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Effects of bee bread, Cornelian cherries treatment on the femoral bone structure using Zucker diabetic fatty rats as an animal model

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Abstract: This is a pilot study dealing with the reduced femoral bone structure in Zucker diabetic fatty (ZDF) rats following a 10 weeks treatment with bee bread and Cornelian cherries. The adult ZDF rats were divided into 4 groups: the E1 group received bee bread (500 mg/kg b.w. daily), the E2 group received Cornelian cherries (500 mg/kg b.w. per day), the E3 group was simultaneously supplemented by bee bread and Cornelian cherries (500 + 500 mg/kg b.w. daily), and the C group served as an untreated diabetic control. A non-significant decrease in the blood glucose, total cholesterol, triglyceride concentrations, ALP activity and a non-significant increase in the insulin, total body weight of the ZDF rats, femoral length and weight were observed in the E1, E2, and E3 groups. The treatments had no impact on the relative volume of the cortical bone, bone mineral density, bone surface and cortical bone thickness. On the contrary, the relative volume of the trabecular bone, trabecular thickness and trabecular bone surface were significantly increased in the E1 group. The relative volume of the trabecular bone and trabecular thickness were significantly elevated in the E2 group. In the E3 group, the trabecular thickness was found to have significantly increased. Single administrations of either bee bread or Cornelian cherries had more positive effects on the trabecular bone microarchitecture in the ZDF rats than the simultaneous supplementation.

Keywords: bone health; *Cornus mas* L.; diabetes mellitus; experimental animals; honey product

Diabetes mellitus (DM) is a group of chronic diseases manifested by hyperglycaemia. It is estimated that more than 382 million people currently suffer from diabetes (Sen and Chakraborty 2015), and many people will soon have diabetes. Type 2 *diabetes mellitus* (T2DM) represents the most frequent form of diabetes. It is characterised by the failure of the pancreatic β cells to secrete enough insulin in order to offset hyperglycaemia due to the reduced insulin secretion and enhanced hepatic glucose production (Garnett et al. 2005). This harmful disease negatively affects many organs, e.g., the heart, kidneys, eyes, and also adversely influences the bones.

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Bone disease was first reported as an associated complication of diabetes almost 100 years ago. DM affects the bone health through a number of mechanisms, including hyperglycaemia, side effects of pharmacological drugs and concomitant vascular complications (Shah and DiMeglio 2019). Generally, individuals with DM have a higher risk of osteoporotic fractures because they have reduced bone quality. Diabetic bone disease can, therefore, be considered as a very important aspect and a secondary complication of diabetes.

Taking a supportive therapy in the treatment of DM into account, several studies have pointed to the favourable health impacts of bee products, e.g., honey, bee pollen, bee bread. Bee bread is a fermented blend of flower pollen, honey and bee digestive enzymes which the bees apply as food for the larvae and also for the production of royal jelly (Sobral et al. 2017). The beneficial effects of bee bread are the result of several bioactive constituents, especially polyphenols with an anti-inflammatory role, phytosterols and fatty acids with anti-tumour effects, as well as polysaccharides with increased immunological activity (Margaoan et al. 2019). A Cornelian cherry (*Cornus mas* L.) is a type of plant used in traditional cuisine and folk medicine. Its relative plant, the Japanese cherry, includes the iridoid glycosides morroniside and loganin, which have antidiabetic effects and a positive impact on the lipid metabolism in mice (Park et al. 2010). The hypoglycaemic properties of *Cornus officinalis* have also been observed in alloxan-induced diabetic rats (Shamsi et al. 2011). Generally, Cornelian cherries are rich in anthocyanins, polyphenols and vitamins (De Biaggi et al. 2018).

Animal models that reflect the pathogenesis of a disease in humans are necessary for research. The T2DM animal model provides an opportunity to examine the treatment and prevention of certain diseases and also their related complications. Zucker diabetic fatty (ZDF) rats are considered to be a suitable animal model for the T2DM research (Al-Awar et al. 2016).

