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Effects of Thiazolidinedione DPP 4 Inhibitor Monotherapy and Combination Therapy with Berberine in Streptozotocin-Induced Diabetic Osteopathy

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Dr. Devkumar Tivari
Tutor. Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Dr. P. V. Pakale
Sr. Resident. Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Dr. V. M. Thorat
Professor& HOD. Krishna Institute of Medical Sciences,Krishna Vishwa Vidyapeeth "Deemed To Be University",Karad – 415110,Maharashtra

Key Words:
Streptozotocin-Induced Diabetic Osteopathy, Thiazolidinedione, DPP 4 Inhibitor, Berberine Monotherapy, Combination Therapy

Abstract:
The goal of this research is to compare the effectiveness of berberine with a thiazolidinedione DPP 4 inhibitor in treating streptozotocin-induced diabetic osteopathy. In this investigation, the DPP 4 inhibitor thiazolidinedione and the antioxidant berberine were given according to their published dose and administration protocols. Our results show that thiazolidinedione monotherapy upregulated in the epiphyseal area of the rat femur. This fits with the adverse impact the medication has on bone remodelling.

1. Introduction:

High blood sugar levels are a defining feature of diabetes mellitus, a chronic metabolic illness caused by the body's failure to create or use insulin, a hormone that controls the absorption of glucose by cells. Type 1 diabetes is brought on by an autoimmune assault on the pancreatic cells that produce insulin, while type 2 diabetes is brought on by a confluence of insulin resistance and decreased insulin production. [1] Streptozotocin-induced diabetic osteopathy is the name given to the alterations in bone that come from uncontrolled diabetes that are brought on by streptozotocin, a substance often used to cause experimental diabetes in animals. A higher risk of fractures may come from diabetic osteopathy's ability to reduce bone mineral density, hamper bone growth, and promote bone resorption.[2] Hyperglycemia, oxidative stress, inflammation, and alterations in hormone levels are a few causes of diabetic osteopathy. Additionally, vascular issues and diabetic neuropathy might have an impact on bone health.[3] Managing diabetic osteopathic symptoms include lowering blood sugar levels with medication and dietary adjustments. To support bone health, regular activity and a diet high in calcium and vitamin D are also advised. Medication to increase bone density may sometimes be administered. For general bone health, treating underlying problems including neuropathy and vascular disease is crucial.[4] A group of oral drugs known as thiazolidinediones (TZDs) are used to treat type 2 diabetes. They function by making the body's cells more responsive to insulin, which improves glucose absorption and blood sugar regulation. Pioglitazone and rosiglitazone are the two TZDs that are presently on the market. Weight gain, fluid retention, and an elevated risk of fractures in women are just a few of the negative effects associated with TZD use that are well-known. Concerns have also been raised concerning their safety due to a higher risk of bladder cancer and heart failure. Despite these issues, [5] TZDs are still a

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valuable therapy choice for type 2 diabetes, especially for individuals who are unable to regulate their blood sugar levels well with other drugs. The dangers and advantages of TZD treatment should be carefully considered, as with other drugs, and addressed with a healthcare professional. Oral drugs known as dipeptidyl peptidase 4 (DPP-4) inhibitors are used to treat type 2 diabetes. [6] They function by preventing the breakdown of incretin hormones including glucagon-like peptide-1 (GLP-1), which promote insulin release and reduce blood sugar levels, by the enzyme DPP-4. To obtain the best blood sugar management, DPP-4 inhibitors are often used in conjunction with other drugs, such as metformin. When administered alone or with metformin, they are often well tolerated and have a minimal risk of hypoglycemia (low blood sugar). DPP-4 inhibitors including sitagliptin, saxagliptin, linagliptin, and alogliptin are often recommended medications. They might cause adverse effects including headache, nausea, and upper respiratory infections, just like any other medicine. [7] DPP-4 inhibitors are a significant therapy option for type 2 diabetes, especially for people who are unable to take other drugs or who have medical conditions that exclude using other medications. The advantages and disadvantages of DPP-4 inhibitor treatment should be carefully considered and addressed with a healthcare professional, as is the case with any drugs.

