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PLASTRUM TESTUDINIS PROMOTES BONE FORMATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

In vivo studies have shown that extracts from the *plastrum testudines* extract (*PTE*) promote osteoblastic activity in glucocorticoid induced osteoporosis (GIOP). The goal of this research is to look into the preventive effects of *plastrum testudines* extract and to look into the therapeutic targets of *plastrum testudines* extract in diabetic osteoporosis. Normal control rats received saline (NC), diabetic control rats received saline (DC), and two groups of diabetic rats received 1000mg/kg body weight of metformin (MET), and 30mg/kg body weight of *plastrum testudines* extract respectively. The bone mineral density (BMD) and blood glucose levels were assessed. *Plastrum testudines* extract caused a substantial increase in bone quantity, according to the findings. This showed that *plastrum testudines* extract can help prevent and treat diabetic osteoporosis by improving bone mineral density.

KEYWORDS

Diabetic osteoporosis, *Plastrum testudinis* extract and Streptozotocin induced diabetes.

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INTRODUCTION

Diabetes and osteoporosis are common in older individuals, and both are linked to an increased risk of fracture. Many modern medications are derived from natural materials, which serve as sources of inspiration. Diabetes affects an estimated 422

million individuals globally, and its incidence rises with age, reaching 25% among those ≥65 in the United States. Similarly, the risk of developing osteoporosis increases with age; as a result, diabetes and osteoporosis are prevalent among the elderly. Despite having higher bone mineral density (BMD) than those without diabetes, those with diabetes have a higher fracture risk.

Poor bone quality, diabetes problems, physical impairment, and an increased risk of falling are all possible contributors to this seeming contradiction. It's crucial to look at how osteoporosis therapies affect bone health in diabetic patients[1]. In China, *Plastrum testudinis* extract (PTE) is a common treatment for bone disorders. *Plastrum testudinis* extracts have been demonstrated to enhance osteoblastic activity in earlier research. *Plastrum testudinis* extract, on the other hand, is rarely studied on its own in the context of diabetic osteoporosis. As a result, the goal of this study is to see if *Plastrum testudinis* extract can help prevent diabetic osteoporosis from affecting bone quantity[2].

MATERIAL AND METHODS

Animals

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

Induction of diabetes

To induce diabetes in the animals, the pancreatic-cell toxin streptozotocin (STZ) was administered intraperitoneally at a dosage of 65mg/kg body weight (Sigma Chemical Co., freshly dissolved in sterile saline, 0.9 percent)[3,4]. In the control group, all of the rats received the same quantity of vehicle. STZ was weighed individually for each animal, solubilized with 0.1ml of freshly produced cold Na citrate buffered (NaB-0.1 M, pH 4.5), and given within 5 minutes to prevent deterioration. A volume of 1.0ml/kg of STZ was estimated. Rats were administered a 5% glucose solution for 48 hours after the injection to offset the substantial acute hypoglycemia impact of STZ.

Three days following STZ injection, blood was collected from the tail vein, and samples were analyzed for blood glucose using a glucometer (Aqua Check, Roche). Diabetic animals had fasting blood glucose levels (FGLs) more than 250mg/dL. The rats were divided into four groups, each consisting of six individuals. Normal rats received saline (NC), diabetic rats received saline (DC), and two groups of diabetic rats received 1000mg/kg body weight of metformin (MET), and 30mg/kg body weight of *Plastrum testudinis* extract respectively. To evaluate the animals' hyperglycemic state, blood glucose levels were tested once a week for the duration of the study using a Roche Accu Chek advantage glucometer. The study did not include the animals which did not acquire blood glucose levels more than 250mg/dL. The rats administered saline instead of streptozotocin in the control group (n=6) had normal blood glucose levels (120mg/dl).

Determination of fasting blood glucose

Blood samples were collected from the rats' tail veins to measure blood glucose levels using a glucometer after they had been fasted for 12-14 hours. After the rats' tails have been cleaned with 70% (v/v) ethanol, blood will be drawn using a 1-ml needle, placed on a glucose strip, and measured with a glucometer.

Determination of intra-peritoneal glucose tolerance test

All of the rats were fasted for 12-14 hours and blood were collected from the tail vein as a baseline. The rats were subsequently given 2g/kg BW of a 40% (w/v) glucose solution intraperitoneally. Blood will be taken from the tail vein and analysed for blood glucose using a glucometer after 30, 60, 90, and 120 minutes after

starting glucose treatment. Fasting blood sugar values of less than 250mg/dl were used to prove diabetes in these rats.

Determination of hemoglobin A1c

After blood samples from the tail vein are collected and deposited on a test cartridge, haemoglobin A1c (HbA1c) will be analysed using a Clover A1cTM Self-Analyzer. The Clover A1cTM Self-Analyzer's LCD screen will show the percentage of HbA1c in the blood sample.

Bone Mineral Density Measurement

After blood was taken, the BMD of the left femur and lumbar vertebrae (L1–L4) of rats was measured with a dual energy X-ray absorptiometry (DEXA) scanning device (Lunar, WI, USA).

RESULTS AND DISCUSSION

The glucose profiles of the positive control group (STZ) deteriorated over time (Table No.1). However, *Plastrum testudinis* extract were demonstrated to protect against diabetes progression.

