Iraqi Journal of Science, 2026, Vol. xx, No. x, pp: xx
DOI: 10.24996/ijs.2026.67.9.10

ISSN: 0067-2904

Histological Effect of RAD 140 Supplement on the Prostate of Albino Rats

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Received: 15/5/2025 Accepted: 23/11/2025 Published: xx

Abstract

Vosilasarm, known as RAD140, is a new selective androgen receptor modulator with limited data on its pharmacokinetic profile, adverse effects, and interference with body organs. This research paper reports the experimental work prepared to assess the histological impact of RAD 140 on the prostate tissue of albino rats. The study involved 18 six-week-old male albino rats, which were randomly divided into two groups; the control group consisting of three mice that were only fed the regular laboratory diet and did not receive any treatment, and the experimental group that contained 15 mice, divided into five subgroups of three animals, each receiving orally a daily dose of 0.3 ml of RAD140 with the following concentrations: (1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml) respectively. After 6 weeks of dosing, the prostate glands were ablated, treated with a routine histological processing system, and then examined under the light microscope. The results indicated the presence of pathological changes in the prostate tissues in a dose-dependent manner. Different concentrations of RAD 140 led to varying severities of cellular and tissue changes in the prostate. The results of this study indicate prostate damage resulting from the indiscriminate use of RAD 140 without a prescription. Therefore, it is recommended to use the lowest dose of RAD 140 to minimize its toxic effects.

Keywords: Androgenic modulator, RAD140, Adverse effect, SARMS, Prostate hypertrophy

التأثير النسيجي لمكمل RAD 140 على البروستات في الجرذان البيض.

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جامعة بغداد، كلية العلوم، قسم علوم الحياة، بغداد، العراق

الخلاصة

الفوسيلاسارم، المعروف باسم RAD140، هو مُعدّل انتقائي جديد لمستقبلات الأندروجين، ببيانات محدودة للغاية حول حركيته الدوائية، وآثاره الجانبية، وتداخله مع أعضاء الجسم. تُوثّق هذه الورقة البحثية العمل التجريبي المُعدّ لتقييم التأثير النسيجي لـ RAD 140 على أنسجة البروستات في الجرذان البيض. شملت الدراسة 18 جرذًا أبيض ذكرًا بعمر ستة أسابيع، قُسِّموا عشوائيًا إلى مجموعتين: المجموعة الضابطة، التي تضم ثلاثة فئران، والتي تغذّت فقط على النظام الغذائي المختبري المعتاد ولم تتلقَّ أي علاج، والمجموعة التجريبية، التي تضم 15 فأرًا، مُقسَّمة إلى خمس مجموعات فرعية، تضم كل منها ثلاثة حيوانات، تتلقى كل منها جرعة يومية فموية مقدارها 0.3 مل من RAD140 بالتراكيز التالية: (1.25 ملجم/مل، 2.5 ملجم/مل، 5 ملجم/مل، 10 ملجم/مل، و20

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ملجم/مل) على التوالي. بعد ستة أسابيع من تناول الجرعة، تم استئصال غدد البروستات، وعولجت بنظام المعالجة النسيجية الروتيني، ثم فُحصت تحت المجهر الضوئي. أشارت النتائج إلى وجود تغيرات مرضية في أنسجة البروستات، وذلك بناءً على الجرعة. بمعنى أن التراكيز المختلفة من RAD 140 أدت إلى تغيرات خلوية ونسيجية متفاوتة الشدة في البروستات. نتائج هذه الدراسة تدل على تضرر البروستات الناتج عن عشوائية تناول RAD140 بدون وصفة طبية. وعليه يقترح استخدام أصغر جرعة من RAD140 لتقليل آثاره السامة.

Introduction:

Selective Androgen Receptor Modulators (SARMs) are a group of chemicals that have almost the same effect as anabolic steroids in terms of muscle-building, but their side effects related to androgen are minimized. Besides that, the exploitation of substances like anabolic steroids is also linked with the danger of various severe side effects. Vosilasarm or RAD140 is a recent SARMs family member that selectively activates androgen receptors in a few specific tissues [1]. Bones and muscles remain flexible with the help of SARMs, but the impact is minimal in those parts of the body where receptors are very close to testosterone, such as the skin, prostate, and testes. The differences between SARMs are both at the atomic level and largely non-steroidal [2].The mode of action of AR (androgen receptor) antagonist is one of the hallmarks of all SARMs [3].

