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The effect of watery extract Cyminum on the testes of the local Rabbits (Oryctolagus cuniculus)

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***Abstract* — This study was conducted to investigate the effect of watery extract of Cumin plant (200 mg/kg B.W.) on some histological changes of the male reproductive system in albino rabbits. Sixteen adult male rabbits weighting about (200-250 g) and 12-15 weeks age were used in the present study and divided into (2) groups, 8 rabbits per group, group1: serve as a control group and given normal diet with drinking water, group2: Given Cumin extract (200 mg/kg B.W.) through oral tubular feeding, the experiment were continued 60 days for all groups. The results of the current study showed significant decrease (P<0.05) in serum testosterone compared with control group. As for ectopic tissue, water extract (200 mg/kg) has resulted in satisfactory tissue changes of the testes. The water extract of Cumin plant acts as an antioxidant through its important role in curbing the harmful effects of some types of free radicals within the body and histological changes and functions of the male reproductive system of rabbits. The results of histological diagnosis of testes in male rabbits that giving cumin for long periods leads to a decrease in the level of testosterone**
***Keywords* — Testis, effect of Cyminum, Rabbits.**

I. INTRODUCTION

CUMIN is a versatile aromatic plant utilized globally as a food ingredient, exemplifying the widespread appeal of spices to enhance many dishes, including cheese, pickles, soups, beans, and alcoholic beverages. It is utilized in traditional medicine, particularly in veterinary practice (1). Cumin seeds have antioxidant characteristics and are beneficial for indigestion, diarrhea, and cholera, serving as a remedy for indigestion and colic. Additionally, cumin seed oil is utilized as an active agent against microorganisms. The medicinal advantages were primarily attributed to the presence of active constituents and potent compounds, including terpenes, phenols, and flavonoids. Additionally, it encompasses essential fatty acids and diverse classes of compounds, such as terpenes, alcohols, phenols, and aldehydes, specifically cumin aldehyde, eugenol, B-Benin, and several minor constituents. The botanical kingdom is the primary source of the majority of medicines and other active pharmaceutical compounds still to be found. In the last ten years, the therapeutic application of herbal medicines has proliferated globally, prompted by the significant toxicity associated with numerous synthetic drugs, leading to a marked rise in herbal medicine producers. The cumin product exhibits significant antioxidant activity, capable of inhibiting free radicals and lipid peroxides. (6). It has the ability to reduce cholesterol levels in plasma for diabetic rats (7). Cuminum cyminum was used as an antioxidant in male rabbits by measuring the level of testosterone (8) It was noted that the testes of adult rams are located within a specialized pouch of skin known as the scrotum. The testes are encased by a capsule known as the tunica albuginea, which consists of strong collagenous tissue. This capsule is enveloped by the tunica serosa, whose connective tissue merges with the tunica albuginea. The tunica albuginea is continuous with the loose collagenous tissue of the mediastinum testis, partitioning the testis into lobules (lobuli testis). The tubules of the lobule, comprising the seminiferous tubules and rete testis, are encased in loose collagenous tissue abundant in reticular fibers (9,10) reported that the testicular seminal pathway in camel is divided into two portions; an intratesticular and extra testicular portion. The extra testicular portion consist of the ret testis, an extension of the intratesticular portion and an elongated sac from which 6-7 ductuli efferent originated. Studies which are done by (11) revealed that the long straight seminiferous tubules in the male camel are lined with simple cuboidal cells to simple columnar. The different types of germ cells of the sperm lie in seminiferous tubules and represents different phases within the sperm development. The wide base of Sertoli cells stands on the basement membrane of seminiferous tubules and it is usually that the surface of Sertoli cell contains the old spermatids. The interstitial cells (Leydig cells) are being polygonal, organized in groups or nest and well supplied by blood capillaries and occupies the spaces that lies among the seminiferous tubules. The researchers also observed that the rete testis is divided into an internal portion within the testis and an exterior portion. The exterior portion of the rete testis constitutes a sac at the convergence of the ductuli efferentes. Spermatocytogenesis

commences with spermatogonia located on the basement membrane of seminiferous tubules and advances toward the lumen. The emergence of spermatids signifies the conclusion of spermatogenesis and the commencement of spermiogenesis (11).

II. Materials and Methods

A. Animals Housing and Experimental Design

sixteen adult male albino rabbits weighting about (200-250 g) were used in the present study and divided into (2) groups, 8 rabbits per group, group1: serve as a control group and given normal diet with drinking water, group2: given Cumin extract (200 mg/kg B.W.) through oral tubular feeding and the experiment were continued 60 days for all groups.

Collection of Plant and Preparation of Extract:

Cumin powders were obtained from the local market in Kerbala city. The water extract of Cumin powder was prepared based on (14).

