



Potential Protective Effects of *Tribulus terrestris* Extract (TTE) against Reproductive Damage Induced by Cytarabine (Ara-C) Chemotherapy in Male Rats: Histopathological Study

Zahra Mohammed Hashim¹, Mayada S. Hassan², Raed Altaee³

¹M.Sc. Student, College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq

^{2,3} Department of Physiology Biochemistry and Pharmacology, College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq

*Corresponding author: Email: rana.a@uokerbala.edu.iq

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Abstract

The cytotoxic effects of many cancer medications cause different general side effects and defects in spermatogenesis, so still a concern that requires additional enhancement. The study was performed to assess the preventive benefits of *Tribulus terrestris* extract (TTE) against reproductive damage induced by cytarabine (Ara-C) chemotherapy. Twenty mature male rats will be divided into four groups. Each group contains 5 rats. A control group is included in the first group, *T. terrestris* extract (250 mg/kg) is given to the second group, Ara-C (25 mg/kg BW) is given to the third group, and the fourth group receives both an oral dose of TTE (250 mg/kg BW) and an intraperitoneal dosage of Ara-C (25 mg/kg BW) for a total of 28 days. After the end of the experiment tissue samples in testes and epididymis are taken for the necessary histopathological changes that revealed detectable spermatogenesis degeneration. Treatment caused spermatocytes disruption, significant histopathological alterations, rounded, shrunken seminiferous tubules with less cellular epithelium, degeneration in germinal epithelium represented by depletion with damaged Leydig cells, and this may result in reduced rat fertility. The results of the study indicate that *Tribulus terrestris* exhibits significant histological alterations, as evidenced by an increase in sperm growth, in contrast to Ara C group, which displayed some detrimental effects.

Keywords: *T. terrestris* extract, Cytarabine (Ara-C), histopathological studies, testes and epididymis

Introduction:

Chemotherapy is an effective therapy for many cancers, but because it involves a variety of extremely toxic substances, there are still a lot of issues with this treatment. This kind of treatment's low specificity and significant toxicity are most likely its drawbacks [1]. Patients receiving chemotherapy frequently die from pneumonia, common infections, or other cancers due to serious side effects of the drug [2]. Cytarabine, also referred to as Ara-C, is an antimetabolite chemotherapeutic drug that is primarily used for the treatment of white blood cell malignancies, including as acute myeloid leukemia (AML) and non-Hodgkin lymphoma.. By inhibiting DNA polymerase through competing with deoxycytidine triphosphate, it kills

cancer cells by preventing DNA synthesis [3]. One of the most frequent cytotoxic side effects of chemotherapy for men is testicular injury with Leydig cell destruction [4]. Even while important research findings have lessened the cytotoxic effects of many cancer medications, toxicity is still an issue that requires more attention.

Tribulus terrestris (TT) is an herb that is found around the world and is a member of the Zygophyllaceae family. This herbal remedy has anti-inflammatory, antioxidative, hypolipidemic, liver-protective [5]. Properties, cardiogenic, aphrodisiac, antibacterial, anthelmintic, and diuretic properties, among other health advantages [5]. In addition, TTE has been utilized in conventional Chinese medicine for treating a variety of diseases, such as cancer, hypertension, edema, atherosclerosis, myocardial infarction, coronary artery disease, and post-stroke syndrome [5]. Thus, the purpose of this investigation was to determine whether or not administration of TT extract (TTE) after Ara-c exerts protective benefits on male rat testicular spermatogenesis histologically.

Materials and methods:

Laboratory Animals

Male rats, weighing between 150 and 200 grams at 12 to 14 weeks of age, were used in this study. The University of Kerbala's College of Veterinary Medicine served as the animal home, where the perfectly formed plastic cages were held under carefully regulated air conditions. The animals were housed for ten days before to the experiment. They were kept under conventional circumstances for a 12-hour light-dark cycle, with room temperature and relative humidity at $60 \pm 5\%$. Throughout the trial, they were fed a regular pellet meal and had unlimited access to water.