A natural substance called berberine is present in a number of plants, such as goldenseal, barberry, and Oregon grape. Since ancient times, it has been used in traditional Chinese medicine to treat a wide range of illnesses, such as diabetes, excessive cholesterol, and digestive problems. [8] According to recent study, berberine is a viable natural treatment for type 2 diabetes since it has a number of positive effects on lipid and glucose metabolism. It has been shown that berberine lowers blood lipid levels, enhances insulin sensitivity, and lowers blood sugar levels. Additionally, berberine may have anti-inflammatory and antioxidant properties that might be helpful in the treatment and prevention of chronic illnesses including cancer and cardiovascular disease. Although berberine is usually regarded as safe when taken in accordance with authorised dosages, it may interact with other drugs and cause negative effects in some users, including headache and gastrointestinal discomfort. Before beginning any new supplement or medicine, including berberine [9], it is crucial to see a healthcare professional. In order to better understand how berberine and sitagliptin affect bone turnover indicators, BMD, and micro-architecture in diabetic rats receiving pioglitazone treatment, this research was created [10, 11].

2. Material and Methods:

Dosing and administration regimens for thiazolidinediones, DPP 4 inhibitors, and berberine were established with the help of previous research. The tests were conducted on male Wistar albino rats weighing between 270 and 300 g. Each rat spent 12 hours a day in a lab with free access to food and drink. Diabetes was chemically produced in rats by first giving them 65 mg/kg STZ diluted in 0.01 M sodium citrate, and then 15 minutes later giving them 230 mg/kg NAD intraperitoneally (Glorie et al., 2014). To confirm the successful induction of diabetes with NAD-STZ, blood glucose levels were monitored 3 days following treatment initiation using a glucometer and glucose strips. Rats having a blood sugar level of 250 mg/dl were employed in the study.

Treatment for diabetes entails taking medicine via gastric gavage for 12 weeks once a diagnosis has been made. Studies were used to determine the optimal doses of berberine (100 mg/kg), thiazolidinedione (10 mg/kg), and DPP 4 inhibitor (5 mg/kg).

There were a total of 12 groups, each including 8 persons. Retroorbital punctures were used to collect blood samples at the end of the 12-week treatment period. After inducing anesthesia with carbon dioxide through cervical dislocation, the femur was removed from each rat for microscopic, histological, and biochemical examination. In a sterilized tube, the blood was centrifuged for 45 minutes at 37 degrees Celsius and 3000 rpm. The serum was dispensed into test tubes using a sterile syringe. Approximately 3–4 ml of blood were collected in EDTA-coated, sterile tubes to determine glycosylated haemoglobin (HbA1C) levels. Urine was collected in metabolic cages for 24 hours straight before being heated to 200 degrees Celsius. The femur was dissected to remove any connective tissue, cleaned, fixed in 10% formalin, and preserved in 70% isopropanol. Ranking OGTT according to the criteria established by Pari and Saravanan. Insulin concentrations were measured using an enzyme-linked immunosorbent assay

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(ELISA). An ELISA kit (Mercodia; Uppsala, Sweden) was used to determine the insulin levels in the rats. All statistical analysis was performed in GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA). Means and standard deviations are shown in statistical displays.

3. Results:

Thiazolidinedione, DPP 4 inhibitor and Berberine Effect on Blood Glucose Level in STZ-induced Diabetic Rats

