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Histological changes in rabbits' testicles after silymarin treatment

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Abstract

This research was designed to study the effect of silymarin dosage on the histological structure of the testicle in rabbits and the impact it has on the level of sperm production in these animals. Thirty adult male rabbits were used to complete this work. Their ages ranged between 5 to 6 months, while their weight ranged between 1,500 to 1,700 grams. The animals were divided into three groups based on the amount of dose of silymarin given as follows (C- 10 rabbits given free diet served as a control group: T1-10 rabbits treated with an oral daily dose of 0.5 ml per kg B.W. T2-10 rabbits treated with orally daily dose 1 ml per kg B.W.). The various types of cells were identified in this study based on several criteria, the most important of which are the external appearance of these cells during the series of divisions, the size of the cell, and the shape of the nucleus. The results showed an improvement in the architecture of seminiferous tubule epithelium, the disappearance of vacuolar degenerations in more than 95% of this epithelium, and an increase in the number of mature sperm in the center of each tubule in addition to increasing all parameters of Leydig cells. In conclusion, the testicles in rabbits exposed to silymarin are positively affected, leading to improved sperm production.

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Introduction

Herbal medicine was a successful treatment, although it was less well-liked (1). Most herbs are used to treat some diseases, like Flax seeds, which have significant efficacy in attenuating climacteric symptoms, decreasing oxidative stress, and increasing serum levels of leptin in menopausal women (2). A combination of herbs has a robust protective impact against pancreatic damage produced in diabetic rats given alloxan, a diabetes-induced drug, and it also has a therapeutic protective effect against diabetes (3). The pomegranate seed helps the rabbit's skin recover from the damage it causes (4). Historically, silymarin has been used to treat varicose veins, gastrointestinal system difficulties, menstrual abnormalities, and diseases of the liver and biliary tract (5), wound healing (6), and treatment of herpes labialis ulcer (7,8). Because of its effective antioxidant properties, silymarin substantially impacts sperm and oocyte quality to enhance fertilization. It prevents radical production, binds certain radical species, interferes with lipid peroxidation of membranes, and raises the cellular level of scavengers (9). Silymarin has also been found to have aromatase-inhibitory properties, which may help explain why testosterone levels are rising. This suggests that silymarin may enhance spermatogenesis and testicular cell function by preventing oxidative stress (10). The test is mainly comprising seminiferous tubules and interstitial tissue containing Leydig cells. Spermatogenesis occurs in seminiferous tubules, and Leydig cells are in charge of secreting the male sex hormone testosterone to keep spermatogenesis active (11). The seminiferous epithelium includes Sertoli cells, primary and secondary spermatogonia, spermatocytes, and spermatids, which evolve into adult spermatozoa, as well as other stages in the development of germ cells (12).

This research was designed to study the effect of silymarin dosage on the histological structure of the testicle in rabbits and obtain an impact on the level of sperm production in these animals.

Materials and methods

Ethical approve

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine within the University of Baghdad No. P. G. 1917 in 7/5/2024.

Animals

Thirty male healthy mature rabbits, ages 5 to 6 months in weight (1400- 1600) gram, purchased from Iraqi local markets, were housed in the College of Veterinary Medicine laboratory animal house at the University of Baghdad. In the animal house, the rabbits were left for two weeks before starting the treatment to become accustomed to the new environment to which they were transferred. During this period, the light program was 12 hours a day and 12 hours of darkness, and the temperature ranged between 23ºC and 27ºC, emphasizing the animals getting proper ventilation (13), the treatment started from November 22 to January 5.

