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BONE FORMATION ABILITY OF *RADIX DIPSACI* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Radix Dipsaci was kidney tonifying herbal medication that has a long history of being used safely to heal bone fractures and joint disorders. *Radix Dipsaci* extract (RDE) has been shown to protect ovariectomized rats against bone loss. The impact of *Radix Dipsaci* extract on bone loss caused by diabetes is investigated in this study. The impact of *Radix Dipsaci* extract on blood glucose, HBA1C levels and bone mineral density in rats was studied using a rat model. Twenty-four male Sprague Dawley rats (n = 6) were split into four groups: Saline was administered to normal control rats (NC), diabetic control rats (DC), diabetic rats were given 1000mg/kg body weight of metformin (MET) and 500mg/kg body weight of *Radix Dipsaci* extract respectively to two groups. *Radix Dipsaci* extract treatment improved blood glucose levels, HBA1C levels and bone mineral density. *Radix Dipsaci* extract has been shown to reduce bone loss caused by diabetes, implying that it might be used to treat diabetes.

KEYWORDS

Radix Dipsaci extract, Bone and Diabetic rats.

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INTRODUCTION

Because people with Type 2 diabetes tend to have higher bone density as a result of their increased body weight, they may not be diagnosed with osteoporosis or poor bone density, which would prompt them to take precautions to prevent fractures[1]. Diabetes causes bone loss, osteopenia and osteoporosis by promoting osteoclast activity[2]. As a result, a specific attention is needed to

understand the mechanisms behind diabetes related changes in bone microstructure. Several studies have identified STZ-induced diabetes as a viable model for investigating the pathophysiological mechanisms of bone loss in diabetes[3]. Natural products have been proved to be effective and dependable options for bone loss prevention[4-6]. The dried root of Dipsacusasperoides *et al*, *Radix Dipsaci*, has long been utilized as an anti-osteoporosis tonic and anti-aging medicine. Lower back discomfort, acute hematoma and bone fractures have all been treated with it[7]. *Radix Dipsaci* extract (RDE) was found to enhance bone density and change bone histomorphology in mice and rats in recent research[8]. However, there is no direct evidence that RDE has an inhibitory impact on diabetes induced bone loss. The goal of this study was to see if RDE had an osteoprotective impact on bone mineral density (BMD) in STZ treated rats.

MATERIAL AND METHODS

Animals

Healthy male wistar albino rats weighing 180 to 240g and aged 3 to 4 months were utilised in the investigation. The animals were taken from the Central Animal House of King Khalid University in Abha, Saudi Arabia. The animals were housed in cages throughout the experiment and fed a standard pellet meal and filtered water ad libitum under standard conditions (light/dark cycle of 12 h/12 h, 50-70 percent humidity, 25ºC 3ºC). The animals were acclimatised to the laboratory environment for 14 days. The therapy was carried out in accordance with the permission of King Khalid University's animal ethics committee and the National Institute of Health's standards for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996).

Induction of diabetes

To induce diabetes in the animals, the pancreatic cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was administered intraperitoneally at a dosage of 65mg/kg body weight[9,10]. In the control group, all of the rats were given the same quantity of vehicle. STZ was weighed individually for each animal, solubilized with 0.1ml of freshly prepared cold Nacitrate buffer (NaB-0.1 M, pH 4.5) and given within 5 minutes to prevent deterioration.

The volume of STZ injection was determined to be 1.0ml/kg. Rats were administered a 5% glucose solution for hours after receiving STZ to counteract the drug's substantial acute hypoglycemia impact. Three days following STZ injection, blood was taken from the tail vein and analysed for blood glucose using a glucometer (Aqua Check, Roche). Animals having fasting blood glucose levels (FGLs) more than 250mg/dL were classified as diabetic. The rats were divided into four groups, each with six rats.

Saline was administered to normal control rats (NC), diabetic control rats (DC), diabetic rats were given 1000mg/kg body weight of metformin (MET) and 500mg/kg body weight of RDE respectively to two groups. To evaluate the animals' hyperglycemic state, blood glucose levels were tested once a week for the duration of the experiment using a Roche Accu Chek advantage glucometer. The study did not include the animals which did not acquire blood glucose levels more than 250mg/dL. The rats administered saline instead of streptozotocin in the control group (n=6) had normal blood glucose levels (120mg/dl).

Determination of fasting blood glucose

Blood samples were collected from the rats' tail veins to measure blood glucose levels using a glucometer after they had been fasted for 12-14 hours. After the rats' tails have been cleaned with 70% (v/v) ethanol, blood will be drawn using a 1-ml needle, placed on a glucose strip, and measured with a glucometer.

Determination of intra-peritoneal glucose tolerance test

All of the rats were fasted for 12-14 hours before blood was collected from the tail vein as a baseline. The rats were subsequently given 2g/kg body weight (BW) of a 40% (w/v) glucose solution intraperitoneally. Blood will be taken from the tail vein and analysed for blood glucose using a

glucometer after 30, 60, 90 and 120 minutes after glucose treatment. Fasting blood sugar values of less than 250mg/dl were used to diagnose diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are collected and deposited on a test cartridge, haemoglobin A1c (HbA1c) will be analysed with a Clover A1cTM Self Analyzer. On the Clover A1cTM Self Analyzer's LCD screen, the percentage of HbA1c in the blood sample is shown.

