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EUCOMMIA ULMOIDES OLIVER EXTRACT PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Patients with diabetes mellitus are more likely to suffer from bone fractures. *Eucommia ulmoides* has previously been shown to be a reducer in osteoporosis. However, it is yet unknown if *Eucommia ulmoides* can prevent osteoporosis in diabetic rats. The effect of *Eucommia ulmoides* on bone oxidative stress and turnover indicators in diabetic rats is investigated in this study. Diabetic is induced by streptozotocin (STZ). For 8 weeks, diabetic Sprague-Dawley rats (n = 6) were given one of three treatments via gavage: Saline (control), metformin (1000mg/kg bwt), or methanol extract of *Eucommia ulmoides* (500mg/kg bw). As a normal control group, a group of healthy rats was used. ELISA assays were used to evaluate serum levels of insulin, oxidative stress and bone turnover indicators. When diabetic rats were given *Eucommia ulmoides*, insulin and osteocalcin levels were considerably higher than in diabetic control rats. By increasing osteogenesis and decreasing bone oxidative stress, *Eucommia ulmoides* may be able to prevent diabetic osteoporosis. These data support the use of *Eucommia ulmoides* as an osteoporosis treatment in diabetic patients.

KEYWORDS

Eucommia ulmoides and Diabetic osteoporosis.

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INTRODUCTION

In the lower limbs, diabetes has been linked to a number of orthopaedic diseases and issues, all of which have a negative impact on one's quality of life[1]. Diabetes promotes osteoclast function, which increases bone loss, osteopenia and osteoporosis[2]. The occurrence of poor bone quality in diabetes people is supported by reports of delayed bone

healing[3,4] lower growth plate thickness[5] and increased cortical porosity[6]. As a result, unravelling the mechanisms behind diabetes-related alterations in bone microstructure requires special focus. At remodelling sites, two opposing processes are known as bone production and bone resorption. Osteoarthritis and osteoporosis are caused by a breakdown in the delicate balance between these two processes[7,8].

An increase in osteoclast activity and a decrease in osteoblast activity reduces bone strength, according to clinical and preclinical investigations[9]. Several research have suggested that STZ-induced diabetes is a good model for learning about the pathophysiological underpinnings of diabetes-related bone loss[10]. STZ's selective toxicity was employed to develop diabetic osteoporosis animal models in mice[11,12]. And rats[13,14] STZ has been shown to be extremely hazardous not just to the pancreas' insulin-producing beta cells, but also to the skeletal muscles. It's worth noting that skeletal muscle can act as a mediator and predictor of bone quality[15] Other investigations have found that STZ reduces bone formation and increases the number of active osteoclasts[16,17]. STZ reduces BMD and causes trabecular bone loss, according to[18] oxidative stress and hyperglycemia.

In the adult ovariectomy rat, daily oral administration of *Eucommia ulmoides* over a 16-week period has been shown to prevent oestrogen deficiency-induced bone competence. The high amount of polyphenolic substances such as lignans, phenolic acid and flavonoids appears to be responsible for these benefits. As a result, *Eucommia ulmoides*, a traditional medication, could be a possible alternative medicine for postmenopausal osteoporosis treatment. Although *Eucommia ulmoides* has shown substantial anti-osteoporotic actions in a model of osteoporosis, its preventive effect on diabetic osteoporosis is unknown. The effects of *Eucommia ulmoides* treatment on bone oxidative stress and turnover indicators, motivated us to investigate in STZ-treated rats.

MATERIAL AND METHODS

Animals

The researchers used 24 male Sprague-Dawley rats weighing 100-120g from King Khalid University's Central Animal House in Abha, Saudi Arabia, for the experiment. The rats were kept in a temperature-controlled environment (22 ±1^{o}C with a 12 hour light/dark cycle) and fed normal rat chow with unlimited access to water. The experiment protocols, which included diabetes induction and sacrifice, were authorized by the animal ethics committee at King Khalid University and were carried out in accordance 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 utilised (pH 4.5). To prevent drug-induced hypoglycemia, the rats were given 5% glucose water for two days after receiving STZ[19]. Animals having fasting blood glucose levels of higher than 11mmol/L were classed as diabetic after a week of injection[20]. The normal control rats received the same quantity of isotonic NaCl injection as the rats in the experimental group.

