcium and phosphate reabsorption as a result of alterations in renal sodium and glucose reabsorption has been linked to an increased risk of bone fractures with SGLT2 inhibitors[10]. There isn't enough evidence to say that these medicines are directly responsible for bone fractures. Geraniin was discovered to have bone-protective effects in rats[7]. 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. Empagliflozin did not affect 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 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	84.4 0 ±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	Empagliflozin	200mg/kg	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	Empagliflozin +Geraniin	200mg/kg, +40mg/kg	248.33 ±7.3*	227.24 ±9.4*	210.22 ±7.8*	186.26 ±8.2*	165.35 ±8.8*	140.39 ±9.8*	110.33 ±10.2*	90.35 ±9.2*	85.35 ±9.7*

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

Table No.2: Effect 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	Empagliflozin	5.46±0.14*
5	Empagliflozin +Geraniin	5.43±0.10*

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

Table No.3: Effect on the 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	Empagliflozin	155 ± 2.2*	98 ± 2.5*
5	Empagliflozin +Geraniin	167 ± 2.7*	210 ± 2.3*

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, while co-supplementing geraniin with empagliflozin reduced diabetic-induced bone loss. As a result, co-administration of geraniin with empagliflozin as a treatment strategy is likely to decrease bone loss and fracture risk in T2DM patients.

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CONFLICTS OF INTEREST

"According to the authors, they have no competing interests. The funders had no role in the study's design, data collection, analysis, or interpretation, manuscript writing, or publication decision".

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