Although SARMs are tissue-selective, they have been somewhat controversial in the medical field for over two decades. In fact, they are proposed to be the solution in the case of damages or diseases of bones or muscles that would result in atrophy, as they enable the increase in muscle strength and volume, which is an aspect that is highly attractive to the users of performance-enhancing substances, especially in the area of resistance training, such as weightlifting or bodybuilding [4].

SARMs are molecules that directly enter the cell cytoplasm, detach the heat shock protein, which is a constituent of the androgen receptor, and then move into the nucleus, where they physically connect to the nuclear receptor binding areas. Finally, they execute their function as transcription factors [5]. Different co-regulatory proteins help shape and modify transcriptional response, depending on the tissue type and the cell's regulatory environment [6].

SARMs have been investigated as possible therapies for multiple sclerosis, Alzheimer's disease, osteoporosis, cancer, sexual dysfunction, and muscle atrophy, and recently they have been included as supplements targeted at athletes [7].

Potential adverse outcomes, including erythrocytosis, prostate enlargement, hepatotoxicity, aromatization to estrogen, and testicular atrophy, frequently limit their therapeutic usage [8].

SARMs have also been shown to change liver function, lower endogenous testosterone, and impact cholesterol levels [9].

According to recent pharmacological reports, SARMs offer a wide range of potential clinical uses and show promise for the safe treatment of breast cancer, prostate cancer, benign prostatic hyperplasia, cachexia, and hypogonadism [5]. In breast cancer, for instance, researchers have shown that RAD140 is a potent AR agonist in breast cancer cells, with a distinct mechanism of action [10].

However, it is uncertain how SARMs products interact with other substances, such as alcohol and other narcotics, especially when used in high doses over an extended period of time [11]. As such, less is known about their long-term consequences on body organs [12, 13].

The new brand RAD 140, is found to have many advantages because it is tissue-selective, but little is known about its disadvantages since its mechanism in different tissues is not well understood [14]. Therefore, this study has been designed to evaluate the impact of different concentrations of RAD140 on the histology of the prostate tissue of rats.

Materials and methodology

Animal care and study design A cluster of eighteen, six-week-old male *Wistar strain* albino rats, weighing 250-350g, were purchased from the National Center for Drug Control and Research in Baghdad, and kept under standardized environmental conditions; constant temperature, moisture, and a 12-hr light regime without stress factors, and allowed to take laboratory food and water. Animals were randomized into 2sets; the control set of 3 rats received regular lab nutrients without treatment. The experiment set, which contained 15 rats, was divided into 5 subsets of 3 animals.

This study received approval from the Scientific Ethics Committee of the University of Baghdad, College of Science. The Ethical approval number is: CSEC/0525/0064.

Preparing RAD140

The supplement was purchased from medical stores in Baghdad (MZ Bio-labs: concentration:20mg/ml/volume:30ml, SKU: 000509, liquid form). The dosage calculation, preparation and duration of this supplement were done according to Earnest *et al.,* [15].

Each animal in the experiment group was given a daily mouth dosage of 0.3 ml of the following concentrations of drug (1.25 mg/ml, 2.5 mg/ml, 5 mg/ml, 10 mg/ml, and 20 mg/ml), respectively.

Histopathological evaluation

After 45 days (6 weeks) of dosing, the remaining animals were sacrificed by Euthanasia, and prostate glands were isolated, fixed in 4% formaldehyde solution, dehydrated in growing sequences of ethanol, cleared in xylol, then the fixed tissues were embedded in wax and cut into thin section of 5 µm which then were attached to slides and stained with hematoxylin and eosin(H&E) for histological analysis. The slides were examined under a light microscope.

Results

Control animals

The histological investigation of the prostate architecture in control rats revealed a normal histological structure without any pathological changes. The prostate lobes (PL) are normal in shape and size, surrounded by a thin connective tissue capsule(CTC) with normal epithelial lining(EPL), and parenchymal tissue arrangement, as seen in Figure 1 A and B.

