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Protective effect of nano-extract of *Lepidium meyenii* on some functional parameters and epididymal tissue in male albino rats treated with the Oxymetholone

Maryam Ahmed Ali Alrushdy*, Ashwaq Kadhem Obeid

Biology Dep., Education for Pure Sciences College, University of Kerbala, Karbala, Iraq
***Corresponding author e-mail: maryam.a.ali@s.uokerbala.edu.iq**
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Abstract

Due to the widespread use of the Oxymetholone and its widespread health problems, it was necessary to search for an alternative medicine treatment that reduces the harm of the Oxymetholone. Objectives from this study is determine the protective effect of the nano-extract of the *Lepidium meyenii* on the damage caused by the Oxymetholone in male rats. the experimental animals (28) were randomly divided into four groups, with 7 animals for each group The duration of the experiment was 55 days. The animal dosed daily, as follows: control group (G1): The animals were dosed with normal saline,Positive control group (G2): animals dosed orally with Oxymetholone (5 mg/kg B.w.),Group three (G3): Animals were dosed orally with the nano-extract of *Lepidium meyenii* (100 mg/kg B.w.),Group four (G4): It included animals dosed orally with the nano-extract of *Lepidium meyenii* (100 mg/kg B.w.) four hours before oral dosing with the Oxymetholone (5 mg/kg B.w.). The level of (GSH, SOD and number of sperm in epididymal tail) decreased in the Oxymetholone group and rose and was within the normal level in G3, G4.The histological sections of the epididymal in G2 It was noted that there were interstitial spaces between the epididymal tubules, and few sperm in the tubule cavities. With a lack of smooth muscle surrounding the tubules.Also showed in G3 and G4 the normal structure of the epididymis appears with regular epididymal tubules, an increase in the number of sperm in the lumen of the epididymis and the presence of connective tissue between the tubules. Maca root nano-extract has been shown to be effective in inhibiting oxymetholone-induced damage to oxidative stress and epididymal tissue in male rats.

Keywords : Oxymetholone, *Lepidium meyenii*, nano-extract.

Introduction

Medicinal plants have been used for the traditional treatment of diseases that afflict humans in various parts of the world for thousands of years. Human societies have inherited the use of plants to control or prevent diseases over many centuries. The Sumerians and Akkadians were the first to record the use of plants as a treatment for many diseases in about In 2600 BC, medicinal herbs are also considered a food source on the one hand and a medicinal source against various diseases on the other hand, because some of their plant parts contain chemical compounds that are of great importance for their functional effect and therapeutic activity for humans and animals [1].

One of the most famous medicinal plants is Maca (*Lepidium meyenii*). Maca species are famous for their antioxidant activities because they contain biologically active compounds that include secondary metabolites such as alkaloids, sterols, phenols, glucose nanites, alamides, and fatty acids [2] and to treat a variety of diseases, including a stimulant to overcome stress and fatigue, enhance human fertility, rheumatism, respiratory system disorders, anemia, improve memory functions and the immune system, and others [3].

The nutritional value of Maca plant, *Lepidium meyenii* (L.M.) [4] consists of carbohydrates (55-60%), protein (10-14%), dietary fiber (8.5%), and fat (1.2%). It is rich in minerals such as calcium, potassium, iron, manganese, copper, zinc and sodium, in addition to fatty acids and secondary metabolites of medicinal and nutritional importance such as maca, macamides, macaridin and alkaloids that are found only in this plant [5]. In addition, maca has nutritional, stimulant and fertility-enhancing properties. In males and females, it has an effect on sexual dysfunctions, prostate enlargement, alleviates menopausal syndrome, improves memory and learning, improves skin health, prevents osteoporosis [6] and has antioxidant and antitumor properties [7]. Therefore, maca is a wonderful food that improves the health of people who eat it.

Maca tubers have been consumed for centuries by indigenous people for their nutritional and stimulating values [8]. More recently, maca root has gained attention for its properties as an aphrodisiac, aphrodisiac and fertility enhancer, giving Maca international fame. These components have demonstrated Or combined in maca a host of bioactive activities in model systems, including reproductive health promotion, neuroprotection, antioxidant, anti-fatigue, anti-cancer, hepatoprotective, anti-osteoporosis, anti-memory, and immunomodulation [9], and valuable effects such as: increasing sperm count, treating infertility and improving fertility, combating stress [10] , nourishing the body’s glands and preventing osteoporosis [11]. Improving memory and learning in humans and laboratory animals [12] , hormonal regulation [5],treating or eliminating rheumatism and improving endurance [13] , and enhancing sexual ability. Increasing fertility and fetal survival [14] , improving the quantity and quality of sperm [15], protecting the skin from ultraviolet rays [16] , increasing sports performance and energy [17]. Effectiveness in combating anemia, leukemia, protecting the liver, AIDS, combating flatulence and cancer [18].

