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Research Article

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Radix Dipsaci extract protects against Rosiglitazone induced bone loss

Khalid A Asseri[1], Yahya I Asiri[1], Ali Alqahtani[1], Krishnaraju Venkatesan[1]*, Noohu Abdulla Khan[2], Vigneshwaran Easwaran[2], Ester Mary Pappiya[3], Premalatha Paulsamy[4], R.Natarajan[5], Kalpana Krishnaraju[5], Kumar Venkatesan[6], Kumarappan Chidambaram[1]

[1] Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia.

[2] Department of Clinical Pharmacy, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia.

[3] Directorate of General Health Affair, Ministry of Health, Najran, KSA

[4] King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia

[5] Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, India

[6] Department of Chemistry, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia.

Corresponding Author:* * V Krishnaraju, Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, Asir Province, Saudi Arabia. Email: kvenkatesan@kku.edu.sa. ORCID ID:0000-0003-2853-5907.

Abstract

The dried root of Dipsacus asperoides is known as *Radix Dipsaci extract(RDE)*. It's a kidney-toning herbal medication with a lengthy track record of safe usage in the treatment of bone fractures and joint disorders. The drug rosiglitazone (RSG) causes an imbalance in bone remodelling, which results in increased apoptotic death of osteogenic cells and decreased bone production. The goal of this study was to investigate the effects of *RDE* on RSG-induced bone loss in diabetic rats in a systematic way. Five groups of six Wistar albino rats were studied: control (vehicle therapy), Streptozotocin (diabetes) group, *RDE* group, Rosiglitazone, and Rosiglitazone +*RDE* group.

Insulin, oxidative stress, and bone turnover markers in the blood were all detected using ELISA tests. When compared to diabetic control rats, *RDE* therapy significantly raised insulin and osteocalcin levels. *RDE* may be able to prevent diabetic osteoporosis by boosting osteogenesis and lowering oxidative stress in the bone.These findings support the use of *RDE* as a bone loss inhibiting in diabetics. Well-designed clinical trials are likely to yield further scientific evidence on its bone-protective effects and safety.

Keywords: *Radix Dipsaci*, Diabetic osteoporosis, Rosiglitazone.

Introduction

Type-2 diabetic mellitus (T2DM) is characterised by chronic hyperglycemia and increased glucose levels, which can lead to dysfunctions in glycometabolism and lipometabolism. T2DM is the cause of 90 percent of all diabetes cases worldwide.[1-5] For a given BMD, T2DM patients had a higher risk of fractures than non-diabetic individuals. Fragility fractures are more prevalent in diabetics due to micro architectural defects in the bone. These anomalies are difficult to see and are typically unrelated to BMD. As a result, bone fragility in diabetics is a problem that is underappreciated.[6]Diabetics have poor bone turnover indicators, and their real fracture rates are greater than fracture risk assessment techniques indicate.[7]

A disruption in the delicate balance between these two processes causes osteoarthritis and osteoporosis. Osteoporosis develops when osteoclastic bone resorption outnumbers osteoblastic bone growth. STZ-induced diabetes has been demonstrated in several studies to be a helpful model for understanding the pathophysiological mechanisms of diabetes-related bone loss.[8-10]*RDE* extracts are commonly used to treat osteoporosis in postmenopausal women.Despite the fact that *RDE* has shown considerable anti-osteoporotic effects in an osteoporosis model,[11] its effect on rosiglitazone (RSG)-induced bone loss in a diabetes model is unclear. We opted to study the effects of *RDE* treatment on bone oxidative stress and turnover indicators in STZ-treated rats who were also given RSG.

Materials and Methods

Animals:

The study employed Wistar rats weighing 100–120 g that were obtained from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were housed in a temperature-controlled environment (22°C, 12 hour light/dark cycle) and fed standard rat chow with free 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:

To chemically induce diabetes-like hyperglycemia in rats, a single intraperitoneal injection of 60 mg/kg STZ dissolved in 10 mM 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. Rats having fasting blood glucose levels of greater than 11 mmol/L were categorised as diabetic after a week of injection.[12] The experimental group and the control group each got the same quantity of isotonic NaCl injection.

Experimental design:

The rats were split into five groups: non-diabetic control (vehicle, n = 6), diabetic control (STZ group, n=6), *RDE* (500 mg/kg/day, n = 6), rosiglitazone (4 mg/kg/day, n = 6), and combination group (*RDE*500 mg/kg/day + Rosiglitazone 4 mg/kg/day, n = 6). Each medication was given by gastric gavage once a day for 35 days. Throughout the trial, the animals were examined daily for symptoms of illness. There were no animals that were really sick or died before the completion of the trial. The rats administered saline instead of streptozotocin in the control group (n=6) had normal blood glucose levels (120 mg/dl).

At the end of the experiment, all of the animals fasted overnight and their blood glucose levels were measured. Before being killed, the animals were administered ketamine (80 mg/kg) and xylazine (8 mg/kg) anaesthesia. The femur and tibia were separated by cutting near the stifle joint. Through heart puncture, blood samples (10–15 mL) 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 °C after centrifuging the blood samples at 4000 rpm for 15 minutes.

Determination of fasting blood glucose: After the rats had been fasted for 12–14 hours, blood samples were taken from their tail veins to test blood glucose levels using a glucometer. Blood will be taken with a 1-ml needle, put on a glucose strip, and quantified with a glucometer after the rats' tails have been washed with 70% (v/v) ethanol.

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 (50 mM Tris-HCl, 1.15 percent KCl pH 7.4). The homogenates were spun at 9000 rpm for 10 minutes in a cooled centrifuge (4 °C) to remove nuclei and debris. The produced supernatant was monitored using a TBARS test kit for 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 technique,[13] which utilised bovine serum albumin as a standard.

