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POLYGONUM MULTIFLORUM THUNB EXTRACT ATTENUATES BONE LOSS IN DIABETIC RATS

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ABSTRACT

The effects and mechanism of *Polygonum multiflorum Thunb* water extract *(PMT)* on diabetes-related bone loss in rats were studied in this study. For 8 weeks, high-fat diet and streptozotocin induced diabetic rats were given *Polygonum multiflorum Thunb* (300mg/kg body weight) orally. *Polygonum multiflorum Thunb* increased the amounts of osteocalcin and BALP in the blood. Furthermore, *Polygonum multiflorum Thunb* enhanced femoral bone mineral density substantially. These findings show that *Polygonum multiflorum Thunb* may alleviate diabetes related bone problems via modulating osteoclast-related genes, implying that *Polygonum multiflorum Thunb* might be utilised to prevent diabetes-induced bone loss.

KEYWORDS

Polygonum Multiflorum Thunb, Osteoporosis and Bone protective effect.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic illness that can cause retinopathy, neuropathy, nephropathy, cardiovascular disease, and osteoporosis. Diabetic complications are serious public health issues that are becoming more common across the world[1]. Fragility fractures are the major cause of morbidity and death in older individuals with diabetes, and are increasingly recognised as a consequence of both type 1 and type 2 diabetes mellites (T1DM and

T2DM, respectively)[2,3] Low bone mineral density (BMD) is a risk factor for fragility fractures; BMD has been demonstrated to decrease in T1DM and both decline and increase in T2DM when compared to healthy people.

Furthermore, anti-diabetes drugs such as thiazolidinediones and sodium glucose cotransporter 2 inhibitors have been found to cause diabetic bone problems through affecting bone and mineral metabolism[4]. Natural plants have recently garnered a lot of attention as potential sources of alternative medications for diabetes related bone diseases prevention and therapy. PMT is a prominent traditional medicinal plant[5]. It is low in toxicity and possesses anti-tumor, anti-inflammation, nephron-protective, anti-atherosclerosis, and anti-diabetes properties.

In estrogen deficient mice, *Polygonum multiflorum Thunb* hot water extract and tetra hydroxyl stilbene glucoside (THSG), a *Polygonum multiflorum Thunb* bioactive component, were similarly shown to prevent bone loss. In mice with streptozotocin (STZ) induced diabetes, Zhang *et al*. (2019) discovered that THSG protects against diabetes induced osteoporosis[6]. However, it is unclear if *Polygonum multiflorum Thunb* may prevent diabetes-induced bone loss in rats *in vivo* or the mechanism by which it works. In STZ induced diabetic rats, we examined the effects of *Polygonum multiflorum Thunb* water extract on serum bone related indicators, BMD.

MATERIAL AND METHODS

Animals

The experiment was carried out with 24 male Sprague Dawley rats weighing 100-120g obtained from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were maintained in a temperature-controlled facility (22±1°C, 12 hour light/dark cycle) and fed standard rat chow with full access to water. The animal ethics committee at King Khalid University approved the experiment procedures, which included diabetes induction and sacrifice, and they were carried out in compliance with 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

To chemically induce diabetes-like hyperglycemia in rats, a single intraperitoneal injection of 60mg/kg STZ dissolved in 10mM citrate buffer was given (pH 4.5). To prevent drug induced hypoglycemia, the rats were given 5% glucose water for two days following STZ injection. Seven rats were categorised as diabetic after a week of injection if their fasting blood glucose levels were more than 11mmol/L[7]. The rats in the normal control group got the same dose of isotonic NaCl injection as the rats in the experimental group.

Experimental design

Four groups of 24 male rats (n = 6) were created. Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rats received 1000mg/kg of metformin (MET), and diabetic rats received 300mg/kg of *Polygonum multiflorum Thunb*. Oral gavage was used to provide the treatments once a day for 56 days. At the conclusion of the experiment, all of the animals fasted overnight and blood glucose levels were measured. Before being killed, the animals were administered analgesia with ketamine (80mg/kg) and xylazine (8mg/kg). The femur and tibia were cut apart at the stifle joint. By heart puncture, blood samples (10-15mL) were collected from the rats and put in a simple red-top tube with no anticoagulants. The serum was split into aliquots and stored at -80°C after centrifuging blood samples at 4000rpm for 15 minutes.