Experimental design

Depending on the amount of silymarin dose provided to each group, the animals in this study were divided into three equal groups, including ten adult male rabbits. The division was as follows; 1st group (C): Control group: The animals in this group were given food only. 2nd group (T1). The animals of this group were dosed with silymarin orally in a dose of 0.5 ml/kg of body weight daily throughout the experiment. 3rd group (T2), the animals were dosed orally with 1 ml/kg of B.W. daily with Silymarin. The dosed solution of silymarin was prepared by dissolving 1 gram of silymarin in the form of powder in an aqueous solution of 20 milliliters; thus, the concentration of the dosed material was 0.05. The loading dose period was 45 days as one spermatogenesis cycle. When the trial period was completed, high doses of anesthetics were used (15 mill gram/kg. xylazine + 105 mill gram /kg. ketamine) to complete the euthanasia process (14). It is necessary to mention that this study was carried out with the following instructions and ethical controls of animal care sciences and methods of dealing with them, in addition to making the necessary efforts to reduce the danger and suffering during the experiment or during killing through humane methods of injecting each animal with euthanasia materials.

Histological protocol

The testicles were carefully extracted from the animals and then washed in sufficient saline solution to remove the fatty tissues attached to them. The testicles were cut into small pieces, followed by the fixation process, which preserved them with sufficient amounts of neutral buffer saline (15). The samples preserved with fixative materials were transferred to the laboratory to perform the routine histological steps to prepare histological slides.

Results

The present work revealed that the testes in animals of the control group have normal architecture surrounded by a layer of fibrous bundles of connective tissue called tunica albuginea. Many partitions are projected from this capsule to separate the parenchyma of the testes into a high number of divisions called lobules, each involving different compartments named convoluted seminiferous tubules. In treated groups, there is a more pronounced increase in branches of trabeculae from the capsule, leading to increased density and number of compartments of seminiferous tubules (Figures 1 and 2). In the control group, the seminiferous tubules appeared circular or elongated to oval, somewhat dispersed or not aligned with each other, with irregular edges in most of them, encircling by a clear basement membrane that was rich with peritubular myoid cells and acting to separate these tubules from the compartments of interstitial tissue. Inside each tubule were complex stratified epithelium represented by two distinguished populations of cells: the highly proliferating spermatogonia cells, which are arranged in different layers or stages, and the non-proliferating supporting Sertoli cells. Despite this, the germinal epithelium appears in many areas to suffer from degeneration through the presence of many empty vacuoles spread between cells in places of tubular germ cells, which can be described as apoptosis. Sometimes, it may come to the absence or presence of small numbers of mature sperm in the lumen of some seminiferous tubules (Figure 3).

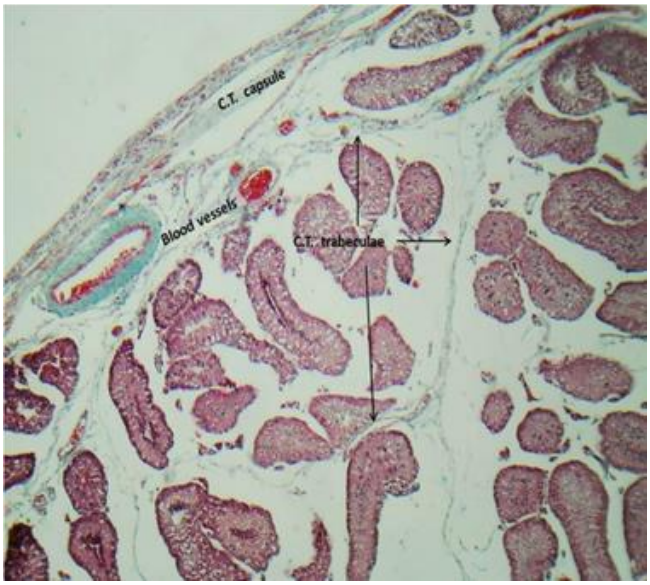

Figure 1: (control group) Many partitions projecting from the C.T. capsule to separate the parenchyma into highly number of lobules, each lobule comprises different numbers of ST. Masson trichrome stain. 100x.

