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Original Research Article

Isolation of 6-Gingerol from the Rhizome of Zinger (*Zingiber officinale*) and Evaluation of its Effect on the Bone Health of Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Several studies have investigated the effects of various natural products on bone mineral density, bone mineral content, and fat content. The present study aims to isolate, characterize 6-gingerol and investigate its effect in diabetic osteoporosis. Isolation and identification of 6-gingerol was done by a combination of column chromatography (CC), thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). The effects of 6-gingerol on diabetic osteoporosis was assessed for 17 weeks (120 days) in streptozotocin (STZ)-induced diabetic rats. The animals were divided into four groups: Group 1 (control rats), Group 2 (diabetic rats), Group 3 (diabetic rats treated with 6-gingerol at 100 mg/kg body weight), and Group 4 (control rats treated with 6-gingerol). Bone mineral density, bone mineral content, fat mass, and total lean mass in all the experimental rats were evaluated at the end of the study using dual-energy X-ray absorptiometry (DEXA). The diabetic rats treated with 6-gingerol showed improved blood glucose level compared to the untreated diabetic rats. Diabetic rats showed fluctuating values of total fat, percentage fat, bone mineral density (BMD), and bone mineral content (BMC). However, following 120 days of treatment with 6-gingerol, all variables were similar to that obtained in the control group. The findings from the present study reveal that 6-gingerol has the propensity to prevent glucotoxicity-induced diabetic osteoporosis while improving bone health.

***Keywords*:** 6-Ginerol, Dual-Energy X-ray Absorptiometry, Bone mineral density, Bone mineral content, Diabetic osteoporosis.

Introduction

Chronic hyperglycemia leads to various microvascular and macrovascular complications. Control of hyperglycemia will delay the onset and progression of such diabetic complications. Apart from these complications, there has been growing evidence of the risk of fragility and fractures in the hip, spine, distal forearm, and other skeletal sites.[1-5] Various studies have confirmed the relationship between muscle and bone by directly measuring bone mass and muscle mass.[6] Recently, the relationship between bone mass and fat consumption has been studied, for example, Ze Bin Fang *et al*. (2023)[7] stated that fatty acid consumption increases adult bone mineral density. As a result, individuals should consume reasonable amounts of fatty acids in order to preserve proper bone mass while avoiding metabolic illnesses. The variation in fat mass does affect bone mineral density unless there is some regulation of fat mass in response to bone mineral density, because increase fat mass is a risk factor for fractures in obesity as well as in metabolic diseases like diabetes. However, studies have revealed that fat protects from osteoporotic fractures.[8,9] Hence, the bone-fat relationship is meaningful clinically.

There is a direct relationship between fat and obesity. Accumulation of fats in the abdominal region increases the risk of chronic diseases such as diabetes, cardiovascular diseases, strokes, etc.[10, 11]

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There are two ways to assess obesity; Body mass index (BMI), and waist-to-hip ratio. While these two parameters are clinically significant, bone mineral density (BMD), bone mineral content (BMC), and fat mass assessments are more precise and critical clinical parameters. Various studies have confirmed the significance of bone mineral density in diabetes especially in elderly diabetic patients where lower bone mineral densities are reported to increase the risk of hip fracture.[12-14] The risk of fracture also increases in other skeletal sites, such as the proximal humerus, foot, and possibly ankle, in diabetes.[15] BMD fluctuations may not be associated with diabetes, but the risk of fracture increases secondary to peripheral neuropathy,[16] showing symptoms like poor gait, a tendency to fall, etc.

Ginger (*Zingiber officinale* Rosc) is an aromatic plant belonging to the family Zingiberaceae, it is used as a flavouring agent in Indian food. The phytochemicals of ginger contribute to its health-promoting properties. The compound 6-gingerol is a non-volatile active constituent of ginger, with many pharmacological properties, including antidiabetic effect. According to Almatroodi*et al*. (2021),[17] 6-gingerol decreases hyperlipidaemic markers, inflammation, and oxidative stress in diabetic rats, ultimately preventing kidney damage. It proved to be a breakthrough therapeutic agent for preventing kidney damage caused by diabetes mellitus. According to Alharbi*et al*. (2022),[18] gingerol do not only aids in the treatment of hyperglycemia but also shows effectiveness in related diseases. The efficacy of 6-gingerol in lowering serum total cholesterol, low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL), as well as raising high density lipoprotein (HDL) in hyperglycemic rats fed a high-lipid diet in a diabetic rat model, has been observed.[19] According to Suzer *et al*. (2022), [20] ginger extract treatment of diabetic rats enhances bone health, and stop the effect of the disease on bone mechanical characteristics. Oxidative stress is a significant risk factor for diabetes and age-related chronic diseases such as osteoporosis.[21]Gingerols, shogaols, and other ketone-phenolic derivatives contribute to ginger's antioxidative abilities. The study of Ajayi*et al*. (2019),[22] has shown that ginger extract reduces

