Pharma

Pharmacological potential of *Angelica sinensis* against invokana-induced bone effects in diabetic rats

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Abstract

The study evaluated the effect of *Angelica sinensis* root aqueous extract (*A. sinensis*) on bone loss induced by Canagliflozin (CGF) in streptozotocin (STZ)-induced diabetic rats. A total of five groups, each comprising six Wistar albino rats, were used: control (vehicle), diabetic control (STZ), *A. sinensis* alone, CGF alone, and CGF + *A. sinensis*. Treatments were administered once daily via gastric gavage for 35 days. Results showed that bone mineral density (BMD) in treated groups was higher than in controls. Diabetic rats receiving *A. sinensis* exhibited significantly elevated insulin and osteocalcin levels compared to diabetic controls. These findings suggest that *A. sinensis* may exert preventive or therapeutic effects against diabetes-associated bone loss, likely through inhibition of bone turnover. Overall, the results support the potential role of *A. sinensis* as an adjunct therapy for managing bone loss in diabetic conditions and postmenopausal osteoporosis.

Keywords: Osteoporosis, Diabetes mellitus, *Angelica sinensis*, Canagliflozin

Introduction

Diabetes is a metabolic disease in which the metabolism of glucose, lipids and proteins metabolism. It is one of the leading causes of morbidity and mortality in Western societies, affecting approximately 6% of the adult population, with a global incidence increasing at an estimated rate of 6% annually. By 2010, the number of cases had reached between 200 and 300 million worldwide. Diabetes and osteoporosis are two major chronic conditions that increasingly affect the elderly population. Epidemiological evidence indicates that individuals with diabetes over the age of 65 have a significantly higher risk of fractures, particularly in the hip, upper arm, and leg.

Type 1 diabetes is generally associated with a mild reduction in bone mineral density (BMD), while type 2 diabetes, more prevalent in older adults, often presents with normal or even elevated BMD. However, experimental studies have revealed alterations in bone microarchitecture in diabetic models, potentially explaining the paradox of increased fracture risk in type 2 diabetic patients despite normal or elevated BMD.

Angelica sinensis (*A. sinensis*), a well-known medicinal plant in traditional medicine, has demonstrated multiple pharmacological properties including hepatoprotective, neuroprotective, antioxidant, anti-osteoarthritic, and anticancer effects. Its bioactive constituents include phthalides, organic acids, polysaccharides, and flavonoids, which contribute to its diverse therapeutic potential.

Canagliflozin (CGF), the first sodium-glucose cotransporter 2 (SGLT2) inhibitor approved in 2013, reduces blood glucose by enhancing urinary glucose excretion through inhibition of renal glucose reabsorption. While effective in improving glycemic control when combined with diet and exercise, treatment with canagliflozin has been associated with an increased risk of fractures. Preclinical and clinical data suggest that *A. sinensis* may exert protective effects against osteoporosis, particularly by reducing fracture incidence. However, its role in diabetic osteoporosis remains unclear. Therefore, the present study was designed to investigate the potential protective effect of *A. sinensis* on streptozotocin (STZ)-induced diabetic bone loss, particularly in combination with canagliflozin.

Materials and Methods

Animals

Male Wistar rats (100–120 g) were obtained from the Central Animal House, King Khalid University, Abha, Saudi Arabia. Animals were housed under controlled environmental conditions (22 ± 2 °C, 12-h light/dark cycle) with free access to standard chow and water. All experimental procedures, including diabetes induction and euthanasia, were approved by the King Khalid University Animal Ethics Committee and conducted in accordance with the *National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg) dissolved in 10 mM citrate buffer (pH 4.5). To prevent drug-induced hypoglycemia, rats were provided with 5% glucose solution for two days post-injection. One week later, animals with fasting blood glucose levels >11 mmol/L were classified as diabetic. Control animals received isotonic NaCl injection under identical conditions.

Experimental design

Animals were randomly divided into five groups (n = 6 per group):

- **Group I:** Non-diabetic control (vehicle)
- **Group II:** Diabetic control (STZ)
- **Group III:** Diabetic + *A. sinensis* (300 mg/kg/day)
- **Group IV:** Diabetic + Canagliflozin (40 mg/kg/day)
- **Group V:** Diabetic + *A. sinensis* (300 mg/kg/day) + Canagliflozin (40 mg/kg/day)

All treatments were administered orally by gastric gavage once daily for 35 days. Animals were monitored daily for signs of illness; no mortality or severe illness was recorded during the study.

At the end of the experimental period, animals were fasted overnight, and blood glucose was measured. Rats were then anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg) before sacrifice. Blood samples (10–15 mL) were collected via cardiac puncture into plain tubes, allowed to clot, and centrifuged at 4000 rpm for 15 minutes. Serum aliquots were stored at –80 °C for biochemical analyses. The femur and tibia were excised at the stifle joint for subsequent examination.

Biochemical assays

Markers of bone formation and resorption were measured in serum samples.

- **Osteocalcin:** Rat Mid Osteocalcin ELISA kit (IDS, UK)
- **Bone-specific alkaline phosphatase (BALP):** Rat BALP ELISA kit (Qayee, Shanghai)
- **Deoxypyridinoline (DPD):** Rat DPD ELISA kit (Qayee, Shanghai)

Optical density was determined at 450 nm using a microplate spectrophotometer (Epoch, BioTek, USA).

