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Ethinyl oestradiol-induced liver damage in female albino rats

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ABSTRACT

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Ethinyl oestradiol (EO) was administered to female albino rats of groups 2, 3 and 4 @ 250, 500 and 750 ¼ g/kg, orally, weekly for 8 weeks, respectively; and the same doses of EO were administered to rats of groups 5, 6 and 7, respectively for 12 weeks. On the 9th week, slight cellular swelling in the liver tissues of group 2 was observed. In group 3, cellular swelling was more marked and the focal areas of hydropic changes were seen. In group 4, the blood vessels including central veins were congested. The hepatocytes revealed nuclear granularity of cytoplasm indicating degenerative changes. On the 12th week, the liver tissues of group 5 showed congestion and perilobular fibrosis. In group 6, severe congestion, focal areas of haemorrhage, and varying degree of degeneration and necrosis were noticed. The central veins were extremely dilated and the fibroblastic proliferation was distinct. In group 7, the histopathological changes were similar to those of group 6; however, the fibroblastic reaction was more intense at the portal triad area and the formation of new bile duct was also evident. The extent and severity of hepatotoxicity were dose and time dependent, indicating that with higher dose and prolonged duration, EO caused severe and extensive damage in the liver tissues. EO at the dose of 500 ¼ g/kg, orally, weekly for 12 weeks is sufficient to cause uniform and optimum liver damage.

Kewwords: Ethinyl oestradiol, female rats, histopathology, liver damage

Oestrogens are widely used (and misused) in small animal practice for the treatment of misalliance, hypogonadal obesity and hormonal urinary incontinence in bitches. In male dogs, they are used to treat anal adenoma, excess libido and prostatic hyperplasia. Adverse effects such as bone marrow suppression, pyometra and infertility have been documented after administration of different oestrogen preparations[1,2]. The synthetic oestrogen, hexoestrol (60 mg/kg, orally daily up to 40 days in female rats) has been reported to induce hepatotoxicity with cellular hypertrophy and hyperplasia[5]. In humans and laboratory rodents, oestrogens have clearly been recognized to be carcinogen[4,6]. In women, oestrogen is most commonly used as a component of oral contraceptives (OCs) to control the birth and as hormonal replacement therapy (HRT). OCs might increase the risk of certain liver cancers (e.g. hepatocellular carcinoma) and benign liver tumours (e.g. hepatic adenoma)[12]. Ethinyl oestradiol (EO), a highly potent semisynthetic oestrogen, has been reported to damage liver, uterus and ovary[7,8,9,11]. Cell proliferation and hyperplasia in the liver of rat have been observed after continuous administration of EO at 10 ppm[10].

The evidences indicate that oestrogen or EO even at therapeutic dose may cause many disorders including cancer after long-term use. The EO is used as OC and HRT by millions of women all over the world. The development of different pathological lesions by EO can give an idea regarding the type of drug action and dosage to be employed for experimental or therapeutic purposes. Such data may be necessary to know the standard toxic dose and duration of EO, so that the females may be warned against the toxic hazards of EO. The standard toxic dose of EO may also be useful to produce experimental carcinogenic model for evaluating the anticarcinogenic activity of drugs[11]. In view of the above facts, the present study was undertaken to induce experimental liver damage in female albino rats by EO at a specific dose and time.

Forty-two healthy inbred female albino rats (100-160 g) were divided into 7 groups, each having 6 rats. The animals were kept in polypropylene cages under standard laboratory conditions with 25±5^{0}C temperature, 45-55% relative humidity and 10 hr light:14 hr darkness. The rats were fed standard pellet diet and drinking water *ad libitum*. The experimental design and protocol were approved by the Institutional Animal Ethics Committee.

EO was purchased as Lynoral tablets (each tablet containing 0.05 mg of EO only), marketed by Organon India Ltd., Kolkata. 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, orally once a week for 8 weeks, respectively; and the same doses of EO were administered to rats of groups 5, 6 and 7, respectively, for 12 weeks. The rats of group 1 were administered normal saline (also prepared in distilled water, and mixed with a pinch of *Gum acacia* powder) to serve as control. After the end of experiment, the rats were sacrificed by humanitarian method (cervical dislocation)

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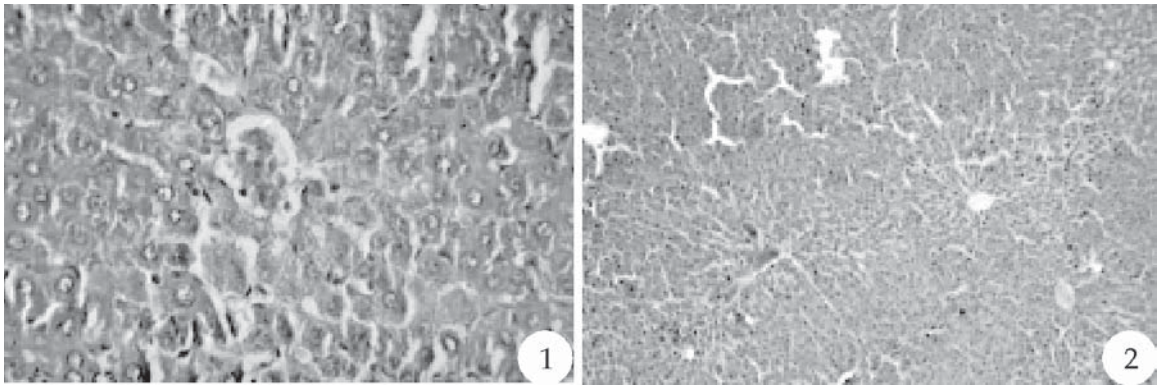