Oxymetholone [17b -hydroxy- 2 -hydroxymethylene17amethyl-5a-androstan-3 one] is a synthetic androgen analogue, and available in the commercial market as Androl. Oxymetholone is classified as an edible steroid when mixed with water. It is known to cause an enlargement in muscle size and a bulky appearance in a short time [19]. AAS induced damage to the reproductive function of males, it leads to testicular atrophy and reduction in sperm production. Also, it causes water and salt retention which leads to skin puffiness that increases blood pressure and strains the kidneys.

Prolonged use of AAS and high-intensity workouts worsen the level of renal damage as a consequence of renal toxicity [20].

Also, it induces a drop-in High-Density Lipoprotein (HDL) levels and a noticeable rise in serum Low-Density Lipoprotein (LDL), these disturbances may result in a stroke, myocardial infarction, edema, or hypertension. It is known that AAS use is attributed to attitude and psychological changes, and mental issues such as anxiety, irritability, depression, suicide, and schizophrenia are possible results [21]. Significant alteration in the activities of antioxidant cellular defenses, such as antioxidant enzymes SOD, CAT and GSH was detected. These changes produce an increase in ROS and induce oxidative stress and lipid peroxidation in cells this imbalance induce significant liver and kidneys damage. Protein powders have a strong market position as dietary supplements. It represents one of the most used supplements among exercising individuals in Fitness Centers [22, 23].

This study aims to determine the effect of the maca plant on the damage caused by the Oxymetholone to antioxidants and the number of sperm in the tail of the epididymis and its effect on the tissue of the epididymis in male rats.

Materials and Methods

Experimental Animals

This study used 28 adult male white rats whose weights ranged from (250-320) grams and their ages ranged between approximately (10-14) weeks. They were raised in the animal house of the College of Pharmacy / University of Kerbala, for the period from the beginning of December 2023 until the end of February 2024 The animals were placed in special plastic cages covered with metal covers, the floors of which were spread with fine sawdust, care was taken to clean the cages, the floor was constantly changed and sterilized with disinfectants, as well as constant care for the cleanliness of the irrigation bottles and the shelter room. All experimental animals were also subjected to appropriate laboratory conditions such as the appropriate temperature of 25 degrees Celsius and the duration of lighting. (12 hours of light and 12 hours of darkness) The animals were provided with water and standard ad libitum ad libitum throughout the research period, and the animals were left for two weeks to adapt to the conditions before conducting the experiment and to ensure that they were free of diseases.

Collection of used plants

The plant used (maca plant) was collected from local market and it was brought to the University of Karbala, College of Education for Pure Sciences, Department of Life Sciences. It was classified by the Prof. Dr. Nepal Emtir Tarad, specializing in plant classification.

Prepare the aqueous extract of the maca plant

A 50 gm of dried and ground maca root powder were added to 250 milliliters of distilled water and the mixture was left in a shaking incubator for (24 h) at a temperature of (37°). Celsius, then filter the solution using several layers of medical gauze, then use a centrifuge at 3000 revolutions for (10) minutes to ensure the removal of unground plant remains. Then pour the liquid into sterile glass dishes to allow it to dry at (25̊) Celsius. And collect it. The dry extract was extracted using a sterilized tool and kept in a dry, cool place until used in the experiment. It was prepared from the weight required in the experiment (200 mg/kg) and according to the weights of the animals, then dosed orally after dissolving it with tap water for each weight using a dosing tool [25].

Preparation of nanocomposites

Preparation of nanocomposites from layers of zinc oxide with maca plant

The method described by [24] was followed, with some modification, in preparing the hybrid nanocomposite, by adding 1 gram of maca to the zinc oxide solution obtained by dissolving 1 gram of zinc oxide in 50 ml of removed distilled water. The ions are extracted and the mixture is stirred using a magnetic stirrer at room temperature for 24 hours. Then the mixture is placed in the Shaker incubator at a temperature of 40°C for 18 hours. The color of the solution changes from pale yellow to reddish yellow. The sediment is separated by a centrifuge at 3000 speed. Cycle for 20 minutes, then wash with distilled, deionized water several times, then dry the precipitate in an electric oven at a temperature of 50°C, then grind it well with a ceramic mortar to obtain a fine powder and store it in the refrigerator until used in the experiment.

Experimental Group

the experimental animals (28) were randomly divided into four groups, with 7 animals for each group, as follows:

Negative control group (G1): The animals were dosed with normal saline daily for 55 days.

Positive control group (G2): animals dosed orally with Oxymetholone (5 mg/kg) body weight daily for 55 days.

Group three (G3): Animals were dosed orally with the nano-extract of maca roots (*Lepidium meyenii*) (100 mg/kg) body weight for a period of 55 days.

Group four (G4): It included animals dosed orally with the nano-extract of maca roots (100 mg/kg) of body weight four hours before oral dosing with the drug Oxymetholone (5 mg/kg) of body weight daily for 55 days

Results and Discussion

Results of the diagnosis of zinc oxide nano-extract from the aqueous extract of maca roots:

Atomic force microscope of Zno (AFM)

The current results of the process of revealing the nature of the surface of the synthesized nanoparticles showed the smoothness of the surface of the nanoparticles, as well as the shape and size of the synthesized particles and the extent of their agglomeration, as in Figure (1). The results of the analysis by AFM showed that the size of the nanoparticles ranges between (1- 52.21) n. The average size of nanoparticles reached (5.335) .

