Effect of Ginger on Bone of Streptozotocin Induced Diabetic Rats

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Abstract

Background: Diabetes has a deleterious effect on bone. Ginger has been used in a wide variety of diseases. This study was designed to clarify changes of the bone of streptozotocin induced diabetic adult male rats and the possible role of ginger in preventing these changes.

Methods: Thirty adult male rats were used. They were divided into three groups: Group I: Control group. Group II: Diabetic group, diabetes was induced in rats by single intraperitoneal injection of freshly streptozotocin 60mg/kg body weight. Group III: Diabetic rat treated with ginger (500mg/kg) orally for 6 weeks. The serum levels of glucose, insulin, calcium, phosphorus, alkaline phosphatase and Osteocalcin (OC) were measured. Both femora of each rat were processed for histological, immunohistochemical and morphometrical studies.

Results: STZ-induced diabetes was characterized by significant increase in serum glucose and alkaline phosphatase levels and significant decrease in serum insulin, calcium, phosphorus and OC levels as well as significant decrease in number of osteoblasts and osteopontin (OPN) protein expression in the femur bone. Also, histological results showed degeneration of osteoblasts and osteocytes, multiple osteoporotic cavities, decreased collagen fibers and irregularity of bone surfaces treatment of diabetic rats with ginger resulted in significant decrease in serum glucose and alkaline phosphatase levels and significant increase in serum insulin, calcium, phosphorus and OC levels as well as significant increase in number of osteoblasts and OPN protein expression in the femur bone and improvement of histological results.

Conclusion: Diabetes could lead to increased incidence of bone loss. Ginger could ameliorate diabetic changes of bone and may represent a promising agent for treating of diabetic osteoporosis.

Key Words: Diabetes mellitus – Ginger – Bone – Osteoblast – Osteocalcin – Osteopontin.

Introduction

THERE is a global epidemic of Diabetes Mellitus (DM), and the number of affected persons is expected to exceed 430 million by 2030 [1]. A clinical syndrome characterized by hyperglycaemia due to insulin deficiency, impaired effectiveness of insulin action, or both, is a diabetes mellitus [2].

Several long-term complications are associated with uncontrolled diabetes (chronic hyperglycemia) [3]. It could cause many bone complications including: Increased risk for osteoporosis besides its well-known complications such as nephropathy, neuropathy, retinopathy and arteriosclerosis [4] decreased bone density (osteopenia) [8], increased fracture rates and delayed fracture healing [6].

In a complex system the components of bone, organic and inorganic are organized. Of the organic components, collagen accounts for around 90%, while matrix proteins, proteoglycans, cytokines and growth factors account for around 10% [7].

Osteocalcin (OC) is a noncollagenous protein of the bone matrix. OC is produced by osteoblasts, osteocytes and odontoblasts. It has two forms, the γ-carboxylated forms bind hydroxyapatite and are found in the bone extracellular matrix, while the majority of the undercarboxylated forms are hormonally active and released in the circulatory system. Its main biological role is bone mineralization and calcium homeostasis, but has also been associated with glucose metabolism and some hormonal actions [8-10].

Osteopontin (OPN) is a non-collagenous bone matrix protein. The suffix 'pontin' is derived from 'pons' the Latin word for bridge that signifies its function as a bridge between cells and minerals [11]. OPN is expressed in preosteoblasts, osteoblasts, osteoclasts, osteocytes, odontoblast, some bone marrow cells, chondrocytes, dendritic cells, hepatocytes, macrophages, lymphocytes, smooth muscle and skeletal muscle, endothelial cells, epithelial cells and extraosseous (non-bone) cells in the inner ear, brain, kidney, and placenta [12].

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is involved in several pathophysiological processes including bone remodelling, immunity, wound healing, adipose tissue inflammation and insulin resistance [13].

Since ancient time, medicinal plants and its constituents have been used for diseases management. Ginger is an underground rhizome of plant Zingiber Officinale. Because of its pungent taste and interesting aroma, ginger has been used as a spice. Ginger contains a number of different pungent and active ingredients. The major pungent compounds found in ginger are the gingerols, the most biologically active components of ginger, which can be converted into shogaol, zingerone and paradol. Zingerone and shogaol are found in small amounts in fresh ginger and in large amounts in stored products. A number of pharmacological activities have been shown to have in gingerol and shogaol [14].

