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SALVIAE MILTIORRHIZAE RADIX PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

The goal of this study was to see if *Salviae Miltiorrhizae Radix (SMR)* affected bone deformities in diabetic rats. In a rat model of osteoporosis, *Salviae Miltiorrhizae Radix* has been found to be beneficial. However, it is uncertain if *Salviae Miltiorrhizae Radix* can prevent osteoporosis in diabetics. The effects of *Salviae Miltiorrhizae Radix* on bone oxidative stress and turnover markers in diabetic rats were examined in this study. Streptozotocin was used to cause diabetes (STZ). Diabetic Sprague Dawley rats (n =6) were administered either saline (control), metformin (1000mg/kg bw), or *Salviae Miltiorrhizae Radix* (5g/kg bw) by gavage for 8 weeks. A healthy rat group was employed as a standard control group. Insulin, oxidative stress and bone turnover markers were measured in the blood using ELISA assays. Insulin and osteocalcin levels were significantly higher in diabetic rats administered *Salviae Miltiorrhizae Radix* than in diabetic control rats. *Salviae Miltiorrhizae Radix* may be able to prevent diabetic osteoporosis by boosting osteogenesis and lowering bone oxidative stress. These findings support the use of *Salviae Miltiorrhizae Radix* in diabetic individuals as an osteoporosis therapy.

KEYWORDS

Salviae miltiorrhizae radix and Diabetic osteoporosis.

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INTRODUCTION

Osteoporosis is a progressive systemic and chronic skeletal disease characterised by low bone mass and rapid degradation of bone micro architecture, resulting in fracture risk[1]. With a rapid increase in the world's elderly population, the prevalence of osteoporosis and its accompanying fractures is on the

rise, putting a significant financial strain on families and society[2,3]. As a result, productive efforts are desperately needed to fulfil the enormous demand for osteoporosis prevention and treatment. With a huge increase in the world's elderly population, the prevalence of osteoporosis and its accompanying fractures is on the rise. The number of individuals with Diabetic osteoporosis (DOP) has risen in the globe[4]. Clinical investigations have revealed that roughly half to two-thirds of diabetic individuals had decreased bone strength and/or an increased risk of fractures, with almost one-third of them diagnosed with osteoporosis[5]. It mostly affects postmenopausal women and the elderly, and it has already established itself as one of the most significant health risks[6,7].

A previous study on *Salviae Miltiorrhizae Radix* (*SMR*) revealed that it has the ability to strengthen bone[8]. *Salviae Miltiorrhizae Radix* has showed promise as an alternative medication for the prevention and treatment of osteoporosis in preclinical studies[9]. Although *Salviae Miltiorrhizae Radix* has demonstrated significant anti-osteoporotic effects in a model of osteoporosis, it is uncertain if it can prevent diabetic osteoporosis. The effects of *Salviae Miltiorrhizae Radix* on bone oxidative stress and turnover markers in STZ-treated rats prompted us to do further research in diabetic rats.

MATERIAL AND METHODS

Extract Preparation

For extraction, two kilogram's of *Salviae Miltiorrhizae Radix* were ground into powder once and then split into five portions. Each component was extracted using 10 litres of distilled water and constant stirring at a low temperature for 48 hours. The supernatants were then collected by centrifugation (3500 rpm at 4^{o}C for 10 minutes), then concentrated and lyophilized to yield a powder under vacuum. All of the powder (400g; 1g contains 5g raw RSM) was combined and kept in the refrigerator until needed.

Animals

The experiment was conducted 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 (21±^{o}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 methods, which included diabetes induction and sacrifice and they were carried out in compliance 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 60mg/kg STZ dissolved in 10mM citrate buffer was used to chemically produce diabetes. The rats were given 5% glucose water for two days after receiving STZ to avoid drug-induced hypoglycemia. After a week of injection[10], animals with fasting blood glucose levels more than 11mmol/L were classified as diabetic[11]. The rats in the control group got the same amount of isotonic NaCl injection as the experimental animals.

Experimental design

A total of 24 male rats (n = 6) were divided into four groups at random. Diabetic Sprague Dawley rats (n = 6) were administered either saline (control), metformin (1000mg/kg bwt), or *Salviae Miltiorrhizae Radix* (5g/kg bwt) by gavage for 8 weeks. At the end of the trial, all of the animals were fasted overnight and their blood glucose levels were tested. After that, the animals were administered ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia. The femur and tibia were separated by cutting near the stifle joint. The rats' blood (10-15mL) was collected by heart puncture into a simple red top tube containing no anticoagulants. The serum was stored in aliquots at 80^{o}C after centrifuging the blood samples at 4000rpm for 15 minutes.

