Protective effect of active and nano-naringenin on methotrexate -induced oxidative stress in male rats' reproductive system

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Abstract

In this study, which aimed to determine the preventive effect of Naringenin extracted from citrus aurantifolial on the damage caused by the methotrexate, in which 30 male rats were used, divided into five groups, which were control group dosed with Normal Saline (G1), second group dosed with methotrexate (G2) , third group dosed with Naringenin (15 to 15.5 mg) (G3), four group dosed with methotrexate and active Naringenin (G4) , fifth group dosed with methotrexate and Naringenin nanoparticles (G5) for two months, it was found that significantly increase the level of sex hormones and sperm parameters in G3 , G5 compared to G2 . It was also found that active Naringenin , Naringenin nanoparticles works to repair damage to the tissues of the testicles, epididymis and accessory glands caused by methotrexate the experimental group administered with G3,G4 and G5 had a typical testicular tissue shape characterized by tubules. Spermatozoa exhibit an elliptical morphology and are organized in a systematic and orderly fashion. the basement membrane envelops each tubule and contains sperm-generating cells, including dispersed Sertoli cells with triangle nuclei sperm progenitors In conclusion, the results of this article revealed It was found that Naringenin alone and Naringenin nanoparticles with the drug gave the best results on the methotrexate -induced male rats reproductive system

Key words : methotrexate, Naringenin, active Naringenin ,nano-Naringenin, reproductive system, sex hormones

Introduction

Oxidative stress refers to a state of imbalance where there is an insufficient level of intracellular reactive oxygen species (ROS) compared to the biological system's capacity to eliminate ROS or restore the ensuing harm. ROS, or reactive oxygen species, are a group of naturally occurring substances generated as a result of regular cellular metabolic activities in organisms. These substances include hydrogen peroxide, hypochlorous acid, and free radicals [1,2].

Under normal physiological settings, cells typically maintain a state of equilibrium between the formation and scavenging of ROS [3]. Nevertheless, the equilibrium can

be disrupted by various inherent elements, such as disease, the process of aging, and individual behaviors like tobacco consumption or alcohol intake[4] .

Antioxidants are bioactive compounds that inhibit the process of oxidation in host molecules. both naturally occurring and artificially produced antioxidants have the ability to remove free radicals and hinder oxidative reactions [5].

Citrus fruits, plants, and algae possess natural chemicals that have substantial antioxidant action while exhibiting few adverse effects. Hence, the examination of natural antioxidants has emerged as a central area of interest within the fields of food science and functional medicine in contemporary times [6,7] .

The Rutaceae family encompasses a variety of citrus fruits, such as lemons, oranges, tangerines, grapefruits, and orange juice, each exhibiting distinct sizes and shape. [8,9]. Citrus fruits and citrus by-products contain medicinal components that exhibit pharmacological effects. Certain phytochemicals present in essential oils derived from citrus peel have demonstrated significant efficacy in scavenging free radicals, as well as exhibiting anti-inflammatory, anti-fungal and anti-oxidative stress properties [10,11] .

Naringenin is classified as a trihydroxyflavanone, a compound derived from gaseous flavones. It is a member of the flavonoid group, which is a subset of polyphenols. This specific flavonoid possesses considerable significance and functions as a vital component in the everyday dietary consumption of individuals. The Nomi plant serves as the principal origin of naringenin, a compound accountable for the plant's pigmentation and its characteristic bitter and sour flavor[11,12].

Naringenin has broad beneficial benefits, such as enhancing glucose metabolism, strengthening antioxidant defenses, enhancing the oxygenation of reactive oxygen species, regulating immune system function, as well as anti-atherosclerosis advantages. Furthermore, it exhibits anti-inflammatory and anti-cancer properties. As well as its beneficial neuroprotective effects and properties that contribute to the prevention of many diseases, including obesity and hyperlipidemia, which cause high blood pressure, atherosclerosis, diabetes, and Alzheimer’s diseasz.[14].

Methotrexate (MTX) is one of the most important folic acid antagonists that has been approved by the US Food and Drug Administration (FDA) for its great effectiveness, as it is often recommended for the treatment of rheumatoid arthritis. Treatment for juvenile idiopathic arthritis can be helpful. Methotrexate is an effective pharmacological intervention for a range of malignancies and lupus erythematosus [15,16].

A study conducted shown that the administration of MTX at specific quantities results in an elevation in the generation of ROS , including hydroxyl radicals, hydrogen peroxide, and superoxide anion, within the human body[17] . Consequently, it induces positive toxicity and undermines the sequence of the sperm formation process through the generation of these specific kinds and the suppression of natural antioxidant production. [18,19].

Sperm is susceptible to oxidative stress due to inadequate cellular repair mechanisms and poor antioxidant defenses resulting from its low cytoplasmic concentration. As a

result, the selective permeability of the flagella is disrupted, causing the flow of Adenosine triphosphate (ATP) to be hindered, leading to impaired flagella movement [20]. Typically, the process of oxidation of membrane lipids results in a significant impairment in both the functionality and morphology of the membrane [21,22].

Continuous fat oxidation in sperm results in the elimination of 60% of fatty acids from the plasma membrane, causing a reduction in membrane fluidity, an increase in non-specific ion permeability, and a decrease in the activity of receptors linked to the membrane[23].

According to [24], elevated levels of ROS harm deoxyribonucleic acid, lipids, and proteins, alter the enzymatic system, and result in permanent alterations that lead to sperm mortality and reduced semen parameters. This is linked to infertility among. [25].

The use of chemotherapy has been established to induce significant and deleterious impacts on several systems and organs. Research findings have demonstrated that guys who undergo chemotherapy experience adverse consequences such as sperm loss or absence, as well as infertility [26]. Methotrexate is a monoclonal antibody employed for the treatment of malignancies. It acts as a folic acid antagonist. Several prior investigations have also reported adverse effects, such as the absence and impairment of the seminiferous tubules in the testicle, a reduction in sperm count, and damage to sperm DNA following the injection of MTX[27].

This study aims to determine the effect of naringenin, active naringenin and nano-naringenin on rats adverse effects of Methotrexate.

Materials and Methods

Experimental animals

Animals

The research included a sample of 30 adult male rats, aged between 8 and 10 weeks, with a weight range of 168 to 178 grams. the animals were transported from the animal facility located at the College of Pharmacy, University of Kerbala .

The subjects were housed in hygienic and well ventilated enclosures throughout the duration of the study, ensuring unimpeded availability of sustenance and hydration.

Groups of experimental animals

In the experiment 30 male rats were divided into five groups, as follows:

1 -control group (G1): which included 6 animals. These animals were dosed with (1 ml) Normal Saline for two months.

2 -second group)G2(:It included 6 healthy animals that were dosed orally with the drug Methotrexate at 1 mg/kg once a week for two consecutive months to cause oxidative stress.