The present study was designated to reveal whether single and simultaneous administrations of bee bread and Cornelian cherry fruit at low doses are able to improve the femoral bone structure in the ZDF rats. We aimed to determine if these natural products have the potential to be used as a supportive therapy in the treatment of T2DM-related complications associated with impaired bone health.

MATERIAL AND METHODS

Animal care

All the experimental procedures were authorised under No. 2288/16-221 by the Ethical Committee and the State Veterinary and Food Institute of the Slovak Republic. Institutional and national guidelines for the care and use of animals were adequately followed.

Sample preparation

The bee bread and Cornelian cherries were supplied by the Institute of Biodiversity Conservation and Biosafety of the Slovak University of Agriculture in Nitra (Slovak Republic). The bee bread was crushed and mixed with distilled water. Fresh ripe cherry fruits were isolated from the stones, crushed and stored at –20 °C. Aliquots were prepared in distilled water (Sigma Aldrich, Darmstadt, Germany) and homogenised to be suitable for application by a gastric gavage.

Animals

Adult ZDF rats [n = 20, 14 weeks of age, pretreatment body weight (b.w.) 360.32 ± 14.67 g] were purchased from the Institute of Experimental Pharmacology and Toxicology (Dobrá Voda, Slovak Republic) and were bred at the Slovak University of Agriculture in Nitra. All the animals (males) were housed under standardised conditions with 12 : 12 h light-dark cycles, fed a normal diet – KKZ-P/M (complete feed mixture for rats) on an *ad libitum* basis and were divided into 4 groups: group E1 (n = 5) received bee bread (500 mg/kg b.w. per day), group E2 (n = 5) received Cornelian cherries (500 mg/kg b.w. per day), group E3 (n = 5) was simultaneously supplemented with bee bread and Cornelian cherries (500 + 500 mg/kg b.w. per day) for 10 weeks. Group C (n = 5) received distilled water in the same way and served as the untreated diabetic control.

Biochemical analysis

The ZDF rats were sacrificed by an intraperitoneal anaesthetic overdose of a xylazine (Ecuphar N.V.,

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Belgium)/Zoletil (Virbac, Carros, France) cocktail and all the samples were taken after deep anaesthesia. The whole blood was processed and the insulin was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Biotech, Bratislava, Slovak Republic). The blood glucose concentrations were determined with a FreeStyle Optium Neo Glucose and Ketone monitoring system (Abbott Diabetes Care Ltd., Maidenhead, UK) using test strips (FreeStyle; Abbott Diabetes Care Ltd., UK). The alkaline phosphatase (ALP), total cholesterol and triglyceride concentrations were measured using commercially available kits (Randox Laboratories Ltd., Crumlin, UK) on a Biolis 24i Premium biochemical analyser (Tokyo Boeki MediSys Inc., Tokyo, Japan).

Macroscopical analysis of the bones

Both femoral bones (n = 40) were weighed and their lengths were measured in each ZDF rat. In addition, the post-treatment body weight of the ZDF rats from all the groups was also measured.

Microstructural 3D analysis of the cortical and trabecular bone tissues

The microstructural 3D analysis of the femoral bone tissue was performed by microcomputed tomography (microCT 50; Scanco Medical, Bruttisellen, Switzerland). In accordance with the generally accepted guidelines (Bouxsein et al. 2010), the regions of interest (ROI) were chosen at such locations where the cortical and trabecular part of the bone could be optimally investigated. The cortical bone structure was assessed in the ROI starting 12.5 mm from the end of the growth plate (distal end) and comprising a section of 2.0 mm. The relative bone volume, bone mineral density (BMD), bone surface and cortical bone thickness were established. The trabecular bone structure was analysed in the ROI starting 2.9 mm from the end of the growth plate and extending for an additional 2.0 mm. The relative bone volume, trabecular number, trabecular thickness and bone surface were evaluated.

