Potent osteogenic action of *Ziziphus spina-christi* through up regulating IGF-1 and bone formation markers in diabetic male rats

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Abstract: Several studies have addressed an association between diabetes and increased bone loss or osteopenia. This study was designed to elucidate the efficacy of ***Ziziphus spina-christi*** **(**ZSC) leaves extract (100 mg/kg b.wt) in preventing diabetes-induced osteopenia. Results exhibited significant increase in glucose level and plasma glycated hemoglobin (HbA1c)%, accompanied by marked decrease in serum insulin, insulin like growth factor-1 (IGF-1) and body weight gain. Significant increases in serum parathyroid hormone (PTH) and bone tartrate resistant acid phosphatase (TRAP), but decreases in serum calcitonin (CT), procollagen type 1 (PC1) and oesteocalcin (OC) were also observed. This goes with further decreases in bone alkaline phosphatase (BALP), bone mineral density (BMD) and levels of Ca and P in both serum and bone. Results also showed increased oxidative stress markers [xanthine oxidase (XOD), hydrogen peroxide (H_2O_2), nitric oxide (NO) and malondialdhyde (MDA)] with decreased antioxidants [glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)] and total antioxidant capacity (TAC) in bone of diabetic rats. Administration of ZSC leaves extract was found to be effective in reducing body weight loss and all diabetes-related bone changes. Thus, ZSC leaves could be approved as a natural therapeutic agent with multiple benefits for management of diabetes-associated bone loss.
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1. Introduction

Diabetes mellitus is a common metabolic disease characterized by deficiency of insulin secretion or action causing chronic hyperglycemia. Diabetes is known to affect different body organs and to cause various complications including nephropathy, neuropathy, retinopathy and cardiovascular disease(Abuohashish *et al*., 2013).Meanwhile, diabetic patients are more likely to suffer from number of bone disorders termed as osteopenia. The association between diabetes and osteopenia was evident in both human and different experimental animals(Rao Sirasanagandla *et al*., 2014).Osteopenia is a condition in which bone resorption exceeds bone formation causing bone loss with increased risk of bone fracture. When bone loss becomes more severe the condition is termed as osteoporosis (Vijayakumar and Büsselberg, 2016). Several factors may play a key role in development of diabetic osteopenia. They include insulin deficiency with impaired mineral metabolism, and alteration in bone remodeling factors (Liang *et al*., 2011)**.**Hyperglycemia was also found to increase bone fragility, through diminished bone matrix microstructure and altered bone cells function (Zhen *et al*., 2010).

Insulin like growth factor-1(IGF-1) is a growth-promoting polypeptide essential for normal growth and development. Many studies found that IGF-1 correlates positively with bone formation markers in patients with type 1 diabetes(Ishikawa *et al*., 2015).In diabetes, impaired bone formation may result from deficiency of IGF-1, which leads to lowered bone mass. Studies have shown that low serum IGF-1 may contribute to pathogenesis of reduced bone mineral density (BMD)(Aboelasrar *et al*., 2010).

Diabetes is also associated with increased generation of reactive oxygen species (ROS) and oxidative stress making disruption of cellular functions, damage of proteins and lipids. Oxidative stress has been suggested as one of the affecting factors on diabetic osteopenia, probably through inhibiting osteoblasts formation and differentiation(Blakytny *et al*., 2011). As a direct result, bones lose their density and become more susceptible to fractures (Ferrucci *et al*., 2014).

In this area of research, studies have been carried out to verify benefits of medicinal plants to diabetic patients with osteopenia and those at risk for developing bone disorders. Medicinal plants are fairly preferred due to their effectiveness, fewer side effects and relatively low cost (Abd Jalil *et al*., 2012). *Ziziphus spina-christi* (ZSC) commonly known as (Sidr, or Wild) has a long history of usage as remedy and for health care. ZSC is a deciduous tree native to the warm-temperate and subtropical regions, including North Africa, Mediterranean Sea region

