

Influence of ovariectomy and flutamide treatment on vaginal tissue and body weight

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ABSTRACT

Objective: To determine the effect of flutamide on vaginal histology, and to ascertain whether flutamide regulates body weight gain in the ovariectomized rat model.

Methods: We chose 36 sexually mature female Wistar-Albino rats in this study. The study took place in the Department of Medical Science Application and Research Center of Dicle University, Diyarbakir, Turkey, in 2002. We divided the model rats into 3 groups: group 1 - control group, group 2 - sham-operated group and group 3 - bilaterally ovariectomized group. In addition, we gave flutamide to group 3.

Results: We found that the body weight was higher in the ovariectomized rats than the control rats. Furthermore, the body weight decreased a little after

flutamide therapy. The thickness in the vaginal epithelium of the control group decreased, moreover, the appearance of stromal tissue was rather loose. After flutamide applications, infiltration of the stromal cells increased, the vaginal epithelial layer thickened and became keratinized. Microscopic papillae and anatomical processes appeared in the flutamide treated group.

Conclusion: We observed that the antiandrogen drug flutamide is hormonally active and may exhibit estrogenic-antiestrogenic activity in rats. Our study may be a source for further research examining the relationship between the androgen receptor antagonist flutamide and reproductive function in female rats.

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Flutamide, an androgen receptor antagonist, and finasteride, a 5 α reductase inhibitor, are antiandrogens used in human clinical practice. Flutamide, used commonly for the treatment of prostate cancer, is also widely used in research on endocrine disruptors.¹ Suppressing effects of flutamide on sexual maturation and behavior have been reported in rats when administered during the perinatal period or to offspring immediately after birth.²⁻⁸ Chemicals that modulate the transcriptional activity of androgen receptor can be divided into 2 structural (steroidal and nonsteroidal) and 2 functional (androgenic and antiandrogenic) classes. Steroidal androgens, mainly testosterone and its derivatives, have been used clinically as

replacement therapies for androgen-deficiency.⁹⁻¹⁰ Antiandrogens are used to counteract the undesirable actions of excessive androgens (example, to treat acne, hirsutism, male-pattern baldness, and androgen-dependent prostates hyperplasia and carcinoma).¹¹⁻¹² Nonsteroidal antiandrogens, such as flutamide, nilutamide, and bicalutamide, are often referred to as 'pure antiandrogens' because they bind exclusively to the androgen receptor, and therefore, are devoid of antigonadotropic, antiestrogenic and progestational effects. These agents are advantageous over steroidal antiandrogens in terms of specificity, selectivity and pharmacokinetic properties.¹³ Antiandrogens are widely used in the treatment of

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women with various hyper-androgenic conditions, including polycystic ovary syndrome, idiopathic hirsutism, acne, seborrhea and hair loss.¹³⁻¹⁵ In recent years, there has been growing interest in the development of nonsteroidal modulators for steroid hormone receptors as therapeutic agents. In addition to the above-discussed nonsteroidal antiandrogens, selective estrogen receptor modulators and nonsteroidal modulators for progesterone receptor have been successfully developed.¹⁶⁻²⁰

Anabolic-androgenic steroids (AAS) are synthetic derivatives of testosterone that has been abused by athletes in an attempt to improve athletic performance.²¹ In contrast to AAS abused by adolescent males, which have remained at a steady level since 1991, AAS abused by adolescent females has actually increased during this same time period.²² The AAS abused by females has been associated with several adverse effects, including menstrual abnormalities and reproductive dysfunction.²³⁻²⁵ Studies have been focused on the adverse effects of AAS abuse in adult females.²³⁻²⁶ However, the physiological effects of AAS abuse by adolescent females are largely unknown, and there are concerns regarding some of the effects, which might be permanent.²⁴ In rat models, there is overwhelming evidence that puberty can be advanced by estrogen and aromatically androgens.²⁷⁻²⁸ Menopause metabolic syndrome may in part be attributable to estrogen deficiency, and may be reversible with estrogen replacement therapy or hormone replacement treatment.²⁹ Previous studies have shown that administration of androgens to ovariectomized rats inhibits estrogen-induced sexual receptivity. Example to this are testosterone propionate, dihydrotestosterone propionate, and 3 androstanediol inhibit the induction of sexual receptivity by estradiol benzoate.³⁰⁻³¹ Recently, synthetic androgens, such as 17 -methyltestosterone, stanozolol and nandrolone decanoate, have also been shown to inhibit estrogen-induced sexual receptivity in ovariectomized rats.³² Evidence that the androgen receptor is involved in the androgenic inhibition of sexual receptivity has been provided by studies using flutamide, an androgen receptor antagonist.³³ Flutamide has been shown to block the inhibitory effects of naturally occurring androgens on estrogen-induced sexual receptivity in ovariectomized rats.³⁴ More recently, however, it was shown that flutamide failed to block the inhibitory effects of dihydrotestosterone on sexual receptivity induced by short-term treatment with unesterified estradiol-17 E₂ in combination with progesterone.³⁵

