

The Protective Effect of Aqueous of *Hylocereus Polyrhizus* Fruits on some Histological Changes of the Testes of Treated Male Rats Carbon Tetrachloride

Alaa Karim Hadi^{1*}, Ashwaq Kadhém Obeid², Heba Alwan Abd - Alsalam Alsalamé³

^{1*}Department of Biology, College of Education for Pure Sciences, Kerbala University, Kerbala, Iraq.

E-mail: alaa.k@s.uokerbala.edu.iq

²Assistant Professor, Department of Biology, College of Education for Pure Sciences, Kerbala University, Kerbala, Iraq.

³Assistant Professor, Department of Biology, College of Education for Pure Sciences, Kerbala University, Kerbala, Iraq.

ABSTRACT

This study aimed to investigate the effect of *Hylocereus polyrhizus* fruits in protecting testis tissue from damage caused by carbon tetrachloride toxicity. In this research, it was suggested that the aqueous extract of red dragon fruit could positively affect the testicular tissue, and to verify this, (24) adult male laboratory rats were divided, weight ranged between (250 - 310) grams and their ages between (11- 13) approximately one week randomly into four groups with (6) animals for each group as follows: the first (negative) control group, the second group, the positive group, were injected with carbon tetrachloride at a concentration of 0.1 ml / 100 g of body weight under the peritoneum and for two days per week, the third group animals I was dosed with aqueous extract of dragon fruits at a concentration of (650) mg / kg of body weight by mouth daily throughout the trial period. The fourth group of animals were dosed with aqueous extract of Red dragon fruits at a concentration of (650) mg / kg by mouth daily an hour before they were injected with carbon tetrachloride at a concentration 0.1 ml / 100 g, for 2 days a week. After 30 days of the experiment, a microscopic examination of the testicular tissue was performed. In the positive group, the presence of interstitial spaces between the seminiferous tubules, the decrease in the size of the germinal epithelial cell layer, fewer Leydig cells, absence of sperm in the lumen, the increase in the diameter of the lumen, the necrosis of the cells lining the tubules and the decrease in the diameters of the seminiferous tubules as well as a decrease in The level of testosterone, luteinizing hormone (LH) and (FSH) when compared with the control group. In the third group, the normal testis structure with the seminiferous tubules appears, increase in the number of epithelial germ layer and sperms, a decrease in the lumen, with the presence of your Leydig cells in the interstitial tissue and an increase in the number of germ cell layers. The formation of the sperm when compared with the control group and the fourth group shows the normal testes structure with the seminiferous tubules, as well as an increase in the number of sperms and a decrease in the lumen with the presence of your Leydig cells in the interstitial tissue, an increase in the number of the germinal epithelial cell layer, an increase in the hormone testosterone and the (LH) and (FSH) when compared to a group of carbon tetrachloride, we conclude from this that the aqueous extract of *Hylocereus polyrhizus* fruits has a positive effect in the process of improving Fertility in male rats is also highly effective in antioxidants and reduces the histological changes caused by carbon tetrachloride.

KEYWORDS

Hylocereus Polyrhizus, CCl₄, Oxidative Stress, Testes, Sex Hormone.

Introduction

Medicinal plants are among the things that have increased interest in the current century by the World Health Organization WHO, despite the development in the fields of chemistry and pharmacology because these chemical drugs have a negative effect that appears after a long period of treatment (am Ende & am Ende, 2019; Jamshidi-Kia *et al.*, 2018), recent studies have also indicated the pharmacological efficacy of plant compounds that have antioxidant qualities as they have become These plants are a safer source for the medicine industry and a preventive source from the occurrence of many diseases, including cancer in humans, as well as studies indicating a decrease in the incidence of chronic diseases among people who depend on fruits and vegetables in their daily diet (Abdullahi *et al.*, 2018; Dwivedi *et al.*, 2019; Siew *et al.*, 2019), researchers have been interested in medicinal herbs and drugs isolated from them to prove their effectiveness and economic feasibility in addition to safety and no side effects

