

Evaluation Alcoholic Extract Effect of *Vitex agnuscastus* on the Testes Histological Structure of Adult White Rabbits *Orectolagus cuniculus*

Hind Tahir Qadir¹, Thekra Atta Ibrahim¹, Mohammed Nsaif Abbas²

Department of Biology, College of Education for Pure Science, Diyala University, Diyala-Iraq¹
Environmental Engineering Department, College of Engineering, Mustansiriyah University, Baghdad-Iraq²



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Vitex agnus castus, chastetree, seminiferous tubes, Leyding cells and Sertoli cells

ABSTRACT

This paper aims to evaluate the effect of the alcoholic extract of the *Vitex agnus castus* on the histological structure of the testes of adult White rabbits. In this study, 12 White male rabbits were used, which were divided into two groups, The first being the control group and its number was 4 rabbits, and the second group included 8 rabbits, and this group in turn was divided into two groups with 4 rabbits for each group. The two groups of rabbits were injected with the alcoholic extract of the *Vitex agnus castus* at a concentration of 100 and 200 mg of extract/kg of body weight. One concentration per group is injected for 30th days. At the 30th days, the treated animals was sacrificed and the testes were removed from them, and then their tissues sections were prepared. The results obtained from this study showed that all the animals of the two groups of experiments that were treated with the extract showed a change in the thickness of the walls of the seminiferous tubes in the testes and their appearance became undulating and irregular. Also it was observed that some germ cells were Shed and depleted and collected in the lumen of seminiferous tubes and the appearance of Phagocytic cells inside the cavity and congestion of blood vessels. This study concludes that *Vitex agnus castus* can reduced the number of sperms in the testes and resulting in damage to the testes which the regularly usage can lead to infertility.



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1. Introduction

The widespread use of medicinal plants in Iraq back to thousands of years due to their rapid effectiveness, low cost, and low side effects compared to chemically prepare medicinal drugs [18]. Therefore, medicinal plants have been used in the treatment of many diseases caused by microorganisms due to the secondary metabolites contained in these medicinal plants, such as tannins, terpenoids, alkaloids and flavonoids [6]. The side and negative effects of chemical drugs used in the treatment of diseases have become known to all. Therefore, researchers' interest has increased in recent years in studying the use of herbs and medicinal plants as alternative therapeutic materials to chemical drugs [4]. This tendency is due to the negligible harm

effects comparing to the side effects of chemical pharmaceuticals. Among these plants is the *Vitex agnus castus* tree or chastetree. Chastetree is considered one of the ancient medicinal plants; it belongs to the deciduous family of the almond family Verbenaceae. But it was included in the Lamiaceae family, and it has a bitter aromatic smell due to the presence of some active substances in it, including flavonoids, glycosides and alkaloids [19]. *Vitex agnus castus* plant is also known by several names, including Chasteberry because it contains flavonoids that are very important for the treatment of many diseases due to its secondary metabolic products that are one of the components of the original plant estrogen that increases and stimulates the flow of menstruation, this plant has been used since ancient times In regulating and treating the female endocrine glands and improving postmenopausal symptoms (PMS), menorrhoea and infertility, it is therefore called the Emmenagogue herb [1]. It was also called monks pepper because monks first used it to lose sexual desire to maintain their chastity, as it is believed that the plant loses sexual desire, hence the name chastetree [23]. The medical and therapeutic importance of *Vitex agnus castus* plant is due to its possession of many active chemical compounds, including essential oil, flavonoids, and glycosides. Flavonoids are among the most important essential compounds of plants, which include casticin, vitexin, isovitexin, linoleic acid [8]. *Vitex agnus castus* plant also contains alkaloids, saponins, compounds such as xacein, vitexin, and precursors of steroid hormones, and all of these substances act on the pituitary gland, which is the gland responsible for regulating and secreting endocrine glands in the human body [15]. Several studies indicated the importance of *Vitex agnus castus* plant, including the study of [3] that *Vitex agnus castus* plant has an effect on the receptors of the neurotransmitter D2 dopamine known as the happiness hormone. Also, the study of [10] indicated that *Vitex agnus castus* plant does not affect the ovaries directly, but rather affects the pituitary gland through its effect on the process of releasing and releasing Follicle Stimulating Hormone (FSH) in females and testosterone in male mice, and thus a reduction in the testosterone hormone, which enters and contributes to the process of spermatogenesis, is the formation of male sperm, and thus its reduction and decrease. As indicated by a study conducted by [24] researcher, in which he showed that a woman with defects in the luteal phase, while progesterone levels remain normal, led to the length of the luteal phase for 3 months after taking nutritional supplements containing the herb vitex. While [5] confirmed that vitamins in vitex increase progesterone levels in addition to higher pregnancy rates in women who take nutritional supplements containing vitex for 3 months because vitex affects the activity of pituitary hormones. The present study aimed to determine the effect of the alcoholic extract *Vitex agnus castus* plant on the histological structure of the testes of adult rabbits.

