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Evaluation of the protective role of *Epimedium grandiflorum* leaf nano-extract in testicular histological changes in male albino rats treated with amlodipine

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Abstract

The current study aimed to investigate the protective role of the nano-extract of *Epimedium grandiflorum* leaves on the testicular tissue structure of male albino rats (*Rattas rattus*) treated with amlodipine. The study was conducted at the Animal Unit, College of Science, University of Kufa. Thirty-five male albino rats, with an average age of 10 weeks and an average weight of 225-250 grams, were used. The animals were divided into seven groups of five animals each and treated as follows: the control group (group 1), which was treated with normal saline solution only; the second group, which was treated with the drug at a dose of 10 mg/kg; the third group, which was treated with the plant extract at a dose of 500 mg/kg; the fourth group, which was treated with both the plant extract and the drug at a concentration of 500 mg + 10 mg/kg; the fifth group, which was treated with the nano-extract at a dose of 1 mg/kg; and the sixth group, which was treated with the extract. The nanoparticles and the drug at a concentration of (1) + (10) mg/kg and the zinc oxide group at a concentration of (1 mg/kg), and all groups were treated for 45 days daily via the Abnub gastric dosing route. The animals were then sacrificed, and the testes were removed and placed in formalin for histological section preparation. Microscopic examination of the testicular tissue in the control group of male rats revealed that the seminiferous tubules had well-filled lumen containing sperm, and that Leydig cells and a germinal epithelial layer were present. In the drug group, however, fibrosis, atrophy, or obstruction were observed, along with degeneration and disintegration of the germinal layer, resulting in few or no sperm in

the seminiferous tubules and separation of the germinal layer from the basement membrane in most tubules. The testicular tissue in the rat group treated with the plant extract had a normal structure, showing normal seminiferous tubules and nuclei. The group treated with the plant extract and the drug exhibited a near-normal structure and was considered a protective group. Spermatogenous cell layers and regular seminiferous tubules were observed, along with the presence of Leydig cells in the interstitial tissue. As for the nano-extract group, the seminiferous tubules appeared normal, indicating the extract's protective and therapeutic effects against the drug. Sertoli cells also appeared. Similarly, the nano-group combined with the drug showed sperm and seminiferous tubules filled with normal sperm, indicating their regularity. However, the zinc oxide group, the toxic group, exhibited a decrease in the size of the epithelial cell layer, almost no sperm in the tubular lumen, cell disintegration, and separation of the germ layer from the basement membrane.

Keywords: *Epimedium grandiflorum*, nano-extract, testicular histological changes, male albino rats, amlodipine

Introduction

Medicinal plants are of great importance, whether used alone or as traditional complementary treatments (1). Numerous studies have explored the use of various medicinal herbs to treat a wide range of ailments. One of the most important of these plants is Epimedium grandiflorum, a member of the *Berberidaceae* family. It grows in the mountainous regions of China, Korea, and Japan, and is known by several names. Its various parts are used in complementary medicine (2). It is characterized by its alkaloid, flavonoid, sugar, and saponin content, in addition to the compound icariin (3). Studies have shown that the presence of flavonoids, the primary bioactive component of Epimedium grandiflorum, contributes to its other benefits. Its extracts also contribute to bone health by improving bone mineral density and preventing osteoporosis, particularly in postmenopausal women.

Research has also demonstrated that its flavonoid compounds, especially icariin, can restore immune balance, thus enhancing the body's resistance to disease. Furthermore, it plays a role in improving sexual function. A recent review suggests that the compound icarine inhibits key cellular signaling pathways involved in tumor

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growth, survival, and metastasis, resulting in reduced tumor cell growth in cancers such as prostate cancer (4). Given that all current treatments are chemical, this means they solve one problem but cause damage to another organ, tissue, or cell. Amlodipine is one such treatment. Amlodipine is a calcium channel blocker widely used to treat hypertension and angina. It works by widening arteries and reducing peripheral resistance, thereby increasing oxygen delivery (5). Despite its ability to treat these disorders, it has side effects, including excessive vasodilation leading to tachycardia and potentially fatal consequences, as well as palpitations, stomach pain, and chest pain (6). Amlodipine works by binding to dihydropyridine and non-hydropyridine sites on cell membranes. Cardiac and vascular smooth muscle contraction depends on the flow of calcium into and out of the cell via ion channels, and amlodipine selectively blocks this ion flow. Amlodipine's effect on smooth muscle is greater than its effect on cardiac muscle cells (5).

