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

PhD

May 2018

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.

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 (*Bixa Orellana*)-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*, *GSK3β* 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.

ACKNOWLEDGEMENT

Alhamdulillah, first of all I would like to praise and thank Allah S.W.T., the Most Gracious and the Most Merciful for granting me the chance to pursue and successfully complete my PhD study.

I would like to express my sincere gratitude and special thanks to my supervisors, Prof. Dr. Mohd Hamim Rajikin and Dr. Sharaniza Ab Rahim for their continuous assistance, guidance, motivation, support and knowledge given throughout this challenging period of study.

Sincere thanks to all the staffs and my fellow lab mates in the Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM) Sg. Buloh Campus for their continuous encouragement, advice and support especially in providing help with the laboratory and research facilities. Also, special thanks to the Ministry of Higher Education (MOHE), Malaysia and UiTM for providing me with the financial assistance throughout my study.

Last but not the least; I would like to thank all my family members for their unconditional love, support and prayers throughout my study and my life in general, especially my mother – Pn. Halimah binti Abdul Kadir. Thank you mum!

Thank you very much.

Wassalam.

TABLE OF CONTENTS