2. Materials and methods

The current study was conducted with the approval of the Scientific Committee of the Department of Biology. The current study was designed based on the half lethal dose (LD50) of the alcoholic extract of the *Vitex agnus castus* which is valued in rabbits is 1650 mg/kg from the alcoholic extract of the *Vitex agnus castus* per kg of body weight [17]. Two concentrations of the extract were chosen to test their toxic effects which were 100 and 200 mg/kg. It was possible to calculate the amount of extract injected into rabbits used in this study, based on the following equation.

$$\frac{x}{D} = \frac{W_{rabbit}}{1000}$$

Where: x : the injected amount of alcoholic extract into rabbits measured by mg, D : the specified dose of the drug measured by mg/kg, W_{rabbit} : the weight of the rabbit used in the experiment measured by g.

Twelve white rabbits were used in this study that obtained from the animal house of the department of

biology –college of education for pure sciences / Diyala University, and their average weight ranged between 1000-1700 kg. The experiments extend from 27/1/2021 to 30/3/2021. No animal was excluded because they were within the conditions of the experimental, and suitable for conducting the experiment, and their ages ranged from 8-12 weeks. These rabbits were randomly divided into two groups, the details of which were as follows The first group is the control group with 3 rabbits, and the second group is the test group, which numbered 9 rabbits, and this group in turn was divided equally into three secondary groups, 3 rabbits for each group. The rabbits were injected with the alcoholic extract of *Vitex agnus castus* at a concentration of 100 and 200 mg/kg of alcoholic extract daily for 30 days. At the end of the experiment, the rabbits were anesthetized with chloroform, then the animals were dissected and the testes were removed from their site. Then Testicular organ samples were fixed with formalin solution for 24 hours and then washed with tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to the method [2]. Which the samples were examined and photographed using a light microscope equipped with a digital camera.

3. Results and Discussion

The results of the current study showed that the histological structure of the white rabbits treated with a concentration of 100 mg/kg of alcoholic extract of the *Vitex agnus castus* during a period of 30 days, there were changes in the histological structure of the testicles of rabbits if the expansion of the lumen of the seminiferous tubes and the occurrence of disintegration and necrosis in some germ layers of the seminiferous tubes as show in Figure 1 and the separation of the epithelial germ layer and its assembly in the lumen of the tubule. Also the results were shown irregular seminal epithelial layer. The results of the current study showed a rupture in some areas of the seminiferous tubule, degeneration in some Sertoli cells, and an increase in interstitial cells vas showed in Figures 2 and 3. The study also showed an increase in the thickness of the vascular wall, congestion, infiltration of inflammatory cells, and an increase in the formation of fibers, as shown in Figure 3.

