

UNIVERSITI TEKNOLOGI MARA

**PRESERVATION OF
CYCLOPHOSPHAMIDE-
COMPROMISED FERTILITY IN
FEMALE MICE BY TOCOTRIENOL
SUPPLEMENTATION**

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Thesis submitted in fulfillment
of the requirements for the degree of
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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 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 supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

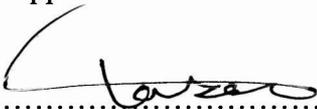
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ABSTRACT

Cyclophosphamide (CPA) chemotherapy has been known to cause ovarian failure. CPA therapy leads to infertility via oxidative stress induced apoptosis of ovarian cells. Tocotrienols (T3), vitamin E isoform, is a much more potent antioxidant compared to tocopherols. T3 is a potent antioxidant and anti-inflammatory agent. The role of T3 in ovarian protection throughout chemotherapy remains un-elucidated, hence this study has been undertaken to investigate the role of T3 in the maintenance of normal female reproductive system from temporal infertility due to CPA administration. The alteration and regulation of ER and PR, genes and proteins, expression were investigated to ascertain the deleterious effect of CPA and whether co-administration with T3 showed any difference. That was hoped to explain the involvement of ER and PR signaling pathway as biomarker in maintaining fertility during chemotherapy treatment. 60 female ICR mice, aged between 8 to 10 weeks were divided into 5 treatment groups: CPA, CPA&T3, normal saline, T3 only, and corn oil only. The treatment was given for 30 consecutive days, followed by administration of pregnant mare serum gonadotrophin and human chorionic gonadotrophin to induce super ovulation. The mice were sacrificed at 14 to 16 hours post induction, after which all animal were euthanized. At dissection, both ovaries were removed, fixed in 10% formalin, processed and embedded in paraffin to form tissue blocks. Histological processing and Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assays were performed on the tissue blocks. Both ovaries were dissected and immediately preserved in liquid nitrogen and then RNA extraction and cDNA synthesis were performed. Real-time quantitative PCR was performed using the syper green probe real time PCR. Concentration of the forward, reverse primers, probes, cDNA template, as well as the annealing temperature were optimized using real time PCR involvement caspase3, Bcl-2, ER, PR, GAPDH and b-actin genes. All IHC reactions included peroxidase for mouse Biotinylated primary antibody code (k3954) and horseradish peroxidase as secondary antibody. All data were subjected to statistical analysis by one-way ANOVA. The histological changes seen in the ovaries of CPA group include increase in degeneration of primary and secondary follicles, with a decrease in their total numbers; large follicles had incomplete and sometimes absent antrum. Whereas in CPA+T3 group, folliculogenesis was near normal, with regular antrum formation in large follicles and increased number of follicles in all stages. There was a significant reduction of TUNEL positive cells in the combined CPA&T3 group compared to CPA group by 11.23% ($p<0.05$). Our results established that combine CPA+T3 administration significantly up-regulated the gene expression of Bcl-2 ($p<0.01$) by 0.36 fold and caspase3 gene expression level was also significantly reduced ($p<0.01$) and suppressed by 2.12 fold, compared to treatment with CPA alone. CPA+T3 also significantly reduced the expression of ER, PR genes and protein in the ovary of CPA-exposed mice ($p<0.05$). The results therefore indicated that co-administration of T3 with CPA confers protection against apoptosis and modulate altered genes and protein expression in ovaries. T3 is a potential candidate for ovarian preservation in chemotherapy-associated damage.

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CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Infertility has become a global problem affecting approximately 15% of both men and women of reproductive age. Anti-cancer therapy is one of the factors leading to decreased fertility (Poirot et al., 2002). According to the recent cancer statistics, the most common cancers diagnosed in women under the age of 40 are breast cancer, melanoma, cervical cancer, non-Hodgkin's lymphoma and leukemia (Jemal et al., 2005). Current therapy of childhood cancer makes long-term survival a realistic outcome for most patients. The majority of young female cancer patients are expected to be cured and become long-term survivors (Jemal et al., 2011).

The improved long-term survival of adolescents and young women treated for cancer has resulted in increased focus on the effects of chemotherapy on ovarian function and its preservation. No established method for preservation of female fertility is currently available. Thus, investigation on ways to preserve fertility of women who had undergone such treatment is of utmost importance. In addition, increasing numbers of patients with autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus (Sonmezer and Oktay, 2004) are being treated successfully with chemotherapy.

Fertility management is recommended for women of fertile age undergoing chemotherapy, as the treatment has implications on family formation (Kirkman et al., 2013). Chemotherapy leads to reduced fertility in women by inducing or hastening menopause (Hickey et al., 2009). In general, all chemotherapeutic drugs act by interrupting vital cell processes and arresting normal cell proliferation. The ovary is commonly affected by these drugs as it contained many rapidly dividing cells, especially those involved in oogenesis.