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SESAMIN PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is now known to be linked to an increased risk of osteoporosis-related fractures and osteoarthritis in recent years. *Sesamin indicum L* has been shown to reduce bone damage in the past. However, it is still unknown if *Sesamin indicum L* may prevent diabetics against osteoporosis. The impact of *Sesamin indicum L* 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 through gavage: saline (control), metformin (1000mg/kg bw), or extract of *Sesamin indicum L* (100mg/kg bw). As a normal control group, a group of healthy rats was used. ELISA assays were used to assess blood levels of insulin, oxidative stress, and bone turnover indicators. *Sesamin indicum L* treatment of diabetic rats resulted in considerably higher insulin and osteocalcin levels than diabetic control rats. By increasing osteogenesis and reducing bone oxidative stress, *Sesamin indicum L* may be able to prevent diabetic osteoporosis. These data support the use of *Sesamin indicum L* as an osteoporosis treatment in diabetic patients.

KEYWORDS

Sesamin indicum L and Diabetic osteoporosis.

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INTRODUCTON

Hyperglycemia is a symptom of diabetes mellitus, a metabolic disease. Diabetes has been linked to a number of lower extremity orthopaedic problems and consequences that negatively impact quality of life[1]. Diabetes promotes osteoclast activity, which increases bone loss, osteopenia, and osteoporosis[2]. As a result, understanding the processes behind

diabetes-related alterations in bone microstructure requires special focus. At remodelling sites, two opposing processes are known as bone production and bone resorption. 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[3,4]. STZ-induced diabetes has been proposed as an useful model for studying the pathophysiological processes of bone loss in diabetes by several research[5]. *Sesamin indicum L.* (Sesamin) has been shown to enhance osteoblastic development of rat bone marrow stromal cells (BMSCs) and improve rat bone structure via modulating the Wnt /-catenin pathway. Sesamin has the potential to treat and prevent osteoporosis[6]. *Sesamin* has demonstrated significant anti-osteoporotic properties in an osteoporosis model, however its preventive impact on diabetic osteoporosis is unknown. The effects of *Sesamin* therapy on bone oxidative stress and turnover indicators encouraged us to investigate its benefits in STZ-treated rats.

MATERIAL AND METHODS

Sesamin preparation

Sesamin (S9314; Sigma-Aldrich LLC, USA) was dissolved in dimethylsulfoxide (DMSO; Sigma) and administrated as a 14.22mm solution and stored at -20°C.

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°C with a 12 hour light/dark cycle) and fed normal rat chow with unlimited access to water. The experiment methods, which included diabetes induction and sacrifice, were authorised by the animal ethics committee at King Khalid University and were carried out in accordance with the US National Institute of Health's standards for the care and use of laboratory animals (NIH Publication No.85-23, revised 1996).

Induction of diabetes

A single intraperitoneal injection of 60 mg/kg STZ dissolved in 10mm citrate buffer was used to chemically produce diabetes-like hyper glycemia in rats (pH 4.5). The rats were given 5 percent glucose water for two days after STZ injection to avoid drug-induced hypoglycaemia. After a week of injection[7], animals with fasting blood glucose levels more than 11mmol/L were classified as diabetic[8]. The rats in the normal control group received the same amount of isotonic NaCl injection.

Experimental design

A total of 24 male rats (n = 6) were split into four groups. Normal control rats were given saline (NC), diabetic control rats were given saline (DC), diabetic rats were given 1000mg/kg body weight of metformin (MET), and diabetic rats were given 100mg/kg body weight of *Sesamin*. Treatments were administered once a day for 56 days by oral gavage. All of the animals fasted overnight at the end of the experiment, and their blood glucose levels were assessed. The animals were subsequently given ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia before being sacrificed. Cutting at the stifle joint separated the femur and tibia. Blood samples (10-15mL) were taken from the rats by cardiac puncture and placed in a simple red-top tube with no anticoagulants. After centrifuging the blood samples at 4000g for 15 minutes, the serum was separated into aliquots and kept at -80°C.

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 9000g for 10 minutes in a chilled centrifuge (4°C). TBARS assay kit for monitoring lipid per oxidation, 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[9] technique, which used bovine serum albumin as a reference.

Marker of bone formation and bone resorption

In serum, all indicators of bone production and resorption were assessed. The osteocalcin level was measured using the Rat-Mid Osteocalcin ELISA kit

(IDS, UK), whereas the BALP level was assessed using the rat BALP ELISA kit (Qayee, Shanghai). DPD was tested using a rat deoxypyridinoline (DPD) ELISA Kit to evaluate bone resorption (Qayee, Shanghai). According to Abdul-Majeed *et al*, all samples were run in triplicate and the optical density was measured at 450nm using a microplate reader (Epoch Microplate Spectrophotometer, Bio Tek, USA) (2012)[10].

