Effect of Estrogen Administration on The Prostate of the Adult Albino Rat  
(Histological, Ultrastructural and Immunohistological Studies)

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Abstract: Background: The prostate is considered to be an androgen dependent organ. On the other hand, the presence of estrogen receptors among different tissue components of the prostate indicates the direct effect of estrogen on the prostate, beside the known indirect effect through the hypothalamic-gonadal axis. Estrogen has been incriminated in the pathogenesis of certain prostatic diseases, namely, cancer prostate, benign prostatic hyperplasia and prostatitis. Methods: 57 adult male albino rats were used in this study. The rats were divided into two groups. Control groups (normal and sham) and experimental groups which were exposed to different estrogen treatments and were further subdivided into 3 subgroups; group LA (repeated low doses group), group TA (Therapeutic dose group) and group HA (single high dose group). The animals were sacrificed and the prostatic specimen were collected and prepared for light, electron microscopically and immunohistological studies. Results: The histological examination revealed the presence of epithelial and stromal hyperplasia in all the experimental groups in a dose dependant manner, the signs of epithelial hyperplasia included papillary projections, vesicular nuclei, marginated nucleoli and double nucleoli. Other common changes included inflammatory infiltration, apoptosis and acinar dilatation. Only the (TA) and (HA) groups showed signs of dysplasia in the form of pleomorphism and hyperchromatism. The ultrastructure examination of different experimental groups revealed variable degrees of degenerative changes. The changes included irregular cell membranes, poor cytoplasmic organelles, proliferated rough endoplasmic reticulum and the appearance of cytoplasmic vacuolations and fatty like inclusions. Nuclear changes included irregular nuclear membranes and pyknotic nuclei. Again, the profound degenerative changes were marked in both the (TA) and (HA) groups. Immunohistological examination revealed decreased immunostaining reactivity in all the experimental groups compared to the control groups. A further decrease was noted in both the (TA) and (HA) groups accompanying the severe dysplasia of these groups. Conclusion: It was concluded that estrogen induces prostatic hyperplasia and prostatic inflammation. Only therapeutic or high doses of estrogen produce dysplastic changes. Within certain limits, estrogen acts in a dose dependent manner. Recommendation: It could be recommended that estrogen treatment for cancer prostate should be restricted to specific cases. Maximum attention to serious side effects has to be ensured during estrogen treatments. Further investigations are needed to prove or exclude the role of estrogen in different prostatic diseases.

Key words: Estrogen, Prostate, Rats.

INTRODUCTION

The normal anatomical and functional growth of the prostate is mainly controlled by androgens Niu et al., (2003). However, estrogen plays an important role in prostate physiology Singh and Handelsman, (1999) indicated by the stimulatory effect of low dose estrogen on the prostate Putz et al., (2001) and the presence of estrogen receptors (ERs) in prostatic tissues Weihua et al., (2002).

A role of estrogen in the development of benign prostatic hyperplasia (BPH) has been suggested; administration of estrogen has been shown to stimulate BPH in dogs Bruenger et al., (1983). BPH in man is accompanied by decreased testosterone and elevated estrogen levels Prins et al., (1996).

The relationship between estrogen and cancer prostate is controversial. Kastendieck and Altenahr, (1975) suggested that epithelial metaplasia of the prostate may be caused by estrogen-androgen imbalance; Risbridger et al. (2001) found that estrogen causes a metaplastic response in prostatic tissue and Ricke et al. (2006)
concluded that estrogen accompanied by testosterone promotes prostatic cancer. On the other hand, a number of therapeutic strategies including estrogens are actively being investigated in the treatment of prostate cancer, estrogens of various types have exhibited antitumor activities both in vitro and in vivo Smith et al., (1998). It has been postulated that different responses of prostatic tissue to estrogen may be mediated by different hormone receptors Mobley et al., (2004).

The aim of the present work was to study the different effects of estrogen on prostatic tissue the adult albino rat using histological, ultrastructural and immunohistological techniques, trying to clarify the role of estrogen in the development of certain prostatic diseases as well as to explore the estrogen effect that might be used as a guide in the treatment of cancer prostate.

MATERIAL AND METHODS

The present study was carried out on 57 adult male rats weighing 236- 370 gm obtained from animal house, Faculty of Medicine, Cairo University. The rats were maintained in a controlled environment with a free access to food and water. The animals were divided into the following groups

Control Groups (Normal and Sham):
 each group included 6 rats. The sham group received oily injections at the same regimen of the experimental groups. The rats of the control groups were sacrificed according to the same schedule of the experimental group.

