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ANTICANCER EFFECT OF A HERBAL DRUG PROIMMU ON THE EXPERIMENTAL UTERINE CANCER IN RAT

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ABSTRACT

The present study was conducted to evaluate the anticancer effect of ProImmu, a herbal drug on ethinyl oestradiol (EO, an oestrogen) induced uterine cancer in albino rats. Rats of groups 2 to 5 were administered with EO @ 750 µg/kg, orally, weekly for 24 weeks. However, the rats of group 1 (normal) were given normal saline alone. ProImmu was administered @ 500 mg/kg, orally, daily for 4, 8 and 12 weeks after 20, 16 and 12 weeks of the administration of EO in groups 3, 4 and 5, respectively. The normal activities of serum transaminases, viz., serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) estimated in group 1 were significantly ($P<0.05$) increased by EO in group 2, which decreased significantly after treatment with ProImmu in groups 3 to 5 and returned to normal in group 5. The uterine tissues damaged by EO in group 2 revealed fibroblastic bundles as collagens, focal hyperplasia of endometrial lining and other cancerous changes such as hyperchromasia, enlargement of nuclei, anisokaryosis, anisocytosis and new angiogenesis. On the contrary, the uterine tissues of rats administered with ProImmu and EO both showed the increasing order of regeneration and normalization consequently after 4, 8 and 12 weeks of ProImmu treatment. The results suggest that ProImmu possess the anticancer effect, which may be due to immunostimulatory, antioxidant, phagocytic and other tissue protective activities of ProImmu or its plant-ingredients.

KEY WORDS: Anticancer effect, ethinyl oestradiol (oestrogen), ProImmu, rat, SGOT, SGPT, uterine cancer.

INTRODUCTION

EO (a highly potent semisynthetic 17β estradiol oestrogen) is most commonly used as oral contraceptive (OC) and hormonal replacement therapy (HRT) in women. Oestrogen has been reported to cause cancers of many organs, including uterus, ovary, mammary gland, liver, kidney and colon (Hertz, 1976; Madhuri, 2008; Pandey et al., 2010). In bitches, oestrogens and synthetic oestrogenic drugs are widely used for the treatment of misalliance, hypogonadal obesity and hormonal urinary inconsistence. In male dogs, they are used to treat anal adenoma, excess libido and prostatic hyperplasia; however, oestrogens have been reported to be carcinogenic in rodents and humans (Madhuri, 2008). Serum transaminases are elevated during organ dysfunction and cancer pathogenesis. The enhanced levels of SGOT and SGPT have been reported by Bradley et al. (1972), Shar and Kew (1982), and Somkuwar (2003) during carcinogenesis. Many drugs are prevalent for the treatment of cancer but there is no perfect remedy of this disease and still a plenty of research is being carried out throughout the world.

Some herbal products have been prepared from certain medicinal plants. ProImmu (a herbal formulation manufactured by Indian Herbs Research & Supply Co. Ltd., Saharanpur, U.P.) has been reported to possess immunomodulatory and genoprotective effects (Madhuri, 2008; Nemmani et al., 2002). ProImmu contains extracts of four medicinal plants, viz., *Emblica officinalis* (Amla fruit), *Ocimum sanctum* (Tulsi leaf), *Tinospora cordifolia* (Giloe stem bark) and *Withania somnifera* (Ashwagandha root). These plants are traditionally used for the treatment of several diseases.

Hence, the present study was performed to evaluate the anticancer effect of ProImmu on ethinyl oestradiol (EO) induced uterine cancer in albino rats by estimating the activities of serum transaminases, viz., SGOT and SGPT enzymes *vis-a-vis* histopathological study of uterus.

MATERIALS AND METHODS

Thirty female albino rats (100-150 g) equally divided into five groups were kept in polypropylene cages under standard laboratory conditions. Rats were fed on standard pellet diet and drinking water *ad libitum*. The experimental designs and protocols in the study received the approval of Institutional Animal Ethics Committee.

