ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2024; 5(2):84-90. https://dergipark.org.tr/tr/pub/ntms

The Toxic Effects of Flutamide vs. Bicalutamide vs. Cyproterone Acetate on the Testis: An Experimental Rat Study

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Article History
Received 28 Feb 2024
Accepted 22 May 2024
Published Online 30 May 2024

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Doi:10.56766/ntms.1441182

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Abstract: The aim of this study was to investigate the toxic effects on the rat testis of flutamide, bicalutamide, and cyproterone acetate using histopathological methods. Twenty-four male Sprague-Dawley rats were randomly divided into four groups, control (Group 1), flutamide (Group 2), bicalutamide (Group 3), and cyproterone acetate (Group 4). Physiological saline solution or anti-androgens were administered via oral gavage for 14 days. At the end of the study, the testes were harvested for histological toxic effect scoring. The mean histopathology scores were 0 in Group 1, 0.33±0.81 in Group 2, 1.66±1.36 in Group 3, and 2.93±0.98 in Group 4. The histopathology score in Group 4 was significantly higher than that in Group 1 (p=0.002), but was not significantly different to those in groups 2 and 3 (p=0.317 and p=0.028, respectively). No significant difference was also observed between the other groups. Cyproterone acetate, a steroidal antiandrogen, resulted in significant impairment of testis histology relative to the non-steroidal antiandrogens flutamide and bicalutamide. A non-steroidal agent such as flutamide or bicalutamide should therefore be selected if antiandrogen therapy is to be initiated for reasons such as acne, hirsutism, and paraphilias, particularly in young males. ©2024 NTMS.

Keywords: Flutamide; Bicalutamide; Cyproterone Acetate; Testis; Histopathology.

1. Introduction

Antiandrogens are agents that bind to intracellular androgen receptors. They compete with both dihydrotestosterone and testosterone at the receptor level in the cell nucleus, thus obviating the effects of endogenous androgens on target tissues including the testes, hair follicles, hypothalamus, pituitary and prostate glands, and ovaries. They are employed to treat a range of hyperandrogenic states, such as acne,

Cite this article as: Gur M, Dil E, Akdeniz E, Cobanoglu U, Kalyoncu NI, Topbaş M and Ozyavuz R. The Toxic Effects of Flutamide vs. Bicalutamide vs. Cyproterone Acetate on the Testis: An Experimental Rat Study. New Trend Med Sci. 2024; 5(2):84-90. Doi:10.56766/ntms.1441182.

hirsutism, and paraphilias, although their principal use is in the treatment of prostate cancer [1]. Anti-androgens are classified as steroidal (such as cyproterone acetate, medroxyprogesterone acetate, and megestrol acetate) and non-steroidal (including nilutamide, bicalutamide, and flutamide). Members of both classes compete with androgens at the receptor level, and non-steroidal anti-androgens are limited to this. However, steroidal anti-androgens also exhibit progestational characteristics by crossing the blood-brain barrier, resulting in central inhibition of the pituitary gland. Non-steroidal anti-androgens therefore do not reduce testosterone levels, which remain either normal or else mildly elevated [2].

The onset and progression of prostate cancers seem to be linked to the aberrant activation of androgen signaling. The activation of such signaling has been shown to exhibit a positive effect on prostate cancer cell growth both in vitro and in vivo [3]. Because of androgens on prostate cancer, anti-androgens have long been used in treatment. Cyproterone acetate, flutamide, and bicalutamide were approved for use in the treatment of prostate cancer by the Food and Drug Administration in 1989, 1989, and 1996, respectively [4]. The European Association of Urology guideline currently recommends the use of anti-androgens in the treatment of prostate cancer to prevent the 'flare-up' phenomenon or for maximum androgen deviation therapy [2]. However, anti-androgens have a number of toxic effects on the body. The principal pharmacological effects of steroidal anti-androgens occur secondary to castration (gynecomastia is unusual), while their non-pharmacological side-effects involve cardiovascular toxicity (4–40% for cyproterone acetate). The principal reported pharmacological side-effects of non-steroidal anti-androgens are gynecomastia (70%) and breast pain (68%) [2].

Androgen signaling is also of crucial importance to the development and preservation of the male reproductive organs and to pathological events concerning these [3,5,6]. Moderate androgen signaling is also essential for normal testis development and function [5]. Anti-androgen drugs exhibit adverse effects on spermatogenesis and toxic effects on testis tissue [1,7]. No direct comparisons have been performed between the anti-androgens in terms of toxic effects on the testes. The present study was intended to determine the toxic effect of anti-androgens on the testis tissue using histopathological methods.

