Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

https://doi.org/10.36673/AJRPSB.2021.v09.i02.A10

TAXUS YUNNANENSIS EXTRACT ENHANCE BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Using streptozotocin (STZ) induced diabetic rats, the impact of *Taxus yunnanensis* aqueous extract *(TYE)* on bone loss was studied. Streptozotocin causes diabetes (STZ). Diabetic Sprague Dawley rats (n = 6) were administered one of three treatments through gavage: saline (control), metformin (1000mg/kg/day), or *Taxus yunnanensis* extract (200mg/kg/day) for eight weeks. When compared to controls, the bone mineral density (BMD) was higher. These findings show that *Taxus yunnanensis* extract might be beneficial in the treatment of postmenopausal osteoporosis, particularly in the prevention of bone fracture in diabetic rats caused by STZ. When diabetic rats were given *Taxus yunnanensis* extract, their insulin and osteocalcin levels were much greater than in diabetic control rats. The inhibition of bone turnover appears to be the cause of *Taxus yunnanensis* extracts prevention or therapeutic actions on diabetic rats bone loss. These findings support the use of *Taxus yunnanensis* extract in diabetic individuals to treat osteoporosis.

KEYWORDS

Osteoporosis, Diabetes mellitus and *Taxus yunnanensis*.

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INTRODUCTION

Diabetes mellitus is a metabolic disease in which glucose, lipid and protein metabolism are disrupted. It is a significant cause of human death, affecting an estimated 6% of the adult population in Western civilization and its global prevalence is predicted to rise by 6% each year, reaching 200-300 million

cases in 2010. Sulfonylureas, which stimulate pancreatic islet cells to secrete insulin; metformin, which reduces hepatic glucose production; alpha-glucosidase inhibitors, which interfere with glucose adsorption and insulin itself, which suppresses glucose production and augments glucose utilisation, are some of the current approaches to reduce hyperglycemia. All of these treatments have limited efficacy and a variety of adverse effects, thus finding new classes of chemicals to address these issues is critical[1].

Diabetes mellitus and osteoporosis are chronic illnesses that affect the elderly in increasing numbers. Elder diabetics have a higher incidence of hip, humerus and foot fractures, according to recent epidemiological research. While type 1 diabetes is connected to a slight drop in bone mineral density (BMD), type 2 diabetes, which is more common in older people, is often associated with a normal or high BMD. Studies on diabetic experimental models have revealed a changed bone structure that might explain the increased risk of fractures seen in these animals, as well as the paradox of an increased risk of fractures in type 2 diabetic seniors with normal or raised BMD.

Furthermore, diabetic seniors are more likely to fall due to impaired eyesight, peripheral neuropathy and weakened muscle function, to name a few factors[2]. *Taxus yunnanensis* extracts enhanced bone growth and dramatically reduced bone resorption in recent trials, with no adverse effects on uterine tissue[3]. These findings show that *Taxus yunnanensis* extracts might be beneficial in the treatment of postmenopausal osteoporosis, particularly in terms of preventing bone fractures caused by oestrogen deprivation. It is uncertain if *Taxus yunnanensis* extracts has an effect on diabetic osteoporosis. As a result, the goal of this study is to see how *Taxus yunnanensis* extracts affects diabetic osteoporosis produced by STZ.

MATERIAL AND METHODS

Animals

The experiment was carried out 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 housed in atemperature-controlled environment (22±^{o}C with a 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 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

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. 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 split into four groups at random. Normal control rats received saline (NC), diabetic control rats received saline (DC), diabetic rats received 1000mg/kg body weight metformin (MET) and the other diabetic rat group received 200mg/kg body weight *Taxus yunnanensis* extracts. Patients received oral gavage treatments once a day for a total of 56 days. At the completion of the trial, all of the animals were fasted overnight and blood glucose levels were tested. Before being killed at the end, the animals were administered ketamine (80mg/kg) and xylazine (8mg/kg) anaesthesia. The femur and tibia were cut apart at the stifle joint. The rats blood samples (10-15mL) were collected by heart puncture into a simple red top tube with no anticoagulants. The serum was separated from the blood samples and stored at 80^{o}C in aliquots after centrifugation at 4000 rpm for 15 minutes.