damage caused by oxidative stress in rats and mice. The ethanol extract of ginger as well as its 6-gingerol and 6-shogaol components have been shown to reduce body weight and prevent hyperglycaemia in diabetic rats.[23,24] The present study reports the effect of 6-gingerol on the bone health of Streptozotocin-induced diabetic rats. In this study, we look specifically at fat and bone cell interactions, their role in the pathophysiology of osteoporosis, and the possible therapeutic applications of 6-gingerol as a novel pharmacologic treatment for osteoporosis.

Materials and Methods

Chemicals and Reagents
Streptozotocin was procured from Sigma Chemicals (USA). Petroleum Ether (extra pure SLR grade) was product of Fisher chemicals, Ethyl Acetate (HPLC grade, 99.5%) was product of Thermo Scientific chemicals. All other chemicals were received from SRL Biochemical, INDIA.

Extraction and Isolation of 6-Gingerol
Fresh ginger rhizomes were obtained from Wargal mandal (https://maps.app.goo.gl/vRgSNhhNTTpQf3HEA) in the Medak district of Telangana State (India) between the month of July and August 2023 (Voucher No. 021). The rhizomes were cut into small pieces and air-dried. The dried rhizomes were crushed into fine powder by means of a mechanical grinder. The powdered rhizomes were extracted with methanol by maceration at room temperature for 24 h. The extract was filtered using Whatman No.1 filter paper. The residue was re-extracted in methanol for another 24 h, filtered, and the combined filtrate was concentrated using a rotary evaporator at reduced pressure. The crude methanol extract was purified by column chromatography (CC) using silica gel (100 - 200 mesh) as the stationary phase, and 15% ethyl acetate in petroleum ether as the mobile phase. The eluates were monitored by thin-layer chromatography (TLC) using 40% ethyl acetate in petroleum ether as the mobile phase. The column fraction with a spot at Rf 0.4 in the TLC profile corresponding to 6-gingerol was further purified with HPLC under the following conditions; Mobile phase: Acetonitrile:Water 55:45, Column: IC3, Detector: SPD-M 10Avp Photodiode array, Flow rate: 1.3 mL/min, Wavelength: 280 nm, and Injection volume: 10 µL.[25] Pure 6-gingerol was used as internal standard.

Animals
Male albino rats (Wistar strain) aged 45 - 60 days, weighing between 250 to 300 g were purchased from the National Institute of Nutrition, Hyderabad, India. The animals were maintained individually in plastic cages under standard laboratory conditions. They were fed with rodent pellets (NIN, Hyderabad) and allowed access to drinking water *ad libitum*. The animals were acclimatized to the laboratory conditions for one week prior to the start of the experiment. The experiment was performed in compliance with the Institutional Animal Ethics Committee's protocol and standards (CPCSEA No. 383/01/a/CPCSE).

Induction of diabetes
100 mM sodium citrate buffer (pH4.5) was injected intraperitoneally into overnight fasted rats to induce diabetes. Rats were classified as diabetes if their blood glucose levels were over 250 mg/dL on day three post induction.

Animal Groupings and Treatments
The animals were divided into four groups of 6 rats each, they were treated as follows;
Group 1: The normal control group, received saline orally.
Group 2: STZ-induced diabetic group (50 mg/kg body weight of STZ),
Group 3: d+gingerol group, STZ-induced diabetic rats treated with 6-gingerol (100 mg/kg body weight) orally, once daily.
Group 4: Non-diabetic control rats, received 6-gingerol (100 mg/kg body weight), which served as the gingerol group.
Treatments were given once daily for 120 days.