The rats of group 1 were administered with normal saline and treated as control group. In order to induce uterine cancer, Lynoral tablets (Organon India Ltd, Kolkata) which contains EO were administered to the rats of groups 2 to 5 @ 750 µg/kg b.w., orally for 24 weeks. ProImmu was administered @ 500 mg/kg b.w., orally, daily for 4, 8 and 12 weeks after 20, 16 and 12 weeks along with administration of EO to the rats of groups 3 to 5, respectively in order to find out the stage at which supplementation of ProImmu subside the effect of EO administration. After end of the experiments (i.e., 1^{st} week in group 1 and 25^{th} week in groups 2 to 5), the blood for biochemical study from rats of group 1 to 5 was collected from the eye vein and serum was separated. The activities (IU/L) of SGOT and SGPT were determined as per the method described by Bradley et al. (1972). The biochemical data were analyzed statistically to find out the significance of difference among different groups in Duncan's new multiple range test at P=0.05 (5% level of significance) as per the method cited by Steel and Torrie (1980).

For histopathological study, the rats were sacrificed by cervical dislocation (euthanized scientifically) after collection of blood. Uteri were collected and preserved in 10% buffered formalin. The uterine tissues were processed and stained with H & E stain as per the method of Culling (1963), and then the tissues were examined, microscopically.

RESULTS AND DISCUSSION

BIOCHEMICAL STUDY:

The average values (IU/L) of SGOT and SGPT estimated in the rats of groups 1 to 5 are presented in Table 1. The activities of the enzymes increased significantly ($P<0.05$) in groups 2 to 4 as compared to group 1 (normal) of rats. The SGPT activities of groups 2 to 5 differed significantly with each other. Whilst, the SGOT activities of groups 2 to 5 differed significantly

with both groups 3 and 4, but the activities of latter groups did not differ with each other. However, there was no significance difference between the SGOT and SGPT activities of groups 1 and 5. The activities of transaminases were increased significantly by administration of EO, are decreased and returned to normal range by supplementation of ProImmu.

Bradley et al. (1972) and Shar and Kew (1982) reported enhanced levels of transaminases in cancer affected animals and women. ProImmu reversed the effect of EO administration as the activities of transaminases (SGOT and SGPT) returned to normal range. The repair has been started after 4 weeks of supplementation of ProImmu, but the maximum effect was seen after supplementation of ProImmu for 20 weeks. Our findings are supported by Das et al. (2000) who after supplementation of Immu 21 (a research name of ProImmu), and also by Sultana et al. (2005) who after supplementation of *E. officinalis* (an ingredient of ProImmu) against carbon tetra chloride induced toxicity observed the normal SGOT and SGPT activities.

HISTOPATHOLOGICAL STUDY:

On the 25th week, the uterine tissues damaged by EO (Group 2) revealed the fibroblastic bundles made up of mature fibrocytes (as collagens). Focal hyperplasia of endometrial lining (epithelium) was quite evident. Other cancerous changes such as hyperchromasia, enlargement of nuclei, anisokaryosis, anisocytosis, angiogenesis and glandular polarity or no glandular structures were observed. The smooth muscles were reactive and damaged. Disarray, leading to severe malignancy in the whole area was also seen (Fig. 1). The uterine tissues of group 3 (dosed with EO for 24 weeks and ProImmu for 4 weeks) revealed mild histopathological changes, including vacuolar degeneration, necrosis and fibrosis; however, the tissue regeneration in some areas was also observed (Fig. 2). In group 4 (administered with EO for 24 weeks and ProImmu for 8 weeks), the necrobiotic changes were rather inconspicuous and reappearance of several normal tissues were seen (Fig. 3). In group 5 (also dosed with EO for 24 weeks and ProImmu for 12 weeks), much better signs of improvement as evident from the reappearance of virtually normal histological profiles in the uterine tissues were noticed (Fig.4).

The histopathological changes, leading to uterine cancer (Fig. 1) produced by EO may be correlated with the report of Meissner et al. (1957) who noticed the uterine cancer after administration of stilbestrol oestrogen. Newbold and Liehr (2000) observed the uterine adenocarcinoma at 12 and 18 months after administration of EO and other oestrogens in mice. After oestrogen binds to its receptors in a cell, it turns on hormone-responsive genes that promote DNA synthesis and cell proliferation. In the reaction, 'free oxygen radicals' are produced that can damage the cell's fats, proteins and DNA. Unrepaired DNA damage can turn into a mutation, leading to cancer (Hertz, 1976; Madhuri, 2008; Madhuri and Pandey, 2010; Pandey et al., 2010). Many workers (Das et al., 2000; Madhuri, 2008; Madhuri and Pandey, 2010; Madhuri et al., 2011b; Nemmani et al., 2002) reported that ProImmu possesses immunomodulatory, antitumour and phagocytic activities. Nemmani et al. (2002) observed that ProImmu causes restoration of normal tissues by increasing natural killer (NK) cell activity and proliferation of splenic leucocyte against K 562 cells in mice. Immunostimulatory and antitumour effects of *E. officinalis* (ingredient of ProImmu) have been reported by Jeena et al. (2001). *E. officinalis* inhibits the growth and spread of many cancers, including uterine cancer