3 -The third group)G3): included 6 healthy animals that were dosed orally with the active ingredient naringenin, at 15 to 15.5 mg of naringenin (28), depending on the weight of the rat for 60 days.

4 -The fourth group (G4): It included 6 animals in which oxidative stress was induced with the drug methotrexate. They were dosed simultaneously with the drug once a week at 1 mg / kg and were dosed with the active ingredient naringenin depending on weight orally for 60 days.

5 -The fifth group (G5):It included 6 animals that were dosed with nano- naringenin and simultaneously with the drug methotrexate orally for 60 days.

Preparing the aqueous extract of the citrus aurantifolial

This study involved the procurement of dried citrus aurantifolial from local markets in Karbala Governorate. The extraction process consisted of numerous sequential processes, which are outlined below:

1- pproximately 250 gm of citrus aurantifolial were immersed in 1 liter of boiling water and thereafter left undisturbed until the water underwent evaporation, followed by drying at ambient temperature.
2- Following the drying process, the extract underwent grinding using an electric mixer grinder. The resulting powder was subsequently stored in plastic containers until the active constituents were identified through the utilization of HPLC technology.

Synthesis of nanoparticles

The nano-extract was prepared by dissolving 1 gram of zinc oxide in 50 milliliters of deionized water, then adding 1 gram of the active compound derived from citrus leaves (naringenin) to the solution, then stirring the mixture using a magnetic stirrer for 24 hours at room temperature. Then place the mixture in a shaker for 18 hours at 40°C. Then separate the sediment using centrifugation at 3000 rpm for 20 minutes. Then wash the sediment using deionized water several times. After drying at 50°C, it was ground well using a ceramic mortar until it obtained a fine powder texture. After that, it was placed in an electric oven at a temperature of 300 °C according to the proposed technique (29), and the powder was kept in a refrigerated environment until it was consumed.

Results and Discussion

Results of the nano-extract diagnosis of the active ingredient Naringenin extracted from the aqueous extract of the citrus aurantifolial plant :-

Atomic force microscope

After being loaded onto a zinc oxide nanocomposite, the active component naringenin was examined using an atomic force microscope. The measurement of surface roughness was conducted, along with the determination of the sizes, diameters, and aggregates of the nanoparticles. According to the approach [30], the models were dispatched to the Islamic Republic of Iran for the purpose of assessment in order to ascertain their shape. The sizes of the nanoparticles are of interest. A deposit of 100 microliters of the nanoparticle sample was applied onto the glass slide, resulting in the

formation of a thin film. The slides were subjected to a drying period of 5 minutes, following which they were subjected to scanning using an atomic force microscope.

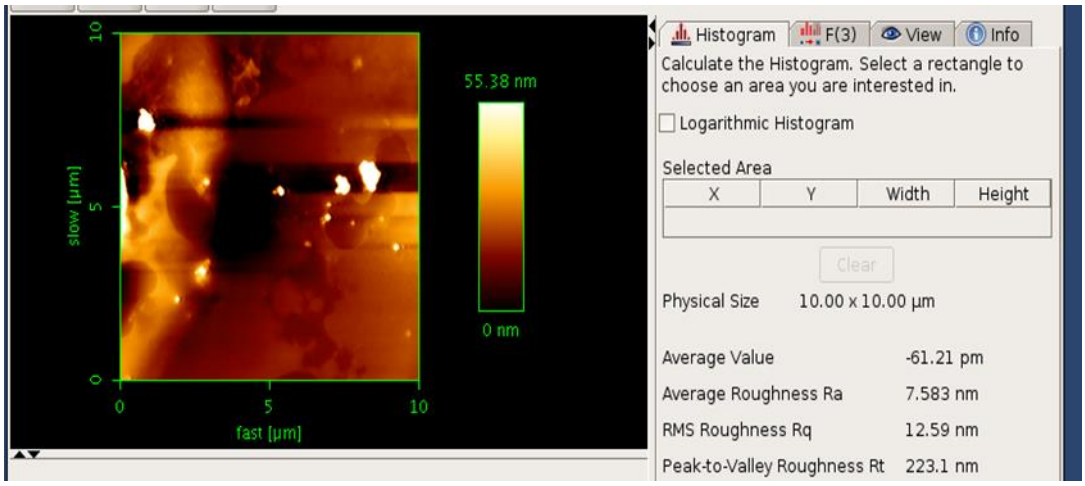

Figure (1): shows the analysis of nanoparticles using an atomic force microscope for the xrd Naringenin nano composite.

Scanning Electron Microscope (SEM)

The findings of the present investigation, as evidenced by pictures captured using a scanning electron microscope, revealed that the nano-extract composed of Naringenin and zinc oxide exhibited particle sizes ranging from 44.85-80.46 nm, with an average size of 65.68 nm. the particles exhibited spherical forms and were either single or clustered, as depicted in Figure(2,3)

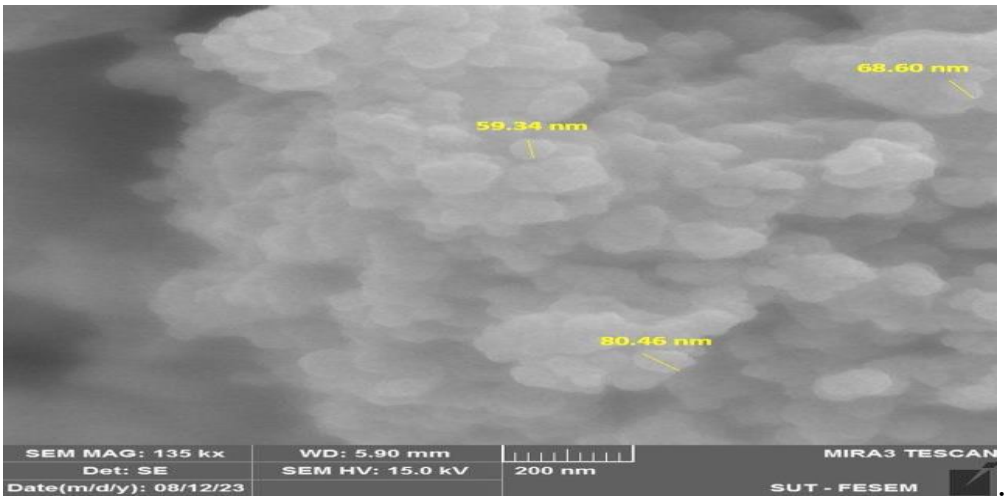

Figure (2): A scanning electron microscope (SEM) image of Naringenin nanoparticles extracted from the aqueous extract of the citrus aurantifolial plant at around 200 nanometers

