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Toxicol. Int.Vol. 16, No. 1, 2009 pp 05-07

Oestrogen induced uterine damage in rats

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Abstract

The present study was undertaken to assess the oestrogen induced uterine damage in albino rats. Ethinyl oestradiol (EO) as a semisynthetic oestrogen was administered to the rats of groups 2, 3 and 4 @ 250, 500 and 750 ?g/kg body weight, respectively orally once a week for 8 weeks. The same doses of EO were administered to the rats of groups 5, 6 and 7, respectively for 12 weeks. On the 9th week, vacuolar degeneration of columnar epithelial cells in the mucosa and endometrial glands was observed in rats of groups 2 to 4. The blood vessels in the endometrium were dilated and congested. In group 3, the necrosis and desquamation of epithelial cells were also evident. At certain places, proliferation of fibrovascular connective tissues was distinct in groups 3 and 4. The degeneration and necrosis of mucosa and endometrial gland with infiltration of inflammatory cells were marked in group 4. On the 13th week, these changes were more marked in the respective group. The extent and severity of uterine damage were found to be dose and time dependent, which indicated that at higher dose and prolonged period, EO (oestrogen) causes severe damage in the uterus. EO has produced the optimum and standard uterine damage at the dose of 750 ?g/kg, orally weekly for 12 weeks.

Keywords: Histopathological study, oestrogen, rats, uterine damage

Introduction

Oestrogen is the most commonly prescribed drug as a component of oral contraceptives (OCs) and hormonal replacement therapy (HRT) in women. Increased incidence of endometrial carcinoma as a result of uterine damage in premenopausal women receiving OCs has been observed by Vessey *et al.* (1983). The long-term use of oestrogen may cause cell damage leading to cancers of many organs including uterus, vagina, ovary, breast and liver in human beings and animals (Estrogen and cancer website, 2006; Loose and Stancel, 2006; Satoskar *et al.*, 2005). Stilbesterol, a synthetic oestrogen caused uterine cytotoxicity such as hyperplasia, carcinoma, adenomyosis and endometriosis in rabbits (Meissner *et al.*, 1957). According to Hertz (1976), the "oestrogen cancer" hypothesis postulates that both exogenous and endogenous oestrogens and their metabolites play a significant and possibly an etiologic role in the pathogenesis of cancer in the oestrogen-responsive tissues. Studies indicate that oestrogen administration is followed by reproducible tumors of the breast, cervix, endometrium, ovary, pituitary, testicle, kidney and bone marrow in either mice, rats, rabbits, hamsters or dogs. Jick *et al.* (1979) stated that long-term use of oestrogen promotes the development of endometrial cancer/ cytotoxicity.

It is disconcerting to think that a natural hormone (oestrogen) circulating in significant amounts through the bodies of half of the world's population (women) is a carcinogen, but it is now official. Thus, the U.S.A. Government's National Toxicology Program and the National Institute of Environmental Health Sciences have added oestrogen to the list of known human carcinogens (Estrogen and cancer website, 2006).

Ethinyl oestradiol (EO), a semisynthetic 17 b-oestradiol, is the highly potent oestrogen. Most of the OCs contain 0.02 to 0.1 mg (20-100 μg) of EO. As little as 0.02 mg of EO daily serves as HRT in the menopause; while 0.015 mg daily can prevent vasomotor symptoms in menopause and doses of 0.015 to 0.25 mg daily are required to prevent bone loss in postmenopausal women. For the treatment of prostate cancer, it is administered @ 0.5 to 3 mg, orally daily (Loose and Stancel, 2006; Satoskar *et*

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al., 2005). Lynoral tablet containing 0.05 mg of EO is prescribed 1 to 3 times, orally daily for ORT. The median lethal dose (LD_{50}) of EO has been determined to be more than 1000 μg/kg body weight, orally by Pandey and Madhuri (2006) in rats. EO induced cytotoxicity has been seen in liver (Pandey *et al.*, 2006) and ovary (Madhuri *et al.*, 2006, 2007) of rats.

Hence, the present study was undertaken to assess the optimum and standard uterine damage due to EO (oestrogen) administration in albino rat.

Materials and Methods

Forty-two healthy inbred female albino rats (100-150 g) were taken in the study. The rats were divided into seven groups, each having six rats. The animals were kept in polypropylene cages under standard laboratory conditions of photoperiod (12 hr light: 12 hr darkness), temperature (250±5°C) and RH (85±5%) in animal house and fed on standard pellet diet and clean drinking water. However, before experiment the animals were fasted overnight, but water was given ad libitum. The experimental designs in the study received approval of Institutional Animal Ethics Committee.