(2018; Shakya, 2016 Roy *et al.*,) so that medicinal plants are considered one of the main sources. And the main one for extracting medicinal treatments and medicinal drugs (Nankaya *et al.*, 2020), with this, many medicinal plants have been used to treat many diseases and cancers resulting from several reasons, the most important of which is exposure to chemicals, especially carcinogens, to which humans and animals are frequently exposed, and carbon tetrachloride is one of the most important. These chemicals are classified as secondary carcinogens (Manibusan *et al.*, 2007; Qin *et al.*, 2019). The reproductive system is one of the body systems that are affected by these chemicals. Human and animals are exposed through air, water and food (Patel *et al.*, 2015). Recent studies have also shown that oxidative stress, which appears through exposure to toxic chemicals, causes great harm to sperm quality by disrupting the balance of antibodies. Oxidation and reactive oxygen species (ROS), leading to abnormalities in spermatogenesis and male sterility (Maestra *et al.*, 2015; Othman *et al.*, 2014). Previous studies confirmed that carbon tetrachloride (CCL₄) is a chemical that causes oxidative stress due to stimulation of the production of free radicals that causes oxidation of germ cells in the testes (Agarwal *et al.*, 2014) (Gharagozloo & Aitken., 2011). and to maintain and protect cell function, Redundant free radicals should be continuously inactivated by antioxidants in seminal plasma and preventing the formation of new ROS or continued removal of the generated ROS (Agarwal *et al.*, 2014). Recent results indicate that natural products containing antioxidants have the potential to Testicular protection against oxidative damage, lipid peroxidation and weakness in antioxidants caused by carbon tetrachloride (Ge *et al.*, 2015 Al-Olayan *et al.*, 2014 Satyam *et al.*, 2013;), and from plants that are a rich source of phenolic compounds. The red dragon fruit, *Hylocereus polyrhizus*, has active and antioxidant activity, and these compounds are one of the strong and effective antioxidants in scavenging free radicals, and they have an important role in protecting humans (Mehir *et al.*, 2019).

It is a perennial cactus, rocky climbing plant that lives far from the ground level and belongs to the Cactacea family of the genus *Elmarzugui et al* (2016), Red Dragon fruit plays an important role as a fertility factor due to its antioxidant and anti-reproductive properties as it works to increase the number of sperm. And its ability and rate of production (Aziz and Noor, 2010), it also effectively increases the synthesis of the hormone testosterone and plays an important role in the formation of sperm, and therefore it can be used as herbs that enhance sexual activity and male fertility (Kanedi *et al.*, 2016).

Materials and Methods

Experimental Animals in this Study

In this experiment, (24) adult male rats were used, with an average of weights ranging between (250 - 310) grams and their ages between (11-13) weeks approximately, and they were placed in the animal house of the College of Pharmacy / University Karbala for the period from November of 2020 until January 2020, animals were placed in plastic cages of dimensions (40 x 20 x 20 cm) covered with metal covers prepared for this purpose. Sterilizing them while changing their floors continuously, as well as constant care for the cleanliness of the irrigation bottles and the room for the accommodation, and the animals were subjected to adequate laboratory conditions such as the appropriate temperature and reached (22 ± 2) degrees Celsius, and a light cycle was divided into (10) light hours and (14) An hour of darkness, and the experimental animals were provided with water and fodder consisting of (35% wheat, 34% yellow corn, 20% soybeans, 10% animal protein, 1% milk powder, plus 50 grams of preservatives and anti-fungal substances) (Cynthia, 2007), continuously (Ad libitum) and in sufficient quantities throughout the trial period.

Plant Used

The plant used in the research (Red dragon fruits) was collected from one of the local markets in the noble province of Najaf, and it was brought to Karbala University - College of Education for Pure Sciences - Department of Life Sciences and was classified by the teacher specializing in plant classification (Mohd, 2010). The red dragon fruits was washed and cleaned well in running water to remove the impurities and dust from it. Then the fruit was dried from the water and mixed and mashed in an electric mixer to convert it into juice, and then the fruit juice was filtered with multi-layered gauze paper, and then The filtrate was diluted with 20% of distilled water and the mixture was left in a container covered with aluminum foil with continuous stirring of the mixture for 12 hours, after this it was poured into sterile containers after being filtered and the mixture was

left to dry at a temperature of (40 - 45) degrees Celsius in the oven Then the crude extract (crude) was collected by scraping it and put it in opaque, sterile, and clean glassware in the refrigerator under a temperature of (2-4) degrees Celsius to be used in the experiment later, the source Harborne, 1984) was adopted for this preparation method, with modifications according to (Sato, *et al.*, 1990).