Marker of bone formation and bone resorption

All bone production and resorption indicators were measured in serum. The osteocalcin level was determined using the Rat Mid Osteocalcin ELISA kit (IDS, UK) while the BALP level was determined using the rat BALP ELISA kit (IDS, UK) (Qayee, Shanghai). To assess bone resorption, DPD was measured with a rat deoxypyridinoline (DPD) ELISA kit (Qayee, Shanghai). All samples were run in triplicate, according to Abdul-Majeed *et al* and the optical density was measured at 450nm using a microplate reader (BioTek, USA) (2012)[8].

Bone Mineral Density Measurement

After blood was taken, the BMD of the left femur and lumbar vertebrae (L1-L4) of rats was evaluated

using dual energy X-ray absorptiometry (DEXA) scanning equipment.

Statistical analysis

All of the data was analysed using ANOVA. The significance of the findings was determined using Duncan's multiple comparison test. All of the analyses were carried out with a 95% level of confidence.

RESULTS AND DISCUSSION

Bone turnover markers

The STZ injection resulted in significant reductions in blood osteocalcin, but serum DPD was significantly higher than in the NC group (Table No.1). Despite no significant differences in BALP values across all treatment groups, blood osteocalcin levels increased while DPD levels decreased following *Polygonum multiflorum Thunb* therapy.

Discussion

In animal studies, STZ induced diabetic control rats had greater levels of oxidative damage markers. According to the findings, blood DPD levels increased in DC rats, whereas serum osteocalcin and BALP activity decreased.

This discovery is in line with the findings[9] that a reduction in bone turnover is the most prominent characteristic of T1DM related bone deterioration. Our findings are supported by previous reports of increased serum DPD in rats with osteoarthritis[10] and osteopenia[11]. According to prior studies, osteocalcin does not appear to be as sensitive a marker as BALP[12]. BALP activity is still low in *Polygonum multiflorum Thunb* extract treated rats, suggesting that mineral metabolism is still affected. BALP (Bone Specific Alkaline Phosphatase) is a bone specific alkaline phosphatase isoform that is synthesised by osteoblasts for bone remodelling but more accurately represents mineral metabolism.

The ratio of osteocalcin to DPD was nearly similar in the *Polygonum multiflorum Thunb* extract treatment and NC groups, suggesting that bone formation and resorption were nearly balanced. Low bone mass and micro structural damage to the bone tissue define osteoporosis. The bone mineral density (BMD) is the most important quantitative biomarker of osteoporosis. When compared to the control group, *Polygonum multiflorum Thunb* enhanced femoral BMD and tend to increase tibial BMD.

Table No.1: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± SD)

S.No	Groups	Bone formation markers		Bone resorption marker
		Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
1	NC	138.78 ± 6.96^{c}	104.49 ± 7.69^{b}	157.08 ± 5.43^{b}
2	DC	16.35 ± 0.87^{a}	68.06 ± 4.80^{a}	162.10 ± 0.21^{c}
3	MET	57.42 ± 8.64^{b}	83.38 ± 0.45^{a}	156.16 ± 4.18^{ab}
4	*Polygonum multiflorum Thunb*	159.66 ± 4.10^{d}	77.30 ± 8.31^{a}	144.53 ± 0.51^{a}

Values with different superscripts (a, b, c) down the column indicate significant difference at $p < 0.05$.

Table No.2: Effect of *Polygonum multiflorum Thunb* extract 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	NC	179 ± 2.4^{b}	224 ± 2.7^{b}
2	DC	76 ± 2.8^{b}	104 ± 2.6^{b}
3	MET	159 ± 1.8^{a}	210 ± 1.9^{a}
4	*Polygonum multiflorum Thunb*	178 ± 1.6^{a}	225 ± 1.8^{a}

Values with different superscripts (a, b, c) down the column indicate significant difference at $p < 0.05$.

CONCLUSION

In conclusion, these results indicate that *Polygonum multiflorum Thunb* might play a protective role in diabetes induced osteoporosis, with positive impact on positive impact on DPD activity and bone mineral density in diabetic rats.

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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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