

COMPARATIVE THE EFFECTS OF PALM
TOCOTRIENOL MIXTURE AND ALPHA-
TOCOPHEROL ON JOINT HEALTH AND *WNT*
SIGNALLING PATHWAY IN MALE RATS
WITH OSTEOARTHRITIS INDUCED BY
MONOSODIUM IODOACETATE

HIBA MURTADHA HUSSEIN AL-SAADI

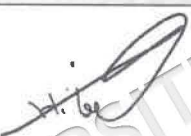
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	BORANG DAN SENARAI SEMAK PENYERAHAN TESIS/DISERTASI SELEPAS PEMBETULAN FORM AND CHECKLIST OF AMENDED THESIS/DISSERTATION SUBMISSION		

F. KELULUSAN DEKAN/PENGARAH / APPROVAL FROM DEAN/DIRECTOR			
Tandatangan: Signature		Tarikh: 24/9/26 Date	
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No. Pendaftaran Pelajar (Student's Registration No.)	P106538	Sesi Akademik (Academic Session)	SEMESTER 1, 2020/2021
Tajuk Tesis / Disertasi (Thesis / Dissertation Title)	COMPARATIVE THE EFFECTS OF PALM TOCOTRIENOL MIXTURE AND ALPHA-TOCOPHEROL ON JOINT HEALTH AND WNT SIGNALLING PATHWAY IN MALE RATS WITH OSTEOARTHRITIS INDUCED BY MONOSODIUM IODOACETATE		
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HIBA MURTADHA HUSSEIN AL-SAAD

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

FACULTY OF MEDICINE
UNIVERSITI KEBANGSAAN MALAYSIA
KUALA LUMPUR

MEMBANDINGKAN KESAN CAMPURAN TOKOTRIENOL SAWIT DAN
ALFA-TOKOFEROL TERHADAP KESIHATAN SENDI DAN LALUAN
ISYARAT *WNT* PADA TIKUS JANTAN DENGAN OSTEOARTRITIS
YANG DIARUH OLEH MONOSODIUM IODOASETAT

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries, which have been duly acknowledged.

28 February 2026

HIBA MURTADHA HUSSEIN AL-SAAD

P106538



ACKNOWLEDGEMENT

First and foremost, praise be to Almighty Allah for all his blessings for giving me patience and good health throughout the duration of this master's research.

I am very fortunate to have Assoc. Prof. Dr. Chin Kok Yong as my research supervisor. He has been a constant source of guidance, encouragement, and support throughout my journey. I am also grateful to my co-supervisors, especially Prof. Dr. Ima for her invaluable advice and strong support, Assoc. Prof. Norliza for her kindness and cooperation. Their collective support has greatly eased my journey as a PhD's student, and I truly appreciate all that they have done for me.

I would like to thank technicians Fadhlullah Zuhair, Juliana Abdul Hamid and Azlan bin Mohd Arlamsyah, and Mr. Mustazil; and all postgraduate students from the Bone Metabolism Research group for their help, friendship and creating a pleasant working environment throughout my two-year journey in Universiti Kebangsaan Malaysia.

I would also like to extend my gratitude to my dearest family: my father Dr. Murtadha AL-Saadi, for his support and unconditional love; my kind mother, Mrs. Ekhlash; my dearest brothers Dr. Mohammed and Ali; my lovely and beautiful sister, Dr. Zainab, and also to my Family-in-law; my father in law Dr. Jaafar; my mother in law Mrs. May; my brother in law Ahmed and; Hassan and my beautiful sister in law Muhaj, all for their emotional and financial support and unconditional love.

I would also like to thank my beloved and supportive husband; and my sons, Haider, Ahmed and Ameer, for their unconditional love, support and patience for me. You are the reason why I stand strong every day. I hope that I will be your backbone when you need my support in the future.