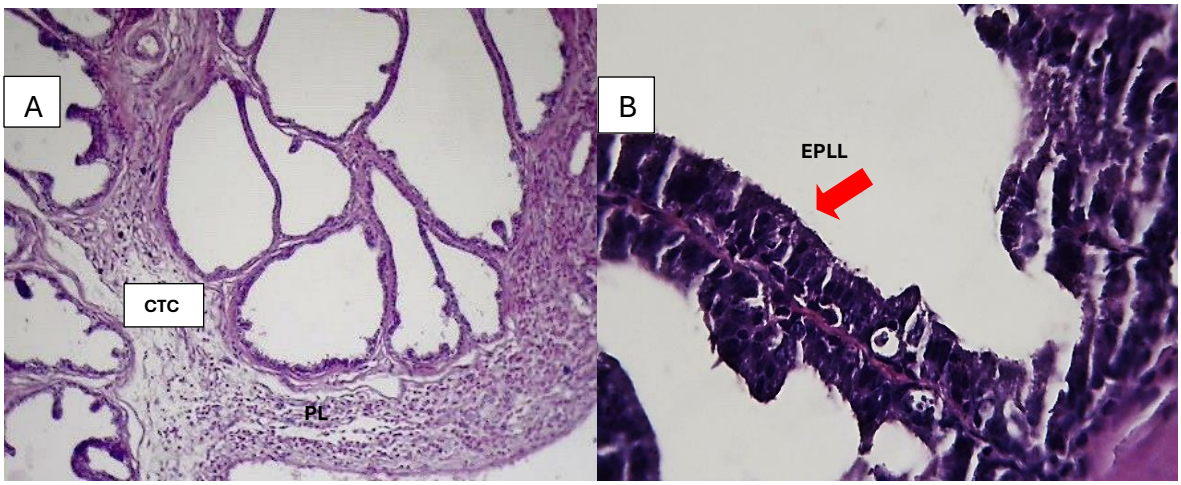

Figure 1: Sections from the prostate of healthy animals showing normal histology of the prostate. A: shows normal prostate lobes(PL) surrounded by a thin connective tissue capsule(CTC) (H and E stain, 10X). B: shows normal epithelial lying (EPL) (H and E stain, 40X).

Effect of 1.25mg/ml dose

The histological examination revealed mild dilatation in the acinar tissue of the prostate, characterized by an increase in the lumen of the acini, associated with destruction of the fibromuscular stroma (Figure 2 A and B). There is a mild increase in the epithelial lining of the glandular part of the tubular acinar, with mild accumulation of proteinaceous material in the lumen of the acinar (Figure 2 C and D).

