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Adverse effect of Canaglifloxacin on bone is reversed by Puerariae radix in rat model of diabetics

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Abstract

The root of Pueraria labata, *Puerariae radix(PR)*, a leguminous plant that grows wild, is one of the first and most significant basic herbs used in traditional medicine for a variety of therapeutic uses. *PR* is high in isoflavonoids including daidzein and genistein, which have been shown to protect against bone loss caused by oestrogen insufficiency. In an osteoporotic animal model, soybean isoflavones have been shown to reduce bone loss. CGF (canagliflozin) appears to raise the risk of fractur. In diabetic rats, the potential function of *PR* in reversing CGF-induced bone loss was investigated.

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A rat model was used to investigate the effects of Puerariae radix extract (*PRE*) on blood glucose, HBA1C levels, and bone mineral density. Control group (vehicle therapy), Diabetic group, `*PRE* group, Canagliflozin (CGF), and CGF +*PRE* group were all made up of six Wistar albino rats. Each medication was given by gastric gavage once a day for 35 days. *PRE* treatment increased bone mass substantially when compared to normal controls. This suggests that *PRE* might be developed as an alternative therapy for osteoporosis caused by anti-diabetic drugs.

Keywords: *Puerariae radix*, Diabetic osteoporosis, streptozotocin, Canagliflozin.

Introduction

Diabetic individuals should have their bone health evaluated as part of their treatment. Because of advancements in medical therapy, an increase in the incidence of osteoporosis has corresponded with an increase in the average life expectancy of people with diabetes. In addition to the conventional causes of osteoporosis, such as age, diabetes may decrease bone health. Studies on bone involvement in persons with diabetes mellitus have produced conflicting results due to the disease's pathogenetic complexity. According to new research, type 1 diabetes patients have lower bone mineral density (BMD) and are more likely to fracture.

Despite having a higher BMD than patients with type 1 diabetes, there is mounting evidence that persons with type 2 diabetes who also have comorbidities are at higher risk of osteoporotic fractures.[1] The root of Pueraria labata (Willd.) Ohwi, *Puerariae radix(PR)*, is one of the most significant basic herbs used in traditional medicine for a variety of therapeutic reasons. *PR* is high in isoflavonoids including daidzein and genistein, which have been shown to protect against bone loss caused by oestrogen insufficiency.[2]

According to new data, the extract of *Puerariae Radix* can boost antioxidant defenses by lowering plasma levels of coenzyme Q9 and MDA. Puerarin has showed promise in the treatment of diabetes, not only by shielding islets cells from oxidative stress by activating antioxidant enzymes, but also by significantly increasing insulin responsiveness in diabetic ratwhen administered as a metabolic disease adjuvant.[3]Canagliflozin is a novel antidiabetic and SGLT2 inhibitor that blocks glucose reabsorption in the kidneys, although its effect on renal structure and function is unknown.

Weight loss and reductions in serum estradiol levels were seen in women treated with canagliflozin, which may explain the increases in bone turnover and decreases in total hip BMD. Previous studies have shown a link between weight loss, decreased estradiol levels, increased bone turnover, and decreases in BMD, possibly due to decreased oestrogen production; weight loss and reductions in serum estradiol levels were seen in women treated with canagliflozin.[4]

Although a number of factors influence the probability of fractures, such as the frequency and kind of falls, visual impairment, neuropathy, and reduced muscular strength, the strength of the bone appears to be the most significant predictor. In rats,[5,6]*PRE* has been found to increase bone density and alter bone histomorphology.[2] However, there is no direct evidence that *PRE*prevents diabetes-related bone loss when co-treated with CGF. The purpose of this study was to investigate if *PRE* had an osteoprotective effect on BMD in STZ-treated rats that were also given CGF.