ABSTRAK

Osteoarthritis (OA) ialah penyakit sendi degeneratif yang dicirikan oleh kemerosotan rawan, kesakitan, and gangguan fungsi. Kedua-dua campuran tokotrienol and alfa-tokoferol telah dicadangkan mempunyai kesan perlindungan terhadap OA. Walau bagaimanapun, kos campuran tokotrienol yang lebih tinggi memerlukan perbandingan langsung dengan alfa-tokoferol bagi giustifikasi penggunaannya. Kajian ini bertujuan untuk membandingkan keberkesanan campuran tokotrienol sawit and alfa-tokoferol dalam model tikus OA yang diaruh oleh monosodium iodoasetat (MIA) serta menilai kesannya terhadap fungsi sendi, histologi rawan, and isyarat Wnt. Tikus jantan Sprague-Dawley berumur tiga bulan (~250 g) diagihkan secara rawak kepada kumpulan kawalan normal (sham), kumpulan OA, kumpulan glukosamin sulfat (250 mg/kg/hari), kumpulan campuran tokotrienol sawit (100 mg/kg/hari), atau kumpulan alfa-tokoferol (100 mg/kg/hari). OA diaruh melalui suntikan intra-artikular MIA pada semua kumpulan kecuali kumpulan sham sebelum rawatan dimulakan. Kumpulan kawalan normal and OA menerima air minuman sepanjang tempoh eksperimen, manakala kumpulan rawatan menerima rawatan oral masing-masing selama empat minggu. Sepanjang tempoh rawatan, fungsi sendi dinilai setiap minggu menggunakan aktiviti menegak tikus, kekuatan cengkaman digital, and ujian dawai jaring terbalik. Berikutan proses eutanasia and penuaian sendi, integriti rawan dinilai melalui analisis histologi, manakala perubahan molekul dalam lapisan rawan dikaji menggunakan PCR masa nyata kuantitatif and analisis Western blot. Kajian mendapati bahawa kumpulan OA menunjukkan gangguan fungsi yang ketara (peningkatan lebar sendi and kehilangan kekuatan cengkaman) and kemerosotan rawan (skor struktur Mankin) berbanding dengan kumpulan sham ($p < 0.05$). Tokotrienol sawit memperbaiki histologi rawan and mengurangkan pengepresan gen *Mmp13* and *Il1b*, manakala kebanyakan penanda berkaitan Wnt menunjukkan perubahan yang terhad pada penamat kajian ($p < 0.05$). Walau bagaimanapun, alfa-tokoferol meningkatkan pengepresan mRNA *Sost* ($p < 0.05$ berbanding semua kumpulan lain) and *Mmp13* ($p < 0.05$ berbanding OA), manakala perubahan ini tidak pemerhatian dalam kumpulan tokotrienol sawit. Kesimpulannya, tokotrienol sawit and alfa-tokoferol menunjukkan kesan yang berbeza pada tikus dengan OA yang diaruh oleh MIA. Tokotrienol sawit dikaitkan dengan pemeliharaan struktur rawan yang lebih baik, peningkatan fungsi sendi, and pengepresan gen *Mmp13* yang lebih rendah, manakala alfa-tokoferol menunjukkan kesan yang lebih ketara terhadap pengepresan gen *Sost*. Penemuan ini mencadangkan bahawa kedua-dua isomer vitamin E mungkin mempengaruhi OA melalui mekanisme yang berbeza. Kajian lanjut diperlukan untuk lebih memahami mekanisme ini and potensi peranan mereka dalam pengurusan OA.

ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease characterised by cartilage deterioration, pain, and functional impairment. Both tocotrienol mixtures and α -tocopherol have been suggested to exert protective effects against OA. However, the higher cost of tocotrienol mixtures necessitates direct comparison with α -tocopherol to justify its use. This study aimed to compare the efficacy of palm tocotrienol mixture and α -tocopherol in a monosodium iodoacetate (MIA)-induced rat model of OA and to evaluate their effects on joint function, cartilage histology, and Wnt signalling. Three-month-old male Sprague–Dawley rats (~250 g) were randomly assigned to a normal control (sham) group, an OA group, a glucosamine sulphate group (250 mg/kg/day), a palm tocotrienol mixture group (100 mg/kg/day), or an α -tocopherol group (100 mg/kg/day). OA was induced by intra-articular injection of MIA in all groups except the sham group before the treatments commenced. The normal control and OA groups received drinking water throughout the experimental period, whereas the treatment groups received their respective oral treatments for four weeks. Throughout the treatment period, joint function was assessed weekly using rats' rearing activity, digital grip strength, and inverted-mesh wire tests. Following euthanasia and joint harvest, cartilage integrity was evaluated by histological analysis, while molecular changes in the cartilage layer were examined using quantitative real-time PCR and Western blot analysis. The study found that the OA group exhibited significant functional impairment (increased joint width and loss of grip strength) and cartilage degeneration (Mankin's structural score) compared with the sham group ($p < 0.05$). Palm tocotrienol improved cartilage histology and reduced *Mmp13* and *Il1b* gene expression, while most Wnt-related markers showed limited changes at the study endpoint ($p < 0.05$). However, α -tocopherol upregulated *Sost* ($p < 0.05$ vs all other groups) and *Mmp13* mRNA expression ($p < 0.05$ vs OA), whereas these changes were not observed in the palm tocotrienol group. In conclusion, palm tocotrienol and α -tocopherol showed different effects in rats with MIA-induced OA. Palm tocotrienol was associated with better preservation of cartilage structure, improved joint function, and lower *Mmp13* gene expression, whereas α -tocopherol showed a more noticeable effect on *Sost* gene expression. These findings suggest that the two vitamin E isomers may influence OA through different mechanisms. Further studies are needed to better understand these mechanisms and their potential role in OA management.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADAMTS-5	A disintegrin and metalloproteinase with thrombospondin motifs-5
AT	α -Tocopherol
β -catenin (Ctnnb)	Beta-catenin
CGRP	Calcitonin gene-related peptide
CK1	Casein kinase 1
DAMPs	Damage-associated molecular patterns
Dkk1	Dickkopf-related protein 1
DW	Distilled water
ECL	Enhanced chemiluminescence
ECM	Extracellular matrix
FZD	Frizzled receptor
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GSK-3 β	Glycogen synthase kinase-3 beta
HRP	Horseradish peroxidase
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
JNK	c-Jun N-terminal kinase
LRP5/6	Low-density lipoprotein receptor-related protein 5/6
MAPK	Mitogen-activated protein kinase
MIA	Monosodium iodoacetate
MMP-9	Matrix metalloproteinase-9
MMP-13	Matrix metalloproteinase-13
NC	Normal control

