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Histological changes of extract cumin cyminum on the leydig cells, seminiferous tubules and epididymis in adult rabbits (*Oryctolagus cuniculus*)

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Abstract

The primary histological alterations caused by using the cumin plant and associated with rabbit fertilization are the subject of the present investigation. The current study used ten mature, healthy male rabbits have been readied for testing, with five rabbits in each treatment and control group. The treatment group received an extract from the cumin plant at a dose of 250 mg/kg b.w/orally administered once daily for six weeks, while the control group received a regular diet and water. An extract was made from each 25 g of dried cumin powder, and it was subsequently diluted with 10 ml of boiling distilled water. The extraction process took thirty minutes to be finished. After filtering each extract, its concentration was adjusted to provide 250 mg/ml of sample per milliliter. After that, the solutions are kept in a container that is tightly closed. The primary histological changes detected by hematoxylin and eosin in this study were damage to seminiferous tubules and a decrease in the thickness of the germinal layer of spermatogonia. In addition, the Leydig cells (Interstitial cells) in the interstitial space were weak and deteriorating. Moreover, the arrangement of epithelial cells in the epididymis duct with empty spermatozoa was seen. Cumin consumption in large doses causes a lot of histological changes that show up in the male reproductive system.

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Introduction

Herbs and other components can treat or ward off illness, as is well-known (1-3). Due to their accessibility, safety, and use, some edible herbal plant species, including cumin (*Cuminum cyminum* L.), are frequently employed as food additives. Cumin contains two numbers (14 chromosomes) and is a component of the flowering plant, tribe Ammineae, and subtribe Carinae (4-6). After black pepper, cumin is the second most commonly used seed kind. It is an annual plant with several therapeutic, nutraceutical, and pharmacological characteristics. It is among the oldest and most widely used aromatic and herbaceous natural products. Additionally, cumin is commonly used in the culinary, beverage, alcohol, pharmaceutical, cosmetic, and toiletry sectors (7,8). Despite the common uses of cumin in many medical fields as anti-diabetic and antimicrobial activity, its frequent use damages the male reproductive system. It leads to histological changes (9). Some inflammatory markers that treatments supplemented with C. cyminum have been demonstrated to impact significantly are adiponectin, high sensitivity C-reactive protein (hs-CRP), and TNF (10-12). This article extensively discusses *C. cyminum* generally beneficial properties (13). The use of ingredients to flavor various food compositions, including cheese, pickles, soups, beans, and alcoholic drinks, is widespread. Cumin is an aromatic herb commonly used as a food supplement globally. It is also employed in herbal medicine, particularly veterinary medicine (14-16). Seeds have demonstrated antioxidant qualities and help treat colic, diarrhea, and cholera (17).

Cumin seed extract is also a microorganism-fighting oil (18,19). Most medicines and other active pharmacological compounds now under development originate from the natural kingdom. Given the high toxicity of various synthetic medications, the therapeutic use of herbal medicines has increased over the recent decade, dramatically increasing the number of herbal medicine makers (20,21). The cumin compound has been shown to have active antioxidant properties, which can restrain lipid peroxides and free radicals (22). In diabetic rats, it possesses the capacity to lower plasma lipid profile (23).

Even though cumin is often used in medicine, too much might lead to hormonal abnormalities; therefore, this study was done to recognize the most significant histological changes.

Materials and methods

Experimental design

In the current study, ten adult male local rabbits weighing approximately 1150-1300 g have always been separated into two groups (5 group). The control group received a typical diet and water, whereas the treatment group received cumin extract 250 mg/kg b.w. through stomach tube feeding (24). For all groups, the experiment lasted for six weeks. By administering intramuscular injections of xylazine 0.5 ml and ketamine, all study rabbits were killed 0.5 ml.

Cumin extraction

Cumin powders were purchased from the Kerbala city market. Each 25 g of dry cumin powder was used to prepare an extract, after which it was treated with 10 ml of boiling distilled water. The extraction process required thirty minutes. Each extract had been filtered, and the content was modified so that each ml of sample was equal to 250 mg/ml. Those solutions are subsequently stored in a tightly sealed container (25).

