Protective role of *Epimedium grandiflorum* extract on epididymal tissue and sperm parameters in male rats treated with cisplatin

Rasha Fadhel Musa[1*], Ashwaq Kadhem Obeid [2]

[1] Directorate of education of kerbala, Ministry of Education,Iraq
[2] Department of Biology, College of Education for pure Sciences, University of Kerbala, Kerbala, Iraq
***Corresponding author e-mail: rasha.f@s.uokerbala.edu.iq**
https://doi.org/10.59658/jkas.v12i2.3798

Received:
Feb. 04, 2024

Accepted:
Apr. 15, 2024

Published:
June 20, 2025

Abstract

Given the widespread use of cisplatin and its side effects on health, it was necessary to search for an alternative medicine treatment that reduces the harmful effects of cisplatin. To determine the protective effect of the aqueous extract of *Epimedium grandiflorum* on cisplatin-induced damage in male rats. The experimental animals include 28 adult male mice were used and randomly divided into four groups of (7) animals each. The duration of the experiment was 57 days. They were treated as follows: negative control group (G1), positive control group (G2) was injected with 2 mg/kg of cisplatin subperitoneally, (G3) was given a daily oral dose of the aqueous extract of *Epimedium grandiflorum* leaves at a concentration of 350 mg/kg, (G4) received a daily dose of the aqueous extract followed by a weekly injection of cisplatin. Histological changes of the epididymis in group G2 showed little or no interstitial spaces between the epididymal tubules and spermatozoa in the lumen of the tubules, with epididymal tissue damage and some cellular degeneration. In groups G3 and G4, the epididymis showed normal structure with regularity of the epididymal tubules and increased sperm count. Sperm concentration and percentage of motile sperm were decreased, with an increase in the percentage of spermatozoa (non-motile and deformed heads and tails) in G2 compared to (G1, G3, G4) which showed the opposite, so it could be concluded from this study that using the aqueous extract of horny goat weed leaves has been shown to be effective in inhibiting cisplatin-induced damage to the epididymis tissue of male rats.

Keywords: Cisplatin**,** *Epimedium grandiflorum*, epididymis, sperm criteria.

Introduction

Medicinal plants have biological features and properties that have the ability to cure some diseases in humans, due to their content of some effective chemical compounds with therapeutic pharmacological effects. Alkaloids, flavonoids, polyphenols and ter-

penes are considered chemical compounds with therapeutic properties. Their importance is evident not only in their diversity, but also in the many roles they play, starting from being antioxidants that protect cells from oxidative stress, to being anti-inflammatory agents [1]. In addition to their antioxidant properties, many medicinal plants also have anti-cancer properties, and thus can prevent the growth and spread of cancer cells [2].

Epimedium grandiflorum is a medicinal plant with antioxidant activity because it contains biologically active compounds including secondary metabolites such as flavonoids, alkaloids, tannins, saponins, glycosides, steroids, triterpenoids, and fatty acids [3]. Horny goat weed is considered a natural aphrodisiac that causes an increase in sex hormones and an increase in the activity of antioxidant enzymes [4].It also has a high rate in the treatment of erectile dysfunction [5]. The active compounds in horny goat weed have pharmacological effects such as antibacterial, antiviral, antidepressant, anti-inflammatory, antioxidant, anticancer, and antitumor properties [6].

Epimedium grandiflorum (*Eg*) is a perennial herb belonging to the Berberidaceae family, native to China, Japan, and Korea. It is a large, glossy, evergreen flowering herb, and has many common names such as goat's beard, goryanka, and flax flower [7].

One of the most deadly diseases in the world is cancer, which is characterized by abnormal cell cycle activity and irregular cell proliferation driven by genetic and environmental factors [8]. Chemotherapy drugs have an undeniable important role in the treatment of many diseases, but they cause long-term side effects [9]. Inevitable, such as genetic toxicity and reproductive system damage [10].

Cisplatin (CP) is the first of a class of platinum-containing anticancer drugs that currently includes carboplatin and oxaliplatin, which have been approved by the Food and Drug Administration (FDA) [11]. However, these drugs have their side effects, as these compounds interact with DNA inside cancer cells and bind to its molecules, which prevents cell division and DNA replication, leading to programmed cell death [12].

The death of sperm stem cells after exposure to cisplatin is a result of oxidative stress, which can alter the chemical modification of sperm DNA, so reproductive dysfunction and infertility associated with cisplatin treatment are a major concern [13,14]. Increased oxidative stress in cisplatin treatment is associated with increased production of reactive oxygen species (ROS), lipid peroxidation, which increases inflammation, apoptosis in testicular tissues, and inhibition of testosterone production [15].

Impaired spermatogenesis manifested by azoospermia, and cisplatin induces epididymal toxicity by reducing the number of sperm in the epididymis [16]. A way must be found to secure the use of cisplatin. Many studies have resorted to the use of effective natural plant antioxidants to prevent or reduce oxidative stress and inflammation resulting from the use of cisplatin, which have shown good effects on the pathophysiological condition [11].

