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SOME ALTERATIONS CONCOMITANT WITH PROLONGED ADMINISTRATION OF ESTROGEN

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SUMMARY

The study was done for deeper understanding of adverse effects concomitant with repeated administration of Estrogens. It was found that prolonged treatment, particulary with high doses, leads to deleterious effects represented by immunosuppression, alteration of cellular structure of different organs as well as deviation of hepatorenal performance. These findings motivate us to recommend the cautious use of estrogenic agents to avoid their drastic effect.

INTRODUCTION

Recent advances in steroid chemistry have yielded a variety of estrogenic agents which have been widely involved among livestock for induction of abortion, treatment of postpartum metritis, initiation of lactation, synchronization of estrus and resumption of cyclicity following postpartum (Hemeida et al., 1986 and Hafez, 1987). Moreover, estrogens were recommended to improve growth rate and feed conversion ratio in finishing animals (Stollard et al., 1977 and Sawyer et al., 1987). Recently, a number of studies has incriminated estrogenic compounds to alter immune status (Magnusson and Einarson, 1990 and Farris and Benjamin, 1993), induce histopathological changes (Thomas, 1988 and Cotran et al., 1994) as well as suppress hepatorenal sufficiency (Fakhry et al., 1988).

Because the issue is of clinical implications to merit special attention, we thought it important to precisely characterize some of the adverse effects concomitant with repeated administration of estradiol benzoate "E. B." particularly those related to immune response, cellular structure and hepatorenal performance.

MATERIAL AND METHODS

The present study included 120 mature female Albino rats of an average body weight 180 g. To avoid the influence of endogenous ovarian steroids, all animals were ovariectomized

(Wayneforth, 1980). Two weeks later, animals were classified into 3 comparable groups; the first was injected intramuscularly "i.m" with 0.5 ml corn oil. Each rat of the second and third groups was injected i. m. with 1.0 and 2.0 mg E. B. (Folone 5, Misr Co.) contained in 0.5 ml oil, respectively. Administration was repeated on alternate days for 3 succesive months. To determine cellular immunity (expressed as percentage of suppression), 10 rats of each group were selected and the method of Prescott et al. (1982) was adopted which depends upon intradermal challenge against 250 U/0.1 ml of tuberculin after the animal has been sensitized by 50 ul BCG (Vet. Ser. and Vac. Res. Inst., Egypt). For humoral immunity (expressed as the potency of rat antiserum to agglutinate sheep RBCs), 10 rats of each group were subjected to sheep RBCs inoculation (Prescott et al., 1982). From the remaining rats, individual blood samples were collected to determine total and differential leucocytic counts (Jain, 1993) as well as the sera were used to evaluate levels of urea (Patton and Crouch, 1977), creatinine (Husdan and Rapoport, 1968), alanine aminotransferase "ALT" and asparatate aminotransferase "AST" (Reitman and Frankel, 1957), cholesterol (Waston, 1960) and triglycerides (Fossati and Prencipe, 1982). Postmortem inspection was carried out and samples from liver, kidneys, lungs, heart, spleen, regional lymph nodes "L.N." and uterus were placed in 10% neutral formalin, dehydrated, cleared and embedded in in paraffin wax. Sections of 4 u thickness were stained with hematoxylin and eosin as well as mucicarmin (Clayden, 1971). Results were subjected to statistical analysis (Snedecor and Cochran, 1980).

RESULTS

It appears from Table 1 that either low or high doses of E. B. led to a significant depression in both cellular and humoral immunity of ovariectomized rats as compared with the control group. Also, administration of low dose resulted in a significant esinopenia accompanied with increased levels of creatinine, ALT and AST. On the other side, high doses of E. B. led to a significant decrease in total WBCs count, esinophils and small lymphocyte percents concomitant with relative increase in both neutrophil and monocyte percents as well as elevated levels of urea, creatinine, ALT, AST, cholesterol and triglycerides as compared with their corresponding control values.

