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GERANIIN PREVENTS DIABETES-INDUCED BONE LOSS IN RATS BY REDUCING BLOOD GLUCOSE AND SUPPRESSING BONE TURNOVER

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ABSTRACT

Diabetic osteoporosis (DOP) is a prevalent metabolic bone disease marked by low bone mineral density (BMD) and microstructure degradation. It has been reported that geraniin is good for osteoporosis; however, it has not been reported whether geraniin protects against diabetes-induced osteoporosis. In this study, a rat model of diabetic osteoporosis was established by streptozotocin injection, the bone protective effects and potential mechanism of geraniin on diabetes-induced boneloss was observed. Wistar albino rats were divided into three groups; control group (vehicle treatment), Streptazocin (diabetic) group and Geraniin group (diabetic rats treated with geraniin), 6 rats in each group. After 8 weeks of geraniin treatment, the bone mineral density (BMD) was measured. The results demonstrated that consuming 40mg/kg of geraniin reduced blood glucose and improved BMD in diabetic rats. All of the evidence suggests that taking geraniin orally can help prevent diabetic osteoporosis; the impact is mostly due to its capacity to raise BMD and lower blood glucose.

KEYWORDS

Diabetic osteoporosis, Blood glucose and Bone mineral density.

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INTRODUCTION

Diabetes, a chronic metabolic disease that impacted 422 million people globally in 2015, was an epidemic. Diabetes is associated to nephropathy, neuropathy, retinopathy and foot ulcers, among other issues. Furthermore, bone thinning is an understudied diabetic complication[1,2]. T2DM has been associated to an increased risk of osteoporotic

fractures, which can result in disability and death[3]. T2DM patients were 1.7 times more likely to experience hip fractures than age-matched non-diabetic controls[4]. Geraniin is a dehydro ellagitannin that can be found in geraniums and has a variety of bioactivities. Geraniin not only inhibited but also accelerated bone formation, according to studies and its antiresorptive activity was linked to the down regulation of matrix metalloproteinase-9 and carbonic anhydrase II[5]. Although geraniin has been proven to have a bone-protective function, its role in STZ-induced diabetic bone injury is unknown. As a result, the goal of this study was to see if geraniin (98 percent pure) could protect against diabetic drug-induced bone injury.

MATERIAL AND METHODS

Animals

The study used healthy male wistar albino rats that were 3- to 4-months-old and weighed 180 to 240g. The animals were obtained from King Khalid University's Central Animal House in Abha, Saudi Arabia. During the trial, the animals were kept in cages and fed a standard pellet diet and filtered water ad libitum under standard settings (light/dark cycle of 12 h/12 h with 50-70 percent humidity, at 25ºC 3ºC). For 14 days, the animals were acclimatised to the laboratory setting. The treatment was carried out in compliance with King Khalid University's animal ethics committee's approval and the US National Institute of Health's guidelines for the care and use of laboratory animals (NIH Publication No.85-23, revised 1996).

Induction of diabetes[6,7]

The pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was injected intraperitoneally at a dose of 65mg/kg body weight to cause diabetes in the animals. The rats in the control group were given the same amount of vehicle. To avoid degradation, STZ was weighed separately for each animal, solubilized with 0.1ml of freshly made cold Na-citrate buffered (NaB-0.1 M, pH 4.5) and delivered within 5 minutes. The STZ injection volume was calculated to be 1.0ml/kg. To counteract the significant acute hypoglycemia effect of STZ, rats were given a 5 percent glucose solution for 48 hours following the injection.

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). Blood glucose levels were measured once a week for the course of the trial using a Roche Accu-Chek advantage glucometer to determine the animals' hyperglycemic status. The animals that did not develop blood glucose levels greater than 250mg/dL were not included in the study. The rats were put into three groups of six animals each (Group-1 (Non-Diabetic control), Group-2 (Diabetic control) and Group 3 (Geraniin40mg/kg body weight). Blood glucose levels were measured once a week for the course of the trial using a Roche Accu-Chek advantage glucometer to determine the animals' hyperglycemic status. The animals that did not develop blood glucose levels greater than 250mg/dL were not included in the study. The rats in the control group (n=6) who were given saline instead of streptozotocin had normal blood glucose levels (120mg/dl).

Determination of fasting blood glucose

The rats were fasted for 12-14 hours before blood samples were taken from their tail veins to assess blood glucose levels using a glucometer. Blood will be obtained with a 1-ml needle, put on a glucose strip and quantified using a glucometer after the rats' tails have been cleansed with 70% (v/v) ethanol.