Ginger has been used in a wide variety of diseases, specially in gastrointestinal disorders, such as diarrhea, anorexia, colic, dyspepsia, nausea, vomiting and motion sickness. In addition, it possesses antioxidative characteristic, anti-cancer [15], anti-arthritis [16], anti-migraine [17], anti-thrombotic, anti-inflammatary and hypolipidaemic activities [18,19].

However, few studies about the effect of ginger on diabetes-related bone disorders were reported. Thus, the present study aims to clarify changes of the bone of streptozotocin-induced diabetic adult male rats and assess the possible role of ginger in preventing these changes.

**Material and Methods**

**Experimental animals:**

Thirty (30) adult male albino rats weighing (150-200) gm were used in this study. Rats were brought from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on a balanced diet with water supply freely in clean containers. The experimental procedures were carried out according to Guidelines of care and use of laboratory animals and approved by Ethical Committee at Faculty of Medicine, Assiut University, Egypt.

**Chemicals and kits:**

Streptozotocin (STZ) was purchased from MP Biomedicals, France. Blood glucose kit was purchased from Egyptian Company for Biotechnology, Egypt. Insulin kit was purchased from Immunospec Corporation, Canoga Park, CA. Calcium and phosphorus kits were purchased from Lab-Care Diagnostics, India. Alkaline phosphatase kit was purchased from DiaSys Diagnostic Systems, Germany. Osteocalcin kit was purchased from Immunodiagnostic systems, Germany.

**Induction of diabetes:**

Diabetes was induced in rats by single intraperitoneal injection of freshly streptozotocin 60mg/kg body weight [20].

The animals were fasted for overnight 12 hours before STZ injection. After injection, the rats were kept for next 48 hours on oral 10% glucose solution on top of their chaw. Administration of glucose is to prevent hypoglycemia as STZ is capable of producing fatal hypoglycemia due to destruction of β cells which in turn results in to massive pancreatic insulin release [21].

After 3 days, blood glucose levels were measured by glucometer (Bionime, Taiwan). Animals were considered diabetic when fasting blood glucose was over 250mg/dl [22].

**Preparation of ginger aqueous extract:**

**Extract preparation:**[18]:

Aqueous ginger extract was prepared from locally available ginger roots. The ginger roots were peeled on crushed ice, and 50g ginger were cut into small pieces and homogenized in 75ml cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 2min bursts for a total of 12min. The homogenized mixture was filtered three times through cheesecloth. The filtrate was centrifuged at 2000 relative centrifugal force for 10min and the clear supernatant fraction was made up to 100ml with normal saline. The concentration of this ginger preparation was considered to be 500mg/ml on the basis of the weight of the starting material (50g/100ml). The aqueous extract of ginger root was stored in small samples at 20ºC until use. Lower concentrations of ginger were prepared by dilution of this solution with cold, sterile 0.9% NaCl.

**Experimental design:**

After one week of acclimatization, the animals were randomly divided into three groups (10 rats each) as follow:

**Group I (control):** Served as control group. They received saline orally.

**Group II (diabetic):** The rats were injected with a single dose of streptootocin (60mg/kg, intraperitoneally) to induce diabetes [20].
**Group III (diabetic rats treated with ginger):**
The diabetic rats were treated with ginger in the dose of 500mg/kg body weight orally [18] for 6 weeks.

**Biochemical estimations:**
The serum levels of glucose, insulin, calcium (Ca++,), phosphorus (P), Alkaline Phosphatase (ALP) and Osteocalcin (OC) were measured using enzymatic colorimetric assay kits.

**Sample collection:**
Animals were fasted for 12 hours and blood samples were obtained before the animals were killed by cervical dislocation. Blood was collected from the retro-orbital venous plexus. The serum was collected by centrifugation of blood sample at 3000 revolution per minute (rpm) for 10min at 4ºC. The separated serum was stored frozen at 20ºC in aliquots until use for measurement of the biochemical parameters.