Histological preparations

The testis served as the source of the anatomical samples. The samples, which were about 0.5 cm in size, were then preserved in 10% Neutral buffered formalin for 48 hours. Hematoxylin and eosin satin were used with conventional histological methods to distinguish distinct tissue components (26). The germinal layer of the seminiferous tubules and the interstitial space occupied by Leydig cells and all tissue sections have been incorporated into the histological measurements. The histological characteristics were analyzed with the help of a digital USB microscopic camera (Canon 550D, 18 Megapixel, Japan), which was attached to a Novel microscope. A stage micrometer has been employed to correlate the objective lenses with the program (27).

Ethical approve

Under the reference number UOK.VET.AN.2022.052, this research was carried out in the anatomical laboratory of the College of Veterinary Medicine at the University of Kerbala.

Results

Histological features

The illustration shows the typical characteristics of the testes in the control group, including regular histological features with well-developed spermatids and a germ cell lining performing cell proliferation. Furthermore, we observed Sertoli cell activity and their presence in their usual location in the basement membrane of seminiferous tubules, which revealed the arrangement of spermatogonial stages. The mean diameters of seminiferous tubules were approximately 98.14 µm (Figure 1). In the control group, Leydig cells were observed to be active; these cells appeared as a massive regular, with non-granular and spherical cytoplasm and abundant nuclei with visible nucleoli. The majority of the nuclei are located in the cytoplasm's middle. The mean thickness of the Leydig area was 152.21 µm (Figure 2). Moreover, the histological features of the rabbit epididymis duct in the control group were lined by pseudostratified columnar epithelium and a large number of spermatozoa in the epididymis lumen (Figure 3).

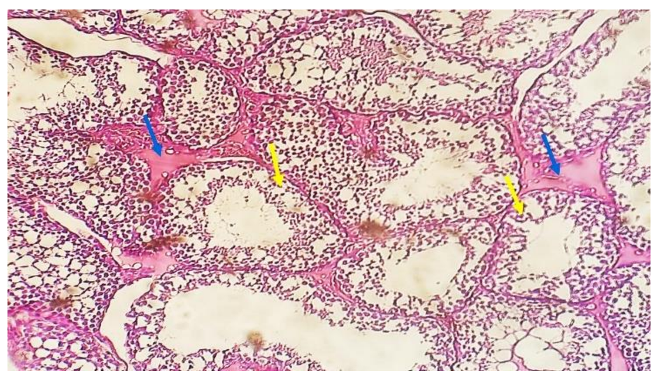

Figure 1: Photomicrograph of control testis in rabbits showing the normal histological features in semiferoius tubules (yellow arrows), and activity secretion in interstitial space (blue arrows). H&E stain 10x.

When the histological testis characteristics of the extract-cumin group were examined under the microscope, numerous changes were observed, including the destruction of the seminiferous tubules, a significant number of damaged cells, the absence of spermatozoa in the tubule lumen output, spermatogonia detaching from the basal membrane in some areas, and an increase in the space between the seminiferous tubules, the mean diameters of seminiferous tubule about 50.22 µm (Figure 4). In this Group, we observed the decrease in the number of Leydig cells an appearance of inactivity, limited cytoplasm, and psychotic nucleus the mean thickness of Leydig area was 92.45 µm (Figure 5). The epididymis duct appears empty in this group due to spermatozoa in the epididymis lumen (Figure 6).

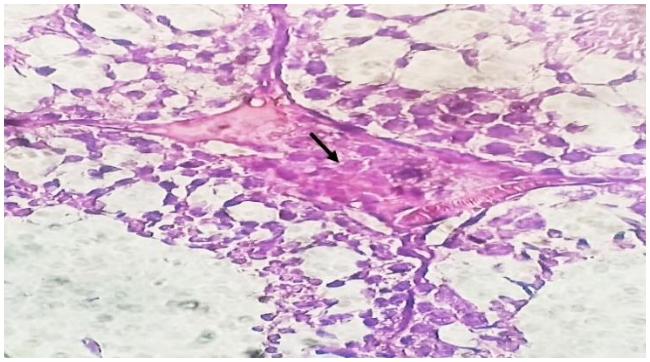

Figure 2: Photomicrograph of control testis in rabbits showing the crowded and activity in leydig cells (black arrow). H&E stain 40x.

