HEPATIC CELL INJURY BY ETHINYL OESTRADIOL ESTROGEN
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ABSTRACT:
Estrogens are the most commonly used as oral contraceptives (OCs) to control birth and as hormonal replacement therapy (HRT) by millions of women all over the world. In men, they are mainly used for the treatment of prostate cancer. However, the excessive and prolonged use of estrogens may cause severe cytotoxicity and cancer of many organs. Most of the OCs contain a highly potent estrogen, ethinyl oestradiol (EO) as semisynthetic 17\textsubscript{β}-oestradiol. Hence, the present study was undertaken to assess the hepatic cell injury after administration of EO in female albino rats. EO was administered @ 500 µg/kg, orally, weekly for 8, 12, 16, 20 and 24 weeks to the rats of groups 2 to 6, respectively. However, the rats of group 1 were given saline served as control. On the 9\textsuperscript{th} week (Group 2), the hepatic tissues showed cellular swelling and focal areas of hydropic changes, including congested central veins. The hepatocytes revealed nuclear granularity of cytoplasm, indicating the degenerative changes. On the 13\textsuperscript{th} week (Group 3), the hepatic tissues showed focal areas of haemorrhage, varying degree of degeneration, necrosis and perilobular fibrosis. The central veins were extremely dilated and the sinusoids were distended. On the 17\textsuperscript{th} week (Group 4), 21\textsuperscript{st} (Group 5) and 25\textsuperscript{th} (Group 6) weeks, the hepatic cell injury was more severe, leading to severe hyperaemia, degeneration, necrosis, fibrosis and vacuolization. The extent and severity of hepatic cell injury were time dependent, which indicated that the EO @ 500 µg/kg, orally, weekly for 20 to 24 weeks may cause severe hepatic cell injury.

KEYWORDS: Ethinyl oestradiol (EO) estrogen, female albino rat, hepatic cell injury, liver.

INTRODUCTION
Estrogens are the most commonly prescribed drugs, by far the two major uses are as a component of OCs and HRT in women. OCs used to prevent fertilization or control birth, have influenced the lives of untold millions of women. Estrogen is responsible for many illnesses in women as well as men, and its long-term use may cause deleterious effects on liver as well. Excessive and long-term use of estrogen has been reported to cause cancers of uterus, ovary, cervix, vagina, pituitary gland, mammary gland, testicle, liver, kidney, lymphoid system and bone marrow in humans as well as animals\textsuperscript{1-7}. The USA Government’s National Toxicology Program and the National Institute of Environmental Health Sciences have added estrogen to the list of known carcinogens. It is disconcerting to think that a natural hormone (estrogen) circulating in significant amounts through the bodies of about half of the world’s population (women) is a carcinogen, but it is now official\textsuperscript{3,5,8}. Most of the OCs contain ethinyl oestradiol (EO), a highly potent semisynthetic 17\textsubscript{β}-oestradiol estrogen. The median lethal dose (LD\textsubscript{50}) of EO has been determined to be more than 1000 µg/kg body weight, orally in female albino rats\textsuperscript{6}. EO (@ 250, 500 and 750 µg/kg, orally, weekly for 8-24

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weeks) induced cytotoxicity, resulting into cancer in the uteri and ovaries of albino rats has been reported\(^3\,^4\). Furthermore, the liver damage in female albino rats after administration of EO (@ 750 µg/kg, orally, weekly for 8-24 weeks) has also been observed\(^5\).

In view of the above facts, the present study was undertaken to assess the hepatic cell injury due to EO (estrogen) administered at a lower dose of 500 µg/kg, orally, weekly for 8 to 24 weeks in female albino rats. This investigation has great importance as no other literature could be available on the EO induced experimental hepatic cell injury, and probably no such research works have been conducted in India.