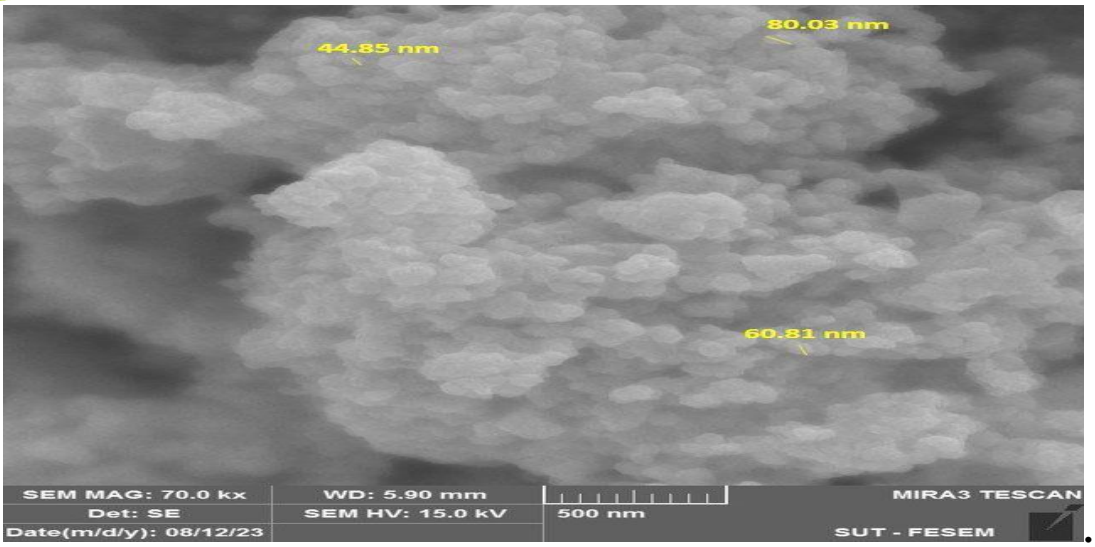

Figure (3) : A scanning electron microscope (SEM) image of Naringenin nanoparticles extracted from the aqueous extract of the citrus aurantifolial plant at a size of 500 nanometers.

Changes of sperm parameters

Sperms count of epididymis cauda

The findings shown in (Table 1) indicate a statistically significant reduction ($p<0.05$) in the quantity of sperm located in the tail of the epididymis among the participant group receiving the treatment, as compared to the control group.

The findings presented in (Table 1) indicate that there are no statistically significant differences ($p<0.05$) observed between G4 and G1. However, there are significant differences ($p<0.05$) observed between G4 and G2, which are higher than those shown between G1 and G2.

The findings presented in Table 1 indicate a statistically significant increase ($p<0.05$) in the G3 and G5 groups when compared to the G1 and G2.

Percentage of motile sperms

The findings presented in (Table 1) demonstrate a statistically significant reduction ($p<0.05$) in the proportion of motile sperm in the G2 as compared to the G1 phase.

The findings presented in Table 1 indicate that there are no statistically significant differences ($p<0.05$) observed between G3 and G5 in comparison to G1. However, substantial differences ($p<0.05$) are observed when comparing G3 and G5 to G2.

According to the findings shown in (Table 1), a statistically significant increase ($p<0.05$) was observed in the naringenin group in comparison to G2.

Percentage of live sperms

The findings presented in Table 1 indicate a statistically significant reduction ($p<0.05$) in the proportion of viable sperm in G2 as compared to G1.

According to the findings presented in Table 1, there are no statistically significant differences ($p<0.05$) observed in G3, G4, and G5 when compared to G1. However, a substantial rise ($p<0.05$) is shown when comparing G3 to G2.

Percentage of died sperms

The results in (Table1) showed that there was a significant increase ($p<0.05$) in the percentage of dead sperm in G2 compared to G1.

The results in (Table1) also showed that there were no significant differences ($p<0.05$) in G3, G4 and G5 compared with G1, and significant differences ($p<0.05$) when compared with G2.

Percentage of abnormal sperms

The results in (table1) showed a significant increase ($p<0.05$) in the percentage of abnormal sperm in G2.

The results in (table1) also showed that there were no significant differences ($p<0.05$) in the percentage of abnormal sperm in G3, G4 and G5 compared to G1, and there was a significant difference ($p<0.05$) decrease when compared with G2.

Table (1): shows the sperm standards of male rats in the experimental groups

	stderr± Means				
	Percentage of abnormal sperms	**Percentage of died sperms**	**Percentage of live sperms**	**Percentage of motile sperms**	**Sperms count of epididymis cauda 1ml*10^6**
G1	4.96 ± 1.08 BC	18.40 ± 2.27 B	92.96 ± 1.42 AB	31.04 ± 0.59 A	79.58 ± 2.23 C
G2	98.69 ± 0.79 A	98.35 ± 0.92 A	4.38 ± 2.43 C	1.00 ± 0.36 C	2.83 ± 1.15 D
G3	4.51 ± 1.36 BC	15.86 ± 1.29 B	91.86 ± 2.04 AB	31.22 ± 0.56 A	82.40 ± 2.37 BC
G4	8.07 ± 2.10 B	16.97 ± 2.03 B	86.78 ± 2.61 AB	29.30 ± 0.76 B	75.33 ± 3.78 C
G5	2.85 ± 0.40 C	13.38 ± 2.64 B	91.03 ± 2.38 AB	31.48 ± 0.66 A	93.99 ± 6.24 AB
LSD 0.05	3.6004	5.70	7.7606	1.7335	1.2257

Measuring levels of reproductive hormones

Level of testosterone hormones

The results in (Table 2) indicate a statistically significant reduction ($p<0.05$) in testosterone levels within the G2 group. The findings shown in the table indicate that there are no statistically significant differences ($p<0.05$) observed between G4 and G1. However, substantial differences ($p<0.05$) are observed when comparing G4 to G2.

The findings presented in (Table 2) indicate a statistically significant increase ($p<0.05$) in G3 and G5 in comparison to G1 and G2.

Level of LH hormone

Table 2 demonstrates a statistically significant reduction ($p<0.05$) in the LH hormone levels in the G2 phase. The table provides evidence of a statistically significant rise ($p<0.05$) in the levels of G3 and G5, as well as the LH hormone, in comparison to G1 and G2.

The findings presented in (Table 2) indicate that there were no statistically significant differences ($p<0.05$) in the LH hormone levels between G1 and G4. However, substantial differences ($p<0.05$) were observed when comparing G4 to G2.

Level of follicle-stimulating hormone

The data presented in (table 2) demonstrate a statistically significant reduction ($p<0.05$) in the FSH level in G2 compared to G1.

Table 2 demonstrates a statistically significant rise ($p<0.05$) in the FSH level in the preventative G5 group compared to G1, as well as compared to the G2.

Table (2): shows an estimate of the level of the testosterone, LH, and FSH hormones among the treated groups.