2. Material and Methods

2.1. Animals

The experimental protocol employed in this study was approved by the Karadeniz Technical University Animal Care and Ethics Committee (no. 2009/15-2). Twenty-four Sprague-Dawley male rats aged 9-12 weeks were obtained from the Karadeniz Experimental Animals Laboratory (Trabzon, Türkiye). These were housed in individual cages in a specific-pathogen-free environment at 22±1˚C, at a relative humidity of 40-70%, and in a 12 h/12 h light/dark cycle. Ad libitum access was permitted to food and water.

2.2. Experimental Design

Following a seven-day adaptation period, the 24 male rats were randomly and equally assigned into control, and three experimental groups (n = 6) as follows:

Group 1 (Control): Physiological saline solution was administered via oral gavage for 14 days.

Group 2 (Flutamide): Flutamide dissolved in physiological saline solution was administered via oral gavage at a dose of 50 mg kg^{-1} day^{-1} for 14 days as recommended by Wang et al. [8].

Group 3 (Bicalutamide): Bicalutamide dissolved in physiological saline solution was administered via oral gavage at a dose of 25 mg kg^{-1} day^{-1} for 14 days as recommended by Singh et al. [9].

Group 4 (Cyproterone acetate): Cyproterone acetate dissolved in physiological saline solution was administered via oral gavage at a dose of 25 mg kg^{-1} day^{-1} for 14 days as recommended by Gual et al. [10].

Fifteen days after the commencement of the experiment, all rats were fasted for six hours before being anesthetized via intramuscular injection of 60 mg kg^{-1} ketamine hydrochloride. The abdominal region was first shaved and sterilized using povidone iodine solution. A 3-cm midline incision was then made (Figure 1), and the abdominal viscera were extended upward to reveal the bladder. This was then pulled upward, and the prostate and bilateral seminal vesicles were located and excised. The testes were delivered through the inguinal canal, and bilateral orchiectomy was performed. The rats were finally euthanized with a lethal intraperitoneal dose of ketamine.

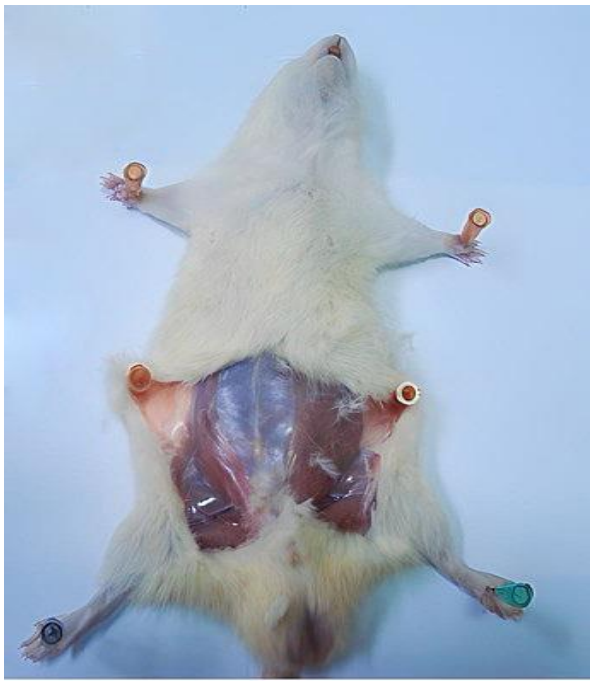

Figure 1: Surgical incision of the rat under general anesthesia for the removal of male reproductive organs.

2.3. Macroscopic evaluation

The isolated organs were weighed using sensitive scales before being placed into the solutions.

2.4. Histopathological examination

The extracted testes were fixed in formalin, and sections from the upper, middle and lower parts were embedded in paraffin. Multiple three-micrometer sections were cut and stained with hematoxylin/eosin (H&E). Evaluation of the effects on the testes of the three distinct anti-androgen drugs was based on the criteria specified by Dianne M. Creasy, and pathologies observed in testis tissues were scored accordingly [11].

This scoring system is employed to show the toxic effect on the testis of a drug, chemical, or herbal agent:

Score 0: Normal.

Score 1: Spermatid retention emerging with chemicals or hormonal disturbance.

Score 2: Missing germ cell layers in seminiferous tubules.