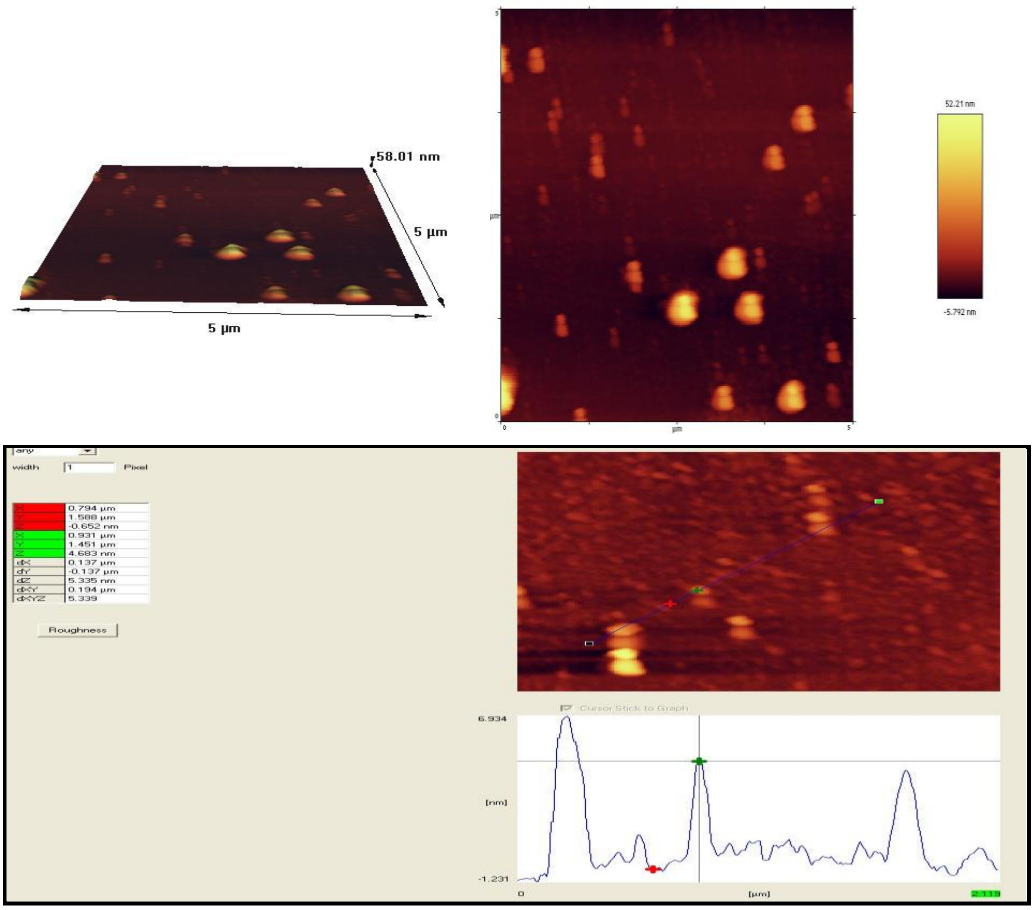


Figure (1): AFM analysis of zinc oxide nanoparticles (ZnO NPs) manufactured from the roots of the maca plant *Lepidium meyenii*.

X-ray diffraction (XRD) analysis

The XRD study confirms the Wurtzite structure of the zinc oxide nanoparticles, and the formation of a narrow peak at the Bragg angle indicates the spherical nature of the zinc oxide nanoparticles. Stabilization of the nanoparticles occurs by some capping agents, which is confirmed by the sharp peaks. The pattern was observed for X-ray diffraction of maca plant roots and was not observed. Any other stage as shown in Figure(2) .

