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Histometric evaluation of testis in rabbits treated with Silymarin

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Abstract

This study aimed to demonstrate the expected positive effect of Silymarin on the histometrical parameters of the testis in rabbits, which is reflected in the process of sperm production. A total of 30 adult healthy male rabbits, weighted 1500- 1700 g and aged 5- 6 months, were used and divided into three comparable groups according to treatment doses of Silymarin. dentification of germ cells or Sertoli cells is done based on many considerations: the size of these cells, their outline features within the sequences of division, the level of their acrosome, and the shape of their nucleus. The results show that the thickness of the capsule, number of germinal cells, Sertoli cells, and Leydig cells were significantly increased in the first and second groups as compared with the control group, while the average diameter of the seminal tubule was higher in the control group than the treated groups. In conclusions, the silymarin has a very clear effect on the histological measurements in the testicle and the number of cells present in it, which leads to improving the process of sperm production.

Keywords: Testis, Silymarin, Leydig cells, Sertoli cells, sperm production.

Introduction

Medical treatments have utilized medicinal herbs since ancient times. Many studies have been conducted across the world to confirm their effectiveness, and some of the results have prompted the development of plant-based medications (1). Medicinal plant products have a yearly global sales value of more than $100 billion (2). The rabbit, a

domestic animal that is widely available, is a workable model if the conditions are met (3). Silybum marianum is frequently used in the treatment of a variety of disorders (4, 5). Silymarin (C25H22O10), a polyphenolic and flavonoid molecule, is isolated from silybum marianum seeds (5-7). Silymarin is useful for a variety of conditions, such as male and female infertility (8). These substances have various biological characteristics, but they are also known to be antioxidants (9, 10). In 1959, silybin, the main structure and active component of silymarin was identified as the earliest member of a recently discovered family of naturally occurring chemicals known as flavonolignans (5, 11, 12). Numerous studies have proven silymarin's antioxidant and protective qualities against the negative effects of chemotherapy medications and environmental pollutants on sperm (13). The testes' natural functions include the synthesis of sex steroid hormones and the generation of spermatozoa for reproduction. (14). The creation of spermatozoa for reproduction and the synthesis of sex steroid hormones are two of the testes' physiological functions (15). Thus, disruption of seminiferous tubules by histological changes in the testis stroma may be deleterious to spermatozoa produced (16). However, there is a complicated relationship between male fertility, the testis, and herbal entities. Many different medical traditions have used herbal medicines to increase fertility for thousands of years, but studies have shown that some botanical entities, including herbal medicines, are toxic to the testis and/or suppress male fertility (17, 18).

Materials And Methods

Animals

30 adult healthy male rabbits obtained from the Iraqi market, weighing 1500- 1700 g and aged 5-6 months, were maintained in the animal house unit (University of Baghdad - College of Veterinary Medicine). They were housed at a controlled temperature 25◦C ± 2◦C, with a light program of 12 hours at light, 12 hours at dark, with a suitable ventilated system. Before loaded doses, the animals were left for two weeks to acclimate to the laboratory environment (19).

Experimental Design

An equal design of grouping was done; 30 rabbits were divided into three comparable groups, each group contained 10 male rabbits according to treatment doses of Silymarin as follows:

C- Rabbits were given free diet served a control group.

T1- Daily doses rabbit treated 0.5 ml per Kg B.W.

T2- Daily doses mice treated 1 ml per Kg B.W.

We dissolved 1 gram of silymarin in 20 ml of saline solution, resulting in a concentration of 0.05. e loading dose period spanned 45 days, representing one cycle of spermatogenesis. Following the conclusion of the dosage period, an overdose of anesthetics (105 mg/kg ketamine + 15 mg/kg xylazine) led to the rabbits' euthanasia. (20). The experiment was achieved in correspondence with the ethical

regulations of the sciences on animal's care and handling. Each animal was humanely injected with solutions of euthanasia and all necessary efforts were made to decrease the risk of suffering. The testes were extirpated gently after injection of euthanized solutions, from anterior to posterior border, and immediately carefully washed with enough normal saline to remove any adipose tissue that might have attached to them, the testes were placed in large amounts of fixation liquid (neutral buffer solution). The preserved samples were transferred to be subjected to routine histological techniques (21).

