UNIVERSITI TEKNOLOGI MARA

THE EFFECT OF TOCOTRIENOL-RICH FRACTION SUPPLEMENTATION IN AGING MICE ON THE EMBRYO DEVELOPMENT AND ANTI-AGING GENE EXPRESSION

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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 acknowledge as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree of 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

Female reproductive aging resulted from oxidative stress that happened due to the relentless formation of free radicals in body system, which lead to ovarian aging. Tocotrienol (TCT), one component of vitamin E is a powerful antioxidant that is able to overcome the detrimental effects of oxidative stress and delay the consequences of aging in female reproductive system. Therefore, this study aims to determine the effects of tocotrienol rich fraction (TRF) supplementation in aging mice on the quality and development of preimplantation embryos and to study the expression of anti-aging gene, SIRT1. Six to eight months old female mice, *Mus musculus* were given either tocopherol-stripped corn oil (vehicle control) or supplemented with tocotrienol at the doses of 120, 150 and 180 mg/kg body weight (BW) orally per day for 30 (one month) or 60 (two months) or 120 (four months) days according to their respective groups. Young mice (6 weeks old) were used as negative control while aging mice (7 or 8 or 10 or 12 months old) were used as positive control. At the end of the TRF supplementation period, mice were sacrificed by cervical dislocation, embryos were retrieved and cultured whereas ovaries were used for gene expression study. The morphology and *in vitro* development of embryos were monitored and recorded. Total RNA were isolated from the mice ovaries for gene expression analysis using QuantiGene Plex 2.0 Assay kits. Results confirmed that aging caused significant reduction on the percentage of normal embryos and caused embryonic retardation to develop until blastocyst stage. On the other hand, TRF supplementation in aging mice at the dose of 120 mg/kg BW for one and four months was able to increase the percentage of normal embryos as compared to their respective controls. In addition, TRF supplementation at the dose of 120 mg/kg BW for four months was able to improve the embryonic development until blastocyst stage as compared to its control. In gene expression study, the expression of SIRT1, anti-aging gene was significantly lower in aging group and CDKN2A and E2F, the two genes that down regulate the expression of SIRT1 gene were significantly higher in those groups. This finding documented that TRF supplementation was able to improve the quality and development of embryos retrieved from aging mice thus confirmed that it delays the consequences of reproductive aging in female reproductive system. It was suggested that the effect of TRF in protecting, thus delaying the consequences of reproductive aging is by up regulating the expression of anti-aging gene, SIRT1.

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