Experimental Groups:
 were further subdivided into 3 subgroups:

Group LA (Repeated Low Doses Group):
 included 15 rats, each rat received a weekly therapeutic dose (140 µg/kg body weight) of estradiol benzoate as subcutaneous injections for four successive weeks and were sacrificed two days after the last injection Kohler-Samouilidis et al., (1998).

Group TA (Therapeutic Dose Group):
 included 15 rats, each rat received a daily therapeutic dose (1 mg/kg body weight) of estradiol benzoate as subcutaneous injections for 21 days and were sacrificed two days after the last injection Chrouses et al., (2001).

Group HA (Single High Dose Group):
 included 15 rats, each rat received a single high dose (25 mg/kg body weight) of estradiol benzoate as subcutaneous injection and were sacrificed two days after the injection Garcia-Florez et al., (2005).

In all the experimental subgroups, the time of sacrifice was based on the finding that the peak plasma level of estrogen was reached two days after estradiol benzoate injection Oriowo et al., (1980).

The drug used was estradiol benzoate (folone®) in the form of 5 mg/ml oily solution, produced by Misr Co. For Pharm Ind SAE. If needed, the drug was diluted in corn oil and injected subcutaneously.

Rats were sacrificed by cervical dislocation, dissected and prostate specimens were collected.

Light Microscopic Study:
 Part of the prostatic specimens was fixed in buffered formol saline, processed for paraffin sections of 5µm thickness and sections were stained with haematoxylin and eosin (Hx. and E.) and Masson's trichrome stains Bancroft and Gamble, (2002) for histological study.

Ultrastructural Study:
 The other part of the prostatic specimens was cut into small pieces, fixed in 4% glutaraldehyde then washed in phosphate buffer and post fixed in 1% osmium tetraoxide. Fixation was followed by dehydration and embedding in epoxy resins. Semithin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate according to Dawes (1980) then examined using Philips CM100 transmission electron microscope. The examination was done in Anatomy department, Faculty of Medicine, Ain Shams University.
Immunohistological Study:

For detection of estrogen receptors, estrogen receptor Ab-14 (Clone 1D5+6F11) Mouse Monoclonal Antibody Neo Markers, Fermont, USA was used for immunohistology using formalin/paraffin specimens. Two tissue control slides was used, a positive control specimen from breast known to be positive to estrogen receptors was processed exactly as other samples, the specimen was provided by the National Cancer Institute, Cairo University, another negative control specimen was prepared from one of the control prostatic tissue specimens but instead of using a primary antibody, a non immune solution was used.

Scoring of ER staining intensity was done, it was classified as negative (no staining), weak (+), moderate (++), or strong (+++). Specimens were reported negative when the staining did not differ from the negative control sections Makela et al., (2000).

RESULTS AND DISCUSSION

A- Histological results:

Control groups:

The histological examination of the different control groups revealed nearly the same findings. Examination of the control group specimens revealed variable sized acini, abundant secretions, and minimal stroma. The acini were lined by normal columnar or pseudostratified columnar epithelium (figs. 1-4).

![Fig. 1](image.jpg)

Fig. 1: A photomicrograph of a section in the prostate of a normal control rat showing normal appearance of acini with variable sizes, abundant secretions (Se) and minimal stroma (S). (Hx. & E.; × 40).

![Fig. 2](image.jpg)

Fig. 2: A photomicrograph of a section in the prostate of a normal control rat showing normal appearance of acini with variable sizes (è). Hx. & E.; × 100).

![Fig. 3](image.jpg)

Fig. 3: A photomicrograph of a section in the prostate of a normal control rat showing normal pseudostratified columnar epithelial lining (E). (Hx. & E.; × 1000).
Fig. 4: A photomicrograph of a section in the prostate of a normal control rat showing minimal stroma (S) and abundant secretions (Se). (Masson's trichrome; × 100).

Experimental groups:

Low dose effect group (LA):

Examination of the (LA) group revealed acini full of inflammatory cellular infiltrate (fig. 5). There was a moderate acinar dilatation and the acini were filled with secretion with low epithelial lining (fig. 6). The signs of the epithelial hyperplasia were observed in this group by the appearance of vesicular nuclei, double and marginated nucleoli (fig. 7). Also stromal hyperplasia was observed in this group (fig. 8).

Fig. 5: A photomicrograph of cross section in the prostate of (LA) group showing acini full of inflammatory cellular infiltrate (arrows). (Hx. & E.; × 40).

Fig. 6: A photomicrograph of a section in the prostate of (LA) group showing moderate acinar dilatation (→), the acini are filled with secretion (Se) with low epithelial lining (E). (Hx. & E.; ×100).