Experimental Design

The animals in this study were split up into four groups, each with five animals. The divisions of the groups are as follows:

1. A control group received distilled water treatment.
2. Ara-C (25 mg/kg BW) treatment group.
3. TTE (250 mg/kg) (obtained from the local market).
4. A mixed group was given a treatment consisting of TTE (250 mg/kg) and Ara-C (25 mg/kg BW) for 28 days. After the end of the experiment tissue samples are taken for the necessary histopathological changes.

Sample collection and tissue preparation:

After 28 days, all of the animals were given a deep chloroform anesthesia, sacrificed, and testes and epididymis were removed from their surrounding connective tissue and fat. They were then quickly immersed in 10% formal saline after a quick rinse with tap water. Each animal's organs from one side was examined for histological alterations [6]. Standard hematoxylin and eosin (H&E) staining protocols were used to the sections. In order to examine the sections under inspection, microscopy images of each section were acquired with a light microscope and a digital imaging device (Canon, Japan).

Results

Histological analysis revealed that Ara-C caused damage in the testes, as evidenced by an increase in cell death and a decrease in seminiferous tubule growth. In comparison to the control group, the histopathological images showed that the seminiferous tubules of the Ara-c treated group had considerable cellularity together with degenerative alterations and parenchymal congestion with focal degeneration (Figure 2). While the histological images of the group treated with TTE and Ara-c show no substantial degradation and a relative increase in cellularity with a few mature sperm positioned in the center (image 3). There is a noticeable increase in spermatogenic activity in this group as compared to the fourth, and there are more mature sperm in this group (figure 4).