After sacrificing animals, the femur were dissected out and cleaned of excess muscle and soft tissues. Then were fixed in 10% neutral buffered formalin for an additional 24h. The specimens were then decalcified in 14% EDTA [23] and processed for paraffin blocks. 5-µm cross sections were cut and stained with Hematoxylin and Eosin (H & E) for histological study and Masson trichrome for collagen fibers [24].

**Immunohistochemical study:**
Five µm paraffin sections were incubated with a rabbit anti-human osteopontin (OPN) polyclonal antibody which has cross reactivity with rats (Thermoscientific, USA), ready to use using the avidin biotin peroxidase method [25].

**Morphometrical study:**
H & E sections of femur from all groups were examined under light microscopy at magnification of 400 and by using the image analyzer in Histology Department, Faculty of Medicine, Assiut University. The number of osteoblasts were measured in 10 fields of each specimen.

**Statistical analysis:**
Statistical analysis was performed using the GraphPad Prism software version 4 (GraphPad Software, San Diego California, USA). The results were presented in the form of mean ± Standard Deviation (SD) for ten rats in each experimental group. One way analysis of variance (ANOVA) was done to compare between the studied groups. Bonferroni’s test was used to compare between each two means. *p*-values <0.05 were considered as significant.

**Results**

**Biochemical parameters:**
Table (1) summarizes the biochemical parameters in the studied groups. The glucose levels were significantly increased (*p*<0.001) while serum insulin levels were decreased significantly (*p*<0.001) in Group II in comparison to Group I. Ginger administration ameliorate this effect as it decreased significantly (*p*<0.001) the glucose level and significantly increased (*p*<0.001) serum insulin levels in Group III when compared to Group II. In comparison to Group I, the levels of glucose were significantly increased (*p*<0.05) while serum insulin levels were decreased significantly (*p*<0.05).

The serum calcium and phosphorus levels were significantly decreased (*p*<0.001) after STZ administration in Group II compared with Group I, and the decrease was significantly prevented (*p*<0.05 and *p*<0.01, respectively) after administration of ginger in Group III compared with Group II but the levels sill significantly decreased (*p*<0.01) compared to Group I.

Serum ALP levels of Group II were significantly higher (*p*<0.001) than in Group I. Ginger treated rats in Group III showed significantly lower (*p*<0.001) of serum ALP than in Group II but the levels sill significantly higher (*p*<0.01) than in Group I.

Significant lower levels (*p*<0.001) of serum osteocalcin were found in Group II compared with Group I. Ginger treated rats in Group III showed significantly higher levels (*p*<0.001) of serum osteocalcin than in Group II but the levels sill significantly higher (*p*<0.01) than in Group I.

**Histological results:**

**Light microscopy:**

**Group I: Fig. (1A-C):**

Haematoxylin and eosin-stained sections of femur diaphysis of control rats revealed that it was formed of an outer shell of cortical bone to which the periosteum was attached to its external surface and endostium was attached to its internal surface. The periostium was composed of a thick outer fibrous layer that was formed of dense collagenous fibers with fibroblasts in between the fibers and an inner osteogenic layer. The endosteal surface of the cortical bone appeared smooth and was lined with osteoprogenitor cells, osteoblasts and osteoclasts. Active osteoblasts having cubical nuclei
appeared on bone surfaces. Osteocytes in their lacunae were present between Haversian canals. With masson trichrome collagen fibers were regularly arranged.

**Group II: Fig. (1D-F):**

Haematoxylin and eosin stained femoral sections from diabetic rat: Revealed highly significant decrease in number of osteoblasts. Most of osteocytes appeared degenerated with pyknotic nuclei. There were some empty lacunae devoid of cells and multiple osteoporotic cavities of different sizes. Periosteum and endosteum appeared irregular with multiple notches. With masson trichrome collagen fibers were apparently decreased.

**Group III: Fig. (1 G-I):**

Sections from treated diabetic rats with ginger showed nearly normal osteoblasts. Most of osteocytes were normal but few cells were degenerated. There were few small osteoporotic cavities and some cement lines indicating bone repair. Collagen fibers were more or less normal. Outer and inner bone surfaces appeared slightly irregular.