Score 3: The presence of multinucleate giant cells formed from cell cytoskeletal disintegration and cytoplasm fusion due to a slow degenerative process

Score 4: Impairment of the spermatogenic cycle due to the slow degenerative process and sloughing of spermatogenic cells into the lumen.

Score 5: Increased interstitial space volume associated with seminiferous tubule cell loss [11].

A minimum of 10 high-power fields (magnification, x200) were examined per section for each sample.

2.5. Statistical analysis

All statistical analyses were performed on computerized software (IBM SPSS version 25, Chicago, IL, USA). Data are presented as mean±standard deviation at a significance level of 0.05. Differences were analyzed using the Kruskal Wallis-H test. The Bonferroni-corrected Mann-Whitney U test was employed to identify the source of significance in variables identified as significant.

3. Results

The mean weight of the 24 rats was 293±19.17 g. The mean weight of the right testis was 1.37±0.32 g, the mean weight of the left testis was 1.36±0.34 g, the mean weight of the prostate was 0.73±0.26 g, and the mean weight of the bilateral seminal vesicles was 0.68±0.25 g. The mean weights of the rats were 299.33±25.1 g in Group 1, 293.66±22.5 g in Group 2, 291.83±20.1 g in Group 3, and 294.16±12.1 g in Group 4 (p=0.980). No differences were found in terms of right testis, left testis, prostate, or seminal vesicle weights. The groups' weight data are shown in Table 1.

No pathological findings were observed in Group 1 (control). Figure 2 shows the histological image of normal testicular tissue in the control group. The most severe pathological change in Group 2 (flutamide) was missing germ cell layers in the seminiferous tubules. The worst pathological change in Group 3 (bicalutamide) was the presence of multinucleate giant cells, while that in Group 4 (cyproterone acetate) was sloughing of spermatogenic cells into the lumen (Figure 3). An increased interstitial space volume, the worst pathological finding of the scoring system used, was not encountered in any rat.

Mean score values were 0 in Group 1 (control), 0.33±0.81 in Group 2 (flutamide), 1.66±1.36 in Group 3 (bicalutamide), and 2.93±0.98 in Group 4 (cyproterone acetate). The elevation in Group 4 was significant compared to Group 1 (p=0.002), but not compared to groups 2 (flutamide) or 3 (bicalutamide) (p=0.317 and p=0.028, respectively). No significant difference was observed between groups 2 and 3 (p=0.071). The groups' histological scores are shown in Table 2.

Table 1: Rat and organ weights.

Weight (gram)	Group 1 (Control)	Group 2 (Flutamide)	Group 3 (Bicalutamide)	Group 4 (Cyproterone)	p*
Rat	299.33 ± 25.1	293.66 ± 22.5	291.83 ± 20.1	294.16 ± 12.1	0.980
Right testis	1.35 ± 0.42	1.49 ± 0.35	1.23 ± 0.37	1.43 ± 0.42	0.436
Left testis	1.37 ± 0.45	1.5 ± 0.3	1.13 ± 0.39	1.44 ± 0.53	0.288
Prostate	0.88 ± 0.3	0.81 ± 0.19	0.5 ± 0.16	0.73 ± 0.23	0.085
Seminal vesicle	0.75 ± 0.25	0.67 ± 0.16	0.51 ± 0.15	0.78 ± 0.35	0.196

*Kruskal Wallis-H Test.

Table 2: Mean histopathological scores in groups.

Histology	Group 1 (Control)	Group 2 (Flutamide)	Group 3 (Bicalutamide)	Group 4 (Cyproterone)	p*
Mean score	0	0.33 ± 0.81	1.66 ± 1.36	2.93 ± 0.98[#]	0.002

*Kruskal Wallis-H Test, [#]significantly different from the Control group (p=0.002).

