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ACHYRANTHES BIDENTATA PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIAETIC RATS

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ABSTRACT

The current pharmacological diabetic medications have a number of negative side effects. Herbal medication is in high demand for this chronic illness since it has fewer side effects and is more effective. As a result, the current study looked at the effects of *Achyranthes bidentata (AB)* root extract on bone properties in diabetic rats. In a rat model of osteoporosis, *Achyranthes bidentata* was shown to be beneficial. However, it is uncertain if *Achyranthes bidentata* can prevent osteoporosis in diabetics. The effects of *Achyranthes bidentata* 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 bwt), or *Achyranthes bidentata* (100mg/kg bwt) 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 greater in diabetic rats administered AB than in diabetic control rats. *Achyranthes bidentata* may be able to prevent diabetic osteoporosis by boosting osteogenesis and lowering bone oxidative stress. The use of *Achyranthes bidentata* as an osteoporosis therapy in diabetic individuals is supported by these findings.

KEYWORDS

Achyranthes bidentata and Diabetic osteoporosis.

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INTRODUCTION

In today's culture, diabetes is the most common chronic disease and leading cause of mortality[1]. It's a complex metabolic disease defined by excessive blood glucose levels caused by the body's cells' failure to effectively use glucose[2]. Although insulin therapy and other chemical treatments can manage

certain features of diabetes, many complications remain a regular occurrence. Because high glucose levels directly harm cells and promote lipid peroxidation, hyperglycemia is the primary cause of severe diabetic complications[3].

Tissue antioxidant level has been found to play a function in the genesis of diabetes in studies[4-7] and oxidative stress may be a common route connecting many processes that cause diabetic problems[6]. Multiple therapeutic treatments, as well as therapeutic synergy, may be focused on oxidative stress. Previous research on (*Achyranthes bidentata*) has revealed that it can boost the immune system, prevent tumour metastasis, raise leucocytes, protect liver cells, and activate human thoracic cavity macrophages, as well as have anti-diabetic properties[7,8]. The number of individuals with Diabetic osteoporosis (DOP) has risen with the global prevalence of DM[9]. 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[10].

It mostly affects postmenopausal women and the elderly, and it has already established itself as one of the most significant health risks[11,12]. Preclinical study suggests that *Achyranthes bidentata* might be useful as an alternative medication for osteoporosis prevention and treatment[13]. Although *Achyranthes bidentata* has demonstrated significant anti-osteoporotic effects in a model of osteoporosis, it is uncertain if it can prevent diabetic osteoporosis. This study is aimed to examine the effects of *Achyranthes bidentata* therapy on bone oxidative stress and turnover markers in STZ-treated rats.

MATERIAL AND METHODS

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 (22±°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 in rats (pH 4.5). The rats were given 5% glucose water for two days after receiving STZ to avoid drug-induced hypoglycemia. After a week of injection[14], animals with fasting blood glucose levels more than 11mmol/L were classified as diabetic[15]. 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. Saline was administered to normal control rats (NC), diabetic control rats (DC), and the other two diabetic rat groups were given 1000mg/kg bw of diabetes medication or *Achyranthes bidentata* (100mg/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 (8mg/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°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 (4±°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[16], 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 450nm with a microplate reader (Epoch Microplate Spectrophotometer, BioTek, USA)[17].

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 *Achyranthes bidentata* significantly reduced fasting blood glucose levels while significantly raising serum insulin levels in diaetic rats.

Oxidative stress marker and antioxidant enzymes in bone

Table No.2 summarises the effects of *Achyranthes bidentata* on bone 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 found with the *Achyranthes bidentata* 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 *Achyranthes bidentata* treatment.

Discussion

The effects of *Achyranthes bidentata* on bone deformities in STZ-induced diabetic rats were examined in this study. Due to persistent and chronic hyperglycemia, which depletes the function of the antioxidative defence system, diabetics and experimental animal models experience high levels of oxidative stress, resulting in increased amounts of oxygen free radicals[18]. Previous research has shown that *Achyranthes bidentata* has antiosteoporosis properties in rats[14]. These findings imply that the herbs might be useful in the prevention and treatment of osteoporosis.

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. Together, these findings suggest that diabetic rats acquire osteoarthritis-like illness. Osteoarthritis-like symptoms have been observed in both T1DM and T2DM animals[15,19,20]. The activation of oxidative stress is thought to be a contributing factor in these changes.

Measurement of bone turnover indicators makes sense, since oxidative stress might alter the balance between osteoblast and osteoclast activities[21]. 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 *Achyranthes bidentata* treatment while DPD levels decreased (Table No.3). A variety of herbs that have osteoprotective characteristics have shown similar results[22].

Indeed, BALP activity in EU 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. The ratio of osteocalcin to DPD was nearly similar in the *Achyranthes bidentata* and NC groups, suggesting that an equilibrium between bone

formation and bone resorption was almost achieved with *Achyranthes bidentata* treatment.

Table No.1: Effects of *Achyranthes bidentata* on fasting blood glucose level and serum insulin in STZ induced diaetic 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	4.14 ± 3.13c
2	DC	19.00 ± 3.24b	30.11 ± 2.65b	50.61	1.55 ± 0.13a
3	MET	31.30 ± 3.60c	21.73 ± 3.74c	−34.25	1.66 ± 0.34a
4	*Achyranthes bidentata*	29.87 ± 6.02c	19.27 ± 4.87c	−36.03	2.59 ± 0.28b

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	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	76.60 ± 9.20c	44.04 ± 0.88b	0.42 ± 0.14
4	*Achyranthes bidentata*	74.79 ± 0.24c	43.40 ± 0.47bc	0.64 ± 0.19

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	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	58.30 ± 8.24b	84.38 ± 0.45a	152.26 ± 4.58AB
4	*Achyranthes bidentata*	156.69 ± 4.20d	75.30 ± 8.31a	143.63 ± 0.41a

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

CONCLUSION

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

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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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