Materials and Methods

Collection and Classification of plant

The dried leaves of *Epimedium grandiflorum* were imported from China and identified in the College of Education for Pure Sciences, University of Karbala by Professor Dr. Nepal Imteer Trad, a specialist in plant taxonomy

Preparation of aqueous extract of plant leaves *Epimedium grandiflorum*

A cold aqueous extract was prepared from *Epimedium grandiflorum* herb leaves based on the method of Hernandezi *et al*., [17]. (40g) of dried and ground E herb leaves were added to 800 milliliters of distilled water, and the mixture was left in a shaker incubator for 24 h at a temperature of (37 °). The solution was then filtered using several layers of medical gauze, followed by filtration using high-precision filter paper. Afterward, the centrifuge was used at (3000) rpm for (10) minutes to obtain a clear solution. The solution was placed in sterile glass dishes and dried in an oven at 40 ° to produce an extract in powder form. The powder was scraped with a sterile tool and stored in a refrigerator in sterile, airtight glass containers until use. It was prepared from the required weight in the experiment (350 mg/kg) according to the animals' weights and then administered orally after dissolving it in distilled water for each weight using a Gavage feeding tool.

Preparing a dose of cisplatin

Cisplatin was obtained in the form of a liquid package at a concentration of (50 mg/50 ml), which is the dose used for humans. The required dose was prepared according to[18]. At a concentration of (2 mg/kg) of body weight by calculating the required concentration based on the average weight of the animal's body.

Preparation of Experimental Animals

This study used (28) adult male white rats whose weights Approximately (225-250) gram and their ages ranged between (12-14) weeks. They were raised in the animal house of the College of Pharmacy / University of Kerbala, for the period from mid-September 2024 to mid-November 2024.All experimental animals were also subjected to appropriate laboratory conditions such as the appropriate temperature of 25 ° and the duration of lighting. (12 h of light / 12 h of darkness) and The animals were given a week period to acclimate to the surroundings prior to the experiment, in order to guarantee their disease-free status.

Groups of experimental animals

The animals were randomly divided into four groups, with 7 animals for each group, and treated with different treatments for 57 days as follows:

1- Negative Control Group (G1): The animals were dosed with normal saline daily for 57 days.

2- Positive Control Group (G2): Animals were injected intraperitoneally with Cp at a concentration of 2 mg/kg body weight once a week for 57 days.

3- Group 3 (G3): Animals were given daily oral doses of the aqueous extract of *Epimedium grandiflorum* at a concentration of (350 mg/kg) of body weight for 57 days.

4- Group 4 (G4): Animals were given daily oral doses of the aqueous extract of *Epimedium grandiflorum* at a concentration of (503 mg/kg) of body weight, then injected with Cp at a concentration of (2 mg/kg) of body weight four hours later once a week from the first treatment for 57 days.

Study of sperm parameters

Sperm Concentration in Epididymis

Immediately after dissection of the animal, the left epididymis was removed and crushed in a Petri dish after adding (1) ml of normal saline, then placed in an incubator at 37 °C. Then a drop of the mixture was taken using a Pasteur pipette and placed on the glass slide for counting Improved Hemocytometer after sterilizing and warming it in the incubator. After that, the cover slide was placed on the glass slide and examined under an Olympu light microscope at a magnification of (40x). The sperm concentration was calculated according to the method described in [19], where the calculation was done in the five small fields designated for counting red blood cells, and the readings were recorded after multiplying the result by 106 to determine the sperm concentration in (1 ml) of the epididymis.

Sperms Motility Percent

A drop of epididymal fluid was taken and placed on a clean glass slide, and the average percentage of motile sperm was calculated in ten random fields, and all sperm were viewed individually under an Olympu microscope at a magnification of (40x) and were considered motile if they showed any movement according to the following equation [19].

Motile sperm % = 100x (Motile sperm number) / (Total sperm number)

The percentage of abnormal sperms

A clean glass slide was used and a drop of epididymal fluid was placed on it to calculate the percentage of normal sperm by studying the deformities in the head, tail, location of the cytoplasmic drop, and deformities of the midpiece of the sperm. The slide was examined at a magnification of (40x) and the abnormal sperms were counted among 200 sperms according to the following equation: [20]

Percentage of normal sperm = 100x (abnormal sperm number) / (total sperm number)

Statistical analysis

The results of the statistical analysis were subjected to the SPSS Program (V.25) to find out the differences between the rates of the studied criteria for the different groups based on finding the least significant difference (Least Significant Differences, LSD). Then the results were expressed in the form of the rate ± standard error and the rate ± standard deviation. A probability value (0.05 (P≤) was adopted as a significant difference between the rates for all the studied criteria [21].

Results and Discussion

The results of the statistical analysis in the current study shown in Table (1) showed that there were statistically significant differences (P<0.05) between the four groups

with respect to sperm concentration, motility, and morphology (abnormal head, abnormal tail). This study observed a significant decrease in various sperm parameters, including concentration, motility, and morphology. It showed significant increases in head and tail abnormalities in the cisplatin-treated group.

Table (1): Effect of cisplatin and aqueous extract of *Epimedium grandiflorum* leaves on sperm parameters (sperm concentration, percentage of motile and non-motile sperm, and percentage of abnormal sperm with deformed head and tail) of male albino rats.

