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BONE FORMATION EFFECT OF *RHIZOMA DRYNARIAE* EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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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* Extract *(RDE)* is one of the most often used herbal remedies for osteoporosis therapy. The goal of this study is to see how *Rhizoma Drynariae* Extract affects diabetic osteoporosis. Diabetic Sprague Dawley rats (n=6) were administered one of three treatments through gavage for eight weeks: saline (control), metformin (1000mg/kg/day), or *Rhizoma Drynariae* Extract (100mg/kg/day). In comparison to the control group, *Rhizoma Drynariae* Extract treatment enhanced fatty acid profile and bone development. The use of *DRE* cataplasm in the treatment of osteoporosis may be beneficial.

KEYWORDS

Diabetic osteoporosis and *Rhizoma Drynariae*.

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INTRODUCTON

Osteoporosis is a condition characterized by a loss of bone mass and degradation of bone microstructure, both of which lead to increased fragility and consequent fragility fractures, particularly in the elderly. According to a recent study, 50% of women and 20% of men over the age of 50 may have osteoporosis related fractures, such as hip and spine fractures, resulting in disability, death, and financial burden. Due to oestrogen

deficiency induced bone catabolism, postmenopausal women are thought to have a greater risk of osteoporosis and subsequent fractures. Menopause accelerates bone loss, which leads to an increase in the frequency of spine, hip, and wrist fractures[1]. Diabetes mellitus (DM) is a set of severe disabling metabolic disorders characterised by persistent hyperglycemia caused by impaired insulin production and/or insulin activities.

Chronic hyperglycemia causes organ dysfunction and failure, especially in the eyes (diabetic retinopathy and cataract), kidneys (diabetic nephropathy), nerves (diabetic neuropathy), heart (diabetic cardiomyopathy) and blood vessels (diabetic cardiomyopathy) (microangiopathy). DM has also been linked to metabolic bone disorders, osteoporosis, and low-impact fractures, as well as other bone related occurrences in senior individuals, such as falls. Indeed, DM is one of the "causes" of both osteopenia (T-scores between -1 and -2.5, as measured by dual energy X-ray absorptiometry; DXA) and osteoporosis (T-scores -2.5). However, bone degeneration differs significantly between type 1 and type 2 diabetes mellitus, and this may be due to distinct cellular and molecular processes[2].

In a previous study, *Rhizoma Drynariae* Extract therapy enhanced callus development and bone union when compared to a control group. Furthermore, as compared to the control group, *Rhizoma Drynariae* Extract improved bone strength at the femoral diaphysis in osteoporotic fractures in rats by increasing ultimate load and stiffness. In addition, as compared to the control group, *Rhizoma Drynariae* Extract restored trabecular bone mineral density in the femur. In this rat model, *Rhizoma Drynariae* Extract cataplasm administration improved the therapeutic benefits against osteoporotic fracture. There is currently no data on the effect of *Rhizoma Drynariae* Extract on diabetes-induced bone damage. The impact of *Rhizoma Drynariae* Extract on diabetes induced bone damage is the subject of this research.

MATERIAL AND METHODS

Animals

The experiment was carried out with 24 male Sprague Dawley rats weighing 100-120g from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were housed in a temperature controlled environment (22± °C with a 12 hour light/dark cycle) and given 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. 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 Streptozotocin (STZ) dissolved in 10mM citrate buffer was used to chemically produce diabetes like hyperglycemia in rats (pH 4.5). The rats were given 5% glucose water for two days after being administered STZ to avoid drug induced hypoglycemia. After a week of injection, 20 animals were classified as diabetic if their fasting blood glucose levels were higher than 11mmol/L[3]. The rats in the control group got the same amount of isotonic NaCl injection as the experimental group.

Experimental design

A total of 24 male rats (n=6) were divided into four groups at random. Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rats received 1000mg/kg metformin (MET), and diabetic rats received 100mg/kg *Rhizoma Drynariae* Extract. Patients received treatments through oral gavage once a day for a total of 56 days. At the completion of the trial, all of the animals were fasted overnight and their blood glucose levels were tested. After that, the animals were administered ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia before being killed. The femur and tibia were separated at the stifle joint by cutting. The rats blood samples (10-15mL) were taken by heart puncture into a simple red top tube with no anticoagulants. The serum was stored in aliquots at 80°C after centrifugation at 4000rpm for 15 minutes.

