

HEPATOGENIC EFFECT OF OPTILIV ON ESTROGEN INDUCED LIVER DAMAGE IN FEMALE ALBINO RAT

Govind Pandey

*Ex-Professor/Principal Scientist & Head of Pharmacology (Pharmacy),
presently Officer-In-Charge of Rinder Pest (Veterinary/Animal Husbandry
Department, Govt. of MP), Jabalpur Division, Jabalpur, M.P., India
Email: drgovindpandey@rediffmail.com*

Abstract: The present study was taken to evaluate the hepatogenic effect of OptiLiv (a polyherbal formulation) on estrogen induced liver damage in female albino rat. The rats of groups 2 and 3 were administered with ethinyl oestradiol (EO, a semi-synthetic estrogen) @ 500 µg/kg body weight, orally, weekly for 8 weeks. The rats of group 1 were given saline to serve as normal. From the 9th week, the rats of group 3 were administered with OptiLiv @ 100 mg/kg, orally, daily for 3 weeks; while the rats of group 2 were kept without treatment of OptiLiv for 3 weeks after EO administration. The rats were sacrificed after the experimental periods, and the histopathological study of livers was performed. On the 12th week, the hepatic tissues of group 2 revealed congestion, cellular swelling and focal areas of hydropic changes (vacuolization). The blood vessels, including central veins were also congested. At places, the sinusoids were dilated. Hepatocytes showed nuclear granularity of cytoplasm, indicating the degenerative changes in between the hepatic lobules. However, the hepatic tissues of group 3 showed very mild changes, and regeneration and normalization of many hepatocytes were observed. The results suggest that estrogen (EO) caused the liver damage, which was subsided and repaired to a great extent by OptiLiv.

Keywords: Ethinyl oestradiol (EO, estrogen), Liver damage, OptiLiv, Hepatogenic effect, Female Rat.

INTRODUCTION

Estrogens are the most commonly prescribed drugs, by far the two major uses are as a component of oral contraceptives (OCs) and hormonal replacement therapy (HRT) in women. Numerous studies demonstrate an increased incidence of cytotoxicity and cancer in many organs due to estrogen. In December 2000, the USA Government's National Toxicology Program and the National Institute of Environmental Health Sciences added estrogen to the list of known human carcinogens. The long-term use of estrogen may cause many detrimental effects on liver as well (Hertz, 1976; Loose and Stancel, 2006; Madhuri, 2008; Pandey and Madhuri, 2008).

Ethinyl oestradiol (EO), a semisynthetic 17 β-oestradiol, is the highly potent estrogen. Most of the OCs contain 0.02 to 0.1 mg of EO (Loose and Stancel, 2006; Madhuri, 2008). The median lethal dose (LD₅₀) of EO was determined to be more than 1000 µg/kg body weight, orally in female albino rats (Pandey and Madhuri, 2008). The acute and chronic toxicities of EO have also been recorded by these

authors. EO caused the cytotoxicity, leading to cancer in the uterus and ovary of female albino rat (Madhuri, 2008; Madhuri and Pandey, 2010). Further, EO @ 250, 500 and 750 µg/kg, orally, weekly for 8 and 12 weeks has been reported to cause liver damage in female albino rat (Pandey and Madhuri, 2007).

Many medicinal plants and their formulations are being used for the treatment and prevention of hepatotoxicity. OptiLiv, a polyherbal formulation (Indian Herbs Co. Pvt. Ltd., Saharanpur, UP) contains the standardized extracts of *Andrographis paniculata* (Kiryat), *Picrorhiza kurroa* (Kutki), *Eclipta alba* (Bhangra), *Boerhaavia diffusa* (Punarnava), *Azadirachta indica* (Nim), *Swertia chirata* (Chirayita), *Solanum nigrum* (Makoi), *Terminalia arjuna* (Arjuna), *Aphanamixis rohituka* (Harinhara), *Terminalia chebula* (Harra) and *Fumaria indica* (Pitpapra). All these plants are known to have beneficial effects on the liver (Chopra *et al.*, 2002; CSIR, 1986; Kumar *et al.*, 2002).

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and thus it possesses hepatoprotective and hepatogenic activities (Biswas *et al.*, 1999; Mathur *et al.*, 1996; Singh, 2000).

In view of above facts, the OptiLiv has been taken in the present study to evaluate its hepatogenic effect against the liver damage caused by estrogen (EO).