Calculation of Carbon Tetrachloride Dose

In this study, the compound carbon tetrachloride was used as a toxicity of English origin, prepared from the Sigma company Office for Chemical Supplies / Baghdad. The name of the supplier is BDH. The animals were injected with it in the peritoneal cavity at a dose (0.1 ml / 100 g BW) (Alhazza *et al.*, 2008).

Experimental Design

Divided (24) of the experimental animals randomly into four groups with (6) animals for each group as follow : Negative control group (G1): animals were dosed with normal saline daily for the duration of the experiment, positive control group (G2): animals were injected under The peritoneum with carbon tetrachloride at a concentration of 0.1 ml / 100 g of body weight for two days per week, group three (G3) included animals dosed orally with aqueous extract of red dragon fruit at a concentration of (550) mg / kg of body weight per day throughout the period of the trial, group four (G4) It included animals dosed orally with aqueous extract of red dragon fruit at (550) mg / kg of body weight per day an hour before they were injected under the peritoneum with carbon tetrachloride at a concentration of 0.1 ml / 100 g of body weight for two days per week.

Preparations of Histological Sections

The animals used were anesthetized using an anesthetic substance (chloroform), then the animals were dissected directly by opening the abdominal cavity with a scalpel and sharp scissors from the bottom, after which the testicles were removed and then washed with water. Then these organs were cut into small pieces in a cross-section and longitudinal way to be preserved In 10% formalin for 48 hours after which the samples were dried in ascending degrees of alcohol. Then they were purified in xylene and mixed in paraffin wax at 56 ° C in an oven and made into blocks. The blocks and sections were placed in a rotating microtome at 5 µm, after which all the tissue sections were stained with the Haematoxylin-Eosin stain (Suvarna *et al.*, 2013).

Results and Discussion

The Histological Study

The histopathological examination of the current study of testes of male rats of the control groups showed that they were treated with physiological saline over the length of the experiment and not injected with carbon tetrachloride, as well as not dosed with red dragon fruit extract in Figure (1) and the group that was dosed with red dragon fruit extract at a concentration of (650) Mg / kg in Figure (3) the absence of any changes in the tissue indicating the presence of a disease or an apparent defect as the tissue appears normally as well as the seminiferous tubules, and the stages of the formation of sperms and the cells that form them can be observed in addition to the normal distribution of the layers of epithelial cells Germs, as well as the normal appearance of Leydig cells and Sertoli cells and the presence of sperm in the middle lumen of the seminiferous tubule. While the histological examination of the testes of male rats treated with carbon tetrachloride at a concentration of (0.1) ml / 100 grams of body weight, figure (2) showed clear damage to the walls of the seminiferous tubules as well as their breakdown, and increase interstitial spaces between the seminiferous tubules, a decrease in the diameter of the seminiferous tubules and the size of the germinal epithelial cell layer, in addition to a absence of sperm lumen, congestion in the interstitial tissue, and appear fewer Leydig cells, and degenerate in the cells, necrosis of cells lining the tubules, and this study was in agreement with the study of (La Maestra *et al.*, 2015) for the effect toxicity exerted by CCL₄ through its production of free radicals, which interact directly with cell membranes upon activation of cytochrome P450, which targets testicular tissue. And also because it causes an imbalance of antioxidants and reactive oxygen species (ROS), and this leads to defects in sperm production and quality and causes great harm (Khan, 2012; Othman *et al.*, 2014). CCl₄ for a period of (20) days to (16) weeks. This exposure leads to major damage to the sperm generating cycle, such as destruction

in the epithelial layer of germinal cells, as well as shrinkage of the seminiferous tubules (Kalla & Bansal, 1975;; Horn *et al.*, 2006; Khan & Ahmed, 2009; Khan, 2012).