Marker of bone formation and bone resorption

All bone formation and resorption indicators were measured in serum. A Rat Mid Osteocalcin ELISA kit (IDS, UK) was used to assess the osteocalcin level, and a rat BALP ELISA kit was used to measure the BALP level (Qayee, Shanghai). The Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) was used to assess bone resorption DPD (Qayee, Shanghai). All samples were run in triplicate, and the optical density was measured at 450nm using a microplate reader (BioTek, USA)[4].

Analysis of bone fatty acid composition

As stated by Nurdiana *et al*, total fatty acids were isolated from bone and identified and measured using a gas chromatography technique (2017). The percentages of total detected fatty acids are used to calculate the fatty acid proportions[5].

Statistical analysis

To analyze all of the data, ANOVA was employed. The significance was calculated using Duncan's multiple comparison test. The 95 percent confidence level was used in all of the studies.

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). *Rhizoma Drynariae* Extract therapy significantly reduced fasting blood glucose levels while significantly raising serum insulin levels in diabetic rats.

Bone turnover markers

Although blood osteocalcin and ALP levels were significantly lower after the STZ injection, serum DPD was significantly higher than in the NC group (Table No.2). Despite the fact that BALP values did not differ significantly across the groups, serum osteocalcin levels increased while DPD levels decreased following *Rhizoma Drynariae* extract treatment.

Bone fatty acid changes

In the femurs of DC rats, the total n-3 PUFA was considerably reduced, but the ratio of n-6 to n-3 PUFA was significantly raised, as shown in Table No.3. The MET group made similar observations. In the *Rhizoma Drynariae* extract group, the total bone n-3 PUFA increased while the n-6 to n-3 ratio dropped.

Discussion

Osteoporosis is characterised by decreased bone mass and an increased risk of fracture due to an imbalance of bone remodelling including the encouragement of bone resorption over bone synthesis. Osteoporosis is linked to increased bone fragility owing to excessive resorption and decreased bone production, which increases the risk of hip and other bone fractures. Bone tissue is rebuilt during fracture healing, and biomechanical function is restored. Osteoporosis, on the other hand, causes this process to be disrupted, resulting in discomfort and physical impairment[6,7]. Although numerous researchers have long tried to figure out how DM causes osteopenia and osteoporosis, the specific mechanism is still unknown. Hyperglycemia, on the other hand, is widely acknowledged as a significant factor that has both direct and indirect negative impacts on osteoblast activity and bone formation.

A recent in vitro study in osteoblast like MG63 cells found that high glucose concentrations suppressed cell growth, mineralization, and expression of various osteoblast related markers, such as Runx2, type I collagen, osteocalcin, and osteonectin, while stimulating the expression of adipogenic markers, such as peroxisomal markers[8,9]. DC rats exhibited greater levels of oxidative damage markers, according to this study. According to the findings of this study, blood DPD levels rose in DC rats, whereas serum osteocalcin and BALP activity fell. Zhukouskaya *et al*, (2015) discovered that bone turnover suppression is a major characteristic of T1DM-related bone disease.

Another intriguing finding from this study is that blood osteocalcin levels rose following *Rhizoma Drynariae* extract treatment while DPD levels decreased (Table No.2). A variety of herbs with osteoprotective characteristics have yielded similar results[10]. The ratio of osteocalcin to DPD was nearly equal to that of the NC groups, suggesting that *Rhizoma Drynariae* extract treatment effectively created a balance between bone formation and bone resorption. When compared to

other STZ treated animals, the *Rhizoma Drynariae* extract rats had a considerably higher BMD and a much lower DPD (Table No.2). This result adds to the growing body of data that *Rhizoma Drynariae* extract therapy can prevent bone loss in STZ treated mice.