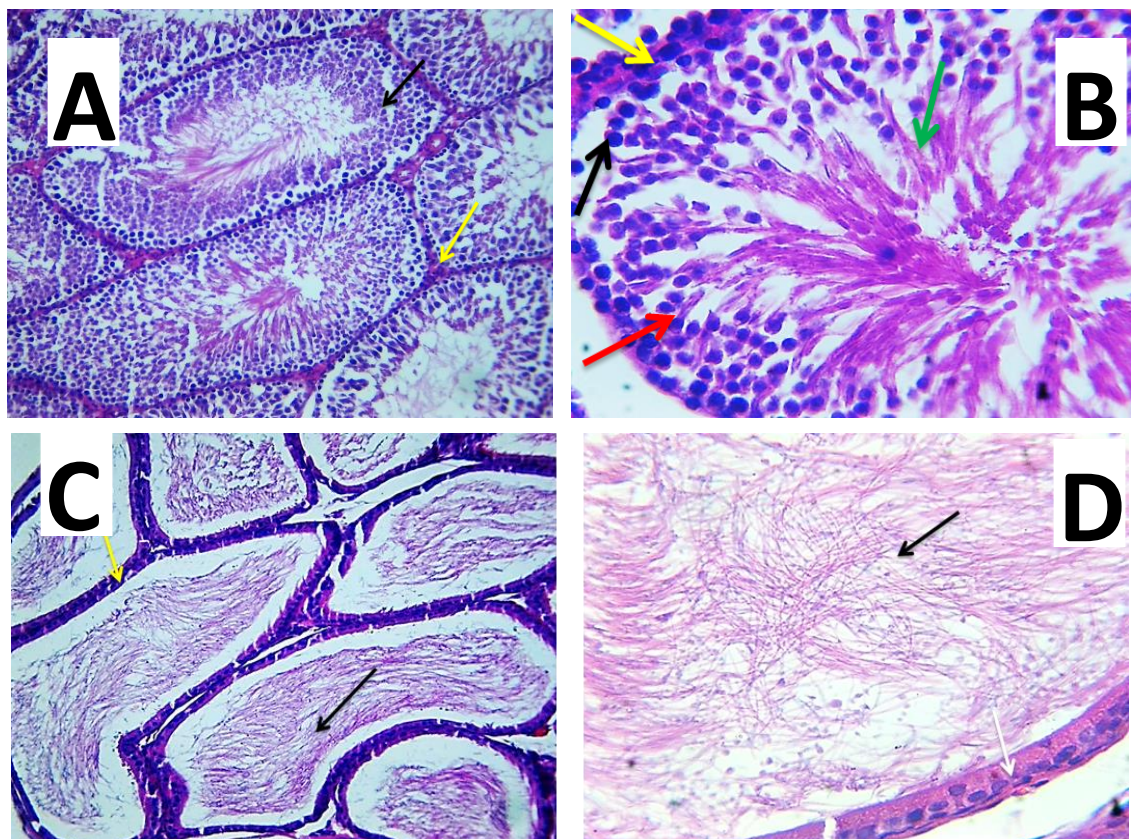
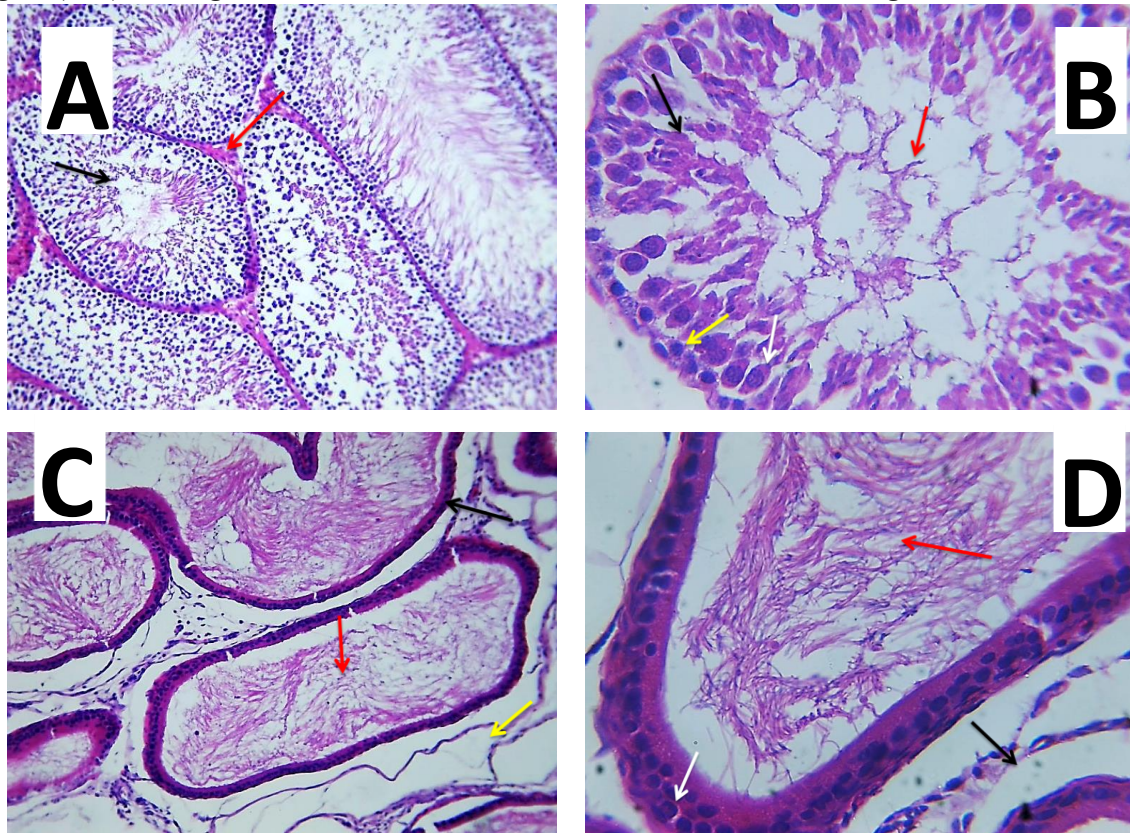


Figure (1,A) Histological examination of a control rat testes showing the normal architecture of testicular tissue, significant rounded to oval shaping of seminiferous tubules (black arrow) , marked and normal surrounding Leydig cells (yellow arrow).(H and E, 10X).**Figure (1,B)** Histological examination of a control rat testes showing the normal structure of seminiferous tubules, normal germinal epithelium , regular spermatogonia (black arrow) , typical surrounding Leydig cells (yellow arrow) ,remarkable spermatocytes (red arrow) and significant long spermatid (green arrow) .(H and E, 40X). **Figure (1,C)** Histological examination of a control rat epididymis showing the normal histoarchitectural appearance of epididymal tubules (yellow arrow), normal spermatid density (black arrow) .(H and E, 10X). **Figure (1,D)** Histological

examination of a control rat epididymis showing the normal histological structure of epididymal tubule, significant high spermatic density (black arrow) with normal lining endothelia (white arrow). (H and E, 40X).