Abbreviation	Definition
OA	Osteoarthritis
OA+AT	Osteoarthritis treated with α -tocopherol
OA+GS	Osteoarthritis treated with glucosamine sulphate
OA+PT	Osteoarthritis treated with palm tocotrienol
PCP	Planar cell polarity
PKC	Protein kinase C
PT	Palm tocotrienol
PVDF	Polyvinylidene difluoride
PVE	Palm vitamin E
qPCR	Quantitative polymerase chain reaction
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SOST	Sclerostin
TBST	Tris-buffered saline with Tween 20
TCF/LEF	T-cell factor/lymphoid enhancer factor
TNF- α	Tumour necrosis factor-alpha
TRF	Tocotrienol-rich fraction
Wnt	Wingless-related integration site

CHAPTER I

INTRODUCTION

RESEARCH BACKGROUND

Osteoarthritis (OA) is a degenerative condition characterized by gradual degeneration of articular cartilage. This degeneration results in persistent joint discomfort, stiffness and limited range of motion, all of which take away from patients' quality of life (Rehman et al. 2024). In the advanced stages, OA typically requires total knee replacement surgery, an expensive and invasive process linked to significant hazards, including infection and postoperative difficulties (Aabedi et al. 2025). Pharmacological approaches currently available for the treatment of OA primarily target symptom management rather than disease progression modification (Cai et al. 2021; Grässel & Muschter 2020). NSAIDs (non-steroidal anti-inflammatory drugs), the most prescribed drugs, are linked to side effects such as gastrointestinal irritation, cardiovascular events and renal dysfunction, which may limit their long-term use. Again along the same lines, slow-acting symptomatic drugs, such as glucosamine, chondroitin, and diacerein, have shown variable clinical efficacy and inconsistent protective effects for cartilage tissue. (Honvo et al. 2019). Consequently, numerous patients with advanced OA at last require total knee arthroplasty, placing an additional burden on health systems (Klug et al. 2021)

The classical understanding of OA as a purely mechanical “wear-and-tear” disorder has been revised to incorporate the role of molecular and cellular mediators in disease pathogenesis (Tong et al. 2022). Proinflammatory cytokines, matrix metalloproteinases (*Mmps*), and key intracellular signalling pathways, such as Toll-like receptors and *Wnt*/β-catenin, have been implicated in chondrocyte hypertrophy, cartilage matrix degradation, subchondral bone remodelling, and osteophyte formation (Tat et al. 2010; Zhou et al. 2017). Among these, dysregulation of the *Wnt* signalling pathway is increasingly recognised as a central mechanism contributing to OA progression, particularly through its role in promoting cartilage degradation and aberrant osteogenesis (Chin et al. 2022; Lories & Monteagudo 2020).

Given these mechanisms, interest has grown in nutraceuticals with both anti-inflammatory and antioxidant properties. Vitamin E, in particular, has attracted attention as a potential chondroprotective agent. Vitamin E is a group of fat-soluble compounds consisting of tocopherols and tocotrienols. Both groups exist in four isoforms (α , β , γ and δ), which differ in their chemical structure and biological activities. Palm oil is one of the major natural sources of vitamin E, particularly tocotrienols. Due to their antioxidant and anti-inflammatory properties, vitamin E isomers have been widely studied for their potential protective effects against various chronic diseases, including OA (Wong et al. 2020; Pang et al. 2021). While both α -tocopherol and tocotrienols belong to the vitamin E family, they differ structurally and functionally. Alpha-tocopherol is widely used due to its availability and affordability. However, tocotrienols, found abundantly in palm oil, have demonstrated superior antioxidant and anti-inflammatory activity in various models (Wong et al. 2020). Recent reports suggest that tocotrienol-rich fractions may help preserve cartilage structure and slow the progression of OA (Liyana et al. 2023; Pang et al. 2021).