and Middle East. Because of easy growing and collection of the plant materials, it is widespread in many countries, including Egypt (Asgarpanah and Haghighat, 2012). Different extracts of ZSC have been used in folk medicine for treatment of pain, fever, wound and ulcers(Alhakmani *et al*., 2014).ZSC also known to have antibacterial, antifungal, antioxidant, anti-hyperglycemic, and anti-conceptive activities due to presence of numerous bioactive phytochemicals (Waggas and Al-Hasani, 2010). Flavonoids, alkaloids and saponins are the main phytochemicals which are reported from this plant(Asgarpanah and Haghighat, 2012). Evidence is provided that flavonoids could have a beneficial effect in preventing or reducing bone loss in osteoporosis conditions(Wattel *et al*., 2003). The increased level of osteocalcin in patients who received ZSC extract confirms the biological activity of these compounds with decreasing of bone resorption (Hussein *et al*., 2009).Most of phytochemicals detected in ZSC extracts have potent antioxidant capacity through their ability to donate H atoms/ electrons from their hydroxyl group to the free radicals. Presence of these phytochemicals would influence oxidative stress and consequently decrease bone resorption(Lamien-Meda *et al*., 2008).

In view of the wide medical activities of ZSC, the present study was carried out to evaluate whether prolonged intake of ZSC leaves extract could play a positive role in reducing diabetes- associated bone loss and also to explore mechanisms underlying biological action of ZSC.

2. Materials and methods

Experimental animals

This study was performed on male albino rats weighing (180±5g), obtained from Center of Serum and Vaccine (Helwan, Cairo, Egypt). They were housed in stainless steel cages under controlled temperature (22-25˚C) and good ventilation for 12 hrs light/dark cycles in the animal house, Faculty of Science, Mansoura University, Egypt. Rats were permitted adequate standard laboratory diet and given water *ad libitum*. They were acclimated for one week prior to the experimental work. All animals were treated in accordance with guidelines of Animal Care Committee of Mansoura University.

Plant material and preparation of the extract

Fresh leaves of *Ziziphus spina-christi* (ZSC) were collected from the local gardens of Mansoura University, Mansoura, Egypt, at the fruiting stage during February, 2015. The fresh plant leaves were washed with clean water, air dried and grinded to get fine powder. Dried powdered leaves were exhaustively extracted using 70% ethanol at room temperature for 72 hrs. Obtained extract was evaporated until dryness. Dry ZSC was dissolved in distilled water to give aqueous solution for oral administration at a dose equal 100 mg/kg b.wt (Abdel-Zaher *et al*., 2005).

Induction of diabetes

For induction of diabetes, overnight fasted rats were i.p. injected by a single dose (50 mg/kg b.wt) of freshly prepared streptozotocin dissolved in citrate buffer (pH 4.5). After three days of injection, successful induction of diabetes was estimated using urine glucose test obtained from Condor-Technology Co. Ltd Netherland for *in vitro* analysis.

Animal groups

After adaptation period, rats were divided randomly into five groups (6 animals/each). The first group was fed a standard diet and served as a normal control. The second group was fed a standard diet and received single dose of citrate buffer (pH4.5), while the third was fed a standard diet and received ZSC leaves extract orally at dose (100mg/kg b.wt) daily for 3 months. The fourth group was fed standard diet and received a single dose of STZ (50 mg/kg b.wt) dissolved in citrate buffer (pH 4.5). In the fifth group, rats received both STZ and ZSC extract at the same mentioned doses and time (Abdel-Zaher *et al*., 2005).

Samples collection and processing

At the end of experimental period (3months), overnight fasted rats were anesthetized and blood was withdrawn from the heart. Two blood samples were collected from each rat, the first in clean heparinized tube for measuring glycated hemoglobin (HbA1c)%, while the second was left to clot, then centrifuged for separating serum. Aliquots were used immediately for assessing glucose levels and the remaining samples were kept at -20 ˚C for later biochemical analysis. After collecting blood, animals were sacrificed, dissected and the two femurs were removed. One femur was homogenized in ice cold saline solution and the homogenate was kept at -20˚C for further analysis. The other one was used for determination of bone mineral density (BMD) by Burker-Micro CT- analyzer, according to Bouxsein *et al*. (2010).