Figure 1: Photomicrograph of histological sections in the femur bone of: (A) Group I showing the periosteum (P) covered the bone from outside. The endosteal surface of the cortical bone appear smooth. Active osteoblasts having cubical nuclei appear on bone surfaces (o). The haversian system is observed with the haversian canal (H) and osteocytes in their lacunae (arrows). (B) Higher magnification of the previous section showing Haversian canal (H) and osteocytes in their lacunae (arrow). (C) Group I showing regularly arranged collagen fibers (c). (D) Group II showing multiple large osteoporotic cavities (O). (E) Group II showing irregular endosteum with notch (N). Notice osteoblast are apparently absent. Many osteocytes are degenerated in their lacunae (arrow). Some lacunae appear empty devoid of osteocytes (*). (F) Group II showing areas of absence of collagen fibers in the matrix (*). (G) Group III showing nearly normal periosteum and decrease in endosteal irregularity. Notice; osteocytes in their lacunae (arrow). (H) Group III showing active osteoblasts on the endosteal surface (O) and osteocytes in their lacunae (arrow). Some osteocytes appear degenerated (*). (I) Group III showing normal distribution of collagen fibers (C) with small area of decreased collagen (arrow). H & E x 200 (A, D, G); H & E x1000 (B, E, H) & Masson trichrome x1000 (C, F, I).
**Immunohistochemical results:**

Femoral sections of Group I showing extracellular osteopontin protein expression which appeared in the bone matrix Fig. (2A). Group II showed an apparently decreased osteopontin protein expression Fig. (2B). Group III showed increased osteopontin protein expression than in diabetic non treated rats Fig. (2C).

![Image of immunohistochemical results](image)

**Morphometrical changes:**

There was a highly significant decrease in osteoblast number in diabetic rats (Group II) compared to controls (Group I) (4.3 ± 1.3 vs 11.1 ± 2.2, \( p < 0.001 \)). Diabetic rats treated with ginger (Group III) showed a highly significant improvement in osteoblast number compared with diabetic rats (Group II) (8.8 ± 2.3 vs 4.3 ± 1.3, \( p < 0.001 \)). However, the number of osteoblast is still significantly decreased in diabetic rats treated with ginger (Group III) when compared to controls (Group I) (8.8 ± 2.3 vs 11.1 ± 2.2, \( p < 0.05 \)) Fig. (3).

![Image of morphometrical changes](image)

**Table (1): Biochemical parameters in the studied group.**

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>87.7±1.2</td>
<td>266.4±4.5***</td>
<td>92.1±3.9###,*</td>
</tr>
<tr>
<td>Insulin (mg/dl)</td>
<td>3.5±0.6</td>
<td>1.6±0.3***</td>
<td>2.85±0.4###,*</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.4±0.5</td>
<td>8.2±0.6***</td>
<td>8.8±0.4#,*</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>4.5±0.3</td>
<td>3.8±0.2***</td>
<td>4.2±0.2##,*</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>210.6±23.9</td>
<td>484.3±77.3***</td>
<td>309.0±69.9##,**</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>3.7±0.5</td>
<td>2.7±0.1***</td>
<td>3.2±0.3#,*</td>
</tr>
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Data are the mean ± SD for ten rats in each group.

\( *: p<0.05; **: p<0.01; \) and \( ***: p<0.001 \); vs. Group I.

\( #: p<0.05; ##: p<0.01; \) and \( ###: p<0.001 \); vs Group II.

**Discussion**

Diabetes causes burden to patients due to its acute and chronic complications which include bone metabolic abnormality [26]. Zhen et al., [27] have demonstrated that new bone formation and bone microarchitecture, and thus, bone quality, are altered in both type 1 and type 2 diabetes.

In accordance, the present study showed that STZ-induced diabetes was characterized by significant increase in serum glucose and alkaline phosphatase levels and significant decrease in serum insulin, calcium, phosphorus and OC levels as well as significant decrease in number of osteoblasts and OPN protein expression in the femur bone. Also, histological results showed several bone changes which might indicate osteoporosis, there were degeneration of osteoblasts and osteocytes with some empty lacunae devoid of cells, multiple erosion cavities, irregular bone surfaces and de-
creased collagen fibers. These results were consistent with Duarte et al., [28]; Sultan et al., [29]; Shady & Nooh [30]; Chen et al., [31] and Al-Saeed & Mohamed [32].

