INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)

www.ijcrcps.com

DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 8, Issue 9 - 2021

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2021.08.09.004

Rhizoma Drynariae protects against Canagliflozin induced bone loss

Ali Alqahtani[1], Khalid A Asseri[1], Yahya I Asiri[1], Krishnaraju Venkatesan[1]*, Noohu Abdulla Khan[2], Sirajudeen Shaik Alavudeen[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. E-mail: kvenkatesan@kku.edu.sa.
ORCID ID: 0000-0003-2853-5907.

Abstract

Osteoporosis is a condition marked by a loss of bone mass and degradation of the bone microstructure, both of which lead to increased fragility and consequent fragility fractures, particularly in the elderly. *Rhizoma Drynariae (DRE)* is one of the most often used herbal remedies for osteoporosis therapy. Transdermal drug administration is a well-established new method for drug delivery that offers numerous benefits over conventional routes.

Wistar albino rats were split into five groups of six rats each: vehicle control, diabeticgroup, *DRE* group, Canagliflozin (CGF), and CGF + DRE group. Each medication was given by gastric gavage once a day for 35 days. The drug canagliflozin appears to raise the risk of fractur. When compared to the control group, *DRE* treatment increased bone strength at the femoral diaphysis in osteoporotic fractures in rats by increasing ultimate load and stiffness.

The goal of this study is to investigate the anti-osteoporosis effects of *DRE* in diabetic rats co-treated with CGF. Blood glucose levels and bone mineral density (BMD) were measured. According to the data, *DRE* produced a significant increase in bone amount. *DRE* may help prevent and cure diabetic osteoporosis by increasing bone mineral density, according to one study.

Keywords: Rhizoma Drynariae Diabetic osteoporosis, Streptozotocin induced diabetes, Canagliflozin.

Introduction

Diabetes and osteoporosis are both connected to an increased risk of fracture in the elderly population. Natural materials, which serve as sources of inspiration, are used in many modern treatments. Diabetes affects an estimated 422 million people worldwide, and its prevalence increases with age, reaching 25% among people over 65 in the United States. In the same way, the chance of getting osteoporosis rises with age; as a result, diabetes and osteoporosis are common among the elderly. Diabetes patients had a greater fracture risk despite having higher bone mineral density (BMD) than those without the disease. This apparent contradiction might be caused by poor bone quality, diabetic issues, physical disability, or an increased chance of falling. It's critical to investigate how osteoporosis treatments influence bone health in diabetics.[1]Canagliflozin (CGF), a sodium glucose cotransporter[2] (SGLT2) inhibitor used to treat people with T2DM (type 2 diabetes mellitus), reduces plasma glucose by increasing glucose excretion in the urine.

Results from a phase[3] study in older T2DM patients aged 55 to 80 years showed that canagliflozin was associated with a small but statistically significant reduction in bone mineral density (BMD) at the total hip over 104 weeks, as well as increases in the bone turnover markers.[4] Rhizoma Drynariae (*DRE*) is one of the most commonly prescribed herbal remedies for postmenopausal women with osteoporosis.

DRE has been found to increase the amount of Foxc2 expression in bone marrow mesenchymal stem cells, triggering osteogenic differentiation, and it has also been shown to have a higher binding affinity for oestrogen receptor (ER), resulting in osteogenesis.[5] Furthermore, a prior study found that a combination of *DRE* and alendronate improved fracture healing and callus development in a rat model of osteoporotic fracture.[6] Despite its efficacy, *DRE* has the potential to cause gastrointestinal adverse effects,[7] necessitating the development of a method to mitigate these side effects.[8-10]

In previous studies, *DRE* extracts were shown to increase osteoblastic activity. In the setting of diabetic rats co-treated with CGF, *DRE* on the other hand, is rarely investigated on its own. As a result, the objective of this study is to investigate if *DRE* may help prevent bone loss in diabetic rats.