Statistical analysis

The statistical analysis was conducted using SPSS Statistics v26.0 software. The data were expressed as the mean ± standard error of the mean (SE). The differences in the examined parameters were detected by an analysis of variance (ANOVA) with Games-Howell's and/or Tukey's post-hoc tests. The statistical significance was assessed at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

RESULTS

Biochemical analysis

A non-significant decrease in the blood glucose, total cholesterol, triglyceride levels, ALP activity and a non-significant increase in the insulin were observed in all the treated groups (E1, E2, E3) compared with the diabetic control one. The results are summarised in Figure 1A–E.

Macroscopical analysis of the bones

We identified a non-significant increase in the total b.w. of the ZDF rats, the femoral length and weight in the E1, E2, E3 groups versus the C group (Figure 1F–I).

Microstructural 3D analysis of the cortical and trabecular bone tissues

The microcomputed tomography showed that the single and simultaneous administrations of bee bread and Cornelian cherries had an insignificant effect on the relative volume of the cortical bone, BMD, bone surface and cortical bone thickness. The results are shown in Figure 2A–D.

On the contrary, the relative volume of trabecular bone, trabecular thickness, and trabecular bone surface were significantly increased in the E1 group. The relative volume of the trabecular bone and trabecular thickness were significantly elevated in the E2 group. In the ZDF rats from the E3 group, a significantly higher trabecular thickness was found. In addition, the ZDF rats from the E3 group had lower values for the relative volume of the trabecular bone, trabecular thickness, and trabecular bone surface as compared to the E1 group. The results are provided in Figure 2E–H. Figure 3 shows representative 3D images of the femoral bone tissue.

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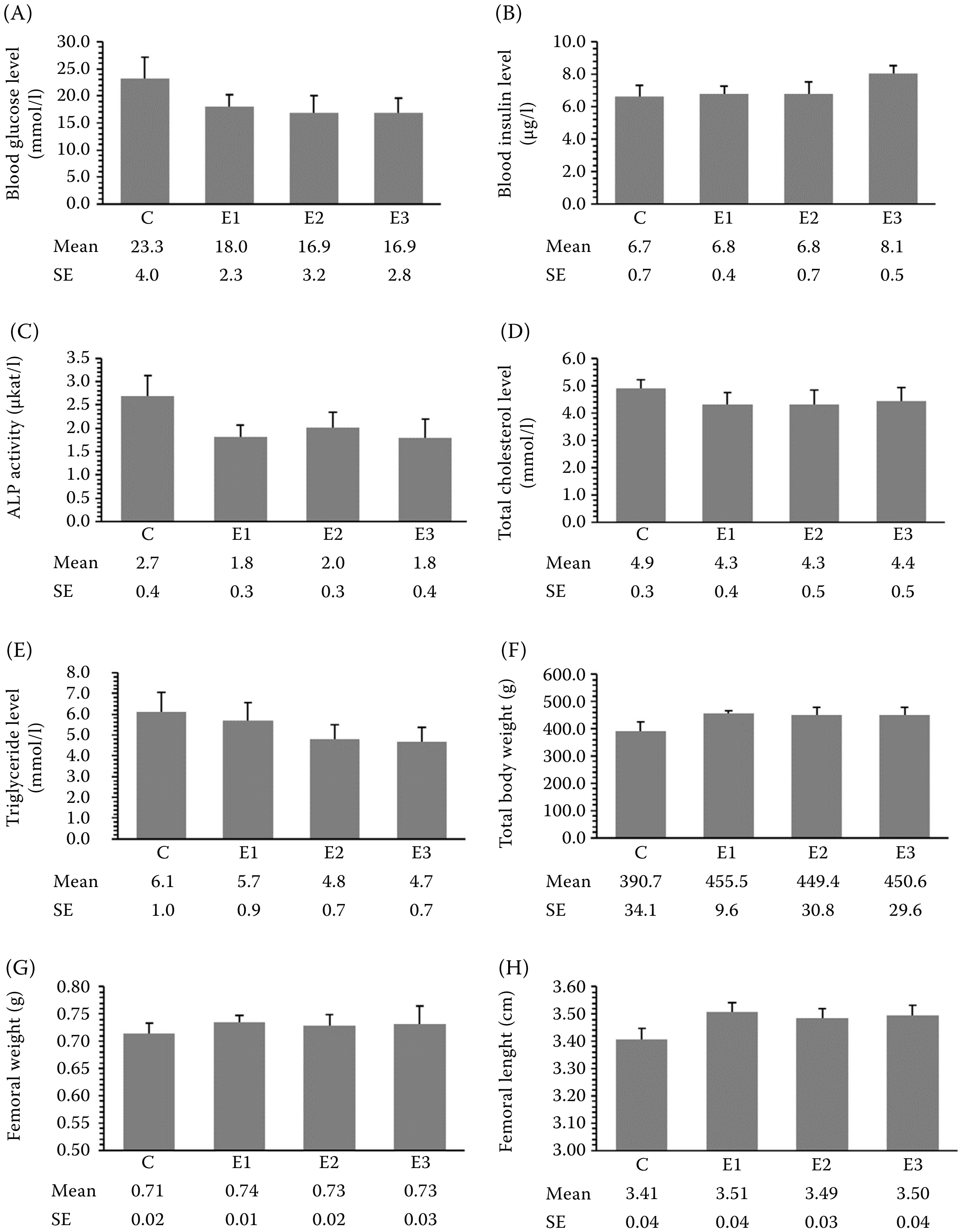