Biochemical analysis

Levels of glucose, calcium (Ca) and phosphorous (P) were measured using commercial kits obtained from Spin React Co.(Santa Coloma, Spain). Plasma glycated hemoglobin (HbA1c)% was analyzed by ELISA kit obtained from Cusabio Biotech Company, while analyzing insulin and tartrate resistant acid phosphatase (TRAP) was performed by ELISA kits purchased from [BioVendor Co. (Shibayagyi, Ltd, Japan), and Blue Gene Biotech Co. (Shanghai, China), respectively]. Other biochemical parameters, including [insulin like

growth factor-1 (IGF-1), parathyroid hormone (PTH), calcitonin(CT), osteocalcin (OT), procollagen type 1 (PC1) and bone alkaline phosphatase (BALP)] were estimated using ELISA kits obtained from MyBioSource (MBS) Co. (Sandiego, Calif, USA). Antioxidants [reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT)] and total antioxidant capacity (TAC), besides oxidative stress markers [xanthine oxidase (XOD), hydrogen peroxide (H_2O_2), nitric oxide (NO) and malondialdehyde (MDA)]were also measured using ELISA kits, supplied from MyBioSource (MBS) Co. (Sandiego, Calif, USA).

Statistical analysis

Present results were analyzed by one-way ANOVA procedure using the Graphpad prism software (version 5). Data were expressed as mean±SE and the values were considered statistically significant at $p \leq 0.05$.

3. Results

As shown in Tables1,2 and 3, obtained data did not show any significant changes between normal untreated control and normal rats administered ZSC leaves extract, indicating non toxicity of this plant. In diabetic animals, significant decrease in the body weight gain with increase in serum glucose and plasma HbA1c%were noticed on comparing to control group. However, administration of ZSC extract to diabetic rats daily for 3 months caused marked improvement through normalizing the recorded changes in the body weight, serum glucose and plasma HbA1c% (Table 1).This goes with significant decrease in serum IGF-1, CT, OC, PC1 and minerals (Ca & P), accompanied with significant increase in PTH in the diabetic animals as compared with the control ones. Conversely, administration of ZSC leave extract to diabetic rats was found to modulate the above all described changes (Table 2). Results also showed significant decrease in bone minerals (Ca & P), bone mineral density (BMD) and BALP, in association with increase in bone TRAP in the diabetic animals. Further changes, including significantly increased values of XOD, H_2O_2, NO and MDA, accompanied by significant decrease in GSH, SOD, CAT and TAC were recorded. Meanwhile, administration of ZSC leaves extract daily for 3 months to diabetic rats was found to exhibit protective effect against diabetes-induced changes in bone minerals, enzymes and oxidative status on comparing to the diabetic group (Table 3), indicating anti-diabetic and anti-osteopenic effects of ZSC leaves extract.

Table1.Body weight gain, serum glucose and plasma glycated hemoglobin (HbA1c)% in control and different experimental groups

Tested Variables	Animal groups				
	Control	Citrate	ZSC	Diab.	Diab.+ZSC
Body weight gain (g)	310.8±8.67	302.3±5.74	295.8±4.44	192.7±5.24[a]	283.7±6.21[b]
Glucose (mg/dl)	81.83±2.30	79.50±2.43	73.58±0.76[a]	364.3±25.13[a]	145.8±7.22[a,b]
HbA1c%	5.01± 0.08	5.06± 0.04	4.80± 0.04[a]	8.16±0.24[a]	6.34±0.14[a,b]

Data are presented as means ±SE (n=6). Diab=diabetic. ZSC = *Ziziphus spina-christi*. a: is significant change at $p \leq 0.05$ on comparing with control group. b: is significant change at $p \leq 0.05$ on comparing with diabetic group.