Materials and Methods

Animals:

Healthy male Wistar albino rats, aged 3 to 4 months and weighing 180 to 240 g, were utilized 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, 25 °C 3 °C). The animals were acclimatised to the laboratory environment for 14 days. The therapy was carried out with the consent of King Khalid University's animal ethics committee and in accordance with 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:

The pancreatic-cell toxin streptozotocin (STZ) was given intraperitoneally at a dose of 65 mg/kg body weight to cause diabetes in the rat (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent).[11] All of the rats in the control group got the same amount of vehicle. To avoid deterioration, STZ was weighed separately for each animal, solubilized with 0.1 ml of freshly generated cold Na citrate buffered (NaB-0.1 M, pH 4.5), and administered within 5 minutes. STZ was calculated to have a volume of 1.0 ml/kg. To counteract the significant immediate hypoglycemia effect of STZ, rats were given a 5% glucose solution for 48 hours following the injection.

Blood was drawn from the tail vein three days after STZ injection, and samples were tested for blood glucose using a glucometer (Aqua Check, Roche). Fasting blood glucose levels (FGLs) in diabetic rats were greater than 250 mg/dL. Animals were divided as follows. Control (vehicle, Non- diabetic control, n = 6), Diabetic control (STZ), *DRE* (100/kg/day, n = 6), CGF(40 mg/kg/day, n = 6), and combination group (*DRE* 100/kg/day and CGF40 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. Blood glucose levels were measured once a week for the course of the research using a Roche Accu Chek advantage glucometer to assess the animals' hyperglycemic condition. The rats in the control group (n=6) that were given saline instead of streptozotocin had normal blood glucose levels (120 mg/dl).

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.

Determination of hemoglobin A1c:

Haemoglobin A1c (HbA1c) will be measured using a Clover A1cTM Self Analyzer after blood samples from the tail vein are obtained and placed on a test cartridge. The proportion of HbA1c in the blood sample will be shown on the Clover A1cTM Self Analyzer's LCD screen.

Bone Mineral Density Measurement:

The BMD of the left femur and lumbar vertebrae (L1–L4) of rats was evaluated using a dual energy X-ray absorptiometry (DEXA) scanning system after blood was collected (Lunar, WI, USA).

Results

The positive control group's (STZ) glucose profiles worsened with time (Table-1). DRE, on the other hand, has been shown to protect against diabetes development.

Table-1: Effect of *DRE* on Fasting blood glucose level:

Treatment Group	Dose	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
Normal Control	5 mL/ kg	78.12±3 .2	76.32± 2.3	77.61±2 .6	75.40± 1.7	78.60 ±1.4	83.36± 1.7	82.40± 1.06	84.40± 1.02	86.40± 1.12
Positive Control	65 mg/kg	263.54± 8.2*	296.35± 7.8*	316.21± 10.62*	37.73± 8.8*	352.7 2±8.4 *	372.76 ±11.5*	394.75 ±10.5*	413.73 ±10.4*	437.75 ±8.6*
DRE	100 mg/kg	267.33± 6.3	262.25± 8.6*	268.12± 7.8*	274.28 ±7.2*	291.3 5±7.5 *	302.34 ±8.8*	306.36 ±10.5*	323.35 ± 7.2*	324.32 ±8.7*
CGF	40 mg/kg	263.33± 8.6	247.25± 11.4*	235.22± 7.5*	213.28 ±7.6*	182.2 8±7.6 *	152.55 ±8.7*	125.45 ±8.2*	103.15 ± 7.2*	93.035 ±7.7*
DRE +CGF	100 +40 mg/kg	262.33± 8.6	246.25± 10.5*	234.22± 8.6*	212.28 ±7.6*	183.2 8±7.6 *	152.55 ±7.7*	125.45 ±6.2*	103.15 ± 7.2*	92.135 ±9.7*

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

HBA1C levels were higher in the positive control group than in the normal control group ($p<0.05$), as indicated in table 2. In contrast to the positive control group, *DRE* was shown to lower HBA1C levels, implying a favourable effect.