Determination of intra-peritoneal glucose tolerance test

As a baseline, all of the rats were fasted for 12-14 hours and blood were drawn from the tail vein. The rats were then intra-peritoneally administered 2g/kg body weight (BW) of a 40% (w/v) glucose solution. At 30, 60, 90 and 120 minutes following glucose therapy, blood will be drawn from the tail vein and tested for blood glucose using a glucometer. Diabetes was proven in these rats by fasting blood sugar levels of less than 250mg/dl.

Determination of hemoglobin A1c

Hemoglobin A1c (HbA1c) will be measured using a Clover A1c™ Self Analyzer after blood samples from the tail vein are taken and put on a test cartridge. The percentage of HbA1c in the blood sample will be displayed on the Clover A1c™ Self Analyzer's LCD screen.

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.

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). Treatment with geraniin, on the other hand, was found to slow the progression of diabetes.

The IPGTT was performed at the beginning of treatment. In all groups of animals, glucose levels at120 min glucose load were above 250mg/dL in IPGTT (Table No.2) which confirms the development of diabetes.

As shown in Table No.3, HBA1C levels were higher in the STZ group than in the normal control group (p 0.05). In contrast to the STZ group, geraniin was shown to have a lower HBA1C level, implying a positive effect for geraniin.

The findings of bone mineral density study revealed that diabetic rats had lower lumbar (L1-L4) and femoral bone mineral density (BMD), which was regained by geraniin treatment ($p < 0.05$). Between the STZ and Geraniin groups, there was a substantial difference in BMD (Table No.4). These findings imply that geraniin can help diabetic rats boost their BMD.

Statistical analysis

The results must be expressed in terms of mean and standard deviation (SD). One way analysis of variance (ANOVA) and Tukey's multiple comparison test will be used to statistically analyse data from distinct groups.Statistical significance is defined as a ‘p’ value of less than 0.05.

Discussion

Reduced bone mineral density characterises microstructural alterations in diabetic bone disease, resulting in bone fragility and an increased risk of fracture[8]. Many studies have demonstrated that diabetes affects bone turnover and bone integrity[9-11] and that bone loss and turnover are accelerated in diabetic patients[12]. High blood glucose levels impact osteoblast differentiation, bone production and mineralization[13], as well as causing osteoblast apoptosis, which is thought to be a major cause of diabetic osteopenia[14-15]. In ratsgeraniin, geraniin was found to have bone-protective properties[5]. However, no studies have been done to see if geraniin can protect against diabetes-induced osteoporosis. Our findings showed that 8 weeks of geraniin therapy can reduce bone loss in diabetic rats.

STZ rats in this study had lower BMD and higher blood glucose, showing that the rat model had been properly developed. These indicators significantly improved after 8 weeks of geraniin administration, implying that geraniin protects rats from bone loss caused by diabetes. Sustained hyperglycemia can block osteoblast proliferation and enhance osteoclast development and it's now thought that elevated glucose levels in the bone marrow microenvironment can lead to enhanced osteoclast differentiation, which could be a pathogenesis of diabetic osteoporosis[16]. In this investigation, a trend toward lower blood glucose was observed after 8 weeks of geraniin treatment as compared to the STZ alone treated group.

Table No.1: Effect of Geraniin 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	STZ	65mg/kg	261.54 ±10.2*	296.35 ±9.8*	314.21 ±12.6*	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	STZ+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*

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 on oral glucose tolerance test

S.No	Treatment Group	Dose	0 min	30 min	60 min	90 min	120 min
1	Normal Control	5 mL/kg	95 ± 2.1	99 ± 2.2	97 ± 2.1	93 ± 2.1	90 ± 2.1
2	STZ	65 mg/kg	261.54±9.2*	266.45±9.6*	269.21±9.7*	273.72±9.8*	275.72±9.4*
3	STZ+Geraniin	40 mg/kg	256.33±8.3	261.25±8.4*	263.22±8.8*	266.28±7.2*	268.35±8.4*

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 on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.42±0.14
2	STZ	5.80±0.06*
3	STZ+Geraniin	5.68±0.03*

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

Table No.4: Effect of Geraniin 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	STZ	78 ± 2.6*	100 ± 2.3*
3	STZ+Geraniin	158 ± 1.5*	200 ± 1.7*

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

CONCLUSION

The results of this study show that taking geraniin orally protects against diabetic-induced osteoporosis. Increased BMD, lower HBA1C and lower blood glucose levels were evidence of this. As a result, the current findings imply that geraniin could be used as a medicine or supplement to treat osteoporosis in diabetic patients.

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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."

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