Materials and Methods

Preparation of Aqueous extract:

The dried Radix Puerariae were crushed into powder, and 500 g (1:1 w/w; 250 g each) was dissolved in 6,000 ml water at 25°C for 30 minutes, followed by 45 minutes of extraction at 100°C. The extraction operation was carried out once again. The extract was made into powder and kept at 4°C after freeze drying.

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Animals:

In the study, healthy male wistar albino rats weighing 180 to 240 g and aged 3 to 4 months were used. The animals were received from King Khalid University's Central Animal House in Abha, Saudi Arabia. Throughout the experiment, the animals were kept in cages and fed a normal pellet diet and filtered water ad libitum under controlled circumstances (light/dark cycle of 12 h/12 h, 50–70 percent humidity, 25 °C 3 °C). For 14 days, the animals were acclimatised to the laboratory setting.The study followed King Khalid University's animal ethics committee's permission as well as 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:

The pancreatic-cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was given intraperitoneally at a dose of 65 mg/kg body weight to cause diabetes in the animals.[7,8] The vehicle dose was the same for the control rats. To avoid deterioration, STZ was weighed separately for each animal, solubilized with 0.1 ml of freshly prepared cold Na citrate buffer (NaB-0.1 M, pH 4.5), and administered within 5 minutes. The STZ injection volume per kilogramme was calculated to be 1.0 ml/kg. To offset the drug's significant acute hypoglycemia effect, rats were given a 5% glucose solution for 48 hours after receiving STZ.

Animals were divided as follows: Control (vehicle, Non-Diabetic, n=6), Diabetic control (STZ treated, n=6), *PRE*(200/kg/day, n = 6), Canagliflozin (CGF) (40 mg/kg/day, n = 6), and combination group (*PRE* 200/kg/day + CGF 40 mg/kg/day, n = 6). Each medication was given by gastric gavage once a day for 35 days. Throughout the trial, the animals were examined daily for symptoms of illness. There were no animals that were really sick or died before the completion of the trial. Blood glucose levels were measured once a week for the course of the research using a Roche Accu Chek advantage glucometer to assess the animals' hyperglycemic condition.

Blood was drawn from the tail vein three days after STZ injection and tested for blood glucose using a glucometer (Aqua-Check, Roche). In animals, fasting blood glucose levels (FGLs) more than 250 mg/dL were deemed diabetic. The animals that did not have blood glucose levels more than 250 mg/dL were excluded from the research. The rats in the control group (n=6) that were given saline instead of streptozotocin had normal blood glucose levels (120 mg/dl).

Determination of fasting blood glucose:

Blood samples from the rats' tail veins were taken to test blood glucose levels using a glucometer after they had been starved for 12–14 hours. Blood will be taken with a 1-ml needle, put on a glucose strip, and quantified with a glucometer after the rats' tails have been washed with 70% (v/v) ethanol.

Determination of hemoglobin A1c:

Haemoglobin 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 proportion of HbA1c in the blood sample will be shown 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 measured using dual energy X-ray absorptiometry (DEXA) scanning equipment after blood was collected (Lunar, WI, USA).

Results

The glucose profiles of the positive control group (STZ) deteriorated over time (Table-1). However, *PRE* were demonstrated to protect against diabetes progression.

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Table-1: Effect of *PRE* on Fasting blood glucose level:

Treatment Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Normal Control	73.32± 3.2	74.42± 3.2	75.81± 3.4	78.40± 2.6	77.30±2.5	81.46± 1.7	85.40± 1.15	83.40± 1.13	85.40± 1.52
Positive Control	263.54 ±9.1*	297.35 ±8.7*	317.31 ±10.62 *	333.74 ±7.6*	356.78± 8.4*	378.72 ±11.5*	394.72± 10.6*	417.72 ±11.2*	435.75± 9.7*
PRE	267.36 ±7.3	276.27 ±8.6*	285.26 ±8.7*	288.26 ±8.2*	293.34± 6.8*	307.34 ±12.8*	313.36± 8.2*	323.34 ±9.2*	323.37± 9.7*
CGF	266.35 ±8.4	247.15 ±12.4*	235.24 ±8.6*	212.29 ±8.6*	186.28± 8.4*	156.54 ±8.0*	124.45± 10.3*	101.14 ±9.6*	93.135± 8.7*
CGF +EHE	264.35 ±8.3	246.15 ±10.4*	235.24 ±8.8*	212.29 ±8.5*	186.28± 8.5*	152.54 ±8.6*	127.45± 10.2*	104.14 ±8.5*	94.135± 7.7*