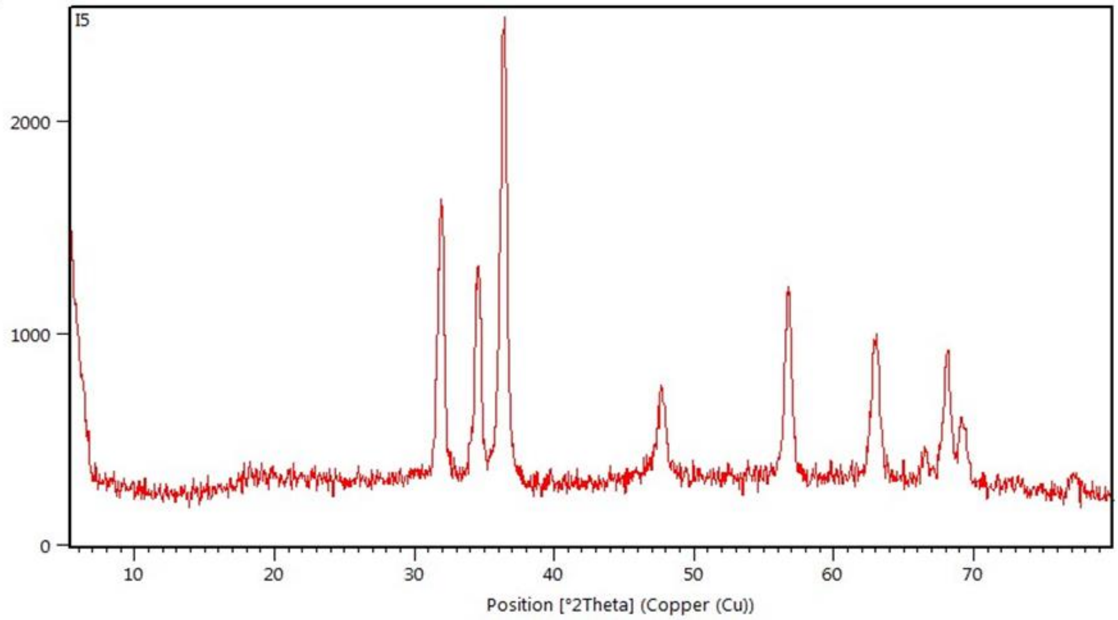


Figure (2): The XRD analysis of the zinc oxide nanocomposite and shows the composition and size

The results of the study in Table (1) showed a significant increase ($P<0.05$) in the levels of MDA for male rats dosed with oxymetholone at a concentration of 5 mg/kg dosed daily over the duration of the experiment of 55 days compared to the control group, and a decrease was also noted. In antioxidants (SOD, GSH) compared to the control group

The results of the current study showed that there were no significant differences for the MDA and SOD (6.64±0.32 and 438.90±1.43) compared to the control group for both. As for the GSH, it showed a significant increase of (40.10±0.58) compared to the control group (37.67±0.61). For the group of nano-extracts of maca roots only at a concentration of 100 mg/kg.

As for the nano-extract group treated with the drug oxymetholone at a concentration of 5 mg/kg, it was observed that there was no significant difference in (MDA, SOD) compared to the control group, while GSH showed a significant increase compared to the control groups.

Table (1): The concentration of SOD, GSH and MDA in the oxymetholone group and the maca roots group

Groups	S.E ± Means		
	GSH	SOD	MDA
Control G1	37.67 ± 0.61 B	435.36 ± 1.52 A	7.38 ±0.39 C
Oxymetholone (5 mg/kg B.W.) G2	9.28 ±0.56 D	297.74 ± 1.94 C	23.53 ±0.66 A
Nano extract of Maca root *Lepidium meyenii* (100 mg/kg B.W.) G3	40.10 ± 0.58 A	438.90 ± 1.43 A	6.64 ±0.32 C
Nano extract of Maca roots, *Lepidium meyenii* (100 mg/kg B.W.) + oxymetholone (5 mg/kg B.W.)	38.62 ±1.48 AB	436.53 ±0.90 A	0.49 ±6.57 C
LSD	2.3391	4.3805	1.2721
P (VALUE)	0.05	0.05	0.05

The results of the current study (Table 2) showed a significant increase ($p<0.05$) in the level of sperm concentration rates (1.36±88.60) for the group of rats that were dosed with the nano-extract at a concentration of 100 mg/kg for 55 days compared to the control group (1.35±81.80). The results of the current study also showed that there was no significant difference in the level of sperm concentrations (1.06±83.20) for the group of rats that were dosed with the nano-extract of maca roots at a concentration of 100 mg/kg for 55 days and those dosed with the drug oxymetholone at a concentration of 5 mg/kg of body weight. The length of the experiment period compared to the control group (1.35±2.80). A significant decrease was found in the second group compared to the control group.

Table (2): The concentration of sperm in the tail of the epididymis

Groups	S.E ± Means Concentration of sperm in the tail of the epididymis (1 ml * 10^{6})
control G1	81.80 ± 1.35 B
Oxymetholone (5 mg/kg) G2	2.80 ± 1.35 D
Nano extract of Maca root Lepidium meyenii (100 mg/kg) G3	88.60 ±1.36 A
Nano extract of Maca roots, Lepidium meyenii (100 mg/kg) + oxymetholone (5 mg/kg)	83.20 ± 1.06 B
LSD	4.4426
P (VALUE)	0.05

Histological Changes in Epididymis

The results of the histological examination in the current study of the epididymis in male rats of the negative control group showed that the tissue is normal, as the epididymal tubules show that their cavities are filled with sperm, the presence of smooth muscles around the tubules, and are lined with pseudostratified columnar epithelial cells as in Figure (3). The results also showed Histological examination in the current study of epididymal tissue in male rats dosed with oxymetholone (5 mg/kg B.W.) for 55 days, as in Figure (4,5). It was noted that there were interstitial spaces between the epididymal tubules, and few or no sperm in the tubule cavities. With a lack of smooth muscle surrounding the tubules.