The required amount of EO as Lynoral tablets (each tablet containing 0.05 mg of EO only) was purchased, and its suspension was prepared in distilled water mixed with a pinch of Gum acacia powder. The rats of groups 2, 3 and 4 were administered EO @ 250, 500 and 750 μg/kg, respectively orally once a week for 8 weeks. The same doses of EO were administered to the rats of groups 5, 6 and 7, respectively for 12 weeks. However, the rats of group 1 were administered normal saline (also containing a pinch of Gum acacia powder) to serve as control. After the end of the experiments, the rats were sacrificed on the 1st week (group 1), 9th week (groups 2-4) and 13th week (groups 5-7), and the uterus of each rat was collected and preserved in 10% buffered formalin. Later on, the uterine tissues were processed and stained with haemotoxylin and eosin (H&E) stain as per the method described by Culling (1963). Then the uniform and optimum damage as standard cytotoxicity in the uterine tissues was observed, microscopically.

Results and Discussion

The uterine tissues of the rats of group 1, which served as control, showed no histopathological changes on the 1st week of the experiment. However, on the 9th week, the histopathological changes in the uteri of the rats of groups 2 to 4 administered with EO for 8 weeks revealed vacuolar degeneration of columnar epithelial cells in the mucosa and endometrial glands. The blood vessels in the endometrium were dilated and congested. In group 3, the necrosis and desquamation of epithelial cells were also evident. At certain places, proliferation of fibrovascular connective tissues was distinct in groups 3 and 4. The degeneration and necrosis of mucosa and endometrial gland with infiltration of inflammatory

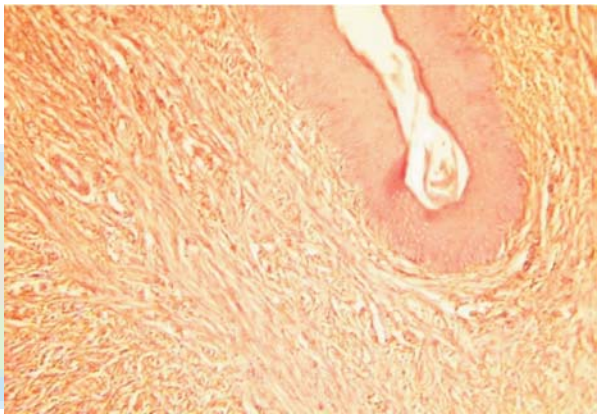


Fig. 1. Uterus of rat (Group 4) at 9th wk of ethinyl oestradiol (EO, 750 μg/kg, orally weekly for 8 wks) administration showing degeneration and necrosis of mucosa and endometrial glands with infiltration of inflammatory cells (H&E,x400).

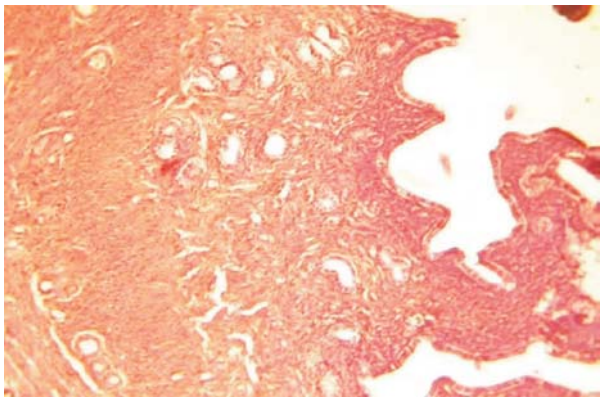


Fig. 2. Uterus of rat (Group 7) at 13th wk of EO (750 μg/kg, orally weekly for 12 wks) administration showing extensive fibrosis resulting into compression of endometrial glands (H & E, x100).

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cells were marked in group 4 (Fig. 1). On the 13th week, these changes were more marked in groups 5, 6 and 7, respectively. In group 7, the fibrosis was extensive resulting into compression of endometrial glands (Fig. 2).

The results of the present study may be correlated with the reports of various authors (Estrogen and cancer website, 2006; Hertz, 1976; Jick *et al.*, 1979; Loose and Stancel, 2006; Meissner *et al.*, 1957; Satoskar *et al.*, 2005; Vessey *et al.*, 1983) who elucidated the oestrogen induced cytotoxicity and carcinogenesis in the uterus. EO induced cytotoxicity (250, 500 and 750 μg/kg, orally weekly for 8 and 12 weeks) induced cytotoxicity has also been found by Pandey *et al.* (2006) and Madhuri *et al.* (2006, 2007) in liver and ovary of rats, respectively. All these reports corroborate with the results of the present study. However, no other literature could be available on the oestrogen induced experimental uterine damage and probably no such research works have been conducted in India.

Excessive oestrogen is trapped in the uterus, ovary, breast or liver due to stagnation in the blood circulation and overstimulates the cell division leading to cytotoxic changes e.g. fibroids, cysts or cancers (Estrogen and cancer website, 2006; Hertz, 1976). The extent and severity of uterine damage observed in the present study were dose and time dependent, which indicated that at higher dose and prolonged period, EO (oestrogen) causes severe damage in the uterus. EO has produced the optimum and standard uterine damage at the dose of 750 μg/kg, orally weekly for 12 weeks.

Acknowledgements

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, Jabalpur for providing laboratory facilities. The first author as a Ph.D. scholar also acknowledges gratefully to the R.D.V.V., Jabalpur, M.P., India.

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Received: 18.07.2007
Accepted: 28.02.2008