Preparation of experimental animals

Thirty-five adult male albino rats, weighing 225-250 grams and aged 10 weeks, were used in this study. The animals were housed in plastic cages filled with wood shavings at the animal house of the College of Science, University of Kufa. The cages were cleaned twice a week. The animals were kept under controlled laboratory conditions, including temperature (22-28°C), lighting (12 hours of light followed by 12 hours of darkness), and proper ventilation. Water and feed were provided, and the animals were allowed to acclimate for two weeks before the start of the experiment.

Collecting the leaves of the Epimedium grandiflorum plant

Dried goat's vine leaves were collected from local markets in the holy city of Karbala, and the plant was classified at the College of Education for Girls at the University of Kufa. The leaves were ground into a powder and placed in airtight containers for use.

Preparation of the aqueous extract of Epimedium grandiflorum leaves

The aqueous extract was prepared by dissolving 40 g of the ground powder in 1000 mL of water at 37°C for 24 hours. The solution was first filtered through high-precision filter paper and then placed in bottles. The bottles were centrifuged for 10 minutes at 4500 rpm at a temperature of 4, and the liquid was separated and placed in

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glass containers. The solution was then incubated in a convection oven for 24 hours to obtain the extract. The process was repeated to obtain a sufficient quantity to complete the experiment (7).

Preparation of the Nano-Extract of Epimedium Grandiflorum Leaves

The nano-extract was prepared by dissolving 40 g of the ground powder in 1000 mL of water at room temperature for 24 hours. The solution was first filtered through high-precision filter paper, then zinc acetate (chemical formula CH3COO)2Zn2H2O was added at a concentration of 2.5 g and left in a shaker incubator for 24 hours. The solution was then divided into equal samples and centrifuged at 4°C for 30 minutes at 4500 rpm. The samples were then washed to remove impurities using the same method, and the washing process was repeated three times. The precipitate was then placed in a convection oven for drying at 40°C for 48 hours to obtain the extract (8).

Amlodipine

Amlodipine was purchased at a dosage of 5 kg/m² from pharmacies in tablet form, and the dose given to each animal was determined based on weight.

Experimental Groups

Thirty-five male albino rats were divided into seven groups of five animals each, as follows: **Group 1:** Five male albino rats were given water and feed. **Group 2:** Five male albino rats were given amlodipine 10 mg orally daily for 45 days .Group **3:** Five male albino rats were given 500 mg orally of an aqueous extract of Epimedium leaves daily for 45 days. **Group 4**: Five male albino rats were given 500 mg orally of an aqueous extract of Epimedium leaves and 10 mg orally daily for 45 days. **Group 5:** Five male albino rats were given 1 mg orally of a nano-extract of Epimedium leaves daily for 45 days. **Group 6:** Five male albino rats were given a nano-extract of Epimedium leaves. Group 7: Five male albino rats were given 1 mg orally and 10 mg of amlodipine daily for 45 days. **Group 7:** This group included five male albino rats that were given 1 mg orally daily for 45 days.

After the 45-day trial period, and 24 hours after the last dose, the animals were anesthetized by intraperitoneal injection. The lower abdomen was opened; the testicle was extracted, washed with water, and placed in a 10% formalin solution for use in

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preparing tissue samples. The tissue samples were prepared and stained with eosin and hematoxylin according to the method of (9).

Histological Study

After dissecting the animals and removing the testicles, the histological sections were prepared according to the method of (Suravan et al., 2018). The histological sections were examined by a compound microscope using a Sony camera and positions were taken with different magnification powers (40x-400x).