	Sperm concentration per 1 ml10^6x	Motile sperm%100	Non-motile sperm %100	Abnormal sperm %100	Abnormal head %100	Abnormal tail %100
Control (G1)	202.6 ±5.6 A	82.1 ±9.7 A	17.9 ±2.3 C	4.1 ±0.2 C	1.8 ±0.1 C	2.2 ±0.1 C
CP drug at a concentration of 2mg/kg (G2)	58.6 ±1.5 C	40.4 ±5.6 D	59.5 ±2.1 A	18.5 ±0.4 A	9.4 ±1.01 A	9.3 ±0.4 A
Epimedium grandiflorum aqueous extract 350mg/kg (G3)	212 ±8.3 A	79.8 ±5.4 A	20.1 ±7.6 BC	3.4 ±0.1 C	1.6 ±0.1 C	1.7 ±0.1 C
Epimedium grandiflorum aqueous extract 350mg/kg + CP drug 2mg/kg (G4)	130 ±1.8 B	68.9 ±3.8 B	31.1 ±0.7 B	10.4 ±0.2 B	4.7 ±0.3 B	5.69 ±0.1 B
LSD	16.15	7.8	13.18	0.82	0.87	2.62
P-VALUE	0.05	0.05	0.05	0.05	0.05	0.05

* Different letters represent significant differences at the probability level ($p<0.05$).
*Similar letters represent no significant differences at the probability level ($P<0.05$).

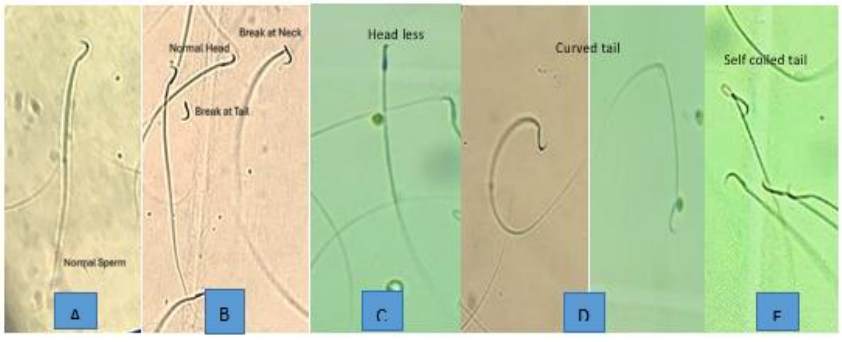

Figure (1): Some types of abnormalities in rat sperm, including normal sperm, sperm with a broken neck, sperm with a head without a tail, a weak head, a bent head, and a coiled tail.

Histological Changes in epididymis

Effect of cisplatin drug group at a concentration of (2 mg/kg) on the epididymal tissue of male white rats.

The results of the current study of the histological examination of the epididymis in male white rats of the negative control group G1 showed the normal histological structure of the epididymal duct, as the epididymal tubules appear cohesive and the tubule cavities are filled with sperm, with the presence of smooth muscles around the tubules and lined with false stratified columnar epithelial cells with the presence of stationary cilia, as seen in (Fig2).

show the results of the histological examination of the epididymis in male white rats treated with cisplatin at a concentration of 2 mg/kg of body weight G2 for 57 days. There were significant histological changes compared to the negative control group G1, represented by the presence of interstitial spaces between the epididymal tubules and sperm, few or no in the lumen of the tubules, with the presence of histological damage represented by the destruction of the epithelial cells lining the tubules, reduced epithelium, lack of smooth muscles surrounding the tubules, and degeneration of some cells (Fig 3) and (Fig 4).

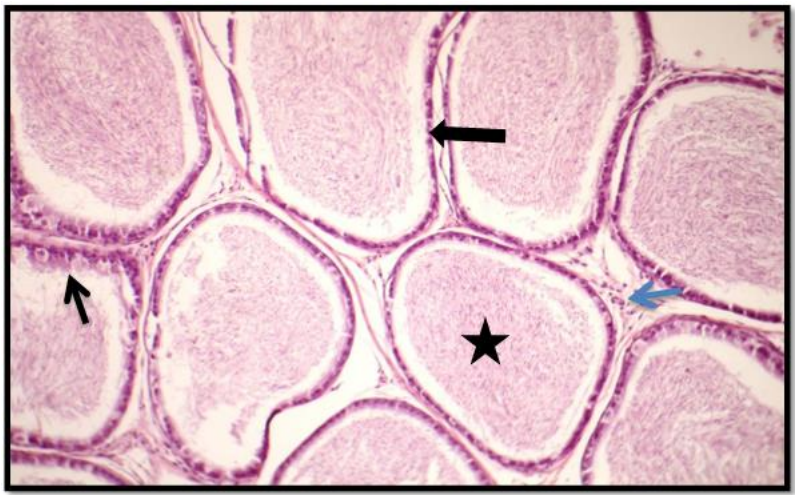

Figure (2) :A transverse histological section of the epididymis of a rat from the control group showing intact epididymal tubules (⬅), lumen filled with sperm (★), smooth muscle around the tubules (←), and pseudostratified columnar epithelium (←). (H & E 100X)