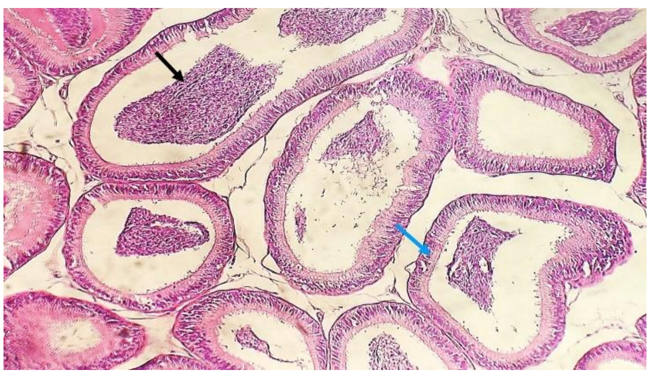

Figure 3: Photomicrograph of control epididymis in rabbits showing a lot of sperms in ducts of epididymis (black arrow), and activity lining epithelia (blue arrow). H&E stain 10x.

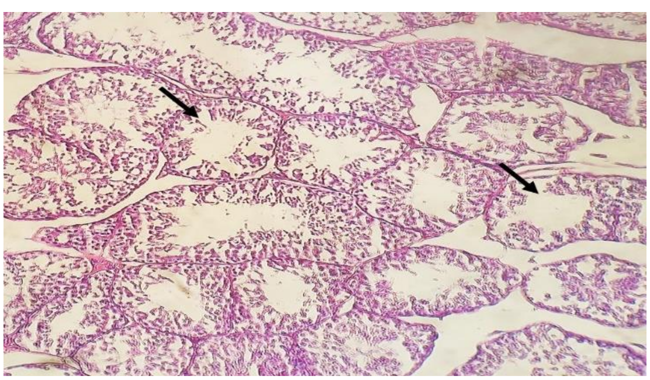

Figure 4: Photomicrograph of extract cumin testis in rabbits showing the damage in semiferoius tubules (black arrows). H&E stain 10x.

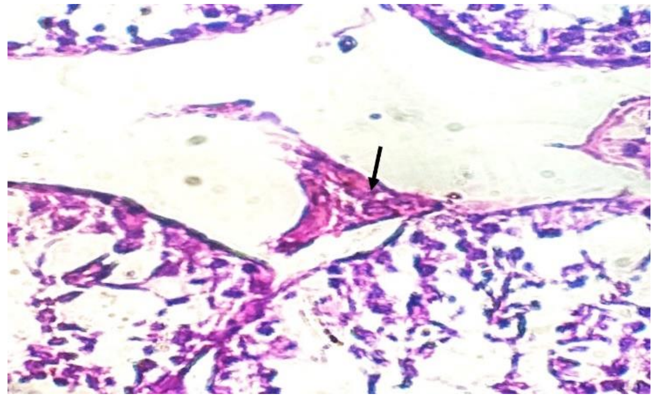

Figure 5: Photomicrograph of extract cumin testis in rabbits showing the leydig cells in active and smaller area size (black arrow). H&E stain 40x.

Figure 6: Photomicrograph of extract cumin epididymis in rabbits showing of ducts of epididymis from sperms (black arrow), and inactivity lining epithelia (blue arrow). H&E stain 10x.

Discussion

The current study shows the typical appearances of the testis in the control group, including regular histological features with very well-developed germ cell lining performing cell proliferation. In contrast, the current study found numerous modifications in the extract-cumin group, including destruction to the seminiferous tubules, a significant number of damaged cells, the absence of spermatozoa in the tubule lumen output, spermatogonia detaching from the basal membrane in some areas, and an increase in the space between the seminiferous tubules. This outcome is similar to that of Abdi *et al.* (28) and Taha (29), which mentions that in a healthy condition, spermatogonia and the basement membrane are nearby in the seminiferous tubules, where germ cells are alive and appear active. Unnoticed changes also included the degradation of specific spermatogenic cells and an accumulation in the tubular hollow. These significances might have resulted from excessive consumption of cumin extract, which is widely used as a body slimming agent and may have caused weight loss.