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). Rat deoxypyridinoline (DPD) ELISA Kit (Qayee, Shanghai) was used to assess bone resorption DPD (Qayee, Shanghai). The optical density of all samples was measured at 450nm using a microplate reader (Epoch Microplate Spectrophotometer, Bio Tek, USA)[4].

Analysis of bone fatty acid composition

The total fatty acids were extracted from bone, identified and quantified by gas chromatography method as described by Nurdiana *et al*. (2017). The fatty acid proportions are expressed as the percentage of total identified fatty acids[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 showed high fasting blood glucose and low insulin levels compared to the NC animals (Table No.1). Treatment significantly decreased fasting blood glucose levels while significantly raising serum insulin levels in diabetic rats.

Bone turnover markers

Although blood osteocalcin was 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 treated groups, serum osteocalcin levels increased while DPD levels decreased following *Taxus yunnanensis* extracts treatment.

Bone fatty acid changes

The recorded data in Table No.3 shows the total *n* - 3 PUFA was significantly decreased while the ratio of *n* - 6 to *n* - 3 PUFA was significantly increased in the femur of DC rats. Similar observations were noticed in the MET group. Remarkably, the total bone *n* - 3 PUFA increased and the *n* - 6 to *n* - 3 ratio decreased in the *Taxus yunnanensis* extracts group.

Discussion

By influencing the activity of osteoclasts and osteoblasts, oxidative stress and hyperglycemia have been shown to influence bone metabolism and architecture[6]. According to the findings of this study, blood DPD levels increased 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. Our findings are supported by previous observations of increased serum DPD in rats with osteoarthritis[7] and osteopenia[8]. Another intriguing finding from this study is that blood osteocalcin levels rose following *Taxus yunnanensis* extracts treatment while DPD levels decreased (Table No.2). A variety of herbs with osteoprotective characteristics have yielded similar results[9].

Despite the fact that osteocalcin is a specific osteoblast marker that correlates well with histological changes[10] blood OC levels tended to fluctuate with meal intake. According to prior studies, osteocalcin does not seem to be as sensitive as BALP[11]. BALP activity is still low in *Taxus yunnanensis* extracts rats, suggesting that mineral metabolism is still affected. BALP is a bone specific alkaline phosphatase isoform that is generated by osteoblasts for bone remodelling, but it also reflects mineral metabolism[12]. The ratio of osteocalcin to DPD was nearly equal in the *Taxus yunnanensis* extracts and NC groups, suggesting that equilibrium between bone formation and bone resorption was achieved with *Taxus yunnanensis* extracts treatment.

With biochemical indications of bone loss and osteoarthritis like disease, the DC and MET rats had a substantial drop in total bone n-3 PUFA and an increase in the n-6: n-3 ratio (Table No.3). However, with *Taxus yunnanensis* extracts therapy, these abnormalities have vastly improved. Longo and Ward have discovered that consuming a large

amount of n-3 PUFA can boost BMD and lower the incidence of fragility fractures[13]. PUFA (n-3) supplementation has also been shown to preserve bone metabolism by lowering bone resorption indicators in previous studies[14]. In fact, as compared to other animals treated with STZ, the BMD was considerably greater (Table No.2) and the DPD was much lower (Table No.2). This discovery adds to the growing body of data that *Taxus yunnanensis* extracts therapy can help STZ treated rats avoid bone loss.