Figure (2, A) Histological examination of testes for TTE treated animal revealing the normal structure of



testicular tissue (black arrow), with blood vessels Figure congestion (red arrow). (H and E, 10X). Figure (2, B) Histological examination of testes for TTE treated animal showing the normal structure of a seminiferous tubule (black arrow), with mild germinal epithelium vacuolation (red arrow), noticeable newly formed spermatocytes (white arrow) and normal spermatogonia (yellow arrow). (H and E, 40X). Figure (2, C) Histological examination of epididymis of TTE treated rat showing the normal epididymal tubules morphology (black arrow), regular spermatoc density (red arrow), with widening in interstitial spaces (yellow arrow). (H and E, 10X). Figure (2, D) Histological examination of TTE treated rat epididymal tubule revealing normal histology, high spermatoc density (red arrow), with widening in interstitial spaces (black arrow) and normal epithelial lining (white arrow). (H and E, 40X).

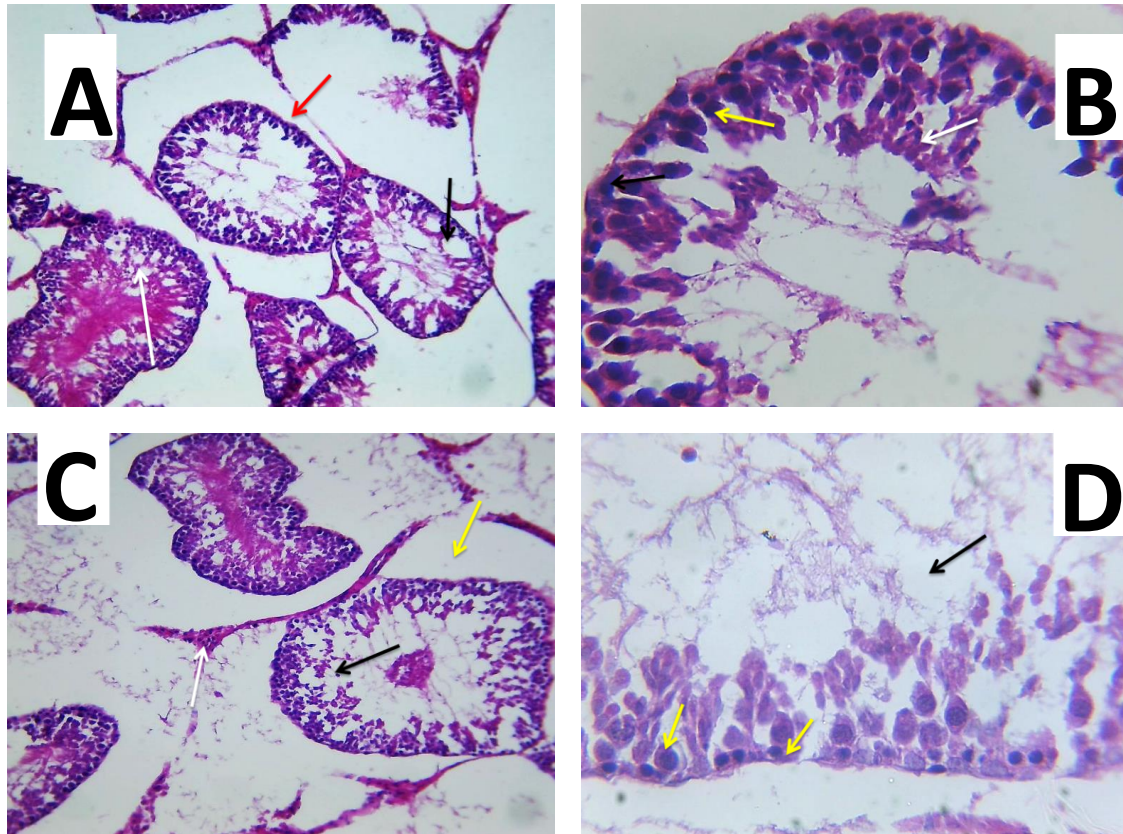


Figure (3,A) Histological examination of testicular tissue for Ara-C treated rat showing characteristic histopathological alterations manifested by, marked seminiferous tubules atrophy (red arrow) , with severe widening in interstitial spaces (black arrow) and vacuolated epithelial lining (white arrow) .(H and E, 10X). **Figure (3,B)** Histological examination of testicular tissue for Ara-C treated rat showing characteristic histopathological alterations in seminiferous tubule , few spermatogonia (black arrow) with pyknotic spermatocytes(yellow arrow) and vacuolated primary spermatids (white arrow) .(H and E, 40X). **Figure (3,C)** Histological examination of testicular tissue for Ara-C treated rat showing significant histopathological alterations in germinal epithelium, sever spermatid degeneration (black arrow) with atrophied tubules (yellow arrow) and congested interstitial spaces with dilation (white arrow) .(H and E, 10X). **Figure (3,D)** Histological examination of testicular tissue for Ara-C treated rat showing significant histopathological alterations in germinal epithelium, sever spermatid degeneration and vacuolation (black arrow) , few spermatogonia and spermatocytes (yellow arrow) .(H and E, 40X).