Marker of bone formation and bone resorption:

All markers of bone formation and resorption were measured using 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 450 nm using a microplate reader, according to Abdul-Majeed et al.[13]

Statistical analysis:

All of the data was analysed using ANOVA. The significance of the means was determined using Duncan's multiple comparison test. The average minus one standard deviation was used to report the results. A 95 percent confidence level was used for all of the analyses.

Results

Fasting blood glucose and serum insulin:

RDE treatment decreased fasting blood glucose levels while dramatically increasing serum insulin levels in diabetic rats,.In comparison to the NC rats, the DC rats had greater fasting blood glucose and lower insulin levels (Table 1).

Table 1: Effects of RDE on fasting blood glucose level and serum insulin in STZ induced diabetic rats (data represent mean ± 1SD).

Groups	Fasting blood glucose (mmol/L)		Serum insulin (µIU/mL)
	Before	After	
NC	5.60 ± 0.40a	4.92 ± 0.21a	3.66 ± 3.04c
DC	22.00 ± 3.15b	26.03 ± 2.79b	1.58 ± 0.26a
RSG	25.30 ± 4.60c	20.63 ± 3.85c	1.98 ± 0.24a
RDE	26.87 ± 7.13c	18.17 ± 4.87c	2.91 ± 0.28b
RDE+ RSG	27.65 ± 7.33c	17.87 ± 4.97c	2.72 ± 0.17b

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

Oxidative stress marker and antioxidant enzymes in bone:

The effects of *RDE* on bone lipid peroxidation and antioxidant enzyme activity are summarised in Table 2. When compared to the NC rats, the DC rats had a significant increase in MDA levels, but no significant changes in GPx or SOD activity. The *RDE*-treated rats show a similar pattern of behaviour.

Table 2: Oxidative stress marker and antioxidant enzymes of various experimental groups (data represent mean ± 1SD).

	Oxidative stress marker	Antioxidant enzymes	
Groups	TBARS (nmol MDA/mg protein)	GPx (U/mg protein)	SOD (mU/mg protein)
NC	32.83 ± 0.49a	45.55 ± 0.68ab	0.50 ± 0.01b
DC	58.74 ± 0.66b	44.40 ± 0.80bc	0.367 ± 0.04bc
RSG	40.51 ± 9.30c	42.16 ± 0.97b	0.49 ± 0.02a
RDE	38.79 ± 0.14c	46.40 ± 0.43bc	0.48 ± 0.19b
RDE+ RSG	38.79 ± 0.14c	45.32 ± 0.56bc	0.56 ± 0.28a

Different superscripts ^{a,b,c} in a column differed significantly at($p < 0.05$).

Bone turnover markers:

Despite the fact that BALP values did not differ significantly between treatment groups, blood osteocalcin levels increased while DPD decreased after *RDE* therapy.Although the STZ injection reduced blood osteocalcin, serum DPD was significantly higher in the STZ group than in the NC group (Table 3).

Table 3: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± 1SD).

Groups	Bone formation markers		Bone resorption marker
	Osteocalcin (ng/ml)	**BALP (ng/ml)**	**DPD (ng/ml)**
NC	135.68 ± 6.82c	102.79 ± 7.39b	165.08 ± 5.43b
DC	111.35 ± 0.37a	65.06 ± 4.60a	164.10 ± 0.21c
RSG	96.42 ± 8.64b	66.38 ± 0.35a	187.16 ± 4.28ab
RDE	136.66 ± 4.11d	85.50 ± 8.31a	175.53 ± 0.31a
RDE+ RSG	141.66 ± 4.01d	84.30 ± 8.31a	171.53 ± 0.41a

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

Discussion

Radix Dipsaci has been used to treat fractures and joint disorders for thousands of years. Although traditional users regard these herbal remedies to be a cost-effective option, international recognition as an alternative therapeutic regime for the prevention and treatment of osteoporosis will need considerable study utilising contemporary science. For the first time, we showed that the crude extract of *Radix Dipsaci* can prevent bone loss in diabetic rats treated with RSG.[11]

A decrease in chondrocyte counts and an increase in tidemark roughness in the femoral articular cartilage have been linked to STZ injection. [13] These data suggest that diabetic rats may experience osteoarthritis-like symptoms. Osteoarthritis-like symptoms have been observed in both T1DM and T2DM rat.[13-14] The activation of oxidative stress is thought to have assisted these changes. In Asia, *RDE* is frequently used to treat renal and hepatic diseases, as well as to strengthen bones. According to studies, *RDE* has the potential to be used as an antioxidant and anti-neoplastic treatment.

RDE may be able to prevent OVX-induced bone loss and trabecular microarchitecture degradation, preserving bone structural integrity and biomechanical quality.[11] Oxidative damage markers were shown to be greater in STZ-induced diabetic control rats in animal studies. Furthermore, oxidative stress in the presence of hyperglycemia has been shown to affect bone metabolism and form through altering osteoclast and osteoblast activity.[15]

According to the findings of this study, blood DPD levels in DC rats rose, whereas serum osteocalcin and BALP activity decreased. This outcome is consistent with the findings that bone turnover is a key characteristic of T1DM-related bone degeneration. BALP (Bone-Rat Alkaline Phosphatase) is a bone-rat alkaline phosphatase isoform produced by osteoblasts for bone remodelling, but it more accurately represents mineral metabolism.[16-17] The *RDE* groups exhibited nearly the same osteocalcin to DPD ratio as the NC groups, suggesting that *RDE* treatment effectively balanced bone formation and resorption.

Conclusion

RDE has the ability to prevent bone loss in STZ-treated rats, according to our data. After *RDE* 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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