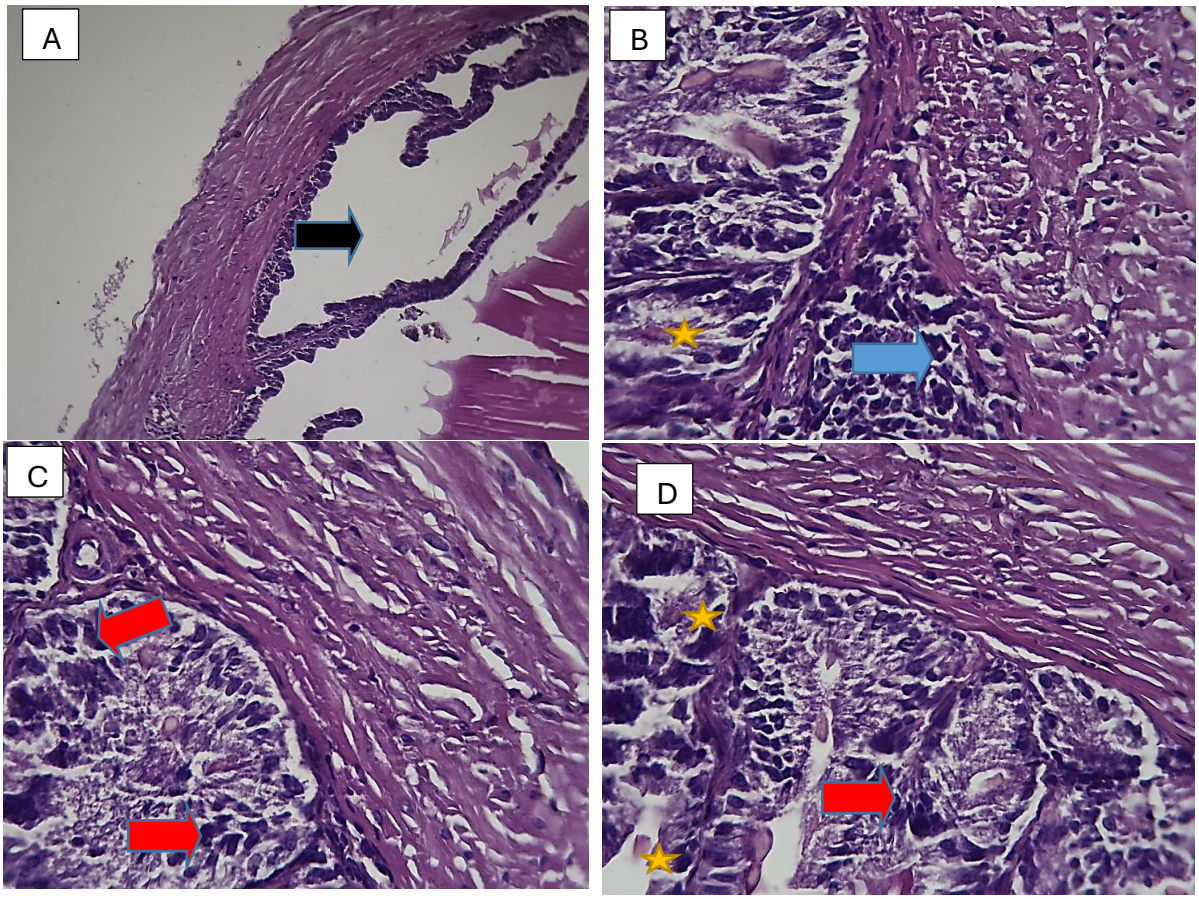

Figure 2: Sections from the prostate of subgroup 1(1.25mg/ml) showing different histopathological effects A: increasing in the lumen of acinar (black arrow). B: destruction of the fibromuscular stroma (blue arrow). C and D: mild increase of the epithelial lining of the glandular part of the tubular acinar (red arrow) with mild accumulation of the proteinaceous material in the lumen of the acinar (yellowish star), (H and E stain, 40X).

Effect of 2.5 mg/ml dose

The section of prostate tissue taken from animals in this group showed the occurrence of prostatic hyperplasia in the columnar epithelium that lined the acini, which organized into papillary projections (Figure 3 A, B, C, and D).