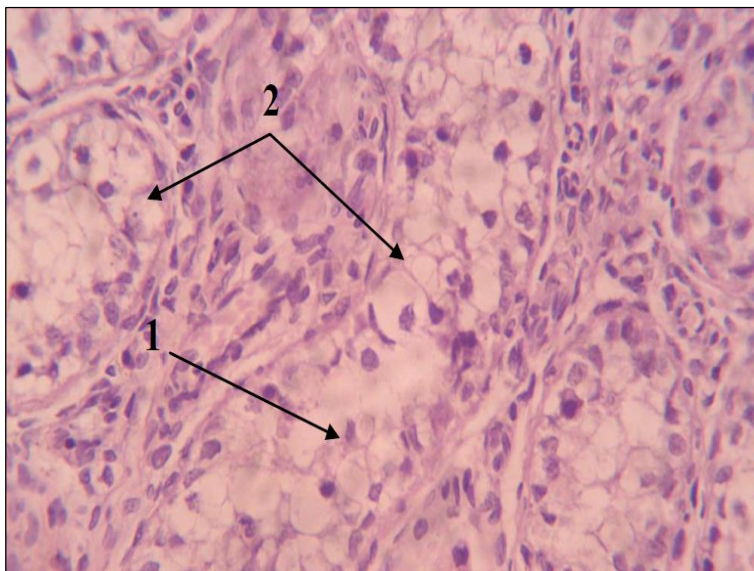


Figure 1 A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 100mg/kg. Note: 1- Expansion of the lumen of the seminiferous tubules and 2- Necrosis in the wall of seminiferous tubules. H&E, 40X

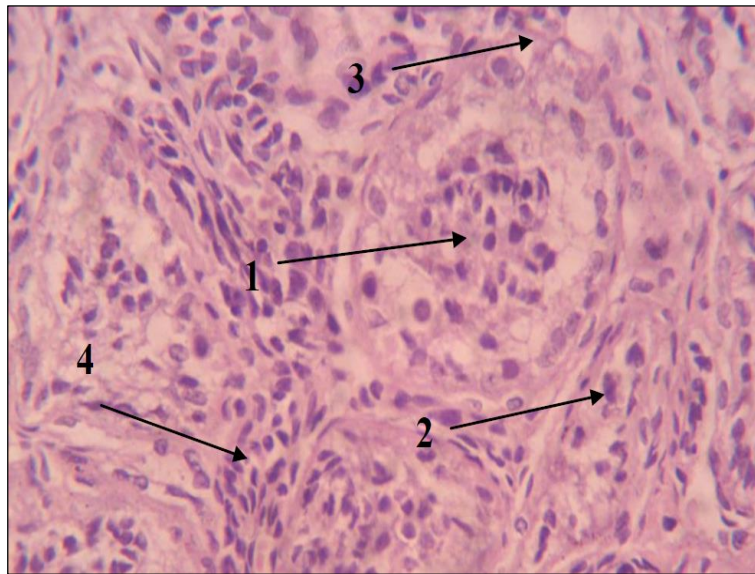


Figure 2 A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 100mg/kg. Note: 1- Dissection of germ cells and their collection in the lume of the tubes 2- Degeneration or Lysis of Sertoli cells 3- Vaculation 4- Interstitial cell. H&E, 40X

The results of the current study agreed with the findings of the researchers [16]. That a disturbance in the Sertoli cells will inevitably affect the germ cells and eventually lead to a defect in the testicular tissues. While [21] mentioned that the Sertoli cell has a necessary role in the development of germ cells through the formation of the testicular blood clot that protects the germ cells, and the transfer of nutrients and hormones to the germ cells. It is believed that all these pathological signs are due to a defect in the structure and function of Sertoli cells.