Experimental design

A total of 24 male rats (n = 6) were randomly assigned to one of four groups. Normal control rats were given saline (NC), diabetic control rats were given saline (DC) and diabetic rats were given 1000mg/kg b.w. of metformin and diabetic rats were given 500mg/kg body weight of *Eucommia ulmoides*. For a total of 56 days, patients were administered treatments via oral gavage once a day. All of the animals were fasted overnight at the conclusion of the experiment and their blood glucose levels were measured. The animals were subsequently given ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia before being sacrificed at the end. Cutting at the stifle joint separated the femur and tibia. Blood samples (10-15mL) were obtained from the rats through cardiac

puncture into a simple red-top tube containing no anticoagulants. The blood samples were centrifuged at 4000g for 15 minutes and the serum was kept in aliquots at 80ºC.

Measurements of bone oxidative stress and antioxidant activities

Mortar and pestle were used to grind the femur bone fragments. A Teflon pestle was used to homogenise bone tissues in a 10% (w/v) homogenising buffer (50mM Tris-HCl, 1.15 percent KCl pH 7.4). To remove nuclei and debris, the homogenates were spun at 9000rpm for 10 minutes in a chilled centrifuge (4ºC). TBARS assay kit for monitoring lipid peroxidation, glutathione peroxidase (GPx) assay kit for GPX activity and superoxide dismutase (SOD) assay kit for SOD activity were used to test the generated supernatant. The protein concentration was calculated using the[21] technique, which used bovine serum albumin as a reference.

Marker of bone formation and bone resorption

Serum was used to measure all bone formation and resorption markers. The osteocalcin level was determined using a Rat-Mid Osteocalcin ELISA kit (IDS, UK), while the BALP level was determined using a rat BALP ELISA kit (Qayee, Shanghai). Bone resorption DPD was measured by using Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) (Qayee, Shanghai). All samples were run in triplicate and the optical density was read at 450nm with a microplate reader (Epoch Microplate Spectrophotometer, Bio Tek, USA)[22].

Statistical analysis

ANOVA was used to assess all of the data. Duncan's multiple comparison test was used to determine the significance. All of the analyses were done with a 95% confidence level.

RESULTS AND DISCUSSION

Fasting blood glucose and serum insulin

In comparison to the NC rats, the DC rats had high fasting blood glucose and low insulin levels (Table No.1). In diabetic rats, treatment with *Eucommia ulmoides* dramatically lowered fasting blood glucose levels while considerably increasing serum insulin levels.

Oxidative stress marker and antioxidant enzymes in bone

The effects of *Eucommia ulmoides* on bone lipid peroxidation and antioxidant enzyme activities are summarised in Table No.2. When compared to the NC rats, the DC rats showed a substantial rise in MDA levels with no significant differences in GPx and SOD activity. In the EU rats, a similar observation is made.

Bone turnover markers

The STZ injection caused a considerable decrease in blood osteocalcin and ALP, but serum DPD was considerably greater than in the NC group (Table No.3). Despite the fact that there were no significant variations in BALP values across the treated groups, serum osteocalcin levels increased while DPD levels dropped after *Eucommia ulmoides* therapy.

Discussion

Because articular cartilage is responsible for lubricating the ends of bones, alterations in it can lead to osteoarthritis. Furthermore, STZ injection has been linked to a decrease in femoral articular cartilage thickness, reduced chondrocyte numbers and higher tidemark roughness. Together, these data point to the development of osteoarthritis-like disease in diabetic rats. T1DM and T2DM rats have both been shown to exhibit osteoarthritis-like symptoms[23-25]. These alterations are considered to be caused in part by the activation of oxidative stress.