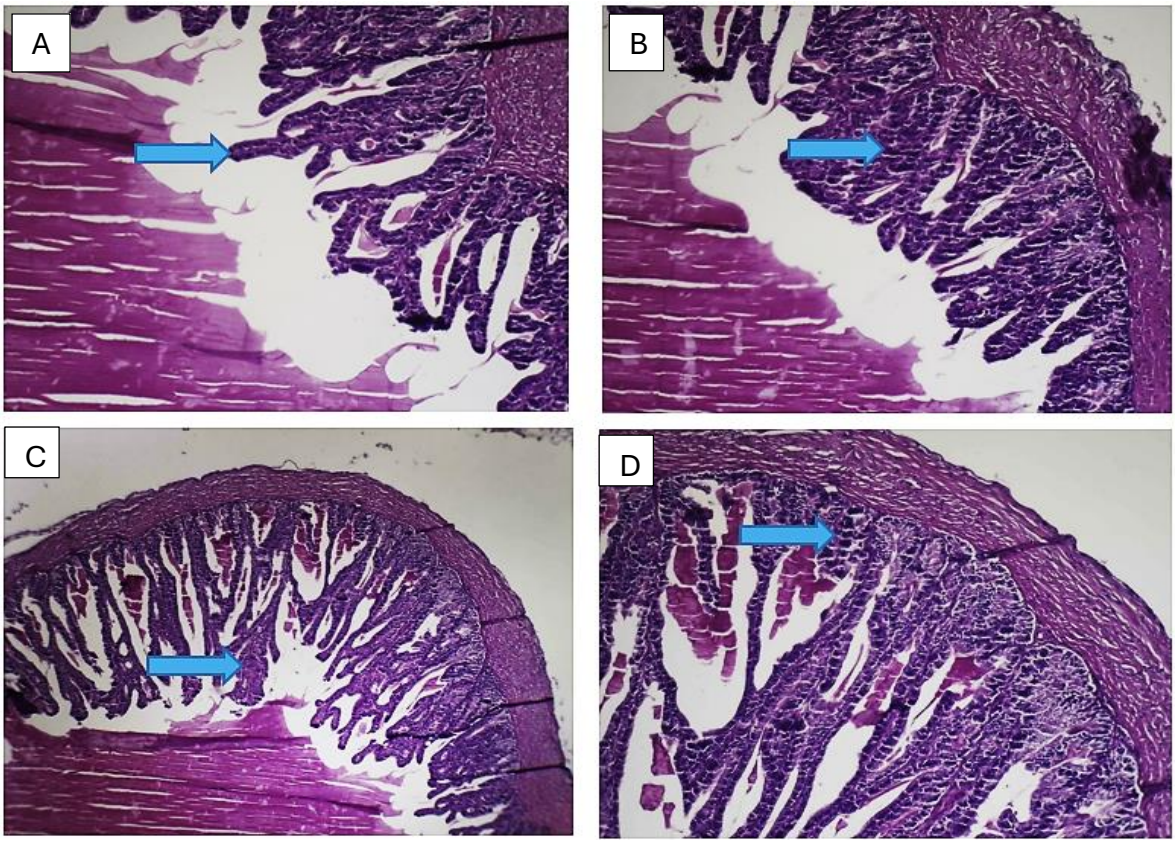

Figure 3: Sections from the prostate of subgroup 2(2.5mg/ml) showing hyperplasia of columnar epithelium which organized into papillary projections (blue arrows). (H and E stain, 40X).

The effect of a 5 mg/ml dose of the drug

The microscopic analysis of prostate sections from this group revealed histopathological changes characterized by epithelial hyaline degeneration, which appears as a prominent proteinaceous material accumulated in the lumen of acini (corpora amylacea) and also seen between acini (Figure 4A and B).

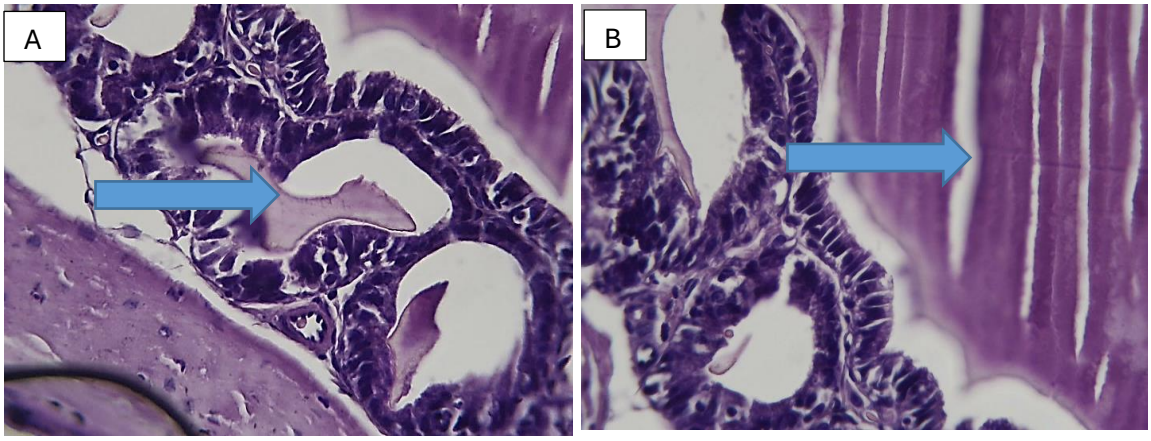

Figure 4: Sections from the prostate of subgroup 3(5mg/ml) showing epithelial hyaline degeneration appears as proteinaceous material (corpora amylacea) accumulated in the lumen of acinar and between the acini (blue arrows). (H and E stain, 40X).

The effect of the 10 and 20 mg/ml doses

There were roughly similar pathological changes in the prostate tissues in animals of these groups included severe hyperplasia in both of stroma and glandular part (Figure 5 A and B), the glandular tissue was highly differentiated (Figure 5 C and D), in addition, destruction of fibromuscular stroma was noticed, the corpora amylacea also still appear with fibrosis present but in little amount, (Figure 5 E and F).