(A)

	C	E1	E2	E3
Mean	23.3	18.0	16.9	16.9
SE	4.0	2.3	3.2	2.8

(B)

	C	E1	E2	E3
Mean	6.7	6.8	6.8	8.1
SE	0.7	0.4	0.7	0.5

(C)

	C	E1	E2	E3
Mean	2.7	1.8	2.0	1.8
SE	0.4	0.3	0.3	0.4

(D)

	C	E1	E2	E3
Mean	4.9	4.3	4.3	4.4
SE	0.3	0.4	0.5	0.5

(E)

	C	E1	E2	E3
Mean	6.1	5.7	4.8	4.7
SE	1.0	0.9	0.7	0.7

(F)

	C	E1	E2	E3
Mean	390.7	455.5	449.4	450.6
SE	34.1	9.6	30.8	29.6

(G)

	C	E1	E2	E3
Mean	0.71	0.74	0.73	0.73
SE	0.02	0.01	0.02	0.03

(H)

	C	E1	E2	E3
Mean	3.41	3.51	3.49	3.50
SE	0.04	0.04	0.03	0.04

Figure 1. Effects of the bee bread and Cornelian cherry supplementation on the biochemical and macroscopical bone parameters in the ZDF rats

ALP = alkaline phosphatase; C = control group; E1, E2, E3 = treated groups ; SE = standard error of the mean; ZDF = Zucker diabetic fatty

