

UNIVERSITI TEKNOLOGI MARA

**PROTECTIVE ACTIONS OF
ANNATTO (*Bixa orellana*)-DERIVED
 δ -TOCOTRIENOL AND
SOY-DERIVED α -TOCOPHEROL
AGAINST NICOTINIC DNA
DAMAGES DURING EARLY
PREIMPLANTATION EMBRYONIC
DEVELOPMENT IN MICE**

SITI SYAIRAH BINTI MOHD MUTALIP

Thesis submitted in fulfillment
of the requirements for the degree of
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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a panel of examiners has met on 17th November 2017 to conduct the final examination of Siti Syairah binti Mohd Mutalip on her Doctor of Philosophy thesis entitled “Protective Actions of Annatto (*Bixa Orellana*)-Derived δ -Tocotrienol and Soy-Derived α -Tocopherol against Nicotinic DNA Damages During Early Preimplantation Embryonic Development in Mice” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

Musalmah Mazlan, PhD
Professor
Faculty of Medicine
Universiti Teknologi MARA
(Chairman)

Zulkhairi Hj. Amom, PhD
Professor
Faculty of Health Sciences
Universiti Teknologi MARA
(Internal Examiner)

Norfilza Mohd Mokhtar, PhD
Professor
Faculty of Medicine
Universiti Kebangsaan Malaysia
(External Examiner)

Budi Wiweko, PhD
Professor
Department of Obstetrics and Gynecology
University of Indonesia
(External Examiner)


**PROF SR DR HJ ABDUL HADI
HJ NAWAWI**

Dean
Institute of Graduate Studies
Universiti Teknologi MARA
Date: 23 May 2018

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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.

Name of Student	:	Siti Syairah binti Mohd Mutalip
Student I.D. No.	:	2012481512
Programme	:	Doctor of Philosophy – MD990
Faculty	:	Medicine
Thesis Title	:	Protective Actions of Annatto (<i>Bixa Orellana</i>)-Derived Delta-Tocotrienol and Soy-Derived Alpha-Tocopherol against Nicotinic DNA Damages during Early Preimplantation Embryonic Development in Mice
Signature of Student	:	
Date	:	May 2018

ABSTRACT

Growing incidences of birth defects remains as a global issue. Vitamin E, which was first discovered as vitamin for reproduction in 1922, has been reported as a potential reproductive protectant that could play protective roles against birth defects during the regulations of embryonic development. The preimplantation embryonic growths are regulated by phosphatidylinositol-3-kinase (PI3K)/Akt and cell cycle signaling pathways. Present study was conducted to determine the effects of annatto (*Bixa orellana*)-tocotrienols (TCTs) and soy-alpha tocopherol (α -TOC) on the regulations of PI3K/Akt and cell cycle pathways in murine preimplantation embryos. Female balb/c mice of 6-8 weeks old (23-25g) were randomly divided into eight groups (G1-G8; n=6). The groups were treated daily for 7 consecutive days: G1 (control) received 0.1 ml tocopherol stripped corn oil, G2 received 3mg/kg bw/day of nicotine, G3 was concurrently treated with 3mg/kg bw/day of nicotine and 60mg/kg bw/day of mixed-TCTs (90% delta & 10% gamma), G4 was concurrently treated with 3mg/kg bw/day of nicotine and 60mg/kg bw/day of pure δ -TCT (>98% purity), G5 was concurrently treated with 3mg/kg bw/day of nicotine and 60mg/kg bw/day of α -TOC, G6 was given 60mg/kg bw/day of mixed-TCTs alone, G7 was given 60mg/kg bw/day of pure δ -TCT alone and G8 received 60mg/kg bw/day α -TOC alone. Following the treatments, all females were superovulated by injection of 5IU of Pregnant Mare's Serum Gonadotropin (PMSG) and 5IU of human chorionic gonadotropin (hCG). Females were mated with males and sacrificed by cervical dislocation at 48 hours post-coitum. Embryos from each group were collected for chromosome karyotyping, DNA sequencing and gene expression analyses. Present results showed a significant decrease in the mean number of embryos produced following nicotine treatment (G2) compared to that of control (G1) ($p=0.000$). Intervention with mixed-TCTs (G3) and pure δ -TCT (G4) significantly ($p<0.05$) increased the number of produced 2-cell embryos by 127% and 79% compared to that of G2, yet it was still lower than that of control (G1). Embryonic growths were attenuated throughout the developmental period in G3, and were arrested at morula stage in G4. Intervention with α -TOC decreased the number of 2-cell embryos by 7%. Meanwhile, supplementations with annatto-TCTs and soy α -TOC alone in G6-G8 were significantly increased ($p<0.05$) the number of 2-cell embryos compared to that of G1. The decreases in the number of retrieved embryos in G2-G5 were shown to be associated with the dysregulations of the PI3K/Akt and cell cycle signaling pathways. Exposure to nicotine induced the DNA damages with chromosomal disruptions, nucleotides deletions in the genes sequences and dysregulations of both pathways. Interventions with annatto-TCTs and soy α -TOC resulted in the anti-proliferative effect, which attenuated the embryonic growths. Dysregulations of the pathways were mainly caused by the upregulations of *PTEN*, *GSK3 β* and *cdkn1b* (p21) genes that control cells growths. The promising effect of annatto-TCTs as equal as α -TOC on embryonic developments in normal conditions was also observed. Present study was the first to provide the novel findings on the anti-proliferative effect of annatto-TCTs and soy α -TOC against nicotinic murine embryonic cells.

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