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Bone formation ability of the wood of *Taxus yunnanensis* in pioglitazone treated diabetic rats

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Abstract

Using streptozotocin (STZ)-induced diabetic rats co-treated with pioglitazone, Methanolic (MeOH) extracts of Taxus yunnanensis (TYE) wood were studied for their impact on bone loss. Pioglitazone (PGZ) has been shown in animal experiments to inhibit bone growth and promote bone resorption. Control (vehicle treatment), Streptozotocin (diabetes), Taxus yunnanensis extract (TYE), Pioglitazone (PGZ), and PGZ +TYE were the five groups of six Wistar albino rats investigated. Each drug was administered through gastric gavage for a total of 35 days. In rats, the effects of TYE on blood glucose, HBA1C, and bone mineral density were investigated. Normal control rats were given saline to drink (NC). Blood glucose levels, HBA1C levels, and bone mineral density all improved after TYE therapy. These findings show that TYE might be beneficial in the treatment of anti-diabetic drug induced bone loss.

Keywords: *Taxus yunnanensis*, pioglitazone; bone resorption, BMD

Introduction

Because persons with Type 2 diabetes have higher bone density as a result of their increased body weight, they are less likely to be diagnosed with osteoporosis or low bone density, which would urge them to take care to avoid fractures [1]. Diabetes promotes osteoclast activity, which leads to bone loss, osteopenia, and osteoporosis [2]. As a result, understanding the processes behind diabetes-related alterations in bone microstructure requires special focus. STZ-induced diabetes has been found as a promising model for studying the pathophysiological processes of bone loss in diabetes in several investigations [3]. Natural products have been shown to be safe and helpful in the treatment of bone loss [4-6]. Synthetic peroxisome proliferator-activated receptor gamma (PPAR) agonists, known as thiazolidinediones (TZDs), are used to treat type 2 diabetes. Clinical investigations, on the other hand, have found that utilizing TZDs to modulate PPAR activity therapeutically might have detrimental consequences for bone metabolism. Pioglitazone has been shown in animal experiments to inhibit bone growth and promote bone resorption. As a result of the ensuing imbalance in bone metabolism, BMD is reduced, and bone fragility rises [13]. Taxus yunnanensis is a species of Taxus that grows in China. The bark and leaves of T. yunnanensis are rich in taxane-type diterpenes, including paclitaxel (Taxols), a potential anticancer drug utilized therapeutically for the treatment of different cancers [14]. We studied the impact of TYE on bone mineral density (BMD) in STZ-treated diabetic rats co-treated with pioglitazone since it is used as an antidiabetic drug in traditional medicine.

Materials and Methods

Animals

In the study, healthy male wistar albino rats weighing 180 to 240 g and aged 3 to 4 months were used. The animals were received from Central Animal House, King Khalid University Abha, Saudi Arabia. Throughout the experiment, the animals were kept in cages and fed a normal pellet diet and filtered water ad libitum under controlled circumstances (light/dark cycle of 12 h/12 h, 50–70 percent humidity, 25 °C 3 °C). For 14 days, the animals were acclimatized to the laboratory setting. The treatment was carried out with the approval of King Khalid University's animal ethics committee and in compliance with the National Institute of Health's guidelines (NIH Publication No. 85-23, revised 1996) for the care and use of laboratory animals.

Induction of diabetes

The pancreatic cell toxin streptozotocin (STZ) (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent) was given intraperitoneally at a dose of 65 mg/kg body weight to cause diabetes in the animals [9, 10, 15]. The identical amount of vehicle was given to all of the rats in the control group. To avoid degradation, each animal's STZ was weighed separately, solubilized with 0.1 ml of freshly produced cold Na citrate buffer (NaB-0.1 M, pH 4.5), and administered within 5 minutes. The STZ injection volume was found to be 1.0 ml/kg. To offset the drug's significant acute hypoglycemia effect, rats were given a 5% glucose solution for hours after ingesting STZ. Blood was drawn from the tail vein three days after STZ injection and tested for blood glucose using a glucometer (Aqua Check, Roche). Diabetic animals were defined as those with fasting blood glucose levels (FGLs) more than 250 mg/dL. Non-diabetic control (vehicle, n = 6), diabetes control (STZ), TYE (100 mg/kg/day, n = 6), PGZ (10 mg/kg/day, n = 6) and combination group (TYE 100 mg/kg/day + PGZ 10 mg/kg/day, n = 6) rats were studied. For 35 days, each drug was administered via gastric gavage once a day. The

animals were checked daily for signs of sickness during the study.