Results and Discussion

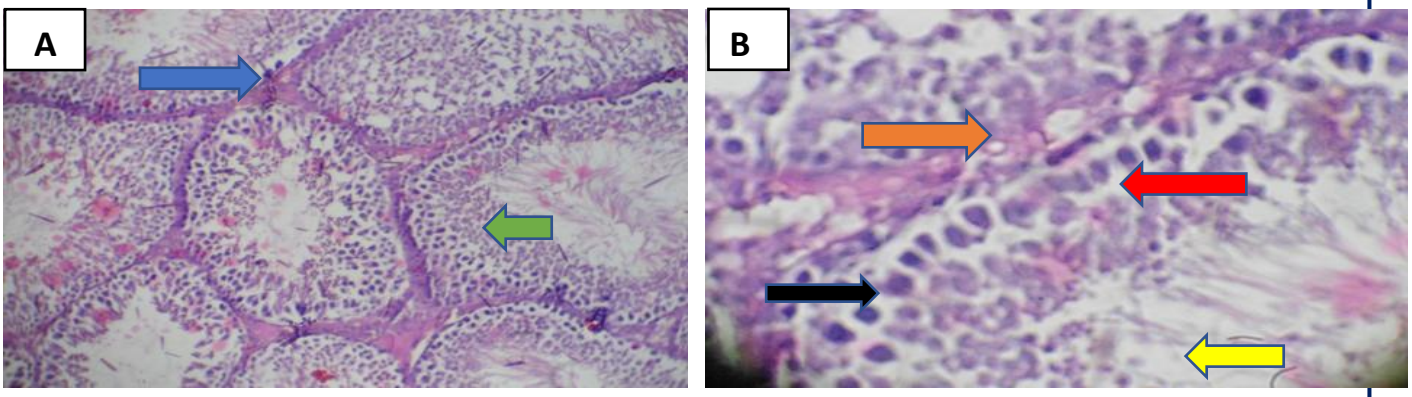

Figure (1) A cross-section of testicular tissue from a male rat in the control group for 45 days showing (blue arrow) seminiferous tubules and (green arrow) sperm, (red arrow) Sertoli cells, (orange arrow) Leydeck cells, (black arrow) germinal epithelium, (yellow arrow) seminiferous tubule lumen (eosin and hematoxylin A: 40x and B: 400x).

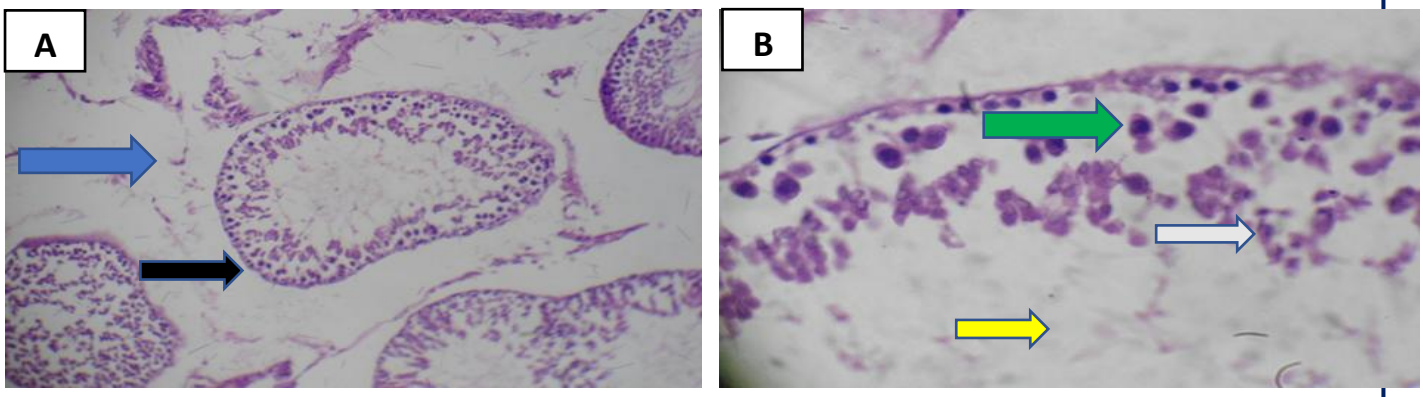

Figure (2) A cross-section of testicular tissue from a male rat treated with amlodipine at a concentration of (10 mg) for (45) days. The (blue arrow) shows the interstitial tissue, the (black arrow) the seminiferous tubule, the (green arrow) primary spermatozoa, the (white arrow) secondary spermatozoa, the (yellow arrow) seminiferous tubule lumen (eosin and hematoxylin A: 40x and B: 400x).