Nevertheless, a comparative study assessing the effects of palm tocotrienol versus α -tocopherol in an identical OA model, as well as their individual impacts on *Wnt* signalling in articular cartilage, has yet to be performed.

The purpose of the current study was to address this research gap by directly comparing palm-derived tocotrienols with α -tocopherol in a rat model of MIA-induced

OA. In addition to evaluating structural and symptomatic outcomes, the study examines the expression of key regulators in the *Wnt* pathway, such as β -catenin, *Sost*, and *Dkk-1* (Chin et al. 2022). In order to confirm this pathway contribution at the protein level, Western blot analysis was conducted to measure *Wnt* signalling activation in articular cartilage. This integrative approach strives for a comprehensive understanding of the effects of vitamin E isoforms on joint health and, more importantly, whether palm tocotrienol provides mechanistic advantages over α -tocopherol that could be beneficial for the prevention of OA.

PROBLEM STATEMENT

OA is a progressive degenerative joint disorder characterised by cartilage degradation, subchondral bone remodelling, synovial inflammation, and functional impairment. Although oxidative stress and inflammatory mediators such as *Il-1 β* and *TNF- α* are recognised contributors to disease progression, current pharmacological therapies remain largely symptomatic and do not effectively modify the underlying molecular mechanisms driving cartilage degeneration. Increasing evidence indicates that dysregulation of intracellular signalling pathways, particularly *Wnt*/ β -catenin signalling, plays a central role in chondrocyte hypertrophy, matrix degradation, and abnormal bone remodelling in OA. However, the interaction between oxidative stress and *Wnt* signalling in OA cartilage remains incompletely clarified.

While α -tocopherol is widely used due to its availability and lower cost, palm-derived tocotrienols exhibit greater antioxidant potency and additional gene-regulatory effects. Nevertheless, existing studies have not directly compared palm tocotrienol and α -tocopherol within the same experimental OA model, particularly with respect to their effects on *Wnt* signalling regulators such as β -catenin, *Dkk1*, and *Sost* in vivo. Furthermore, it remains uncertain whether modulation of these molecular markers translates into measurable improvements in joint function and cartilage integrity.

Therefore, a controlled comparative in vivo study integrating functional assessment, histological evaluation, gene expression analysis, and protein

quantification is required to clarify whether palm tocotrienol offers mechanistic and therapeutic advantages over α -tocopherol in the prevention of OA progression.

RESEARCH QUESTION

Is palm tocotrienol better than α -tocopherol in improving joint health in rats with MIA-induced osteoarthritis?

RESEARCH OBJECTIVE

1.4.1 Main Objective

To compare the efficacy and mechanism of action of palm tocotrienol and α -tocopherol in preventing OA in rats with osteoarthritis induced by MIA.

1.4.2 Specific Objectives

- 1 To compare the effects of palm tocotrienol and α -tocopherol on joint function (rearing and crossing activity, grip strength (inverted mesh wire and T-bar), joint width and body weight in rats with OA induced by MIA.
- 2 To compare the effects of palm tocotrienol and α -tocopherol on cartilage histology in rats with OA induced by MIA.
- 3 To compare the effects of palm tocotrienol and α -tocopherol on gene expression related to inflammation (*Il1b*, *Il6* and *Tnfa*) and metalloproteinases (*Mmp9* and *Mmp13*) in cartilage in rats with OA induced by MIA.
- 4 To compare the effects of palm tocotrienol and α -tocopherol on the protein expression levels of *Wnt* signalling markers (*Wnt*, *Ctnnb1*, *Dkk1* and *Sost*) and the activation of this pathway by the ratio of phosphorylated/total β -catenin in cartilage in rats with OA induced by MIA.

HYPOTHESIS

1. Palm tocotrienol is better than α -tocopherol in protecting joint function (rearing activity, crossing activity, grip strength, digital grip test), improving body weight, and reducing joint width in rats with OA induced by MIA.
2. Palm tocotrienol is better than α -tocopherol in protecting cartilage histology in rats with OA induced by MIA.
3. Palm tocotrienol is better than α -tocopherol in reducing gene expression related to inflammation (*Il1b*, *Il6* and *Tnfa*) and metalloproteinases (*Mmp9* and *Mmp13*) in rats with OA induced by MIA.
4. Palm tocotrienol is better than α -tocopherol in suppressing the expression levels of *Wnt* and *Ctnnb1*, increasing the expression of *Dkk1* and *Sost*, thereby inhibiting activation of this pathway by reducing the ratio of phosphorylated/total β -catenin in cartilage in rats with OA induced by MIA.

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