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ANGELICA SINENSIS ROOT EXTRACT PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The anti-osteoporotic properties of *Angelica sinensis* root *(ASR)* extract in diabetic rats treated with streptozotocin (STZ) were investigated in the current study. *Angelica sinensis* root extract (300mg/kg, p.o. once daily) was given to the STZ treated rats for eight weeks. The BMD of the bone (femur) mineral density (BMD) of rats given the extract (300mg/kg) was considerably greater than that of the control group. Blood osteocalcin and ALP levels were significantly lower after the STZ injection, but serum DPD levels were significantly higher than in the normal control group. Despite no significant differences in BALP values across all treatment groups, blood osteocalcin levels increased while DPD decreased during *Angelica sinensis* root therapy. The findings show that *Angelica sinensis* extract can help diabetic rats that have been exposed to STZ.

KEYWORDS

Angelica sinensis root, Osteoporosis and Bone damage.

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INTRODUCTION

Angelica sinensis (Oliv) *Angelica sinensis* root (*ASR*) extract has a long history of usage in China as a traditional medicine. In Europe and North America, it is used as a dietary supplement[1]. Hepatoprotective[2] neuroprotective[3], antimoxidant[4] anti osteoarthritis[5] and anti cancer[6] properties have also been found with *Angelica sinensis* root extract.

Phthalides, organic acids, polysaccharides, and flavones are the main active chemicals in *Angelica sinensis* root that are responsible for its varied biological activity[7]. Interleukin-1 (IL-1), IL-6, and tumour necrosis factor-α (TNF-α) are well known pro-inflammatory cytokines that regulate bone metabolism.

These cytokines are recognised as very powerful bone resorption cytokines[8-11] and they have the ability to induce enhanced bone turnover indicators[12]. The anti-inflammatory activity of *Angelica sinensis* root extract[13-15] has been postulated to have potential anti-osteoporotic effects in an animal model via suppression of bone turnover indicators, based on the aforementioned findings. The anti-osteoporotic properties of *Angelica sinensis* root extract were investigated in STZ-induced diabetic rats in the current study. Dual energy X-ray absorptiometry was used to calculate the femur's bone mineral density (BMD) (DXA). ELISA was used to measure the levels of osteocalcin (OC), BALP and DPD.

MATERIAL AND METHODS

Animals

The experiment was conducted on 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 environment (22±1°C with a 12 hour light/dark cycle) and were 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 line with the National Institute of Health's criteria 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 intra-peritoneal injection of 60mg/kg STZ dissolved in 10mM citrate buffer was employed (pH 4.5). To avoid drug induced hypoglycemia, the rats were given 5% glucose water for two days after receiving STZ. After a week of injection[16], rat were categorised as diabetes if their fasting blood glucose levels were more than 11mmol/L[17]. The rats in the normal control group got the same quantity of isotonic NaCl injection as the rats in the experimental group.

Experimental design

24 male rats (n = 6) were divided into four groups. Diabetic control rats received saline (DC), normal control rats received saline (NC), diabetic rats received 1000mg/kg body weight of metformin (MET), and the other diabetic rat group received 300mg/kg body weight of *Angelica sinensis* root. Oral gavage was used to deliver treatments once a day for 56 days. At the end of the experiment, all of the animals fasted overnight and their blood glucose levels were measured. The animals were then administered anaesthesia with ketamine (80mg/kg) and xylazine (8mg/kg) before being killed. 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 4000g for 15 minutes.

Marker of bone formation and bone resorption

All markers of bone formation and resorption were measured in the serum. The osteocalcin level was determined using the Rat Mid Osteocalcin ELISA kit (IDS, UK), while the BALP level was determined with the rat BALP ELISA kit (Qayee, Shanghai). To assess bone resorption, DPD was evaluated using a rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai). All samples were run in triplicate and the optical density was measured at 450nm using a microplate reader (Epoch Microplate Spectrophotometer, Bio Tek, USA), according to Abdul Majeed *et al* (2012)[18].

Bone Mineral Density Measurement

After blood was taken, the BMD of the left femur and lumbar vertebrae (L1-L4) of rats was measured with a dual energy X-ray absorptiometry (DEXA) scanning device.

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

Blood osteocalcin were significantly lower after the STZ injection, although serum DPD levels were significantly higher than in the NC group (Table No.1). Despite no significant differences in BALP values across the treatment groups, blood osteocalcin levels enhanced while DPD decreased following ASR therapy.

Discussion

In comparison to the normal control group, eight weeks of *Angelica sinensis* root extract therapy reduced BMD loss in the femur and suppressed bone turnover indicators. These findings show that *Angelica sinensis* root extract inhibits bone turnover triggered by STZ, therefore reducing bone loss. Ferrulic acid, a strong antioxidant and free radical scavenger, is found in *Angelica sinensis* root extract[19]. Free radicals produced by oxidation have been shown to promote osteoclastic development, which increases bone resorption[20]. OVX induced serum interleukin 1 (IL-1β) and tumour necrosis factor (TNF-α) levels are inhibited by ligustilide from *Angelica sinensis* root essential oil[21]. TNF-α seems to synergize with IL-1β to promote bone resorption. IL-1β is a well-known bone resorptive cytokine.

The high amount of ferulic acid orligustilide in *Angelica sinensis* root appears to be linked to its bone damaging actions. Before *Angelica sinensis* root extract can be utilised in medication or supplement development, its toxicity must be determined. *Angelica sinensis* root extract was given orally to rats at dosages of 1000 and 2000mg/kg/day in the described rat toxicity tests. There were no modifications in the *Angelica sinensis* root extract that might be deemed toxicologically significant. There were no toxicological alterations in any of the clinical symptoms, body weight changes, serum biochemistry, necropsy findings, or relative organ weights after repeated oral treatment of *Angelica sinensis* root extract to rats for 4 weeks. As a result, the stated No Observable Adverse Effect Level (NOAEL) of *Angelica sinensis* root extract for both male and female rats was estimated to be 2000mg/kg/day[22].

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	136.68 ± 7.02^{c}	122.09 ± 6.69^{b}	159.18 ± 6.03^{b}
2	DC	16.25 ± 0.87^{a}	64.16 ± 4.80^{a}	166.20 ± 0.11^{c}
3	MET	57.42 ± 7.84^{b}	83.18 ± 0.55^{a}	152.26 ± 4.18^{ab}
4	*Angelica sinensis* root	154.66 ± 4.01^{d}	75.20 ± 8.41^{a}	133.63 ± 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 *Angelica sinensis* root 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	176 ± 3.0^{b}	210 ± 2.4^{b}
2	DC	75 ± 2.7^{b}	103 ± 2.2^{b}
3	MET	148 ± 2.5^{a}	210 ± 1.8^{a}
4	*Angelica sinensis* root	174 ± 2.4^{a}	215 ± 1.7^{a}

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

CONCLUSION

Finally, *Angelica sinensis* root extract can prevent STZ induced bone loss with a level of effectiveness equivalent to that of the control group. The results show that *Angelica sinensis* root extract may be a viable natural option for diabetic osteoporosis prevention. The findings reported here lay a platform for clinical assessment and show that *Angelica sinensis* root extract has potential as a herbal medicine.

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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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