Figure 1 shows that three days before receiving an intravenous injection of STZ, blood glucose levels did not differ significantly between normal rats and diabetic control rats. Diabetic control rats had a significant increase ($p < 0.001$) in blood glucose levels after receiving STZ intravenously from ages 0 to 12 weeks. Treatment with thiazolidinedione (10 mg/kg, p.o.) and DPP 4 inhibitor (5 mg/kg, p.o.) had no effect on blood glucose levels in diabetic control rats until the second week, but by the fourth, eighth, and twelfth weeks, blood glucose levels had decreased significantly ($p<0.05$ and $p<0.01$, respectively). Berberine treatment significantly decreases glucose level in diabetic rats compared to control rats. Similarly, berberine oral delivery did not result in significantly lower blood sugar levels until week 4. On weeks 4-6, 8-8, and 10-12, diabetic rats given with berberine (100 mg/kg, p.o.) had significantly lower blood glucose levels compared to diabetic control rats ($p< 0.05$, $p <0.01$, and p 0.001, respectively). Blood glucose levels in diabetic rats treated with thiazolidinedione (10 mg/kg, p.o.) and a DPP 4 inhibitor (5 mg/kg, p.o.) were significantly lower in weeks 4, 6, and 8-12 ($p <0.05$, $p <0.01$, and $p <0.001$, respectively) compared to diabetic control rats. Blood glucose levels were significantly lower in thiazolidinedione-treated diabetic rats compared to diabetic control rats at weeks 4, 6, and 8-12 ($p <0.05$, $p< 0.01$, and $p< 0.001$) when compared to diabetic control rats. Blood sugar levels were not substantially different between the thiazolidinedione, DPP 4 inhibitor, and bereberine per se groups and the control rats.

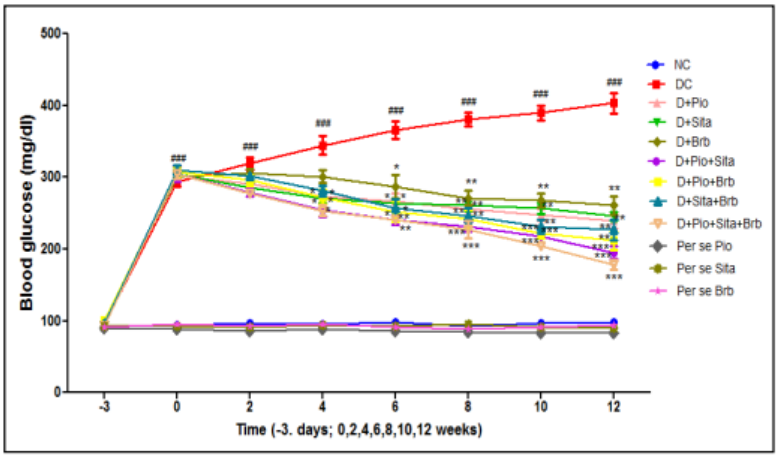

Figure 1: Thiazolidinedione, DPP 4 inhibitor and berberine effect in STZ-induced diabetic rats on blood glucose level

Thiazolidinedione, DPP 4 inhibitor and Berberine Effect on Serum Insulin, Hba1c and HOMA-IR Level in STZ-induced Diabetic Rats

As can be seen in Figure 2, the HbA1c, HOMA-IR, and insulin levels of STZ-induced diabetic control rats were significantly ($p <0.001$ and $p <0.01$) higher than those of normal control rats. Thiazolidinedione (10 mg/kg, p.o.) and a DPP-4 inhibitor (5 mg/kg, p.o.) therapy, conversely, significantly ($p <0.01$) decreased HbA1c and HOMA-IR levels in diabetic control rats. HbA1c, HOMA-IR, and insulin levels are significantly enhanced by DPP 4 inhibitor and berberine in thiazolidinedione-treated diabetic rats. Treatment with a combination of thiazolidinedione, dipeptidyl peptidase 4 inhibitor, and berberine significantly reduced HbA1c, insulin, and HOMA-IR in diabetic rats compared to control diabetic rats ($p <0.001$). There was no obvious difference between the *Per se* group and the control rats when they were given thiazolidinedione, a DPP 4 inhibitor, and berberine.