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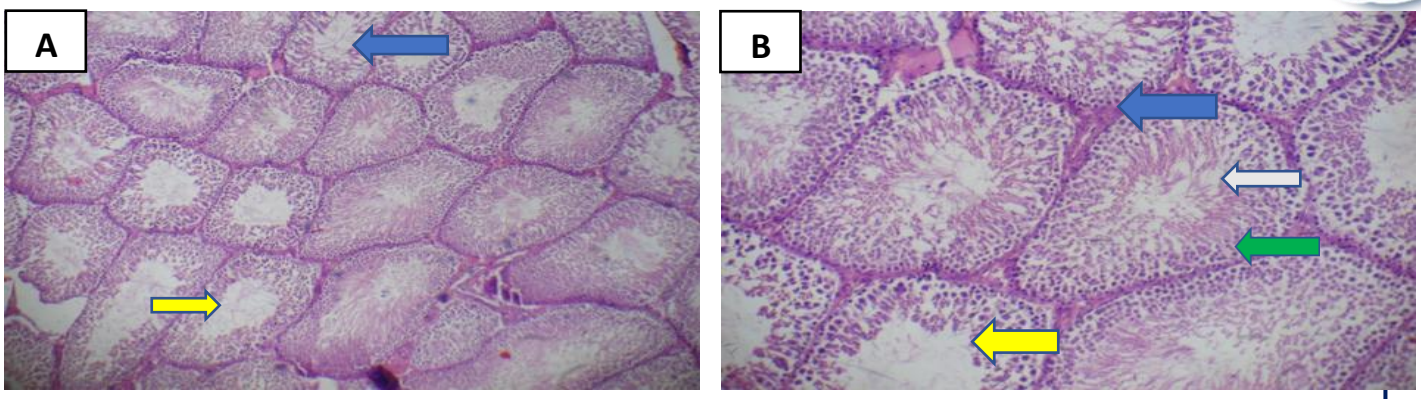

Figure (3) A cross-section of the testicular tissue of a male rat from the group treated with the aqueous extract of the horny goat plant at a concentration of (500 mg) for (45) days. The (blue arrow) shows the seminiferous tubules, the (yellow arrow) the seminiferous tubule lumen, the (green arrow) primary spermatozoa, and the (white arrow) secondary spermatozoa (eosin and hematoxylin A: 40x and B: 400x).

