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ROLE OF GERANIIN IN PREVENTING PIOGLITAZONE INDUCED BONE LOSS IN RATS

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ABSTRACT

As a PPAR gamma agonist, pioglitazone is used to treat type 2 diabetes mellitus. It has been associated with a decrease in bone mass and a higher risk of fracture in those with type 2 diabetes. The goal of this study was to look into the effect of geraniin in preventing pioglitazone-induced bone loss. For diabetes induction, streptozotocin was used. For eight weeks, diabetic rats were given pioglitazone (300mg/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). Glycoslyted haemoglobin serum and serum glucose were also examined. Pioglitazone and geraniin, both alone and in combination, dramatically lowered high blood glucose levels. When compared to the positive control, pioglitazone treatment dramatically reduced HBA1C levels. The combination of geraniin and pioglitazone reduced blood glucose and HBA1C levels considerably. Pioglitazone had negative effects on BMD in the femur and lumbar vertebrae, while geraniin therapy significantly improved these effects. This study suggests that geraniin supplementation in diabetic individuals using pioglitazone could be an appropriate method for reducing pioglitazone-induced bone loss.

KEYWORDS

Geraniin, Pioglitazone and Bone loss.

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INTRODUCTION

According to the World Health Organization, around 422 million people have been diagnosed with diabetes mellitus, compared to 180 million diabetic individuals in 1980[1]. The prevalence of type 2 diabetes (T2DM) is increasing, and the obesity epidemic is spreading[2]. T2DM causes

serious systemic complications in uncontrolled or poorly controlled situations, including vision loss, neuropathy, kidney impairment, and a decreased life expectancy[3]. Due to osteoblastic dysfunction, both kinds of diabetes (T1DM and T2DM) are associated with low bone mineral density (BMD) and a significant risk of bone fracture, particularly at the hip[4].

Insulin-sensitizing thiazolidinediones (TZDs) have been used to supplement traditional diabetes management strategies such as diet, exercise, and metformin[5]. Pioglitazone and rosiglitazone are two primary PPAR-r thiazolidinediones that are used as oral glucose-lowering antidiabetic agents[6]. Long-term use of both TZDs can cause bone loss, and the risk of bone fractures has been found to be considerably higher in women with T2DM who take both drugs[7].

Pioglitazone and rosiglitazone medication has been linked to an increased incidence of fractures in all T2DM patients, particularly young women, according to some meta-analyses of randomized clinical studies[8-10]. In addition, a long term observational cohort analysis found that rosiglitazone use is associated with an increased risk of bone fracture in women (but not in men)[11]. It appears that TZDs cause certain stem cells to differentiate into adipocytes rather than osteoblasts, resulting in decreased bone production and increased bone resorption[12]. Geraniin has been proven in recent studies to help with bone growth, resorption, and microstructure changes. The goal of this study was to see how geraniin affected BMD in pioglitazone-treated rats.

MATERIAL AND METHODS

Animals

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 administered intraperitoneally to the rats at a dose of 65mg/kg body weight to induce diabetes[13-14]. 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-0.1M, 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 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 (Pioglitazone 200mg/kg body weight) and Group 5 (Pioglitazone 200mg/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 collected and dropped 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

After blood collecting, the bone mineral density (BMD) of the left femur and lumbar vertebrae (L1–L4) of rats was measured using a dual energy X-ray absorptiometry (DEXA) scanning system (Lunar, WI, USA).

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, pioglitazone 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, pioglitazone 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 Pioglitazone 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 shall be given as mean ± standard deviation (SD). Data obtained from distinct groups will be statistically examined using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Statistical significance is defined as a 'p' value of less than 0.05.

Discussion

For the first time, evidence of geraniin's preventive impact against pioglitazone-related bone loss is presented in this work. The preventive impact of geraniin on pioglitazone-induced bone loss in diabetic male rats was investigated in this study. The findings suggest that geraniin, in conjunction with pioglitazone, can increase BMD and bone quality while also controlling blood glucose.

Many clinical and preclinical studies have previously shown that pioglitazone reduces trabecular bone volume, BMD, and BMC[15,16]. As a result, it appears that this antidiabetic drug (and other insulin-sensitizing TZDs) can enhance bone resorption while decreasing bone formation by blocking osteoblast differentiation[17]. Particularly in postmenopausal women[18,19]. This *in vivo* animal study confirmed that pioglitazone has an effect on BMD and trabecular bone volume, which is linked to increased bone resorption and decreased bone formation, which is consistent with prior major research.

In rats, geraniin was found to exhibit bone-protective properties[20]. 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 pioglitazone, especially in the femur and lumbar vertebrae. After therapy with geraniin, the negative effects of pioglitazone on femur-BMD were completely reversed.

This research has certain drawbacks. The mechanism of geraniin's impact on pioglitazone-induced bone loss has not been studied in depth. As a strength, this study provides some evidence for the preventive effect of geraniin against pioglitazone-related bone loss for the first time. Further, *in vivo* investigations and clinical trials are recommended to be undertaken to find the vast characteristics of this combo therapy and their mechanism.

Table No.1: Effect of Geraniin in combination with pioglitazone 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	Pioglitazone	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	Pioglitazone +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 of Geraniin in combination with pioglitazone 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	Pioglitazone	5.49±0.14*
5	Pioglitazone +Geraniin	5.45±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 of Geraniin in combination with pioglitazone 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	Pioglitazone	70 ± 2.2*	90 ± 2.5*
5	Pioglitazone +Geraniin	140 ± 2.7*	180 ± 2.3*

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, while co-supplementing geraniin with pioglitazone prevented pioglitazone-induced bone loss. As a result, it is expected that co-administration of geraniin with pioglitazone as a therapeutic strategy will reduce bone loss and fracture risk in T2DM patients using pioglitazone.

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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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