Blood Sampling:

At the end of each experiment, the animals were anesthetized with ketamine and xylazine for several minutes, after that the front and hind limbs of the animal were fixed with pins. blood samples were taken directly from the heart by cardiac stenosis, collected about 8-10 ml of blood, placed in Test tubes free of anticoagulants left for about a quarter of an hour at room temperature (15) In the centrifuge at 3000 / rpm for 15 minutes and take serum, and kept at (-20 °C) in new, clean plastic tubes (Plane Tubes) until required biochemical tests were carried out.

Dissection and Removal of Organs:

The testis was extracted using specialized forceps and severed with scissors at the groin duct level, subsequently placed in a Petri dish containing a saline solution (0.9% NaCl) to isolate it from the surrounding adipose tissue, and preserved in 10% formalin for histological analysis.

Histological Sections Preparation:

Samples of testes were directly fixed in 10% buffered formalin for 24 hours and then processed for paraffin method by dehydrating through ascending concentrations of ethanol (60%, 70%, 80%, 90%, 95% and 100%), cleared in xylene, infiltrated in paraffin wax and finally embedded in paraffin wax. Sections were cut at 5µm thickness with a rotary microtome (Hunting Don, Bright.UK). The sections were stained by hematoxylin and eosin (H&E) method (16).

Statistical analysis:

The outcomes were statistically examined utilizing the Statistical Analysis Package for Social Sciences (SPSS). To delineate the differences between the experimental groups, it is essential to emphasize these distinctions by calculating the standard error (SE). Statistical analyses were performed in accordance with Duncan *et al.* (17).

III. Results and Discussion

The choose of rabbit in this research is considered as an important animal for an anatomical representative of class Mammalia in comparative anatomy classes and its size makes it a convenient research animal. Each testis is found in a large sac or scrotum just ventral to the anus (fig 1,2). The scrotum when cut shows the following layers from the exterior to the inner

wall: (1) Skin, (2) Cremaster muscle and fascia, and (3) Tunica vaginalis, a thick portion of the peritoneum that lines the wall of the scrotum or parietal lamina and covers the testes or visceral lamina. This membrane is continuous with the serous membranes of the peritoneal cavity (fig 1,2) A thick fibrous connective tissue capsule (fig 3), the tunica albuginea,

covers each testis. The inner part of this capsule is the tunica vascular, a loose connective tissue layer that contains blood vessels. Each testis is divided into lobules by incomplete connective tissue septa and there is frequently intercommunication between the lobules. Each lobule is occupied by seminiferous tubules (fig 3) enmeshed in a web of loose connective tissue that is rich in blood and lymphatic vessels, nerves and interstitial or Leydig cells. All phases of event cycle of spermatogenesis and spermiogenesis in rabbit are observed within the seminiferous tubules. The seminiferous epithelium or germ cells show a regular organization, with the basally located spermatogonia, together with spermatocytes, occupying half of the layer of the spermatogenic epithelium. A great number of spermatids and spermatozoa are embedded in the cytoplasmic prolongations of the Sertoli cells. Early spermatids possess a central, rounded nucleus, with granular chromatin. The differentiation of the spermatids into spermatozoa involves the events of nuclear elongation, formation of the acrosomal and axonemal complexes and elimination of residual cytoplasm.

Histological effect of treatment on testis:

The normal feature of the testis in the control group were showed in the figure (3,4,5,6,7,8) in which healthy histological structure of rabbit testis having a germinal epithelium undergoing cell division and well-formed spermatids are seen. While microscopic examination of the testis tissues of the cumin group observed many changes included degenerative in some spermatogenic cells, large number of cellular debris was collected in the tubular cavity, seminiferous tubules showed few spermatozoa in the lumens, detaching of the spermatogonia from the basal lamina in

some places, an increase in the space between the seminiferous tubules, hemorrhage and decreasing in the number of Leydig cells between them, highly vacuolated

spermatogonia also were seen (fig. 9,10,11,12).

These results were disagreed with [18]. The appearance of degeneration and necrosis was explained by the inhibition of cumin for mitochondrial action by disruption of energy production and thus the effect on the sodium pump and the effect of the plasma membrane or the decrease in the pH of the cells through the increase in the production of lactic acid, produces proteins and thus inhibits the formation of the plasma membrane and the occurrence of necrosis. and decreasing in the number of Leydig cells between them, highly vacuolated

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Effect of treatment on Testosterone hormone:

A significant decrease in the level of testosterone in serum of male rabbits was observed in treatment group compared to the significant events ($P<0.05$) in serum compared to control group.