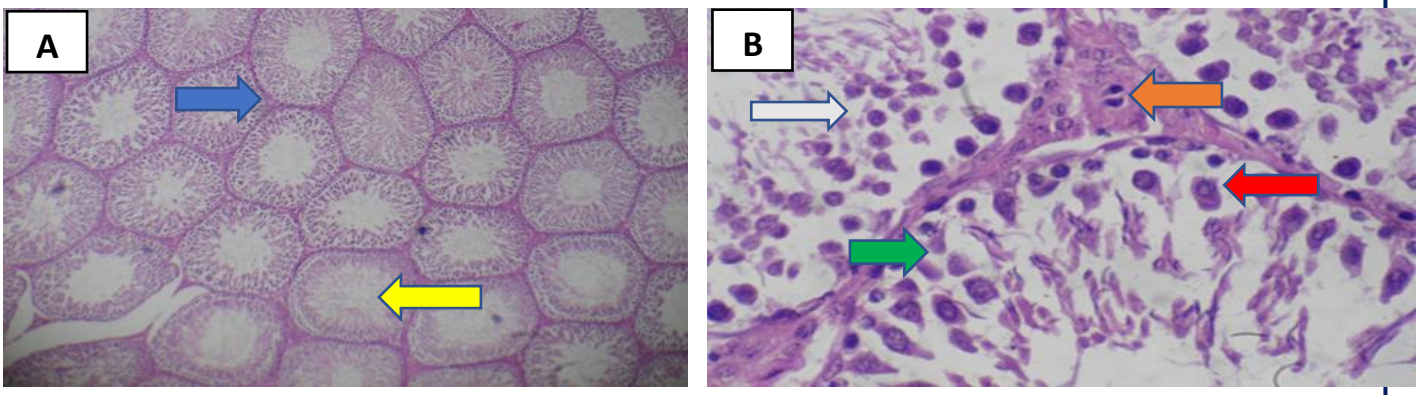

Figure (4) A cross-section of testicular tissue from a male rat in the group treated with an aqueous extract of horny goat's plant at a concentration of (500 mg) and amlodipine drug at a concentration of (10 mg) for (45) days. The (blue arrow) shows the seminiferous tubules, the (yellow arrow) the seminiferous tubule lumen, the (white arrow) secondary spermatozoa, the (orange arrow) Leydeck cells, the (red arrow) Sertoli cells, the (green arrow) primary spermatozoa (eosin and hematoxylin A: 40x and B: 400x).

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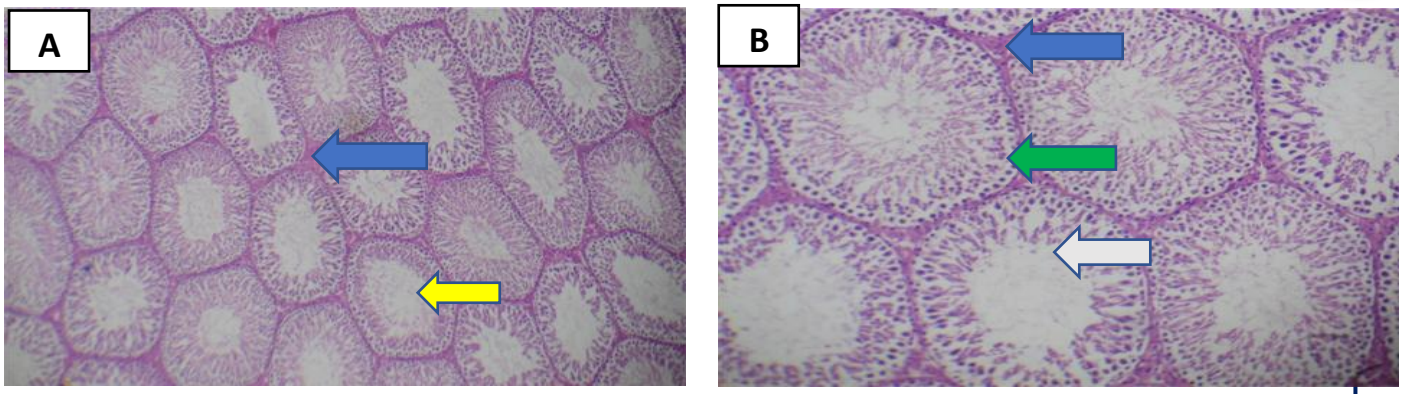

Figure (5) A cross-section of testicular tissue of a male rat from the group treated with the nano-extract of the horny goat plant at a concentration of (1 mg) for (45) days. The (blue arrow) shows the seminiferous tubules, the (yellow arrow) the seminiferous tubule lumen, the (green arrow) primary spermatozoa, and the (white arrow) secondary spermatozoa (eosin and hematoxylin A: 40x and B: 400x).