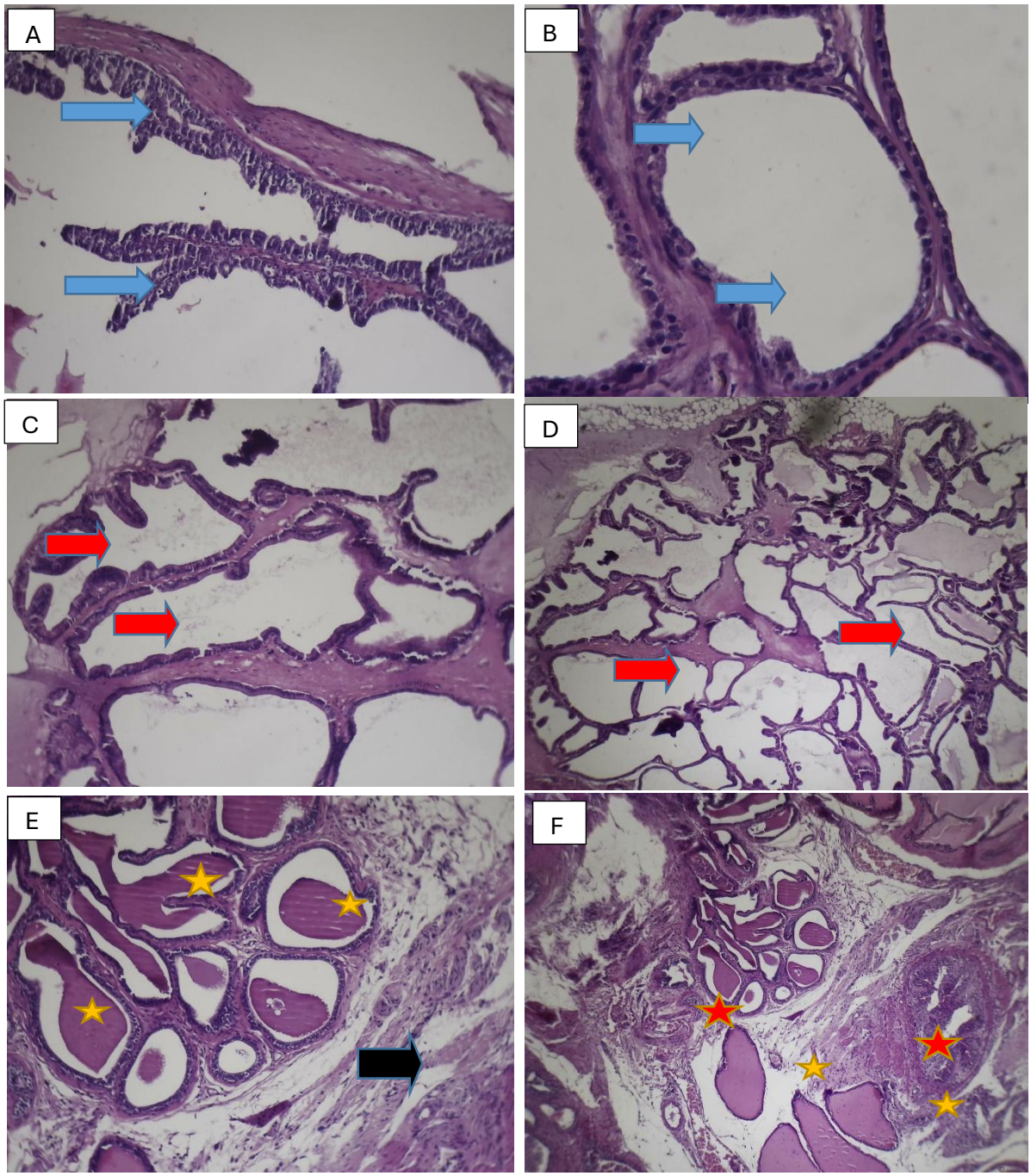

Figure 5: Sections from the prostate tissue of subgroups 4 and 5(10 and 20mg/ml doses) showing different histopathological effects. A and B: severe hyperplasia in both the stroma and glandular part (blue arrows), C and D: the glandular tissue is highly differentiated (red arrows), E and F: the corpora amylacea still appear (yellowish star), fibrosis present in a small amount (red star), with destruction of fibromuscular stroma (black arrow), (H and E stain, 40X).