MATERIALS AND METHODS

Eighteen healthy inbred female albino rats (100-160 g) were kept in colony cages under standard laboratory conditions in the animal house of the place of research work (N.S.C.B. Medical College, Jabalpur). The rats were divided into three groups (each had 6 animals). They were fed on standard pellet diet and drinking water *ad libitum*. The experimental designs and protocols received the approval of Institutional Animal Ethics Committee (IAEC) in accordance with the 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 from the medical shop. The suspension of powdered EO was prepared in distilled water, mixed with a pinch of *Gum acacia* powder. The normal rats (Group 1) were given saline (also mixed with a pinch of *Gum acacia*). However, the rats of groups 2 and 3 were administered with EO @ 500 µg/kg body weight, orally, weekly for 8 weeks. From the 9th week, the rats of group 3 were administered with OptiLiv @ 100 mg/kg, orally, daily for 3 weeks; while the rats of group 2 were kept *placebo* without OptiLiv treatment for 3 weeks after the administration of EO. Then for the histopathological studies of livers, the rats of group 1 were sacrificed on the 1st week; while the rats of groups 2 and 3 were sacrificed on the 12th week. The livers of rats were collected and preserved in 10% buffered formalin. Later on, the hepatic tissues were processed and stained with Harris's haemotoxylin and eosin (H & E) stain as per the methods described by Culling (1963), and the hepatic tissues were examined, microscopically.

RESULTS AND DISCUSSION

On the 12th week, the hepatic tissues (Fig. 1) of rats of group 2 administered with EO alone revealed congestion, cellular swelling and focal areas of hydropic changes (vacuolization). The blood vessels, including central veins were also congested. At

places, the sinusoids were dilated. Hepatocytes showed nuclear granularity of cytoplasm, indicating the degenerative changes in between the hepatic lobules. However, the histopathological changes in the liver tissues (Fig. 2) of rats of group 3 administered with EO and OptiLiv both were very mild, and regeneration and normalization of many hepatocytes were noticed.

The results of group 2 may be correlated with the citation of Loose and Stancel (2006), Madhuri (2008), and Pandey and Madhuri (2008) who reported that the estrogen causes hepatotoxicity. Madhuri (2008), and Madhuri and Pandey (2010) have observed the EO (250, 500 and 750 µg/kg, orally, weekly for 8 to 24 weeks) induced uterine and ovarian cytotoxicity; while Pandey and Madhuri (2007) have noticed the hepatotoxicity caused by EO (250, 500 and 750 µg/kg, orally, weekly for 8 and 12 weeks) in female albino rat. All these reports are in collaboration with the results of the present study. However, probably no research works on estrogen induced experimental liver damage induced by EO (estrogen) has been done in India since no literature could be traceable in this regard.

Excessive estrogen is trapped in different specific target organs (e.g., uterus, ovary, breast, liver, kidney, etc.) due to stagnation, which over-stimulates the cell division, leading to abnormal growth or tissue damage. 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 metabolizing carcinogenic oestrogen, 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 (Madhuri, 2008; Pandey and Madhuri, 2008).

The results of group 3 of the present study correspond with the findings of Biswas *et al.* (1999), Kumar *et al.* (2002), Mathur *et al.* (1996) and Singh (2000) who reported that OptiLiv can optimize hepato-biliary function and protect the liver from toxicity. A similar herbal formulation, Livol which contained six ingredients, viz., *A. paniculata*, *B. diffusa*, *E. alba*, *S. nigrum*, *T. arjuna* and *T. chebula* of OptiLiv, was evaluated by Pandey (1990) against

Figures & Legends

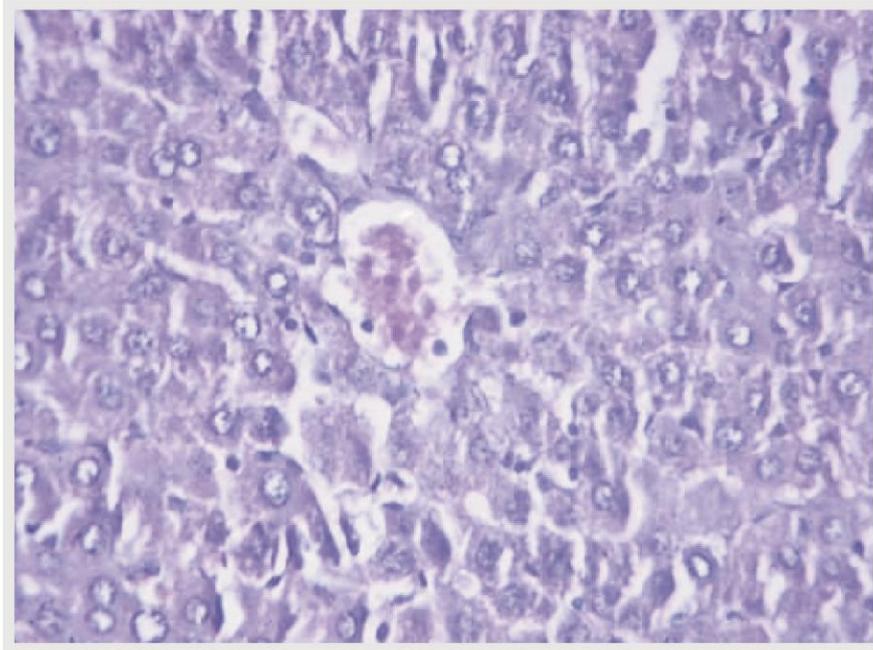


Fig. 1: Liver of rat (Group 2; EO @ 500 $\mu\text{g}/\text{kg}$, orally, weekly for 8 weeks) on 12th week showing congestion, cellular swelling and focal areas of hydropic changes; hepatocytes reveal nuclear granularity of cytoplasm, indicating the degenerative changes in between the hepatic lobules (H&E, x400).