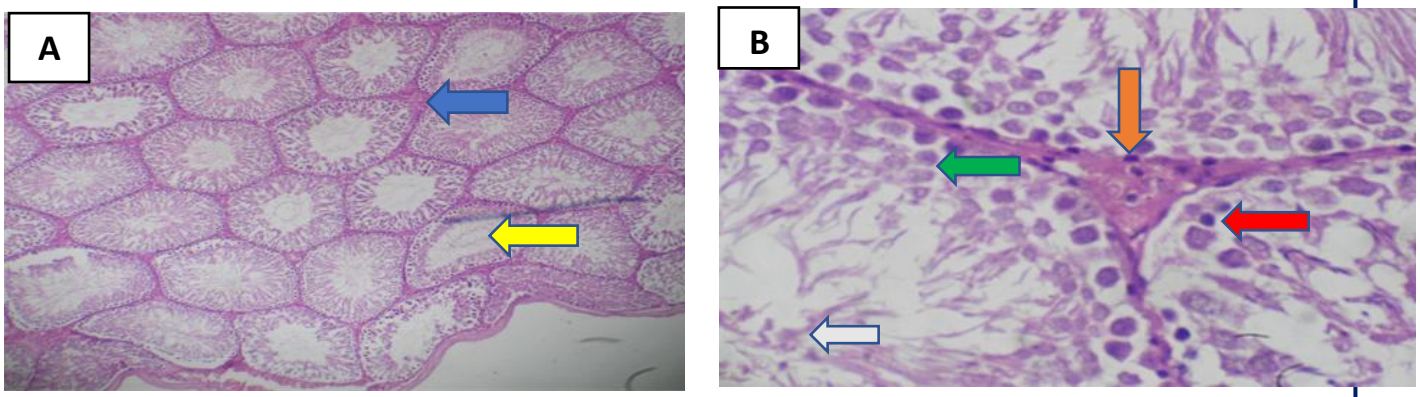

Figure (6) A cross-section of testicular tissue from a male rat in the group treated with the nano-extract of horny goat plant at a concentration of (1 mg) and the drug amlodipine at a concentration of (10 mg) for (45) days. The (blue arrow) shows the seminiferous tubules, the (yellow arrow) the lumen of the seminiferous tubule, the (orange arrow) Leydeck cells, the (red arrow) Sertoli cells, the (green arrow) primary spermatocytes, the (white arrow) secondary spermatocytes (eosin and hematoxylin A: 40x and B: 400x).

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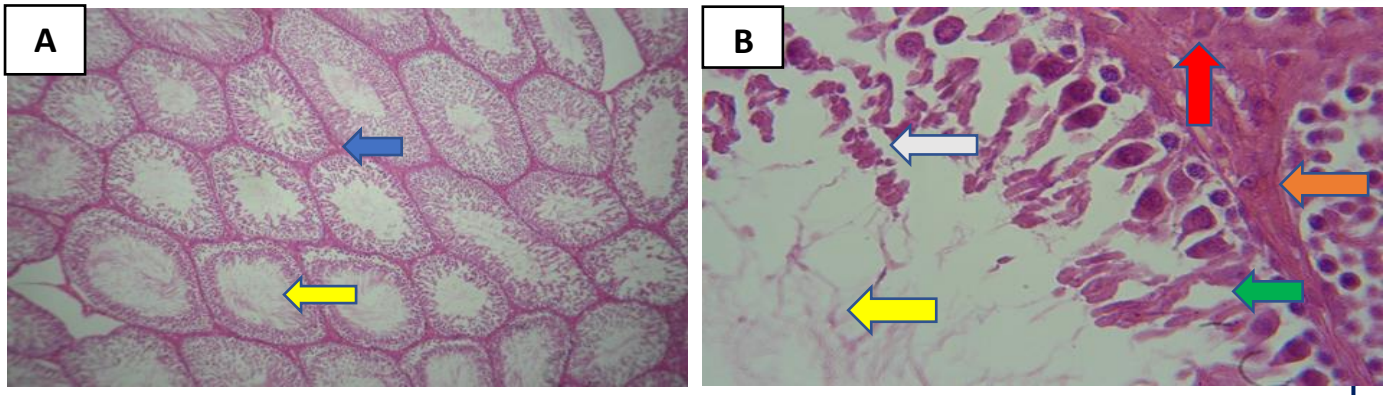

Figure (7) A cross-section of testicular tissue from a male rat in the group treated with zinc oxide at a concentration of (1 mg) for (45) days. The (white arrow) shows the seminiferous tubules, the (yellow arrow) the seminiferous tubule lumen, the (orange arrow) Leydeck cells, the (red arrow) Sertoli cells, the (green arrow) primary spermatocytes, the (white arrow) secondary spermatocytes (eosin and hematoxylin A: 40x and B: 400x).