Fig. 1. Liver of female albino rat (Group 3) on 9th week of EO administration (500 µg/kg, orally, weekly for 8 weeks) showing congestion, cellular swelling and focal areas of hydropic changes (HE x400). **Fig. 2.** Liver of female albino rat (Group 6) on 13th week of EO administration (500 µg/kg, orally, weekly for 12 weeks) showing severe congestion, focal areas of haemorrhage, and varying degree of degeneration and necrosis; central veins are extremely dilated and at places, sinusoids are distended (HE x100).

on the 1st week (group 1), 9th week (groups 2-4) and 13th week (groups 5-7). The liver was collected, preserved in 10% buffered formalin, processed and stained with H & E stain as per the method of Culling[3] for microscopic examination.

The liver tissues of the rats of group 1 (control) administered normal saline showed no histopathological changes. On the 9th week, the liver tissues of the rats of group 2 (EO @ 250 µg/kg, orally weekly) revealed slight cellular swelling. In group 3 (EO @ 500 µg/kg, orally weekly), cellular swelling was more marked and focal areas of hydropic changes (vacuolization) were seen (Fig. 1). In group 4 (EO @ 750 µg/kg, orally weekly), the blood vessels including central veins were congested. At places, sinusoids were dilated with the presence of red cells. The hepatocytes showed nuclear granularity of cytoplasm indicating degenerative changes. On the 13th week, the liver tissues of the rats of group 5 (EO @ 250 µg/kg, orally, weekly) showed congestion and perilobular fibrosis. In group 6 (EO @ 500 µg/kg, orally, weekly), severe congestion, focal areas of haemorrhage, and varying degree of degeneration and necrosis were noticed. The central veins were extremely dilated and few of them were congested. The fibroblastic proliferation was distinct and at certain places, the sinusoids were distended (Fig. 2). In group 7 (EO @ 750 µg/kg, orally, weekly), the histopathological changes were similar to those of group 6; however, the fibroblastic reaction was more intense at the portal triad area and the formation of new bile duct was also evident.

Similar to the present study, various authors[1,2,5,8,9,10,11] have also elucidated that oestrogen or EO may cause liver damage including hepatic hyperplasia and tumour; however, no detailed histopathological lesions have been reported. In the present study, the extent and severity of liver damage were dose and time dependent, indicating that with higher dose and prolonged duration, EO caused severe and extensive damage in the liver tissues. EO at the dose of 500µg/kg, orally, weekly for 12 weeks is sufficient to cause uniform and optimum liver damage.

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REFERENCES

1. Acke E, Mooney CT and Jones BR (2003). Oestrogen toxicity in a dog. *Irish Vet. J.,* **56**(9): 465-468.
2. Cain JL (2001). Rational use of reproductive hormones. In: D.M. Boothe (Ed.), *Small Animal Clinical Pharmacology and Therapeutics*. Saunders, Philadelphia. pp. 677-690.
3. Culling CFA (1963). *Hand Book of Histoliology. Techniques.* 2nd Edn. Butterworth & Co. Ltd., London. pp. 25-172.
4. Estrogen and cancer website (2006). www.womenshealt.com; www.amazon.com.
5. Hart J (1986). Hepatotoxicity of the synthetic oestrogen hexoestrol in the female rat. *Arch. Toxicol.,* **59**(4): 216-220.
6. Liehr JM (2000). Is estradiol a genotoxic mutagenic carcinogen? *Endocrine Reviews,* **21**(1): 40-54.
7. Loose DS and Stancel GM (2006). Estrogens and progestins. In: L.L. Brunton (Ed.), *Goodman & Gilman's The Pharmacological Basis of Therapeutics.* 11th Edn. McGraw-Hill Co., New York. pp. 1541-1571.
8. Madhuri S, Pandey Govind and Khanna A (2007). Toxicity study of ethinyl oestradiol in female rats. *Nat. J. Life Sci.,* **4**(1): 67-70.
9. Madhuri S, Pandey Govind, Shrivastav AB and Sahni YP (2007). Ovarian cytotoxicity by oestrogen in rat. *Toxicol. Int.,* **14**(2): 143-145.
10. Mayol X, Neal GE, Davies R, Romero A and Domingo J (1992). Ethinyl estradiol-induced cell proliferation in rat liver. Involvement of specific populations of hepatocytes. *Carcinogenesis,* **13**(12): 2381-2388.
11. Pandey Govind and Madhuri S (2008). Median lethal dose, acute and chronic toxicities of ethinyl oestradiol estrogen. *Nat. J. Life Sci.,* **5**(2): 291-294.
12. Tao LC (1991). Oral contraceptive-associated liver cell adenoma and hepatocellular carcinoma. *Cancer,* **68:** 341-347.