The risk of CCL_4 toxicity is concentrated in the active transformation through the cytochrome P450 system and this leads to the emergence of trichloromethane (CCl_3) radicals which are also converted to the trichloromethyl peroxy radical (CCl_3O_2). CCl_4 metabolism reacts with polyunsaturated fatty acids (PUFA) and forms Covalent approaches to lipids and proteins. This process leads to lipid oxidation, destruction of cell membranes, and testicular injury (Szymonik-Lesiuk *et al.*, 2003). Testicular cells are well equipped with enzymatic antioxidants and with low molecular weight in order to maintain redox balance as well as prevent the formation of reactive oxygen species (ROS) (Bauche *et al.*, 1994). This over-formation of ROS may override the antioxidant defenses and produce It causes oxidative stress, which in turn causes permanent damage to testicular cells. The high content of polyunsaturated fatty acids in the testis cell membrane readily undergoes lipid oxidation and leads to oxidative stress (Alvarez *et al.*, 1987) (Koizumi, 1992). Consequently, oxidative stress results in abnormal sperm production and function (Shen & Ong, 2000). While we notice a clear improvement in the seminiferous tubules, as well as an increase in the number of sperm with the presence of clear Leydig cells and a decrease in the median lumen of the tubules and an increase in the number of the germinal epithelial cell layer in the protective group treated with CCL_4 at a concentration of (0.1 ml / 100 g of body weight and dosed with an extract Red Dragon fruits at a concentration of (650) mg / kg of body weight (Figure 4). Red dragon fruit is rich in powerful antioxidant compounds that are effective in scavenging free radicals such as phenolic compounds (Mihir *et al.*, 2019) and betaecanin, which is an excellent electron donor and this leads to stabilization of free radicals, thus demonstrating its role as an antioxidant (Kanner *et al.*, 2001; Mihir *et al.*, 2019, and ascorbic acid, vitamin C (Choo & Yong., 2011) is a very powerful antioxidant that can effectively remove free radicals and reduce excess ROS production, thus protecting the testicle from damage (Yousef *et al.*, 2003).

The Hormonal Study

The results of the current study to measure the sex hormones of male rats with positive control (G2) showed that treatment with carbon tetrachloride for 30 days showed a significant decrease ($P < 0.05$) in testosterone (T) (0.01 ± 0.14), ICSH, hormone (LH). (0.006 ± 0.04) and FSH (0.007 ± 0.06) compared to the negative control group (G1). Carbon tetrachloride caused advanced cirrhosis that may cause hypogonadism and low testosterone in the blood (Castilla-Cortazar *et al.*, 2000). Carbon tetrachloride toxicity affected the pituitary gland resulting in a decrease in the level of FSH and LH resulting in inhibition of steroid biosynthesis by Leydig cells causing a decrease in testosterone (Ismail and Al-nahri 2009). Carbon tetrachloride caused a decrease in the level of FSH, which may be attributed to the effect of carbon tetrachloride on the pituitary gland, which led to a decrease in the concentration of FSH, and this result is in agreement with (Ismail and Al-nahri, 2009). who showed that CCL_4 caused a decrease in the concentration of FSH. That the oxidative stress induced by carbon tetrachloride may cause a decrease in the concentration of FSH (Khan and Ahmed 2009; Bansal and Bilaspuri, 2011). While the results of the group treated with red dragon fruit aqueous extract (G3) showed a significant increase ($P < 0.05$) in testicular lipogenic hormone T (0.07 ± 7.11), intercellular stimulating hormone (ICSH), luteinizing hormone (LH) (0.06 ± 4.12 .) And FSH. (60.0 ± 3.53) compared to negative control group (G1). The results also showed a significant increase ($P < 0.05$) in testicular lipogenic hormone T (0.06 ± 5.11), ICSH (luteinizing hormone LH) (0.10 ± 12.3) and FSH (0.07 ± 2.72) compared to the control group. Cation (G2). Dragon fruit extract effectively boosts androgen concentration and increases sperm count, sperm vitality, sperm motility and sperm count (Kanedi *et al.*, 2016). The androgenic effect is related to blood testosterone levels (Amini & Kamkar, 2005). Fat peroxide causes a decrease in the production and secretion of GnRH in the hypothalamus, and this leads to the failure of the pituitary gland to produce and secrete both FSH and LH hormones, thus Leydig cells fail to manufacture testosterone and Sertoli cells are unable to function properly. The Natural (Nugroho, 2007). Red dragon fruits (*Hylocereus polyrhizus*) increases the antioxidants in the body, this leads to a gradual improvement of the hypothalamus and an increase in the secretion of GnRH. Increased secretion of GnRH leads to an increase in the pituitary gland secretion of LH and FSH. The LH function stimulates the Leydig cells to produce hormone testosterone. FSH stimulates Sertoli cells to synthesize and release the androgen-binding protein. ABP is a testosterone receptor in the blood. Increased secretion of LH and FSH leads to increased ABP and testosterone. This study showed that a dose of 250 mg / kg of body weight / day is the maximum dose of red dragon fruits that can increase testosterone levels (I'tishom *et al.*, 2020).