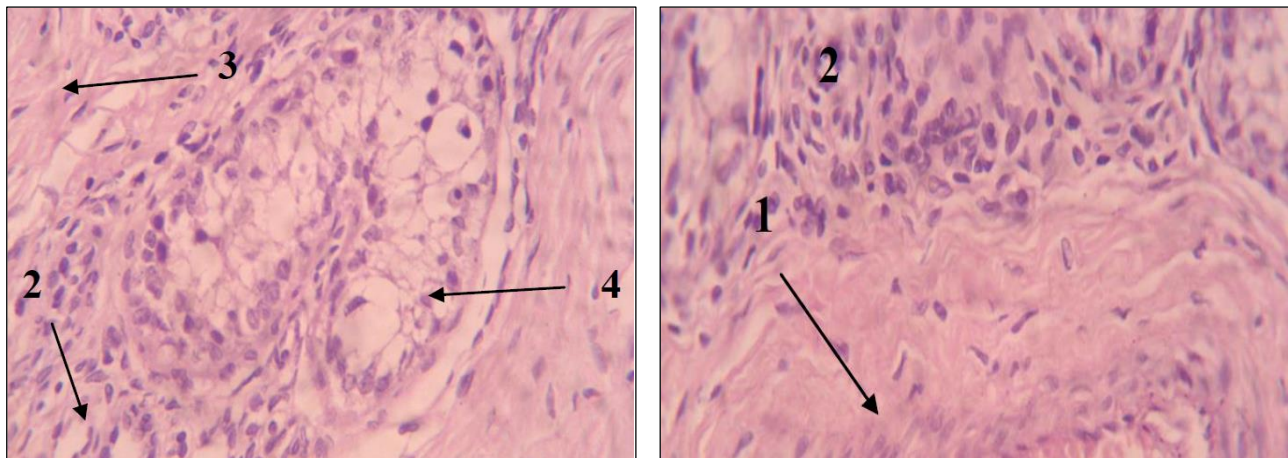


Figure 3: A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 100mg/kg. Note: 1- An increase in the thickness of blood vessel wall and its congestion 2- Infiltration of inflammatory cells, 3- Increase in fiber formation, 4- Sertoli cells degeneration. H&E, 40X

The results of the current study revealed that the extract of the *Vitex agnus castus* has an effect on Leyding cells and interstitial tissue. The results of the current study agreed with the findings of [13] that Leyding cells are a center for fertility regulation, through the production of the hormone testosterone. While [20] mentioned that Leyding cells are stimulating arachidonic acid and testosterone. As for the group of rabbits injected with a concentration of 200 mg /kg for a period of 30 days, it was possible to observe the changes

caused by the *Vitex agnus castus* on the composition of the testicles, in addition to the previous histological changes Which were the noticeable increase in the thickness of the walls of the seminiferous tubes and their contraction, where their general appearance became wavy and irregular [12]. As well as the occurrence of atrophy in the seminiferous tubes epithelium, its collection in the lumen of the tubule, the appearance of a space between Sertoli cells and the collapse of the cells, and the appearance of large phagocytes inside the lumen of the seminiferous tubules. As shown in Figure 4, the result of the current study agreed with the findings of the researchers [22] as they indicated that the basal Lamina that covers the seminiferous tubule plays an important function in the process of transporting materials between the interstitial villi and the germinal epithelium and preserving it in terms of shape, Structure and function where the researcher [25], as they indicated that Sertoli cells secrete type IV collagen fibers, Which probe the thickness of the walls of the seminiferous tubule, in thus leads to Weak spermatozoa. The study showed the depletion, Lysis, rupture and death of cells in some germ layers of the seminiferous tubes, the return of the spermatids and the mature sperm to the inside of the seminiferous tubes.

