International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 13-08-2021, Accepted: 28-08-2021, Published: 13-09-2021
Volume 6, Issue 3, 2021, Page No. 31-33

Role of *Tetrahydroxystilbene glucoside* on bone formation in streptozotocin-induced diabetic rats

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Abstract
Polygonum multiflorum Thunb is a traditional medicinal herb that has long been used to treat age-related ailments. This herb contains tetrahydroxystilbene glucoside (TSG), also known as 2, 3, 5, 4-tetrahydroxystilbene-2-O—D-glucoside. The goal of this trial was to see if TSG could prevent bone loss in diabetic rats who were also given Canagliflozin. *Polygonum multiflorum* Thunb. is a traditional Chinese medicinal herb that has been widely used to treat age-associated diseases. Tetrahydroxystilbene glucoside (TSG), also known as 2, 3, 5, 4-tetrahydroxystilbene-2-O-β-D-glucoside, is a major component of this herb. The present study was designed to investigate the bone loss preventing activity of TSG in diabetic rats co-treated with Canagliflozin (CGF). The Wistar albino rats were placed into five groups, each with six rats: control (vehicle therapy), diabetic group, TSG group, Canagliflozin (CGF), and CGF + TSG group. For a period of 35 days, each medication was given through gastric gavage. TSG-treated diabetic rats had considerably higher levels of insulin and osteocalcin than diabetic control rats. TSG's effects in preventing or treating CGF induced bone loss in diabetic rats appear to be linked to a reduction in bone turnover. These data support the use of TSG as an osteoporosis treatment in diabetics.

Keywords: *Polygonum multiflorum* thunb, osteoporosis, bone protective effect, canagliflozin

Introduction
Bone metabolism includes matrix deposition, mineralization, and resorption, among other things. Numerous studies have demonstrated that dietary components and Phytoconstituents can influence these processes by decreasing bone resorption and so providing skeletal advantages. Many traditional herbal formulae used in ayurveda and Chinese medicine have shown to be effective in pharmacological models of osteoporosis. P. multi florum Thunb. is a traditional Chinese medicinal herb that has been used to treat age-related ailments in China for thousands of years [1]. Male rats with osteoporosis (OP) treated with P. multiflorum Thunb. showed improvements in bone mass, kidney 1-hydroxylase activity, maximal load of the lumbar region and femur, and bone density [2].
One of the key bioactive chemicals in P. multiflorum Thunb is tetrahydroxystilbene gluco side (TSG), also known as 2, 3, 5, 4-tetrahydroxystilbene-2-O—D-glucoside [3]. TSG's anti-OP, anti-inflammatory, anticancer, cardiotoxicity protective, neurotoxicity protecting, and anti-cardiovascular disease actions have all been studied [4]. According to a meta-analysis of 78 randomized controlled trials (RCTs) involving 85,122 individuals, canagliflozin (CGF) appears to increase the risk of fracture, but other SGLT2 (Sodium-glucose transport protein 2) do not [5]. The current study looked at the effects of TSG therapy on bone oxidative stress and turnover indicators in STZ-treated rats who were also given CGF.

Materials and Methods
Animals
The experiment was carried out with Sprague Dawley rats weighing 100–120 g from King Khalid University's Central Animal House in Abha, Saudi Arabia. The rats were housed in a temperature-controlled environment (21±°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
To chemically induce diabetes-like hyperglycemia in rats, a single intraperitoneal injection of Streptozotocin (STZ) dissolved in 10 mM citrate solution was utilized (pH 4.5). To avoid drug-induced hypoglycemia, the rats were given 5% glucose water for two days after receiving STZ. If the animals' fasting blood glucose levels were more than 11 mmol/L after a week of injection, they were categorized as diabetic. The control rats received the same dose of isotonic NaCl injection as the experimental rats [6].

Experimental design
Control (vehicle, Non-Diabetic control, n=6), Diabetic control (STZ), TSG (60/kg/day, n = 6), Canagliflozin (CGF) (40 mg/kg/day, n = 6) and combination group (TSG 60/kg/day and CGF 40 mg/kg/day, n=6) were the five groups. Each medicine was given by gastric gavage once a day for 35 days. Throughout the experiment, the animals were checked daily for signs of illness. There were no animals that grew really ill or died before the completion of the experiment.
All of the animals were fasted overnight and their blood glucose levels were evaluated at the end of the trial. The animals were given ketamine (80 mg/kg) and xylazine (8

mg/kg) anaesthesia before being killed at the end. At the stifle joint, the femur and tibia were separated. Blood samples (10-15 mL) were obtained from the rats through heart puncture into a plain red top tube with no anticoagulants. After centrifugation at 4000 rpm for 15 minutes, the serum was kept in aliquots at 80 °C.