Therapeutic dose effect group (TA):

Examination of the (TA) group revealed the presence of intraluminal inflammatory infiltration (fig. 9), moderate stromal hyperplasia and acinar hyperplasia in the form of papillary projections with abundant secretions (figs. 10, 11, 13). The examination also revealed the appearance of signs of dysplasia, in the form of deeply stained nuclei (hyperchromatism) and different types and shapes of cells (pleomorphism) (figs. 12, 14). Also some desquamated cells were noted in the acinar lumen (fig. 14).
Fig. 7: A photomicrograph of a section in the prostate of (LA) group showing signs of hyperplasia (vesicular nuclei (VN), double nucleoli (DNu) and nuclei with marginated nucleolus (MNu). (Hx. & E.; ×1000).

Fig. 8: A photomicrograph of a section in the prostate of (LA) group showing mild stromal hyperplasia (S). (Masson's trichrome; ×100).

Fig. 9: A photomicrograph of a section in the prostate of (TA) group showing inflammatory infiltrates (arrows). (Hx. & E.; ×100).

Fig. 10: A photomicrograph of a section in the prostate of (TA) group showing moderate stromal hyperplasia (S) and acinar papillary projections (arrows). (Hx. & E.; ×100).
Fig. 11: A photomicrograph of a section in the prostate of (TA) group showing acinar hyperplasia in the form of papillary projections (arrows) with abundant secretions (Se). (Hx. & E.; × 200).

Fig. 12: A photomicrograph of a section in the prostate of (TA) group showing signs of dysplasia; deeply stained nuclei (hyperchromatism) (arrow) and different types and shapes of cells (pleomorphism) (circle). (Hx. & E.; ×1000).

Fig. 13: A photomicrograph of a section in the prostate of (TA) group showing marked stromal hyperplasia (S). (Masson's trichrome; ×100).

Fig. 14: A photomicrograph of a section in the prostate of (TA) group showing dysplastic indented nuclei (arrows); some desquamated cells were noted in the acinar lumen (circle). (Toluidine blue; ×1000).
High dose effect group (HA):
   Examination of the (HA) group revealed the presence of acinar dilatation and epithelial hyperplasia in the form of papillary projections (fig. 15). Also marked epithelial dysplasia (fig. 16) and stromal hyperplasia (fig. 17) were observed.

B- Ultrastructural results:
Control groups:
   Examination of the control groups revealed a normal appearance of the cells; the nuclei appeared normal with apparent nucleoli and regular nuclear membranes. There were many cytoplasmic secretory granules (fig. 18).

Experimental groups:
Low dose effect group (LA):
   The examination of the (LA) group revealed a patchy effect, some fields showed normal cells with normal regular cellular membranes, well developed cytoplasmic organelles, secretory granules, regular nuclear membranes and apparent nucleoli. Other fields showed cellular changes in the form of appearance of cytoplasmic vacuolation, proliferated dilated rough endoplasmic reticulum (RER), nuclear membranes irregularity and heterolysosomes (figs. 19, 20).

Therapeutic Dose Effect Group (TA):
   The examination of the (TA) group revealed the presence of degenerative changes in both the cytoplasm and the nuclei. The cytoplasmic degenerative changes included pale cytoplasm, poor cytoplasmic organelles and cytoplasmic vacuolations. Another noticeable finding was a thickening of basal lamina. The nuclear degeneration was in the form of severe irregularity in the nuclear membranes, pyknotic nuclei and increased nuclear density (figs. 21, 22).

High Dose Effect Group (HA):
   The examination of the (HA) group revealed the presence of massive degeneration in the prostatic cells. The degeneration was diagnosed by the presence of vacuolated cytoplasm, necrotic debris, decreased cytoplasmic organelles. The nuclei showed irregular nuclear membranes with dense heterochromatism (figs. 23, 24).
   Some areas showed secretory granules, exocytosis (fig. 23) indicating that there were some areas of the prostate where the secretory function was still active.

C-Immunohistological results:
   Immunohistological examination of estrogen receptor beta (ERβ) in different groups revealed that the control groups showed strongly positive expression (++++) of ERβ in the epithelium of the acini (fig. 25), while the negative control specimen shows no reaction (fig. 26). As regards the examination of the experimental groups, the appearance of ERβ was moderate (+++) in the (LA) group (fig. 27) while the (TA) and (HA) groups showed mild levels of ERβ (+) (fig. 28).