Table2. Serum biochemical variables in control and different experimental groups

Tested Variables	Animal groups				
	Control	Citrate	ZSC	Diab.	Diab.+ZSC
Insulin(µIU/ml)	14.97±0.32	15.01±0.46	16.05±0.11[a]	4.64± 0.11[a]	11.81±1.30[a,b]
IGF-1 (ng/ml)	7.05±0.06	6.96±0.05	7.07±0.04	2.55±0.10[a]	5.76±0.51[a,b]
PTH (pg/ml)	18.55±0.57	17.19±0.69	16.29 ±0.55	53.87±0.90[a]	34.35±0.50[a,b]
CT (pg/ml)	6.67 ± 0.10	6.51± 0.11	6.74 ±0.04	3.30 ± 0.06[a]	4.45 ± 0.04[a,b]
OC (pg/ml)	42.48±0.35	40.90±0.54	44.4 ± 1.13	16.7 ± 0.45[a]	36.35± 3.35[b]
PC1 (pg/ml)	88.84±1.83	87.07±1.60	91.7 ± 1.30	39.04±0.82[a]	61.16±1.10[a,b]
Ca (mg/dl)	10.03±0.18	10.08±0.21	10.51±0.28	6.82±0.13[a]	8.87±0.22[b]
P (mg/dl)	4.65±0.28	4.47±0.26	5.14±0.36	2.21±0.15[a]	3.84±0.15[a,b]

Data are presented as means ±SE (n=6). Diab=diabetic. ZSC = *Ziziphus spina-christi*. a: is significant change at $p \leq 0.05$ on comparing with control group. b: is significant change at $p \leq 0.05$ on comparing with diabetic group.

Table3.Bone biochemical variables in control and different experimental groups

Tested Variables	Animal groups				
	Control	Citrate	ZSC	Diab.	Diab.+ZSC
Ca (mg/g)	9.12±0.12	9.18±0.19	9.62±0.13	6.82±0.13[a]	8.87±0.22[b]
P (mg/g)	6.41±0.34	6.16±0.36	7.10±0.42	3.26±0.25[a]	5.69±0.31[b]
BMD (g/cm^{-3})	3.05±0.03	3.06±0.04	3.17±0.04[a]	2.01±0.04[a]	2.73±0.03[a,b]
BALP (ug/g)	44.12±0.63	43.10±1.67	45.41 ±0.73	19.94 ±1.38[a]	36.93±1.26[a,b]
TRAP (ng/g)	1.63±0.05	1.67±0.07	1.49±0.09	2.34±0.03[a]	1.72±0.08[a]
XOD (ng/g)	0.56±0.01	0.55±0.01	0.51±0.01[a]	0.93±0.02[a]	0.64±0.01[a,b]
H_2O_2(Mmol/g)	10.00±0.10	10.07±0.18	9.14±0.18	24.55±0.54	12.97±0.69[a,b]
NO (Mmol/g)	58.65±1.05	53.73±1.34	49.10±0.88[a]	123.7±3.67[a]	76.12±0.88[a,b]
MDA (Mmol/g)	63.84±2.79	65.55±3.03	52.52±2.37[a]	159.2±12.00[a]	71.68±2.81[b]
GSH (ng/g)	8.08 ±0.39	8.18 ±0.41	8.49 ±0.10	1.92 ±0.11[a]	6.29 ±0.44[a,b]
SOD (u/g)	46.43±0.80	46.52±0.52	48.81±0.21[a]	14.13±0.72[a]	37.28±1.34[a,b]
CAT (Mmol/g)	55.19±1.04	58.62±0.80	60.11±0.67[a]	19.04±0.56[a]	37.93±0.61[a,b]
TAC (ng/g)	6.44±0.30	6.89±0.18	7.22±0.38[a]	3.10±0.04[a]	5.72±0.21[b]

Data are presented as means ±SE (n=6). Diab=diabetic. ZSC = *Ziziphus spina-christi*. a: is significant change at $p \leq 0.05$ on comparing with control group. b: is significant change at $p \leq 0.05$ on comparing with diabetic group.

