UNIVERSITI TEKNOLOGI MARA

EFFECT OF NICOTINE WITH TOCOTRIENOL RICH FRACTION SUPPLEMENTATION ON CYTOSKELETAL STRUCTURE, DNA DAMAGE, PI3K/AKT AND CELL CYCLE SIGNALLING PATHWAY GENES ON PREIMPLANTATION MOUSE EMBRYOS

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Thesis submitted in fulfilment of the requirements for the degree of
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(Physiology)

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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 indivated 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

Tocotrienol-rich fraction (TRF) has been credited as a fertility-enhancing vitamin. However, the mechanism involved in TRF modulation of preimplantation embryo development is still not well understood. This study aims to examine the effects of TRF on cytoskeletal structures and regulations of PI3K/Akt as well as cell cycle signaling pathway related genes in preimplantation embryos induced by nicotine. Twenty-four 6-8 week old Balb/c female mice, weight between 20-25 g were divided into four groups (G1-G4) and were treated for 7 consecutive days (Day 1-7): G1, NaCl (s.c), G2, 3 mg/kg bw/day nicotine (s.c), G3, 3 mg/kg bw/day nicotine (s.c) and 60 mg/kg bw/day of TRF (oral) and G4, 60 mg/kg bw/day of TRF (oral). The female mice were superovulated with 5 IU PMSG on Day-8 followed by 5 IU hCG on Day-10. They were mated with fertile males and euthanized by cervical dislocation at 48 hours post-coitum. Plasma malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were measured. Embryos at the 2- and 8-cell stages were harvested, fixed, and stained to visualize actin and tubulin distributions by using confocal laser scanning microscope (CLSM). Embryonic DNA was extracted, purified and amplified for gene expression analyses. Results showed that at the 2-cell stage, the actin and tubulin fluorescence intensities in the nicotine group (G2) was reduced, however, the intensities in the nicotine + TRF group (G3) was minimized. A similar trend on actin and tubulin fluorescence intensity was observed at 8-cell stage both in G2 and G3. Both actin and tubulin fluorescence intensities at 2- and 8-cell stages in the TRF groups (G4) were enhanced compared to their respective controls. The levels of SOD, GPx and CAT for G2 were lower, while MDA level was higher ($p<0.05$). With TRF, the levels of SOD, GPx and CAT in G3 and G4 respectively were not different from their respective controls while MDA level was lower ($p<0.05$). At the 2-cell stage, in the G2 group, *Pten, Pi3kca, Atm, p53, p21, p27, Cdk2, Cyclin E, Cdk4, Cdk6* and *Cyclin D* genes were significantly upregulated while *Akt1, Gsk3β, Mapk1, Pdpk1, Stat3, Mapk3, Jak1, Mdm2* and *Cdc25a* genes were significantly downregulated. Intervention with TRF (G3) resulted in a significant downregulation of *Pten, Pi3kca, Atm, p53, p21, p27, Cdk2, Cyclin E, Cdk4, Cdk6* and *Cyclin D* genes followed by a significant upregulation of other genes. The same pattern was seen at the 8-cell stage. This study concludes that actin and tubulin damage, as well as modulation of PI3K/Akt and cell cycle signaling pathway related genes by nicotine was prevented by TRF through the transition in Gap1/Synthesis checkpoint.

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