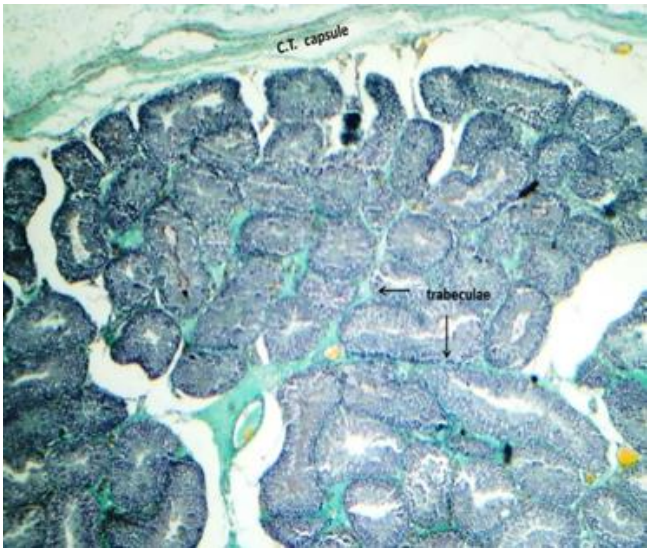

Figure 2: (3rd group). Obvious increase in of density and number of compartments of seminiferous tubules (ST) within each lobule. Masson trichrome stain. 100x.

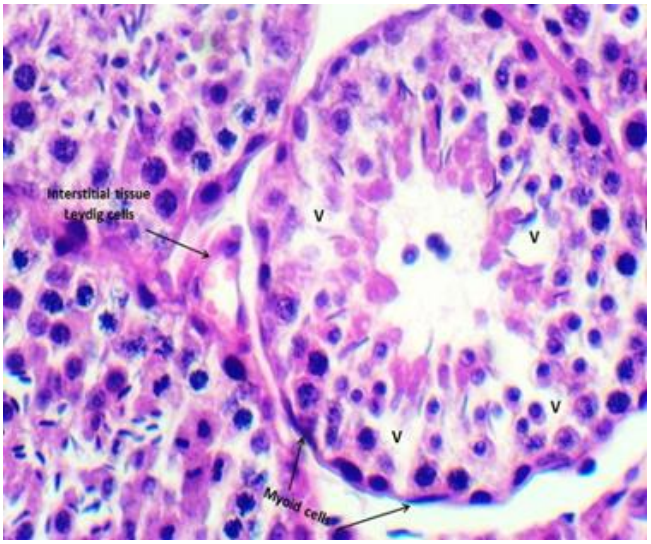

Figure 3: (control group) Circular ST with germinal epithelium appears to suffer from degeneration in many areas through the presence of many empty vacuoles spread between cells. H&E stain. 40x.

In the group of animals that were treated with silymarin, the results of these groups showed that there was a slight improvement in the architecture of seminiferous tubule epithelium of 2nd group through the appearance of vacuoles between the cells of the tubular epithelium, but with a smaller number of despite the increase in the height of the germinal epithelium in these tubules. All the same, some seminiferous tubules still contain a small number of mature sperm, some of which do not contain any of these sperms, but in a lesser way than the tubules in the control group. The seminiferous tubules appeared more compact in this group than in the control group. These tubes were close to one another through the basement membrane surrounding them, which contained myoid cells with almost the same density, and this membrane appeared more regular in its outline (Figures 4 and 5).

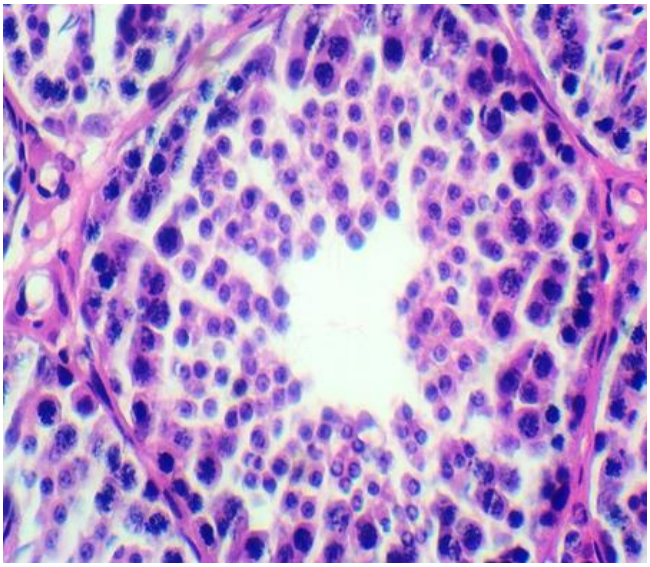

Figure 4: (2nd group). Appearance of vacuoles between the cells of the tubular epithelium, with a smaller number, than in the control group, in addition to increase in the height of the germinal epithelium. H&E stain. 400x.