4. Discussion

Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemia, which is a leading cause for number of health problems or complications (Ozougwu *et al*., 2013). STZ injection to rodents is a well know diabetic model involving pancreatic β-cell destruction, diminished insulin level and significant hyperglycemia (Lenzen, 2008). Similar findings were observed in the present study, where STZ induced diabetes exhibited significant decrease in serum insulin level with marked elevation in serum glucose and plasma HbA1c%. This goes with significant reduction in the body weight gain of diabetic rats, confirming the evidence that abnormalities of serum glucose and insulin levels in diabetes is particularly related to loss of body weight (Choudhary *et al*., 2014).

Several studies have described that diabetes is closely associated with impaired bone formation in the experimental animals (Lu *et al*., 2003), and in human, diabetes was found to be associated with prolonged fracture union time and delayed healing(Jiao *et al*., 2015). Diabetic patients are commonly featured with skeletal disorders named osteopenia, characterized by deranged Ca and P levels, reduction in bone mass and increased risk of bone fracture. Results of the present study similarly indicated decreased serum and bone minerals (Ca &P) with consequent decline of BMD in the diabetic rats, indicating reduced bone formation (Zhang *et al*., 2013).

In other studies, alterations of mineral metabolism and bone remodeling factors are claimed to be possible mechanisms of diabetes induced osteopenia (Ward *et al*., 2001). Osteopenia is a condition in which bones lose minerals like Ca and P. This results in weak bones that become prone to fractures(Ward *et al*., 2001. Diabetic bone disease or osteopenia is thought to result in part from either impaired PTH secretion or deficient osteoblast response to PTH. By this regard, daily administration of PTH to diabetic rats after STZ injection tends to restore the decreased trabecular bone mass and bone turnover (Lozano *et al*., 2009). The effect of PTH on bone is complex; it stimulates bone resorption or formation depending on the used concentration, duration, and the administration method (Tsuchida *et al*., 2000). An increase in PTH secretion may occur to correct for any possibility of a reduction in serum Ca. However, the complete disturbance of mineral metabolism observed in diabetes also involves alterations in Ca homeostasis, with an impaired PTH response to Ca. Most studies suggested that high level of PTH is closely related to prevalence of bone loss or osteopenia in diabetes (Motyl *et al*., 2012).

Developed bone loss in diabetes could be related also to decreased CT level which is essential for inhibiting osteoclasts (the cells causing bone breakdown). When given to patients with osteoporosis, CT produces modest increase in bone mass (Al-Hariri, 2016). Results of the present study exhibited that diabetic rats attained decreased CT level, coupled with higher PTH which together lead to decreased bone mineralization.

Although the established role of PTH and CT, other pathogenic factors have to be elucidated. The most important is insulin deficiency. Reports have revealed that insulin was found to directly induce osteogenic action through increasing osteoblasts proliferation and differentiation, ALP activity and expression of PC1 and OC (Wongdee and Charoenphandhu, 2011). Several studies indicated

that collagen plays a substantial role in the mechanical properties of bone, while the mineral content is mainly involved in determining bone stiffness(Viguet-Carrin *et al*., 2006).Type1 collagen fibrils secreted by osteoblasts are arranged into the organic matrix osteoid, which is subsequently mineralized by Ca and P in the presence of BALP and OC (Harada and Rodan, 2003). OC is another osteoblast-specific protein, has several hormonal features and is secreted in the general circulation (Patti *et al*., 2013). As support, the present study showed decreased levels of serum insulin, accompanied by reduction in PC1, OC and BALP in the diabetic rats. This goes with increased levels of the bone resorption marker TRAP, indicating increased osteoclastic activity(Kini and Nandeesh, 2012).

Evidence offers that many of bone anabolic effects are mediated by IGF-1. Both the systemic circulating, as well as the locally synthesized IGF-1 tend to increase bone formation which is mediated through IGF-1 receptors widely expressed on osteoblasts (Klein, 2014). IGF-1 is regarded as a regulator of bone metabolism that increases both bone minerals deposition and decreases collagen destruction and bone loss (Starup-Linde and Vestergaard, 2015). In patients with uncontrolled diabetes, the levels of free IGF-1 are low due to an increase in IGF-1 binding proteins, particularly IGFBP3 (Moyer-Mileur *et al*., 2008). Insulin may increase circulating IGF-1/IGFBP3 ratio through up-regulating hepatic IGF-1 synthesis or reducing hepatic secretion of IGF-1 binding proteins, resulting in higher free or bioactive IGF-1. Hence, low IGF-1 as a result of insulin deficiency with diabetes may lead to altered osteoblasts differentiation and decreased BMD (Wongdee and Charoenphandhu, 2011). As such, the presently recorded decline of IGF-1 may be considered as a key mediator in the progression of bone loss developed in the diabetic rats.