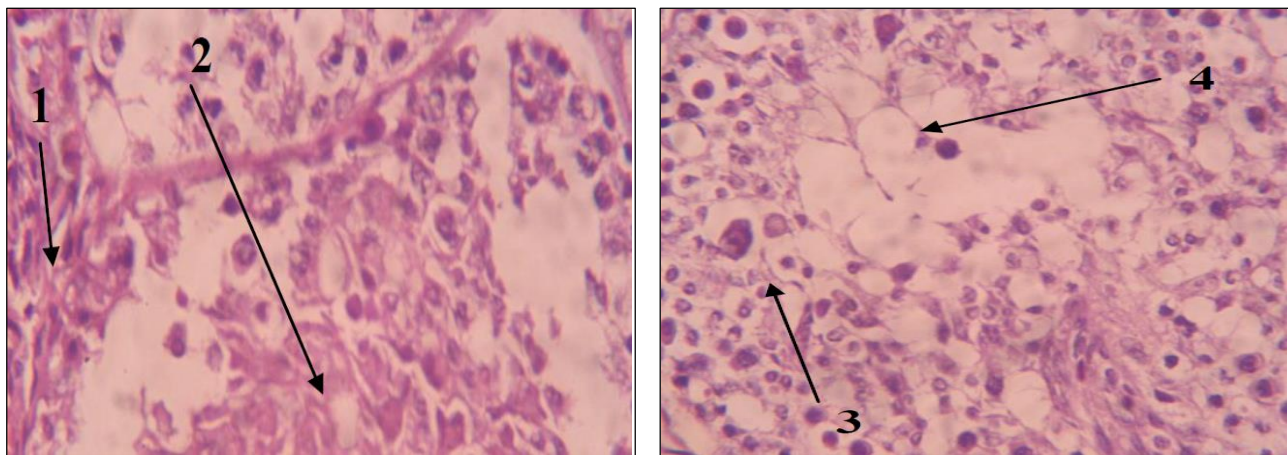


Figure 4 A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 200 mg/kg. Note: 1- The noticeable increase in the thickness and shrinkage of the walls of the seminiferous tubes, 2- Dissection of germ cells and their collection in the lumen of seminiferous tubes, 3- Necrosis of seminiferous tubes wall, 4- The appearance of large phagocytes in the lumen of seminiferous tubes. H&E. 40X

As shown in Figures 5 and 6, the study also showed congestion of blood vessels. The results of the current study agreed with the researchers study [9]. If they indicated that the increase in the thickness of the wall of seminiferous tubules weakens the relationship between it and the interstitial tissue with the increase in wall thickness, many pathological disorders appear within the testicle, mainly in the function of Sertoli cells, Which affects the differentiation of germ cells and inhibits the formation of spermatozoa. It is believed that the reason for this may be attributed to a defect in Sertoli cells, and this defect will in turn affect the essential proteins required in the synthesis process and necessary for the differentiation of germ cells, where these proteins are secreted at their highest level during the stage of spermatid differentiation. This result agrees with what was reached by [14] through their study related to the characteristics of sperm and the micro- structure of testes in mice after long term treatment with methanol. Also with What the researcher [7] mentioned that the retrograde movement of the spermatids and the mature sperm within the wall of the seminiferous tubes may be the result of its alerting to testicular toxicity by the aqueous extract of the turkey raisin plant.