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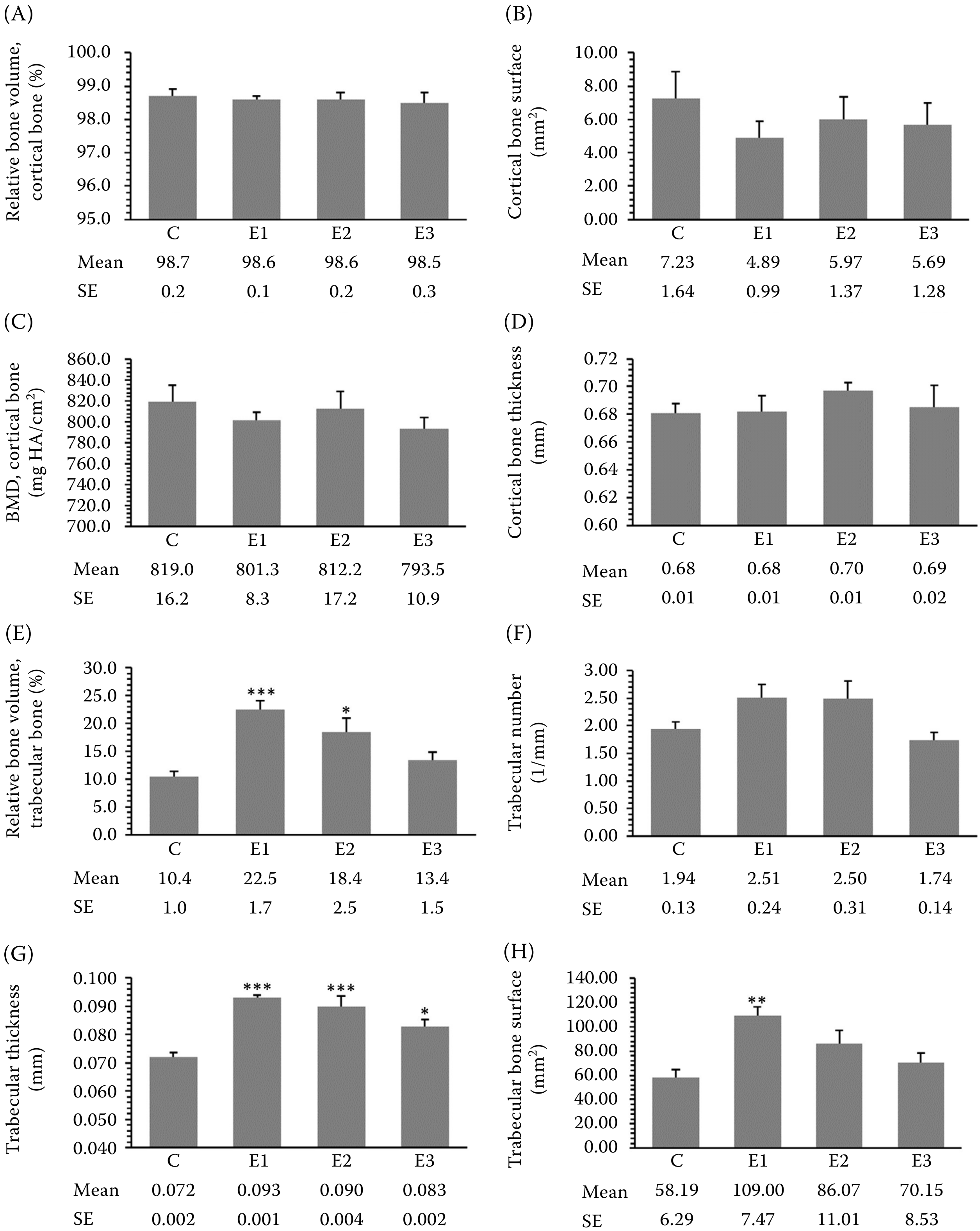

Figure 2. Effects of the bee bread and Cornelian cherry treatment on the microstructural 3D bone parameters in the ZDF rats

BMD = bone mineral density; C = control group; E1, E2, E3 = treated groups; SE = standard error of the mean; ZDF = Zucker diabetic fatty

*Significant difference (P < 0.05), **significant difference (P < 0.01), ***significant difference (P < 0.001)