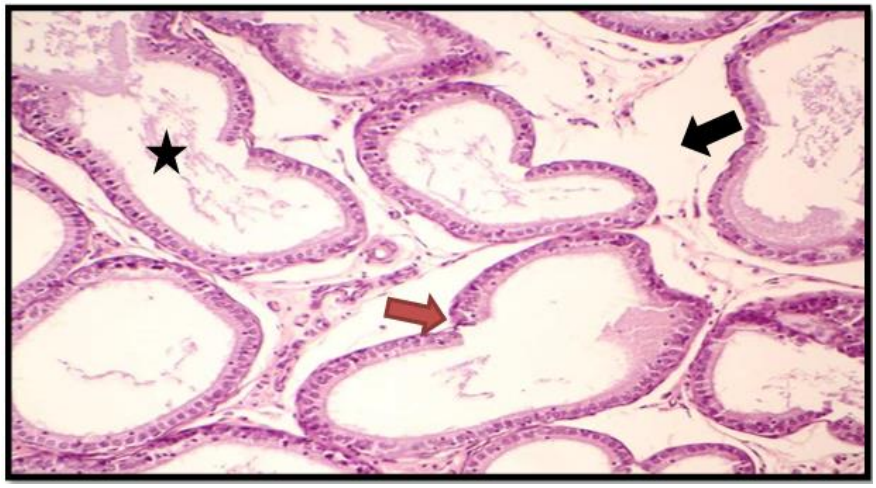

Figure (3): A cross-sectional histological section of the epididymis of a rat in the group treated with cisplatin at a concentration of 2 mg/kg of body weight, showing a group of histological changes represented by the presence of interstitial spaces between the epididymal tubules (⬅), very few or no sperm in the tubule cavities (★), and histological damage represented by the destruction of the epithelial cells lining the tubule (⇦) (H & E 400X).

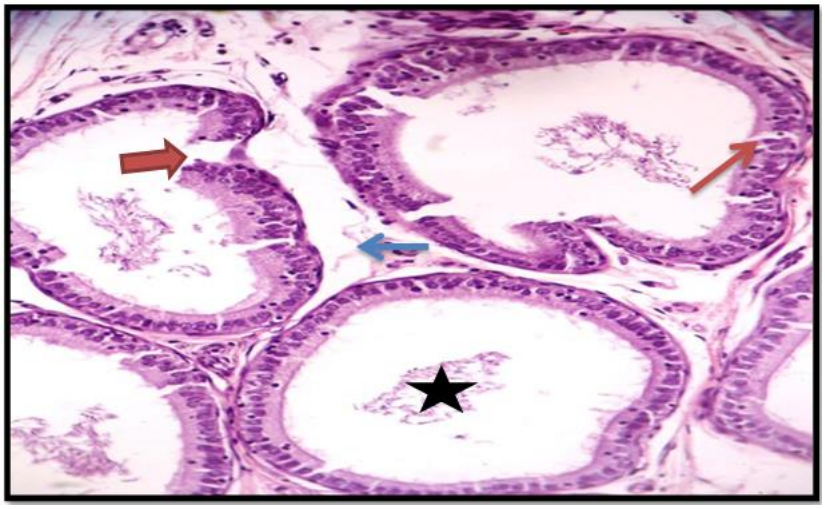

Figure (4): A cross- histological section of the epididymis of a rat in the group treated with cisplatin at a concentration of 2 mg/kg of body weight, showing insufficientin the lumen of the tubules (★) and tissue damage represented by the destruction of the epithelial cells lining the tubule (⇦) and the lack of smooth muscles surrounding the tubule (←) and degeneration of some cells (←) (H & E 200X).

The results of histological investigations of the epididymis of the group of male rats dosed with aqueous extract of horny goat weed leaves at a concentration of 350 mg/kg of body weight for 57 days in (Fig 5) showed that the structure of the epididymis was normal with regular epididymal tubules and the cavities were filled with mature sperm with the presence of smooth muscle cells around the tubules and the presence of static cilia.

the current study finding of the tissue sections of the epididymis of the preventive group that was dosed with the aqueous extract of horny goat weed leaves at a concentration of 350 mg/kg, simultaneously with cisplatin at a concentration of 2 mg/kg of body weight for 57 days, showed in (Fig 6) the structure of the normal epididymis with regular epididymal tubules, an increase in the number of sperm in the epididymal cavity, and the presence of connective tissue between the epididymal tubules.

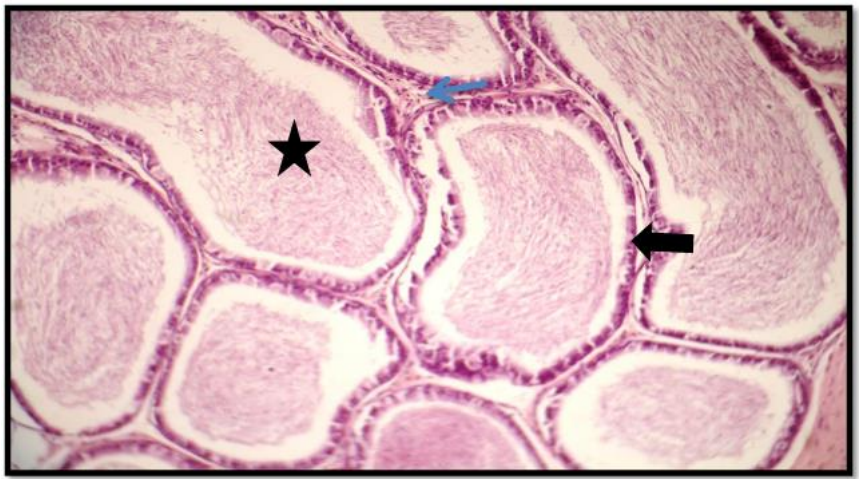

Figure (5): A cross-sectional histological section of the epididymis of a rat in the group treated with the aqueous extract of the horny goat weed plant at a concentration of (350 mg/kg of body weight), showing the normal structure of the epididymis with regular epididymal tubules (⬅) and the cavities filled with mature sperm (★) and the presence of smooth muscles (←) (H & E 100X) .