Diabetes could cause osteoporosis through impairment of bone matrix as hyperglycemia might lead to increased non-enzymatic glycosylation of various bone proteins, forming a variety of chemically modified proteins known as Advanced Glycation End products (AGEs). AGEs accumulate in tissues, including bone leading to bone tissue damage [33]. Also, inhibition of osteoblastic activities or proliferation diabetes decreased bone formation through [34]. Inhibition of osteoblast might be due to absence of the anabolic effects of insulin [35], diminished production of growth factors that regulate osteoblast differentiation [36] and apoptosis of osteoblasts [37]. In addition, high glucose intensifies reactive oxygen species which play important roles in osteoblast differentiation [38]. Moreover, Arikan et al., [39] stated that insulin is an important factor for bone formation, maintaining of bone mass, and preventing bone loss.

Alteration of calcium homeostasis have been reported previously to correlate with the abnormality of fasting serum glucose, insulin level and, B-cell function [40]. Xiao et al., [26] suggested that the decrease in serum calcium and phosphorus in diabetic rats could be resulted from impaired renal reabsorption and increased excretion due to osmotic diuresis by hyperglycemia as well as severe impairment of active transport in small intestine.

Alkaline phosphatase is an enzyme secreted by the osteoblasts as part of the formation of mineral matrix [41]. Raised value of serum ALP in diabetic patients has been reported by Siddiqui et al., [42]. Also, Hasan et al., [43] suggested that increased serum ALP level in type I diabetic patients indicating an increase in bone turnover.

Lu et al., [44] stated that chronic hyperglycemia showed inefficiency of the bone matrix to mineralize as a result of incompetent osteoblasts to synthesize OC and collagen. Also, reduction of the OC level could result from inhibition of osteoblast function due to impaired insulin secretion and increase in insulin resistance leading to hyperglycemia [45]. Osteocalcin expression in osteoblasts not only was suppressed by hyperglycemia but also serum OC secreted from osteoblasts into the circulation could improve glucose metabolism and modulate pancreatic cell function by enhancing the expression of insulin genes and proliferation markers in pancreatic cells [46-47].

In addition, the marked apparent decrease in OPN protein expression in this study indicates decreased bone formation as OPN is known to play a key role in bone formation [48]. Guan et al., [49] found that high glucose could inhibit the expression of osteogenesis genes as OPN in bone marrow stromal cells.

Ginger is a widely used spice and well known as an important medicinal herb. It has numerous bioactive components and is used in management, control and/or treatment of diseases [50].

Biochemical, morphometrical histological and immunohistochemical pictures have shown that an aqueous extract of raw ginger has potential hypoglycaemic properties that reflected on bone formation in diabetic rats treated with ginger. This was evident by significant decrease in serum glucose and alkaline phosphatase levels and significant increase in serum insulin, calcium, phosphorus and OC levels as well as significant increase in number of osteoblasts and OPN protein expression in the femur bone and improvement of histological pictures.

Chakraborty et al., [51] stated that 6-gingerol showed a protective effect on pancreatic 0-cells and restored the plasma insulin level in conjunction with lowered blood glucose in arsenic-induced type 2 diabetic rats. Shidfar et al., [52] also reported that 3 months supplementation of ginger improved glycemic indices in patients with type 2 diabetes.

Zhang and Tan [53] explained the possible effects of ginger by prevention of glycogen breakdown in liver, inhibition of hepatic glucose phosphatase enzyme and increases the activity of the enzymes involved in glycogen synthesis. In addition, Li et al., [54] attributed the action of ginger to facilitation of insulin-dependent glucose uptake by increasing translocation of glucose transporter GLUT4 to the muscle cell plasma membrane surface, together with small increase in total GLUT4 protein expression.

Fan et al., [55] suggested that 6-gingerol may have beneficial effects on bone formation as it stimulated osteoblast differentiation. Also, Kim et al., [56] showed that 6-gingerol inhibited 2-deoxy-D-ribose induced damage and caused a significant improvement of ALP activity, collagen content, and OC secretion in the osteoblastic MC3T3-E1 cells. Furthermore, Mustafa et al., [57] demonstrated that ginger administrations caused improvements in bone microarchitectures and structure, and decreased the osteoporotic changes in femur diaphysis and metaphysis.
Acknowledgments:

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References


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