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BENEFICIAL EFFECT OF GERANIIN IN PREVENTING ROSIGLITAZONE INDUCED BONE LOSS IN DIABETIC RATS

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ABSTRACT

The insulin-sensitizing medication rosiglitazone (RSG) is used to treat type 2 diabetes mellitus. According to the A Diabetes Outcome Progression Trial (ADOPT), women who took RSG had more fractures than those who took other type 2 diabetes medications. The fractures were not osteoporotic spinal fractures, but rather limb fractures. The goal of this study was to see if geraniin could help prevent bone loss caused by the drug Rosiglitazone. For diabetes induction, streptozotocin was used. For eight weeks, diabetic rats were given orally Rosiglitazone (10mg/kg) and geraniin (40mg/kg) alone or in combination. At the end of the experiment, BMD of the femur and lumbar vertebrae was measured by dual-energy X-ray absorptiometry (DXA). Glycosylated Haemoglobin serum and serum glucose were also examined. Rosiglitazone and geraniin, both alone and in combination, dramatically lowered high blood glucose levels. When compared to the positive control, rosiglitazone therapy dramatically reduced HBA1C levels. The combination of geraniin and rosiglitazone reduced blood glucose and HBA1C levels considerably. The combination of geraniin and rosiglitazone reduced blood glucose and HBA1C levels considerably. Rosiglitazone had negative effects on BMD in the femur and lumbar vertebrae, while geraniin therapy significantly improved these effects. This study demonstrates that geraniin supplementation in diabetic patients taking Rosiglitazone could be an effective technique for reducing bone loss caused by the drug.

KEYWORDS

Rosiglitazone, Geraniin and Diabetic rats.

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INTRODUCTON

Type 2 diabetes mellitus is becoming more common[1] necessitating the use of medication when lifestyle and dietary improvements are insufficient. Thiazolidinediones (TZDs) are a new class of oral anti-diabetic medications that are used to treat the symptoms of type 2 diabetes[2]. Rosiglitazone (RSG), an insulin sensitizer that improves glycemic

management, belongs to the TZD class of medicines[3]. Although TZDs are helpful for type 2 diabetes, they have been linked to bone problems, particularly in older women[4-6].

This was demonstrated in the A Diabetes Outcome Progression Trial (ADOPT), which found that female patients on RSG had more fractures than those receiving other type 2 diabetes medications[7,8]. These were not your typical osteoporotic spine and hip fractures; instead, they occurred in the upper and lower limbs. Women experienced a considerable rise in fractures, whereas males did not. Bone loss has also been observed in mice and rats following RSG therapy[9,10]. RSG is a peroxisome proliferator–activated receptor (PPAR-) agonist[10-15]. RSG activates this receptor, allowing insulin-responsive genes to be regulated. It also encourages mesenchymal cells to develop into adipocytes rather than osteoblasts in the bone marrow[16]. As a result, the skeletal consequences of RSG in type 2 diabetics are a source of worry. Even if bone mineral density (BMD) is high, a consequence of type 2 diabetes is an increased riskof osteoporotic fractures[17]. RSG has been demonstrated to lower BMD and trabecular structural characteristics, as well as the number of osteoblasts, while increasing fat content[18]. Other research, on the other hand, suggests that RSG activation regulates osteoclastogenesis in vivo, which would effect bone resorption[19]. Following RSG treatment, ovariectomized (OVX) rats showed an increase in osteoclast number and eroded surface (ES)[20]. Furthermore, RSG-induced bone loss in vivo was linked to an increase in osteoclastogenesis[21].

Geraniin has been proven in recent studies to help with bone growth, resorption, and microstructure changes[22]. The goal of this study was to look into the bone-protective effects of geraniin as a co-treatment in diabetic rats treated with Rosiglitazone.