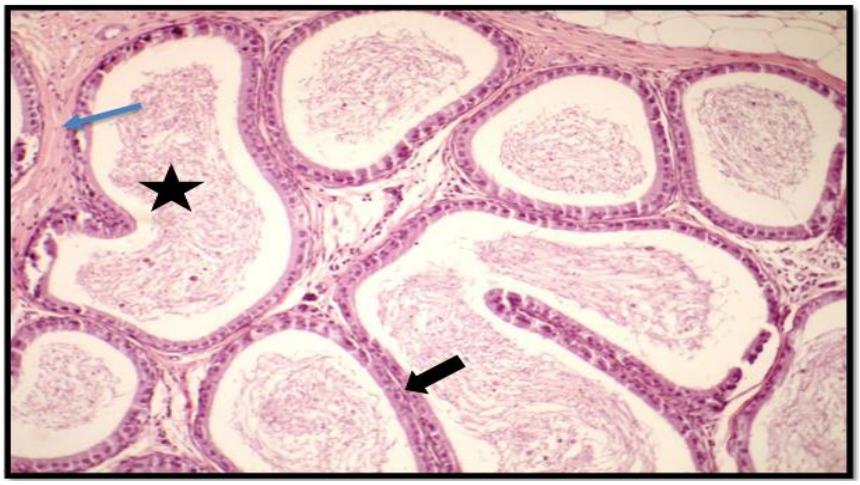

Figure (6): A cross-sectional histological section of the epididymis of a rat in the preventive group treated with the aqueous extract of the horny goat weed plant at a concentration of 350 mg/kg with the drug cisplatin at a concentration of 2 mg/kg of body weight, showing the normal structure of the epididymis with regular epididymal tubules (⬅), an increase in the number of sperm in the epididymal cavity (★), and the presence of connective tissue between the tubules (←) (H & E 100X).

The results of cisplatin treatment in the current study showed a decrease in sperm concentration and impaired sperm motility. Treatment with the drug also led to an increase in the percentage of sperm deformities, which included deformities in the shapes of sperm heads and tails. This was consistent with the study by Azouz et al. [22], which showed that cisplatin causes a decrease in sperm motility and concentration and an increase in the percentage of abnormal cases, which include separate and deformed heads and coiled and curved tails. The study by Yassien et al. [23]showed that the decrease in sperm concentration and motility and the increase in the percentage of abnormal sperm are associated with cisplatin treatment. The current study showed a significant decrease in sperm concentration and motility in the tail of the epididymis of male rats treated with cisplatin (G2), and a significant increase in the percentage of sperm deformities. Cisplatin causes excessive production of free radicals, which in turn leads to oxidative stress and testicular damage. The accumulated free radicals cause DNA strand breaks, which impedes the normal sperm formation process [24]. Increased production of reactive oxygen species (ROS) causes abnormalities in sperm parameters, as this increase damages sperm membranes and DNA and reduces the production of adenosine triphosphate (ATP) in sperm mitochondria, which affects sperm motility [25]. Damage to sperm DNA caused by oxygen species (ROS) may affect sperm function and motility [26]. Cisplatin also causes a decrease in the production of the testicular lipid hormone testosterone [27]. Which supports protein synthesis in sperm cells, so it has an important role in sperm formation [28]. Oxidative stress impairs sperm motility because tail proteins and plasma membrane lipids are oxidized, which makes them lose their ability to perform their function, and high levels of lipid peroxide have harmful effects on sperm function [29]. Lipid peroxide plays a toxic role on sperm, affecting the fluidity of the plasma membrane, sperm shapes, and disrupting key enzymes involved in the molecular mechanisms of sperm motility [30].

Treatment of laboratory animals with the aqueous extract of the leaves of the *Epimedium grandiflorum* plant (*Eg*) led to a clear improvement in sperm parameters in the current study, as Suo and his group [31] mentioned in their study that the compounds of the horny goat weed plant work to raise energy metabolism levels, which enhances sperm motility, as these compounds work directly on the sperm cell membrane, which improves its fluidity, which leads to enhancing sperm motility. The horny goat weed plant (*Eg*) is considered one of the most important antioxidants used in the treatment of various diseases caused by free radicals, and antioxidants contribute to combating the damage of free radicals that cause DNA damage and oxidative stress, which leads to various disorders. These antioxidants are essential to reduce the production of excess free radicals that exceed the body's natural ability to control it [32]. Zhao et al. [33] also showed that horny goat weed extract (*Eg*) can restore apoptosis in sperm cells in male rats, by increasing the level of SOD enzyme and reducing the levels of reactive oxygen species (ROS).

Horny goat weed extract improves sperm abnormalities and inhibits the activity of reactive oxygen species in the testes, as well as inhibits oxidative damage to DNA in

spermatogenic cells [34]. The active compounds and their derivatives in horny goat weed can restore the deficiency in Sertoli cells and reactivate sperm, and they also have the ability to reduce oxidative damage to DNA in the testes of adult rats [35].

