



Faculty of Medicine and Health Sciences

Investigating the Preventive Effects of *Tinospora crispa* and *Tinospora cordifolia* on Methylglyoxal-induced Cytotoxicity and Metabolic Dysregulation in Osteoblast Cells

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Investigating the Preventive Effects of *Tinospora crispa* and *Tinospora cordifolia* on Methylglyoxal-induced Cytotoxicity and Metabolic Dysregulation in Osteoblast Cells

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Patients with type 2 mellitus (T2DM) typically exhibit significantly increased methylglyoxal (MGO) levels. MGO is a reactive carbonyl compound and a byproduct of glycolysis, serving as a precursor of advanced glycation end products (AGE). AGE enters cells via its receptor of advanced glycation end products (RAGE) leading to oxidative stress, inflammation and consequently death of the cells. In osteoblasts, high AGE level can induce inflammatory response and oxidative stress (OS), as well as reducing osteoblastogenesis. *Tinospora cordifolia* (*T. cordifolia*) and *Tinospora crispa* (*T. crispa*) have been traditionally used to treat diabetes. In modern medicine, metformin serves as the primary treatment to reduce hyperglycaemia when lifestyle changes have failed. Currently, little is known about the effects *T. cordifolia* and *T. crispa* on bone remodelling when bone cells are exposed to high MGO levels. In addition, there is no data available on the interaction of metformin with *T. crispa* and *T. cordifolia* extracts. Therefore, this study aimed to evaluate the effects of *T. crispa* and *T. cordifolia* on bone metabolism in MGO-stimulated osteoblast cells (hFOB1.19) and explore the potential synergistic interactions of these two plants with metformin. The plants underwent extraction using methanol and hot water (aqueous). The phytochemical content of the four extracts were determined using GC-MS, the antioxidant activities were assessed via DPPH assay while anti-glycation properties were assessed via BSA-MGO assay. Then, the effects of the extracts on MGO-stimulated osteoblast cells were assessed with or without metformin for antioxidant capacity, anti-apoptotic activity, and anti-inflammatory properties as well as the impact on the expression and activity of the osteoblastic insulin receptors. The results showed that the plant extracts contained antioxidant and anti-inflammatory bioactive compounds, with notable scavenging activity and anti-apoptotic effects. However, the extracts showed limited anti-glycation activity as

shown in BSA-MGO and AGE formation in MGO-stimulated osteoblasts. Furthermore, the extracts significantly reduced the secretion of IL-6 and IL-18 suggesting their anti-inflammatory properties. The extracts also increased the expression of Runx2 in influencing the osteoblastogenesis. In addition, they increased expression of GLUT4, and insulin receptor in MGO-stimulated hFOB1.19, suggesting positive effects on diabetic bone metabolism. In conclusion, this study highlights the potential of *T. cordifolia* and *T. crispa* extracts in mitigating T2DM-related complications, particularly bone loss.

Keywords: *T. cordifolia*, *T. crispa*, Type 2 diabetes mellitus, bone metabolism

***Menyelidik Kesan Pencegahan Tinospora crispa dan Tinospora cordifolia Terhadap
Sitotoksiti dan Disfungsi Metabolik yang Disebabkan oleh Metilglioksal dalam Sel
Osteoblas***