There were no animals that became seriously ill or died before the study was completed. The rats in the control group (n=6) that were given saline instead of streptozotocin had normal blood glucose levels (120 mg/dl). Blood glucose levels were measured once a week for the course of the trial using a Roche Accu Chek advantage glucometer to assess the animals' hyperglycemic condition.

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 with 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 is shown on the Clover A1cTM Self Analyzer's LCD screen.

Bone Mineral Density Measurement

Using dual energy X-ray absorptiometry (DEXA) scanning equipment, the BMD of the left femur and lumbar vertebrae (L1–L4) of rats was evaluated after blood was drawn (Lunar, WI, USA).

Result

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

Table 1: Effect of *TYE* 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	72.22±3.1	73.32±2.1	75.81±2.9	77.39±1.8	81.32±1.6	82.48±1.9	83.40±1.2	85.40±1.06	87.40±1.14
Positive Control	65 mg/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*
TYE	10 mg/kg	259.33±6.2	287.25±8.4*	289.22±8.8*	291.28±7.2*	292.35±8.8*	294.35±8.5*	296.35±8.4*	298.35±9.5*	303.35±9.6*
PGZ	100 mg/kg	254.33±8.3	215.25±9.3*	200.22±7.8*	170.28±9.2*	130.25±8.3*	115.45±7.8*	105.45±10.4*	99.15±8.2*	95.235±8.7*
TYE+ PGZ	100/10 mg/kg	262.33±7.2	244.25±8.4*	236.22±8.6*	214.28±8.4*	176.25±8.2*	157.45±8.7*	133.45±9.5*	104.15±9.2*	92.235±8.6*

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

Table 2 shows that HBA1C levels were greater in the positive control group than in the normal control group (p0.05). TYE was observed to reduce HBA1C levels in comparison to the positive control group, indicating a beneficial impact.

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

Treatment Group	Day 28
Normal Control	5.39±0.13
Positive Control	5.76±0.05*
TYE	5.67±0.02*
PGZ	5.37±0.04*
TYE+ PGZ	5.43±0.13*

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

Diabetic rats showed decreased lumbar (L1–L4) and femoral bone mineral density (BMD), which was restored by TYE therapy (p 0.05). The positive group's BMD differed considerably from the other treatment groups (Table-3). These findings imply that TYE may able to protect bones from the effects of hyperglycemia.

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

	Bone Mineral density(mg/cm3)	
Treatment Group	**Lumbar Vertebrae**	**Femur**
Normal Control	159 ± 2.7	222 ± 2.6
Positive Control	75 ± 2.7*	104 ± 2.2*
TYE	168 ± 1.6*	206 ± 1.6*
PGZ	134 ± 2.2*	166± 2.5*
TYE+ PGZ	169 ± 1.6*	212 ± 1.9*

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

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

This study looks at the impact of TYE on bone quality in a STZ-induced type 2 diabetes animal model. Isotaxiresinol, the primary lignan extracted from the aqueous extract of Taxus yunnanensis wood, was found to have a modest increase in bone production and a substantial inhibitor of bone resorption without having any negative effects on uterine tissue. These findings show that isotaxiresinol might be beneficial in the treatment of postmenopausal osteoporosis, particularly in preventing bone fractures caused by estrogen deprivation [16].

Another research found that water extract of T. yunnanensis had a strong hypoglycemic effect in diabetic rats caused by streptozotocin (STZ). Three lignans, isotaxiresinol, secoisolariciresinol and taxiresinol were isolated as main constituents corresponding to the effect. The antioxidative effects of these lignans were thought to be linked to their hypoglycemic impact [17]. Thiazolidinediones (TZDs) are agonists and insulin sensitizers that target the peroxisome proliferator-activated receptor-r (PPAR-r) in the treatment of T2DM. In women with T2DM, long-term use of TZDs is linked to bone loss and an increased risk of fracture [18].

There have been no trials to investigate if TYE can protect against osteoporosis caused by diabetes and pioglitazone treatment.

TYE treatment reduced bone loss in diabetic rats, according to our findings. In this study, the positive control rats had a lower BMD and a higher blood glucose level, showing that the rat model had been effectively constructed. BMD was improved in the combination group, indicating a protective effect against diabetes-induced bone loss in rats.

Conclusion

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

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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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