UNIVERSITI TEKNOLOGI MARA

OSTEOPROTECTIVE EFFECT OF PHYTOESTROGENS AGAINST BISPHENOL A-INDUCED BONE LOSS

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of the requirements for the degree of
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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Phytoestrogens act as agonists in bone formation and differentiation which not only improves the osteoblast formation, but also affects the bone metabolism, in general. Strong bones depend on the ability of osteoblasts to mineralize the newly formed tissue and osteoclasts in removing damaged and dysfunctional bone tissue. Loss of mineralization leads to a weak bone structure and increase fracture risk. Bisphenol A (BPA), a known xenoestrogen, disturbs the osteoblast proliferation, differentiation and mineralization via changes in receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) expression and associated with binding to the non-classical oestrogen related receptor gamma (ERRG). However, the effect of phytoestrogens against the deteriorative effect of BPA related to the bone health is not yet addressed, to date. The present study was aimed to investigate the potential protective effects of soy phytoestrogens on BPA-exposed osteoblast-like cells, hFOB 1.19 cells. Following 24h of incubation with 12.5 μg/mL of BPA, the cells were treated with daidzein (Dz), genistein (Gt) and equol (Eq) at different concentrations for 24h. The important bone biomarkers; RANKL, OPG and low-density lipoprotein receptor-5 (LRP-5) along with inflammatory biomarkers and transcription factors were analysed. Cell mineralization capacity of phytoestrogens was investigated by evaluating calcium, phosphate contents and alkaline phosphatase activity. Bone related markers; osteocalcin and osteonectin, responsible in maintaining mineralization were also measured. Cells incubated with BPA 12.5 μg/mL alone showed a decrease in bone formation and bone mineralization. Following treatment with phytoestrogens, there was increased cell viability in BPA induced hFOB 1.19 cells. The suppression of RANKL and expression of OPG and LRP-5 levels in phytoestrogens-treated cells were observed. There was a decrease in IL-6 and TNF-α; increase in osterix (Osx) and Runt-related transcription factor 2 (RUNX2) expression following phytoestrogens treatment. The enhanced mineralization efficacy of Dz and Gt (particularly at a dose of 5 and 40 μg/mL, respectively) was evidenced by increasing calcium and phosphate content with higher ALP activity, compared to the untreated BPA group. Osteocalcin and osteonectin levels were increased. It was observed that the protein expression of ERRG was high in the untreated groups whereas ER alpha (ERα) and beta (ERβ) were relatively increased with phytoestrogens treatment under BPA exposure. There was upregulation of MAPK3 and GPR30 expressions which are responsible for osteoblast differentiation. The present findings indicate that phytoestrogens directly improve the osteoblast formation via RANKL/OPG pathway and revert the demineralization process in hFOB 1.19 cells by significantly downregulating the ERRG and upregulating the ERα and ERβ receptors under BPA exposure. The synergistic effects are observed in ESR1 and ESR2 activations. Treatment with phytoestrogens (specifically, low dose of Dz and high dose of Gt) significantly revert the deteriorative effect of BPA on hFOB 1.19 cells.

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