Histometrical Technique of Cells

The histometrical techniques used to count the cells in the testicular section were analyzed quantitatively and investigated at high power (x 100) objective on a MEIJI microscope MT4200 L (Tokyo, Japan) with an ocular micrometer calibrated previously, then captured at different magnifications. All these investigations were done at stage VII of spermatogenic cycle, which is almost characterized by regular divisions of spermatogenic cells and has at least 5 layers of cell types in the epithelia of tubule, these are spermatogonia's, preleptotine, leptotine, (zygotine if present), pachetine and spermatid. (22). This manner gives attributions to fixed the allocated sites of sterio-measurments of quantitative spermatogenic cells to avoid variations of stages differences. Approximately 25-40 at least transverse sections of seminiferous tubules that were ring-shaped or nearly circular were selected randomly and measured for each group of animals. identification of germ cells or Sertoli cells done based on many considerations: the size of these cells, their outline features within the sequences of division, level of their acrosome and shape of their nucleus (23).

Results and Discussion

Capsule Thickness

The present work shows that the measurements of capsules were determined by the composition of their components; the thickness of dense regular connective tissue was 133.4 ±619.4 μm in the control group, while it was 248.2 ±972.8 and 398.7 ±972.8 in the first and second treated testis, respectively (Table 1). Treated groups are more likely to increase branches of trabeculae and increase the density and number of compartments of seminiferous tubules. It is clear that the thickness of the capsule of the testicles of animals treated with the drug silymarin increased with a significant difference from that of the control group animals, which leaves no room for doubt that this increase occurred as a result of this material, and that considered a positive change because this part of the testicle (the capsule) helps a lot in regulating the passage of blood towards the testicle or supporting the movement of sperm towards the epididymis, in addition to many other physiological functions.

The testicular capsule serves as more than just a barrier between the testicular parenchyma and other tissues, as evidenced by its pharmacological and physiological functions. The testicular capsule's capacity to contract and relax in response to various

stimuli unmistakably points to a more complex function for the testicles. Further research is required to solidify the testicular capsule role, even though the primary functions associated with testicular capsule motor functions are the transfer of sperm throughout the testis toward the epididymis, Interstitial pressure, and testicular blood flow regulation (24).

Diameter of the Seminal Tubule

The current study showed that the average diameter of the seminal tubule in the control group was 288.15 + 14.26 µm, while the average diameter of these tubules in the first and second dosing groups was as follows: (265.29 + 11.93 and 259.91+ 9.17) (Table 1). The hypothesis of this study suggests that the material's impact led to an increase in enzyme activity, resulting in higher testosterone hormone levels. This, in turn, positively influenced the growth of various germ cell types within the tubules, leading to a decrease in tubule diameter. From a scientific standpoint, the above-mentioned hypothesis can be considered acceptable in explaining the decrease in the diameter of the seminiferous tubules of the testes of animals dosed with silymarin, as it follows a path parallel to what (25). The effect of quercetin (as flavonoids) on the diameter and thickness of the seminiferous tubules in the testes of rats was studied by (26), she mentioned that quercetin stimulate testosterone synthesis in testis through increase the activity of enzymes which are responsible for synthesis testosterone hormone which is essential for normal growth of testis that result to increase the primary and secondary spermatocyte and spermatid, therefore increasing seminiferous tubules thickness and decrease seminiferous tubules diameter.

Spermatogenic and Sertoli Cells

As showed in (Table 2) the differentiated and numerical quantity of different spermatogenic cells were expressed as 1 cell: the number of cells branched or generated there from. The results showed a significant increase in all types of measured cells in the treated groups compared to the control group.

In the current study, Although there is no clear difference in the values of the numbers of Sertoli cells in both groups dosed with silymarin, the results showed in (Table 1) that there is a significant difference in the average value of these cells from their counterparts in the control group. This difference may be due to the role that silymarin played in increased numbers of Sertoli cells because it plays a prominent role in controlling the environment surrounding the seminiferous tubules by facilitating the process of transforming the germ cells in the epithelium of these tubules into mature sperm through the effect of the hormone's testosterone and FSH, which act on the Sertoli cells.

The predominant somatic cells found in seminiferous tubules are called Sertoli cells (SCs), and they are thought to be the primary regulators of spermatogenesis. The overall number of Sertoli cells controls the potential to produce sperm as every Sertoli cell may maintain a certain number of germinal cells (27).

Interstitial (Leydig) Cells

All numerical measurements related to Leydig cells showed a significant increase in the groups dosed with silymarin the control group. These measurements included three criteria, which are: the number of Leydig cells / S.T., the numbers of Leydig cells present within the clusters and the number of clusters of Leydig cells / S.T. (Table 3). This study places a very high probability that the dosing of silymarin is the reason for the improvement in the criterion for increasing the number of Leydig cells, which has a very effective role in improving fertility standards and protecting sperm.