We hypothesised that *Eucommia ulmoides* therapy delays the pathogenetic course of osteoarthritis in diabetic rats based on these findings. Oxidative stress has been shown to impact the pathophysiology of osteopenia, osteoporosis and osteoarthritis[16,26,27] in both clinical and preclinical research. As a result, additional research into the relationship between oxidative stress and bone quality is warranted. In this study, we discovered that DC rats had higher levels of oxidative damage indicators. In animal experiments, a substantial rise in MDA level was also detected in all treated

animals, which increased sensitivity to STZ-induced bone problems[28]. Oxidative stress and hyperglycemia have been found to impair bone metabolism and architecture by affecting the function of osteoclasts and osteoblasts[26]. This was significant since the EU rats, which have a large amount of chondrocyte hypertrophy, had some of the highest MDA levels. Furthermore, elevated plasma MDA concentrations have been linked to the early stages of osteoarthritis[27]. Supporting the idea that *Eucommia ulmoides* can slow the course of the disease. Because oxidative stress can disrupt the equilibrium between osteoblast and osteoclast processes, measuring bone turnover markers makes sense[29] In the DC rats, blood DPD levels increased but serum osteocalcin and BALP activity decreased, according to the results of this study. This results is consistent with Zhukouskaya *et al*, (2015), who found that bone turnover suppression is a key feature of T1DM-related bone disease. Previous results of elevated serum DPD in rats with osteoarthritis[30] and osteopenia[31] corroborate our findings. Another interesting finding from this study is that after *Eucommia ulmoides* therapy, serum osteocalcin levels increased while DPD levels fell (Table No.3).

Similar findings have been obtained on a number of plants that exhibit osteoprotective properties[32].

Despite the fact that osteocalcin is a particular osteoblast marker that strongly corresponds with histological alterations[33] blood OC levels tended to vary with meal consumption[33] Osteocalcin does not appear to be as sensitive a marker as BALP, according to previous research[34]. Indeed, BALP activity is still low in EU rats, indicating that mineral metabolism is still being harmed. BALP is a bone-specific isoform of alkaline phosphatase that is produced by osteoblasts for bone remodelling, but it also reflects mineral metabolism[35]. The ratio of osteocalcin to DPD was approximately identical to that of the NC groups, implying that with *Eucommia ulmoides* therapy, an equilibrium between bone production and bone resorption was virtually established.

Table No.1: Effects of *Eucommia ulmoides (EU)* on fasting blood glucose level and serum insulin in STZ induced diabetic rats (data represent mean ± SD)

S.No	Groups	Fasting blood glucose (mmol/L)		% Changes	Serum insulin (µIU/mL)
		Before	After		
1	NC	4.82 ± 0.30a	4.91 ± 0.11a	2.70	4.14 ± 3.13c
2	DC	19.00 ± 3.24b	30.11 ± 2.65b	50.61	1.55 ± 0.13a
3	MET	28.30 ± 3.60c	19.73 ± 3.74c	-32.22	1.76 ± 0.24a
4	EU	26.87 ± 6.02c	17.27 ± 4.87c	-37.03	2.39 ± 0.18b

Different values a, b, c in a column differed significantly at ($p < 0.05$).

Table No.2: Oxidative stress marker and antioxidant enzymes of various experimental groups (data represent mean ± SD)

S.No	Groups	Oxidative stress marker	Antioxidant enzyme	
		TBARS (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
1	NC	29.63 ± 0.50a	43.55 ± 0.78ab	0.52 ± 0.01
2	DC	60.64 ± 0.66b	44.44 ± 0.80bc	0.30 ± 0.04
3	MET	73.50 ± 8.20c	42.04 ± 0.98b	0.32 ± 0.04
4	EU	75.78 ± 0.14c	45.40 ± 0.46bc	0.54 ± 0.18

Different values a, b, c in a column differed significantly at ($p < 0.05$).

Table No.3: 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	136.76 ± 6.9c	100.49 ± 7.59b	167.08 ± 5.13b
2	DC	13.34 ± 0.87a	65.06 ± 4.72a	164.10 ± 0.11c
3	MET	56.40 ± 8.14b	81.38 ± 0.35a	151.16 ± 4.48ab
4	EU	153.64 ± 4.10d	76.30 ± 8.21a	145.53 ± 0.31a

Different values a, b, c in a column differed significantly at ($p < 0.05$).

CONCLUSION

Our findings show that *Eucommia ulmoides* can help prevent bone loss in STZ-treated rats. *Eucommia ulmoides* treatment lowered fasting blood glucose levels, enhanced DPD activity, and enhanced insulin production.

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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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