Recent studies confirmed previous observations that estrogen deficiency significantly increased the weight gain in ovariectomized rats, an effect that

was attenuated by estrogen treatment.³⁶ Compared to nonsteroidal androgens, which were only recently conceptualized, nonsteroidal antiandrogens were used clinically for many years.

Nonsteroidal antiandrogens were extensively reported; some of these (namely, flutamide³ and bicalutamide⁴) are successfully used in clinics for the treatment of androgen receptors dependent prostate cancer. There are 3 nonsteroidal antiandrogens that are currently available bicalutamide, flutamide and nilutamide. These drugs antagonize the actions of androgens at the receptor level and thereby inhibit tumor growth. Nonsteroidal antiandrogens block the negative feedback mechanism that regulates testosterone levels.³⁷

We aim to explore the effects of ovariectomy and treatment with flutamide on vaginal tissue in this study.

Methods. Thirty-six, 3-month-old Wistar-Albino female rats, each weighing approximately 200-210 g, obtained from the Department of Medical Science Application and Research Center of Dicle University, Diyarbakir Turkey were used in this study. The rats were acclimated to the local vivarium conditions (22°C and 12h/12h light-dark cycle) for 2 months. They were singly housed in 34 cm x 47 cm x 17 cm cages during the experimental period. All rats were allowed free access to water and a pelleted commercial diet containing 0.97% calcium, 0.85% phosphorus, and 1.0.5 IU/g of vitamin D3. The rats were divided into 3 groups of 12.

Animals in group 1 (control group) was also established with administration of carrier A [1:3 mixture of 100% ethanol and 0.9% saline W/V (Boots Co., Australia)] each day for 3 days. The animals of this group did not receive ovariectomy nor did they receive flutamide treatment. Carrier A injection was given to create the stress environment as in the 2 other animal groups. Surgical ovariectomy was performed to group 2 and 3 rats.

Ovariectomy. All rats in group 2 and 3 were food-deprived prior to surgery. For the ovariectomies, the rats were anesthetized by intraperitoneal injection of Ketamine (50 mg/kg body wt. Parke-Davis) and xylazine HCL 2% (100 mg/kg body weight Rompun - Bayer).³⁸ Bilateral ovariectomies were performed in 24 animals using a dorsal approach in a sterile surgical theater. After bilateral ovariectomy, the rats were allowed to recover for 6-8 weeks before treatment. The control rats (Group 1) were subjected to a sham operation in which the ovaries were exteriorized but left intact. Group 2 was designated as sham-operated group. The rats were subcutaneously injected with

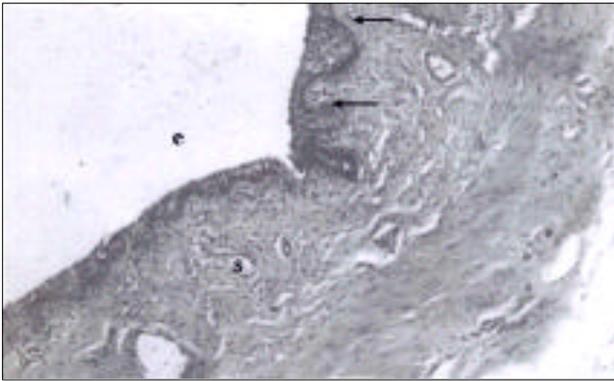


Figure 1 - The appearance of vaginal epithelium (e) (microscopic papilla (→) and stroma (s) was normal in the control group. (Hematoxylin-eosin, original magnification x 100).



Figure 3 - Vaginal epithelium of experimental rat given flutamide. Increased in the thickness of the epithelium layer and keratinized epithelium (→). (Hematoxylin-eosin, original magnification x 200).



Figure 2 - Vaginal epithelium of sham-operated group animals showing typical atrophied appearance of the epithelium non-keratinized (black arrows), and disappearance of microscopic papilla, the appearance of stromal tissue was rather loose (white arrows). (Hematoxylin-eosin, original magnification x 100).