In (2002), it was asserted (28) that testosterone's activity is necessary for spermatogenesis. This hormone can either diffuse into the tubule or connect to a carrier, such as albumin, which carries testosterone across lymph gaps and into the seminiferous tubule (29). In 2002, it was asserted (28) that testosterone's activity is necessary for spermatogenesis. It is believed that the intratesticular levels of testosterone required in rats to sustain spermatogenesis are between 25 and 45 percent of the normal levels.

Table (1): Capsule Thickness, Diameter of the seminiferous tubule and number of Sertoli cells/ S.T. in control and treated groups.

Parameter	Control	T1	T2
Capsule Thickness	133.4 ± 619.4 a	248.2 ± 972.8 a	398.7 ± 972.8 a
Diameter of the seminiferous tubule	288.15 ± 14.26 b	265.29 ± 11.93 b	259.91± 9.17 b
Sertoli cells	3.05 ± 0.071 c	4.32 ± 0.031 c	4.24 ± 0.081 c

Table (2): Numerical quantity of different spermatogenic cells. 1 cell: number of cells branched or generated therefrom it.

Parameter	Control	T1	T2
Spermatogonia to Preleptotene	2.39±0.170 a	2.784±0.465 b	3.280±0.721 c
Preleptotene to leptotene	3.57±0.111 a	3.856±1.201 b	3.984±1.136 c
leptotene to Pachytene	3.168±0.082 a	3.305±0.952 b	3.612±0.656 c
Pachytene to Spermatid Step VII	0.819±1.126 a	16.789±0.701 b	18.991±1.786 c
Spermatid: to Spermatozoa	44.731±5.304 a	57.621±3.748 b	39.816±2.417 c

Table (3): Number of Leydig cells / S.T. , numbers of Leydig cells within the clusters and number of clusters of Leydig cells / S.T.

Parameter	Control	T1	T2
number of Leydig cells / S.T.	3.124±1.1+0 a	4.184±0.212 b	4.752±0.140 c
numbers of Leydig cells within the clusters	2.752±0.128 a	3.981±0.067 b	4.305±0.023 c
number of clusters of Leydig cells / S.T.	0.440±0.017 a	1.051±0.311 b	1.666±0.473 c

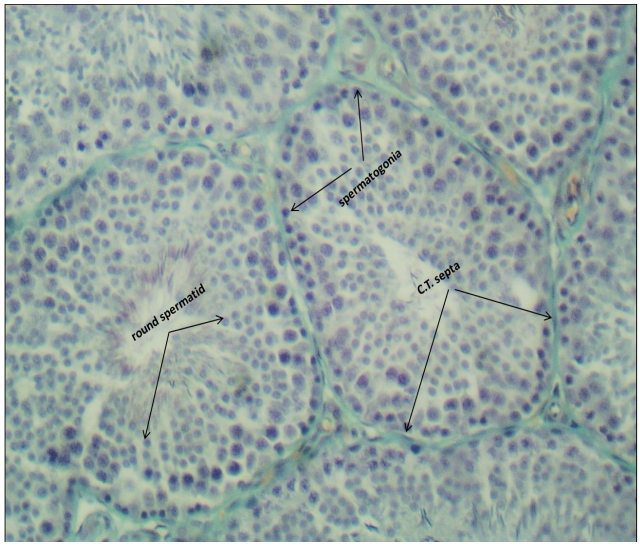

Figure 2: Different layers of spermatogenic cells with C.T. septa that separate between seminiferous tubules. 2nd treated group. Masson trichrome stain 200x

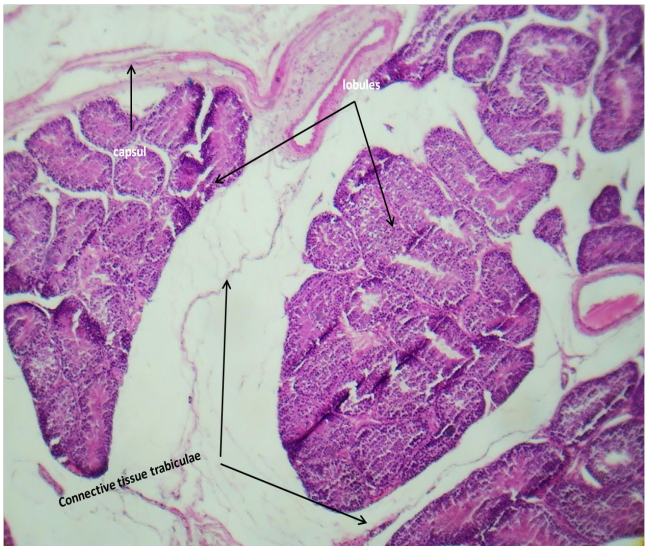

Figure 1: Capsule in testis of 1st treated group with C.T. trabeculae that divided the testis into many lobules. HE stains. 100 x.