Table-2: Effect of *DRE* on Glycoslyted Haemoglobin (HBA1C)

Treatment Group	Day 28
Normal Control	5.45±0.17
Positive Control	5.71±0.06*
DRE	5.59±0.04*
CGF	5.42±0.05*
DRE +CGF	5.44±0.03*

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

DRE treatment improved lumbar (L1–L4) and femoral bone mineral density (BMD) in diabetic rats ($p<0.05$). The BMD of the positive group was significantly higher than that of the other treatment groups (Table-3). These findings show that *DRE* may be able to protect bones from the effects of hyperglycemia.

Table-3: Effect of *DRE* on the bone mineral density of the lumbar vertebrae and femur bone

	Bone Mineral density(mg/cm3)	
Treatment Group	**Lumbar Vertebrae**	**Femur**
Normal Control	176 ± 2.2	223 ± 2.3
Positive Control	78 ± 2.4*	104 ± 2.5*
DRE	156 ± 1.5*	204 ± 1.8*
CGF	96 ± 2.6*	106± 2.4*
DRE +CGF	162 ± 1.5*	199 ± 1.8*

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

Statistical analysis:

The information was presented in the form of a mean and standard deviation (SD). One way analysis of variance (ANOVA) and Tukey's multiple comparison test will be used to statistically analyse data from various groups. Statistical significance is defined as a 'p' value of less than 0.05.

Discussion

Some studies have found an increase in cortical porosity in persons with type 2 diabetes, although not all, which might explain why this cohort has a higher fracture risk while having higher BMD. Pharmacological effects[12] of *DRE* on osteoporosis, bone fractures, and joint disorders have been discovered.[13]*DRE* enhances the ability of recombinant alendronate to inhibit osteoclast bone resorption, according to a previous study.

In pre-osteoblasts,[14] *DRE* treatment promoted osteoblast development and mineralization, while inhibiting bone loss in rodent osteoclasts.[15] In rats, *DRE* therapy has been shown to enhance BMD and trabecular number. In a rabbit bone defect model, *DRE* extracts were found to stimulate bone growth on the edges of bone lesions. Moreover, numerous pharmacological investigations have shown that *DRE* has a variety of biological effects, including immunoregulatory,[16,17] anti-inflammatory, and neuroprotective properties.[19,20]

Canagliflozin had no effect on calcium homeostasis or hormones that regulate calcium homeostasis in clinical trials. Canagliflozin caused increases in bone turnover indicators and reductions in BMD at the whole hip, but not at other locations, which were linked to weight loss. Within 12 weeks, canagliflozin was linked to a greater fracture rate, especially in the distal extremities.[21]In the current research, we found that *DRE* had positive effects on glycosylated haemoglobin and BMD.

Conclusion

Our results suggest that *DRE* may promote bone mass in diabetic osteoporosis and it could be used as an alternative supplement in the prevention and treatment of bone loss induced by Canagliflozin in diabetic rats.

Acknowledgments

The authors are grateful to Deanship of Scientific Research, King Khalid University for sponsoring this study through the Large Research Group Project under grant number RGP 2/186/42.