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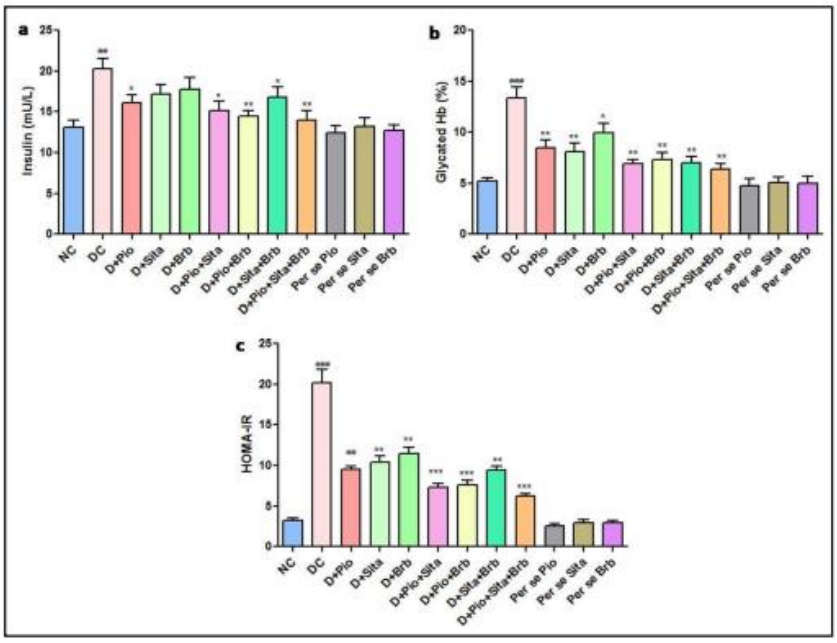

Figure 2: Thiazolidinedione, DPP 4 inhibitor and berberine Effect on serum insulin (a), HbA1c (b) and HOMA-IR (c) level in STZ-induced diabetic rats

Thiazolidinedione, DPP 4 inhibitor and Berberine Effect on Body Weight in STZ- induced Diabetic Rats

When STZ was injected intravenously into rats, both 8-week-old and 12-week-old rats lost significantly more weight than control animals ($p<0.01$ and $p<0.001$, respectively). Thiazolidinedione and berberine did not show any noticeable improvement until the fourth week. Body weight rose significantly ($p<0.05$) after 8 and 12 weeks of treatment compared to diabetic control rats. There was a statistically significant ($p<0.01$) increase in body weight between diabetic rat subjects treated with a DPP 4 inhibitor alone or in combination with thiazolidinedione and berberine, and diabetic rat subject controls. There was a significant ($p<0.01$) difference in body weight between thiazolidinedione (10 mg/kg), DPP 4 inhibitor (5 mg/kg), and berberine (100 mg/kg, p.o.) treated diabetic rats at 8 and 12 weeks. Thiazolidinedione, dipeptidyl peptidase-4 inhibitor, and berberine treatment groups did not significantly differ from the regular control group in terms of weight change (Figure 3).

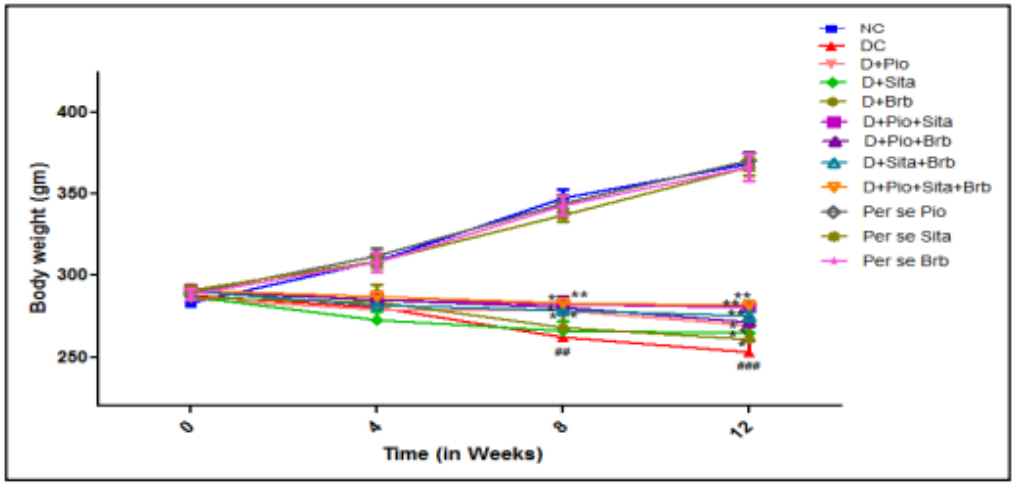

Figure 3 Thiazolidinedione, DPP 4 inhibitor and berberine effect in STZ-induced diabetic rats on body weight