Statistical analysis

Data were expressed as mean ± SEM. Statistical significance was evaluated using one-way ANOVA followed by Duncan's multiple range test. A p-value <0.05 was considered statistically significant.

Results

Blood glucose and serum insulin

Compared with non-diabetic controls, diabetic rats exhibited significantly elevated fasting blood glucose levels and reduced serum insulin concentrations (Table 1). Treatment with *A. sinensis*, canagliflozin, or their combination markedly reduced fasting glucose levels and significantly improved serum insulin compared with the diabetic control group.

Table 1: Effects of *A.Sinensis* on fasting blood glucose level and serum insulin in STZ induced diabetic rats (data represent mean ± 1SD).

Groups	Fasting blood glucose (mmol/L)		Serum insulin (μIU/mL)
	Before	After	
NC	3.91 ± 0.40^{a}	4.43 ± 0.11^{a}	4.26 ± 3.13^{c}
DC	19.00 ± 3.24^{b}	33.14 ± 2.45^{b}	1.45 ± 0.19^{a}
CGF	25.30 ± 3.20^{c}	17.72 ± 3.49^{c}	1.69 ± 0.32^{a}
A.Sinensis	24.85 ± 6.12^{c}	17.26 ± 4.77^{c}	2.38 ± 0.24^{b}
A.Sinensis + CGF	22.85 ± 6.33^{c}	16.54 ± 4.64^{c}	2.37 ± 0.28^{b}

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

Bone turnover markers

Despite the fact that serum DPD was substantially greater in the STZ group than in the NC group, blood osteocalcin was significantly lower (Table 2). Although BALP readings did not change substantially between the treated groups, serum osteocalcin levels increased while DPD levels dropped after *A. Sinensis* therapy.

Table 2: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± SD)

Groups	Bone formation markers		Bone resorption marker
	Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
NC	134.87 ± 6.2^{c}	101.48 ± 7.89^{b}	167.08 ± 5.22^{b}
DC	14.24 ± 0.96^{a}	65.06 ± 4.79^{a}	166.11 ± 0.29^{c}
CGF	61.40 ± 8.34^{b}	86.38 ± 0.47^{a}	176.19 ± 4.59^{ab}
A.Sinensis	144.64 ± 4.15^{d}	79.40 ± 7.31^{a}	138.53 ± 0.41^{a}
A.Sinensis + CGF	147.64 ± 4.17^{d}	74.40 ± 8.31^{a}	153.53 ± 0.41^{a}

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

Discussion

Oxidative stress and hyperglycemia are known to influence bone metabolism and architecture by altering the activity of osteoclasts and osteoblasts. In the present study, diabetic control (DC) rats exhibited increased blood DPD levels, while serum osteocalcin and BALP activity were significantly reduced. These findings are consistent with the observations of Zhukouskaya et al. (2015), who reported that suppression of bone turnover is a hallmark of T1DM-related bone disease. Previous studies have also demonstrated elevated blood DPD levels in animal models of osteoarthritis and osteopenia, further supporting this relationship.

A notable outcome of this study is that treatment with *A. sinensis* resulted in a decrease in blood DPD levels

accompanied by an increase in osteocalcin concentration (Table 2). Similar osteoprotective effects have been reported with other medicinal herbs, suggesting that *A. sinensis* may possess comparable therapeutic potential. Although osteocalcin is a specific osteoblast marker that correlates well with histological changes, its circulating levels are influenced by dietary intake and are generally less responsive than BALP. In our study, BALP activity remained low in *A. sinensis*-treated rats, indicating persistent impairment in mineral metabolism. Since BALP is an osteoblast-derived bone-specific alkaline phosphatase isoform crucial for bone remodeling and mineral metabolism, this reduced activity suggests that complete restoration of bone function may require longer or adjunctive therapy.

Interestingly, the osteocalcin-to-DPD ratio in the *A. sinensis* group was nearly comparable to that of the normal control group, indicating that *A. sinensis* therapy helped achieve a balance between bone formation and resorption.

Canagliflozin, an SGLT2 inhibitor, has been reported to negatively impact bone microarchitecture, likely due to diabetes-associated reductions in bone structural strength and toughness. In male diabetic DBA/2J mice, 10 weeks of canagliflozin treatment impaired cortical and trabecular microarchitecture, reducing femoral and vertebral strength. This was characterized by decreased trabecular volume, trabecular number, and tissue mineral density, along with increased trabecular spacing in the femurs of non-diabetic mice ($p<0.0001$). Other animal studies suggest that poor glycemic control exacerbates these structural deficits, whereas osteocyte cell lines show no direct involvement. Although limited, available data indicate that SGLT2 inhibitors may compromise bone quality, highlighting the need for further preclinical and clinical research on their effects on matrix mineralization and collagen distribution.

In contrast, in our study, bone mineral density (BMD) was significantly higher in *A. sinensis*-treated rats compared to STZ-induced diabetic rats (Table 2). Moreover, DPD levels were markedly lower, providing additional evidence that *A. sinensis* protects against diabetes-related bone deterioration.

Conclusion

The aqueous extract of *A. sinensis* demonstrated significant bone-protective effects against CGF-induced bone loss in STZ-diabetic rats. These findings provide experimental validation for its traditional use in preventing diabetes-associated bone loss and suggest that *A. sinensis* may serve as a potential complementary therapeutic option for managing diabetic osteopathy.

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