Postmortem examination of rats received low dose of E. B. revealed congestion and hemorrhagic patches on the examined organs. In addition, the liver showed greyish-white lentil-sized raised areas with few focal areas of yellowish discoloration. The heart was flabby with focal areas of greyish white discoloration. The apical lobe of the lung showed areas of emphysema while the cardiac lobe was consolidated with greyish-white pin-headed foci. This picture was exaggerated in rats that received high dose of E. B. Besides, the uterus exhibited multiple greyish-white foci.

Histopathological examination of rats that received low dose clarified congestion and hemorrhage of all examined organs. Moreover, the liver showed areas of vacuolar and/or granular degeneration, few areas of necrosis with severe

mononuclear cell aggregation as well as thrombosis of the portal blood vessels and newly formed bile ducts (Fig. 1). The renal cortex revealed granular degeneration and necrosis of tubular epithelium associated with hyaline casts and few interstitial mononuclear cell aggregation besides, thrombosis of some renal blood vessels. The lung showed intravascular hemolysis, hemosidrosis as well as degeneration of the endothelium lining some pulmonary capillaries accompained with thrombosis. Some bronchi, bronchioles and alveoli were filled with neutrophil and mononuclear cells. Focal areas of emphysema and thickening of the alveolar wall due to alveolar cell proliferation were occasionally demonstrated. The heart showed multiple focal myocardial degeneration with mononuclear cell infiltration (subacute focal non-suppurative myocarditis). The spleen and regional L. N. revealed inconsistent depletion of the lymphoid elements. The uterus showed vacuolar degeneration of some endometrial glands accompanied with necrosis and infiltration of inflammatory cells particularly polymorphs. High dose of E. B. led to similar changes but the picture was more drastic. In addition, the liver showed severe vacuolar degeneration and necrosis (Fig. 2) accompanied with mononuclear cell infiltration, newly formed bile ducts and thrombosis of portal blood vessels. The lungs showed bronchiectasis in which the bronchi and bronchioles were dilated, still lined by epithelium, the lumen contained muco-pus (Fig. 3) which was mucicarmin-posiitive (Fig. 4) and the underlying lamina propria was infiltrated by inflammatory cells particularly polymorphs, plasma cells and macrophages. Moreover, 2 cases showed aggregation of large number of foam cells as well as mononuclear and binuclear cells inside the alveoli which were filled by mucous exudate (Fig. 5 & 6). The spleen and regional L. N. showed severe depletion of the lymphoid elements (Fig. 7). The uterus revealed hyperplasia of lining epithelium of some endometrial glands which formed solid masses in the advanced stages (Fig. 8). Moreover, the endometrium showed vacuolation of glandular epithelium with diffuse areas of necrosis and massive infiltration of inflammatory cells.

DISCUSSION

Despite of the widespread usage of estrogenic agents, recent studies have questioned the validity of their application due to the accompanied adverse effects. Clinical significance of the subject motivated us to carry out this study.

Table 1 clarifies that both low and high doses of E. B. led to inhibit cellular and humoral immunity (T & B- cell responses); the degree of suppression is dose - dependent. These results confirm previous reports of Waltman et al. (1971); Bilder (1976) and Sherblom et al. (1985). This estrogenic effect could be referred to intervention with oxygen uptake of lymphocytes (Hulka et al., 1965); reduction of DNA synthesis (Sljvic and Warr, 1973); alterations of lymphocyte membrane leading to inability for mitosis (Morgan et al., 1976 and Wyle and Kent, 1977) or the production of α 2 macroglobulin by

the liver and leucocytes which has immunosuppressive properties (Horne et al., 1978). In this respect, the current study adds the depletion of lymphoid elements of the spleen and L. N. This assumption receives a support from the histopathological examination particularly in animals receiving the high dose. Furthermore, it seems that estrogens enhance the redistribution of circulating lymphocytes to aggregate in the parenchymatous organs; an effect which is dose-dependent. These findings beside the report of Farris and Benjamin (1993) who recorded myelopoiesis-inhibiotory factor resulting in bone marrow hypoplasia following estrogen therapy elucidate the resultant fall in both absolute and relative lymphocytic counts specially following high dose administration. This drop is similar to that reported by Sobhen and Jirasattam (1974) in ovariectomized rats treated with E. B. "7.0 mg/kg/day for 21 days" while lymphocytopenia has been recorded by Franks et al. (1975) in mice treated with estradiol "1.0 mg/day for 5 days". Table 1 also exhibits that high dose of E. B. led to a significant relative increase in monocyte % ; a finding which matches that of Diesselhoff-den Dulk et al. (1979). On the other hand, the resultant relative esinopenia with low and high doses could be attributed either to a direct effect of E. B. or indirectly through stimulating corticosteroid release (Jain, 1993)