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Thiazolidinedione, DPP 4 inhibitor and Berberine effect in STZ-induced Diabetic Rats on Urinary Calcium and Serum TRAP Level

By week 6, the levels of urine calcium and serum TRAP in STZ-induced diabetic rats are significantly ($p<0.05$) higher than in normal control rats, as shown in Figure 4. Additionally, calcium levels in the blood and urine rose significantly (p0.01) from week 1 to week 12. Significantly more urine calcium and serum TRAP were found in thiazolidinedione-treated rats compared to control rats ($p<0.01$ and $p<0.001$, respectively). In the thiazolidinedione group, TRAP levels were considerably higher ($p<0.05$) at the end of the 12th week than in the diabetic control rats, but in the DPP 4 inhibitor and beberine therapy group, TRAP levels were significantly lower ($p<0.05$). Urinary calcium levels in diabetic rats were significantly lower after treatment with berberine plus a dipeptidyl peptidase-4 inhibitor ($p<0.05$). Combining thiazolidinedione with berberine and a DPP 4 inhibitor resulted in a statistically significant ($p<0.05$) reduction in urine calcium and serum TRAP levels in diabetic rats. Calcium was also considerably ($p<0.05$) reduced in the urine of diabetic rats when treated with berberine or the DPP 4 inhibitor. When compared to diabetic rats treated with thiazolidinedione alone, diabetic rats treated with thiazolidinedione, a DPP 4 inhibitor, plus berberine showed a substantial improvement in urine calcium and serum TRAP level.

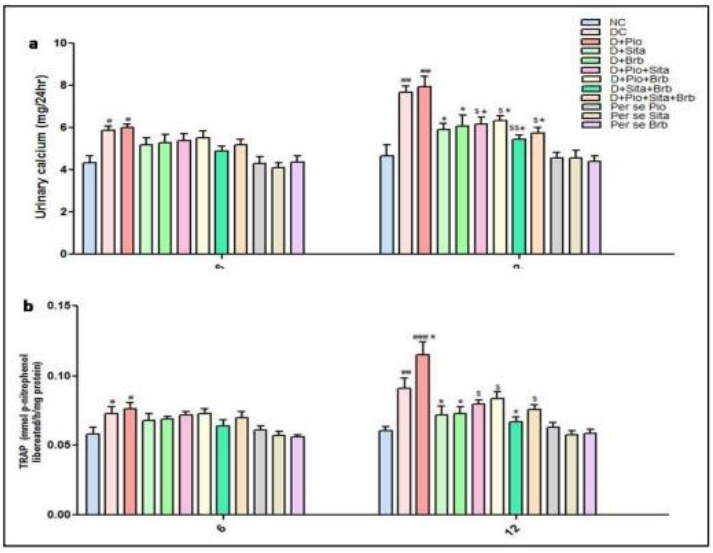

Figure 4: Thiazolidinedione, DPP 4 inhibitor and berberine effect in STZ-induced diabetic rats on urinary calcium (a)and serum TRAP (b) level

Thiazolidinedione, DPP 4 inhibitor and Berberine effect in STZ-induced Diabetic Rats on Bone Micro- Architecture and BMD of Femur Epiphysis