Table No.1: Effects of *Rhizoma Drynariae* Extract 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	5.82 ± 0.40^{a}	5.91 ± 0.21^{a}	2.80	5.24 ± 3.23^{c}
2	DC	19.10 ± 3.34^{b}	32.11 ± 2.85^{b}	52.61	1.85 ± 0.23^{a}
3	MET	26.30 ± 3.70^{c}	20.73 ± 3.84^{c}	-47/33.32	1.86 ± 0.44^{a}
4	*Rhizoma Drynariae* Extract	28.87 ± 6.22^{c}	18.27 ± 4.97^{c}	-38.13	2.59 ± 0.58^{b}

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

Table No.2: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± SD)

S.No	Groups	Bone formation markers		Bone resorption marker
		Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
1	NC	138.76 ± 7.9^{c}	102.49 ± 8.59^{b}	168.08 ± 6.13^{b}
2	DC	14.34 ± 0.97^{a}	64.06 ± 5.72^{a}	167.20 ± 0.21^{c}
3	MET	57.40 ± 9.14^{b}	82.38 ± 0.65^{a}	156.26 ± 4.58^{ab}
4	*Rhizoma Drynariae* Extract	154.64 ± 5.10^{d}	78.30 ± 9.21^{a}	148.63 ± 0.41^{a}

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

Table No.3: Fatty acid composition (percentage of total identified fatty acids) of the bone of the experimental groups (data represent mean ± SD)

S.No		NC	DC	MET	*Rhizoma Drynariae* extract
1	Myristic acid (C14:0)	1.60 ± 0.37^{c}	0.40 ± 0.06^{a}	1.20 ± 0.18^{bc}	0.71 ± 0.13^{ab}
2	Palmitic acid (C16:0)	26.38 ± 4.98	25.88 ± 4.47	29.17 ± 2.18	24.34 ± 14.78
3	Stearic acid (C18:0)	7.17 ± 1.06^{a}	8.98 ± 0.82^{b}	7.43 ± 0.90^{a}	9.93 ± 1.62^{b}
4	Palmitoleic acid (C16:1)	3.52 ± 0.81	1.67 ± 0.58	1.88 ± 0.61	1.96 ± 0.74
5	Oleic acid (C18:1n9)	21.62 ± 8.99	26.02 ± 5.73	28.61 ± 3.12	24.66 ± 3.20
6	Linoleic acid (C18:2n6)	3.24 ± 1.67	3.27 ± 0.55	5.19 ± 0.52	3.69 ± 0.69
7	Arachidonic acid (C20:4n6)	1.10 ± 0.48	2.78 ± 0.24	1.54 ± 0.56	3.12 ± 0.74
8	α- Linolenic acid (C18:3n3)	1.85 ± 0.45	1.26 ± 0.49	1.64 ± 0.62	1.46 ± 0.86
9	Eicosapentaenoic acid (C20: 5n3)	0.82 ± 0.15	0.15 ± 0.15	0.47 ± 0.15	0.78 ± 0.25
10	Docosapentaenoic acid (C22: 5n3)	0.58 ± 0.02	0.67 ± 0.14	0.40 ± 0.44	0.48 ± 0.35
11	Docosahexaenoic acid (C22: 6n3)	0.75 ± 0.26	0.34 ± 0.01	0.38 ± 0.03	0.51 ± 0.06
12	Total SFA	35.84 ± 5.96	36.26 ± 5.42	39.49 ± 3.93	39.58 ± 16.01
13	total MUFA	24.13 ± 7.61	26.59 ± 4.72	29.29 ± 2.76	25.43 ± 3.54
14	total n-6 PUFA	4.14 ± 1.86	4.86 ± 0.67	5.63 ± 0.99	4.89 ± 1.49
15	total n-3 PUFA	2.91 ± 0.70^{c}	1.54 ± 0.28^{a}	1.88 ± 0.34^{ab}	2.44 ± 0.77^{bc}
16	n-6 : n-3	1.64 ± 0.79^{a}	3.74 ± 0.83^{d}	2.84 ± 0.54^{cd}	2.13 ± 0.40^{ab}

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

CONCLUSION

In DM patients, osteoporosis and osteopenia are important debilitating problems in DM patients. DM complications and Osteoporosis increase the risk of low impact fractures, fragility and falls. It is known that increased blood glucose directly suppresses bone formation, accumulate fat in the bone marrow, and promote osteoclast mediated bone resorption, all of which decrease bone strength and quality and enhance susceptibility to fracture. Hence, control on bone damage and effective glycemic control should be the main goal of prevention and treatment of DM induced osteoporosis.

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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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