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Research paper

Histological Analysis of Murine Ovaries and Uteruses Following Supplementation with Alpha-Tocopherol in Nicotine Injected Mice

Mohd Azmil Amirudin, Muhammad Shahrizal Sanudin, Mohd Shahrul Nizam Mohd Isa, *Siti Syairah Mohd Mutalip

Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) Puncak Alam Campus, 42300, Selangor, Malaysia
**Corresponding author: syairah@puncakalam.uitm.edu.my*

Abstract

Vitamin E can be classified into eight different lipophilic subtypes; namely alpha(α)-, beta(β)-, delta(δ)- and gamma(γ)-tocopherol (TOC) as well as alpha(α)-, beta(β)-, delta(δ)- and gamma(γ)-tocotrienol (TCT). From these isoforms, α-TOC has been reported to have the most promising health benefits. The purpose of this study was to determine the effect of α-TOC on the histological structures of murine ovaries and uteruses in nicotine-treated females. Thirty ICR female mice were divided into 5 groups (Group A – E) with 6 mice each. Group A received 0.1 ml of tocopherol-stripped corn oil, group B given 0.1 ml of 0.9% normal saline, group C treated with 3 mg/kg/day of nicotine, group D treated with 60 mg/kg/day of alpha-tocopherol and group E was concurrently treated with 3 mg/kg/day of nicotine and 60 mg/kg/day of alpha-tocopherol. Following the treatments, all females were euthanized and the studied organs were collected and processed for histological analysis. Obtained results indicated that exposure to nicotine reduced the numbers of ovarian follicles and endometrium thickness (Group C). However, these adverse effects have been reduced in the group concurrently treated with nicotine and α-TOC (Group E). This result suggested that α-TOC might be able to minimize the adverse effects of nicotine in the ovaries and the uterus. Further studies are needed to confirm on these effects from the physiological aspects of the ovaries and uteruses.

Keywords: *Vitamin E, Alpha-Tocotrienol, Tocotrienols, Ovary, Uterus*

1. Introduction

Vitamin E was discovered in 1922 (Evan & Bishop, 1922). Since then, it has been extensively studied and becomes a well-recognized lipid-soluble antioxidant. Although many study reports on the health benefits of vitamin E are available, its effects on the reproductive health are still understudied.

Vitamin E can be classified into eight different lipophilic subtypes; namely alpha (α), beta (β), delta (δ) and gamma (γ) forms of tocopherol (TOC), and alpha (α), beta (β), delta (δ) and gamma (γ) forms of tocotrienol (TCT). All of the vitamin E subtypes possess a chromanol ring and a 16-carbon phytyl-like side chain. Tocopherols are present in saturated form while tocotrienols have three double bonds in their tails (unsaturated). The main difference in the TOCs and TCTs structures is the presence of either an hydrogen (H) or a methyl (CH3) group in the chromanol ring (IUPAC-IUB, 1982).

Vitamin E is mostly derived from the vegetables, plants and plant oils. The distribution of tocopherols in the plant kingdom is primarily α-TOC in green leafy plants and γ-TOC in non-green plants parts such as the fruits and seeds. Different subtypes of vitamin E can be found in different sources. For instance, α-TOC can be found in almonds, avocados, hazelnuts, peanuts and sunflower seeds; β-TOC can be found in oregano and poppy seeds; γ-TOC can be found in pecans, pistachios, sesame seeds and walnuts and δ-TOC can be found in edamame and raspberries (USDA Food Composition Databases, 2015).

The health benefits of vitamin E have been widely reported, especially on its antioxidant and anticancer activity (Saleh et al. 2014). Besides, vitamin E was also reported as an anti-proliferative (Alawin et al. 2016), anti-angiogenic (Weng-Yew et al. 2009) and anti-inflammatory (Wu et al. 2008) agent. Despite these reports, studies on the effects of vitamin E on reproductive system remain largely unknown, with only a few attempts was made recently (Nasibah et al. 2012a; 2012b; Kamsani et al. 2013; Syairah et al. 2016).

Cigarette smoke has been reported to contain around 4000 toxic compounds. These compounds include nicotine, polycyclic aromatic hydrocarbons (PAH), nitrosamines, heavy metals, aromatic amines and others (Dechanet et al. 2011). Many studies have been reported on the adverse relationships between smoking and reproductive health (Patil et al. 1998, Hughes et al. 2000).