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

HBA1C levels were higher in the positive control group than in the normal control group (p<0.05), as indicated in table 2. In contrast to the positive control group, *PRE* was shown to lower HBA1C levels, implying a favourable effect.

Table-2: Effect of PRE on Glycoslyted Haemoglobin (HBA1C)

Treatment Group	Day 28
Normal Control	5.53±0.22
Positive Control	5.83±0.13*
PRE	5.75±0.14*
CGF	5.48±0.13*
CGF +*PRE*	5.47±0.15*

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

The positive group's BMD differed considerably from the other treatment groups (Table-3). PRE may be able to protect bones from the consequences of hyperglycemia, according to these data. Diabetic rats showed decreased lumbar (L1-L4) and femoral bone mineral density (BMD), which was restored by PRE treatment (p<0.05).

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Table-3: Effect of PRE on the bone mineral density of the lumbar vertebrae and femur bone

	Bone Mineral density(mg/cm3)	
Treatment Group	**Lumbar Vertebrae**	**Femur**
Normal Control	176 ± 2.4	224 ± 2.6
Positive Control	78 ± 2.7*	105 ± 2.2*
PRE	157 ± 1.4*	205 ± 1.8*
CGF	92 ± 2.3*	105 ± 2.4*
CGF +PRE	155 ± 1.4*	202 ± 1.8*

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

Statistical analysis:

The data should be represented using a mean and standard deviation (SD). To statistically analyse data from diverse groups, one-way analysis of variance (ANOVA) and Tukey's multiple comparison test will be utilised. A “p” value of less than 0.05 is considered statistically significant.

Discussion

According to certain studies, PRE possesses antiosteoporotic effects.[2,9] The mechanism of action of this extract in the case of osteoporosis is unknown. In this work, the influence of PRE on STZ-induced diabetic osteoporosis in rats co-treated with CGF was investigated for the first time. After being exposed to OVX, PRE has been demonstrated to protect rats from acquiring osteoporosis-like symptoms (Ovariectomy).[2] Diabetic rats had considerably higher amounts of BCAAs, glutamate, arginine, and tyrosine. Treatment with PRE may help to restore the amino acid metabolism that has been disrupted.

As a result, PRE has a lot of therapeutic promise in the treatment of diabetes mellitus (DM) since it improves metabolism and prevents oxidative damage. PRE has a more potent inhibitory effect on bone growth and resorption. Furthermore, PRE was found to completely prevent decreases in trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th) in ovariectomized (OVX) rat and restored the increase in trabecular separation (Tb.Sp) in ovariectomized (OVX) rat in histological analysis of the femoral metaphysis in ovariectomized (OVX) rat. Fracture risk was greater in diabetic individuals treated with canagliflozin who were older, had a prior history or risk of cardiovascular disease, had a lower baseline estimated glomerular filtration rate, and used more diuretics.

Falls may have a role in the rise in fractures, but the reason of the increased fracture risk with canagliflozin is unclear.[4] This study looks at the impact of PRE on bone quality in a STZ-induced type 2 diabetic animal model.The effects of herbal products on bone loss, production, and resorption, as well as bone structure and content, and bone quality as assessed by mechanical properties, have been studied before.[10-14]PRE had a beneficial effect on unloading, as evidenced by increases in BMD. The direct effect of PRE on bone formation and inhibition of osteoclast growth might explain its bone-protective qualities.[10]

Conclusion

Our study provides evidence that aqueous *PRE* may have potential use in the complementary and alternative treatments of diabetic bone loss induced by canaglifoxacin.