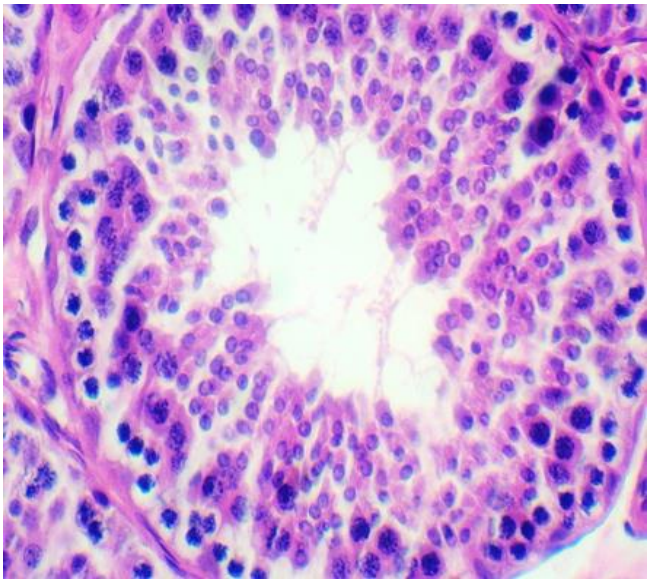

Figure 5: (2nd group) Despite the increase in the height of the germinal epithelium. Some ST still contain a small number of mature sperm. H&E stain. 40x.

In a related, this study showed the finding in the animals of the 3rd group that the germ cells in the epithelium of the seminiferous tubules of the testis witnessed a more significant development compared to what happened in the 2nd group through the disappearance of vacuolar degenerations in more than 95% of the seminiferous tubules. These degenerations are no longer seen except in very few tubules that can be considered abnormal compared to their counterparts in the same group. The significant improvement and the remarkable positive difference in the animals of this group was in the almost complete disappearance of the seminiferous tubules devoid of mature sperm. These tubes appeared to be closed to each other as in the first treatment group, but all of them contained medium or large numbers of mature sperm in the lumen of the seminiferous tubule. The great convergence between the seminiferous tubules in the animals of this group was evident through the contact of the basal lamina of the adjacent tubules with each other, which led to the miniaturization of the interstitial tissue between the tubules compared to the control group (Figures 6 and 7). This study also showed that the interstitial tissue of the testicle in animals dosed with silymarin does not differ significantly from its counterpart in the control group in terms of shape. Still, the number of cells within it varies, especially Leydig cells, which increase in number, singly or in cluster form. Even the clusters appeared in greater numbers in the treatment groups than in the control group. This means increased active Leydig cells within interstitial tissue (Figure 8).

