

UNIVERSITI TEKNOLOGI MARA

**OSTEOPROTECTIVE EFFECTS OF
GREEN AND BLACK TEA
POLYPHENOLS IN OSTEOBLAST
CELLS IN TNF- α INDUCED
INFLAMMATION AND
COMPARISON WITH
OSTEOCLAST CELLS**

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Thesis submitted in fulfilment
of the requirements for the degree of
Master of Science

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 1st December 2015 to conduct the final examination of Husna binti Zulkipli on her Master of Science thesis entitled “Osteoprotective of Green and Black Tea Polyphenols in osteoblast cells in TNF- α induced inflammation and comparison with osteoclast cells” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The panel of Examiners recommends that the student be awarded the relevant degree. The panel of Examiners were as follows:

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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 result of my own, unless otherwise indicated or acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

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ABSTRACT

Chronic inflammatory diseases are often linked with bone loss. The underlying mechanisms involve disturbance of remodelling cycle which include imbalance activity of osteoblasts and osteoclasts involving receptor activator of NF-KB ligand (RANKL)/receptor activator of NF-KB (RANK)/osteoprotegerin (OPG) signalling system. This study aimed to evaluate and compare the effects of green (GTE) and black tea extracts (BTE) on osteoblasts and osteoclasts in the presence of TNF- α -induced inflammation. Total Phenolic Content (TPC) of both extracts was measured using Folin-Ciocalteu reagent. Quantification of catechin and derivatives and caffeine in the extracts was performed by Ultra Performance Liquid Chromatography (UPLC). Normal human osteoblasts (NH_{ost}) were cultured in non-inflammatory and inflammatory conditions to study osteoblasts. Human Peripheral Blood CD14⁺ monocytes were cultured to study osteoclasts. MTS assay was used to measure cell viability in both cells. Osteoblasts functions were evaluated using Alizarin Red S (ARS) staining, Alkaline Phosphatase (ALP) activity, RANKL and OPG protein expression. Microarray was used to screen novel gene expression in osteoblasts. Tartrate-Resistant Acid Phosphatase (TRAP) staining assay and gene expression of osteoclast markers were done to investigate GTE and BTE effects on osteoclastogenesis. All concentrations of GTE and BTE showed viability of NH_{ost} above 80% at all-time points in both conditions. On the contrary, BTE- and GTE-treated osteoclasts showed decrease in cell viability in concentration-dependent manner with greater magnitude observed in GTE-treated cells. RANKL was significantly reduced at day 2 with 5 and 50 μ g/ml BTE in non-inflammatory condition. BTE also reduced the level at day 5 with 50 and 100 μ g/ml and at day 10 with 100 μ g/ml concentration. At day 5, all GTE treatments significantly reduced RANKL. In inflammatory condition, 100 μ g/ml GTE and BTE reduced RANKL at day 5 and 10. OPG was significantly elevated by 5 μ g/ml each GTE and BTE at all-time points in non-inflammatory condition. During inflammation, 5 μ g/ml each GTE and BTE significantly enhanced OPG at day 5 and 10. Microarray and validation experiment indicated that the genes of ASPN, BIRC3, CTSK, CCL2, ICAM-1, IL6, IL8, OMD, SFRP4, SOD2, TLR3, TNFAIP3 and TNFAIP6 have potential role in bone metabolism and inflammation. GTE and BTE significantly decreased the number of multinucleated and tartrate-positive cells compared to control. GTE- and BTE-treated osteoclasts also showed a significant decrease of ACP5 and RANK mRNA levels. Other osteoclast markers including DC-STAMP, ADAM8, Atp6v0d2, CTSK, ITGAV and CALCR were increased in BTE treatment but were decreased in GTE. This study has demonstrated that BTE in comparison to GTE may have the potential as supplementary therapy to the current treatment of bone loss associated with inflammatory bone diseases even though the effects are not prominent as that of GTE.

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