Groups	testosterone	LH	FSH
G1	1.40 ± 0.17 C	1.40 ± 0.14 B	1.41 ± 0.21 CD
G2	0.22 ± 0.07 D	0.36 ± 0.13 C	0.50 ± 0.12 E
G3	2.23 ± 0.27 B	2.56 ± 0.30 A	1.98 ± 0.20 ABC
G4	1.42 ± 0.19 C	1.13 ± 0.12 B	1.26 ± 0.21 D
G5	2.40 ± 0.29 AB	2.60 ± 0.28 A	2.21 ± 0.29 AB
LSD 0.05	0.7368	0.6381	0.6385

Histopathological changes

Pathological changes in testicular tissue:

The existence of Leydeck cells and normal tubules packed with sperm was observed in microscopic examinations of histological sections obtained from the testicles of animals in the control group (G1) (Figure 4). The experimental group administered with the active compound naringenin (G3) had a typical testicular tissue shape. Spermatozoa

exhibit an elliptical morphology and are organized in a systematic and orderly fashion. The basement membrane envelops each tubule and contains sperm-generating cells, including dispersed Sertoli cells with triangle nuclei. Sperm progenitors, which are circular cells that are purified and lie on the basement membrane, are the cells responsible for sperm generation. The cells had a greater size and included black, circular nuclei, along with spermatids that displayed a round or rectangular shape in close proximity to the cavity. Sperm was observed to be present within the cavities of the seminal tubules. Additionally, it was noticed that the interstitial tissue occupied the interstitial spaces between the seminiferous tubules. Figure 6 shows an increase in the number of cell layers, which included Leydeck cells and blood vessels. (Figure 6).

The Figure in G2 (Figure 5) also illustrates a distorted structure of the testicular tissue. This is characterized by an enlargement of the spaces between the seminiferous tubules, resulting in increased diameters. Additionally, there is a deficiency of Leydeck cells, a reduction in the size of the epithelial cell layer, cell necrosis, and a scarcity or absence of sperm. Furthermore, there is evidence of blood congestion and the presence of interspaces. Besides the marked absence of communication between Sertoli cells and spermatogenic cells, the observed phenomenon revealed the collapse of the germinal layer, and furthermore, it was shown that the germinal layer separates from the basement membranes. Testicular tissue damage in the prophylaxis group (G4) was not found to be statistically significant compared to the G2 group, as observed through histological investigation. The histological structure of the testis in the G4 group had typical features within the seminiferous tubules, which were characterized by intact basement membranes that remained attached to the germinal layer. . With a limited amount of interstitial spaces. In addition, these organisms are distinguished by the high concentration of cells responsible for sperm production, as well as the presence of sperm inside the seminiferous tubules and the presence of blood arteries and Leydeck cells within the interstitial tissues. G5 groups were also characterized by the presence of normal testicular structure, intact basement membranes, normal sperm content, and the presence of interstitial tissue and blood vessels (Figures 7, 8).

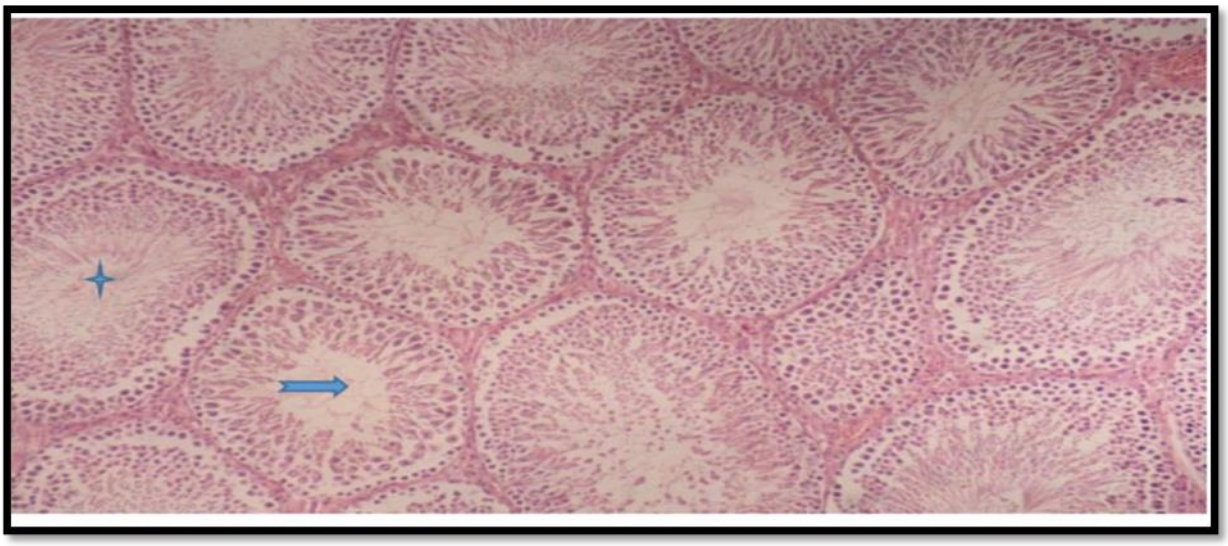

Figure (4): A cross-section of the testicle of a male rat from the control group showing the normal structure of the testicular tissue (✦) the sperm and the middle cavity (⟹) (E and H stain , 100X)

