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The ability of hesperidin compared to that of insulin for preventing osteoporosis induced by type I diabetes in young male albino rats: A histological and biochemical study

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ABSTRACT

Background: Patients with type I diabetes are at increased risk of osteoporosis even after insulin therapy in adult stage. This study was conducted to compare the efficacy of hesperidin (hesp) therapy versus that of insulin alone in the alleviation of osteoporosis arising from type I diabetes mellitus (T1DM) in young rats.

Materials and methods: Hesperidin was administered orally to STZ-induced diabetes. The animals were evaluated morphologically and biochemically and compared with that received daily SC injections of long-acting insulin.

Results: Histologically, we observed the degeneration of osteoblasts and osteocytes, decreased collagen fibers, and disturbed bone turn over markers in untreated DM rats. Hesperidin+ insulin supplementation to diabetic rats caused significant improvement of most of the bone histological and morphometric parameters compared with the insulin-treated group. Furthermore, hesp treatment significantly reduced pro-inflammatory mediators TNFα and NF-κB and increased serum biochemical markers of bone turnover, including osteopontin (OPN), osteocalcin (OC) and decreased serum alkaline phosphatase (ALP).

Conclusion: These data demonstrated that hesp could be considered to be a beneficial drug for preventing diabetic osteoporosis in growing age.

1. Introduction

Diabetes is one of the most important metabolic disorders in the world that causes death, lifelong disability and complications (Gispen and Biessels, 2000). Insulinopaenia in T1DM is associated with osteoporosis (Nicodemus and Folsom, 2001). Type 1 diabetic patients are at high risk for bone loss, bone fracture and delayed fracture healing with significantly lower bone mineral density (Heap et al., 2004; Bayat et al., 2013). Disastrous outcomes could be associated with simple fracture in diabetic patients even after insulin treatment (Mann et al., 2010; Birdee and Gloria, 2010).

Oxidative stress has been implicated in pathogenesis of diabetes-related bone diseases. In recent years, many studies demonstrated potential efficacies of polyphenols on metabolic disorders and complications induced by diabetes. Flavonoids are present in several types of vegetables and fruits, and may be associated with potential health benefits (Sharma et al., 2008; Cao et al., 2007).

Hesperidin (hesp) is considered as a citrus bioflavonoid exclusively found in citrus fruits and called sometimes “Vitamin P”. It acts as an antioxidant and proved by other investigator to have anti-cancer and anti-inflammatory because of its antioxidative activities (Mahmoud et al., 2012).

Hesperidin acts on bone to increase the anabolic process and increase bone production (Lister et al., 2007). Studies published in the last few years have shown that citrus compounds may promote bone health. Although the hypoglycemic and hypolipidemic effects of hesperidin in rodents are well studied, the actual molecular mechanism of this effect is not well established (Akiyama et al., 2010). They act early in life when maximal bone mass is reached

Abbreviations: ALP, Alkaline phosphatase; BTM, bone turnover marker; BW, Body weight; DM, Diabetes Mellitus; Hesp, Hesperidin; H&E, Hematoxylin & Eosin; OPN, Osteopontin; OC, Osteocalcin; STZ, Streptozotocin; T1DM, Type 1 diabetes mellitus.

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and later in life during post-menopausal osteoporosis (Martin et al., 2016). Many studies detected beneficial role of hesp on postmenopausal osteoporosis (Chiba et al., 2003; Martin et al., 2016).

Therefore, the aim of this study was to investigate whether hesperidin can be an effective regimen for ameliorating bone complications in growing rats with diabetes and comparing the results with that receiving long acting insulin.

2. Materials and methods

2.1. Chemical and drugs

Streptozotocin (STZ, Cat # S0130) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lantus (insulin glargine) was manufactured by Sanofi–Aventis (Frankfurt am Main, Germany). Hesperidin (Cat # H5254) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Eighty male albino rats aged 3 weeks (just after weaning; 70 ± 12 g body weight) were included in the study. The rats were fed a laboratory pellet diet and provided tap water ad libitum. The animals were left for two weeks accommodation before start of the experiment.

The experiment was performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' (Institute of Laboratory Animal Resources, 1996) approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Zagazig University, Egypt.