Figure 4 - Increased in the thickness of the epithelium layer (←), keratinized (white thin arrow) and appearance of microscopic papilla and papillary process (white thick arrow), can be seen. (Hematoxylin-eosin, original magnification x 200).

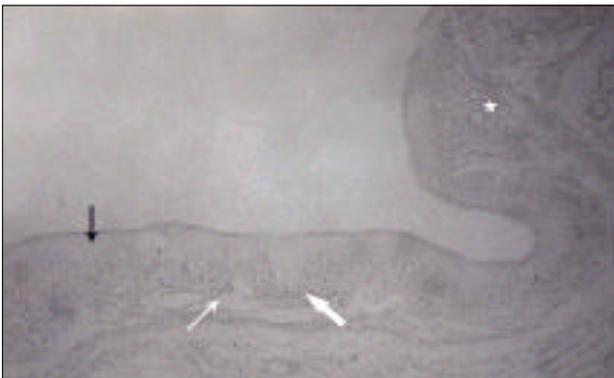


Figure 5 - Increased in the thickness of the epithelium layer, keratinized (black arrow) and appearance of microscopic papilla (white arrows), infiltration of the stromal cells (star) was increased. (Hematoxylin-eosin, original magnification x 100).

Table 1 - Comparison of weights of the body.

Groups	At the start of experiment	After 2 months (ovx)	Following 3 days of drug administration
Group 1 (n=12)	204.83 ± 1.1 ^a	222.08 ± 0.70 ^a	222.08 ± 0.7 ^a
Group 2 (n=12)	203.50 ± 0.96 ^a	284.58 ± 2.71 ^{b,d}	284.58 ± 2.71 ^{b,d}
Group 3 (n=12)	205 ± 1.03 ^a	284.58 ± 2.60 ^{c,d}	279.75 ± 1.59 ^{c,d}
Means within a column not followed by the same letter differ significantly ($p=0.05$).			

Table 2 - Comparison of the live weight means of the control group with other groups after the second month.

Groups	At the start of experiment	After 2 months
Group 1 (n=12)	204.83 ± 1.1 ^a	222.08 ± 1.2 ^a
Group 2, 3 (n=24)	203.33 ± 0.71 ^a	284.58 ± 1.8 ^b
Means within a column not followed by the same letter differ significantly ($p=0.05$)		

equivalent amount 0.1 ml of a 1:3 mixture of benzyl alcohol and peanut oil (carrier B), each day for 3 days. Carrier B injection was given in order to create the stress environment as in the 2 other animal groups. Group 3 was designated as sham-operated and flutamide group. Animals in this group were given oral gavage 10 mg /100g flutamide (Eulexin 250 mg tablet) each day for 3 days.

After approximately 2 months of ovariectomy, the animals were anesthetized and killed by cardiac exsanguinations. The pelvic region was seen to be far too much fatty at autopsy. Vaginae were removed, fixed in formalin fluid and embedded in paraffin. Five-micron sections were cut using a Leica RM 2125 RT and the sections were stained with hematoxylin-eosin. The stained sections were later evaluated with light microscopy.

In all experiments, body weights were measured daily using top-loading balance and the person measuring the body weight was blinded to each treatment group. Food intake was determined by estimating the amount of food consumed by the mice throughout the treatment period.

Statistical analysis. Data are expressed as mean ± SEM. Statistics were calculated using Minitab. The analysis of variance (ANOVA) test followed by Fisher's was used to compare the differences between groups.¹⁶

Results. Vaginal histology. We observed no pathology in the vaginal epithelium of the control

group. The lumen of the vagina was lined by a thick stratified squamous non-keratinized epithelium. The lamina propria of mucosa is composed of a loose fibro elastic connective tissue containing a rich vascular supply in its deeper regions (**Figure 1**).

In the sham-operated group, the thickness in the vaginal epithelium (typical atrophied appearance of the epithelium) decreased and disappearance of microscopic papilla, moreover, the appearance of stromal tissue was rather loose (**Figure 2**).

We observed increase in the thickness of the epithelium and keratinization, appearance of microscopic papilla and papillary processes in the flutamide treated group. After flutamide applications, infiltration of the stromal cells increased (**Figure 3-5**).

As a result, bilateral ovariectomy was determined to cause atrophy in vaginal epithelium of rats. When flutamide was administered later, thickening and keratinization due to hyperplasia in vaginal epithelium were observed. In this study, we found out that the antiandrogen drug flutamide is hormonally active and may exhibit estrogenic-antiestrogenic activity.