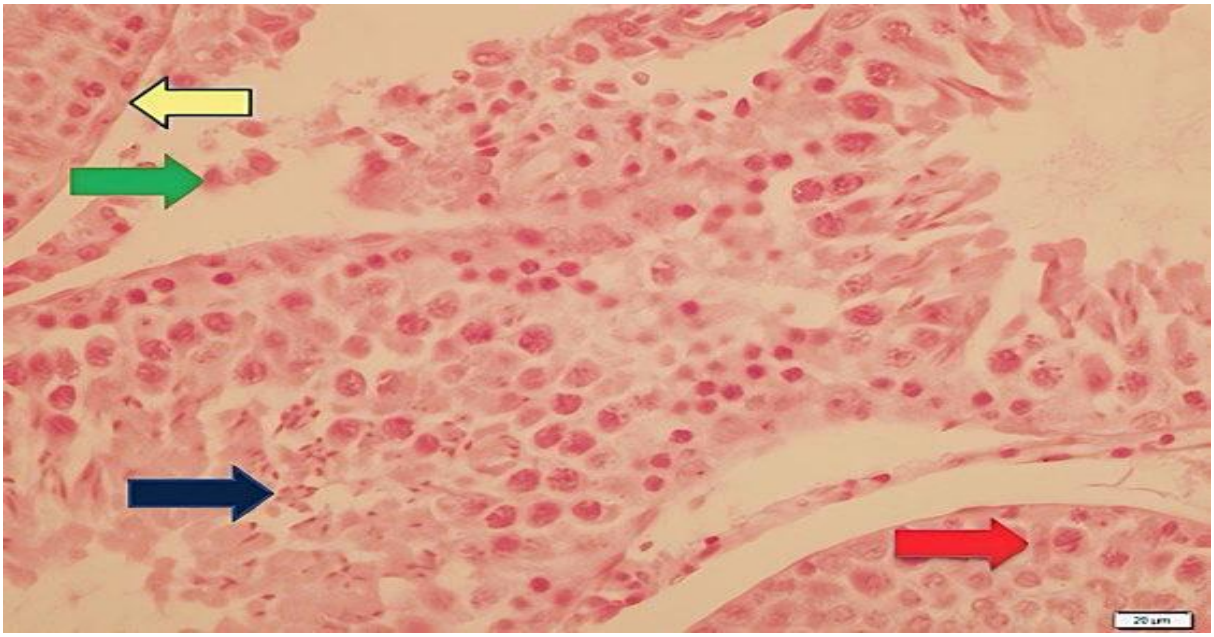

Figure 2: Normal histological findings of rat testis (yellow arrow, basement membrane; green arrow, Leydig cell; blue arrow, spermatocyte; red arrow, Sertoli cell) (x40).