ABSTRAK

Pesakit diabetes mellitus jenis 2 (T2DM) biasanya menunjukkan peningkatan yang ketara dalam tahap metilglioksal (MGO). MGO adalah sebatian karbonil reaktif dan produk sampingan glikolisis, yang berperanan sebagai pendahulu produk akhir glikasi lanjutan (AGE). AGE memasuki sel melalui reseptor produk akhir glikasi lanjutan (RAGE) yang menyebabkan stres oksidatif, keradangan dan akhirnya kematian sel. Dalam osteoblas, kadar AGE yang tinggi boleh meningkatkan respons inflamasi dan tekanan oksidatif (OS), serta mengurangkan osteoblastogenesis. Tinospora cordifolia (T. cordifolia) dan Tinospora crispa (T. crispa) telah digunakan secara tradisional untuk merawat diabetes. Dalam perubatan moden, metformin berfungsi sebagai rawatan utama untuk mengurangkan hiperglisemia apabila perubahan gaya hidup gagal. Pada masa kini, sedikit maklumat yang diketahui tentang kesan T. cordifolia dan T. crispa terhadap pembentukan semula tulang dalam sel tulang yang terdedah kepada tahap MGO yang tinggi. Selain itu, tiada data yang tersedia mengenai interaksi metformin dengan ekstrak T. crispa dan T. cordifolia. Oleh itu, kajian ini bertujuan untuk menilai kesan T. crispa dan T. cordifolia terhadap metabolisme tulang dalam sel-sel osteoblas (hFOB1.19) yang dirangsang dengan MGO dan menilai potensi interaksi sinergistik dua tumbuhan ini dengan metformin. Tumbuhan-tumbuhan ini telah diekstrak menggunakan metanol dan air panas. Kandungan fitokimia keempat-empat ekstrak ditentukan menggunakan GC-MS, aktiviti antioksidan dinilai melalui ujian DPPH manakala sifat anti-glikasi dinilai melalui ujian BSA-MGO. Kemudian, kesan ekstrak terhadap sel-sel osteoblas yang dirangsang dengan MGO dinilai dengan atau tanpa

metformin untuk kapasitas antioksidan, aktiviti anti-apoptosis, sifat anti-keradangan serta dampak terhadap ekspresi dan aktiviti reseptor insulin di dalam osteoblas. Hasil kajian menunjukkan bahawa ekstrak tumbuhan mengandungi sebatian bioaktif antioksidan dan anti-keradangan dan kesan anti-apoptosis yang tinggi. Walau bagaimanapun, ekstrak menunjukkan aktiviti anti-glikasi yang terhad seperti yang ditunjukkan dalam BSA-MGO dan pembentukan AGE dalam osteoblas yang dirangsang dengan MGO. Selain itu, ekstrak secara signifikan mengurangkan pengeluaran IL-6 dan IL-18, menunjukkan sifat anti-keradangan mereka. Ekstrak juga meningkatkan ekspresi Runx2 yang mempengaruhi osteoblastogenesis. Tambahan pula, ekstrak-ekstrak tersebut meningkatkan ekspresi GLUT4 dan reseptor insulin dalam hFOB1.19 yang dirangsang dengan MGO, menunjukkan kesan positif terhadap metabolisme tulang ketika diabetes. Secara kesimpulannya, kajian ini menekankan potensi ekstrak T. cordifolia dan T. crispa dalam mengurangkan komplikasi berkaitan dengan T2DM, terutamanya kesan terhadap osteoblas dan kesihatan tulang.

Kata kunci: *T. cordifolia, T. crispa, Diabetes mellitus jenis 2, metabolisme tulang*

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AG	Aminoguanidine
AGE	Advanced glycation end products
AO/PI	Acrymidine orange/propidium iodide
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BAK	Bcl-2 antagonist killer
BAX	Bcl-2 associated X protein
BSA	Bovine serum albumin
COC	Carboxylated osteocalcin
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DPBS	Dulbecco's Phosphate Buffered Saline
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
ETC	Electron transport chain
FADD	Fas-associated death domain
FRAP	Ferric reducing ability of plasma
GC-MS	Gas chromatography mass spectrometry
HAT	Hydrogen atom transfer
HBSS	Hanks' Balanced Salt Solution
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intercellular Adhesion Molecule 1

IKK	Inhibitor of nuclear factor- κ B (I κ B) kinase
IL	Interleukin
IRS	Insulin receptor substrate
ISO	International Organization for Standardization
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage colony-stimulating factor
MGO	Methylglyoxal
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NADPH	Nicotinamide-adenine dinucleotide phosphate
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIST-17	National institutes of standard and technology library ver. 2017
NLR	Nucleotide-binding oligomerization domain-like receptors
NY	New York
OC	Osteocalcin
OPG	Osteoprotegerin
OS	Oxidative stress
PBS	Phosphate buffered saline
PRRs	Pattern recognition receptors
RANKL	Receptor activator of nuclear factor kappa-B ligand
RFU	Relative fluorescence unit
ROS	Reactive oxygen species
RUNX2	Runt-related transcription factor 2
SET	Single electron transfer
SOST	Sclerostin