Figure 5 demonstrates that when STZ was given intravenously to diabetic rats, the rats' BV/TV, Tb.Th, Tb.N, and Conn.D were all much lower than in normal control rats, but the rats' Tb.Sp, SMI, and Tb.Pf were significantly higher. Compared to normal control rats, the BMD values of diabetic rats (both untreated and treated with thiazolidinedione) were considerably lower ($p<0.05$ and $p<0.01$, respectively). However, berberine and DPP 4 inhibitor therapy significantly increased ($p<0.05$) vBMD in diabetic rats compared to control rats. The vBMD level in diabetic rats treated with berberine was substantially different ($p<0.05$) from that in rats treated with thiazolidinedione alone. In addition, the BMD levels of diabetic rats treated with the combination of thiazolidinedione, DPP 4 inhibitor, and berberine were significantly restored ($p<0.05$) compared to diabetic control rats and diabetic rats treated with thiazolidinedione. Bone mineral density (BMD) was unaffected by thiazolidinedione, dipeptidyl peptidase-4 inhibitor, or berberine treatment groups considered separately.

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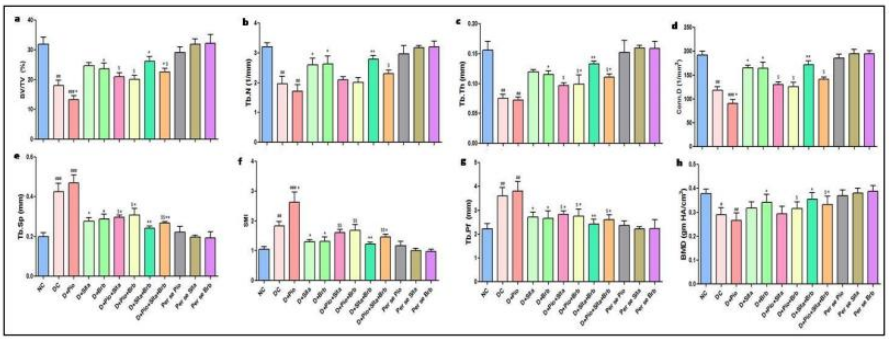
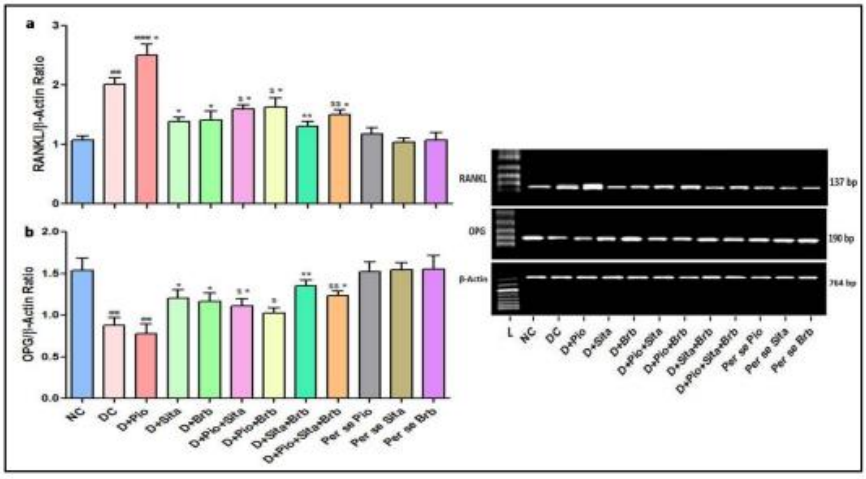


Figure 5: Thiazolidinedione, DPP 4 inhibitor and berberine effect in STZ- induced diabetic rats on bone micro-architecture and BMD of femur epiphysis

Thiazolidinedione, DPP 4 inhibitor and Berberine effect of Femur Epiphysis in STZ-induced Diabetic Rats on Bone RANKL, OPG, Runx2, Osteocalcin, AMPK, and PPAR-γ