The active compounds in this herb can improve sperm quantity and quality by activating the ERK1/2 signaling pathway [36]. Wang et al. [37] also showed that horny goat weed has effects similar to sex hormones in males via the hypothalamic-pituitary-gonadal axis. This plant is used to treat diseases resulting from a deficiency of sex hormones such as decreased sperm motility and low sperm concentration. Thanks to its fertility-enhancing properties, horny goat weed (*Eg*) works to increase testosterone levels and improve sperm formation [38].

The results of the current study in the group of male rats injected with cisplatin G2 showed tissue damage in the epididymal ducts represented by the destruction of the epithelial cells lining the tubule, the lack of smooth muscles surrounding the tubule, and the degeneration of some cells with the presence of few or no sperm in the tubule cavities. These results were consistent with the study [39]. There are many explanations that attribute the cause of tissue damage to the oxidative stress resulting from cisplatin, which stimulates oxidative stress by generating reactive oxygen species that cause further damage to DNA, proteins, and fats, leading to cell malfunction [40]. Pathological changes in the epididymis tissue may be due to increased levels of lipid peroxide and decreased antioxidant activities resulting from treatment with cisplatin, which leads to injury to the testicular and epididymal tissues and activation of programmed cell death pathways [41]. Tchounwou et al. [42] reported that cisplatin generates excessive amounts of free radicals, which leads to depletion of antioxidant activity, increased lipid peroxidation, structural protein deformation, and enhanced apoptosis. Cisplatin also induces epididymal toxicity by reducing sperm count [43], which is consistent with our current study. Narayana [44] also reported that the cause of epididymal damage was attributed to cisplatin's ability to cause double-strand breaks in DNA, as cisplatin induces structural changes, oxidative stress, and irreparable double-strand breaks in DNA.

The results of the current study, which were observed in a group of male rats that were dosed with the aqueous extract of horny goat weed leaves at a concentration of 350 mg/kg of body weight for 57 days, showed a positive effect on the epididymal tissues. This demonstrates the role of the aqueous extract of horny goat weed in improving the structure of the male reproductive system and the activities of the reproductive endocrine glands, as it contains a wide range of phytochemical compounds and their antioxidant potential and ability to eliminate free radicals [3]. Horny goat weed and its biologically active compounds protect the reproductive system by enhancing the synthesis of testosterone and protecting Leydig cells from injury caused by chemicals [45]. This improvement may be attributed to the ability of horny goat weed to regulate reproductive hormones and increase the activity of antioxidants, which play an important role in maintaining the health and function of the epididymis and fertility in males in general [4]. This is consistent with our study.

It has been proven that *Epimedium grandiflorum* extract has a protective role and effectiveness in inhibiting the damage caused by cisplatin to epididymal tissues and sperm parameters in male albino rats.

References

1) Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, *28*(4), 1845.

2) Foroughi-Gilvaee, M., Martirosyan, D., Mashayekhnia, M., Maadi, M., Sarvendani, M., & Maghsoumi, M. (2024). Exploring the potential of bioactive compounds in preventing cancer growth and progression: A comprehensive review. *Bioactive Compounds in Health and Disease-Online ISSN: 2574-0334; Print ISSN: 2769-2426*, *7*(6), 302-324.

3) Munir N, Mahmood Z, Yameen M, Mustafa G.(2020). Therapeutic response of Epimedium gandiflorum's different doses to restore the antioxidant potential and reproductive hormones in male albino rats. Dose-response,18(3):1559325820959563.

4) Nasef, A. Z., & El-Sheikh, N. A. (2023). Potential Effects of Horny Goat Weed (Epimedium grandiflorum) on The Level of Fertility in Male Rats Infected with Cadmium Chloride: Biochemical and Histopathological Study. Alexandria Science Exchange Journal, 44(3), 433-443.

5) Ariyo, O. C. (2023). Ethno-Botanical Survey of Medicinal Plants used in Traditional Treatment of Erectile Dysfunction in Akinyele Local Government Area, Ibadan, Oyo State, Nigeria. *Nigeria Agricultural Journal*, *54*(1), 156-172.

6) Ding, J., Li, C., Wang, G., Yang, Y., & Li, J. (2024). Cancer-Related Therapeutic Potential of Epimedium and Its Extracts. *Nutrition and Cancer*, *76*(10), 885-901.

7) Bustonova, S. S., & Gulomova, M. M. (2024). SOME INFORMATION ABOUT GORYANKA "MOUNTAIN GRASS" DISTRIBUTED IN THE MOUNTAINS OF HUMID REGIONS. Экономика и социум, (1 (116)), 112-113.

8) Brown, J. S., Amend, S. R., Austin, R. H., Gatenby, R. A., Hammarlund, E. U., & Pienta, K. J. (2023). Updating the definition of cancer. Molecular Cancer Research, 21(11), 1142-1147.

9) Sharma, A., Jasrotia, S., & Kumar, A. (2024). Effects of chemotherapy on the immune system: implications for cancer treatment and patient outcomes. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(5), 2551-2566.

10) Jin, Z., Zhao-Xia, L., Fan-Ke, P., Wen-Juan, Z., Min-Li, W., & Han-Yi, Z. (2024). Progress in the study of reproductive toxicity of platinum-based antitumor drugs and their means of prevention. Frontiers in Pharmacology, 15, 1327502.