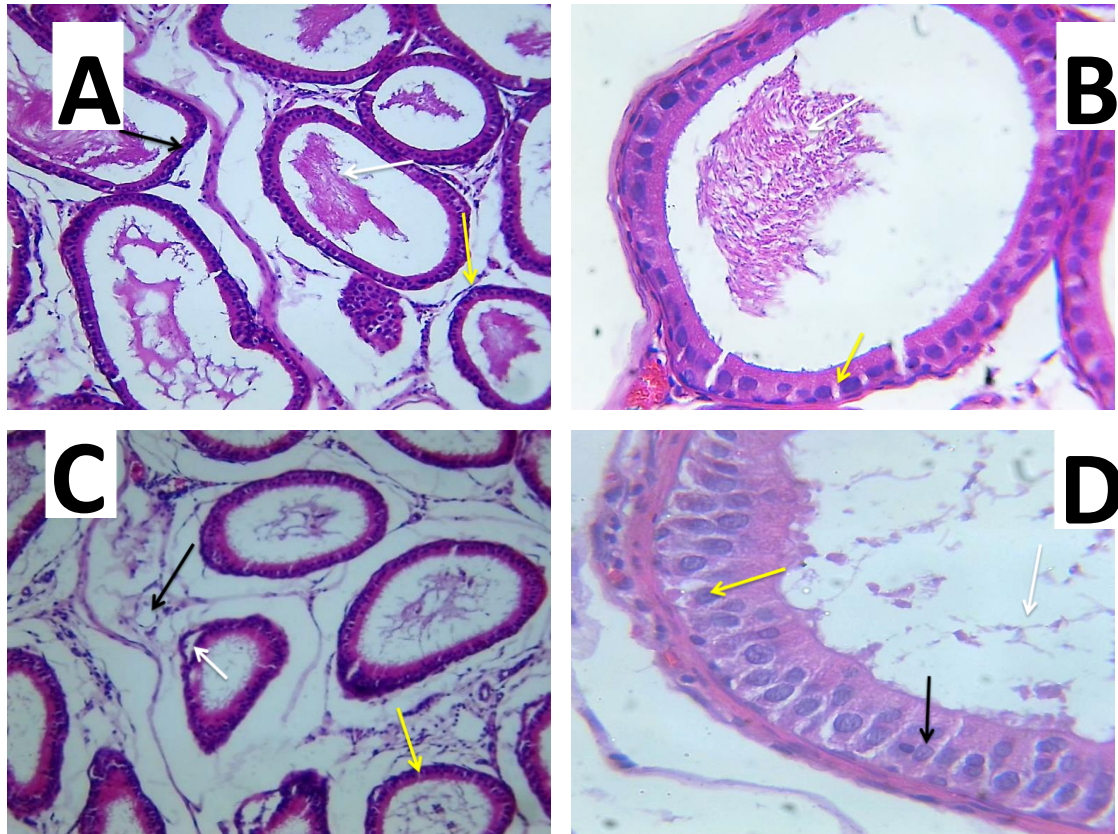


Figure (4,A) Histological examination of epididymis for Ara-C treated rat showing significant histopathological changes , marked increase in interstitial spaces (black arrow) with atrophied tubules (yellow arrow) and low sperm density in the lumen of the tubule (white arrow) .(H and E, 10X). **Figure (4,B)** Histological examination of epididymis for Ara-C treated rat showing significant histopathological changes increase in epithelial lining thickening (black arrow) with few vacuolation (yellow arrow) and low sperm density in the lumen of the tubule (white arrow) .(H and E, 40X). **Figure (4,C)** Histological examination of epididymis for Ara-C treated rat showing significant widening in interstitial spaces (black arrow) with atrophied tubules (yellow arrow) and noticeable sloughing in epithelial lining from the basement membrane (white arrow) .(H and E, 10X). **Figure (4,D)** Histological examination of epididymis for Ara-C treated rat showing significant increase thickness in epithelial lining (hyperplasia) (black arrow) with few vacuolation (yellow arrow) and low or no sperm density in the lumen of the tubule (white arrow) .(H and E, 40X).