BMD and BMC Measurements
The BMD and BMC of the rats were measured using Dual-Energy X-ray Absorptiometry (DEXA). DEXA Scan was performed at the National Institute of Nutrition, ICMR Institute, Hyderabad, Telangana State, India, using the Hologic manufacturer Discovery A and the QDR400W Elite model. The system was calibrated according to the manufacturer's instructions before the commencement of the measurement.
Rats in all the groups were laid flat on the instrument with the dorsal side exposed. DEXA scan was done from the anterior region to the posterior region of the animals without any regional specialization. Fat mass, lean mass, fat percentage, BMD, and BMC (whole-body) were evaluated.
Serum glucose was estimated using the glucose oxidase-peroxidase (GOD-POD) method with a glucose testing kit (Beacon Diagnostics Pvt. Ltd., New Delhi, India).

Statistical analysis
Data were presented as mean ± SD of six replicates. Data were analysed using Two-Way ANOVA, followed by multi-regression analysis, and the null hypothesis test. The SPSS program version 2020 was used for the analysis.

Results and Discussion

Isolation and identification of 6-Gingerol
Pure as6-gingerol was isolated from the rhizomes of zinger by column chromatography. The isolated compound was identified by TLC and HPLC analyses. The TLC and HPLC chromatograms are shown in Figures 1 and 2. From the HPLC analysis, the percentage of 6-gingerol in the dried ginger extract was 5%.

Effect of 6-Gingerol on body weight of rats
The body weight of rats in the diabetic control group (Group 2) at the 60th day of the experiment showed 27.10% decrease in comparison to the normal control group (Group 1). On the other hand, the body weight of rats in the d+gingerol group (Group 3) decreased by 6.90%, while that of the gingerol group (Group 4) showed a 5.60% increase in body weight. On the 90th day, the body weights of diabetic rats further decreased by 68.14%, while those of other groups, such as d+gingerol and gingerol groups decreased by 18.87% and 11.44%, respectively. However, at the end of the experimental period, i.e., on the 120th day, the body weights of d+gingerol had significantly increased by 0.012% compared to the diabetic groups, which had further decreased by 0.188% (Figure 3).

Effect of 6-Gingerol on serum glucose
Figure 4 shows the serum glucose levels of the four experimental groups of rats. Rats in the diabetic group remained consistently hyperglycemic throughout the experimental period, their serum glucose concentrations increased drastically by 158.75%; 357.37%; 505.86% on the 60th, 90th, and 120th day, respectively compared to the normal control group. A significant antidiabetic effect was evident in the gingerol-treated diabetic rats (d+gingerol) from the 60th day onwards.

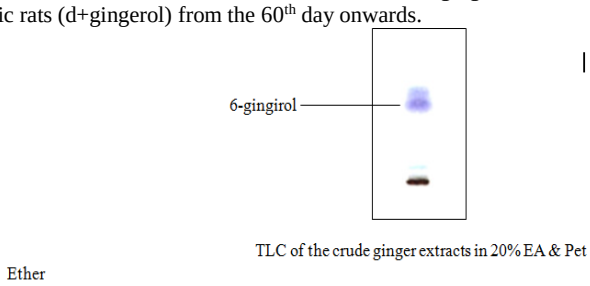

Figure 1: TLC chromatogram of the crude ginger extracts in 20% ethyl acetate in petroleum ether.

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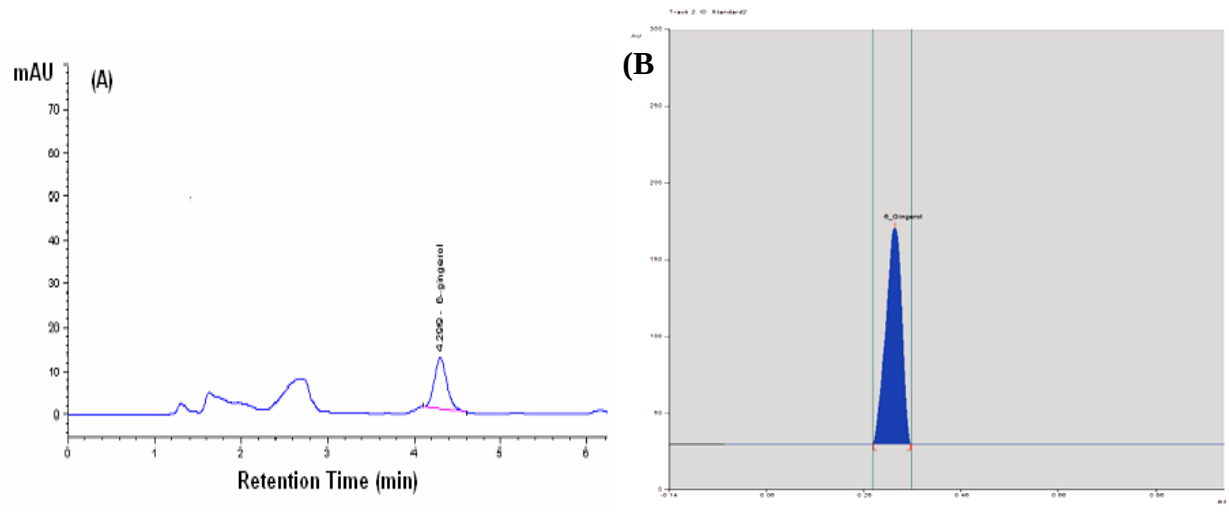