11) Andrés, C. M. C., Pérez de la Lastra, J. M., Bustamante Munguira, E., Andrés Juan, C., & Pérez-Lebeña, E. (2024). Anticancer activity of Metallodrugs and metallizing host defense peptides—current developments in Structure-Activity relationship. *International Journal of Molecular Sciences*, *25*(13), 7314.

12) Yang, Y., Liu, L., Tian, Y., Gu, M., Wang, Y., Ashrafizadeh, M., ... & Zou, J. (2024). Autophagy-driven regulation of cisplatin response in human cancers: exploring molecular and cell death dynamics. *Cancer Letters*, *587*, 216659.

13) Zavattaro, M.; Lanfranco, F.; Salvagno, F.; Motta, G.; Sestero, M.; Marinelli, L.; Canosa, S.; Revelli, A.(2021). Gonadal failure and infertility in cancer survivors: Clinical management and strategies for prevention. Endocr. Metab. Late Eff. Cancer Surviv., 54, 59–69.

14) Razavi, S.; Hashemi, F.; Khadivi, F.; Bakhtiari, A.; Mokhtarian, A.; Mirzaei, H.(2021). Improvement of rat sperm chromatin integrity and Res. Part C Embryo Today Rev, 75, 112–129.

15) Keshta, A. T., Fathallah, A. M., Attia, Y. A., Salem, E. A., & Watad, S. H. (2023). Ameliorative effect of selenium nanoparticles on testicular toxicity induced by cisplatin in adult male rats. Food and Chemical Toxicology, 179, 113979.

16) Chiang, Y. F., Chen, Y. T., Huang, K. C., Hung, W. L., Chung, C. P., Shieh, T. M., ... & Hsia, S. M. (2024). Protective Effects of L-Cysteine Against Cisplatin-Induced Oxidative Stress-Mediated Reproductive Damage. Antioxidants, 13(12), 1443.

17) Hernandezi , M; Lopez, R; Abanas, R.M.;V and Arias, A.(1994) .Antimicrobial activity of visnea mocanera leaf extracts .j. Ethno pharma cology ,41 ;115-119.

18) Kmosh, S. M., & Al-Naely, A. J. (2024). The Effects of Different Concentrations of Vitamin D3 on Immunological Parameters of Immunosuppressed Rats Induced. *World's Veterinary Journal*, (1), 145-150.

19) Ranawat, P., and Bansal, M. P. (2009). Delineating the molecular mechanism behind regulation of spermatogenesis by selenium: Involvement of mitogen activated protein kinase; JNK. Am. J. Biomed. Sci, 1(3): 226-241.

20) Hinting, A. (1989). Methods of semen analysis in: assessment of human sperm fertilizing ability (Doctoral dissertation, Ph. D. thesis by Hinting, A., University of Michigan state).

21) Muhsin, S.S. 2022. Histopathological and Molecular Study on Effects Dandelion Leaf Aqueous Extract and Dandelion Silver Nanoparticles on Diabetic Wister Rats. M.Sc.Thesis. Faculty of Veterinary Medicine. University of Kufa, 70 pp.

22) Azouz, R. A., & Hassanen, E. I. (2020). Modulating effect of gum arabic on cisplatin-induced testicular damage in albino Wister rats. Revista Brasileira de Farmacognosia, 30(1), 90-98.

23) Yassien, E. E., Bikheet, M. M., Zaki, A. H., Shaban, M. M., Almuraee, A. A., Almehmadi, A. M., ... & Abdel-Aleem, W. M. (2024). Therapeutic effects of bee drone milk-fortified soymilk on cisplatin-induced testicular toxicity: Mechanisms and potential benefits. Journal of Functional Foods, 123, 106618.

24) Wei, Y. S., Chen, Y. L., Li, W. Y., Yang, Y. Y., Lin, S. J., Wu, C. H., ... & Tsai, P. S. (2023). Antioxidant Nanoparticles Restore Cisplatin-Induced Male Fertility Defects by Promoting MDC1-53bp1-Associated Non-Homologous DNA Repair Mechanism and Sperm Intracellular Calcium Influx. International Journal of Nanomedicine, 4313-4327.

25) Lahimer, M., Mustapha, H., Bach, V., Khorsi-Cauet, H., Benkhalifa, M., Ajina, M., & Ali, H. B. (2023). Oxidative stress in male infertility and therapeutic approach: A mini-review. Asian Pacific Journal of Reproduction, 12(6), 249-255.

26) Rashki Ghaleno, L., Alizadeh, A., Drevet, J. R., Shahverdi, A., & Valojerdi, M. R. (2021). Oxidation of sperm DNA and male infertility. *Antioxidants*, *10*(1), 97.

27) Gad, F. A., Abdelghaffar Emam, M., Eldeeb, A. A., Abdelhameed, A. A., Soliman, M. M., Alotaibi, K. S., ... & Abughrien, B. (2024). Mitigative Effects of l-Arginine and N-Acetyl Cysteine against Cisplatin-Induced Testicular Dysfunction and Toxicity through the Regulation of Antioxidant, Anti-inflammatory, and Antiapoptotic Markers: Role of miR-155 and miR-34c Expression. ACS Omega.