Histopathological examination of the kidney revealed degenerative changes of renal tubules and glomeruli with interstitial mononuclear cell aggregation. The severity of changes is dose-dependent. These changes reflected upon serum levels of urea and creatinine which indicate kidney function insufficiency. This estrogen effect was reported by Fakhry et al. (1988). Also cases with renal insufficiency were associated with depressed cellular and humoral response (Raska et al., 1980). Additionally, Table 1 shows elevated serum ALT and AST activities following E. B. administration which reflects deviation of hepatic functions. This effect of E. B. was reported by Fakhry et al. (1988) in ovariectomized rats. Histopathology revealed areas of vacuolar and/or granular degeneration with hepatic necrosis and mononuclear cell infiltration in addition to newly-formed bile ducts (Fig. 1). It seems that repeated administration of E. B. results in excessive production of highly toxic metabolites specially cholesterol which was reported to participate in the pathogenesis of liver cell damage (Ramaswamy and Smith, 1976). This suggestion is evidenced by the hypercholestrolemia concomitant with high dose injection (Table 1).Moreover, E. B. led to thrombosis of the portal, renal and pulmonary blood vessels; a lesion which may be due to direct effect of estrogens upon platelet function (Jones et al., 1977).

The current study showed that E. B. led to subacute focal non-suppurative myocarditis. It was previously reported that damage of the liver, kidneys and myocardium as well as oral contraceptives led to increased levels of triglycerides (Fischbach, 1992). Also, the same author recorded that all conditions that cause hypercholesterolemia lead to an increase in triglycerides. These findings may clarify the increased level of triglycerides following high dose of E. B. (Table 1).

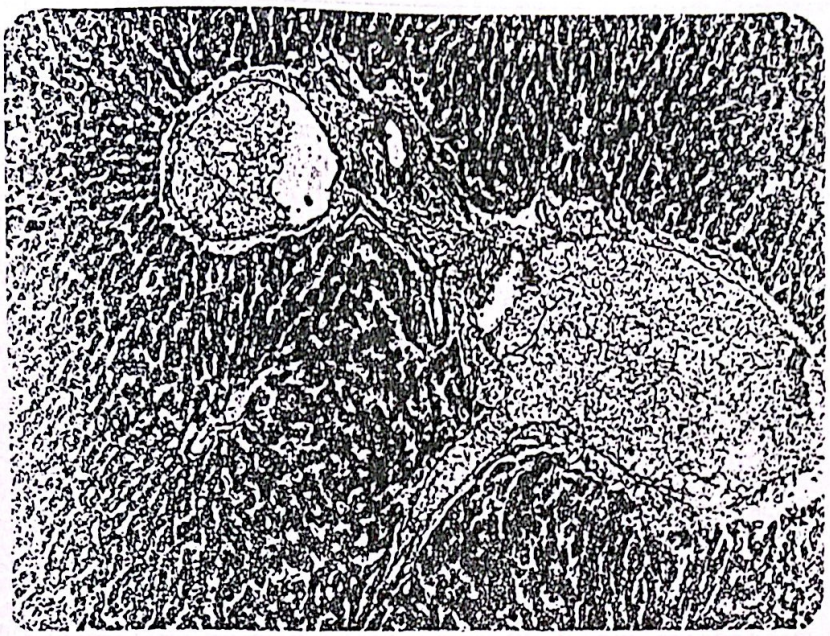

Fig. 1: Liver of rat receiving low dose level of estradiol benzoate "E. B." reveals large portal thrombosis associated with newly formed bile ducts. (H & E X 100).

