The preservation of fertility during cancer therapy remains a major challenge in women in the reproductive age. The adverse effects of alkylating chemotherapeutic agent cyclophosphamide on folliculogenesis in the ovary are well-known. On the other hand, resveratrol, a polyphenolic phytoalexin, has been shown to exert antioxidant, anti-inflammatory, cardioprotective, DNA protective and neuroprotective effects in a variety of clinical and experimental settings. This study aims to evaluate the effects of resveratrol on cyclophosphamide-induced ovarian damage in rats.

For this purpose, 28-day-old immature Wistar Albino female rats were treated with pregnant mare serum gonadotrophin (PMSG) to develop the first generation of preovulatory ovarian follicles. Then, the animals in experimental groups were treated with cyclophosphamide (100 mg/kg, i.p), resveratrol (25 mg/kg/day, i.p), cyclophosphamide + resveratrol or vehicle ethanol. Forty eight hours after PMSG injection, rats were sacrificed, the ovaries were removed and embedded in paraffin. Cleaved caspase-3 immunohistochemistry and TUNEL staining were performed on paraffin sections to determine the apoptotic process in the ovarian follicles. To compare the intensity of cleaved caspase-3 expression and the extent of TUNEL labeling between experimental groups, a semiquantitative assessment of immunostaining in granulosa and theca cells of the follicles at different development stages was performed by h-scoring on four sections obtained at regular intervals from serial sections of the ovaries.

Cleaved caspase-3 immunoreactivity and TUNEL labeling were significantly increased in granulosa and theca cells of the multilaminar primary, secondary and Graafian follicles in cyclophosphamide-treated rats. In these animals, resveratrol treatment significantly reduced the increased cleaved caspase-3 immunoreactivity in granulosa and theca cells of the multilaminar primary follicles and in cumulus and mural granulosa cells of the Graafian follicles (Figure 1). In addition, a significant decrease of the increased TUNEL labeling was observed in granulosa and theca cells of the multilaminar primary follicles and in mural granulosa cells of the Graafian follicles (Figure 2).

In conclusion, our data suggest that resveratrol treatment may provide a choice of pharmacologic approach in the preservation of fertility owing to its beneficial effects on chemotherapy-induced apoptotic process in ovarian follicles at both early and late phases of development.

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Fig. 1: Representative images showing cleaved caspase-3 immunostaining of Graafian follicles from ovaries of rats in cyclophosphamide (a) and cyclophosphamide + resveratrol (b) groups. Scale bars = 100 µm.

Fig. 2: Representative images showing TUNEL labeling of multilaminar primary follicles from ovaries of rats in cyclophosphamide (a) and cyclophosphamide + resveratrol (b) groups. Scale bars = 25 µm.