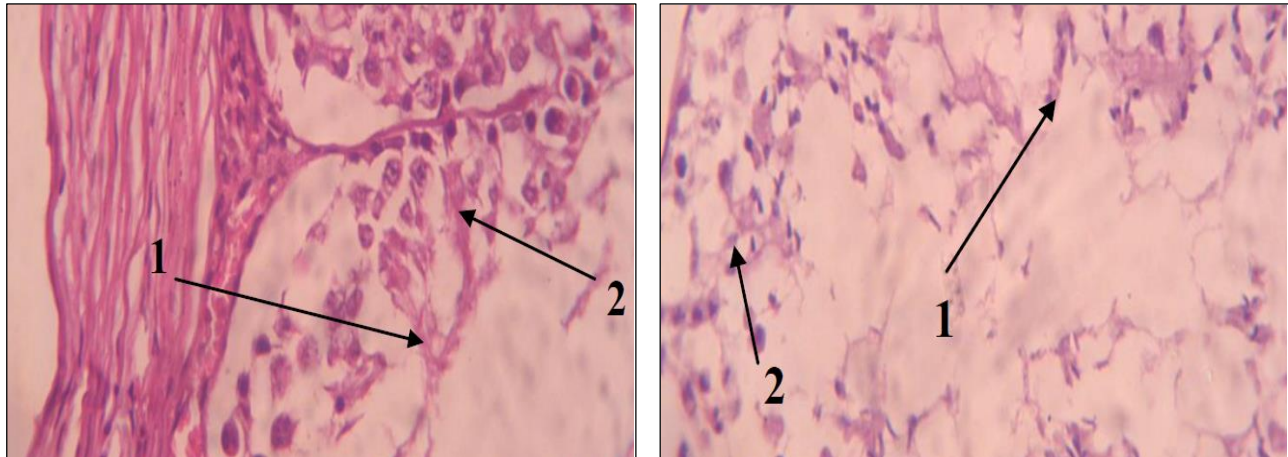


Figure 5 A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 200mg/kg. Note 1- Depletion decomposition and rupture occur in some germ Layers of seminiferous tubes, 2- Necrosis of the seminiferous tubes H&E. 40X

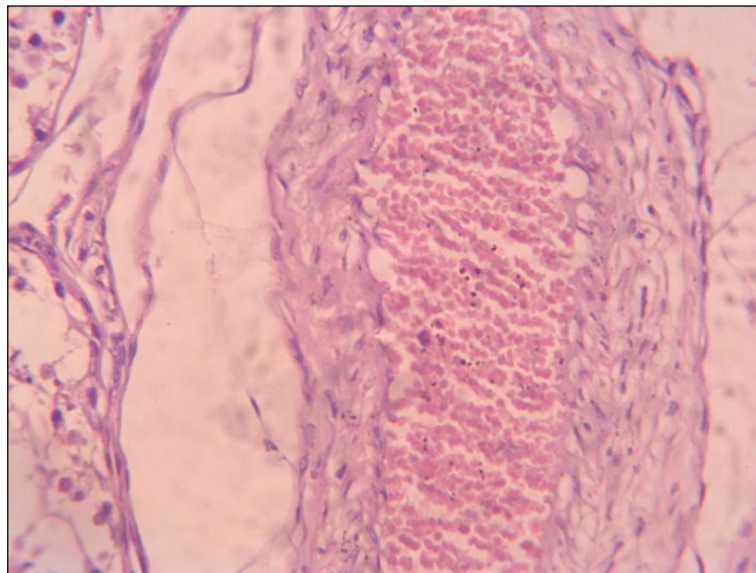


Figure 6 A cross section passes in rabbits injected with the alcoholic extract of *Vitex agnus –castus* at a concentration of 200mg/kg. Note 1- Vascular congestion. H&E. 40X.

The result of our study did not agree with a study conducted by [11] where they indicated that the methanol extract used in the process of extracting the active compounds from the leaf is very safe and no signs of toxicity of the extract were observed, and there was no death for the injected rabbits during. A period of 30 days Where they indicated a normal density in the seminiferous tubules in the testicular section and the process of generating normal sperm, spermatogenesis and there is no tubular atrophy, neither enlargement of Leyding cells, no enlargement of the basement membrane of the seminiferous tubule [12].

4. Conclusion

Vitex agnus castus caused several histopathological changes, including the appearance of vacuolation between the cells of the germ layer and Lydic cells. It also caused sloughing of the germ layer and its collection in the central lumen of the tubule, expansion of some lumen of seminiferous tubules, dissociation of spermatogenic cells and separation of the epithelial layer from the basement membrane, as well as contraction of the walls of seminiferous tubules, where their appearance became the general in some

sections is wavy and irregular, and the intensity of the effects increases with increasing dose.

5. References

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