Bone Mineral Density Measurement

After blood was taken, the BMD of the left femur and lumbar vertebrae (L1-L4) of rats was measured using dual energy X-ray absorptiometry (DEXA) scanning equipment (Lunar, WI, USA).

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, *Radix Dipsaci* were demonstrated to protect against diabetes progression.

HBA1C levels were higher in the positive control group than in the normal control group ($p < 0.05$), as indicated in Table No.2. In contrast to the positive control group, *Radix Dipsaci* was shown to lower HBA1C levels, implying a favourable effect.

The findings of bone mineral density study revealed that diabetic rats had lower lumbar (L1-L4) and femoral bone mineral density (BMD), which was recovered by *Radix Dipsaci* treatment ($p < 0.05$). The BMD of the positive group and the other treatment groups differed significantly (Table No.3). These findings imply that *Radix Dipsaci* may able to protect bones from the effects of hyperglycemia.

Statistical analysis

The data should be expressed as a mean and standard deviation (SD). To statistically analyse data from different groups, one way analysis of variance (ANOVA) and Tukey's multiple comparison test will be employed. A “p” value of less than 0.05 is considered statistically significant.

Discussion

The effects of *Radix Dipsaci* extract on bone quality in a STZ-induced type 2 diabetes animal model are investigated in this work. *Radix Dipsaci* extract influence on bone loss and formation/resorption, bone structure and composition and bone quality as measured by bone mechanical characteristics has been studied in the past[4-8]. *Radix Dipsaci* extract treatment had a positive effect on bone, as indicated by increases in BMD. RDE's bone protective benefits may be owing to its direct influence on bone production and suppression of osteoclast development, according to reports[4].

Reduced levels of bone turnover markers and changes in urine calcium and phosphorus excretion were the primary indicators of RDE's bone loss prevention efficacy. *Radix Dipsaci* extract therapy has also been shown to improve bone biomechanical strength and prevent the degradation of trabecular bone microarchitecture in previous studies. *Radix Dipsaci* extract also suppressed osteoclastogenesis *in vitro* by increasing osteoprotegrin and decreasing NF-kB ligand expression[11]. *Radix Dipsaci* extract are often utilised in individuals with fractures and osteoporosis[12]. Furthermore, their osteoprotective properties have been demonstrated in hip fracture patients and *Radix Dipsaci* extract has been shown to play a protective effect in reducing RANKL-induced osteoclastogenesis *in vitro* by decreasing bone resorption-related gene expression[11].

Table No.1: Effect of *Radix Dipsaci* on Fasting blood glucose level

S.No	Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
1	Normal Control	5mL/kg	75.22 ±3.2	74.32 ±2.3	76.81 ±3.5	78.40 ±1.7	79.30 ±1.5	80.46 ±1.9	82.40 ±1.05	83.40 ±1.02	84.40 ±1.12
2	Positive Control	65mg/kg	261.54 ±10.2*	296.35 ±9.8*	314.21 ±12.62*	336.72 ±9.6*	351.72 ±8.4*	375.72 ±11.5*	398.72 ±10.5*	412.72 ±10.2*	435.72 ±9.6*
3	Radix *Dipsaci*	500mg/kg	266.33 ±7.3	286.25 ±9.4*	291.22 ±7.8*	296.28 ±8.2*	304.35 ±8.8*	307.35 ±9.8*	310.35 ±10.2*	320.35 ±9.2*	330.35 ±9.7*
4	Metformin	1000mg/kg	265.33 ±8.3	245.25 ±9.3*	238.22 ±7.8*	216.28 ±9.2*	170.25 ±8.3*	150.45 ±7.8*	130.45 ±10.4*	102.15 ±8.2*	90.235 ±8.7*

Values are expressed as mean ± standard error of the mean (n=6).
*p<0.001 compared with normal control.

Table No.2: Effect *of Radix Dipsaci* on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.42±0.14
2	Positive Control	5.80±0.06*
3	*Radix Dipsaci*	5.68±0.03*
4	Metformin	5.39±0.04*

Values are expressed as mean ± standard error of the mean (n=6).
*P<0.001 compared with normal control.

Table No.3: Effect of *Radix Dipsaci* on thebone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density(mg/cm3)	
		Lumbar Vertebrae	Femur
1	Normal Control	178 ± 2.5	220 ± 2.5
2	Positive Control	78 ± 2.6*	100 ± 2.3*
3	*Radix Dipsaci*	158 ± 1.5*	200 ± 1.7*
4	Metformin	130 ± 2.1*	185± 2.4*

Values are expressed as mean ± standard error of the mean (n=6).
*P<0.001 compared with normal control.

CONCLUSION

In a diabetes-induced rat model, *Radix Dipsaci* extract enhanced bone mass. These results suggest the therapeutic effect of *Radix Dipsaci* extract as an alternative supplement to be applied in the prevention and treatment of bone loss induced by diabetes.

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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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