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PSORALEAE FRUCTUS PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

In recent years, diabetes mellitus has been linked to an increased risk of osteoporosis related fractures and osteoarthritis. In the past, *Psoraleae Fructus (PF)* has been found to decrease bone damage. However, it is uncertain if *Psoraleae Fructus* can protect diabetics from developing osteoporosis. This study looks at the effects of *Psoraleae Fructus* on bone oxidative stress and turnover markers in diabetic rats. Streptozotocin induced diabetes (STZ) Diabetic Sprague Dawley rats (n = 6) were administered one of three treatments a through gavage: Saline (control), metformin (1000mg/kg bw), or *Psoraleae Fructus* (1000mg/kg bw) over an 8-week period. 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. Diabetic rats given *Psoraleae Fructus* therapy had significantly greater insulin and osteocalcin levels than diabetic control rats. *Psoraleae Fructus* may be able to prevent diabetic osteoporosis by boosting osteogenesis and lowering bone oxidative stress. These findings support the use of *Psoraleae Fructus* in diabetic individuals as an osteoporosis therapy.

KEYWORDS

Psoraleae fructus and Diabetic osteoporosis.

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INTRODUCTION

Diabetes now affects about 415 million people worldwide, with the number expected to rise to 640 million by 2040. Reduced bone mass and altered bone micro architecture define osteoporosis, leading to lower bone strength and an increased risk of fractures. Fragility fractures are frequent in people with diabetes, both type 1 and type 2. Hip fractures

are six times more common in people with type 1 diabetes mellitus (T1DM) than in the general population. Hip fractures are 2.5 times more common in those with type 2 diabetes (T2DM). When compared to vertebral fractures, hip fractures are more frequent among diabetics. For a given bone mass density (BMD), patients with T2DM had a higher risk of fractures than non-diabetic patients. Fragility fractures are more likely in diabetic individuals due to micro architectural defects in the bone. These anomalies are difficult to detect and are frequently unrelated to BMD. As a result, bone fragility is an underappreciated issue in diabetes individuals. Patients with diabetes have poor bone turnover indicators and real fracture rates in diabetics are greater than those indicated by fracture risk assessment tools[1].

A disruption in the delicate balance between these two processes causes osteoarthritis and osteoporosis. Several studies have suggested that[2,3]. STZ-induced diabetes is a good model for understanding the pathophysiological mechanisms of bone loss in diabetes[4]. *Psoraleae Fructus* is the dried fruits of Psoralea corylifolia Linn, which are commonly employed in the treatment of postmenopausal osteoporosis. Although *Psoraleae Fructus* has shown substantial anti-osteoporotic effects in an osteoporosis model[5], its influence on diabetic osteoporosis prevention is unclear. We decided to look into the effects of *Psoraleae Fructus* treatment on bone oxidative stress and turnover markers in STZ-treated rats.

MATERIAL AND METHODS

Psoraleae Fructus preparation

The dried matured fruits of *P. corylifolia L.* were added with 70% ethanol for reflux extraction twice, with 1.5 h each time. Afterwards, the extracted solutions were combined, filtered and then concentrated by a rotary evaporator under reduced pressure.

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 environment (22±1^{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 procedures, which included diabetes induction and sacrifice and they were carried out in line with the US 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 hyper glycemia in rats, a single intraperitoneal 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 following STZ injection. Seven rats with fasting blood glucose levels of greater than 11mmol/L were categorised as diabetic after a week of injection[6]. The normal control rats got the same quantity of isotonic NaCl injection as the rats in the experimental group.

Experimental design

Twenty four male rats (n = 6) were divided into four groups. Saline was administered to normal control rats (NC), the other one as diabetic control rats (DC) and the other two diabetic rats were given 1000mg/kg body weight of metformin (MET) and 100mg/kg body weight of *PR* extracts. Oral gavage treatments were given once a day for 56 days. At the conclusion of the experiment, all of the animals fasted over-night and their blood glucose levels were measured. Before being sacrificied, the animals were administered ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia. The femur and tibia were separated by cutting near the stifle joint. By heart puncture, blood samples (10-15mL) were obtained from the rats and put in a simple red-top tube with no anticoagulants. The serum was split into aliquots and stored 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 9000rpm for 10 minutes in a cooled centrifuge (4ºC) 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 using the method, which utilised bovine serum albumin as a standard.

Marker of bone formation and bone resorption

All markers of bone formation and resorption were measured in serum. The BALP level was determined using the rat BALP ELISA kit, whereas the osteocalcin level was determined using the Rat Osteocalcin ELISA kit. To assess bone resorption, DPD was measured using a Rat deoxypyridinoline (DPD) ELISA Kit. All samples were run in triplicate and the optical density was determined at 450nm using a microplate reader, according to Abdul-Majeed *et al*[7].