28) Ismail, H. Y., Hussein, S., Shaker, N. A., Rizk, H., & Wally, Y. R. (2023). Stem cell treatment trials for regeneration of testicular tissue in laboratory animals. Reproductive Sciences, 30(6), 1770-1781.

29) O'Flaherty, C., & Scarlata, E. (2022). Oxidative stress and reproductive function: The protection of mammalian spermatozoa against oxidative stress. *Reproduction*, *164*(6), F67-F78.

30) Walters, J. L., Gadella, B. M., Sutherland, J. M., Nixon, B., & Bromfield, E. G. (2020). Male infertility: shining a light on lipids and lipid-modulating enzymes in the male germline. *Journal of clinical medicine*, *9*(2), 327.

31) Suo J, Wang J, Zheng Y, Wang Y, Wang L, Wang X, Xiao F, Li R, Shen M, He J and Gao Q,.(2024). Effects of epimedium on the reproductive system and semen quality in male animals. In: Khan A, Mohsin M, Khan AMA and Aziz S (eds), Complementary and Alternative Medicine: Chinese/Traditional Medicine. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 9- 15.

32) Asif, N., Amjad, S., Hussain, K., Salman, M., Qureshi, F., Bukhari, N. I., ... & Zahid, F. (2016). Physicochemical Characterization and Antioxidant Activity of Extract of Epimedium Grandiflorum. Journal of Pharmacy and Chemistry.

33) Zhao, H., Song, L., Huang, W., Liu, J., Yuan, D., Wang, Y., et al. (2017). Total f lavonoids of Epimedium reduce ageing-related oxidative DNA damage in testis of rats via p53-dependent pathway. Andrologia 49 (10), e12756. doi:10.1111/and.12756

34) **34**)Zhao, H., Zhao, T., Yang, J., Huang, Q., Wu, H., Pan, Y., ... & Qian, Y. (2022). Epimedium protects against dyszoospermia in mice with Pex3 knockout by exerting antioxidant effects and regulating the expression level of P16. *Cell Death & Disease*, *13*(1), 69.

35) He, W., Liu, H., Hu, L., Wang, Y., Huang, L., Liang, A., et al. (2021). Icariin improves testicular dysfunction via enhancing proliferation and inhibiting mitochondria dependent apoptosis pathway in high-fat diet and streptozotocin-induced diabetic rats. Reprod. Biol. Endocrinol. 19 (1), 168. doi:10.1186/s12958-021-00851-9.

36) Nan, Y., Zhang, X., Yang, G., Xie, J., Lu, Z., Wang, W., et al. (2014). Icariin stimulates the proliferation of rat Sertoli cells in an ERK1/2-dependent manner in vitro. Andrologia 46 (1), 9–16. doi:10.1111/and.12035.

37) WANG, H., ZHANG, Q., CHEN, W., YANG, X., CHEN, F., ZHUANG, W., & ZHU, L. (2022). Research progress on sex-hormone-like effects of epimedium. International Journal of Traditional Chinese Medicine, 465-468.

38) Erasmus, L. J. C., & Leisegang, K. (2021). Herbal medicines (Zingiber officinale and Epimedium grandiflorum) used to treat andrological problems: Asia and Indian subcontinent. In *Herbal Medicine in Andrology* (pp. 123-128). Academic Press.

39) Makled, M. N., & Said, E. (2021). Tranilast abrogates cisplatin-induced testicular and epididymal injuries: An insight into its modulatory impact on apoptosis/proliferation. *Journal of Biochemical and Molecular Toxicology*, *35*(8), e22817.

40) Elmorsy, E. A., Saber, S., Hamad, R. S., Abdel-Reheim, M. A., El-Kott, A. F., AlShehri, M. A., ... & Youssef, M. E. (2024). Advances in understanding cisplatin-induced toxicity: molecular mechanisms and protective strategies. *European Journal of Pharmaceutical Sciences*, 106939.

41) Kohsaka, T., Minagawa, I., Morimoto, M., Yoshida, T., Sasanami, T., Yoneda, Y., ... & Sasada, H. (2020). Efficacy of relaxin for cisplatin-induced testicular dysfunction and epididymal spermatotoxicity. *Basic and clinical andrology*, *30*, 1-13.

42) Tchounwou, P. B., Dasari, S., Noubissi, F. K., Ray, P., & Kumar, S. (2021). Advances in our understanding of the molecular mechanisms of action of cisplatin in cancer therapy. Journal of experimental pharmacology, 303-328.

43) Chiang, Y. F., Chen, Y. T., Huang, K. C., Hung, W. L., Chung, C. P., Shieh, T. M., ... & Hsia, S. M. (2024). Protective Effects of L-Cysteine Against Cisplatin-Induced Oxidative Stress-Mediated Reproductive Damage. Antioxidants, 13(12), 1443.

44) Narayana, K. (2010). Cisplatin induces duplex 3′ overhangs and 5′ blunt ends in epididymal epithelium in a Bax-dependent manner without any protection from L-ascorbic acid. *European journal of pharmacology*, *641*(2-3), 238-245.

45) Liu, S. P., Li, Y. F., Zhang, D., Li, C. Y., Dai, X. F., Lan, D. F., ... & Zhang, J. D. (2025). Pharmacological actions of the bioactive compounds of Epimedium on the male reproductive system: current status and future perspective. *Asian Journal of Andrology*, *27*(1), 20-29.