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Original Article

EFFICACY OF PROIMMU ON OESTROGEN INDUCED UTERINE DAMAGE IN RAT

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Abstract

In the present study, effect of ProImmu on oestrogen induced uterine cytotoxicity has been evaluated. Three groups of healthy adult female albino rats having six rats in each group were taken. The rats of groups 2 and 3 were administered ethinyl oestradiol (EO, a semi-synthetic oestrogen) @ 750 µg/kg body weight, orally once a week for 12 weeks. However, the rats of group 1 (normal) were given saline alone. After 12 weeks, the rats of group 3 were administered ProImmu @ 100 mg/kg, orally daily for 3 weeks. After the experimental periods, the rats were sacrificed and the histopathological study of uteri was performed. The uterine tissues of the rats of group 2 showed marked vascular congestion, epithelial necrosis and fibrous tissue proliferation. The fibrosis was extensive resulting into compression of endometrial glands. Desquamation of glandular epithelium was also observed. However, in group 3, endometrial tissues revealed mild pathological changes as compared to those observed in group 2. Regeneration and normalization of some uterine tissues with mild congestion were also seen at places, indicating that the ProImmu has some cytogenic effects on oestrogen induced uterine damage.

Key words: Uterine damage, oestrogen (ethinyl oestradiol), *Proimmu*, cytogenic effect, rats.

INTRODUCTION

Oestrogen is the most commonly prescribed drug as a component of oral contraceptives (OCs) and hormonal replacement therapy (HRT) in women. There is evidence that long-term use of oestrogen may cause cytotoxicity leading to cancers of uterus, ovary, mammary gland, liver, kidney, colon etc. in human and animals (Qestrogen and cancer, 2006-website; Hertz, 1976; Loose-Mitchell and Stancel, 2001). Oestrogen (stilbestrol) has been reported to cause uterine abnormalities such as endometrial polyp, hyperplasia, metaplasia, carcinoma, adenomyosis and endometriosis etc. in rabbits (Meissner *et al*, 1957). Oestrogen induced cytotoxicity has been observed in liver (Pandey *et al*, 2006) and ovary (Madhuri *et al*, 2006) of rats.

Numerous medicinal plants and their formulations are being used for the treatment and prevention of cytotoxicity caused by various aetiological agents. One of the indigenous drugs, *Proimmu* (Immu-21) manufactured by Indian Herbs/Envin Bioceuticals Pvt. Ltd., Saharanpur (U. P.) has been reported to possess immunomodulatory effect. *Proimmu* restored the normal histoarchitecture of the damaged tissues (Agrawala *et al*, 2001; Madhuri *et al*, 2006). It contains four immunoactive plants viz. *Tinospora cordifolia* (Giloe), *Withania somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi) and *Emblica officinalis* (Amla).

Pretreatment with Immu-21 (10 and 30 mg/kg, IP once a day for 7 days) in mice increased the natural killer (NK) cell activity and proliferation of splenic leucocyte to B-cell mitogen and cytotoxic activity against K 562 cells (Kumar *et al*, 2002). Several workers (Arondekar, 1999; Somkuwar, 2003; Sultana *et al*, 2005; Thatte and Dahanukar, 1989) reported the immunomodulatory and cytogenic effects including anticancer activity of the plant, 2002). Several workers (Arondekar, 1999; Somkuwar, 2003; Sultana *et al*, 2005; Thatte and Dahanukar, 1989) reported the immunomodulatory and cytogenic effects including anticancer activity of the plantingredients of *Proimmu*.

In view of above facts, the present study has been undertaken to evaluate the cytogenic effect of *Proimmu* on oestrogen induced uterine damage in albino rat.

MATERIALS AND METHODS

Eighteen healthy adult female albino rats weighing 100 to 150 gm were undertaken in the present study. The animals were kept in colony cages under identical managemental condition and fed on uniform balanced diet. The rats were divided into three groups (1, 2 and 3) having six rats in each group.