Figure 2: HPLC chromatogram (A): Gingerol isolated from the test ginger extract (B): Standard gingerol

The serum glucose concentration in this group decreased by 28.15% on the 60th day, with further decrease of 10.98% on the 90th and 0.47% on the 120th day. The control gingerol group showed consistent glucose levels throughout the experimental period, but with a slight decrease of 12.46% on the 60th day, 6.69% on the 90th day, and 3.80% on the 120th day compared to that of the normal control group.

Effect of 6-Gingerol on bone mineral density (BMD)
Figure 5 shows the BMD of all the experimental groups of rats. The BMD value of the whole body scan of the diabetic rats was 14.285% lower than that of the control rats on the 120th day. Treatment of the diabetic rats with 6-gingerol resulted in an appreciable improvement in the BMD, with only a 4.729% decreased when compared with the normal control group.

Effect of 6-Gingerol on whole body bone mineral content (BMC)
The whole bodyBMC of all the experimental groups of rats is shown in Figure 6. The BMC value of the whole body scan of diabetic rats was lower than that of the control rats on the 120th day. The percentage variation was 39.342%, but on treatment with 6-gingerol, the BMC increased significantly with only a 7.631% variation from that of the control animals. Control animals treated with 6-gingerol (Group 4) showed 15.131% decrease in BMC on the 120th day compared to the normal control group (Group 1).

Effect of 6-Gingerol on lean bone mineral content (BMC)
The lean BMC of all the experimental groups of rats is presented in Figure 7. The lean BMC of the diabetic rats was 34.754% lower than that of the control rat on the 120th day. Treatment of the diabetic rats with 6-gingerol resulted in an increase in lean BMC with only a 3.0% variation from that of the control animals. Control animals treated with 6-gingerol showed 29.952% decrease in lean BMC as at the 120th day.

Effect of 6-Gingerol on total fat content
Figure 8 shows the total fat content of the rats in all the experimental groups. The results show that the total fat content of the diabetic rats was 73.562% lower than that of the control rats on the 120th day. Treatment with 6-gingerol resulted in an increase in the fat content of the diabetic rats with only a 1.349% variation from that of the normal control group. On the other hand, the control animals treated with 6-gingerol had a 25.473% lower fat content on the 120th day than the normal control group.

Effect of 6-Gingerol on percentage fat content
Figure 9 shows the percentage fat content of the experimental groups of rats. On the 120th day, results show that the percentage fat content of the diabetic rats was 56.059% lower than that of the rats in the control group. However, the 6-gingerol-treated diabetic rats showed a significant increase in the percentage fat content with only a 5.193% variation compared to that of the normal control rats, while the control rats treated with 6-gingerol showed a 4.122% difference in the percentage fat content from that of the normal control as at the 120th day.

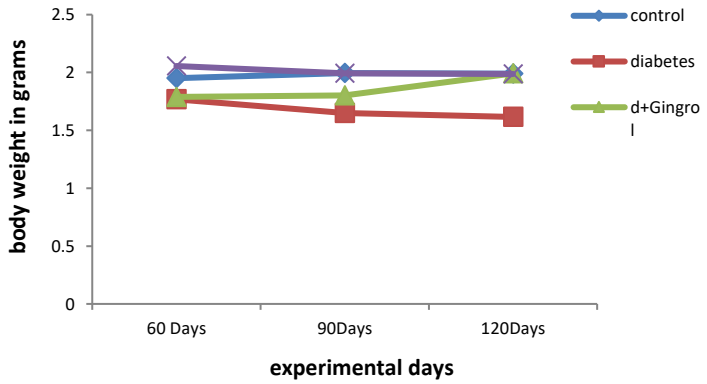

Figure 3: Effect of 6-gingerol on body weight of rats. Values represent Mean ± SD, n = 5.