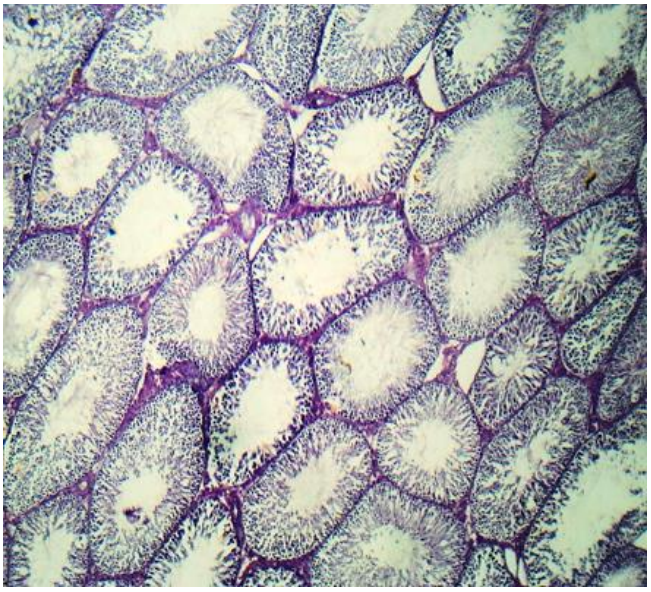

Figure 6: (3rd group). Contact of the basal lamina of the adjacent ST with each other, led to the miniaturization of the interstitial tissue between the tubules compared to the control group. H&E stain. 20x.

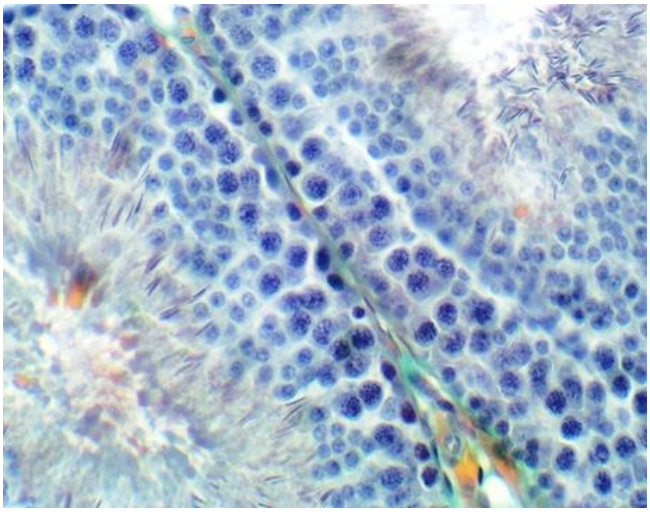

Figure 7: (3rd group). Disappearance of vacuolar degenerations in epithelia of seminiferous tubules as well as containing large numbers of mature sperm in the center of each tube. Masson trichrome stain. 40x.

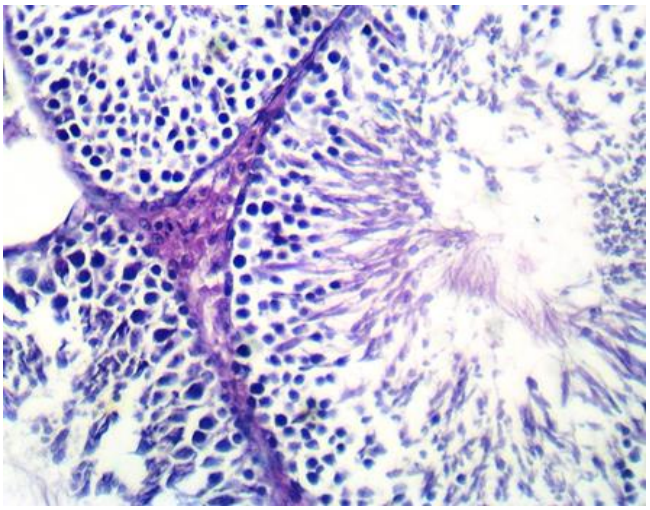

Figure 8: (3rd group). Increase in number of Leydig cells, whether singly or in clusters. H&E stain. 40x.