Marker of bone formation and bone resorption

Serum was used to determine all bone formation and resorption parameters. The osteocalcin level was measured using a Rat Mid Osteocalcin ELISA kit (IDS, UK), whereas the BALP level was measured using a rat BALP ELISA kit (Qayee, Shanghai). The bone resorption DPD was measured using a rat deoxypyridinoline (DPD) ELISA kit (Qayee, Shanghai) (Qayee, Shanghai). All samples were run three times, and the optical density was determined at 450 nm using a microplate reader (BioTek, USA).

Statistical Analysis

ANOVA was used to assess all of the data. Duncan's multiple comparison test was used to determine the significance. All of the analyses were done with a 95% confidence level.

Results

Fasting blood glucose and serum insulin

The DC rats had higher fasting blood glucose and lower insulin levels than the NC animals (Table 1). Treatment with TSG significantly reduced fasting blood glucose levels while significantly boosting serum insulin levels in diabetic rats.

Table 1: Effects of TSG 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	4.79 ± 0.31a	4.94 ± 0.13a	4.17 ± 3.15c
DC	19.04 ± 3.37b	30.17 ± 2.66b	1.58 ± 0.16a
CGF	27.29 ± 3.54c	20.74 ± 3.74c	1.88 ± 0.34a
TSG	25.91 ± 5.45c	17.37 ± 4.84c	2.29 ± 0.16b
CGF + TSG	29.32 ± 3.64c	19.73 ± 3.65c	1.78 ± 0.27a

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

Bone turnover markers

Although serum DPD was significantly greater in the STZ group than in the NC group, blood osteocalcin was significantly lower following the STZ injection (Table 2). Despite no significant variations in BALP values between the treated groups, serum osteocalcin levels increased while DPD levels decreased after TSG treatment.

Table 2: Changes in serum osteocalcin, BALP and DPD of various experimental groups (data represent mean ± SD).

Groups	Bone formation markers		Bone resorption marker
	Osteocalcin (ng/ml)	BALP (ng/ml)	DPD (ng/ml)
NC	129.81 ± 6.8^{c}	98.49 ± 7.61^{b}	161.08 ± 5.06^{b}
DC	14.43 ± 0.87^{a}	68.16 ± 4.62^{a}	189.17 ± 0.12^{c}
CGF	59.40 ± 8.27^{b}	82.92 ± 0.36^{a}	119.16 ± 4.57^{ab}
TSG	146.66 ± 4.10^{d}	74.29 ± 8.31^{a}	146.54 ± 0.32^{a}
CGF + TSG	156.62 ± 4.23^{d}	73.30 ± 8.41^{a}	147.54 ± 0.29^{a}

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

Discussion

Oxidative stress has been shown to play a role in the etiology of osteopenia, osteoporosis, and osteoarthritis in both clinical and preclinical studies [7-9]. As a result, additional research is needed into the relationship between oxidative stress and bone quality. According to this study, DC rats had higher levels of oxidative damage indicators. Oxidative stress and hyperglycemia have been found to affect bone metabolism and architecture by altering the activity of osteoclasts and osteoblasts [8].

Blood DPD levels increased in DC rats, while serum osteocalcin and BALP activity decreased, according to the findings of this study. Bone turnover suppression is a key feature of T1DM-related bone disease, according to Zhukouskaya *et al*. (2015) [10]. Previous results of elevated serum DPD in rats with osteoarthritis [10] and osteopenia confirm our findings [11]. Another fascinating discovery from this study is that after TSG treatment, blood osteocalcin levels increased but DPD levels decreased (Table 2). Similar findings have been seen with a range of herbs that have osteoprotective properties [12].

Despite the fact that osteocalcin is a particular osteoblast marker that is tightly linked to histological changes [13], blood OC levels fluctuate with meal intake [14]. Osteocalcin does not appear to be as sensitive a marker as BALP, according to previous research [14]. BALP activity of TSG treated rats is still low, implying that mineral metabolism is still compromised. BALP is a bone-specific alkaline phosphatase isoform that is produced by osteoblasts and reflects mineral metabolism [15]. The ratio of osteocalcin to DPD was virtually identical in the TSG and NC groups, implying that TSG treatment had nearly established an equilibrium between bone production and bone resorption.

The TSG rats exhibited a much higher BMD and a significantly lower DPD as compared to other STZ-treated animals (Table 2). This finding adds to a growing body of evidence that TSG therapy can assist STZ-treated rats avoid CGF-induced bone loss.

Conclusion

CGF appears to have a significant bone-protective effect in STZ-treated rats, according to our findings. By increasing bone mineral density, CGF appears to drive osteoblast genesis and bone production.

Acknowledgments

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.

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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