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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."

References

1. Krishnaraju Venkatesan et al. Plastrum testudinis promotes bone formation in streptozotocin-induced diabetic rats, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*,2021;9(1):85-89.
2. Wang X, Wu J, Chiba H, Umegaki K, Yamada K, Ishimi Y. Puerariae radix prevents bone loss in ovariectomized *rat*. *J Bone Miner Metab*. 2003;21(5):268-75.
3. Zhang Y, Wang P, Xu Y, Meng X, Zhang Y. Metabolomic Analysis of Biochemical Changes in the Plasma of High-Fat Diet and Streptozotocin-Induced Diabetic Rats after Treatment with Isoflavones Extract of Radix Puerariae. *Evid Based Complement Alternat Med*. 2016;2016:4701890.
4. Watts NB, Bilezikian JP, Usiskin K, et al. Effects of Canagliflozin on Fracture Risk in Patients With Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab*. 2016;101(1):157-166.
5. Zhang ZG, Bai D, Liu MJ, Li Y, Pan JH, Liu H, Wang WL, Xiang LH, Xiao GG, Ju DH. Therapeutic effect of aqueous extract from Ecliptae herba on bone metabolism of ovariectomized rats. *Menopause*.2013; 20(2): 232-40.
6. Saller A, Maggi S, Romanato G, Tonin P, Crepaldi G. Diabetes and osteoporosis. *Aging Clin Exp Res*.2008;20(4):280-9.
7. Reddy GK, St*EH*no-Bittel L, Hamade S, Enwemeka CS. The biomechanical integrity of bone in experimental diabetes. *Diabetes Res Clin PRact*.2001;54(1):1-8.
8. Erdal N, Gürgül S, Demirel C, Yildiz A. The effect of insulin therapy on biomechanical deterioration of bone in streptozotocin (STZ) induced type 1 diabetes mellitus in rats. *Diabetes Res Clin pract*.2012;97(3):461-7.
9. Qin H, Zhao W, Jiao Y, Zheng H, Zhang H, Jin J, Li Q, Chen X, Gao X and Han Y (2021) Aqueous Extract of *Salvia miltiorrhiza* Bunge-*Radix Puerariae* Herb Pair Attenuates Osteoporosis in Ovariectomized Rats Through Suppressing Osteoclast Differentiation. *Front. Pharmacol.* 11:581049.
10. Zhang R, Liu ZG, Li C, et al. Du Zhong (*Eucommia ulmoides* Oliv.) cortex extract prevent OVX induced osteoporosis in rats. *Bone*.2009; 45:553–9.
11. Zhang Y, Li Q, Wan HY, et al. Study of the mechanisms by which *Sambucus williamsii* HANCE extract exert protective effects against ovariectomy induced osteoporosis *in vivo*. *Osteoporosis Int*.2011; 22:703–9.
12. Hung TM, Na M, Thuong PT, et al. Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacus asper* Wall. *J Ethnopharmacol*. 2006; 108: 188–92
13. Wong RW, Rabie AB, Hägg EU.The effect of crude extract from Radix Dipsaci on bone in rat. *Phytother Res*.2007; 21:596–8.

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14. Chi-Fung Cheng, Jeff Chien Fu Lin, Fuu Jen Tsai, Chao Jung Chen, Jian Shiun Chiou, Chen Hsing Chou, Te Mao Li, Ting Hsu Lin, Chiu Chu Liao, Shao Mei Huang, Ju Pi Li, Jung-Chun Lin, Chih Chien Lin, Bo Ban, Wen Miin Liang, Ying Ju Lin, protective effects and network analysis of natural compounds obtained from Radix dipsaci, Eucommiae cortex, and Rhizoma drynariae against RANKL induced osteoclastogenesis in vitro. *Journal of Ethnopharmacology*. 2019; 244:112074.

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