UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF PALM OIL TOCOTRIENOL-RICH FRACTION (TRF) ON ENDOMETRIOSIS-RELATED PRO-INFLAMMATORY AND OXIDATIVE STRESS MARKERS OF HUMAN ENDOMETRIAL STROMAL FIBROBLAST CELLS

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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

Endometriosis is a gynaecological disease in which endometrial tissues are found outside the uterus. Due to a lack of understanding of its pathophysiology, the global prevalence rate of endometriosis is unknown. Recent findings suggests that inflammation and oxidative stress are involved in this disease pathophysiology and progression. Tocotrienols, a form of vitamin E derived from palm oil, have long been known for their potent antioxidant and anti-inflammatory properties in other inflammatory diseases; however, their effect on endometriosis is not well reported. Therefore, this study aimed to investigate the effect of tocotrienol-rich fraction (TRF) supplementation on pro-inflammatory and antioxidant markers of endometriosis. In here, human primary endometrial stromal fibroblast (eSF) cells isolated from the endometrium of patients with (PEC) and without endometriosis (NEC) were cultured and subjected for cell characterisation by immunocytochemistry staining (vimentin and platelet-derived growth factor receptor beta (PDGFRβ)). NEC and PEC cells were then divided into three groups: control, TRF in long-chain triglyceride (LCT) carrier (TRF) and new enhanced formulation of TRF (ETRF) in medium-chain triglyceride (MCT) (ETRF). After that the cells were subjected to cell viability assay, quantitative real-time polymerase chain reaction (RT-qPCR) for cyclooxygenase-2 (*COX-2*), macrophage migration inhibitory factor (*MIF*), interleukin-6 (*IL-6*), interleukin-8 (*IL-8*), glutathione peroxidase 1 (*GPX1*) superoxide dismutase 1 (*SOD1*), and superoxide dismutase 2 (*SOD2*) gene expression and Luminex assay for MIF, IL-6 and IL-8 protein expression. In treated NEC cells, a dose-dependent increase (more than 10%) in cell viability was observed in TRF and ETRF when compared to control. However, in PEC cells treated with more than 25µg/ml of TRF and ETRF, a significant decreased by 10% in cell viability were observed. Hence, for following study, PEC and NEC cells were treated for 24 hours with 25µg/ml of TRF and ETRF, and their anti-inflammatory and antioxidant properties were investigated and analysed ($p < 0.05$). The results showed that treatment with TRF and ETRF significantly downregulate pro-inflammatory markers gene expression in NEC cells; *COX-2* (0.80 ± 0.11; 0.79 ± 0.04), *MIF* (0.82 ± 0.07; 0.86 ± 0.12), and *IL-6* (0.80 ± 0.05; 0.87 ± 0.07) when compared to control. Whereas, in PEC cells, treatment with TRF and ETRF significantly downregulate pro-inflammatory markers gene expression of *COX-2* (0.82 ± 0.05; 0.69 ± 0.07), *MIF* (0.42 ± 0.05; 0.49 ± 0.07), and *IL-8* (0.78 ± 0.08; 0.69 ± 0.06). For antioxidant genes expression, TRF and ETRF treatment significantly upregulate the expression of *GPX1* (1.77 ± 0.16; 1.69 ± 0.13) in NEC cells but significantly upregulate the expression of *GPX1* (1.78 ± 0.11; 1.69 ± 0.08), *SOD1* (1.45 ± 0.15; 1.50 ± 0.13) and *SOD2* (2.15 ± 0.12; 1.77 ± 0.09) in PEC cells when compared to control. For IL-6, no significance differences were seen at protein level for treated NEC and PEC. However, significance increases in IL-8 protein level were observed for treated NEC and PEC. In accordance, TRF and ETRF treatment at a concentration of 25µg/ml had a significant favourable effect on endometriosis-related pro-inflammatory and antioxidant gene expression. In conclusion, our findings provided a new insight into the effects of TRF and ETRF treatment on human primary eSF cells isolated from NEC and PEC groups and demonstrates their potential as potent anti-inflammatory and antioxidant agent that can reduce endometriosis-associated inflammation and oxidative stress.

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TABLE OF CONTENTS