This study's results indicated a substantial reduction ($P<0.05$) in serum testosterone levels in male rabbits within the treatment group relative to the control group. This outcome contradicts the findings of (18). The reduction in sex hormone concentration may result from an increase in reactive oxygen species in Leydig cells, which impede the expression of steroidogenic acute regulatory protein in the mitochondrial membranes, crucial for hormone synthesis in these cells (19). The treatment group with cumin exhibits a substantial reduction in total sperm count compared to the control group. which contradicts the findings of the researchers (18). The effective oxygen classes degrade Leydig cells, which are responsible for testosterone secretion in the testes. This results in a diminished level of the hormone that regulates prostate and testicular functions, a reduction in sperm count, and the destruction of cells that support spermatozoa (Sertoli cells and spermatogenic cells), thereby hindering sperm maturation (20).

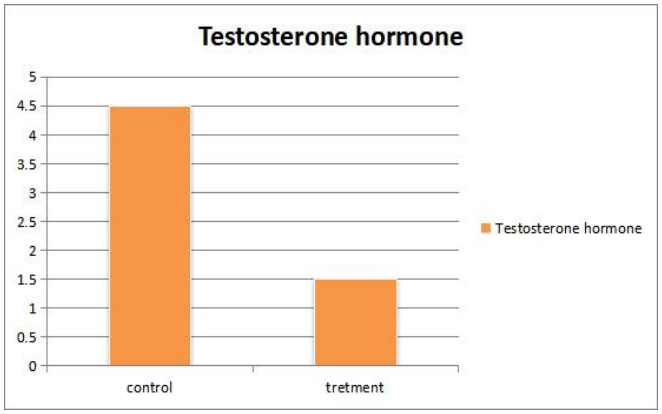

Figure 1: show the significant differences ($P<0.05$) between groups in The Testosterone Hormone serum level

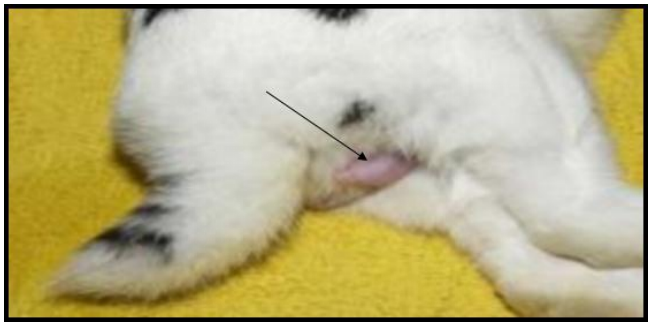

Figure 2: shows the testis of the rabbit

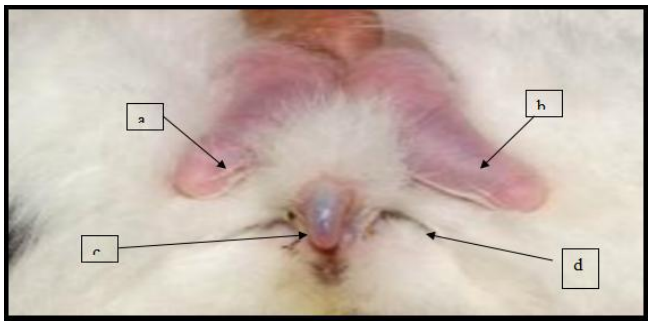

Figure 3: dissecting rabbit shows: a-right testis, b-left testis, c-penis, d-anal gland

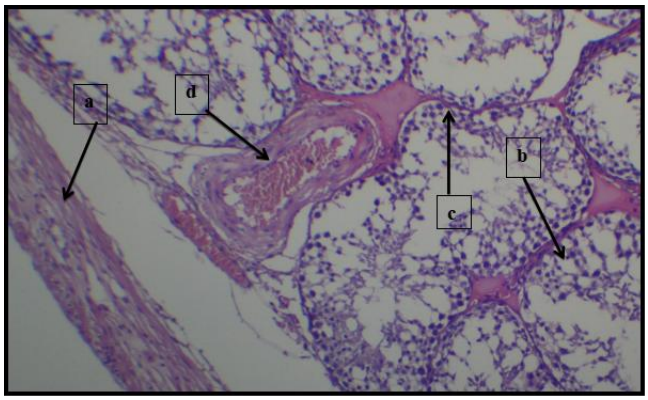

Figure 4: This figure shows a-capsule, b-seminiferous tubules, c- connective tissue septa, d- blood artery, H&E stain, 100x.