Fig. 15: A photomicrograph of a section in the prostate of (HA) group showing moderate acinar dilatation (---) and papillary projections (arrows). (Hx. & E.; ×100).
Fig. 16: A photomicrograph of a section in the prostate of (HA) group showing signs of dysplasia, hyperchromatism (arrows) and pleomorphism (circle). (Hx. & E.; ×400).

Fig. 17: A photomicrograph of a section in the prostate of (HA) showing marked stromal hyperplasia (S). (Masson's trichrome; ×100).

Fig. 18: A photomicrograph of a section in the prostate of a normal control rat showing normal part of prostatic acini, the cells shows normal nucleus (N) with regular nuclear membrane (NM), apparent nucleolus (Nu) and secretory granules (SG). (Uranyl acetate × 3000).
Fig. 19: A photomicrograph of a section in the prostate of (LA) group showing patchy effect, cells with proliferated RER, other cells with dilated RER (arrow), and nuclear membrane irregularity (NM). (Uranyl acetate × 3900).

Fig. 20: A photomicrograph of a section in the prostate of (LA) group showing irregular nuclear membrane (NM) and cytoplasmic vacuolation (V), the neighboring cells shows heterolysosomes (arrows). (Uranyl acetate × 11500).

Fig. 21: A photomicrograph of a section in the prostate of (TA) group showing irregular nuclear membrane (NM), pale cytoplasm (C), poor cytoplasmic organelles and thickening of the basal lamina (BL). (Uranyl acetate × 4000).
Fig. 22: A photomicrograph of a section in the prostate of (TA) group showing pyknotic nuclei (N), there is increased nuclear density, the nuclear membrane shows marked irregularity (NM) and the cytoplasm (C) shows massive vacuolations (V). (Uranyl acetate × 8000).

Fig. 23: A photomicrograph of a section in the prostate of (HA) group showing luminal part of prostatic acini with secretory granules (SG), vacuolated cytoplasm (V), necrotic debris (arrow), decreased cytoplasmic organelles, and exocytosis (Ex). (Uranyl acetate × 4000).

Fig. 24: A photomicrograph of a section in the prostate of (HA) group showing degenerated and rarified cytoplasm (C) and irregular nuclear membrane (NM) with dense heterochromatism (Ch). (Uranyl acetate × 4000).
Fig. 25: A photomicrograph of a section in the prostate of control rat showing strongly positive (+++) immunoreactivity of ERβ (marked by the dark brown stain) (arrows). (× 400).

Fig. 26: A photomicrograph of a section in a negative control slide showing negative immunoreactivity of ERβ. (× 400).

Fig. 27: A photomicrograph of a section in the prostate of (LA) group showing moderately positive (+) immunoreactivity of ERβ (arrows). (× 400).
Fig. 28: A photomicrograph of a section in the prostate of (TA) group showing mildly positive (+) immunoreactivity of ERβ (arrows). (× 400).

**Discussion:**


Simulating previous studies Yoshida, (1975) the histological findings of the control groups revealed the presence of acini variable in size, lined by columnar epithelium with minimal stroma. The ultrastructural findings revealed the presence of normal epithelial arrangement, well developed cytoplasmic organelles as well as abundant cytoplasmic secretory granules. The nuclei showed regular nuclear membrane with apparent nucleoli.

Our experience revealed the presence of intraluminal inflammatory infiltrate in the all experimental groups; this finding agreed with Naslund et al. (1988) who found increased incidence and severity of non bacterial prostatitis after treating adult rats with estrogen, even treatment of neonatal rats with estrogen induced imprinting prostatitis in adulthood. These inflammatory cells migrate to the acinar lumen through the stroma and the epithelium of the prostate (Bianco et al., 2002; Kawamura, 2002).

Apoptosis was another common feature in all the experimental groups ; this finding is shared by Taylor et al. (2005) who suggested the appearance of apoptotic cells to the down regulation effect of estrogen on the androgen receptors.

Comparison between the histological appearances of the different experimental groups showed the appearance of stromal hyperplasia as reported by other authors (Bianco et al., 2002; Holterhus et al., 1993) This was confirmed by the ultrastructural study as there was thickening of the basal lamina of the prostatic acini in the (TA) group. This thickening might be due to marked fibromuscular hypertrophy and wide distribution of collagen and elastic fibers adjacent to the epithelial basal lamina of the prostatic acini Scarano et al., (2005).

Beside the stromal hyperplasia, there was also epithelial hyperplasia in the all experimental groups in the form of vesicular nuclei, double nucleoli and nuclei with marginated nucleoli. These findings were nearly similar to those of Bianco et al. (2002).