(Madhuri et al., 2011b). Another plant-ingredient of ProImmu, i.e., *O. sanctum* (leaf) showed the potent antioxidant, anticancer, chemopreventive and immunomodulatory activities (Pandey and Madhuri, 2010a). Similarly, the methanolic extract (200 mg/kg, ip daily for 5 days) of *T. cordifolia* (ingredient of ProImmu) stem increased the humoral immune response and reduced solid tumour growth of Balb/c mice (Mathew and Kuttan, 1999). Anticancer effect of *T. cordifolia* was also reported by Jagetia and Rao (2006), Madhuri et al. (2011a) and Singh et al. (2005). The root of *W. somnifera* (an ingredient of ProImmu) exhibited antioxidant, immunomodulatary and anticancer properties. It reduced the cancer cell proliferation and increased overall survival time (Madhuri and Pandey, 2009). The immunomodulatory effects of *W. somnifera* and *T. cordifolia* were observed by Thatte and Dahanukar (1989). *O. sanctum* leaves and *W. somnifera* roots were effective against various cancers (Somkuwar, 2003). The immunomodulatory activity of the combined extracts of *O. sanctum*, *W. somnifera* and *E. officinalis* was seen by Arondekar (1999).

Medicinal plants contain certain phytochemical antioxidants such as vitamins, carotenoids, terpenoids, polyphenols, flavonoids, enzymes, minerals, polysaccharides, alkaloids, glycosides, saponins, lignins, pigments and xanthenes. They prevent from cancer and other diseases by protecting cells from damage caused by 'free oxygen radicals' (Madhuri and Pandey, 2010; Pandey and Madhuri, 2010b). Many of these antioxidants are present in the plant-ingredients of ProImmu. It may be, therefore, concluded that ProImmu possesses anticancer effect (Fig. 2-4) due to immunostimulatory, antioxidant, phagocytic and its other tissue protective activities.

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Table 1: Anticancer effect of ProImmu (herbal drug) on SGOT and SGPT activities during EO induced uterine cancer in rat

Group[#]	Treatment	Week of experi-ment	SGOT activity		SGPT activity	
			Mean* ±SE (IU/L)	% decrease (-) against group 2	Mean* ±SE (IU/L)	% decrease (-) against group 2
1	Normal Saline	1st	77.4[c] ±2.0	-37.2	31.7[d] ±1.0	-60.5
2	EO @ 750 µg/kg, orally, weekly for 24 wk	25th	123.2[a] ±2.7	0	80.3[a] ±0.6	0
3	EO @ 750 µg/kg, orally, weekly for 24 wk, and ProImmu @ 500 mg/kg, orally, daily for 4 wk after 20 wk of EO administration	25th	108.5[b] ±4.6	-11.9	71.5[b] ±1.5	-11.0
4	EO @ 750 µg/kg, orally, weekly for 24 wk, and ProImmu @ 500 mg/kg, orally, daily for 8 wk after 16 wk of EO administration	25th	105.1[b] ±4.2	-14.7	53.4[c] ±1.1	-33.5
5	EO @ 750 µg/kg, orally, weekly for 24 wk, and ProImmu @ 500 mg/kg, orally, daily for 12 wk after 12 wk of EO administration	25th	82.3[c] ±0.8	-33.2	34.8[d] ±1.1	-56.7

[#] Number of rats in each group = 6.

[*] Mean with same superscript does not differ significantly (Duncan's new multiple range test at P=0.05).

Figures & Legends

Fig. 1: Uterus of rat (Group 2) on 25th wk of EO (750 µg/kg, orally, weekly for 24 wk) administration showing cancerous changes, including hyperchormasia, enlarged nuclei, anisokaryosis, anisocytosis, angiogenesis and glandular polarity; disarray leading to severe malignancy in the whole area (x100, H & E).