Table No.1: Effects of *Taxus yunnanensis* extracts 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	**4.92 ± 0.40^{a}**	4.98 ± 0.11^{a}	2.90	4.24 ± 3.23^{c}
2	DC	20.00 ± 3.34^{b}	31.11 ± 2.75^{b}	49.71	1.65 ± 0.23^{a}
3	MET	29.30 ± 3.70^{c}	18.73 ± 3.79^{c}	-33.22	1.79 ± 0.34^{a}
4	*Taxus yunnanensis* extracts	27.87 ± 6.12^{c}	16.27 ± 4.97^{c}	-38.03	2.40 ± 0.28^{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	137.86 ± 6.4^{c}	103.49 ± 7.89^{b}	169.08 ± 5.23^{b}
2	DC	14.34 ± 0.97^{a}	67.06 ± 4.82^{a}	165.10 ± 0.31^{c}
3	MET	58.40 ± 8.44^{b}	84.38 ± 0.45^{a}	154.16 ± 4.49^{ab}
4	*Taxus yunnanensis* extracts	155.64 ± 4.17^{d}	76.40 ± 8.31^{a}	146.53 ± 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 (data represent mean ± SD)

S.No		NC	DC	MET	*Taxus yunnanensis* extracts
1	Myristic acid (C14:0)	1.70 ± 0.47^{c}	0.60 ± 0.08^{a}	1.20 ± 0.07^{bc}	0.61 ± 0.03^{ab}
2	Palmitic acid (C16:0)	26.38 ± 4.98	25.88 ± 4.47	26.27 ± 2.98	25.34 ± 13.78
3	Stearic acid (C18:0)	7.17 ± 1.21^{a}	8.88 ± 0.52^{b}	7.43 ± 0.80^{a}	9.93 ± 1.92^{b}
4	Palmitoleic acid (C16:1)	2.82 ± 0.71	1.57 ± 0.36	1.78 ± 0.41	1.9 6 ± 0.74
5	Oleic acid (C18:1n9)	19.62 ± 7.89	26.02 ± 4.83	28.61 ± 3.12	23.56 ± 3.10
6	Linoleic acid (C18:2n6)	3.14 ± 1.57	3.97 ± 0.45	4.29 ± 0.42	2.79 ± 0.49
7	Arachidonic acid (C20:4n6)	1.20 ± 0.18	1.78 ± 0.14	1.14 ± 0.06	2.13 ± 0.24
8	α- Linolenic acid (C18:3n3)	1.65 ± 0.55	1.46 ± 0.39	1.34 ± 0.52	1.39 ± 0.86
9	Eicosapentaenoic acid (C20: 5n3)	0.72 ± 0.35	0.24 ± 0.15	0.37 ± 0.15	0.78 ± 0.25
10	Docosapentaenoic acid (C22: 5n3)	0.48 ± 0.12	0.67 ± 0.24	0.60 ± 0.34	0.39 ± 0.25
11	Docosahexaenoic acid (C22: 6n3)	0.65 ± 0.16	0.24 ± 0.21	0.68 ± 0.13	0.41 ± 0.23
12	total SFA	33.94 ± 5.96	35.16 ± 4.42	36.39 ± 3.73	34.38 ± 13.11
13	total MUFA	24.13 ± 7.61	27.59 ± 4.72	28.29 ± 2.86	25.63 ± 3.64
14	total n-6 PUFA	4.14 ± 1.86	4.86 ± 0.79	5.23 ± 0.88	4.9 1 ± 1.49
15	total n-3 PUFA	2.91 ± 0.67^{c}	1.54 ± 0.28^{a}	1.79 ± 0.34^{ab}	2.39 ± 0.47^{bc}
16	n-6 : n-3	1.46 ± 0.79^{a}	3.49 ± 0.13^{d}	2.95 ± 0.44^{cd}	2.23 ± 0.30^{ab}

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

CONCLUSION

In STZ induced diabetic rats, the aqueous extract of *Taxus yunnanensis* extracts had a strong hypoglycaemic effect. *Taxus yunnanensis* extracts bone protective action was thought to be linked to fatty acid alterations and bone quality. Even though more research on oral administration, mechanistic aspects and minor components is needed, the current findings give scientific proof of traditional usage of this plant for diabetes related bone loss.

ACKNOWLEDGMENT

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

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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Please cite this article in press as: Krishnaraju Venkatesan *et al. Taxus yunnanensis* extract enhance bone formation in streptozotocin-induced diabetic rats, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 9(2), 2021, 92-97.