Table 1. The sex hormones of animals treated with carbon tetrachloride and red dragon fruit

Standards Means \pm S.E Groups	Testosterone hormone (T) mIU / ml	Follicle stimulating hormone (FSH) mIU / ml	Luteinizing hormone (LH) mIU / ml
Control	5.25 \pm 0.22 C	2.92 \pm 0.04 C	3.26 \pm 0.12 C
Carbon tetrachloride	0.14 \pm 0.01 E	0.06 \pm 0.007 F	0.04 \pm 0.006 E
<i>Hylocereus polyrhizus</i> extract 650 mg/kgBW	7.11 \pm 0.04 B	3.58 \pm 0.06 B	4.12 \pm 0.06 B
<i>Hylocereus polyrhizus</i> extract 650 mg/kgBW+ CCL4	5.11 \pm 0.06 C	2.72 \pm 0.07 D	3.12 \pm 0.10 C
L.S.D	0.3075	0.1528	0.2656

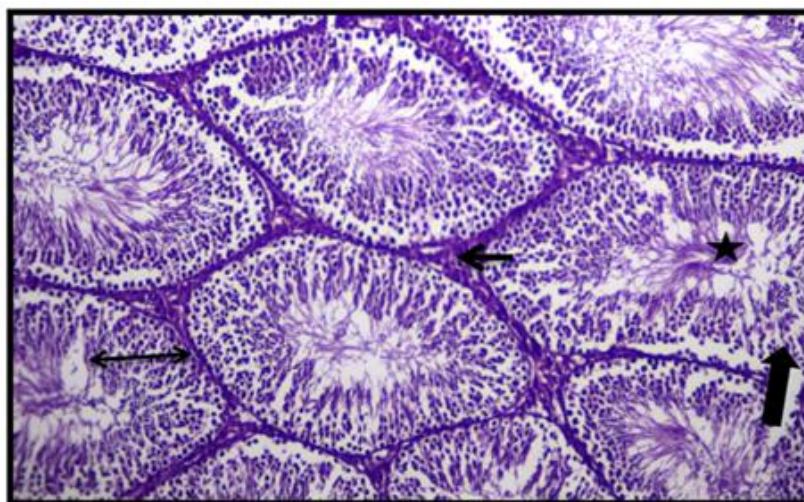


Figure 1. Testis section of rat (control) Showing normal appearance of seminiferous tubules (thick arrow), Leydig cells (thin arrow), with presence of sperm (star), lumen (star blue) (H & E 100X).

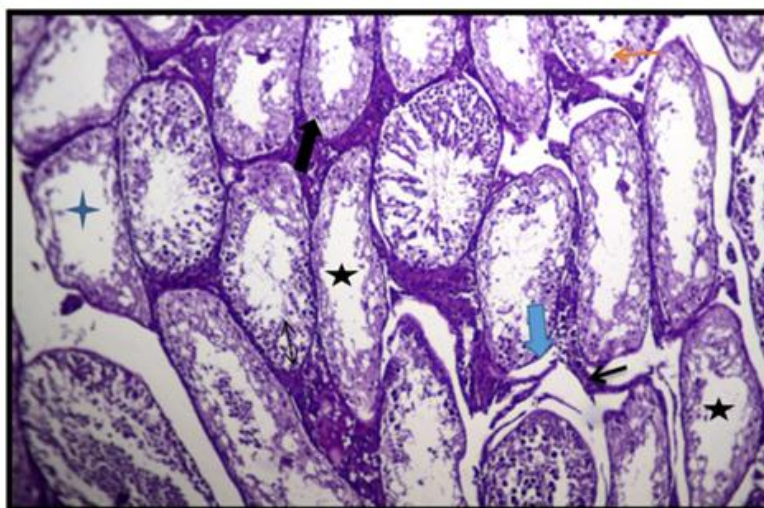


Figure 2. Testis section of rat, treated with carbon tetrachloride 0.1 ml Showing increase interstitial spaces in the seminiferous tubules (thick blue), reduced in the epithelial height (), absence of sperm in the lumen (star), fewer

Leydig cells (thin arrow), necrosis of cells lining the tubules (red arrow), decreased diameters of the seminiferous tubules(thick arrow), sperm (star), increase in the lumen(star blue) (H & E 100X).