Measurements of bone oxidative stress and antioxidant activities

The femur bone fragments were ground with a mortar and pestle. In a 10% (w/v) homogenising buffer, bone tissues were homogenised using a Teflon pestle (50mM Tris-HCl, 1.15 percent KCl pH 7.4). The homogenates were spun at 9000 rpm for 10 minutes in a cooled centrifuge (4^{o}C) to remove nuclei and debris. The produced supernatant was tested using a TBARS assay kit for monitoring lipid

peroxidation, a glutathione peroxidase (GPx) assay kit for GPX activity and a superoxide dismutase (SOD) assay kit for SOD activity. The protein content was determined by the method[12], which utilised bovine serum albumin as a standard.

Marker of bone formation and bone resorption

All bone formation and resorption indicators were measured using serum. A Rat-Mid Osteocalcin ELISA kit (IDS, UK) was used to assess the osteocalcin level, whereas a rat BALP ELISA kit was used to determine the BALP level (Qayee, Shanghai). DPD was evaluated using a Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) to determine bone resorption (Qayee, Shanghai). The optical density was measured at 450 nm with a microplate reader (Epoch Microplate Spectrophotometer, Bio Tek and US[13].

Statistical analysis

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

RESULTS AND DISCUSSION

Fasting blood glucose and serum insulin

The DC rats exhibited higher fasting blood glucose and lower insulin levels than the NC animals (Table No.1). *Salviae Miltiorrhizae Radix* therapy significantly reduced fasting blood glucose levels while significantly raising serum insulin levels in diabetic rats.

Oxidative stress marker and antioxidant enzymes in bone

Table No.2 summarises the effects of *Salviae Miltiorrhizae Radix* on lipid peroxidation and antioxidant enzyme activity. The DC rats had a considerable increase in MDA levels as compared to the NC rats, but no significant changes in GPx or SOD activity. A similar observation is reported with *Salviae Miltiorrhizae Radix* rats.

Bone turnover markers

Although blood osteocalcin was significantly lower after the STZ injection, serum DPD levels were significantly higher than in the NC group (Table No.3). Despite the fact that BALP values did not differ significantly across the groups, serum osteocalcin levels increased while DPD levels decreased following *Salviae Miltiorrhizae Radix* treatment.

Discussion

Salviae Miltiorrhizae Radix has the potential to be a novel source of antiosteoporotic agent by promoting bone formation and preventing bone resorption *in vitro* and *in vivo*, according to emerging data. *Salviae Miltiorrhizae Radix* aqueous extract enhanced bone strength in diabetic rats, according to our present data. However, the active ingredients in *Salviae Miltiorrhizae Radix* that have a bone protective action are mainly unknown. Measurement of bone turnover indicators makes sense since oxidative stress might alter the balance between osteoblast and osteoclast activities[14]. According to the findings of this study, blood DPD levels rose in DC rats, whereas serum osteocalcin and BALP activity decreased.

Another noteworthy finding from this study is that serum osteocalcin levels rose following *Salviae Miltiorrhizae Radix* treatment while DPD levels decreased (Table No.3) a variety of herbs that have osteoprotective characteristics have shown similar results[15]. Indeed, BALP activity in *Salviae Miltiorrhizae Radix* rats is still low, indicating that mineral metabolism is still affected. BALP is a bone specific alkaline phosphatase isoform that is generated by osteoblasts for bone remodelling but also reflects mineral metabolism[16]. The ratio of osteocalcin to DPD was nearly similar in the *Salviae Miltiorrhizae Radix* and NC groups, suggesting that an equilibrium between bone formation and bone resorption was almost achieved with *Salviae Miltiorrhizae Radix* treatment.

Table No.1: Effects of *Salviae Miltiorrhizae Radix* 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	4.82 ± 0.30a	4.91 ± 0.11a	2.70	14 ± 3.13c
2	DC	19.00 ± 3.24b	30.11 ± 2.65b	50.61	1.55 ± 0.13a
3	MET	29.30 ± 4.60c	21.73 ± 4.74c	-34.22	1.96 ± 0.44a
4	*Salviae Miltiorrhizae Radix*	28.87 ± 6.12c	19.27 ± 5.87c	-38.03	2.59 ± 0.28b

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

Table No.2: Oxidative stress marker and antioxidant enzymes of various experimental groups (data represent mean ± 1SD)

S.No	Groups	Oxidative stress marker	Antioxidant enzymes	
		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	76.50 ± 9.20c	45.04 ± 0.88b	0.46 ± 0.06
4	*Salviae Miltiorrhizae Radix*	77.78 ± 0.24c	46.40 ± 0.56bc	0.64 ± 0.28

Different superscripts ^{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 ± 1SD)

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	54.40 ± 8.24b	83.38 ± 0.45a	154.16 ± 4.38ab
4	*Salviae Miltiorrhizae Radix*	154.64 ± 4.20d	77.30 ± 9.21a	146.53 ± 0.41a

CONCLUSION

It can be concluded that *Salviae Miltiorrhizae Radix* can help prevent bone loss in STZ-treated rats. *Salviae Miltiorrhizae Radix* treatment enhanced DPD activity, lowered fasting blood glucose levels 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 design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings".

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