Discussion:

Microscopic examination of testicular tissue in male albino rats in the control group revealed normal structure and no histological changes, as shown in Figure (1). The seminiferous tubules and nuclei appeared normal. However, in the group treated with amlodipine at a dose of 10 mg/kg body weight daily for 45 days, several effects were observed, including germinal layer degeneration and cell breakdown, reduced or absent sperm in the seminiferous tubule lumen, and separation of the germinal layer from the basement membrane in most tubules. These testicular effects are attributed to the amlodipine treatment and its toxicity to various organs, including the testes (10).

The drug, due to a decrease in sperm motility caused by chemical factors, resulted in the breakdown of the blood-testicular barrier (11). It also reduced sperm count due to the drug's ability to interfere with spermatogenesis. A decrease in sperm count is an important indicator of the effect of chemicals on spermatogenesis (12). Amlodipine acts directly or indirectly on the secretory function of the pituitary gland, causing a decrease in epididymal serotonin concentration through reduced androgen secretion (13). Amlodipine creates a different environment in the inner part of the seminiferous tubule wall compared to the outer part (11). Furthermore, the effects are attributed to the drug's impact on Leydig cells themselves, as the atrophy of Leydig cells is due to a decrease

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in LH hormones and testicular fat (14). This effect leads to a decrease in sperm count or delayed sperm maturation. This decrease in sperm cell components is attributed to the drug's inhibition of a stage of spermatogenesis (15).

The results of the histological examination of the group of white rats that were doused with the plant extract and the group of plant extract with the drug for horny goat weed (Eg) leaves at a concentration of (500) mg/kg of body weight for 45 days showed that the normal tissue of the testis appeared with the presence of seminiferous tubules, regularity of the seminiferous tubules, the presence of sperm, and an increase in the thickness of the seminiferous epithelial layer. Dosing with the extract of (Eg) Epimedium grandiflorum leaves led to an improvement in the testicular tissues with seminiferous tubules and their cavities filled with sperm, the presence of Leydig cells in the interstitial tissue, an increase in the level of testosterone, an increase in the activity of the enzyme superoxide dismutase and catalase enzyme, and a reduction in testicular tissue atrophy caused by taxi-stress in male white rats (16).

Through the hypothalamus (17), and also through GC-MASS analysis, our current study identified compounds such as 3-Methyl-2,2-diphenylaziridine and Bis, 2-EP-Aminophenylbenzimidazole (2,6). This demonstrates the protective role of horny goat weed leaf extract (Eg) in improving testicular function due to its bioactive compounds (16). The herb, an antioxidant, treats many diseases caused by free radicals that the body's natural biological systems cannot control (18). The aqueous extract of horny goat weed leaves reverses programmed cell death in sperm by increasing SOD levels and reducing reactive oxygen species in testicular tissue, inhibiting oxidative damage to sperm cells and DNA (19). This herb possesses therapeutic and antioxidant properties due to its content of compounds such as flavonoids, glycosides, alkaloids, steroids, and saponins (18).

Icariin is the active compound in this herb (Eg), as it is a flavonoid glycoside that promotes erectile dysfunction and is used to treat erectile dysfunction and fertility problems. Icariin acts on the function of nitric oxide synthase (NOS), which in turn stimulates the production of nitric oxide (NO), which increases the production of cyclic guanosine monophosphate (CGMP), thus improving blood flow and treating erectile dysfunction (20). The results of the testicular histological examination in the nano-extract group and the nano-extract group with the drug showed normal tissue, with the

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seminiferous tubule lumen and primary spermatocytes observed. Similarly, in the nano-extract group with the drug, Sertoli cells, Leydeck cells, and secondary spermatocytes were present. No abnormal changes in prostate tissue were noted. Furthermore, vital functions were improved due to the presence of numerous antioxidants, such as sterols, vitamins, and phenols, which play a role in reducing oxidative stress in body tissues. This indicates that the nano-extract has a protective effect against amlodipine. The beneficial effect of horny goat weed may be due to its rich antioxidant content, known for its ability to improve the structure of the reproductive glands and the concentration of sperm damaged by amlodipine. Therefore, the protective effect of the herb is closely linked to inhibiting oxidative stress, as observed in our study.

Conclusions

The current study concludes that the nano-extract of horny goat weed leaves has a protective role in safeguarding the testicular tissue structure of male albino rats treated with amlodipine.

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