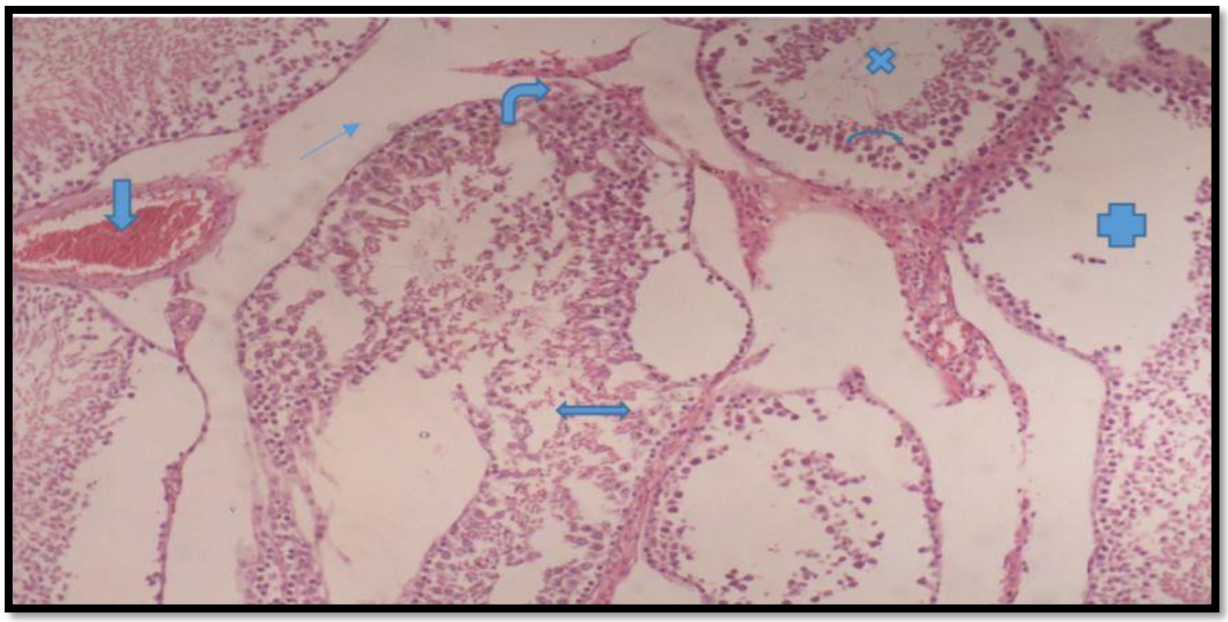

Figure (5): A cross-section of the tissue in the testicle of a rat from the drug group, representing (↱) leydig cell paucity, (↓) represents blood congestion, (↗), interstitial spaces, increased diameters (✚), oligozoospermia (✖), cell necrosis (⟺), and a decrease in the size of the epithelial cell layer. (E and H stain, 100X).

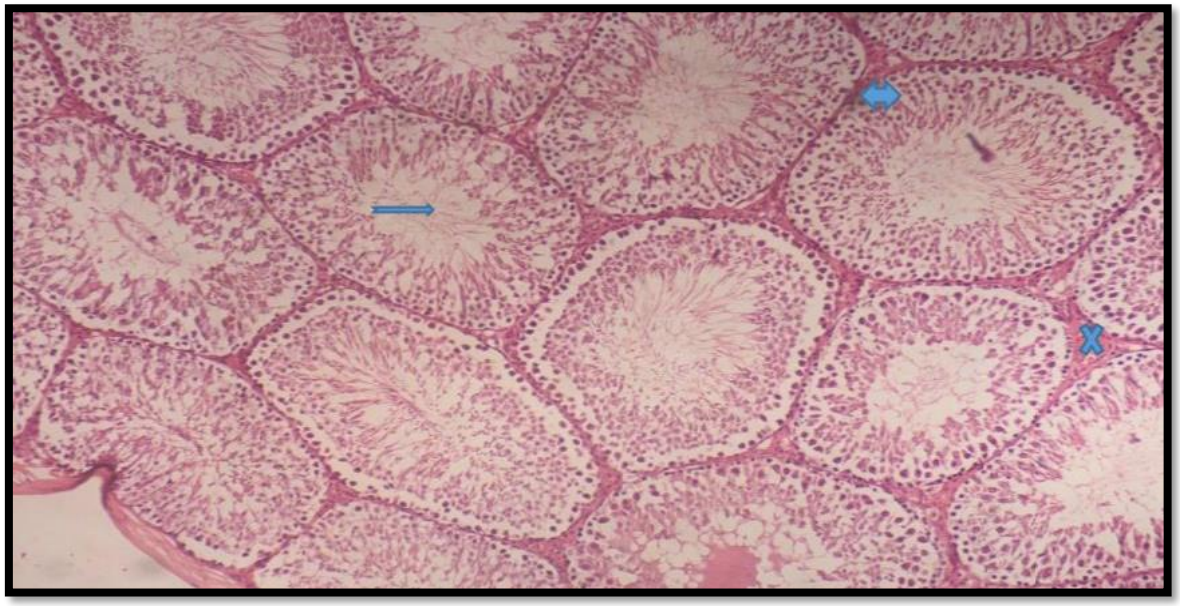

Figure (6): a cross-section of a rat testicle from the group of active substance naringenin ( ) represents normal leydig cells and ( ) normal seminiferous tubules and ( ) represents cells filled with sperm (E and H stain, 100X)

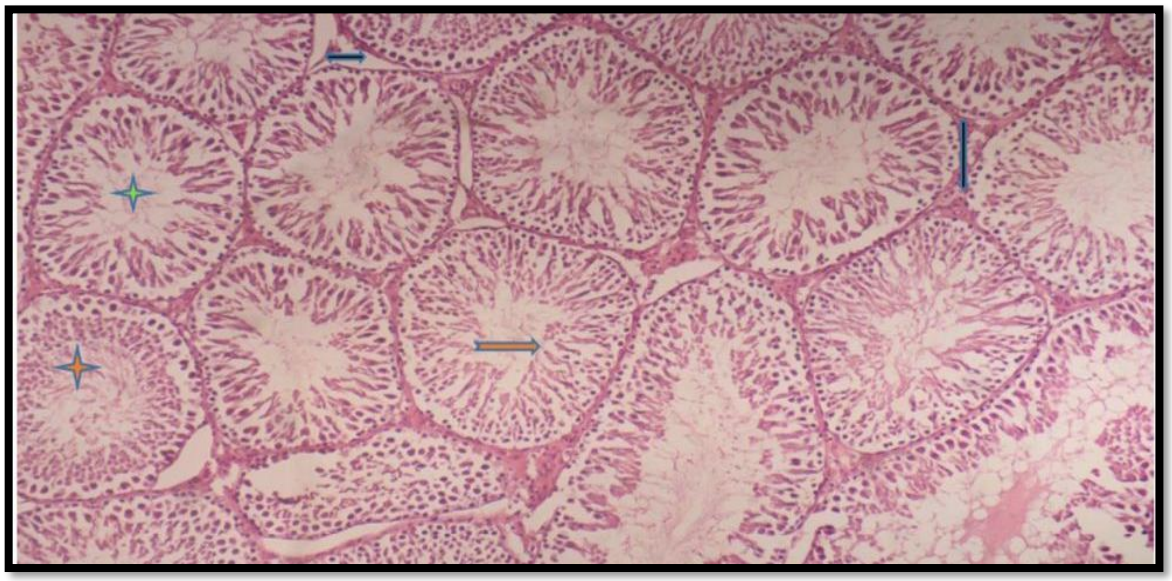

Figure (7): A cross-section of a histological section of the testicle of a rat from the naringenin and drug prophylaxis group showing ( ) the presence of fewer interstitial spaces, an improvement in sperm ( ), a decrease in the diameter of the central lumen ( ), an increase in the germ layer forming the sperm ( ) and an improvement in the seminiferous tubules ( )(E and H stain, 100X)