2. Problem Statement

Cigarette smoking is one of the major unhealthy lifestyle behaviors that adversely affect the reproductive health. Meanwhile, vitamin E has been reported to have the antioxidant and anti-survival properties. Taking together, this study was conducted to determine the possible reductive effects by α-TOC following nicotine treatment in ovaries and uterus of the mice.

3. The Aim of Research

This study was conducted to determine the effect of α-TOC on the histological structures of murine ovaries and uteruses in nicotine-treated females.

4. Method of Research

An approval by the university's Committee on Animal Research and Ethics (CARE) was obtained prior to beginning this study(Approval No. 197/2017). Thirty (30) ICR female mice were divided into 5 groups (Group A – E) with 6 mice each. Group A (control) received 0.1 ml of tocopherol-stripped corn oil, group B given 0.1 ml of 0.9% normal saline, group C treated with 3 mg/kg bw/day of nicotine, group D treated with 60 mg/kg bw/day of alpha-tocopherol and group E was concurrently treated with 3 mg/kg bw/day of nicotine and 60 mg/kg bw/day of alpha-tocopherol. The treatments were given once daily for 7 consecutive days. Following the completion of the treatments, all females were euthanized and the studied organs samples were collected and processed for histological analysis. Histological analysis involve several processes including fixation, dehydration, clearing, embedding, sectioning, staining and mounting.

5. Analysis and Discussion

Ovaries

As shown in Table 1, histological analysis showed that the highest average number of ovarian follicles was in the control group (Group A). Treatment with nicotine (Group C) reduced the number of ovarian follicles, whereas intervention with α-TOC (Group E) showed an increase in the average number of ovarian follicles compared to treatment with nicotine alone.

Uteruses

Histological analysis on the uteruses showed that the average thickness measurement of the endometrium layer in the group treated with 3 mg/kg bw/day of nicotine (Group C) was significantly ($p<0.05$) reduced to 114.28 ± 28.58 µm compared to the group receiving 0.9% normal saline (Group B) (200 ± 57.14 µm). Meanwhile, intervention with α-TOC increased (non-significant, $p=0.18$) the thickness of endometrium (152.38 ± 59.48 µm) compared to treatment with nicotine alone (Group C). Besides endometrium, the thickness of myometrium layer were also measured. Results of the thickness measurements of endometrium and myometrium layers are shown in Table 1. The histological results of Group A, Group C and Group E are shown in Figure 1(a) – (c).

Table 1. Average number of ovarian follicles and uterine thickness following the treatments

Groups	Numbers of ovarian follicles	Average thickness readings (µm)	
		Endometrium	Myometrium
A	66	266.67 ± 43.65	180.94 ± 59.49
B	45	200 ± 57.14	161.9 ±8.25
C	15*	114.28 ± 28.58*	209.52 ± 67.51*
D	48	147.62 ± 21.82	195.24 ± 81.23
E	30	152.38 ± 59.48	200 ± 28.57