Diabetes is often accompanied by increased oxidative stress due to depletion of cellular antioxidants, excessive generation of reactive oxygen species (ROS) or both (Sandukji *et al*., 2011). ROS are known to stimulate bone resorption via increasing the activity of osteoclasts(El-Wakf *et al*, 2013).*In vitro* studies have also shown that oxidative stress inhibits osteoblasts differentiation and induces osteoblasts insult and apoptosis. Thus, oxidative stress could play a causative role in the development of osteopenia in the diabetic cases (Hamada *et al*., 2009). Previous studies indicated an association between IGF-1 and diabetes induced oxidative stress and further evidenced that elevated ROS allow inhibition of insulin/ IGF-1 signaling with reduced IGF-1 receptors (IGF-1R) levels(Ilatovskaya *et al*., 2013).

In this context, XOD has considered as a potential source of ROS. Studies of diabetic human showed increased activity of XOD, which is important for generating ROS, such as hydrogen peroxide (H_2O_2) and superoxide anion (O^-_2)(Granger and Kvietys, 2015). XOD tends also to catalyze production of active nitrogen species (NO) which in turn may react with O^-_2 to form cytotoxic oxidant peroxynitrite ($ONOO^-$) leading to sequence of oxidative damage (Zhao, 2007).

Although different mechanisms have identified, oxidative stress is generally regarded as a direct consequence of chronic hyperglycemia in diabetes. This may occur through diverse of molecular pathways, like glucoxidation, glycation and mitochondrial over production of ROS (Rains and Jain, 2011). ROS can react with cellular unsaturated fatty acids and might cause lipid peroxidation. MDA is one of the most common markers of lipid peroxidation, and an increase in MDA levels indicate impairment of non-enzymatic and enzymatic antioxidant defense system(Sameni *et al*., 2016). In this view, the presently observed increase of bone oxidative stress markers (H_2O_2, NO, MDA and XOD) with lowering of TAC and cellular antioxidants (GSH, SOD and CAT) may thus be involved in developing osteopenia in the diabetic rats.

For long time, several plants have been used as an alternative medicine for controlling diabetes (Kooti *et al*., 2016). Among those plants, *Ziziphus spina-christi* (ZSC) has been recognized. Administration of ZSC leaves extract to diabetic animals have shown to cause glucose lowering action, as typically achieved in the current study and in prior investigations (Parsaeyan and Rezvani, 2014). This effect is probably mediated by increased insulin secretion via blocking K_{ATP} channels in pancreatic beta cell membranes. K_{ATP} channels play a central role in membrane depolarization and activation of voltage-dependent Ca channels which in turn elevates Ca influx and initiates exocytosis of insulin (Zhang *et al*., 2013). Data from current study showed increased insulin levels with subsequent normalization of body weight gain in response to ZSC administration, probably due to presence of active phytochemicals such as quercetin (Gaikwad *et al*., 2014).

Quercetin which is one of the most prevalent polyphenols of ZSC leaves has shown to increase insulin release in STZ-induced diabetic rats by promoting regeneration of islets of Langerhans and increasing its number (Vessal *et al*., 2003). ZSC plant is a rich source of active polyphenolic compounds. The effect of polyphenols on bone metabolism looks promising, and the long term measurements of bone

minerals may confirm bone-protective effect of those natural constituents(Alhakmani *et al*., 2014). Flavonoids as abundant polyphenols were reported to modulate the level of PTH with subsequent reduction of bone resorption and osteoclastic activities (Elkomy and Elsaid, 2015). Polyphenols appear also to enhance the effect of calcitonin on Ca metabolism, which may aid in preventing bone loss(Das and Crockett, 2013). In the present study, administration of ZSC leaves extract to diabetic rats showed reduction of PTH with increased levels of CT which may have an association to enhanced bone mineralization and bone formation, probably due to presence of numerous types of flavonoids.