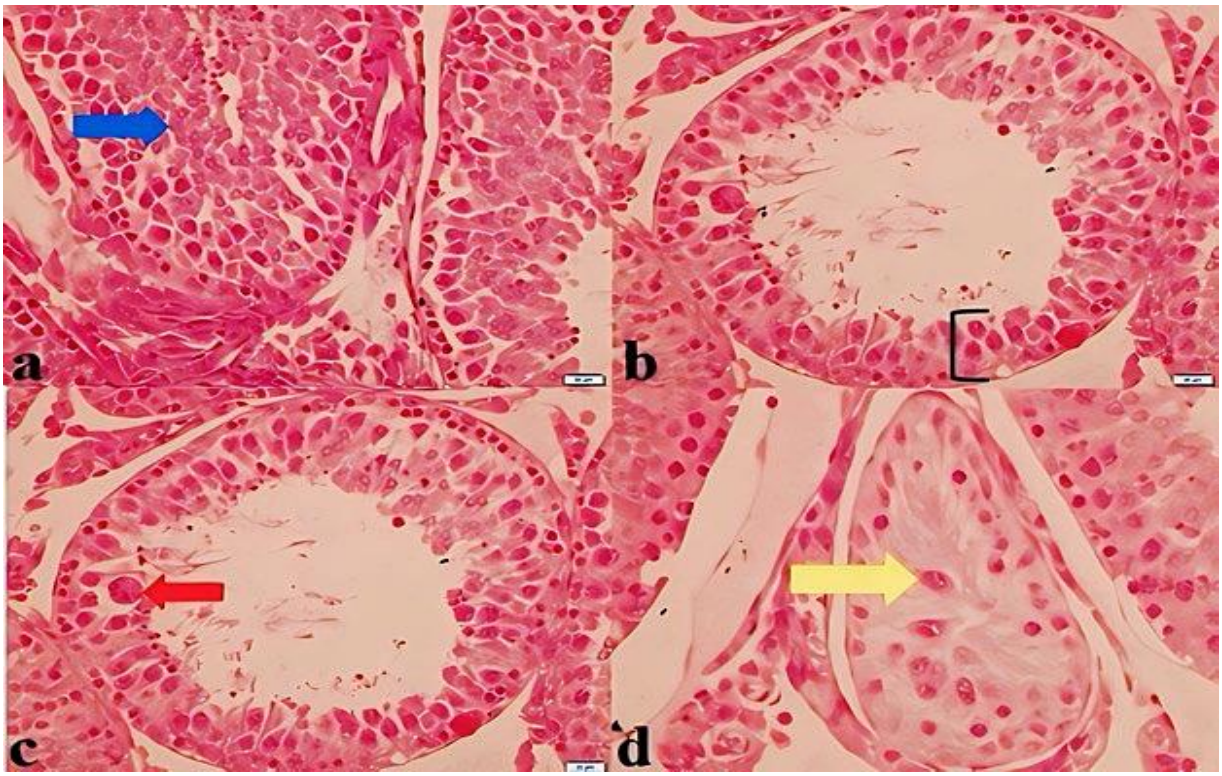

Figure 3: Different morphological changes scored by the pathologist with Hematoxylin-Eosin stain (a: blue arrow, retained spermatid; b: **(**, missing germ cell layers; c: red arrow, multinucleate giant cells; d: yellow arrow, sloughing of spermatogenic cells into the lumen)(x40).

4. Discussion

This study involved a histopathological investigation of the effects of steroid (cyproterone acetate) and non-steroid (flutamide and bicalutamide) anti-androgens on normal testis tissue. The non-steroidal antiandrogens flutamide and bicalutamide had no toxic effect on testis tissue compared with the control group, while cyproterone acetate exhibited a significant toxic effect. Although anti-androgen agents are mainly employed in the treatment of prostate cancer, they are also used in acne, hirsutism, paraphilias and coronavirus-related respiratory diseases [1, 2, 12]. Chemically, antiandrogens are classified as either steroidal (such as cyproterone acetate, medroxyprogesterone acetate, and megestrol acetate) or non-steroidal (including nilutamide, bicalutamide, and flutamide). In addition, anti-androgens are also currently classified as older generation androgen receptor antagonists (non-steroidal and steroidal) and second generation androgen receptor antagonists (enzalutamide, darolutamide, and apalutamide) [2, 13]. Second generation androgen receptor antagonists are currently used in the treatment of prostate cancer, while the European Association of Urology recommends the use of older generation androgen receptor antagonists to prevent the 'flare-up' phenomenon and for maximum androgen deviation therapy [2].

Histopathological examination of testis tissues in the present study revealed no damage in the control group, while the most severe injury was in Group 4 (cyproterone acetate). The seminiferous tubules from the control group rats exhibited a normal histological structure with well-developed spermatozoa in the lumens. The administration of cyproterone acetate resulted in an alteration of the histological testis structure, such as the appearance of retained spermatids in seminiferous tubules and missing germ cell layers of seminiferous tubules into their lumens, which in turn led to a decreased germinal epithelium thickness. In the seminiferous tubules, multinucleate giant cells formed as a result of cell cytoskeletal disintegration and cytoplasm fusion, and spermatogenic cells resulting from the separation of spermatids and disruption of the spermatogenic cycle in their lumen were observed to slough into the lumen and accumulate. Group 2 (flutamide) and Group 3 (bicalutamide) exhibited no significant histopathological difference relative to the control group. The most severe injury caused by flutamide was the observation of missing germ cell layers in the seminiferous tubules. The damage caused by bicalutamide involved the presence of multinucleate giant cells in seminiferous tubules. An experimental rat study involving cyproterone acetate reported that this agent resulted in degeneration of germ cell layers and decreased seminiferous tubular diameters and significant testis damage [14]. An experimental rat study involving bicalutamide reported the appearance of vacuoles and sloughing of germ cells from the germinal layer of seminiferous tubules into their lumens, resulting in a decreased germinal epithelium thickness. Spermatid detachment and accumulation of desquamated spermatocytes together with spermatids and cellular debris were observed in the seminiferous tubule lumens following administration of bicalutamide [15]. Anahara et al. summarized the effects of flutamide on the mouse testis in their mini-review, and concluded that it resulted in structural changes in the spermatid acrosome and nuclei, and increased the numbers of abnormal spermatids, but caused no significant injury to Sertoli cells, Leydig cells, germ cells, or ectoplasmic specialization [7]. In the present study, the agent with the least toxic effect on testis tissue was flutamide, with a mean score of 0.33±0.81. From that perspective, the current research was consistent with Anahara et al.

A general examination of the literature shows that flutamide, bicalutamide, and cyproterone acetate all exhibit histopathological toxic effects on the rat testis [7, 14-17]. These three agents also exhibited toxic effects on the testis in the present study, the most severe injury being observed with cyproterone acetate and the mildest with flutamide. However, a comparison of the three agents revealed no significant difference between them in terms of toxic damage. Our search of the literature revealed no previous studies comparing the toxic effects of flutamide, bicalutamide, and cyproterone acetate on normal testis tissue using histopathological methods. The fact that our research is one of the first studies to investigate the toxic effects of these three anti-androgens on the rat testis in terms of histopathology therefore represents one of its particular strengths.