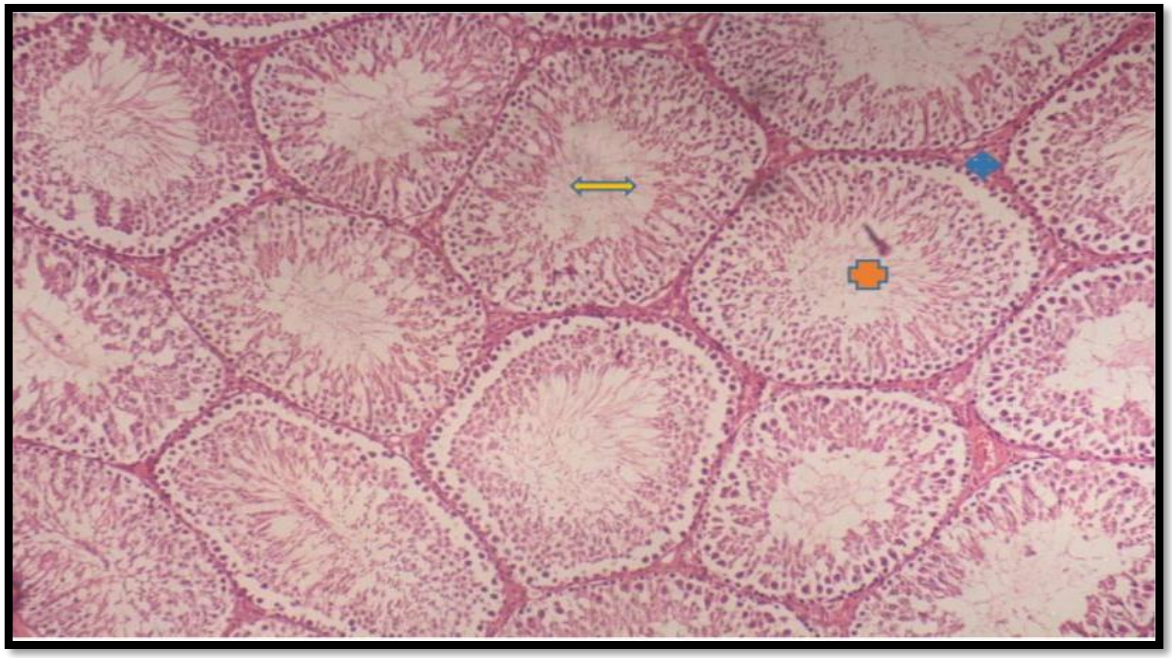

Figure (8): a cross-section of the testicle of a rat from the preventive group naringenin nanoparticles and the drug showing normal tissues of the testicles, increased sperm ( ), decreased central lumen cavity ( ) and the presence of Leydeck cells ( )(E and H stain, 100X)

Histopathological changes in the cauda epididymis

Histological sections and images obtained from the tail of the epididymis of animals belonging to the G1 (Figure 9), along with the group of active substances naringenin G3, revealed a typical histological composition of the epididymal tubules. These tubules exhibited a large and dilated appearance, characterized by normal epithelial tissue and contained a substantial quantity of sperm (Figure 11).

Drug group (Figure 10) exhibits various pathological changes in the epididymal tubules, including atrophy, decreased or absent sperm count within the tubular cavities, reduced thickness of the epithelial tissue lining, detachment of epithelial cells from the basement membranes, irregular distribution of epididymal tubules, and disintegration of the connective tissue.

The histological sections of the protective groups exhibited a notable decrease in pathological histopathological alterations. This was observed through the widening of tubules and the presence of substantial sperm, as well as the thickening of the epithelium lining the tubules, as depicted in (Figure 12).

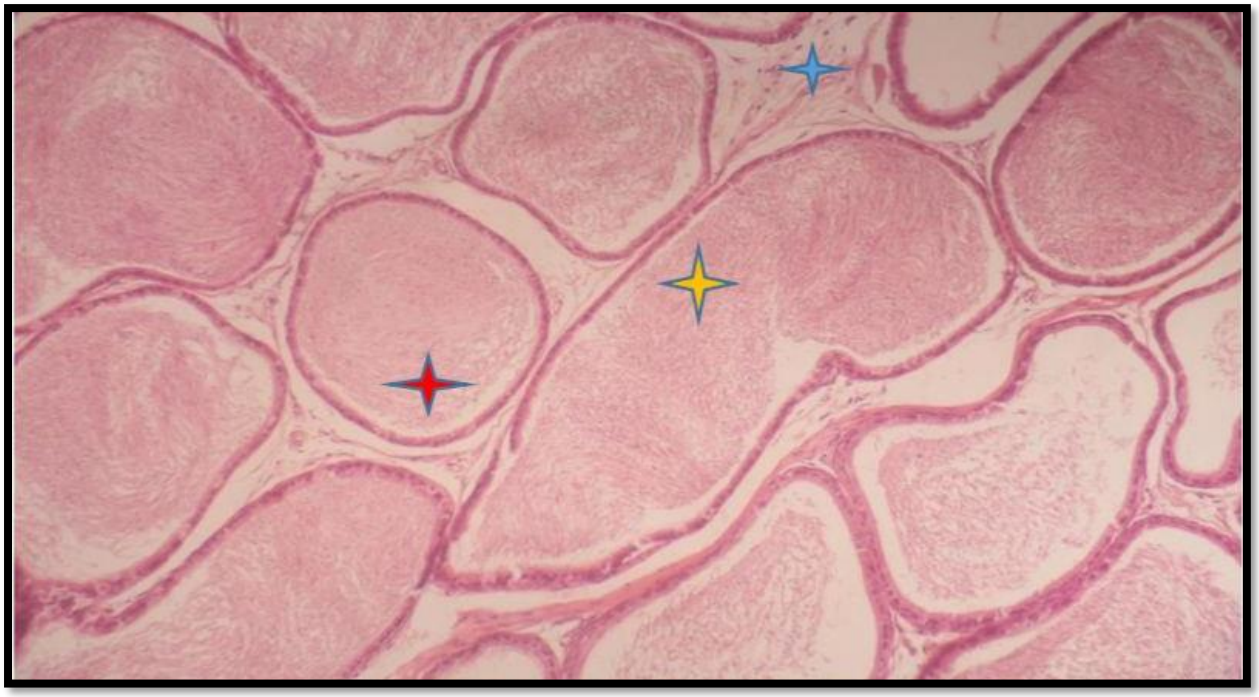

Figure (9): is a cross-section of the epididymis of a rat from the control group, showing the epididymal tubules distributed regularly. ( ) The cavity is filled with sperm. ( ) represents a normal tubule of the epididymal duct. ( ) represents smooth muscle cells around the tubule. (E and H stain, 100X).