The required amount of EO as Lynoral tablets (each tablet contains 0.05 mg of EO) was purchased and the suspension of powdered EO was prepared in distilled water mixed with a pinch of Gum acacia powder. The rats of groups 2 and 3 were administered EO suspension @ 750 µg/kg body weight, orally once a week for 12 weeks. However, the rats of group 1 (normal) were given saline alone. After 12 weeks, the rats of group 3 were administered *Proimmu* @ 100 mg/kg, orally daily for 3 weeks. Then the rats of all the groups were sacrificed after their respective week of the experiment and the uterus of each rat was collected and preserved in 10% buffered formaline. Thereafter, the uterine tissues were processed and stained with Harris's haemotoxylin and eosin (H & E) stain. Microscopically, the histopathological changes in groups 2 and 3 as compared to group 1 were examined.

RESULTS AND DISCUSSION

On microscopic examination, the uterine tissues (Fig. 1) of the rats of group 2 treated with EO alone showed marked vascular congestion, epithelial necrosis and fibrous tissue proliferation as against group 1 (control). The fibrosis was extensive resulting into

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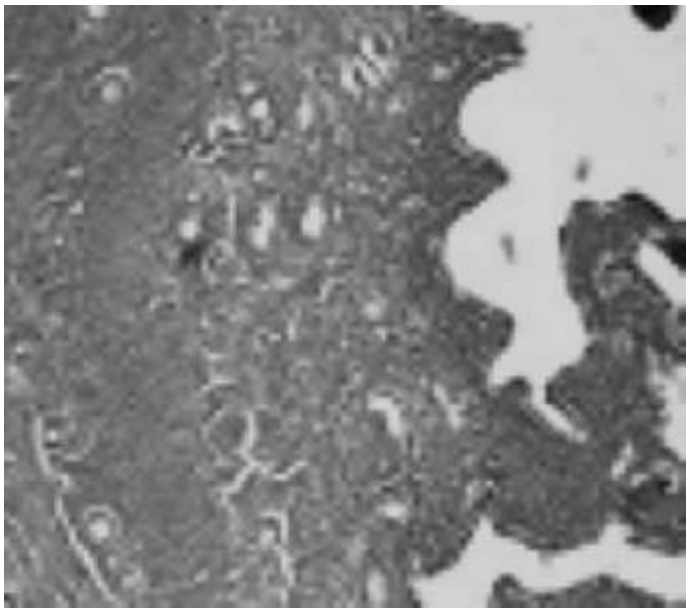

Figure 1: *Uterus of rat (Group 2) on the13th week after administration of EO (750 µg/kg, orally weekly for 12 weeks). The tissues show vascular congestion and extensive fibrosis resulting into compression of endometrial glands with necrosis of some glands (x100, H & E).*

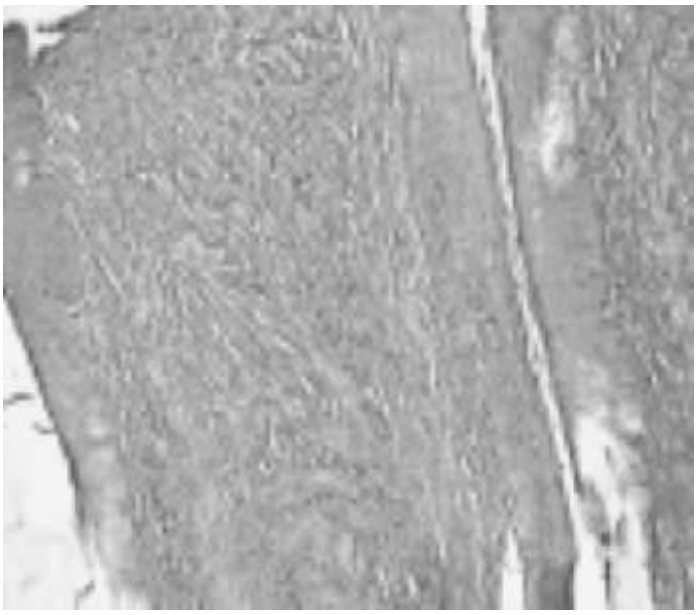

Figure 2: *Uterus of rat (Group 3) on the16th week after administration of EO (750 µg/kg, orally weekly for 12 weeks) and Proimmu (100 mg/kg, orally daily for 3 weeks after 12 weeks of EO dosing). Some tissues show regeneration and normalization (x100, H & E).*

compression of endometrial glands. Desquamation of glandular epithelium was also noticed. However, in group 3 treated with EO and *Proimmu* both, the endometrial tissues revealed mild pathological changes as compared to those observed in group 2. Regeneration and normalization of some uterine tissues with mild congestion were observed, at places (Fig. 2). These findings indicate that the *Proimmu* has some cytogenic effects on oestrogen induced uterine damage in rat.