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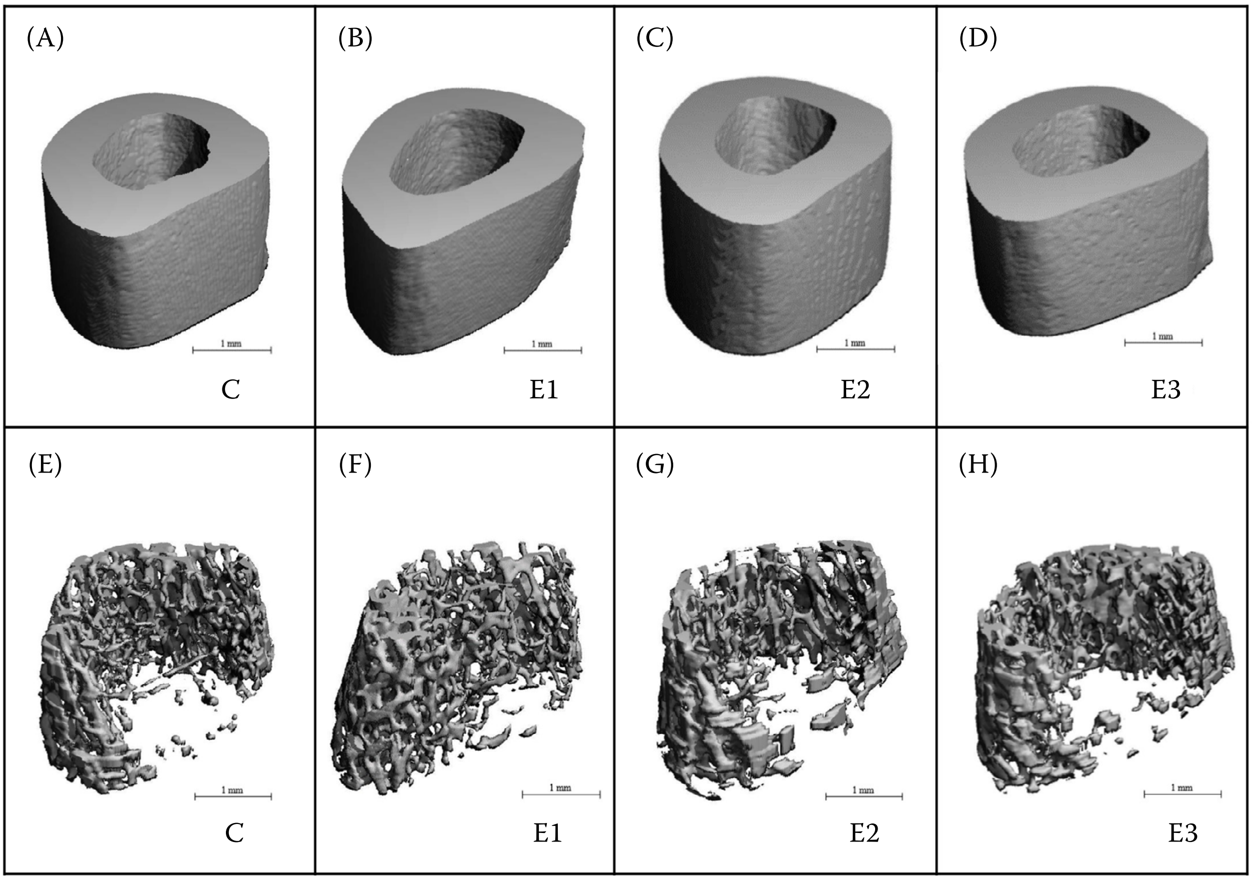

Figure 3. Representative 3D images of the cortical (A–D) and trabecular bone tissues (E–H) in the ZDF rats
C = control group; E1, E2, E3 = treated groups; ZDF = Zucker diabetic fatty

DISCUSSION

Our findings provide initial information on the femoral bone structure of ZDF rats following a bee bread and Cornelian cherries treatment. Although no significant differences in the blood glucose, total cholesterol, insulin, triglyceride concentrations, ALP activity were observed in the E1, E2, E3 groups, there was a tendency to have lower values of the blood glucose, total cholesterol, triglyceride, ALP and higher values of the insulin in all the treated groups. We suppose that a higher number of ZDF rats in each group and/or elevated concentrations of both substances used could lead to statistically significant differences. According to Capcarova et al. (2019), Capcarova et al. (2020), ZDF rats treated with a higher doses of bee bread and *Cornus mas* (700 mg/kg b.w. and 1 000 mg/kg b.w., respectively, for 10 weeks) had significantly lower blood glucose concentrations in comparison with the diabetic control group. However, this treatment did not significantly increase the insulin concentrations in the ZDF rats, which is consistent with our findings.