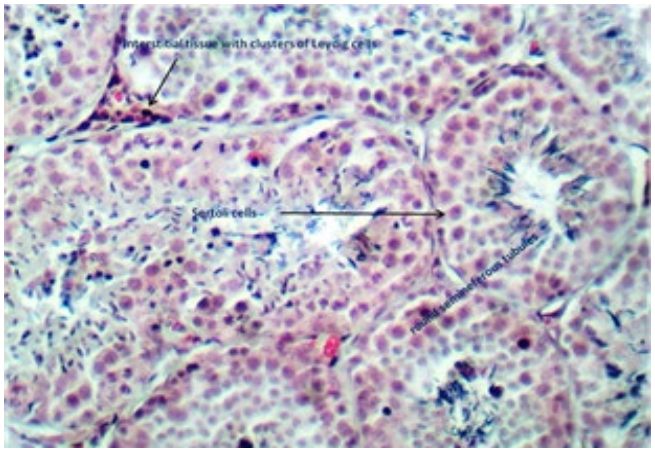

Figure 4 : Different stages of spermatogenesis and cluster of Leydig cells within interstitial tissue . Control group. HE stain 200x

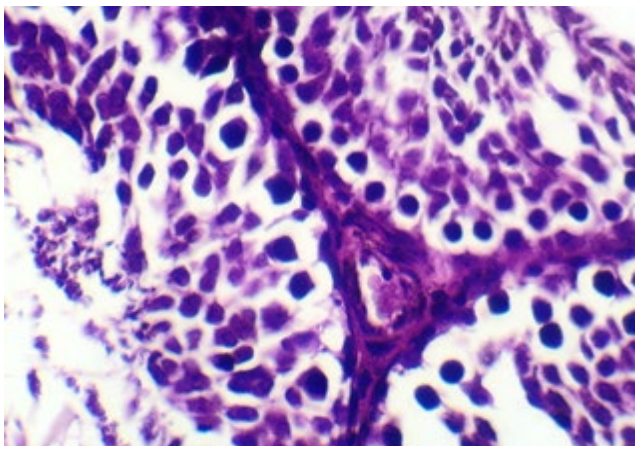

Figure 3 : number of round S.T. in 2nd treated group with Sertoli cell and interstitial tissue have cluster of Leydig cells. 2nd treated group. HE stain 400x

Conclusions

This study concluded that silymarin has a significant effect on histological measurements and cell numbers in the testicle, which in turn positively affects the process of sperm formation.

Conflicts of interest

The authors declare that there is no conflicts of . interest

Ethical Clearance

This work is approved by The Research Ethical . Committee

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التقييم النسيجي للخصية في الأرانب المعالجة بالسيليمارين

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الخلاصة

هدفت هذه الدراسة إلى بيان التأثير الإيجابي المتوقع للسيليمارين على القياسات النسيجية للخصية في الأرانب، والذي ينعكس على عملية إنتاج الحيوانات المنوية. 30 حيوانا من ذكور الأرانب البالغة الأصحاء تم استخدامها، بأوزان 1500-1700 جرام، وأعمار تتراوح بين 5-6 أشهر، وتم تقسيمها إلى ثلاث مجاميع متماثلة حسب جرعات العلاج بالسيليمارين. تم تحديد الخلايا الجرثومية أو خلايا سيرتولي بناءً على العديد من الاعتبارات: حجم هذه الخلايا، وملامحها الخارجية ضمن تسلسل الانقسام، ومستوى الأكروسوم الخاص بها، وشكل نواتها. أظهرت النتائج زيادة معنوية في سماكة الكبسولة، وأعداد الخلايا الجرثومية وخلايا سيرتولي وخلايا لايديغ في المجموعة الأولى والثانية مقارنة بمجموعة السيطرة، في حين كان متوسط قطر النبيبات المنوية أعلى في مجموعة السيطرة منه في المجموعات المعالجة. في الاستنتاجات: للسيليمارين تأثير واضح جداً على القياسات النسيجية في الخصية وعلى عدد الخلايا الموجودة فيها مما يؤدي إلى تحسين عملية إنتاج الحيوانات المنوية.

الكلمات المفتاحية: الخصية، السيلمارين, خلايا لايديغ, خلايا سرتولي, إنتاج النطف.