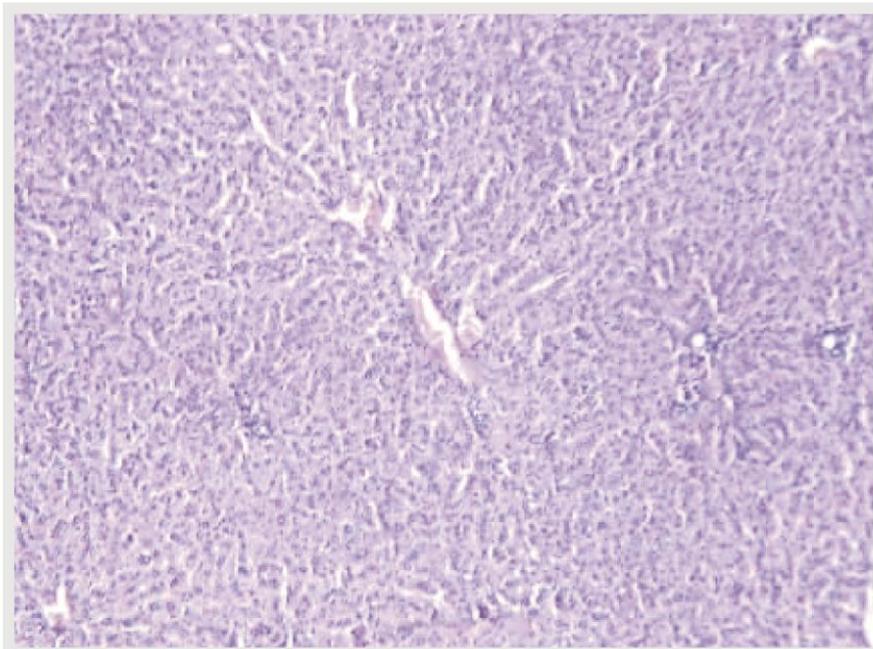


Fig. 2: Liver of rat (Group 3; EO @ 500 $\mu\text{g}/\text{kg}$, orally, weekly for 8 weeks + OptiLiv @ 100 mg/kg , orally, daily for 3 weeks after 8 weeks of EO dosing) on 12th week showing regeneration and normalization of many hepatocytes (H&E, x100).

hepatotoxicity induced by paracetamol in mice and rabbits. The author observed a significant hepatogenic effect as evidenced by restoration of normal biochemical functions and cytoarchitecture of the liver. Sharma *et al.* (2005) also noted the hepatoprotective and hepatogenic activities of a preparation (which had four ingredients, viz., *A. paniculata*, *E. alba*, *S. chirata* and *T. chebula* of OptiLiv, besides other plants) against paracetamol induced liver damage in rats. Furthermore, most of the ingredients of OptiLiv have been stated to be active against various liver diseases (Chopra *et al.*, 2002; CSIR, 1986; Kumar *et al.*, 2002). All these reports confirm the hepatogenic effect of OptiLiv as observed in the present study.

Most of the medicinal plants contain strong antioxidant phytochemicals, e.g., vitamins (A, E, C, K), carotenoids, terpenoids, flavonoids (quercetin, anthocyanins, catechins, flavones, flavonones, isoflavones), polyphenols (ellagic acid, gallic acid, tannins), alkaloids, enzymes (superoxide dismutase, catalase, glutathion peroxidase), minerals (Cu, Mn, Se, Zn), polysaccharides, saponins, lignins and xanthenes. These antioxidants can cure various diseases, including cancer by protecting cells from damage caused by 'free radicals'- highly reactive oxygen compounds (Gupta and Sharma, 2006; Kathiresan *et al.*, 2006; Madhuri, 2008; Madhuri and Pandey, 2010). Since many of these antioxidants are present in the ingredients of OptiLiv, it might have caused the regeneration and normalization of hepatic tissues.

CONCLUSION

EO (500 µg/kg, orally, weekly for 8 weeks) caused the liver damage in rats. On the other hand, OptiLiv (100 mg/kg, orally, daily for 3 weeks after 8 weeks of EO dosing) repaired and normalized the liver damage to a great extent. Hence, OptiLiv possesses hepatogenic activity, and it may also protect the liver from various toxicants and during liver disorders.

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