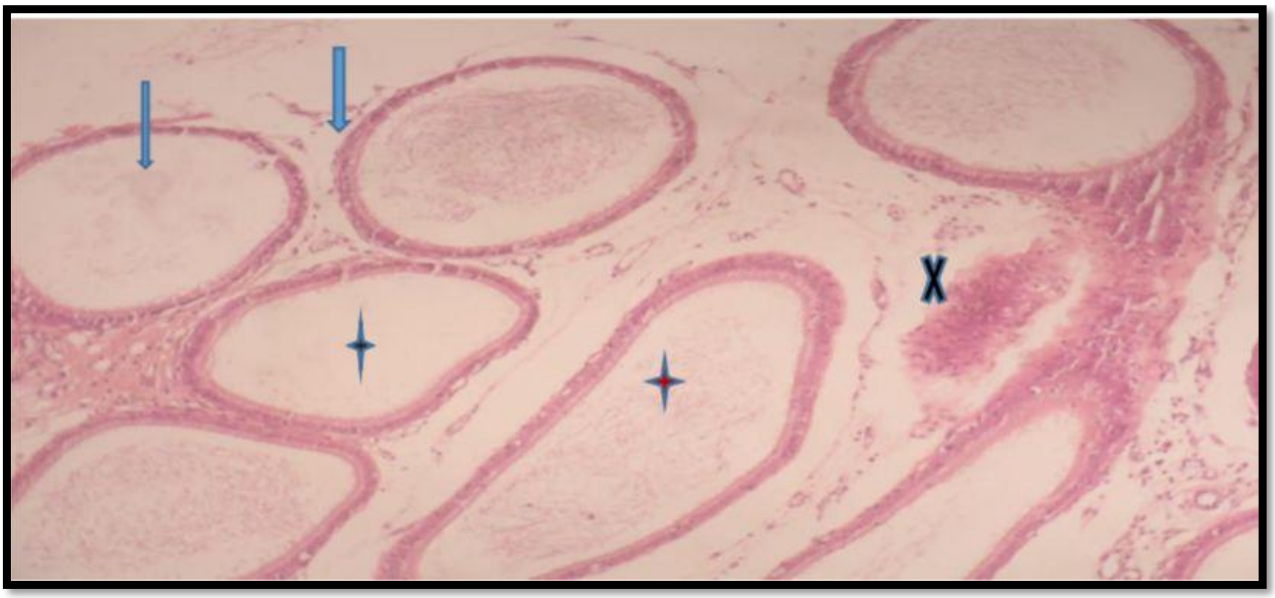

Figure (10): a cross-section of the epididymis of a rat from the drug group showing atrophic and irregularly distributed epididymal tubules ( ) and lack of sperm ( ) or absence of sperm ( ) and lack of smooth muscle ( ) (E and H stain, 100X).

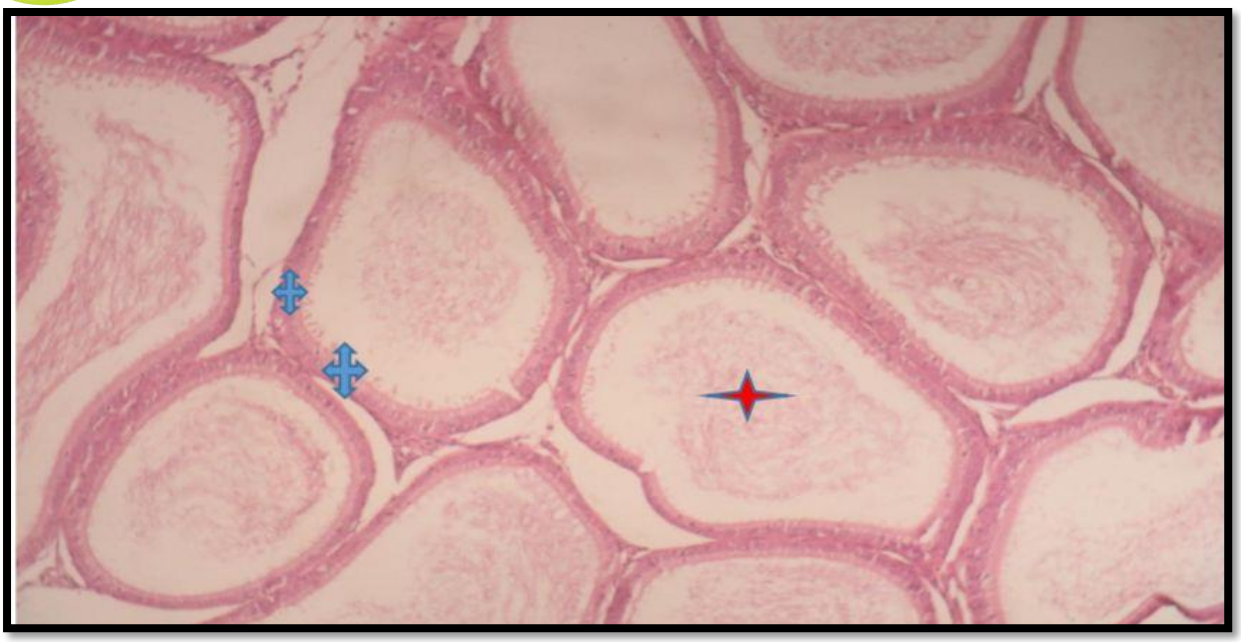

Figure (11): is a cross-section of the epididymis of a rat from the active naringenin group, in which the epididymal tubules appear uniformly distributed and tightly knit together, and the tissue appears normal. ( ) represents the cavities filled with sperm, and ( ) represents with the presence of stationary cilia (E and H stain, 100X).