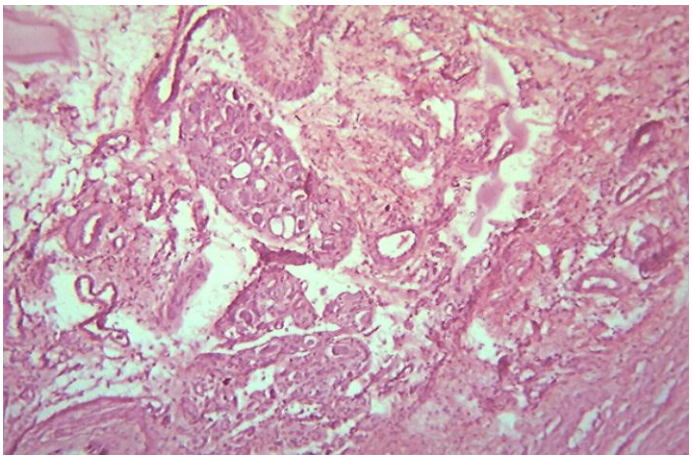

Fig. 2: Uterus of rat (Group 3) on 25th wk of EO (750 µg/kg, orally weekly for 24 wk) and ProImmu (500 mg/kg, orally daily for 4 wk after 20 wk of EO) administration showing mild histopathological changes including vacuolar degeneration, necrosis and fibrosis*;* regeneration of uterine tissues in some areas is also seen (x100, H & E).

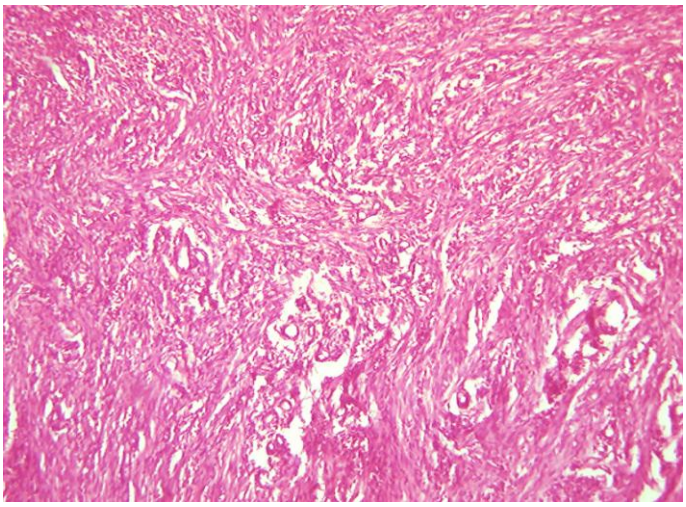

Fig. 3: Uterus of rat (Group 4) on 25th wk of EO (750 µg/kg, orally weekly for 24 wk) and ProImmu (500 mg/kg, orally daily for 8 wk after 16 wk of EO) administration showing lesser degree of pathological changes including degeneration and necrosis with reappearance of several uterine tissues (x100, H & E).

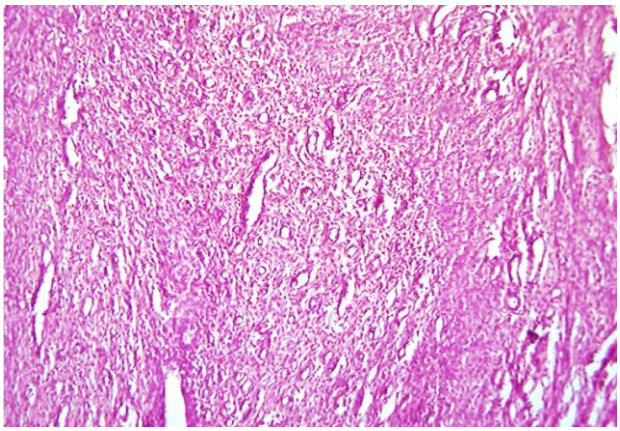

Fig. 4: Uterus of rat (Group 5) on 25th wk of EO (750 µg/kg, orally weekly for 24 wk) and ProImmu (500 mg/kg, orally, daily for 12 wk after 12 wk of EO) administration showing regeneration and reappearance of virtually normal profile (x100, H & E).