

ESTROGEN INDUCED OVARIAN DAMAGE LEADING TO CARCINOGENESIS IN ALBINO RAT

S. Madhuri¹, Pandey Govind^{2*}, Khanna Asha³ and Sahni Y.P.⁴

¹Guest Faculty, Department of Zoology & Biotechnology, Govt. MH College of Home Science & Science for Women, Jabalpur, MP, India

²Officer-In-Charge of Rinder Pest (Animal Husbandry Dept, Govt. of MP), Jabalpur Division, Jabalpur

³Professor & Head of Zoology & Biotechnology, St. Aloysius' College, Jabalpur

⁴Professor & Head of Pharmacology & Toxicology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur

*Officer-In-Charge of Rinder Pest (Animal Husbandry Department, Govt. of MP), Jabalpur Division, Jabalpur-482001, MP, India; E-mail: drgovindpandey@rediffmail.com

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ABSTRACT

In the present study, the ovarian damage, leading to carcinogenesis after administration of estrogen was assessed. A highly potent semisynthetic 17β oestradiol estrogen, ethinyl oestradiol (EO) was administered @500 $\mu\text{g}/\text{kg}$, orally, weekly to the rats of groups 2, 3 and 4 for 12, 16 and 20 weeks, respectively. The rats of group 1 were given saline alone to serve as control. On the 13th week (Group 2), the ovarian tissues revealed severe vascular congestion and fibrosis at many places. Most of the ovarian parenchyma was replaced by fibrovascular connective tissues. On the 17th week (Group 3), more severe histopathological changes were seen. Degeneration, necrosis, fibrosis of follicular tissues, presence of homogenous mass in the lumen, infiltration of lymphocytes and thickening of blood vascular walls were quite conspicuous. On the 21st week (Group 4), these changes were much more severe and marked. Extensive fibrosis, including severe degeneration and necrosis of follicular tissues were observed. Interestingly, the papillary proliferation in surface epithelium and hyperplasia of follicular cells were observed, indicating the ovarian carcinogenesis. The extent and severity of ovarian damage were time dependent, suggesting that EO (estrogen) at the dose of 500 $\mu\text{g}/\text{kg}$, orally, weekly after prolonged period (20 weeks) may cause more severe damage, leading to carcinogenesis in the rat ovary.

KEYWORDS: Cancer/Carcinogenesis, Estrogen (ethinyl oestradiol), Ovarian damage, Rat.

INTRODUCTION

Carcinogenesis is a process which results in the appearance of lesions that meet the requirement of a malignant tumour (cancer). The term 'cancer' is a frightful disease in which the patient suffers pain, disfigurement and loss of many physiological processes. Cancer may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. Estrogen is the necessity of female because without it female is no more female. It is used as oral contraceptive (OC) to control the birth and as hormonal replacement therapy (HRT) by millions of women throughout the world¹⁻². In bitches, estrogen is used for the treatment of hypogonadal obesity, hormonal urinary incontinence and misalliance³⁻⁴. However, excessive and prolonged use of estrogen may cause carcinogenesis (or cancer) of many organs in humans and animals^{1-2,5-7}. In December 2000, the USA Government's National Toxicology Program and National Institute of Environmental Health Sciences have added oestrogen to the list of known human carcinogens. American Cancer Society researchers reported that 0.2% pre- and post- menopausal women had died from ovarian cancer. Among the women who died, about 32% had taken oestrogen replacement therapy². Generally, OCs contain ethinyl oestradiol (EO), a highly potent

semisynthetic 17β oestradiol estrogen. It is a 17β oestradiol. Most of the OCs contain 0.02 to 0.1 mg of EO. The acute and chronic toxicities of EO at different doses have also been observed⁶. EO (@ 250, 500 and 750 $\mu\text{g}/\text{kg}$, orally, weekly for 8 and 12 weeks) induced cytotoxicity has been observed in liver⁸ and uterus⁹ of rat.

In view of the above facts, the present study was undertaken to produce the ovarian damage, leading to carcinogenesis after administration EO in albino rats. However, probably, no experimental works in this regard have been done so far in India as such work could not be traceable in the literature. This experiment has important role to know the standard carcinogenic dose and duration of estrogen (or EO, the most commonly used estrogen). The individuals, especially the women must be warned not to take excessive EO or other forms of estrogens for prolonged period. This experiment is also useful to evaluate the cytogenic or anticancer effect of drugs against estrogen induced cytotoxicity or carcinogenesis (cancer).

MATERIALS AND METHODS

Twenty-four healthy inbred female albino rats (100-150 g) were kept in polypropylene cages under standard laboratory conditions in the animal house of College of Veterinary Science & Animal Husbandry, Jabalpur. The rats were fed on standard pellet diet and drinking water *ad libitum*. The experimental designs and protocols in the study received the approval of Institutional Animal Ethics Committee.