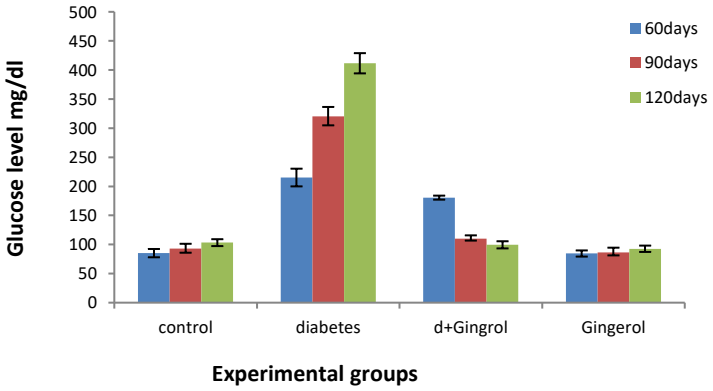

Figure 4: Effect of 6-gingerol on serum glucose of experimental rats. Values represent Mean ± SD, n = 6.

Effect of 6-Gingerol on total lean mass

The total lean mass of the rats in all the experimental groups is presented in Figure 10. On the 120th day, the results show that the total lean mass of the diabetic rats (Group 2) was 41.31% lower than that of the normal control rats (Group 1). After 6-gingerol treatment of the diabetic animals (Group 3), the total lean mass became only 6.699% lower when compared to the control group 1. On the other hand, treatment of the control rats with 6-gingerol resulted in a 29.400% decrease in the total lean mass as at the 120th day.

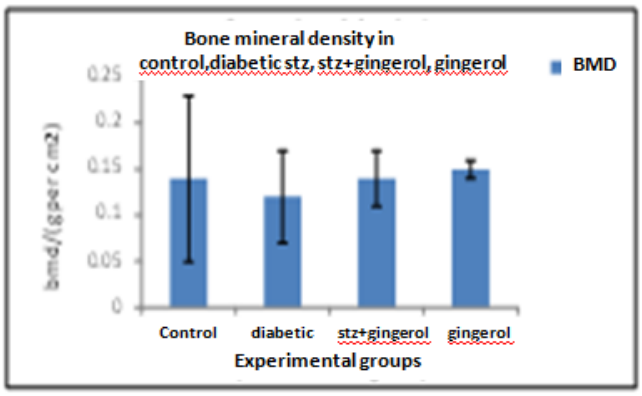

Figure 5: Effect of 6-gingerol on bone mineral density of STZ-induced diabetic rats. (BMD expressed as in bmd/g per cm^2) Values represent Mean ± SD, n = 6.

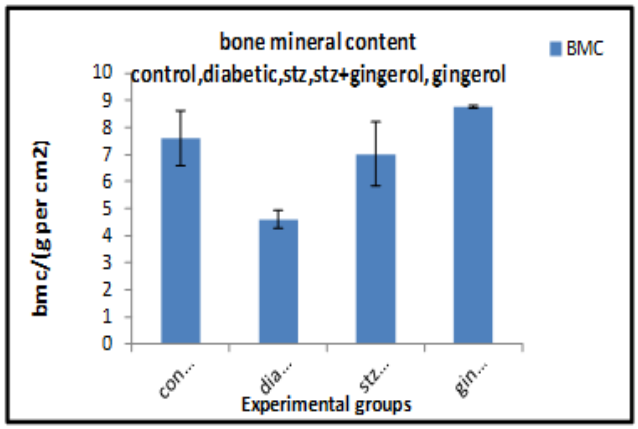

Figure 6: Effect of 6-gingerol on bone mineral content of STZ-induced diabetic rats. Values represent Mean ± SD, n = 6.