Discussion:

In this study, the findings showed that after 45 days of RAD 140 administration, there were dose-dependent alterations in the histology of the prostate tissue; the different concentrations of RAD 140 were associated with varying degrees of structural alterations in the prostate tissue. Higher doses produced more noticeable effects, such as epithelial hyaline degeneration and hyperplasia, whereas lower doses had lesser effects, such as moderate dilatation of acinar tissue.

The observed histological changes, which include fibromuscular stroma breakdown, proteinaceous material buildup (evidence of chronic inflammation), and hyperplasia, suggest that the prostate gland's cellular composition and structural integrity changed in response to RAD 140 exposure due to the agonistic/ antagonistic effects of this medicine, according to previous works [16, 17].

Some studies reported the potential risks of frequent usage of SARMs, including RAD 140, in clinical practice and fitness supplementation due to its androgenic effects [14, 18].

The current outcome is in line with that of Zhang *et al.,* who conducted a study that focused on the rise in the ratio of estrogen-to-androgen E/T that was found in the bloodstream and within the prostate of men. This increase coincided with elevated expression of estrogen receptors in the prostate stromal tissue. The heightened estrogenic effects, which are mediated through the stromal tissue, are observed to be associated with the development of prostatic hyperplasia [19].

The combined findings from several studies suggest that the androgen receptors in the prostate stroma play a crucial role in prostate development. Felix-Patrício *et al.,* emphasized that Androgen/Androgen receptors signaling had a significant impact on various aspects of prostate development, including proliferation, differentiation, and morphogenesis [20].

Additionally, another study examined 84 adult male albino rats and found a decrease in the height of the epithelial cell layer and apoptosis of the epithelium of the prostatic acinus [21].

The observations of current study contradict some previous works, of them, findings by Gao *et al.,* and Christiansen *et al.,* who studied the mechanism of action of SARMs in different rat models and noticed significant effects on prostate tissue and seminal vesicle respectively, they attributed the outcome to the antagonistic activity which cause reducing in prostate weight and inhibiting growth of seminal vesicle, this leads them to consider SARMs as potential candidates for treating of benign prostate hyperplasia because of their ability to reduce prostate weight, those findings specified that SARMs can be of clinical utility, alone or as adjuvant treatment [22, 23].

Also, the present outcome disagrees with Budaya *et al.,* who recorded no histological changes in prostate mass and fibromuscular stroma in rats after the management of RAD140; they interpreted that finding as the selectivity of some types of SARMs for specific tissues that may be linked to the tissue-specific presence of coregulation proteins. The variations in the effect of SARMs can be traced to the inability of some types of SARMs to undergo aromatization [24].

On the other hand, several studies have addressed the impact of RAD140 on prostate cancer, finding that RAD140, as a SARM, has the potential to modulate androgen receptor signaling pathways in prostate cancer cells, leading to the inhibition of cancer progression and suggesting its potential as a therapeutic avenue [25, 26].

Unfortunately, SARMs are currently being marketed and sold as a dietary supplement, although they are unapproved drugs. The US Food and Drug Administration has issued warnings to consumers of the potential dangers of using these drugs in an unregulated or unapproved manner [27].

Case reports have also been published of self-medicating users of RAD140 presenting with acute myocarditis [28], and liver injury [29, 30].

The unregulated use of SARMs is difficult to track, as the dosage and duration are not controlled. Additionally, the source of the SARM may contain various other ingredients, and the reporting of side effects is not entirely reliable. Any future studies investigating the use of SARMs must properly track dosage, duration, and assess side effects [18].
Healthcare providers should be aware of the possible adverse effects on overall health and counsel individuals accordingly, especially those using SARMs without medical supervision [31]. It is worth mentioning that information from other studies examining the effect of RAD140 on experimental animals is limited, which in turn limits further interpretation of the current outcome.

Conclusion

In this study, it was approved that the administration of RAD140 resulted in prostate damage, which leads to suggesting the use of the lowest possible amount of RAD140 to minimize its harmful effects. Further studies are needed to compare the results of this study with those of other studies and to understand the mechanisms behind RAD 140's effects on other body organs, as well as to validate these findings in humans.

Acknowledgments

The author would like to extend gratitude to the employees of the Histology Unit/ Biotechnology Research Center / Al-Nahrain University for their assistance in the achievement of this work.

Conflict of interest: The author affirms that work was completed without any financial or commercial ties that might be seen as a conflict of interest.

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