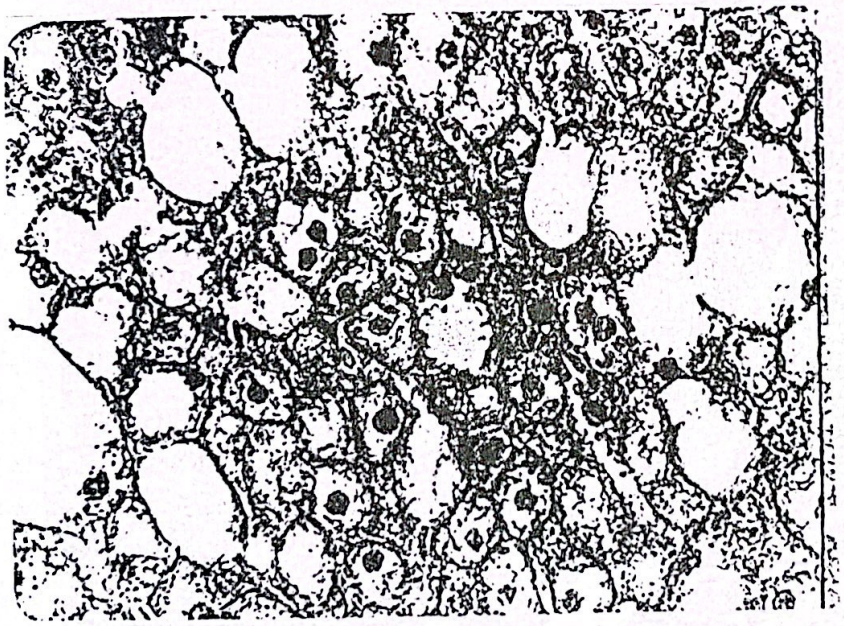

Fig. 2: Liver from rats receiving high dose level of "E. B." shows severe vacuolar degeneration and individual cell necrosis of hepatocytes. (H & E X 500).

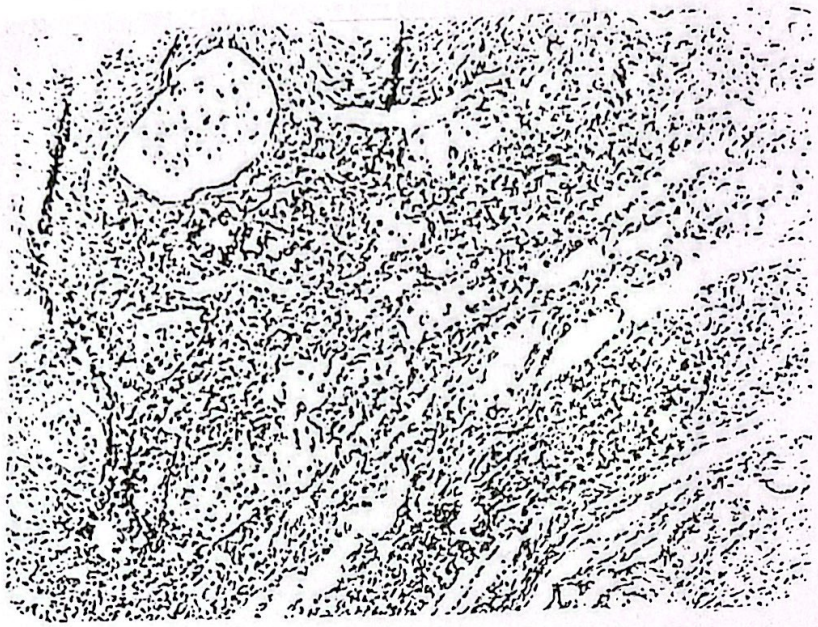

Fig. 3: Lung of rat receiving high dose of "E. B." exhibits bronchiectasis with aggregation of mono clear cells and polymorphs with mucinous exudate inside the bronchi. (H & E X 250).