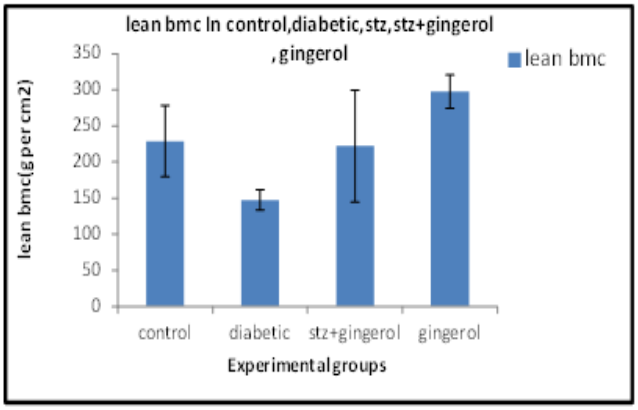

Figure 7: Effect of 6-gingerol on Lean bone mineral content of STZ-induced diabetic rats. Values represent Mean ± SD, n = 6.

Plants make phytochemicals to protect themselves from environmental hazards such as predatory insects, pollution, and diseases. Gingerols are the major compounds present in the rhizomes of ginger (***Zingiber officinale***). This study isolated 6-gingerol from the rhizomes of ginger using column chromatography (CC) and thin-layer chromatography (TLC). The isolated compound was characterized by HPLC analysis. The phytochemical analysis of *Z. officinale* led to the isolation of the pure bioactive compound 6-gingerol, which was confirmed by HPLC and TLC, and by comparison with literature data.[26] Natural products are a rich source of bioactive compounds for the treatment of various ailments. The compound 6-gingerol is the main biochemical marker of *Z. officinale*, it has been shown to have hypoglycemic properties in diabetic mice and improved impaired insulin signaling in arsenic-intoxicated mice.[27] 6-gingerol has also been shown to possess anti-cancer properties by promoting apoptosis through the upregulation of NAG-1 and G1 cell cycle arrest and downregulation of cyclin D1. Kuhad*et al.* (2006)[29] have shown the renoprotective activity of 6-gingerol, the compound alleviated cisplatin-induced oxidative stress and renal dysfunction in rats. Similarly, other studies have confirmed the hypoglycemic effects of 6-gingerol in diabetic rats.[30]

The current study demonstrated the effect of 6-gingerol on the fat content and bone metabolic health of diabetic rats. In diabetic rats, treatment with 6-gingerol at a dose of 100 mg/kg body weight improved the impact of diabetes on bone health and fat content as shown by the DEXA scan (Figure 11). Hyperglycemia is the primary cause of diabetic complications. These complications may be micro- or macro-vascular. Other than these complications, diabetes also causes other complications such as osteoporosis. Diabetic osteoporosis is characterized by reduced BMD and BMC with an increased risk of fracture.[31] Many researchers have alluded to the fact that diabetes affects bone turnover and bone integrity,[32,33] which results in bone loss.[34]

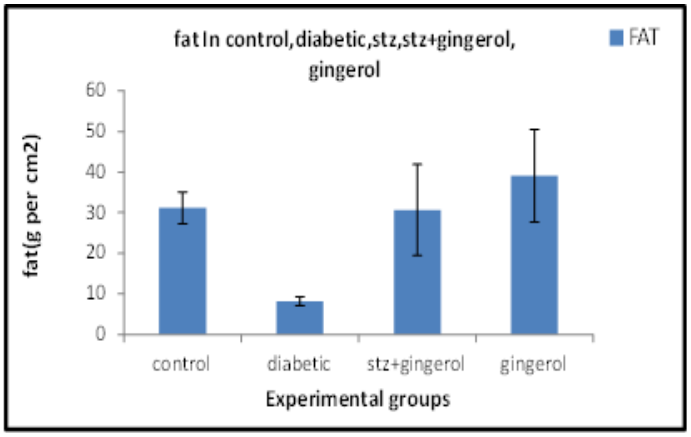

Figure 8: Effect of 6-gingerol on total fat content of STZ-induced diabetic rats. Values represent Mean ± SD, n = 6.