**MATERIALS AND METHODS**

Thirty-six healthy inbred female albino rats (100-160 g) were equally divided into six groups (each group had 6 rats). They were kept in polypropylene cages under standard conditions with 10 hr day light and 14 hr darkness in the animal house of Govt. NSCB Medical College, Jabalpur. The rats were fed on standard pellet diet and drinking water *ad libitum*. The experimental designs and protocols for investigation received the approval of Institutional Animal Ethics Committee as per guidelines provided by the CPCSEA, Govt. of India. 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 (since the drug is insoluble in water). EO was administered @ 500 µg/kg, orally, weekly for 8, 12, 16, 20 and 24 weeks to the rats of groups 2, 3, 4, 5 and 6, respectively. However, the rats of group 1 were given saline (also mixed with a pinch of *Gum acacia* powder) to serve as control. To assess the EO induced liver damage (hepatic cell injury / hepatotoxicity), the rats of groups 1 to 6 were sacrificed by cervical decapitation (euthanized scientifically) on the 1\(^{st}\), 9\(^{th}\), 13\(^{th}\), 17\(^{th}\), 21\(^{st}\) and 25\(^{th}\) week, respectively. The liver of each rat was collected and preserved in 10% buffered formalin. Later on, the liver tissues were processed and stained with Harris’s haemotoxylin and eosin (H & E) stain as per the methods described by Culling\(^10\). Then the histopathological changes in the liver tissues were observed, microscopically.

**RESULTS AND DISCUSSION**

On the 1\(^{st}\) week, the hepatic tissues of group 1 (control) showed normal histological profile. On the 9\(^{th}\) week, the hepatic tissues of group 2 showed cellular swelling, focal areas of hydropic changes (vacuolization) and congested central vein. The hepatocytes revealed nuclear granularity of cytoplasm, indicating the degenerative changes (Fig. 1). On the 13\(^{th}\) week (Group 3), the liver tissues showed focal areas of haemorrhage, varying degree of degeneration, necrosis and perilobular fibrosis. Central veins were extremely dilated and congested. The hepatocytes revealed nuclear granularity of cytoplasm, indicating the degenerative changes (Fig. 1). On the 17\(^{th}\) week (Group 4), the hepatic cell injury was more severe, leading to severe vascular hyperaemia, focal necrosis and fibrosis with dissolution of nuclear material and extreme vacuolization of cytoplasm. The fibroblastic reaction was more intense at the portal triad area (Fig. 3). On the 21\(^{st}\) (Group 5; Fig. 4) and 25\(^{th}\) (Group 6) weeks, the above histopathological changes in the hepatocytes were found to be much more severe and extensive. The findings of the present study may be correlated with our earlier report\(^9\) that EO @ 750 µg/kg, orally,
weekly for 8 to 24 weeks causes liver damage in rats. Other similar studies\textsuperscript{3-5} showed that EO @ 250, 500 and 750 µg/kg, orally, weekly for 8 to 24 weeks produced uterine and ovarian damage which further corroborate with the EO induced hepatic damage observed in the present study. Some other authors\textsuperscript{1-2,6-8,11-12} have also cited the estrogen or OCs induced hepatotoxicity, leading to liver tumour. Besides these, however, no detailed hepatic cell injury induced by EO, similar to the present result, has been reported in the literature.

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**Fig. 1:** Liver of rat (Group 2- on 9\textsuperscript{th} week after administration of EO) showing congestion, cellular swelling, focal areas of hydropic changes and congested central vein (H & E, x400).

**Fig. 2:** Liver of rat (Group 3- on 13\textsuperscript{th} week after administration of EO) showing focal areas of haemorrhage, varying degree of degeneration, necrosis and perilobular fibrosis with extremely dilated central vein and distended sinusoids (H & E, x100).

**Fig. 3:** Liver of rat (Group 4- on 17\textsuperscript{th} week after administration of EO) showing more severe hepatic cell injury, leading to severe vascular hyperaemia, focal necrosis and fibrosis with dissolution of nuclear material and extreme vacuolization of cytoplasm; fibroblastic reaction is more intense at the portal triad area (H & E, x100).
CONCLUSION
EO (estrogen) @ 500 µg/kg, orally, weekly for 20 to 24 weeks caused the optimum or standard hepatic cell injury in female albino rat. The extent and severity of hepatic cell injury were time dependent, which indicated that the EO after prolonged period of administration may cause more severe liver damage. Therefore, the excessive and prolonged use of estrogen should be avoided as it is an unsafe drug. Excessive estrogen is trapped in target organs such as uterus, ovary and liver, etc. due to stagnation, which overstimulates the cell division, leading to abnormal growth. After the hormone binds to its receptors in a cell, it turns on hormone-responsive genes that promote DNA synthesis and cell proliferation. If a cell happens to have cancer-causing mutations, those cells will also proliferate and develop into tumours. In carcinogenic estrogen, the reactions produce intermediates capable of producing oxygen radicals that can damage the cell’s fats, proteins and DNA. Unrepaired DNA damage can turn into a mutation, leading to cancer.3,5,7

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