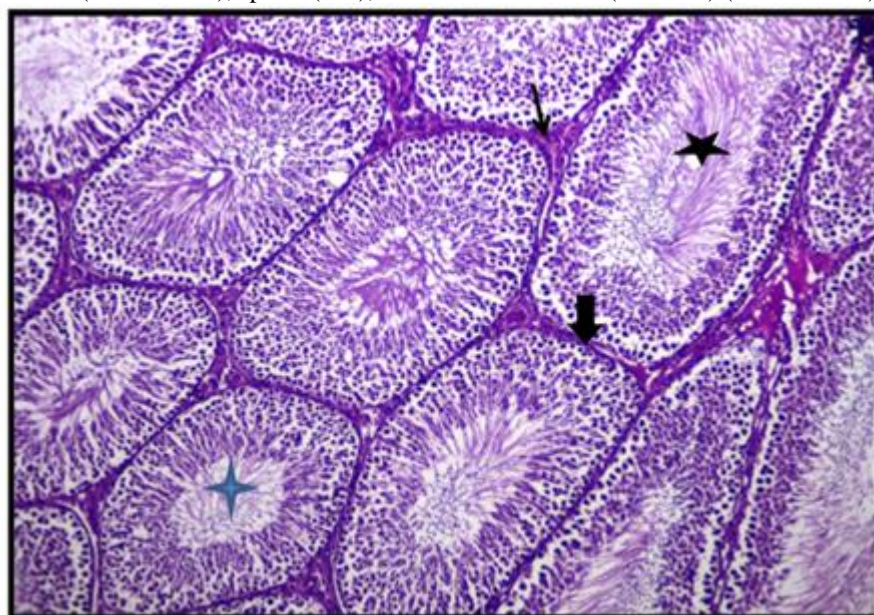


Figure 3. Testis section of rat dosed 650 mg/kg of *Hylocereus polyrhizus* extract Show intact seminiferous tubules (thick arrow), Leydig cells (thin arrow), increase in the number of epithelial germ layer () and sperms (star), decreased in the lumen (star blue) (H & E 100X).

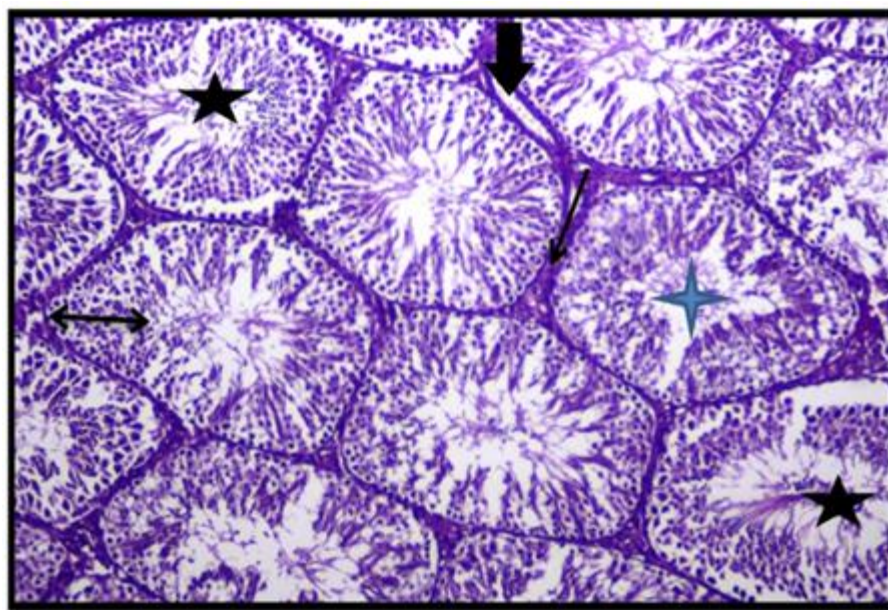


Figure 4. Testis section of rat dosed 650 mg/kg of *Hylocereus polyrhizus* extract and carbon tetrachloride 1.0 ml showing nearly normal seminiferous tubule (thick arrow), increase in the number of sperms (star), and Leydig cells (thin arrow), decreased in the lumen (star blue)), increase in the number of epithelial germ layer(⇔) (H & E 100X).

Conclusions

We conclude from this that the aqueous extract of *Hylocereus polyrhizus* has a positive effect in the process of improving fertility in male rats, as well as highly effective in antioxidants, an increase in the level of sex hormones, and reduces the toxic effect and tissue changes caused by carbon tetrachloride.

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