No difference was determined among the groups in terms of bilateral testis weights. The greatest toxic effect in this study was exhibited by cyproterone acetate. A decrease in testis weight might therefore have been anticipated in the cyproterone acetate group. In terms of testis weights, however, there was no difference between the cyproterone acetate and either the control group or the other study groups. Similarly, to the present study, Aleem et al. also found that cyproterone acetate had no effect on testis weights [16]. A previous study examining the effect of daily treatment with flutamide on testicular function in adult male rats observed no effect on testicular weights [17]. Macleod et al. reported no effect on testis weights calculated in early puberty in male rats exposed to flutamide in utero [18]. In contrast, other studies involving bicalutamide and flutamide have reported that both agents significantly reduced testis weights [15, 19, 20]. However, it should be remembered that the treatment period in the present study was 14 days, compared to 28 days in the majority of these studies.

In this study, the prostate gland and bilateral seminal vesicles were excised, weighed using sensitive scales, and compared between the groups. Unfortunately, due to technical deficiencies, prostate and seminal vesicle tissues could not be subjected to advanced histopathological examination. Only prostate and bilateral seminal vesicle weights were compared

between the groups, and no significant differences were observed (p=0.085 and p=0.196, respectively). No similar previous studies have compared prostate and seminal vesicle weights. However, in a study performed at the histopathological level, Elzoghby at al. showed that flutamide significantly reduced hyperplasic and dysplastic lesions in prostate tissue [21]. Sarrabay et al. investigated the effects of different doses of flutamide on prostate and seminal vesicle tissues, and reported that flutamide causes atrophy in the prostate and also the seminal vesicle beginning from a dosage of 1 mg kg^{-1} day^{-1} [22]. A study of bicalutamide in the rat prostate reported that this agent increased apoptosis in prostate cell lines [23]. A study performed with cyproterone acetate showed that this resulted in glandular atrophy in prostate tissue [24]. An experimental rat study showed that all three anti-androgens caused atrophy and apoptosis in prostate tissue. A decreased weight in these organs may therefore be expected under these conditions. However, no differences were observed between the groups in terms of organ weights in the present study. This may be attributable to our relatively short treatment period (14 days). Similarly, to our hypothesis, Anahara et al. also stated that they believed that flutamide reduced prostate weight in mini-review, but were unable to demonstrate this in an objective manner [7].

5. Conclusion

The primary aim of this study was to investigate the effects of different anti-androgens on testis tissue. The results showed that the steroidal anti-androgen cyproterone acetate exhibited toxic effects on testis tissue relative to the control group. However, the non-steroidal anti-androgens flutamide and bicalutamide had no toxic effects on testis tissue. We therefore think a non-steroidal agent such as flutamide and bicalutamide will represent an appropriate option, especially in young men, if anti-androgen therapy is to be initiated for reasons such as acne, hirsutism, and paraphilias.

Limitations of the Study

This study has a number of limitations due to technical factors. Hormones such as luteinizing hormone, follicle-stimulating hormone, and testosterone could not be investigated in rat sera. In addition, stereological investigation aimed at a more detailed examination of toxic effects on testis tissue could not be performed. Semen analysis was also not carried out with the collection of semen samples from the cauda epididymis of sacrificed rats. Moreover, the tumoral effects of the anti-androgens on testis tissue could not be investigated. However, despite all these limitations and the fact that we were only able to perform histopathological examinations, we think that this study is valuable as experimental research into the effects of steroidal and non-steroidal anti-androgens on testis tissue.

Acknowledgement

None.

Conflict of Interests

The authors declare that there is no potential conflict of interest for the research, authorship, and/or publication of this article.

Financial Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

Author Contributions

Conceived and designed the experiments; MG, ED, NIK, RO. Supervision; UC, RO. Data Collection and/or Processing; MG, ED, UC. Analyzed and interpreted the data; ED, EA, UC, NIK, MT. Literature Review; EA, NIK, MT. Writing; MG, EA, UC, RO. Critical Review; UC, MT, RO. Study of biostatistics; MT. All authors read and approved the final manuscript.

Ethical Approval

Karadeniz Technical University Animal Care and Ethics Committee approved by the study (no. 2009/15-2).

Data sharing statement

None.

Consent to participate

None.

Informed Statement

None.

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