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). Treatment with *Psoraleae Fructus* 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 *Psoraleae Fructus* on bone lipid per oxidation 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 found with the *Psoraleae Fructus* rats.

Bone turnover markers

The STZ injection significantly reduced blood osteocalcin, but serum DPD was significantly higher than in the NC group (Table No.3). Despite no significant differences in BALP values across the treatment groups, blood osteocalcin levels increased while DPD decreased following *Psoraleae Fructus* therapy.

Discussion

Osteoarthritis is caused by changes in articular cartilage, which is responsible for lubricating the ends of bones. STZ injection has also been related to a drop in femoral articular cartilage thickness, a reduction in chondrocyte numbers and an increase in tidemark roughness[8]. Together; these findings suggest that diabetic rats acquire osteoarthritis-like illness. Osteoarthritis-like symptoms have been observed in both T1DM and T2DM animals[9,10]. The activation of oxidative stress is thought to be a contributing factor in these changes.

In animal studies, STZ-induced diabetic control rats were shown to have greater levels of oxidative damage markers. Furthermore, oxidative stress in conjunction with hyper glycemia has been shown to change the activity of osteoclasts and osteoblasts, affecting bone metabolism and morphology[11]. This was important since the *Psoraleae Fructus* rats had some of the highest MDA levels, while having a lot of chondrocyte hypertrophy. Furthermore, higher plasma MDA concentrations have been related to the early stages of osteoarthritis[12], supporting the idea that *Psoraleae Fructus* might help delay the progression of osteoarthritis.

According to the findings of this study, blood DPD levels rose in DC rats, whereas serum osteocalcin and BALP activity decreased. This discovery is in

line with the findings[13] of who discovered that a reduction in bone turnover is a key characteristic of T1DM-related bone degeneration. Previous research has shown that *Psoraleae Fructus* can reduce PINP (Procollagen type I N-terminal propeptide) and CTX (C-terminal telopeptide of type I collegen) levels to improve bone turnover rate and increase OPG/RANKL (nuclear factor-B ligand) mRNA levels to promote osteogenic differentiation, implying that FFP could be a promising anti-osteoporosis drug[14].

Our findings are supported by previous reports of increased serum DPD in rats with osteoarthritis[15] and osteopenia[16]. Another interesting finding from this study is that blood osteocalcin levels rose following *Psoraleae Fructus* therapy while DPD levels decreased (Table No.3). A variety of plants with osteoprotective characteristics have been discovered to have similar findings[17-19]. According to prior studies, osteocalcin does not appear to be as sensitive a marker as BALP[20]. Indeed, BALP activity in EU rats is still low, indicating that mineral metabolism is still affected. BALP (Bone-Specific Alkaline Phosphatase) is a bone-specific alkaline phosphatase isoform that is produced by osteoblasts for bone remodelling but more accurately represents mineral metabolism[21]. The ratio of osteocalcin to DPD in the *Psoraleae Fructus* groups was nearly equal to that in the NC groups, suggesting that *Psoraleae Fructus* treatment effectively balanced bone formation and resorption.

Table No.1: Effects of *Psoraleae Fructus* 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	4.70 ± 0.30a	4.83 ± 0.21a	2.61	3.16 ± 3.03c
2	DC	21.00 ± 3.24b	30.03 ± 2.63b	50.55	1.48 ± 0.16a
3	MET	28.30 ± 3.70c	19.73 ± 3.75c	−31.32	1.68 ± 0.34a
4	*Psoraleae Fructus*	26.87 ± 6.03c	16.17 ± 4.97c	−37.03	2.31 ± 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 ± 1SD)

S.No	Groups	Oxidative stress marker	Antioxidant enzymes	
		TBARS (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
1	NC	31.73 ± 0.50a	41.65 ± 0.78ab	0.50 ± 0.01
2	DC	59.74 ± 0.66b	43.40 ± 0.80bc	0.32 ± 0.04
3	MET	72.51 ± 8.20c	41.06 ± 0.98b	0.33 ± 0.04
4	*Psoraleae Fructus*	74.79 ± 0.14c	44.41 ± 0.46bc	0.56 ± 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 ± 1SD)

S.No	Groups	Bone formation markers		Bone resorption marker
		Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
1	NC	135.78 ± 6.82c	100.49 ± 7.49b	156.08 ± 5.23b
2	DC	12.35 ± 0.87a	66.06 ± 4.60a	165.10 ± 0.21c
3	MET	56.42 ± 8.14b	80.38 ± 0.45a	150.16 ± 4.18ab
4	*Psoraleae Fructus*	153.66 ± 4.01d	76.30 ± 8.21a	144.53 ± 0.31a

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

CONCLUSION

PF has the ability to prevent bone loss in STZ-treated rats. After *PF* treatment, fasting blood glucose levels were lower, DPD activity was higher and insulin secretion was higher.

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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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