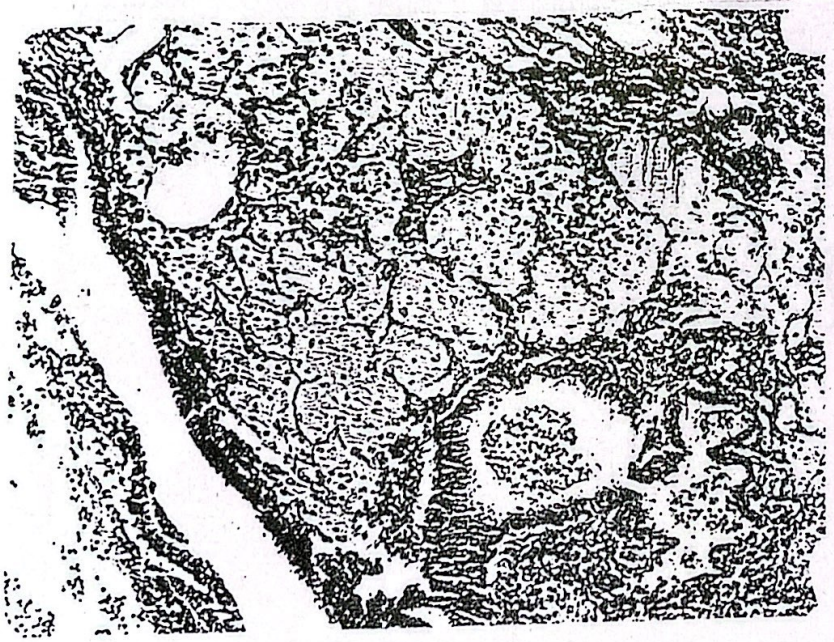

Fig. 4: Lung from rats receiving high dose level of "E. B." showing bronchopneumonia; the bronchi and alveoli are filled with mononuclear and foam cells embedded in mucinous material. (H & E X 250).

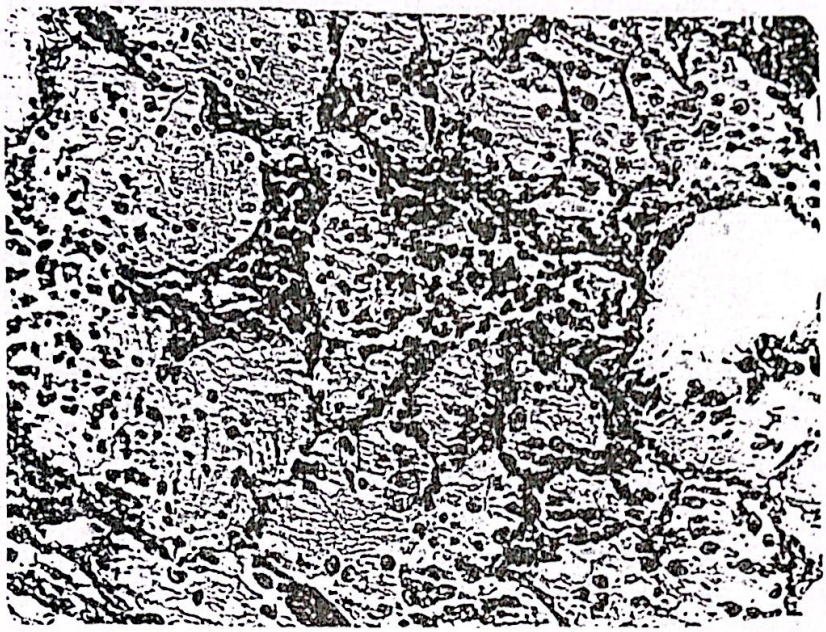

Fig. 5: High power view of Fig. 4. (H & E X 500).