The results of the histological examination of the group Nano extract of *Lepidium meyenii* root (100 mg/kg B.W.) showed that the normal tissue of the epididymis was observed, filled with mature sperm, and the presence of smooth muscle cells around the tubules in Figure (6). As for the group Nano extract of Maca roots treated with oxymetholone (5 mg/kg B.W.), the normal structure of the epididymis appears with regular epididymal tubules, an increase in the number of sperm in the lumen of the epididymis and the presence of connective tissue between the tubules (Figure 7).

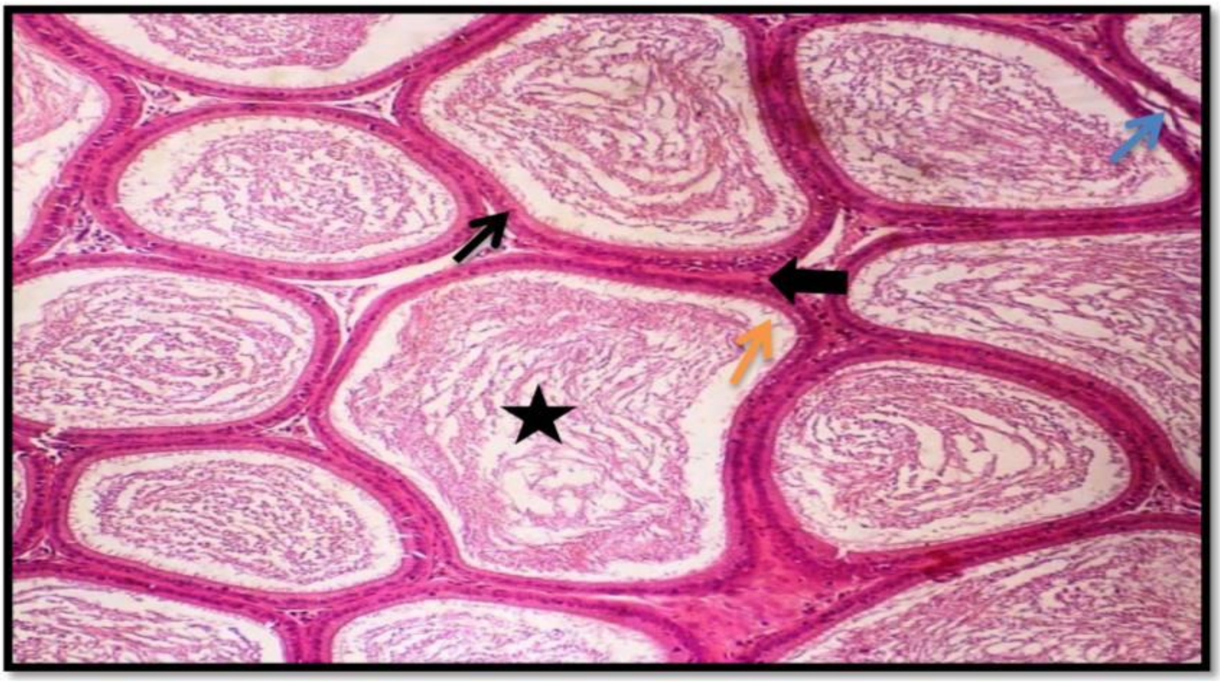
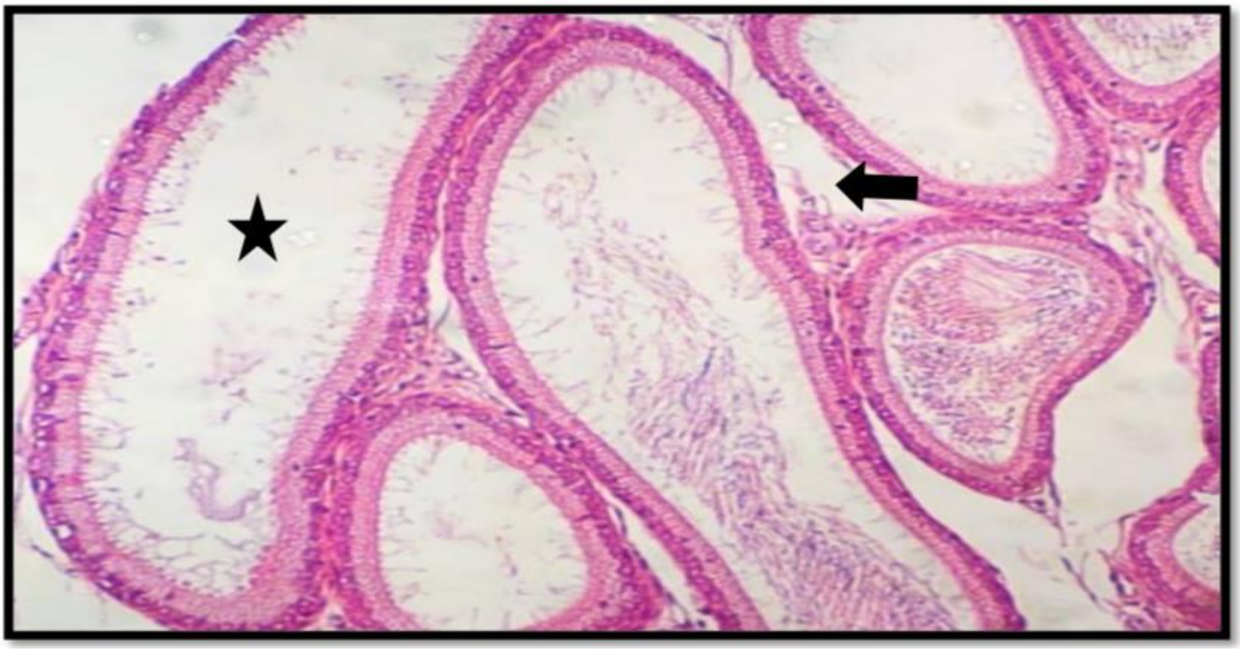