2.3. Animal grouping

Group I (control): included 20 rats randomly assigned from initial 80 rats. They were treated intraperitoneally with vehicle only.

The rest 60 animals received STZ and then randomly assigned to three equal groups. Group II (non-treated DM): The animals with diabetes were untreated.

Group III (insulin-treated DM) and group IV (insulin+ hesp-treated DM): The animals with diabetes were administered daily SC injections consisting of 2 units of long-acting insulin (Kohzaki et al., 2008). In addition, animals of group IV were administered a daily dose of hesperidin at a dose of 200 mg/kg by orogastric tube (Bang et al., 2012).

Another group of animals was used to detect the effect of hesp on normal bone tissues to be sure that this dose is optimal and not causing any changes in normal tissue. No significant changes were detected at biochemical or structure level between this group and control group. Data are not shown.

2.4. Induction of diabetes

For induction of diabetes, rats of groups II, III, and IV were administered a single freshly prepared intraperitoneal injection of STZ dissolved in citrate buffer (pH 4.5) at a dose of 50 mg/kg after 12 h of fasting (Lee, 2006). The rats were have diabetes if the tail blood glucose concentrations were greater than 200–300 mg/dl using a glucometer in fed animals 2 days after STZ injection (Frode and Medeiros, 2008). Tail vein blood glucose samples from the groups with diabetes (II, III, IV) were measured weekly with a glucometer to observe blood glucose levels throughout the experiment.

2.5. Sampling and histological analysis

The general health conditions of the rats were observed and recorded daily. Body weights of all animals were measured weekly and at the end of the experiment.

At the time of sacrifice (8 weeks after the induction of diabetes), the animals were anesthetized. Fasting blood samples (after 12–14 h) were withdrawn from the retro-orbital vein and processed immediately into two tubes. The first tube contained fluoride for the immediate estimation of fasting blood glucose, and the second tube was allowed to clot at room temperature and then centrifuged. The cleared sera were separated into small glass tubes and stored at $-20\,^{\circ}C$ prior to the biochemical analysis.

The femurs were carefully dissected and cleared from the adjacent muscles. Parts of the femurs were snap-frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ to measure TNFα and NF-κB tissue mRNA expression. The other part was decalcified according to the method described by Bancroft and Gamble, 2002.

For the light microscopy study, the decalcified femurs were processed and stained with haematoxylin and eosin (H&E) for routine histological analysis and Masson trichrome to assess the collagen fibers. For the transmission electron microscopy study, small specimens of the decalcified diaphysis were processed according to the method of Glauret and Lewis, 1998. Ultrathin sections were stained and examined with a JEOL-JEM 1010 electron microscope in the histology department of the Faculty of medicine, Zagazig University, Egypt.

2.6. Morphometric analysis

Sections of the femurs from all groups were examined under a light microscope at 400× magnification. Measurements were taken from 10 non-overlapping fields of each specimen using an automatic analysis system and the Leica image analysis software. The number of osteoblasts was recorded; Osteoblasts were identified as single-nucleated, rod shaped cells attached to bone surface.

The mean cortical bone thickness (μm) was measured by drawing vertical lines from just beneath the periosteum to the endosteum (Balena et al., 1993). The coloured area percentage of the blue-stained collagen fibers was measured in the trichrome-stained sections.

Each field was scanned together with a microscale by a "Leica Quin" image analyser computer system (Leica Imaging System Ltd., Cambridge, England). The measuring frame of a standard area is equal to 7286, 78 μm^2. The same method was applied for measuring area percentage of the blue coloured areas for collagen fibers in Masson's trichrome stained sections but the measuring frame was of area 118476, 6 and an objective lens 100 in 10 fields for each specimen.

2.7. Biochemical analysis

2.7.1. Serum alkaline phosphatase (ALP) estimation

Serum alkaline phosphatase (ALP) estimation was measured as an enzyme marker of osteoblasts using a commercial kit (Bio-Diagnostics. Dokki, Giza, Egypt).

2.7.2. Serum osteopontin (OPN) and osteocalcin (OC)

Serum osteopontin (OPN) and osteocalcin (OC) were measured using a rat osteopontin assay kit (Immuno-Biological-Laboratories, Gunma, Japan) and a rat osteocalcin IRMA kit (Immunotopics, San Clemente, CA, USA), respectively.