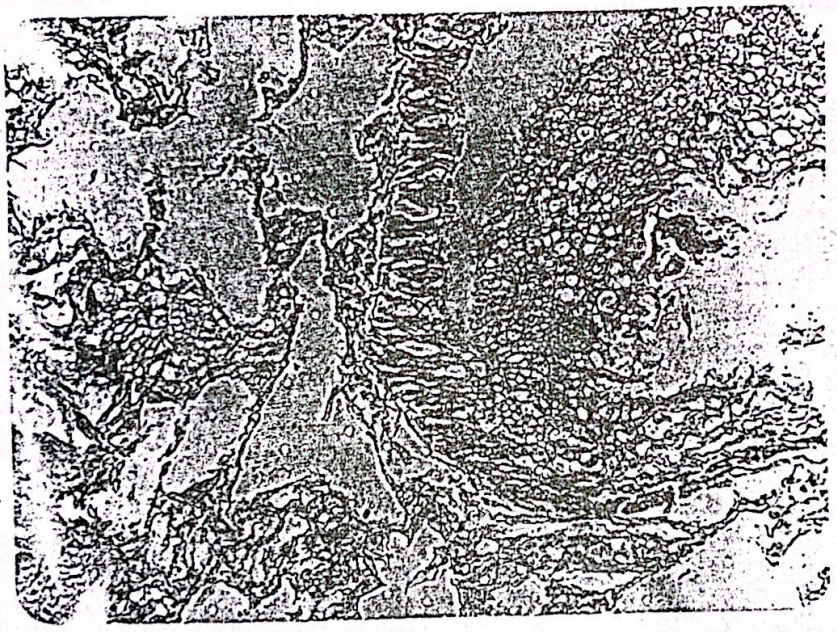

Fig. 6: Lung of the high-dosed group shows the bronchi and alveoli filled with excessive mucinous material (Mucicarmine stain X 500).

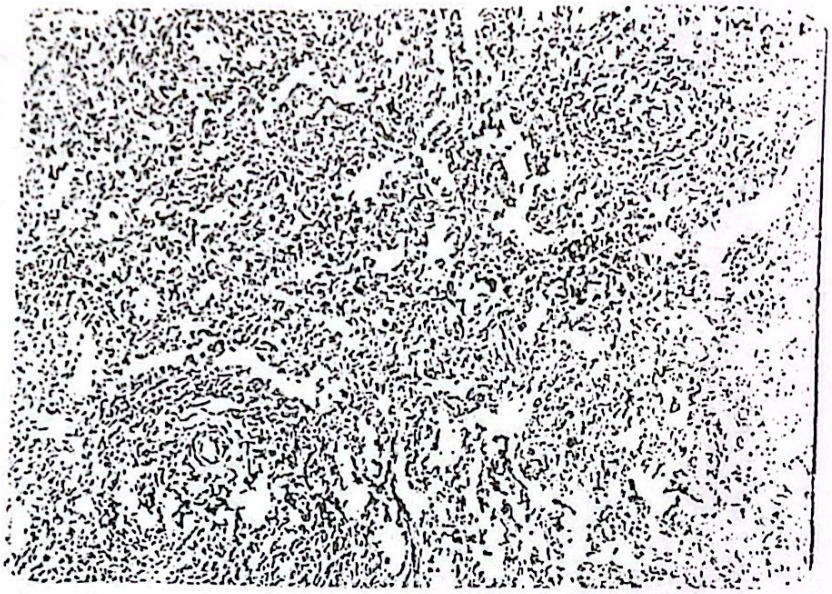

Fig. 7: Spleen from rats receiving high dose of E. B. showing severe depletion of the lymphoid e ments specially in the centre of the lymphoid follicle with an increase of the reticular fibres . & E X 250).