Body Weight. Our results show that body weight significantly increased in ovariectomized rats. Comparison of the starting means of the control group and other 24 animals at the start of trial were found to be insignificant (**Table 1**). Comparison of the live weight means of control group with the other animals after the second month (post ovariectomy) were found to be significant (**Table 2**) ($p<0.05$). According to the Fisher Tukey test, the difference between the last weight means of animal groups having only ovariectomy and then whether receiving the drug or without receiving the drug was found to be insignificant (**Table 1**). The differences between the weight means of animal groups 1 and 2, and also groups 1 and 3 were found to be significant following the administration of the drug (**Table 1**) Paired t-test; the given drug did not cause any difference in the same weight of animals before and after the administration of the drug combinations following Tukey Checking test (**Table 1**).

In this trial, paired t-test was performed in order to clarify whether the administration of the drug was

effective in those animals, and it was determined that administration of the drug was not effective on body weight ($p>0.05$) (-4.83 ± 2.31).

We observed that the body weight increased significantly in the ovariectomized rat. Furthermore, the body weight decreased a little after flutamide therapy.

Discussion. It is well known that vaginal tissues in different animal species, including human vaginal tissue, respond to the action of endogenous or exogenous estrogens. Estrogen receptors (ER) have been reported in vaginal tissues of various species, namely, rabbits, mouse and humans.³⁹ The lamina epithelialis thickness of vagina in rats and domestic animals are less than humans. Furthermore, the form of cell on the surface is related with cycles. Exogenous estrogens have teratogenic and carcinogenic effects on the developing female genital tract of both laboratory animals and humans.⁴⁰ Steroidal and nonsteroidal estrogens such as diethylstilbestrol elicit adverse effects. Certain triphenylethylene compounds, such as clomiphene, tamoxifen, and nafoxidine are hormonally active and may exhibit antiestrogenic activity. However, in many species, including subhuman primates, these compounds also have significant estrogenic activity.⁴¹ Moreover, in developing rodents, clomiphene and tamoxifen elicit the development of genital tract lesions similar to those usually associated with exposure to exogenous estrogens.⁴²

Flutamide is a nonsteroidal antiandrogen that conceivably exerts its major effects by blocking the nuclear binding of androgens to their receptors.⁴³ Some androgens are known to exert both estrogenic/antiestrogenic and progestational effects in female rats.⁴⁴⁻⁴⁶ The studies presented here were carried out to evaluate this effect of flutamide, a potent androgen, which has potential use in male contraception and hormone replacement therapy. The physiological role of androgen action in females is not clear.⁴⁷ In males, however, the ratio of androgen to estrogen is considered important to protect them from the feminizing effects of estrogen.⁴⁸ Several studies have demonstrated that, histologically, the vaginal epithelium showed stratification and cornification in flutamide treated rats. In those rats, vaginal epithelium was hypertrophied and gave the appearance of stratification.⁴⁹

Endometrial and vaginal epithelium undergoes specific transformations under the influence of gestagens. Oral administration of 1 mg flutamide to ovariectomized rats induced antiestrogenic effect on vaginal epithelium. Full antiestrogenic response was observed in rat vaginal tissue after oral administration of flutamide.⁵⁰ Antiestrogens may be

more or less as hormonally active as estradiol depending on parameter and dose. The vaginal epithelium showed stratification and cornification in estradiol treated rats. In tamoxifen treated rats, vaginal mucosa was slightly hypertrophied and gave the appearance of stratification.⁴⁹ The effect of flutamide on the antiestrogenic effect was investigated in ovariectomized rats. When flutamide was given, the flutamide effect induced vaginal cornification. In this study, we observed the antiandrogen drug flutamide was hormonally active in the vaginal epithelium similar to antiestrogens. In earlier studies to establish the relationship between flutamide administration and ovariectomy, we observed that ovariectomy estrogens and antiestrogens can cause some histopathologic changes in female genital system.^{51,52} In this study, we determined that flutamide eliminated the problems experienced following ovariectomy.

Recent research has reported that the body weight was higher in the ovariectomized rats than the intact rats.⁵³ In another experimental study, it was observed that ovariectomy increased body weight.⁵⁴ Further studies have shown that body weight significantly increased in ovariectomized rat.⁵⁵ These results support the hypothesis that alternation of estrogen status may be related to the development of obesity. Estrogen not only participates in the regulation of body weight, but also modulates insulin sensitivity.⁵⁶

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