For induction of ovarian damage/carcinogenesis, the required amount of Lynoral tablets (containing 0.05 mg of EO only in each tablet) was purchased and the suspension of its powdered form was prepared by dissolving in the distilled water mixed with a pinch of *Gum acacia* (since the drug is insoluble in water). The rats were equally divided into 4 groups (each group with 6 rats). The EO suspension was administered @ 500 $\mu\text{g}/\text{kg}$, orally, weekly to the rats of groups 2, 3 and 4 for 12, 16 and 20 weeks, respectively. The rats of group 1 were administered with saline alone (also mixed with a pinch of *Gum acacia* powder) to serve as control. To assess the ovarian damage/carcinogenic effects of EO on the ovary, the rats were sacrificed as follows: group 1 on the 1st week; group 2 on the 13th week; group 3 on the 17th; and group 4 on the 21st week. The ovaries of rats were collected and preserved in 10% buffered formalin. Later on, the ovarian tissues were processed, and stained with H & E stain as per the methods described by Culling¹⁰. Microscopically, the histopathological changes in the ovarian tissues were observed.

RESULTS

Microscopically, on the 13th week (Group 2), the ovarian tissues revealed severe vascular congestion and fibrosis at many places as against the normal ovarian tissues seen in group 1 (control) of rats. Most of the ovarian parenchyma was replaced by fibrovascular connective tissues (Fig. 1). On the 17th week (Group 3), more severe histopathological changes than observed in group 2 were noticed. Degeneration, necrosis, fibrosis of follicular tissues, presence of homogenous mass in the lumen, infiltration of lymphocytes and thickening of blood vascular walls were quite conspicuous. On the 21st week (Group 4), these changes were much more severe and marked. Extensive fibrosis, including severe degeneration and necrosis of follicular tissues were observed (Fig. 2). Interestingly, the papillary proliferation in surface epithelium and hyperplasia of follicular cells were observed, indicating the ovarian carcinogenesis (Fig. 3).

The extent and severity of ovarian damage were time dependent, suggesting that EO (estrogen) at the dose of 500 $\mu\text{g}/\text{kg}$, orally, weekly after prolonged period (20 weeks) may cause more severe damage, leading to carcinogenesis in the rat ovary.

DISCUSSION

The findings of the present study may be correlated with the reports of many authors. Meissner et al.⁷ reported the hyperplasia and carcinoma of ovary after administration of stilbestrol (a synthetic oestrogen) in rabbits. The adverse effects like bone marrow suppression, pyometra and infertility have been noted in dogs after treatment with many estrogen preparations⁴. Our results also correspond with the earlier reports describing that the estrogen causes ovarian damage, leading to cancer^{1-2,5-6}. Madhuri et al.⁶ cited that the excessive estrogen may cause many illnesses, including polycystic ovary syndrome and ovarian

damage. A case of clinical hyperestrogenism in bitch that developed follicular ovarian cysts, and cystic endometrial hyperplasia and pyometra in uterus was recorded¹¹. After continuous exposure of oestradiol, the formation of a papillary ovarian surface resembling human serous neoplasms of low malignant potential in laboratory animals has also been observed¹². Similar to the present study, EO @ 250, 500 and 750 µg/kg, orally, weekly for 8 and 12 weeks has been reported to cause cytotoxicity in the liver⁸ and uterus⁹ of rat.

Both exogenous and endogenous estrogens, and their metabolites may induce cytotoxicity and cancer in the estrogen-responsive tissues of humans and animals⁵. Excessive estrogen is trapped in the uterus, ovary, or breast due to stagnation in the blood circulation, and overstimulate the cell division, leading to cytotoxic changes such as fibroids, cysts or cancers in these organs^{2,6,8-9}.

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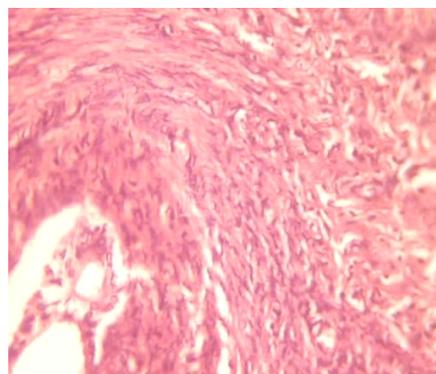


Fig. 1 (x100, H & E): Ovarian tissues of rat (Group 2; EO @ 500 µg/kg, orally, weekly for 12 weeks) showing severe vascular congestion and fibrosis; fibrovascular connective tissues replaced most of ovarian parenchyma.

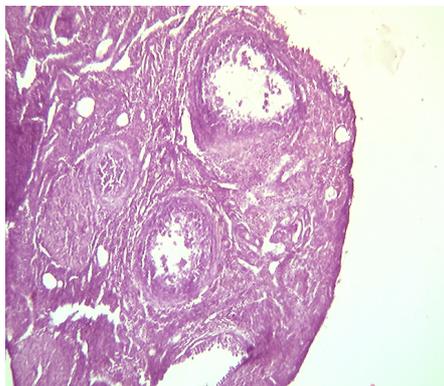


Fig. 2 (x100, H & E): Ovarian tissues of rat (Group 4; EO @ 500 µg/kg, orally, weekly for 20 weeks) showing severe and extensive changes than those of groups 2 & 3.



Fig. 3 (x400, H & E): Ovarian tissues of rat (Group 4; EO @ 500 µg/kg, orally, weekly for 20 weeks) showing papillary proliferation in surface epithelium and hyperplasia of follicular cells, indicating the ovarian carcinogenesis

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