Figure (3): A cross-section of the epididymal duct of a rat from the control group, showing the compact epididymal tubules(⬅), the lumen filled with sperm(★), the presence of smooth muscles around the tubules (←), the pseudostratified columnar epithelium (←) with the presence of stationary cilia (←) (H&E 100X).



Figure(4):A cross-section of the epididymal duct of a rat in the group treated with oxymetholone(5mg/kg b.w),showing agroup of histological changes represented by the presence of interstitial spaces between the epididymal tubules (⬅) Very few or no sperm in the tubule cavities (★) (H&E 100X).

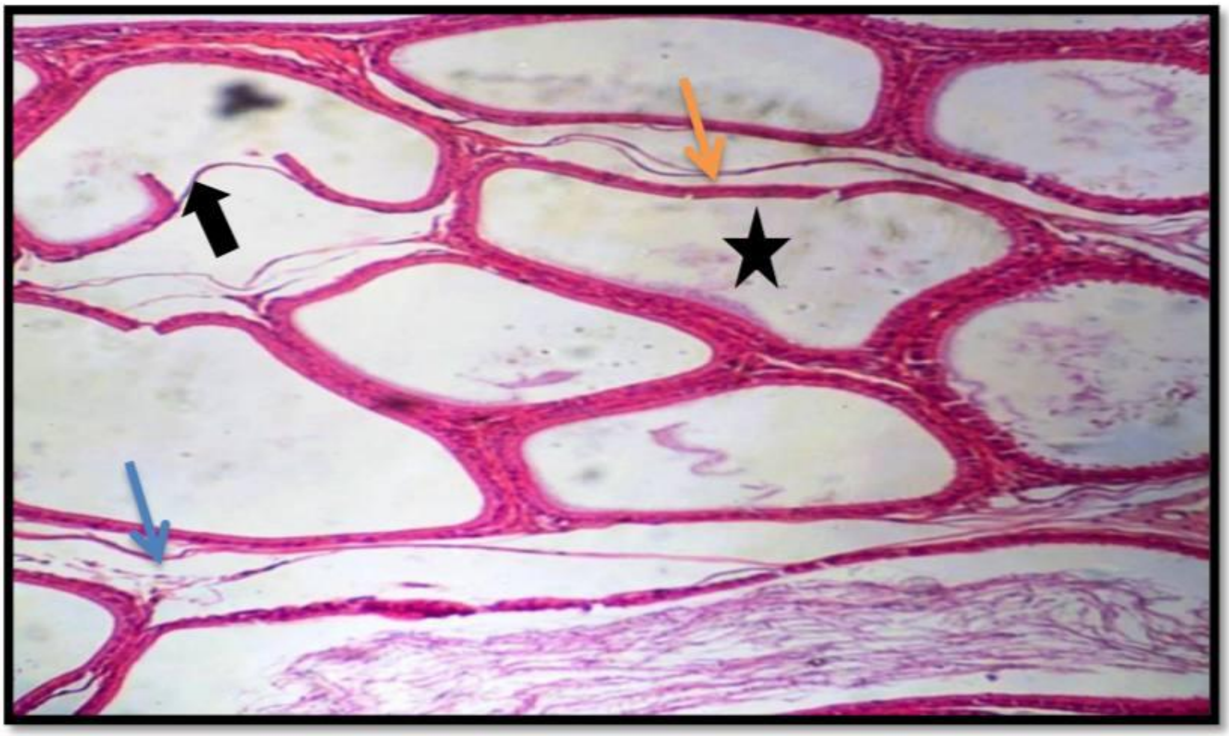


Figure (5): A cross-section of the epididymal duct of a rat in the group treated with oxymetholone (5 mg/kg B.w.), showing a lack or absence of sperm in the lumen of the tubules (★) , tissue damage with the destruction of the epithelial cells lining the tubules (⬅) and the epithelium is reduced (←) and Lack of peritubular smooth muscle (←) (H&E 100X).