Various uterine abnormalities including cytotoxicity after administration of stilbestrol (synthetic estrogen) have been reported by Meissner *et al* (1957) in rabbits. The oestrogen induced uterine cytotoxicity leading to cancer has been further cited by many authors (Estrogen and cancer, 2006-website; Hertz, 1976; Loose-Mitchell and Stancel, 2001). Oestrogen induced damage has also been reported by Pandey *et al* (2006) in liver and Madhuri *et al* (2006) in uterus of rats. All these reports corroborate the results of group 2 of the rats administered oestrogen alone in the present study.

It has been stated that excessive oestrogen is trapped in uterus, ovary, or breast due to stagnation in the blood circulation and overstimulate the cell division leading to cytotoxic effects such as fibroids, cysts or cancers in these organs. Both exogenous and endogenous oestrogens and their metabolites play a significant and possibly an aetiologic role in inducing cytotoxicity and cancer in the oestrogen-responsive tissues of men and animals (Hertz, 1976).

Proimmu administered to the rats of group 3 caused regeneration and normalization of some uterine tissues (Fig. 2). These findings correspond with the reports of several workers. Agrawala *et al*, (2001) reported that Immu-21 (*Proimmu*) possesses good immunomodulatory properties and may restore the normal histo-architecture of the damaged tissues. It can act by stimulating both non-specific and specific (humoral and cellular arms of the host immune system) immunity and can promote host resistance against infection or toxic substances by re-stabilizing body equilibrium and conditioning the body tissues. This herbal drug significantly increased phagocytic activity of macrophages and lymphocytes leading to tumoricidal effect and prevented leucopaenia, genotoxicity/mutogenicity and bone marrow suppression. Kumar *et al* (2002) observed that Immu-21 increased the natural killer (NK) cell activity, splenic leucocyte proliferation to B-cell mitogen and cytotoxic activity against K 562 cells in mice. Madhuri *et al* (2006) reported that oestrogen (EO @ 750 µg/kg, orally weekly for 12 weeks) induced ovarian cytotoxicity in rat has been repaired and normalized to some extent by *Proimmu* (100 mg/kg, orally daily for 3 weeks after 12 weeks of EO administration).

The beneficial effect of *Proimmu* over oestrogen induced uterine damage observed in group 3 of the present study may be further correlated with the findings of many workers, who reported that the ingredients of *Proimmu* have immunomodulatory and cytogenic effects including anticancer activity and thus *Proimmu* can protect/inhibit the cytotoxicity caused by several reasons. Thatte and Dahanukar (1989) observed the immunomodulatory effect of two ingredients of *Proimmu*, viz. *T. cordifolia* and *W. Somnifera*. Similarly, Arondekar (1999) recorded the immunomodulatory activity of the combined extracts of the three ingredients of *Proimmu*, viz. *E. officinalis, O. sanctum* and *W. Somnifera.* The cytogenic effect leading to anticancer activity of O. sanctum and W. somnifera has also been observed in mice with tumours (Somkuwar, 2003). *E. officinalis* (100 and 200 mg/kg for 7 consecutive days) has been reported by Sultana *et al* (2005) to protect liver against carbon tetrachloride (1 ml/kg) induced hepatotoxicity in rats. The prolonged use of oestrogen has been reported to cause cytotoxicity leading to cancers of many organs (Hertz, 1976; Madhuri *et al* (2006). In the present study also, oestrogen caused uterine damage in rat, which was repaired and normalized to some extent by *Proimmu*. Thus, it can be concluded that the *Proimmu* has some beneficial effects on oestrogen induced uterine damage.

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