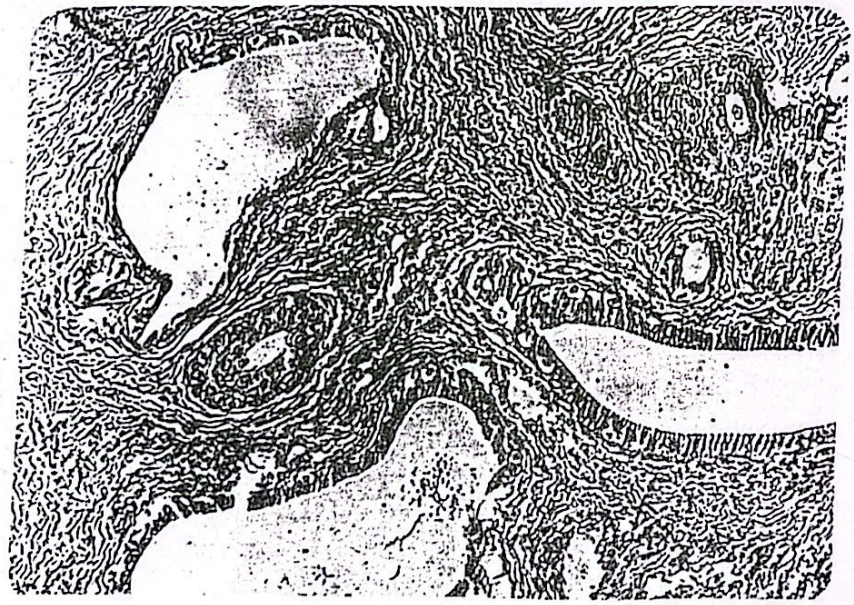

Fig. 8: Uterus of rat from the high-dosed group showing stratification of the lining epithelium of some endometrial glands. (H & E X 250).

Table 1: Evaluation of immune status and blood parameters of ovariectomized rats administered low and high doses of E. B. (Mean ± S. E).

Parameter	*Control*	*Low dose of E.B*	*High dose of E.B*
Percentage of suppresion	0.00^{AB}	28.18 ± 4.31^{aA}	53.81 ± 6.12^{aB}
Anti-sheep RBCs. titre	960.00 ± 64.15^{A}	307.20 ± 28.19^{A}	134.40 ± 12.37^{A}
Total WBCs./ul.	10.70 ± 0.61^{A}	9.48 ± 0.47	8.12 ± 0.35^{A}
Neutrophil %	35.93 ± 3.08^{a}	40.47 ± 4.15	48.86 ± 4.14^{a}
Small lymphocyte %	48.96 ± 2.35^{a}	45.83 ± 5.79	38.56 ± 3.07^{a}
Large lymphocyte %	8.74 ± 1.65	10.41 ± 2.11	7.91 ± 1.35
Monocyte %	1.87 ± 0.24^{A}	2.40 ± 0.32	3.69 ± 0.51^{A}
Eosinopil %	4.43 ± 0.33^{AB}	1.81 ± 0.11^{aA}	1.18 ± 0.24^{aB}
Urea (mg%)	15.51 ± 1.02^{A}	28.53 ± 2.62^{a}	$38.65+3.03^{aA}$
Creatinine (mg%)	7.58 ± 0.66^{A}	13.81 ± 1.33^{A}	20.14 ± 1.05^{A}
ALT (U/ml.)	22.45 ± 2.04^{A}	34.77 ± 2.22^{A}	53.11 ± 4.10^{A}
AST (U/ml.)	48.05 ± 3.48^{A}	134.91 ± 4.12^{A}	191.33 ± 11.99^{A}
Cholesterol (mg%)	122.14 ± 1016^{a}	129.81 ± 9.83	159.92 ± 13.02^{a}
Triglyceride (mg%)	170.69 ± 14.33^{A}	198.01 ± 19.40	239.69 ± 10.14^{A}

In the same row values with identical small letter (a) differ significantly at $P<0.05$ while, those with capital letters (A, B) at $P< 0.001$.

Literature concerning the effects of estrogens upon the lungs are scarce. Histopathology of all treated animals revealed a picture in resemblance to pneumonia, the severity of which was dose-dependent. This effect may be referred to the diminished cellular and humoral responses detected after E. B. administration. It was previously found that estrogens increased the susceptibility to intracellular infection (Rifkind et al., 1973). Moreover, estrogens were found to have a strong hemolytic properties (Tateno and Kilbourne, 1954) as well as stimulatory effect upon the phagocytic activity of the macrophage system (Loose and Di Luzio, 1976) which explain the presence of intravascular hemolysed RBCs and abundance of intra-alveolar foam cells, respectively. The current study also shows drastic effect of estrogen administration on the uterus particularly with high doses which led to hyperplasia of the lining glandular epithelium forming solid masses. This finding indicates the first sign of endometrial cancer and coincides with previous studies (Ashkenazy et al., 1983 and Thomas, 1988).

Thus it could be concluded that the usage of estrogens, specially when high doses for prolonged periods are administered, must be done cautiously to avoid their drastic effects.

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