A relationship between the ALP activity and DM is controversial. According to Chen et al. (2017), the ALP has increased in diabetic patients when compared to non-diabetic subjects. Nevertheless, it is unclear whether the enhanced ALP is associated with liver disease, bone failure or both damage. Other studies did not report any significant relationship between ALP and incident diabetes (Hanley et al. 2004; Nannipieri et al. 2005). We observed an insignificant decrease in the ALP activity in all the treated groups, suggesting a slight protective effect of bee bread and Cornelian cherries against liver and bone disease. In general, T2DM often occurs simultaneously with elevated cholesterol levels or dyslipidaemia. In the presence of insulin resistance, the lipid is used as an alternative cellular energy source leading to increased triglyceride and/or cholesterol concentrations (Lapmanee et al. 2014). In the E1, E2, E3 groups, the values for the total cholesterol and triglycerides were insignificantly de-

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creased. In contrast, alloxan-induced diabetic rats supplemented with Nigerian honey (1.0 or 2.0 g/kg for 3 weeks) had significantly reduced total cholesterol and triglyceride values (Erejuwa et al. 2016). Dietary berries (similar to Cornelian cherries) have also been shown to reduce the total cholesterol and triglyceride concentrations (Basu 2019). The results of Soltani et al. (2015) pointed to the fact that the *Cornus mas* fruit extract improved the glycaemic control by increasing the insulin and decreasing the serum triglyceride concentrations in patients with T2DM.

The decrease in the total b.w. that occurs involuntarily, was set as a warning sign of diabetes. Although no significant differences in the post-treatment b.w. were determined in the E1, E2, E3 groups, there is a tendency to have a higher b.w. in all the treated groups. Capcarova et al. (2019), Capcarova et al. (2020) also noted a negligible increase in the total b.w. of ZDF rats receiving higher doses of bee bread and *Cornus mas*. Similarly, treatment with bee pollen (0.2% concentration for 90 days) had an insignificant effect on the total b.w., femoral weight and length of Wistar rats (Martiniakova et al. 2014). Zaid et al. (2012) also reported no significant differences in the post-treatment b.w. of ovariectomised (OVX) rats after administration of Tualang honey.

Our results from the microcomputed tomography indicate that single administrations of either bee bread or Conrelian cherries had more positive effects on the trabecular bone microarchitecture in the ZDF rats than the simultaneous supplementation. On the contrary, the cortical bone structure was not influenced by the treatment. Zaid et al. (2012) stated a significant increase in the relative volume of the trabecular bone, trabecular thickness and trabecular number in OVX rats receiving Tualang honey (0.2 g/kg b.w. for 6 weeks). According to Yudaniayanti et al. (2019), supplementation of *Apis dorsata* honey (2 g/kg b.w. for 3 months) can inhibit a reduced cortical bone thickness in OVX rats. Shimizu et al. (2018) found that treatment with an anthocyanin-rich blueberry extract (500 mg/kg b.w. for 8 weeks) had no significant impacts on the BMD, relative bone volume, trabecular number, mineralising surface and bone formation rates in OVX rats.

In accordance with our results, the different effects of the bee bread and Cornelian cherries treatment on the cortical and trabecular bone structure of ZDF rats could be caused by their dissimilar bone remodelling levels. In general, cortical and trabecular bones exhibit different metabolic behaviours in a variety of physiological and pathological conditions, such as pharmacokinetics, glycation, and also their dietary response (Li et al. 2017). As a whole, cortical bone remodelling cycles are shorter than those in the trabecular bone, and therefore these two types of bone tissue should be considered as two separate subjects in the treatment of various bone-related diseases, including T2DM. Moreover, the trabecular bone has a large area exposed to bone marrow and blood flow, and bone turnover is higher there. According to Lapmanee et al. (2014), a cholesterol-rich diet was able to mask the T2DM-induced differences in the bone microstructure of rats. Therefore, the non-significant changes in the cortical bone structure in the E1, E2, E3 groups could also be associated with this statement.

This pilot report deals with the treatment of a reduced femoral bone structure in ZDF rats following bee bread and Cornelian cherry administrations. Our results suggest that both natural products could be beneficial and useful in the prevention of T2DM-impaired bone health. However, the expected synergistic effect of the aforementioned substances on the trabecular bone microarchitecture was not confirmed. Additional studies involving higher doses of bee bread and Cornelian cherries and also a longer duration of the experiment are needed to better understand the impacts of these products on diabetic bone diseases.

Conflict of interest

The authors declare no conflict of interest.

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