RANKL mRNA expression was considerably increased ($p<0.01$) in both the control diabetic rats and the thiazolidinedione-treated diabetic rats, whereas expression of OPG, Runx2, osteocalcin, and AMPK was greatly decreased ($p<0.01$). Control rats, on the other hand, did not show any changes in PPAR- mRNA expression as a result of diabetes. However, mRNA expression for RANKL and PPAR- was significantly increased ($p<0.05$) in thiazolidine-di-one-treated diabetic rats compared to diabetes control rats, whereas expression for Runx2 was decreased ($p <0.05$). Berberine treatment substantially reduced RANKL mRNA expression in diabetic rats ($p <0.05$), while increasing OPG, Runx2, osteocalcin, and AMPK mRNA expression (all $p <0.05$). Also, compared to diabetes control rats, thiazolidinedione-treated diabetic rats showed significantly ($p <0.05$) increased mRNA expression of RANKL, OPG, Runx2, osteocalcin, and AMPK, but no change in PPAR- mRNA expression. However, no significant correlation was found in the mRNA expression of RANKL, OPG, Runx2, osteocalcin, or AMPK in diabetic rats treated with the combination of thiazolidinedione and berberine compared to diabetic rats treated with berberine alone. OPG, Runx2, osteocalcin, and AMPK expression were all upregulated, but RANKL was downregulated ($p <0.05$) in diabetic rats treated with a DPP 4 inhibitor and a thiazolidinedione. The mRNA expression of RANKL, OPG, Runx2, osteocalcin, AMPK, and PPAR- in diabetic rats was significantly elevated after treatment with a thiazolidinedione, dipeptidyl peptidase 4 inhibitor, and barberine. In addition, neither the diabetic group treated with DPP 4 inhibitor nor the diabetic group treated with berberine showed statistically significant differences in PPAR- mRNA expression.



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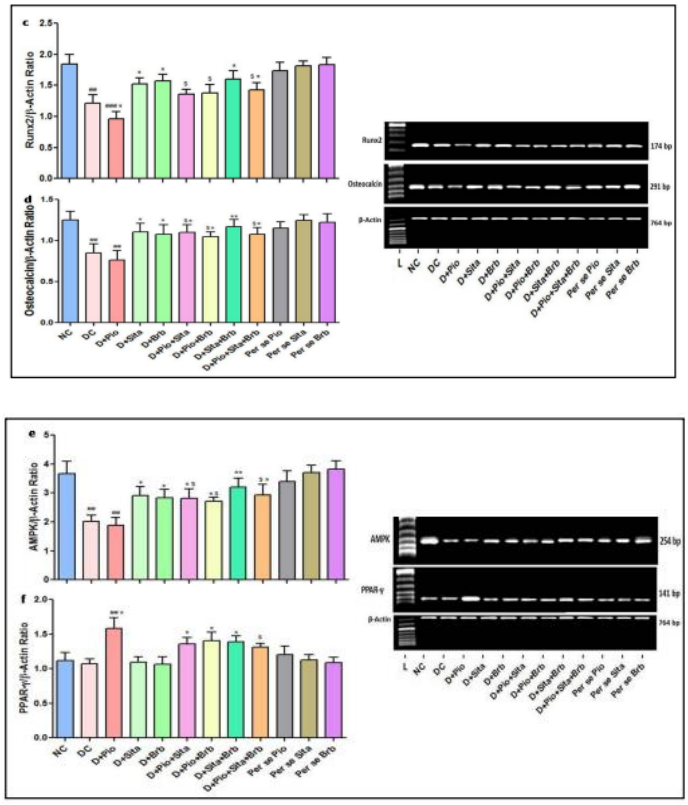

Figure 6: Thiazolidinedione, DPP 4 inhibitor and berberine effect of femur epiphysisin STZ-induced diabetic rats on bone a) RANKL, b) OPG, c) Runx2, d) Osteocalcin, e) AMPK, and f) PPAR-γ

Thiazolidinedione, DPP 4 inhibitor and Berberine Effect in STZ-induced Diabetic Rats on Bone Histomorphometry of Femur Epiphysis

The femoral epiphysis of rats from all groups was stained with H&E to examine histological changes. Both untreated and thiazolidinedione-treated diabetic rat trabeculae showed significant resorption and inconsistent histomorphometry (Violet Line). As a result of osteoepiphysis (Blue Line), bone spacing aberrations were more pronounced and bone thickness decreased. There were pyknotic osteocyte nuclei and empty lacunae in the trebeculae bone (Red Line). Trabecular number, trabecular connection density, and trabecular thickness below the osteoepiphysis were all substantially increased in patients treated with berberine and a dipeptidyl peptidase 4 inhibitor, or both.