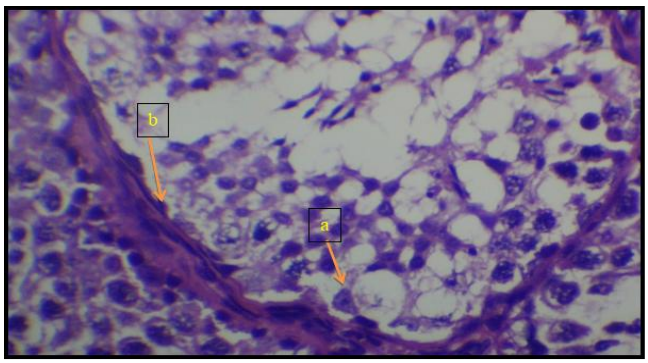

Figure 5: This figure show a-A-Sertoli cell, b-B-spermatogonia H&E stain 400x

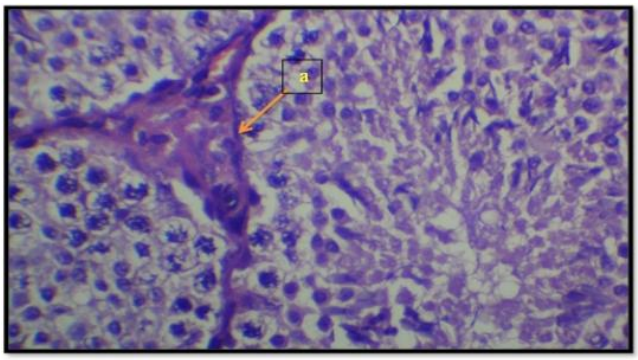

Figure 6: This figure shows a-Leydig cell H&E stain 400x

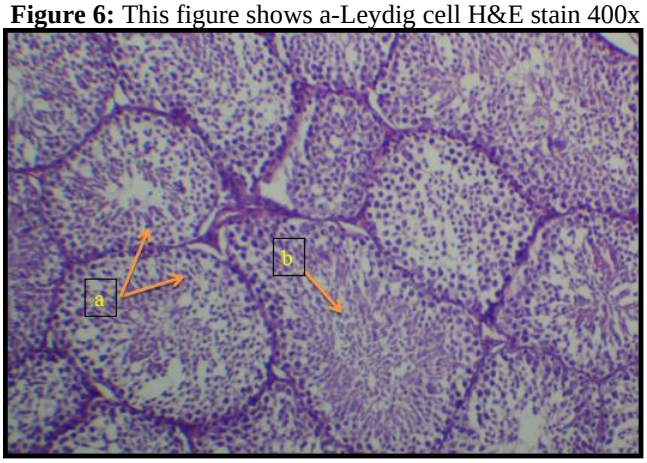

Figure 7: This figure shows a-seminiferous tubules, b–spermatid H&E stain 100x

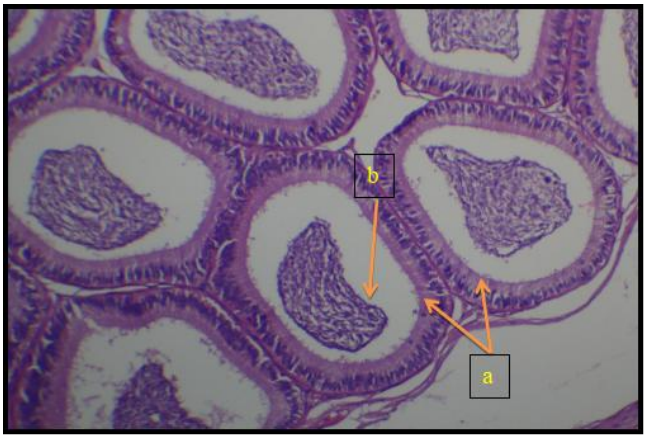

Figure 8: This figure shows a-epididymus, b–sperm H&E stain 100x

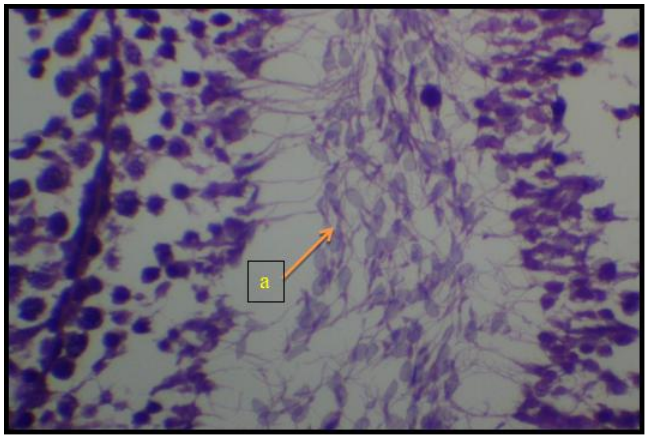

Figure 9: This figure shows a-sperm H&E stain 400x

IV. Discussion

1- The Cumin are harmful on the male genital system.
2- The Cumin are harmful on the testosterone level hormone.

There is a need for further proposing studies which may include:

1- Study the effects of cumin in female genital system in different animals.
2- Study the effects of cumin in male genital system with stress in different animals.

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