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35

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Title :
Preservation of Cyclophosphamide-Compromised Fertility in Female Mice by Tocotrienol Supplementation

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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 unelucidated, 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 caspace3 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.