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THE ENHANCEMENT OF BONE PROTECTIVE EFFECT OF METFORMIN BY GERANIIN

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ABSTRACT

Diabetes mellitus, particularly type 2 diabetes mellitus, is becoming more common all over the world. Diabetics face a higher risk of mortality and morbidity, in addition to cardiomyopathy and nephropathy, due to an increased risk of bone fractures caused by low bone mineral density (BMD). Because of hyperglycemia, the toxic effects of advanced glycosylation end-products (AGEs) on bone tissue, and an altered bone microvascular system, diabetic patients have worse bone quality than non-diabetic patients. Hyperglycemia may potentially play a role in the progression of osteoarthritis and osteoporosis. As a result, in diabetic individuals, glucose management is critical for bone health. Metformin, a commonly prescribed diabetes medication, has been proven to improve bone quality and reduce the incidence of fractures in diabetic patients, in addition to improving glycemic control and insulin sensitivity. After 8 weeks of geraniin treatment, the effect of geraniin on improving BMD in metformin-treated subjects was investigated. The results revealed that consuming 40 mg of geraniin per kilogramme of body weight strengthened the bone-protecting effect of metformin by lowering blood glucose and increasing BMD. All of the evidence suggests that taking geraniin with metformin can help prevent diabetic osteoporosis.

KEYWORDS

Bone protective, Geraniin and Metformin.

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INTRODUCTION

The global prevalence of diabetes, particularly T2DM, is rising, and there is mounting evidence that diabetes is both the cause and a risk factor for osteoporotic fractures[1,2]. Diabetes weakens bones by disrupting glucose metabolism, bone microvascular function, glucose oxidative

byproducts, and muscle endocrine function[3]. Bones are intimately linked to glucose metabolism, and osteoblast growth clearly necessitates glucose[4]. Diabetes affects bone tissue (osteopenia and osteoporosis) due to hyperinsulinemia, decreased serum levels of IGF-1, presentation of advanced glycosylation of end-products (AGEs) specifically in collagen following hyperglycemia, decreased levels of osteocalcin, and renal failure. Hypercalciuria, microangiopathy, and inflammation are all symptoms of hypercalciuria[5].

The production of osteocalcin, which is important for matrix maturation and bone mineralization, is negatively affected by hyperglycemia and its repercussions. Metformin, a biquanide antidiabetic medicine, is a commonly used oral prescription for the treatment of type 2 diabetes (non-insulin dependent). Despite the fact that it has been used for over 40 years, its mechanism of action remains unknown[6]. Metformin has a low cost and is quite safe, with a low risk of hypoglycemia, no weight gain, and few side effects, making it a first-line oral medicine for T2DM[7]. Surprisingly, metformin has been shown to have no effect on glucose levels in non-diabetic persons, which supports the idea of utilising metformin as an adjuvant medication, particularly in the treatment of bone problems[8,9].

Geraniin is a dehydroellagitannin 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 downregulation of matrix metalloproteinase-9 and carbonic anhydrase II[10]. Despite the fact that geraniin and metformin have been found to have a bone-protective effect, its role in STZ-induced diabetic bone injury remains unknown. As a result, the goal of this study was to see if geraniin (98 percent pure) could help metformin 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 25°C 3°C). 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

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 produce diabetes in the animals[11-12]. The rats in the control group were all given the same amount of vehicle. Diabetes was induced in the animals by an intraperitoneal injection of the pancreatic-cell toxin streptozotocin (STZ) at the dose of 65mg/kg body weight. Equal volumes of vehicle were injected into the rats assigned to the control group. To avoid degradation, STZ was weighed separately for each animal, solubilized with 0.1ml of freshly made cold Na-citrate buffered (NaB-0.1M, 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). Diabetic animals were defined as those with fasting blood glucose levels (BGLs) more than 250mg/dL. The rats were separated into three groups of six animals each (Group 1: Non-Diabetic control, Group 2: Diabetic control, Group 3: Geraniin 40mg/kg body weight, Group 4: Metformin 100mg/kg body weight, and Group 5: Metformin 100mg/kg + Geraniin 40mg/kg body weight). Blood glucose levels were measured once a week 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 (IPGTT)

As a baseline, all of the rats were fasted for 12-14 hours and blood was 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 A1cTM 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 A1cTM 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 metformin, geraniin, or metformin + geraniin, on the other hand, was found to slow the course of diabetes.

Effect of Geraniin in combination with Metformin on Fasting blood glucose level

HBA1C levels were higher in the STZ group than in the normal control group (p 0.05), as indicated in Table No.2. In contrast to the STZ group, metformin, geraniin, and metformin + geraniin were shown to have lower HBA1C levels, implying that geraniin plays a positive 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 geraniin and metformin treatment (p 0.05). The BMD of the STZ, metformin, and Geraniin groups differed significantly (Table No.3). These findings imply that geraniin improves metformin's capacity to increase BMD in diabetic rats.

Statistical analysis

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

Discussion

Even if individuals have a normal or greater BMD, diabetes mellitus is linked to poor bone health and an increased risk of fracture. The processes behind the numerous skeletal diseases caused by diabetes mellitus are unknown. Anti-diabetic medications can affect bone metabolism in either a favourable or negative way.With the rising global prevalence of T2DM, which predisposes patients to osteoporosis and an increased risk of fractures[13,14], there is a growing need to assess anti-diabetic drug skeletal effects and explore their impact on osteoporotic fracture healing. Metformin reduces trabecular bone loss[15] and lowers bone mineral density[16] induced by OVX, according to two trials in ovariectomized rats[15,16] Metformin, on the other hand, appears to slow bone production, has no effect on bone mass in rodents *in vivo*, and does not accelerate fracture healing, according to other research. Positive control rats in this study had lower BMD and higher blood glucose, showing that the rat model had been successfully established. These indicators

significantly improved after 8 weeks of therapy for the combination group indicating a protective effect against diabetes-induced bone loss in rats.

Table No.1: Effect of Geraniin in combination with Metformin 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	Metformin	100mg/kg	264.33± 7.3	245.25± 9.4*	235.22± 7.8*	212.28± 8.2*	180.25 ±8.8*	150.55 ±9.8*	120.45 ±10.2*	101.15 ±9.2*	90.035 ±9.7*
5	Metformin+Geraniin	100mg/kg, +40mg/kg	248.33± 7.3	235.25± 9.4*	212.52± 7.8*	187.28± 8.2*	165.35 ±8.8*	140.35 ±9.8*	110.35 ±10.2*	90.035 ±9.2*	84.025 ±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 in combination with Metformin 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	Metformin	5.49±0.14*
5	Metformin+ Geraniin	5.45±0.14*

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 Metformin 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	Metformin	135 ± 2.2*	170 ± 2.5*
5	Metformin+ Geraniin	150 ± 2.2*	190 ± 2.5*

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

CONCLUSION

According to the findings, oral administration of geraniin and metformin, either alone or in combination, protects against diabetic-induced osteoporosis. When geraniin was added to metformin, the positive effects were amplified. 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 supplement in diabetic individuals using anti-diabetic medications.

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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".

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