Flavonoids; such as quercetin was found to exhibit a potent inhibiting effect against *in vitro* bone resorption activity via its inhibitory effect on osteoclasts differentiation with reduced osteoclasts production (Wattel *et al*., 2004).Quercetin can also alleviate diabetic induced impairment in BMD, Ca and P levels, microstructure of the bone, as well as biochemical and mechanical markers of bone turnover, thereby attenuates bone loss (Liang *et al*., 2011). Rutin is another flavonoid caused proliferation and differentiation of human osteoblast-like MG-63 cells. Also, it showed higher increase in the activity of BALP, expression of PC1 and degree of mineralization in osteopenic cases (Hyun *et al*., 2014). Flavonoids as genistein and daidzein have also reported to cause stimulatory effect on protein synthesis and on BALP, with decreased TRAP which may be responsible for inhibited osteoclast activity and increased bone formation(Li *et al*., 2013).In the present study, prolonged administration of ZSC leaves extract to diabetic animals succeeded to reduce diabetic bone loss, through increasing bone OC, PC1, BALP activity and decreasing TRAP with enhancing bone minerals (Ca & P) level, which in all may be related to ZSC enrichment of various phytochemicals.

Earlier studies provided evidence that saponins which are widely identified in ZSC leaves extract possess potent insulin tropic effect and were found to stimulate releasing of insulin in pancreatic cells (Metwally *et al*., 2012). Tannins also increase insulin levels by inhibiting insulin degradation (Hussein *et al*., 2006). In addition, Othman *et al*. (2009)reported that ZSC extract, as a rich source of (tannins and saponins) are effective in increasing serum insulin level, with increased hepatic synthesis of IGF-1 (Kini and Nandeesh, 2012). IGF-1, as a growth regulator promotes body cells proliferation, regulates protein synthesis, and contributes to bone growth, tissues and organs development. Effects of IGF-1 on bone both *in vivo*(Guerra-Menéndez *et al*., 2013) and *in vitro* (Marie and Kassem, 2011) were evidenced, where IGF-1 enhances bone collagen and matrix synthesis and stimulates the replication of cells from the osteoblast lineage. Recent studies presented that flavonoids have performance effects on increasing IGF-1 levels (Qi *etal*., 2017).This agreed with the present findings showing improved insulin levels with up regulating of IGF-1 in the diabetic animals following administration of ZSC, thereby promoting bone formation and inhibiting bone resorption. ZSC has also shown to possess potent antioxidant properties (Abuohashish *et al*., 2013), mostly due to presence of quercetin. Previous study demonstrated that quercetin; as an active flavonoid compound; has a protective effect against diabetes through decreasing different markers of oxidative stress, besides increasing antioxidant enzymatic activities (Tung and Chang, 2010). As such, the present findings showed decreased bone MDA, NO and XOD, along with enhanced bone TAC and antioxidants (GSH, SOD and CAT) in the diabetic rats following ZSC administration. Thus, indicating powerful antioxidant action of ZSC probably due to the effect of flavonoids in competing with free radicals that burden the anti-oxidative function of these compounds(Othman *et al*., 2009). However, this effect may not be related only to direct ROS scavenging, but also to flavonoids ability of increasing cellular antioxidants depending on the structure and substitution pattern of their hydroxyl groups (Pandey *et al*., 2012).

Conclusion

The present study confirmed that induction of diabetes tended to cause remarkable bone loss or osteopenia which may contribute directly to chronic hyperglycemia, hormonal imbalance and lowering of IGF-1 with subsequently increased bone oxidative stress. However, ZSC administration seemed to be potentially involved in preventing diabetes-induced bone loss mainly via normalizing hyperglycemia and the hormonal pattern as an initial events followed by increasing IGF-1 bioavailability.

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