Discussion

In general, the testes of animals in this group revealed an enhanced spermatogenesis process. It is very natural to consider that the apparent histological changes in the seminiferous tubules of the two treatment groups are a result of the effect of silymarin dosed to the animals of these groups, which is considered (silymarin) an antioxidant substance capable of improving the process of sperm formation by reducing the damage generated from oxidative stress. Because testicular tissue has a significantly more significant proportion of unsaturated fatty acids than other

tissues and has a very rapid rate of division of cells and the mitochondrial usage of oxygen, oxidative stress plays a significant role in the incidence of male infertility. Furthermore, there is considerable competition between cells for oxygen because of the low partial pressure of oxygen caused by weakening testicular arteries (16). Consequently, the male reproductive system and testicular tissue are more vulnerable to the negative effects of oxidative stress. On the other hand, oxidative stress increases, and germ cell death and subsequent spermatogenesis are triggered by exposure to X-rays, environmental pollutants and chemicals, and some physical disorders (such as varicocele). However, under normal circumstances, genetic makeup and metabolic processes impact the body's capacity to create antioxidants, which help prevent oxidative stress's harmful consequences. In addition, external elements like nutrition, toxins, and chemicals can impact this ability. As a result, the body's antioxidant system cannot stop all free radicals or prevent the negative effects of oxidative stress. Thus, applying antioxidants and creating antioxidant treatment can disrupt the oxidative chain reaction and significantly enhance the body's capacity to combat oxidative stress caused by free radicals, thereby enhancing the spermatogenesis process (17).

Returning to cell functions, we find that this increase in all parameters of Leydig cells is considered a positive change because it leads to an increase in the secretion of the hormone testosterone, which is necessary to complete the process of sperm formation. Castro *et al.* (18) stated that testosterone's activity is necessary for spermatogenesis. Either this hormone can attach to a carrier like albumin, which transports testosterone over the lymph spaces and into the seminiferous tubule, or it can diffuse to the tubule. Because the seminiferous tubules are so close to the Leydig cells, there are high local testosterone concentrations around them. According to Sharpe *et al.* (19), the level of testosterone required intratesticular in rats to sustain spermatogenesis is estimated to be between 25 and 45 percent of the normal levels. Testosterone is necessary for spermatogenesis to begin and continue during adolescence and maturity. Furthermore, meiosis must be completed, and the spermatids must be differentiated (20).

Conclusion

The testicles in rabbits exposed to silymarin are positively affected, leading to improved sperm production.

Acknowledgments

We express our thanks to College of Veterinary Medicine, University of Baghdad to support current study.

Conflict of interest

There was no conflict of interest.

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التغيرات النسيجية في خصيتي الأرانب بعد العلاج السيليمارين

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الخلاصة

صمم هذا البحث لدراسة تأثير جرعة السيليمارين على التركيب النسيجي للخصية في الأرانب وتأثير ذلك على مستوى إنتاج الحيوانات المنوية في هذه الحيوانات. ولإنجاز هذا العمل تم استخدام ثلاثين أرنباً ذكراً بالغاً تراوحت أعمارهم بين من ٥ إلى ٦ أشهر، فيما يتراوح وزنها بين ١٥٠٠ إلى ١٧٠٠ جرام. تم تقسيم الحيوانات إلى ثلاث مجموعات على أساس كمية جرعة السيليمارين المعطاة على النحو التالي: (١٠- أرانب أعطيت نظام غذائي مجاني كمجموعة مراقبة. ١٠- أرنب تعامل بجرعة يومية عن طريق الفم قدرها ٠,٥ مل لكل كجم من وزن الجسم كمجموعة علاج اولئ.١٠- أرانب تعامل بجرعة يومية عن طريق الفم قدرها ١ مل لكل كجم من وزن الجسم كمجموعة علاج ثانية). تم التعرف على أنواع الخلايا المختلفة في هذه الدراسة بناءً على عدة معايير أهمها المظهر الخارجي لهذه الخلايا أثناء سلسلة الانقسامات، وحجم الخلية، وشكل النواة. أظهرت النتائج تحسنا في بنية ظهارة الأنبوبة المنوية، واختفاء التنكسات الفجوية في أكثر من ٩٥% من هذه الظهارة، وزيادة في عدد الحيوانات المنوية الناضجة في مركز كل نبيبة بالإضافة إلى زيادة جميع معلمات ليديج الخلايا. في الختام: تتأثر الخصيتين في الأرانب المعرضة للسيليمارين بشكل إيجابي، مما يؤدي إلى تحسين إنتاج الحيوانات المنوية.

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