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 acclimatized 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 65 mg/kg body weight to cause diabetes in the animals. The rats in the control group were all 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). Diabetic animals were defined as those with fasting blood glucose levels (BGLs) more than 250mg/dL. The rats were split into five groups of six animals each (Group 1 (Non-Diabetic control), Group 2 (Diabetic control), Group 3 (Geraniin 40mg/kg body weight), Group 4 (Rosiglitazone 40mg/kg), and Group 5 (Rosiglitazone 40mg/kg + Geraniin 40mg/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 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 (Lunar, WI, USA).

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, rosiglitazone and geraniin, both alone and in combination, were demonstrated 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, rosiglitazone and geraniin, alone and in combination, were shown to lower HBA1C levels, implying that geraniin plays a favourable 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 rosiglitazone 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 are to be represented 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

This study examines the effects of RSG on bone quality in an STZ induced animal model of type 2 diabetes. Previous studies examine the effect of RSG on bone loss and bone formation/resorption, bone structure and composition to bone quality determined by bone mechanical properties.

Both cortical and trabecular BMD, as well as bone mineral content (BMC), were reduced in the OVX model after RSG treatment, as expected. In a similar study, Sottile *et al.* (used OVX female Wistar rats and found that RSG therapy increased bone loss in the tibia, femur, and lumbar spine[16]. In mice, Soroceanu *et al.* Found lower trabecular BV and lower spinal BMD following RSG therapy[14]. For the first time, proof of geraniin's preventive action against rosiglitazone-related bone loss is presented in this study. The preventive impact of geraniin on rosiglitazone-induced bone loss in diabetic male rats was investigated in this study. The findings demonstrate that geraniin, when used in conjunction with rosiglitazone, can help regulate blood sugar levels while also improving BMD and bone quality.

In rats, geraniin was found to exhibit bone-protective properties[22]. However, no studies have been done to see if geraniin can protect against diabetes-induced osteoporosis. Our findings showed that an 8-week geraniin therapy can reduce bone loss in diabetic rats.

In previous research, we discovered reduced BMD in diabetic rats when compared to normal rats. BMD was lowered by rosiglitazone, particularly in the femur and lumbar vertebrae. After therapy with geraniin, the negative effects of rosiglitazone on femur-BMD were completely reversed. We discovered that taking probiotics with rosiglitazone lowered the elevated glucose levels induced by STZ in the current trial.

In addition, *in vivo* investigations and clinical trials should be done to learn more about the many features of this combination medicine and how it works.

Table No.1: Effect of Geraniin in combination with rosiglitazone 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	Rosiglitazone	200mg/kg	262.33 ± 6.3*	245.25 ± 8.4*	235.22 ±7.8*	210.28 ± 8.8*	180.35 ±8.6*	150.35 ±9.3*	120.35 ±10.6*	100.35 ±8.2*	90.35 ±9.8*
5	Rosiglitazone + Geraniin	200mg/kg, +40mg/kg	248.33 ±7.7*	235.25 ±9.8*	210.22 ±7.4*	186.28 ±8.1*	165.35 ±7.8*	140.35 ±8.8*	110.35 ±9.2*	90.35 ±9.4*	85.35 ±9.3*

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 rosiglitazone 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	Rosiglitazone	5.39±0.04*
5	Rosiglitazone +Geraniin	5.35±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 Rosiglitazone 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 ± 2.5	220 ± 2.5
2	Positive Control	78 ± 2.6*	100 ± 2.3*
3	Geraniin	158 ± 1.5*	200 ± 1.7*
4	Rosiglitazone	72± 2.6*	97± 1.5*
5	Rosiglitazone +Geraniin	130 ± 2.1*	185± 2.4*

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

CONCLUSION

In a diabetes-induced rat model, geraniin enhanced bone mass and co-supplementing geraniin with rosiglitazone prevented rosiglitazone-induced bone loss. As a result, it is expected that co-administration of geraniin with rosiglitazone as a therapeutic strategy will reduce bone loss and fracture risk in T2DM patients using rosiglitazone.

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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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