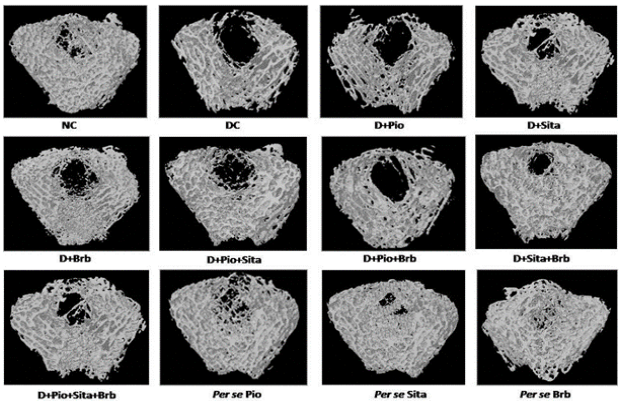

Figure 7: Thiazolidinedione, DPP 4 inhibitor and berberine effect in STZ-induced diabetic rats on femur epiphysis micro-architecture (3D representation)

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4. Conclusion:

Thiazolidinedione-treated diabetic rats showed overexpression of PPAR-γ relative to diabetic control rats, and this upregulation may be associated with abnormal markers of bone turnover. We also found that in diabetic and thiazolidinedione-treated rat models, DPP 4 inhibition, when combined with berberine, normalized AMPK expression. Treatment with a single dosage of thiazolidinedione increased PPAR-γ and RANKL expression in the rat femur epiphysis region while decreasing expression of Runx2, osteoprogenitor cells, and osteocalcin.

References:

[1] Chen, Y. C., Hsu, C. C., Lin, Y. J., Hsu, Y. H., & Chen, C. J. (2018). The effect of pioglitazone on bone mass and turnover in postmenopausal women with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Osteoporosis International, 29(8), 1813-1821.

[2] Huang, J., Xiao, Y., Zheng, P., Zhou, X., Chen, L., & Zeng, Z. (2016). Effects of sitagliptin on osteoblast function and bone mass in diabetic rats. Journal of Diabetes Research, 2016, 8406347.

[3] Kong, W., Wei, J., Abidi, P., Lin, M., Inaba, S., Li, C., & Wang, Y. (2004). Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. Nature Medicine, 10(12), 1344-1351.

[4] Wang, Z. H., Hsu, H. C., Lin, J. H., Wang, C. J., Li, T. M., & Tang, F. C. (2012). Berberine prevents bone loss in streptozotocin-induced diabetic rats: a randomized, controlled trial. Journal of Bone and Mineral Research, 27(3), 746-755.

[5] Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia. 2003;46:3–19.

[6] Samtani MN. Simple pharmacometric tools for oral anti-diabetic drug development: competitive landscape for oral non-insulin therapies in type 2 diabetes. Biopharm Drug Dispos. 2010;31:162–177.

[7] Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. N Engl J Med. 2006;355:2427–2443.

[8] Hanefeld M, Pfutzner A, Forst T, Lubben G. Glycemic control and treatment failure with pioglitazone versus glibenclamide in type 2 diabetes mellitus: a 42-month, open-label, observational, primary care study. Curr Med Res Opin. 2006;22:1211–1215.

[9] Kim DJ, Lee MS, Kim KW, Lee MK. Insulin secretory dysfunction and insulin resistance in the pathogenesis of Korean type 2 diabetes mellitus. Metabolism. 2001;50:590–593.

[10] Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma) J Biol Chem. 1995;270:12953–12956.

[11] Rizos CV, Liberopoulos EN, Mikhailidis DP, Elisaf MS. Pleiotropic effects of thiazolidinediones. Expert Opin Pharmacother. 2008;9:1087–1108.