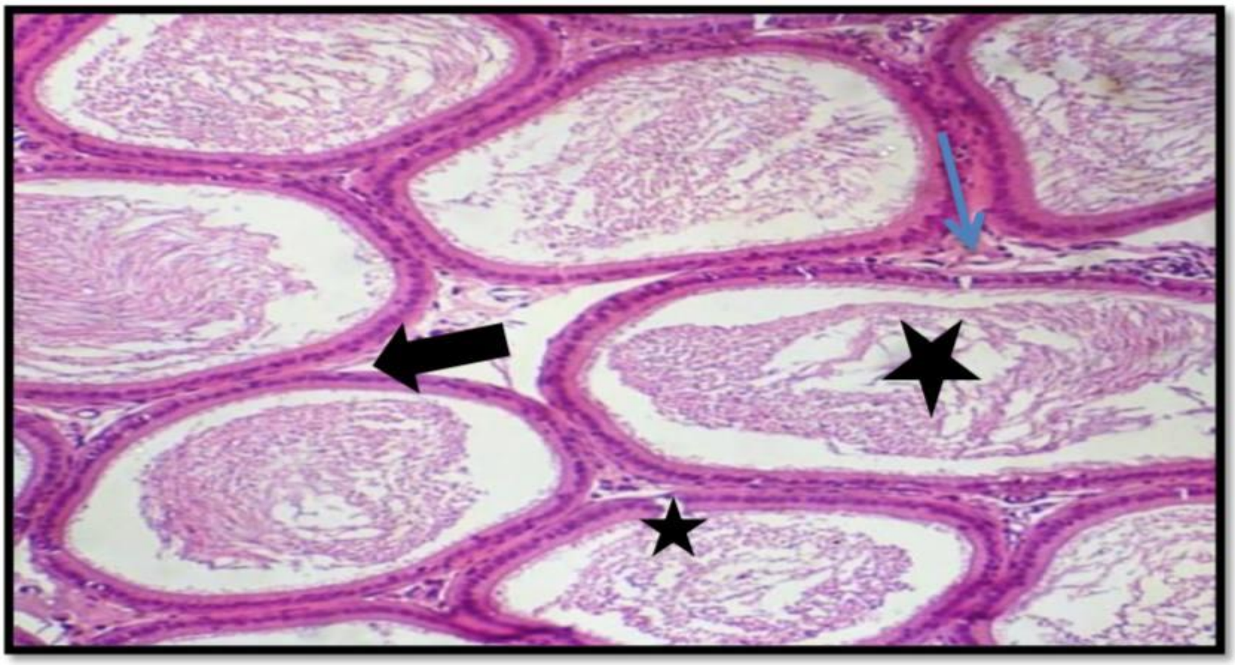


Figure (6): A cross-section of the epididymis of a rat in the group treated with the nano extract of the maca plant (100 mg/kg of b. w.) The normal tissue of the epididymis (⬅), the cavities filled with mature sperm (★), and the presence of smooth muscle cells around the tubules (←) (H& E 100X).