HBA1C levels were higher in the positive control group than in the normal control group ($p<0.05$), as indicated in Table No.2. In contrast to the positive control group, *Plastrum testudinis* extract was shown to lower HBA1C levels, implying a favourable effect.

Diabetic rats showed decreased lumbar (L1–L4) and femoral bone mineral density (BMD), which was restored by *Plastrum testudinis* extract therapy ($p<0.05$). The positive group's BMD differed considerably from the other treatment groups (Table No.3). These data suggest that *Plastrum testudinis* extract may be able to protect bones against hyperglycaemia's consequences.

Statistical analysis

The data was provided 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 utilised. A 'p' value of less than 0.05 is considered statistically significant.

Discussion

Some studies, although not all, have discovered an increase in cortical porosity in people with type 2 diabetes, which might explain why this population has a greater fracture risk while having higher BMD[5,1]. *Plastrum testudinis* extract enhanced BMSC osteogenic development and mineralization substantially after 7 and 14 days in culture, according to an in-vitro research. *Plastrum testudinis* extract and osteogenic induction were shown to have a strong synergistic effect. Tnfr2, Traf2, Pi3k, Akt, and Gsk3 mRNA expression were increased by *Plastrum testudinis* extract, whereas Tnfr2, Traf2, Pi3k, Akt, and Gsk3 mRNA expression were down regulated by *Plastrum testudinis* extract. *Plastrum testudinis* extract reduced the expression of TNFR2, TRAF2, and p CATENIN proteins while promoting the expression of p-PI3K, p-AKT, and p-GSK3.

In 293T cells, Tnfr2 was also found to be a functional target of Let-7f-5p. *Plastrum testudinis* extract enhanced bone quantity and quality, bone strength, and bone turnover in another research, as well as reduced histological damage after glucocorticoid treatment and withdrawal. *Plastrum testudinis* extract decreased bone resorption by lowering CTSK protein expression and promoting Runx2 mRNA and protein expression to different degrees[5]. *Plastrum testudinis* extract's effects on bone quality in a STZ-induced type 2 diabetic animal model are investigated in this work. Previous research has looked at the impact of RSG on bone loss, formation, and resorption, as well as bone structure and content, and bone mechanical characteristics[5]. *Plastrum testudinis* extract treatment had a positive effect on bone, as demonstrated by increases in BMD.

Table No.1: Effect of *Plastrum testudinis* extract 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	5mL/kg	77.22± 4.2	75.32± 3.3	79.81± 3.6	78.40± 1.8	79.60± 1.6	82.46± 1.8	84.40± 1.09	85.40± 1.03	87.40± 1.22
Positive Control	65mg/kg	262.54± 9.2*	297.35± 8.8*	315.21± 11.62*	38.73± 9.8*	350.72± 9.4*	370.76± 10.5*	395.75± 11.5*	414.73± 10.8*	438.75± 9.6*
Plastrum testudinis extract	500mg/kg	268.33± 7.3	288.25± 9.6*	294.22± 8.8*	299.28± 8.2*	314.35± 8.5*	313.34± 9.8*	309.36± 10.6*	322.35± 8.2*	340.32± 9.7*
Metformin	1000mg/kg	261.33± 7.6	248.25± 10.4*	236.22± 7.6*	214.28± 8.6*	181.28± 8.6*	150.55± 8.7*	124.45± 9.2*	102.15± 8.2*	90.035± 8.7*

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

Table No.2: Effect of *Plastrum testudinis* extract on Glycoslyted Haemoglobin (HBA1C)

S.No	Treatment Group	Day 28
1	Normal Control	5.44±0.18
2	Positive Control	5.81±0.07*
3	*Plastrum testudinis* extract	5.69±0.05*
4	Metformin	5.40±0.06*

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

Table No.3: Effect of *Plastrum testudinis* extract on the bone mineral density of the lumbar vertebrae and femur bone

S.No	Treatment Group	Bone Mineral density(mg/cm3)	
		Lumbar Vertebrae	Femur
1	Normal Control	177 ± 2.3	224 ± 2.4
2	Positive Control	79 ± 2.5*	103 ± 2.4*
3	*Plastrum testudinis* extract	157 ± 1.6*	203 ± 1.9*
4	Metformin	133 ± 2.4*	186± 2.5*

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

CONCLUSION

Our results suggest that *Plastrum testudinis* extract 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 diabetes.

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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".

BIBLIOGRAPHY

1. Ay B, Parolia K, Grasselli G, Cooper D M L, Davies J E. Hyperglycemia compromises rat cortical bone by increasing osteocyte lacunar density and decreasing vascular canal volume, *Commun Biol,* 3(1), 2020, 20.

2. Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: Possible cellular and molecular mechanisms, *World J Diabetes,* 2(3), 2011, 41-48.
3. Logar D B, Komadina R, Prezelj J, Ostanek B, Trost Z, Marc J. Expression of bone resorption genes in osteoarthritis and in osteoporosis, *J Bone Miner Metab,* 25(4), 2007, 219-225.
4. Feng X, McDonald J M. Disorders of bone remodelling, *Annu Rev Pathol,* 6(1), 2011, 121-145.
5. 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,* 866, 2020 172805.

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