The control group observed regular histological features with the activity of Sertoli cells in the control group; additionally, the Leydig cells had nuclei located in the cytoplasm's middle, a wide interstitial area that was occupied by Leydig cells, whereas cumin describes a decrease in the number of Leydig cells, inactivity appearance, limited cytoplasm, and psychotic nuclei in the extract group. Findings are akin to Tripepi *et al.* (30) and Malhi *et al.* (31) those that describe the Leydig cells. They were found in groups or cords of various shapes and sizes with round nuclei and granular cytoplasm. These cells seem to be distributed randomly, some being perivascular, others related to vessels. The testicular hormone was markedly lower in these results compared to the control group, comparable to those of Willatgamuwa *et al.* (32), which evaluated the therapy with a water extract of the cumin plant. These studies, however, contradict Smith *et al.* (33), Hipler *et al.* (34) and Fonseca *et al.* (35) that claimed that the Cumin therapy was ineffective when administered alone in sperm characteristics and had a detrimental impact. Gupta *et al.* (36), Sharifi *et al.* (37) and Al-Hafedh *et al.* (38). The testicular tissue may have acquired changes due to all these histological changes caused by hormone changes. Consequently, these variations need the cells responsible for adapting these mechanisms to maintain the new metabolic balance.

The present study explains why the epididymis duct in the extract cumin team appeared to be vacant of spermatozoa, in contrast to the morphological features of the rabbit epididymis duct in the comparison group, which were lined by a significant number of spermatozoa in the epididymis lumen and had a regular appearance of pseudostratified columnar epithelium. Our findings are consistent with those of others who believed that some variables determined by sperm concentration are elements of sperm parameters that significantly affect male fertility Roshankhah *et al.* (39), Al-Allaf *et al.* (40) and Amin *et al.* (41). Herbal medicine may be widely used in many cultures to treat conditions like obesity and to enhance sperm quality. Hence, excessive use always has a harmful impact on the amount and quality of sperm production.

Conclusion

It is clear that eating unbearable cumin in large amounts results in a variety of histological alterations in the male reproductive system. The study concluded that cumin extract 250 g/kg b.w. caused germ cell damage in seminiferous tubules and Sertoli and Leydig cell degeneration, resulting in rabbit infertility.

Acknowledgment

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Conflict of interest

There is absolutely no apparent conflict, according to the author.

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التغييرات النسجية لمستخلص الكمون على خلايا لايدك، النبيبات المنوية والبربخ في الارانب البالغة

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الخلاصة

إن التغيرات النسيجية الأولية الناتجة عن استخدام نبات الكمون والمرتبطة بالإخصاب في الأرانب هي موضوع التحقيق الحالي. أخذت في الدراسة الحالية عشرة أرانب ذكور سليمة ناضجة للاختبار، خمسة أرانب لكل من مجموعة العلاج والسيطرة. تلقت مجموعة العلاج مستخلص نبات الكمون بجرعة ٢٥٠ مجم / كجم من وزن الجسم / عن طريق الفم مرة واحدة يومياً لمدة ٦ أسابيع بينما تلقت المجموعة السيطرة حمية طبيعية وماء. تم تحضير المستخلص ٢٥ غم من مسحوق الكمون المجفف، وتم تخفيفه بعد ذلك بـ ١٠ مل من الماء المقطر المغلي. استغرقت عملية الاستخلاص نحو ثلاثون دقيقة بعد ذلك تم تصفيته للتخلص من الشوائب العالقة، تم ضبط تركيزه لتجهيز ٢٥٠ ملغم/مل من المحلول المراد تجريعه. بعدها، تم حفظ المحلول في حاوية مغلقة بإحكام. اهم التغيرات النسيجية الأولية التي تم الكشف عنها كانت بواسطة استخدام

صبغة الهيماتوكسيلين والأيوسين بينت الدراسة الحالية كان هناك تلف كبير واضح جدا في نسيج الأنابيب المنوية وكذلك انخفاض واضح في سمك الطبقة الجرثومية للحيوانات المنوية. بالإضافة إلى ذلك، كانت خلايا لايديك (الخلايا البينية) في الفراغات النسجية البينية ضعيفة ومنتكسة. علاوة على ذلك، كان ترتيب للخلايا الظهارية غير منتظم وقناة البربخ الخالية من الحيوانات المنوية ومن الواضح أن استهلاك الكمون بجرعات كبيرة يسبب الكثير من التغيرات النسيجية التي تظهر في الجهاز التناسلي الذكري.