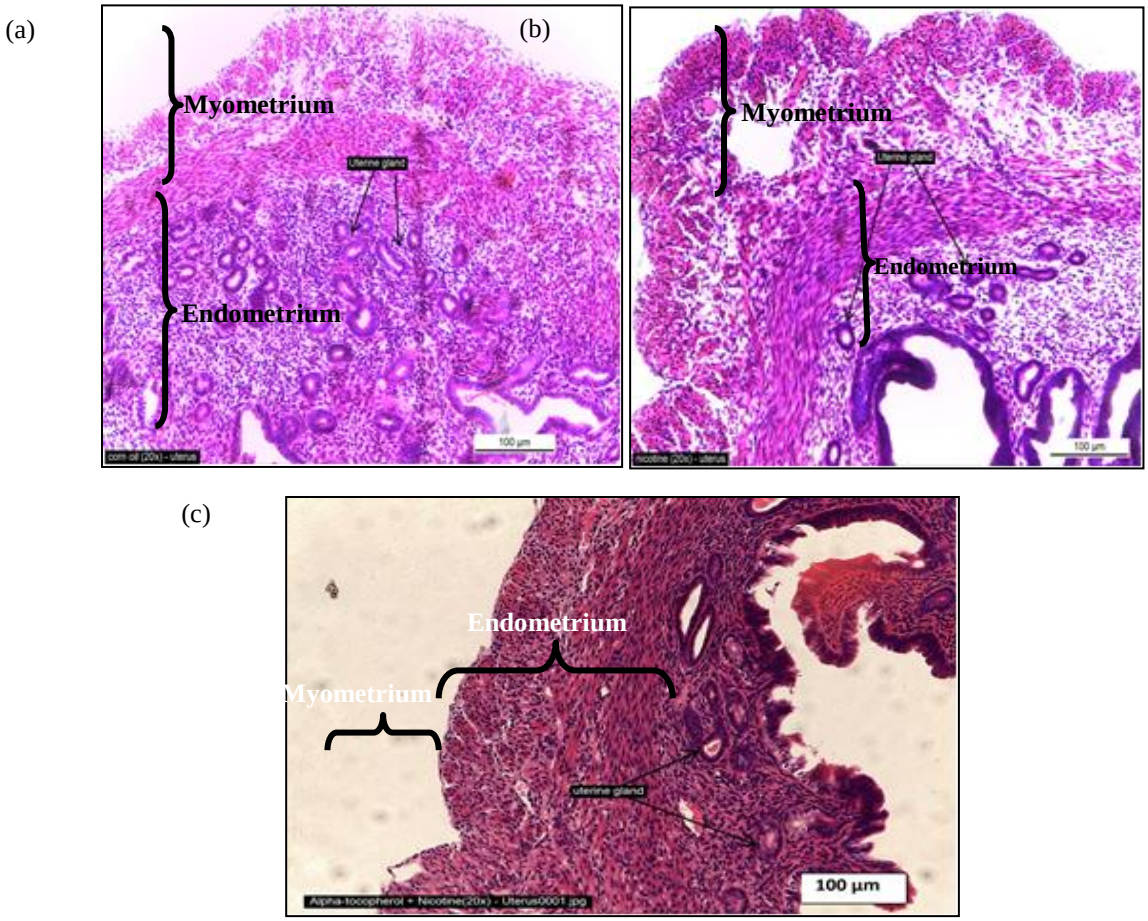

Figure 1: Results of (a) Group A, (b) Group C and (c) Group E. Differences in the thickness of endometrium and myometrium layers were observed. Arrows indicate the uterine glands.

Ovaries

Present findings indicated that treatment with tocopherol-stripped corn oil resulted in higher number of follicles compared to the other four groups, while the group treated with 3 mg/kg bw/day of nicotine showed the lowest number of ovarian follicles. This suggested that exposure to nicotine affected the structural changes of the ovaries and caused reduction in the formation of the primary and secondary follicles.

These results were in line with previously reported studies in which maternal exposure to cigarette smoke or cigarette smoke condensate (CSC) for 4 weeks resulted in the increased oocyte fragmentation or delayed fertilization, thus reduced the embryonic development to blastocysts *in vitro* (Huang et al. 2009). Besides, fragmented oocytes also showed increased production of reactive oxygen species (ROS). Another study on the effects of nicotine on early embryogenesis in murine embryos reported that embryos treated with 3 – 6 µM of nicotine were smaller than control (Zhao & Reece, 2005). Nicotine was also reported to reduce the number of ovarian follicles by triggering oxidative stress (OS) through lipid peroxidation of the ovarian structures (Dumanına et al. 2014).

Follicular developments were observed in group treated concurrently with nicotine and α-TOC. This suggested that α-TOC might be able to reduce the adverse effect of nicotine. High level of ROS induced by nicotine could be neutralized by α-TOC through electron donation from the hydroxyl group of the aromatic ring of tocohromanols (Azzi, 2007). Alpha-TOC is an antioxidant, therefore it could protect the cells from the excessive presence of ROS.

Uteruses

Obtained results indicated differences in the thickness measurements of the endometrium and myometrium layers in all experimental groups. Groups treated with 3 mg/kg bw/day of nicotine resulted in a significant ($p<0.05$) reduction of the endometrial thickness compared to its control. This reduction probably caused by nicotine in which nicotine has been previously reported to decrease uterine blood flow, decrease endometrial stem cell recruitments and decrease the production of estrogen, besides triggering atrophic endometrium (Dumanına et al., 2014).

As observed in the results of the ovaries, intervention with α-TOC also showed increment in the thickness measurement of the endometrium.

6. Conclusion

Present results are suggesting that α-TOC might exerting a beneficial effect against the effects of nicotine. However, these results are superficial, and more studies are needed to further understand the mechanisms of actions by α-TOC against the nicotine-induced cellular damages.

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