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**."**

References

1. Ay B, Parolia K, Liddell RS, Qiu Y, Grassel li G, Cooper DML, Davies JE. Hyperglyce mia compromises rat cortical bone by increasing osteocyte lacunar density and decreasing vascular canal volume. *Commun Biol*.2020; 3(1):20.
2. Wongdee K, Charoenphandhu N. Osteoporo sis in diabetes mellitus: possible cellular and molecular mechanisms. *World J Diabetes*. 2011; 2(3):41–48.
3. Logar DB, Komadina R, Prezelj J, Ostanek B, Trost Z, Marc J. Expression of bone resorption genes in osteoarthritis and in osteoporosis. *J Bone Miner Metab*. 2007; 25(4):219–225.
4. Watts NB, Bilezikian JP, Usiskin K, et al. Effects of Canagliflozin on Fracture Risk in Patients With Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab*. 2016; 101(1):157-166.
5. Wong RW, Rabie B, Bendeus M, et al. The effects of *Rhizoma Curculiginis* and *Rhizoma Drynariae* extracts on bones. *Chin Med.* 2007; 2:13.
6. Chen L, Tao ZS, Chen H, et al. Combined treatment with alendronate and *Drynaria Rhizome* extracts: Effect on fracture healing in osteoporotic rats. *Z Gerontol Geriatr*. 2018; 51(8):873–79.
7. Jang Y, Kim TK, Shim WS, et al. Naringin exhibits *in vivo* prokinetic activity via activation of ghrelin receptor in gastrointestinal motility dysfunction rats. *Pharmacology*. 2013; 92:191–97.
8. Roy SD, Gutierrez M, Flynn GL, et al. Controlled transdermal delivery of fentanyl: characterizations of pressure-sensitive adhesives for matrix patch design. *J Pharm Sci.* 1996; 85:491–95.
9. Schafer-Korting M, Mehnert W, Korting HC. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv Drug Deliv Rev.* 2007; 59:427–43.
10. Souto EB, Wissing SA, Barbosa CM, et al. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. *Int J Pharm.* 2004;278:71–77
11. Feng X, McDonald JM. Disorders of bone remodelling. *Annu Rev Pathol*.2011; 6:121–145.
12. Ying X, Chen X, Wang T, Zheng W, Chen L, Xu Y. Possible osteo protective effects of myricetin in stz induced diabetic osteoporosis in rats. *Eur J Pharmacol.* 2020 ;866:172805.
13. Sung YY, Kim DS, Yang WK, et al. Inhibitory effects of Drynaria fortunei extract on house dust mite antigen-induced atopic dermatitis in NC/Nga rat. *J Ethnopharmacol.* 2012; 144:94–100.
14. Chen LL, Lei LH, Ding PH, et al. Osteogenic effect of *Drynariae Rhizoma* extracts and naringin on MC3T3-E1 cells and an induced rat alveolar bone resorption model. *Arch Oral Biol.* 2011; 56:1655–62.
15. Jeong JC, Kang SK, Youn CH, et al. Inhibition of *Drynariae Rhizoma* extracts on bone resorption mediated by processing of cathepsin K in cultured mouse osteoclasts. *Int Immunopharmacol.* 2003; 3:1685–97.
16. An HJ, Lee GG, Lee KT. *Drynariae Rhizoma* increases immune response in rat. *Nat Prod Commun.* 2012; 7:905–8.
17. Anuja GI, Latha PG, Suja SR, et al. Anti-inflammatory and analgesic properties of *Drynaria quercifolia* (L.) J. Smith. *J Ethnopharmacol.* 2010; 132:456–60.
18. Guo W, Shi K, Xiang G, et al. Effects of Rhizoma Drynariae Cataplasm on Fracture Healing in a Rat Model of Osteoporosis. *Med Sci Monit*. 2019; 25:3133-3139. Published 2019 Apr 28.

19. Wang W, Li H, Yu J, et al. Protective effects of Chinese herbal medicine *Rhizoma Drynariae* in rats after traumatic brain injury and identification of active compound. *Mol Neurobiol.* 2016; 53:4809–20.
20. Maria Alba, John Xie, Albert Fung & Mehul Desai (2016) The effects of canagliflozin, a sodium glucose co-transporter 2 inhibitor, on mineral metabolism and bone in patients with type 2 diabetes mellitus, *Current Medical Research and Opinion*, 32:8, 1375-1385.

Access this Article in Online

Website: www.ijcrcps.com

Subject: Pharmacology

Quick Response Code

DOI: 10.22192/ijcrcps.2021.08.09.004

How to cite this article:
Ali Alqahtani, Khalid A Asseri, Yahya I Asiri, Krishnaraju Venkatesan, Noohu Abdulla Khan, Sirajudeen Shaik Alavudeen, Ester Mary Pappiya, Premalatha Paulsamy, R.Natarajan, Kalpana Krishnaraju, Kumar Venkatesan, Kumarappan Chidambaram. (2021). *Rhizoma Drynariae* protects against Canaglifloxacin induced bone loss. Int. J. Curr. Res. Chem. Pharm. Sci. 8(9): 22-28.
DOI: http://dx.doi.org/10.22192/ijcrcps.2021.08.09.004