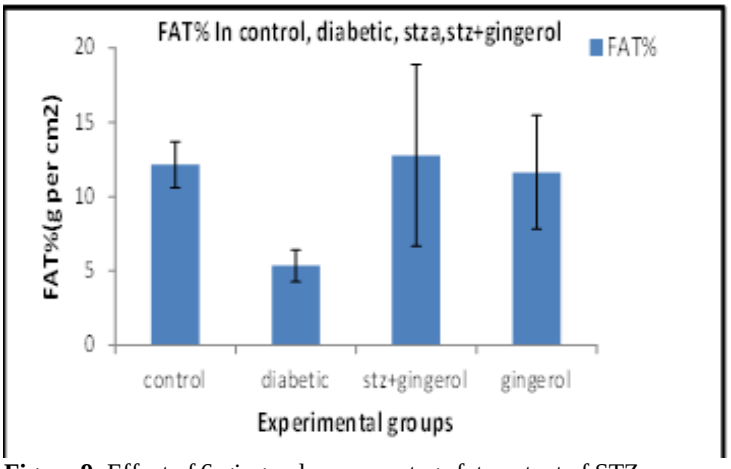

Figure 9: Effect of 6-gingerol on percentage fat content of STZ-induced diabetic rats. Values represent Mean ± SD, n = 6.

As a result, osteoblast differentiation from elevated blood glucose levels characterizes diabetic osteopenia, which inhibits bone mineralization and impairs bone formation.[35]In this type of complication, the age and weight of the patient may play crucial roles. Most of the antidiabetic drugs available in the market target micro- and macro-vascular complications of diabetes, but not complications like osteoporosis. Therefore, the present study aimed to evaluate the effects of 6-gingerol on bone mineral content and overall body fat content using DEXA. Ginger is the most widely used spice worldwide, and 6-gingerol is a major component of ginger, which has many medicinal properties, including antidiabetic properties. The mechanism of action of 6-gingerol as an antidiabetic agent is associated with insulin release and improved carbohydrate and lipid metabolism.[36] Herein we report the effect of 6-gingerol on bone metabolism and fat content in diabetic rats. Complications of diabetes have been found to be associated with age and weight. Hence, body weight plays a crucial role in bone metabolism. The possible mechanism that could lead to bone loss in older diabetic patients is weight loss.[37] On the other hand,Insulin resistance has been linked to obesity.[38] Therefore, neither an increase nor a decrease in weight in diabetic condition is desirable. In the present study, treatment of diabetic rats with 6-gingerol ameliorated diabetes-induced weight loss. This effect may be due to its blood glucose lowering capacity, which in turn improves insulin sensitivity. Older diabetic patients have more significant weight loss, which is strongly associated with bone loss.[39, 40] In the present study, there was a significant decrease in blood glucose levels in STZ-induced diabetic rats treated with 6-gingerol. The mechanism involved in the hypoglycemic effect may be due to potentiation of glucose-stimulated insulin secretion through the GLP-1-mediated pathway. The proposed mechanism of 6-gingerol increases insulin exocytosis and enhances glucose utilization in skeletal muscle.[41]

Studies have shown that obesity is a risk factor for decreased bone mineral density, which leads to fractures.[42] The risk associated with this imbalance increases with comorbidity of diabetes and age-related bone loss.[43] Most studies confirm that fat has an independent effect on bone remodelling, increasing bone mass. One of the studies confirmed that total body weight, fat mass, percentage body fat, and bone mass index are inversely associated with fracture risk.[44]The present study showed that mean BMD and BMC were significantly lower in diabetic rats compared to the normal control rats. However, treatment of the diabetic rats with 6-gingerol increased BMD and BMC significantly up to levels close to that recorded for the control rats. The cellular mechanisms responsible for reduced BMD and BMC in diabetes are not completely understood, but most of the studies related them to advanced glycation end-products (AGEs) and oxidative stress.[43,45] Higher AGEs in the blood will lead to higher glycated haemoglobin levels in the blood and bone tissue.[46] In diabetic conditions, non-enzymatic glycation via AGEs processes may create collagen crosslinks that lead to more brittle bone biomechanically and impaired bone resistance to fracture (deficient toughening mechanisms), which negatively impact bone quality at microstructural and nanostructural levels.[46] Any drug or compound that can neutralize circulating AGEs and has antioxidant properties can help protect against bone fractures in diabetic patients. The compound 6-gingerol proved to have these properties.