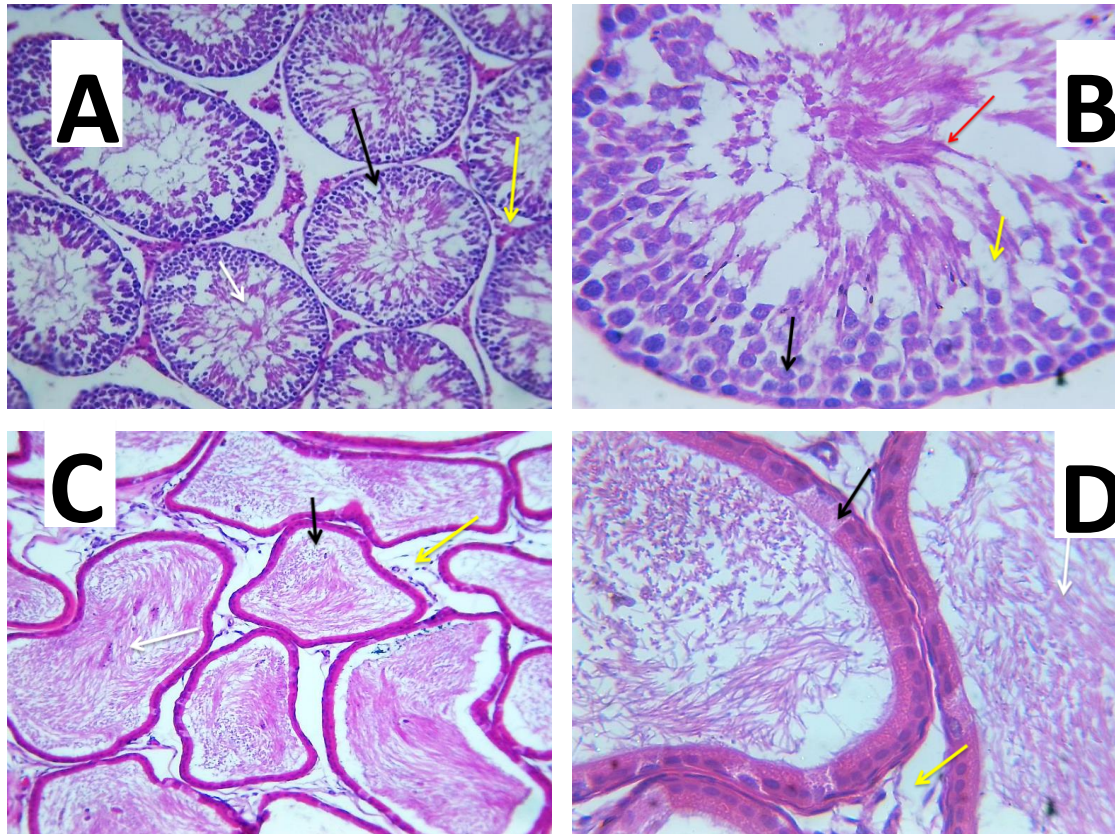


Figure (5,A) Histological examination of testicular tissue for TTE and Ara-C treated rat showing significant histological improvements in seminiferous tubules, normal size and rounded morphology (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal spermatogenesis(white arrow) .(H and E, 10X). **Figure (5,B)** Histological examination of testicular tissue for TTE and Ara-C treated rat showing remarkable regular arranged spermatogonia (black arrow) , few vacuolation in spermatocytes (yellow arrow) with normal spermatid appeared in the lumen (red arrow).(H and E, 40X). **Figure (5,C)** Histological examination of epididymus for TTE and Ara-C treated rat showing significant histological reversible changes in epididymal tubule (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal spermatic density(white arrow) .(H and E, 10X). **Figure (5,D)** Histological examination of epididymus for TTE and Ara-C treated rat showing significant histological reversible changes in epididymal tubule , normal and regular epithelial lining (black arrow) with decreased interstitial spaces (yellow arrow) and noticeable normal and high spermatic density(white arrow) .(H and E, 40X).

Discussion

Depending on the findings of the testicular and epididymal histological examination, Ara C therapy greatly reduced proliferative cells two weeks after treatment. Ara-C caused damage to the testes, as demonstrated by histological analysis; this was further supported by an increase in cell death and a decrease in cell proliferation in the seminiferous tubules. [7]. This was explained by the fact that Ara-C and other chemotherapies might target cells that were rapidly growing. (10). Additionally, in adult mice, Ara-C reduces sperm parameters, increases the proportion of tubules containing apoptotic cells, and impairs spermatogenesis. [8]. In rat testes, Ara-C 's histological effects result in minor vacuolization and tubular deformation [7]. Because of the damage seen in the structure and histology of their seminiferous tubules as well as the observed increase in apoptotic cells, we advise treating rats with Ara-C with extra care to ensure their future fertility. Histological examination revealed a definite detrimental effect of Ara-C on the cellular composition/structure of the seminiferous tubules. As a result, we investigated Ara-C treatment affected the apoptotic process and cell proliferation in the treated groups' seminiferous tubules. Histological study revealed that Ara-C had a definite negative effect on

the cellular composition/structure of the seminiferous tubules. As a result, we investigated how all treatments affected the apoptotic process and cell proliferation in the treated groups' seminiferous tubules. Our results revealed an important rise in the percentage of tubules with positive apoptotic cells in the Ara-C group 28 days after treatment compared to the control group. Furthermore, histologically, Ara-C produces tubular deformation and minor vacuolization in rat testes [7]. The significant increase in spermatogenic activity in *Tribulus terrestris* exhibited in Figure (4) may be attributed to the androgenic effect of the herb. A vital role that androgens play in the development and differentiation of numerous tissues, including the organs of reproduction, androgen also responsible for pubertal development of testes. The development of the testes during puberty is likewise controlled by androgen. Adult rats' testes had significantly more spermatogonia, spermatocytes, and spermatids when *Tribulus terrestris* preparation was administered; these results may indicate that protodioscin stimulated DNA synthesis [9]. According to Abadjieva and Grigorova [10], TTE can have an impact on the weight of the rabbits' testicles and body weight. The current study's findings demonstrated that exposure to TTE results in the dilatation of the epididymis, or an increase in cell density. This could be the outcome of a faster rate of cell division, which would enhance the epididymis's ability to store and mature sperm [11].

Conclusion

We conclude from our study the protective effect of *TTE* on male rats treated with Ara-C to avoid cytotoxic effects of cancer medications.

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