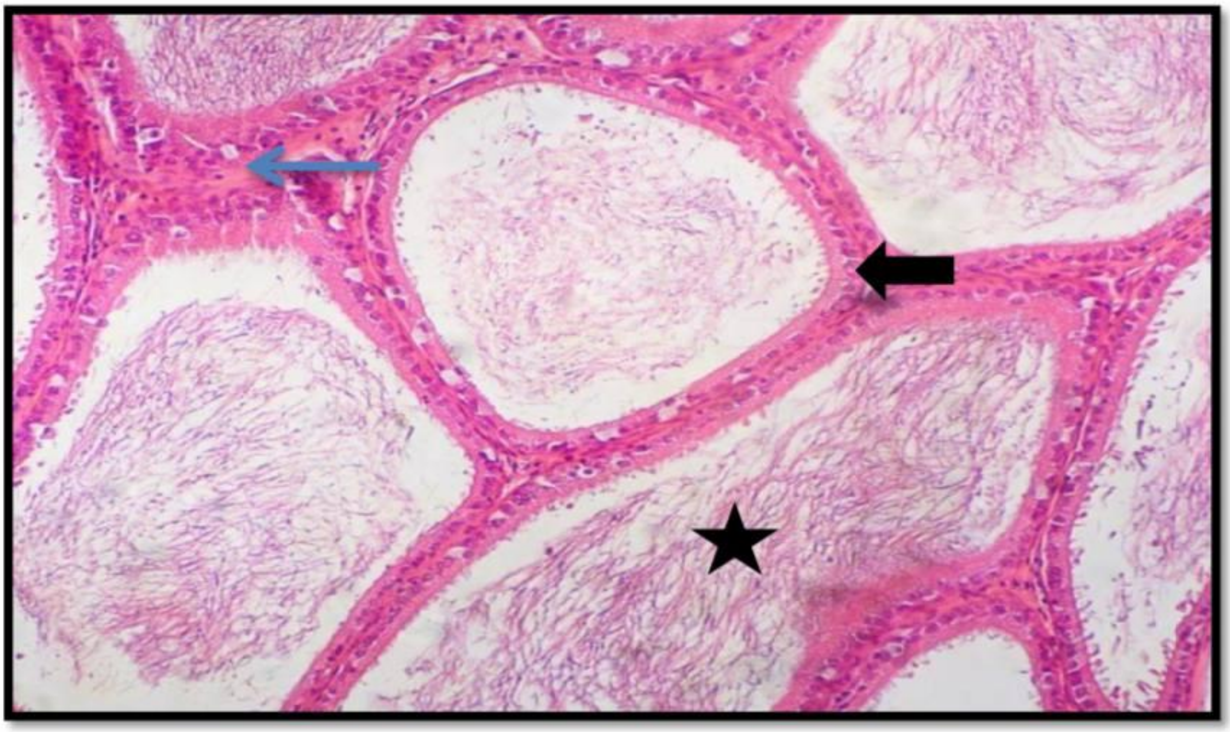


Figure (7): A cross-sectional histological section of the epididymal duct of a rat in the group treated with the nano extract of the maca plant (100 mg/kg) with oxymetholone (5 mg/kg b.w.), showing the normal structure of the epididymis with regular epididymal tubules (★) , increased sperm numbers in The lumen of the epididymis (←) and the presence of connective tissue between the tubules (⬅)(H & E 100X).

The results of the current study agreed with the results of [26] , as a decrease in sperm concentration and its indicators was observed in the oxymetholone group.
The results of this study were consistent with previous studies due to the hormones responsible for sperm formation, which are FSH and LH, which decrease due to the oxymetholone and have a role in starting and ending the process of sperm formation during the natural maturation of rat sperm [27]. Starting the process of sperm formation naturally and qualitatively requires the presence of sufficient levels of the hormone, nutrients for nutrition, and testosterone. Usually, insufficient levels of these hormones are associated with the presence of severe abnormalities in the sperm and may lead to sperm weakness and death.
Androgens also have an important role in the final stages of spermatogenesis (by stimulating them and facilitating their movement to a long distance during the spermatogenesis cycle, and the lack of androgens also delays the process of spermatogenesis by changing them in Sertoli cells, which It leads to premature separation of round sperm from Sertoli cells [28].
The effect of antioxidants in the experimental group is due to and can be attributed to oxymetholone -induced free radicals in the liver [29]. Despite tremendous advances in modern medicine, prevention and treatment of liver diseases still are challenging. However, the role of oxidative stress and inflammation in the pathogenesis of hepatic

diseases is wellestablished. Therefore, blocking or retarding the chain reactions of oxidation and inflammation can be considered as a promising strategy to prevent or treat liver injury[30]. ROS, as a common by-product of oxidative biochemical and physiological processes, is involved in numerous physiological and pathophysiological processes. Furthermore, higher concentrations of ROScan result in cell damage through oxidative modification of proteins, lipids and DNA and,therefore, plays a major role in the pathogenesis of a variety of human diseases[31]. A previous study [32] has reported that liver injury is associated with the increase of ROS generation in liver.

oxymetholone and its metabolites can harm the hepatocyte membrane by triggering an increase in the production of free radicals, consequently impairing normal liver function. This ultimately results in altered levels of liver functional markers. Research suggests that even therapeutic doses of oxymetholone (<5 mg/kg) can cause an elevation in serum levels of ALT, AST, ALP, and both total and direct bilirubin oxymetholone induced oxidative stress [33].

The excessive generation of ROS and Reactive Nitrogen Species (RNS) resulting from the metabolism of oxymetholone by hepatocyte cytochrome P450 and its excessive buildup in the liver and kidneys, surpassing the capabilities of the body's own antioxidant defense system, leads to harmful effects on cell membrane polyunsaturated fatty acids, protein structure, and DNA alkylation and methylation.[34]The glucuronic acid conjugates derived from ROS produced by oxymetholone and its metabolites are excreted, resulting in degradation of glomerular basement membranes, increased apoptosis

oxymetholone (5 mg/kg) resulted in several effects. It induced the production of free radicals, leading to an increase in NO levels and a decrease in the activity of GPx and CAT. These oxidative changes triggered apoptosis[35].

Food supplementation with antioxidants may offer a viable way to avoid oxidative damage to spermatozoa. Maca contains substances such as macamides and glucosinolates that may be broken down into isothiocyanates by myrosinase. This enables Maca to effectively remove free radicals and safeguard cells from Oxidative stress .

Maca includes materials inclusive of macamides and glucosinolates that may be damaged down via myrosinase into isothiocyanates. These components allow Maca to successfully dispose of loose radicals and protect cells from oxidative pressure [36].

The Maca plant has a clear effect in reducing oxidative stress, increasing sperm count, and repairing the epididymal tissue from damage caused by the oxymetholone.

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