Body weight impacts BMC and BMD, influencing bone health which makes it an essential risk factor for bone fracture coupled with advancing age. Both fat mass and lean mass contribute to body weight. The relationship between fat and bone arises from the effect of soft tissue mass on skeletal growth, adipocyte-induced secretion of bone active hormones (leptin and estrogens), fat mass-induced secretion of bone active hormones from the pancreatic beta cell (insulin, amylin, and preptin).[47] In metabolic diseases, including diabetes, there is the need to address the association between fat and bone. Diabetic patients lose weight due to decreased fat and lean mass and loss of proteins in muscles and other tissues. The present study shows similar results of reduced body weight and reduced percentage fat, and total lean mass in diabetic rats. However, after receiving 6-gingerol, body weight, the total lean mass and percentage fat content of the diabetic rats normalized and approached those of the control rats. Therefore, 6-gingerol is considered to have a role in weight maintenance and may prevent osteoporosis. The mechanism involved in maintaining optimal values of percentage fat content, and total lean mass of diabetic rats treated with 6-gingerol could be by enhancing energy metabolism, reducing the extent of lipogenesis through downregulation of SREBP-1c (sterol regulatory element-binding protein 1c) and related molecules, which suppresses body fat accumulation.[46]

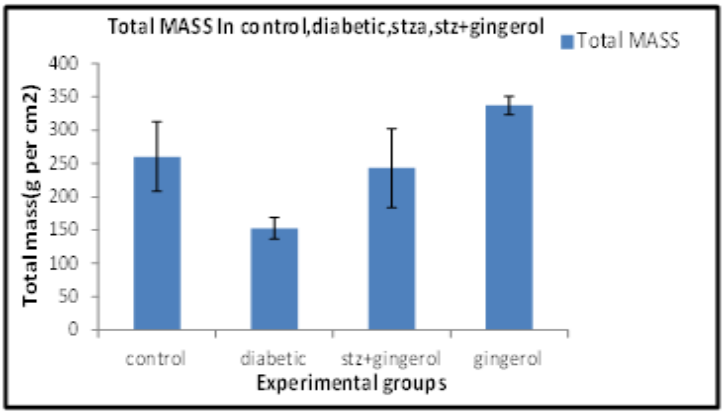

Figure 10: Effect of 6-gingerol on the total lean mass of STZ-induced diabetic rats. Values represent Mean ± SD, n = 6.

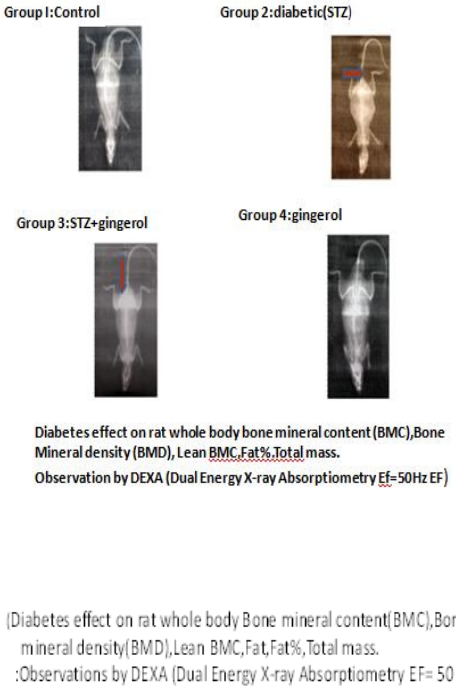

Figure 11: DEXA rat scan of all experimental groups and standard analysis image of total body BMD, BMC, Fat, percentage fat and Total Mass content

Conclusion

Many researchers have focused on the identification of effective plant-derived compounds for treating metabolic illnesses such as diabetes and osteoporosis. This research interest may have arisen from the extreme negative consequences of synthetic medications. The compound 6-gingerol has a wide range of biological properties, contributing to its widespread pharmaceutical use. There are several known methods for 6-gingerol extraction and purification. In the present study, 6-gingerol was isolated and analyzed by a combination of chromatographic technique including column chromatography (CC), thin-layer chromatography (TLC), and high performance liquid chromatography

(HPLC). The study clearly shows that 6-gingerol can improve bone metabolism by normalizing weight and blood glucose levels. 6-gingerol has a role in protecting against diabetic osteoporosis by increasing BMC and BMD. This study reveals that the protective mechanism of 6-gingerols on diabetic rats' bone metabolism is by lowering blood glucose, maintaining ideal body weight, and upregulation of percentage fat and total lean mass in diabetic rats. As a result, 6-gingerol is a potentially viable alternative therapeutic agent for the treatment of diabetic osteoporosis. However, more research is required to produce functional foods supplemented with 6-gingerol with high bioavailability to provide consumers with the most health benefits possible.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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