Statistical analysis

ANOVA was used to evaluate all of the data. Duncan's multiple comparison test was used to determine 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 *Sesamin* substantially decreased fasting blood glucose levels while considerably increasing serum insulin levels.

Oxidative stress marker and antioxidant enzymes in bone

The effects of *Sesamin indicum L* 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 substantial decrease in blood osteocalcin, whereas serum DPD was considerably greater than in the NC group (Table No.3). The levels of blood osteocalcin rose while DPD reduced after *Sesamin* therapy, despite no significant changes in BALP values between all treated groups.

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, decreased chondrocyte numbers, and higher tidemark roughness[11]. Together, these data point to the development of osteoarthritis-like disease in diabetic rats. T1DM and T2DM rats have both been shown to develop osteoarthritis-like symptoms[12,13]. These alterations are considered to be caused in part by the activation of oxidative stress.

STZ-induced diabetic control rats were shown to have higher levels of oxidative damage indicators in animal investigations. Furthermore, oxidative stress in combination with hyperglycemia has been demonstrated to affect bone metabolism and architecture by changing the activity of osteoclasts and osteoblasts[14]. 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 are linked to the early phases of osteoarthritis[15] bolstering the theory that *Sesamin* might slow osteoarthritis development.

In the DC rats, blood DPD levels increased but serum osteocalcin and BALP activity decreased, according to the results of this study. This observation is consistent with the findings[16] that the major feature of T1DM-related bone disease is a decrease in bone turnover. Previous observations of elevated serum DPD in rats with osteoarthritis[17] and osteopenia[18], corroborate our findings. Another noteworthy conclusion from this study is that after *Sesamin* therapy, serum osteocalcin levels increased while DPD levels dropped (Table No.3). Similar findings have been found on a number of plants that exhibit osteoprotective properties[19]. Despite the fact that osteocalcin is a particular osteoblast marker that has a strong correlation with histological alterations, the level of serum OC tends to vary with meal consumption in people[20,21].

Osteocalcin does not appear to be a sensitive marker as BALP, according to previous research[22]. Indeed, BALP activity is still low in sesamin rats, indicating that mineral metabolism is still being harmed. BALP (Bone-Specific Alkaline Phosphatase) is a bone-specific isoform of alkaline phosphatase that is synthesised by osteoblasts for bone remodelling but more precisely reflects mineral metabolism[23]. The ratio of osteocalcin to DPD was approximately identical to that of the NC groups, implying that *Sesamin* therapy virtually reached an equilibrium between bone production and bone resorption.

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

S.No	Groups	Fasting blood glucose (mmol/L)		% Changes	Serum insulin (µIU/mL)
		Before	After		
1	NC	4.60 ± 0.20a	4.83 ± 0.10a	2.51	4.06 ± 3.03c
2	DC	20.00 ± 3.24b	30.13 ± 2.63b	50.65	1.58 ± 0.04a
3	MET	29.10 ± 3.50c	20.83 ± 3.75c	−33.32	1.68 ± 0.14a
4	Sesamin	26.87 ± 6.13c	17.27 ± 4.97c	−34.03	2.21 ± 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 enzymes	
		TBARS (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
1	NC	27.73 ± 0.40a	43.65 ± 0.78ab	0.51 ± 0.01
2	DC	62.74 ± 0.66b	45.40 ± 0.80bc	0.24 ± 0.04
3	MET	73.51 ± 8.20c	43.06 ± 0.98b	0.39 ± 0.04
4	Sesamin	75.69 ± 0.14c	44.41 ± 0.46bc	0.48 ± 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	135.78 ± 6.92c	102.49 ± 7.59b	155.08 ± 5.33b
2	DC	15.35 ± 0.97a	65.06 ± 4.70a	164.10 ± 0.21c
3	MET	56.42 ± 8.24b	80.38 ± 0.45a	151.16 ± 4.08ab
4	Sesamin	152.66 ± 4.11d	76.30 ± 8.31a	143.53 ± 0.41a

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

CONCLUSION

Sesamin has the ability to prevent bone loss in STZ-treated rats, according to our findings. *Sesamin* treatment decreased fasting blood glucose levels, enhanced DPD activity and boosted insulin secretion.

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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".

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