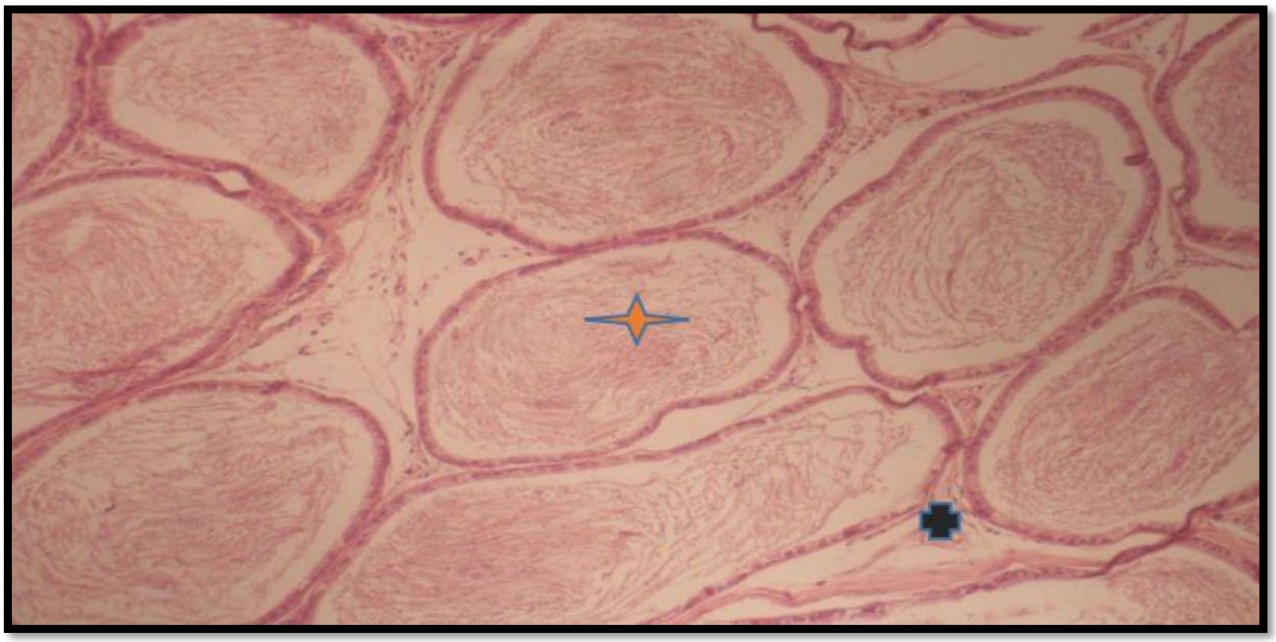

Figure (12): is a cross-section of the tail of the epididymis of a rat from the nano-naringenin preventive group. The sperm appear to be increasing ( ) and the presence of smooth muscles surrounding the tubules ( ), (E and H stain, 100X).

Changes of sperm parameters

The findings of the present investigation are consistent with the research conducted by [31] in establishing that the administration of MTX leads to testicular toxicity and inflicts harm upon testicular tissue. Additionally, it had an impact on the quantity of sperm, the morphological index, and the progressive movement. Furthermore, there was a notable reduction in both the number of sperm and their final shape, which aligns with the findings of the study Present.

The present findings are consistent with previous research [32], which demonstrated a statistically significant ($p<0.05$) reduction in sperm count in both the male tail and testicle. The study found that when men were given a single dose of 20 mg/kg MTX, the addition of albino mice changed sperm quantity and motility, as well as the proportions of viable, abnormal, and dead sperm. The drug also caused increased sperm DNA damage. G2 testicular tissue showed decreased sperm cells and production ($P < 0.05$). When compared to the control group, the results of the current study support this fact.The results of the present investigation are consistent with those reported in a previous study [33]. The previous investigation revealed evidence that management.The results obtained from the current study were in line with prior research conducted by scholars[33, 34].Observations have shown that nanoparticle naringenin provides protective effects against oxidative stress. Due to its strong antioxidant capabilities, it increases body weight, stimulates the production of reproductive hormones, and enhances sperm count. The current study has provided proof of the effectiveness of nanoparticle techniques, as indicated by the observed augmentation in the numbers of mobile and viable sperm, as well as the concentration of sperm in the epididymal tail, in comparison to alternative methodologies. By employing a control group and G2.

Levels of reproductive hormones

The results displayed in Table 2 demonstrate a statistically significant decrease ($p<0.05$) in the levels of testosterone, LH, and FSH among the experimental group that received the medication.

The results of the current study are consistent with prior research [31, 32,35] that has investigated the potential toxicity of MTX in connection to testicular injury. The aforementioned investigations have provided evidence indicating that MTX exerts a deleterious effect on both the testicle and epididymis, leading to a notable reduction in the concentrations of reproductive hormones, including testosterone, LH, and FSH.

Additionally, MTX has been associated with a decrease in sperm count, morphological indicators, and movological shapes of sperm. Furthermore, it has been found to cause damage to sperm DNA and pathological histological alterations.

The findings of the present investigation corroborated a previous study [33, 36, 37, 38] which shown that active naringenin has a safeguarding influence against toxicity and oxidative stress, thereby resulting in enhanced testosterone levels. Prophylactic

administration of LH and FSH in mice. Additionally, it plays a significant part in enhancing the composition of the reproductive organs.

In addition, zinc oxide nanoparticles containing naringenin are utilized due to the fact that zinc oxide enhances the production of reproductive hormones. The research findings indicate that the administration of zinc oxide nanoparticles is associated with a dose-dependent elevation in testosterone and FSH levels, as well as the restoration of testicular structure to its normal state. This effect is attributed to the concurrent increase in androgens, which are a type of hormone. Testosterone has been found to enhance sexual activity, promote weight gain and facilitate the development of reproductive organs [39,40].

Histopathological in testicular tissue

The findings of the present study are consistent with the study conducted by [41], as methotrexate induces histological alterations characterized by the disintegration of germinal tissue, diminished cohesion and connectivity among sperm-generating cells and Sertoli cells, and the detachment and shedding of germinal layers from the basement membrane within the tubules. The testicular cavities exhibit a deficiency of sperm and a dearth of its amount. The study also demonstrated a reduction in the quantities of sperm cells and sertoli cells, which aligns with the findings of the present study.

Methotrexate induces an elevation in oxidative stress, leading to an enhanced generation of free radicals and a diminished efficacy of the antioxidant enzyme system. Consequently, cells become more susceptible to ROS and ultimately experience cellular harm. Abnormal sperm count and infertility can be attributed to heightened levels of oxidative stress within the testicles. Additionally, MTX has been found to enhance the synthesis of the immune interleukins TNF-κ and IL-1β. Additionally, it results in a notable reduction in body weight, testicular weight, and testicular structural abnormalities, alongside a decline in testosterone levels. MTX is known to induce a significant reduction in the quantity of germinal epithelium and inflict harm onto tubule tissue. Sperm and a loss of reproductive hormone production, resulting in the halt of sperm creation [42,43].

The present study's findings regarding the preventative effects of active naringenin and nano-naringenin are consistent with the results reported in previous studies [38, 39, 40, 44]. in the context of naringenin's preventative properties Enhancing the integrity of seminal tubules and restoring testicle weight to normal levels, while also boosting sperm quality in the protective groups, in order to restore the normal tissue structure of male albino rats. The current study demonstrated that treatment with naringenin resulted in a decrease in testicular injury and the restoration of body weight, as depicted in the accompanying images. Groups focused on prevention and nano-prevention. Animals treated with active and nano-preventive naringenin experienced the restoration of their natural testicular structure.

Histological changes in the cauda epididymis: The findings of the present investigation are consistent with the findings of a previous study [45] concerning the toxicity

induced by MTX and its impact on the male reproductive system in albino rats. The study also established that MTX has detrimental effects on the functional integrity of the testicles and epididymis, as evidenced by a decrease in sperm concentration within the epididymis. Furthermore, it resulted in a drop in germ cells and an increase in areas within the interstitial tissue, along with a reduction in epididymal sperm density, which aligns with the findings of the present investigation.

The findings of this study support the previous research conducted by [46,44] which shown the protective effects of naringenin and nano-naringenin against methotrexate-induced toxicity. Furthermore, it has been observed that these chemicals possess the ability to reinstate the typical composition of epididymal tissues through the restoration of sperm levels to their baseline condition, alongside the restoration of testosterone, LH, and FSH levels, which are recognized as typical hormones.

naringenin extract works to suppress the negative effects of the methotrexate and increase the efficiency of the male reproductive system

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