Estrogen was incriminated in the pathogenesis of BPH. In our study, stromal hyperplasia of the prostate was found in all the experimental groups, in a dose dependant manner. The marked fibromuscular hypertrophy observed after estrogen treatment in animals simulates the stromal modifications observed in BPH Scarano et al., (2005). However, regarding estrogen as a causative agent of BPH still needs further investigations.

On the other hand, signs of dysplasia were only found in the (TA) and (HA) groups. The dysplastic signs included hyperchromatism, pleomorphism, increased mitotic figures, and damaged cytoplasmic organelles with the presence of severe nuclear changes. These findings were previously recorded by Bernoulli et al. (2008) who correlated the dysplastic changes to the associating inflammation and stated that estrogen played a significant role in the induction of both features.

It was apparent that the histological picture was more affected in the (TA) and (HA) groups, the hyperplasia was marked and the dysplasia was profound. The more obvious effect noticed in the (TA) and (HA) groups supported the dose dependant manner finding of Putz et al. (2001) who examined the effect of
seven logarithmic ranges of doses and found a dose related increased effect. The correlation of estrogen to cancer prostate is still bidirectional. The appearance of dysplasia strongly suggests the carcinogenicity. Ho et al. (1995) stated that the administration of estrogen with androgen produces a unique lesion in the prostate, characterized by simultaneous occurrence of proliferation and apoptosis disturbing the balance between both processes, this disturbance is often seen during cancer development. Li et al. (2001) referred its cause to epithelial basement membrane and stromal alteration leading to its hydration, a condition which is in favor of tumor growth and invasion. Furthermore, Scarano et al. (2004) reported the pattern of epithelial stratification characteristic of prostatic intraepithelial neoplasia after chronic treatment with estrogen. However, other investigators (Smith et al., 1998; Oh et al., 2004) had proven the anti cancer prostate effect of estrogen. Estrogen has previously been extensively used in cancer prostate treatment, mostly due to its indirect inhibition of androgen through the hypothalamo-pituitary-gonadal axis. Lam et al. (2006) stated that secondary hormonal therapy (including estrogenic compounds) serves as an excellent therapeutic option in patients with androgen insensitive cancer prostate patients even in which primary hormonal therapy has failed.

Like other authors (Scarano et al., 2004), our observation revealed the presence of cellular degeneration in the all experimental groups. The degenerative changes in the (LA) group were mild and patchy while in the (TA) and (HA) groups were marked. These degenerative changes included decreased cytoplasmic organelles, proliferated RER, decreased secretory bodies, appearance of lipid like inclusion and thick stroma. Also, there were irregular nuclear membranes and partial loss of exocytosis of the cell membranes facing the lumen indicating diminished secretory functions. These findings support the previous reports of Merk et al. (1986) who found a regression in the size of the secretory granules after estrogen treatment and the findings of Holterhus et al. (1993) who recorded complete loss of secretory granules.

As suggested by others Makela et al., (2000), the immunohistological examination in the current work proved the presence of estrogen receptor beta (ERβ) in the cells of adult prostate, the presence of ERβ in the prostatic tissues confirms the direct effect of estrogen on the prostate. The control groups showed abundant expression of ERβ in the epithelium of the acini (strongly positive +++) , a decrease in this expression was noted in all the experimental groups, the appearance of ERβ was moderate (++ ) in most groups, further decrease in ERβ level was noted in the (TA) and (HA) groups which showed mild levels of ERβ (+). These results support the previous findings of Lau et al. (1998) who found a decrease in the expression of ERβ following the use of estrogen or orchidectomy. The further decrease of ERβ in both TA and HA groups may be due to the fact that ERβ decreases accompanying the appearance of severe dysplasia or carcinogenesis Leav et al., (2001) due to loss of growth control Yuen et al., (2005).

Immunohistological examination for ERβ was chosen in the present study due to its importance, as different ER predominance was another suggested factor which could play a role in detecting whether estrogen has a carcinogenic effect on prostate or not. Horvath et al. (2001) demonstrated a reduction in ERβ expression in association with the process of carcinogenesis, suggesting that ERβ might be important for the maintenance of normal prostate epithelium and probably guarding against cancer. However, it is not yet proved if reduction in ERβ expression is causally related to the development of neoplasia or just an associated finding Harkonen and Makela, (2004).

It could be concluded that estrogen induces prostatic hyperplasia and prostatic inflammation. Only therapeutic or high doses of estrogen produce dysplastic changes. Within certain limits, estrogen acts in a dose dependent manner. So, it could be recommended that estrogen treatment for cancer prostate should be restricted to specific cases. Maximum attention to serious side effects has to be ensured during estrogen treatments. Further investigations are needed to prove or exclude the role of estrogen in different prostatic diseases.

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