Therapeutic Effect of Nano-Extract of Nigella Sativa Seeds on The Histological Structure of The Testis In Male Albino Rats Induced With Anemia

Noor M. Hasnawi[1], Jabbar Abadi Mohammed[2]

[1]*Al-Furat Al-Awsat Technical University , Najaf Technical Institute , Department of Forensic and Forensic Evidence Technologies ,*
[2]*Universty of Kufa/ Faculty of Science , Department of Biology. Email: Noor.mahdi.inj@atu.edu.iq*

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ABSTRACT
Background: The study explores the effect of nanoparticles derived from the aqueous extract of Nigella sativa seeds on the histological structure of the testis in male albino rats with tartrazine-induced anemia. Nanoparticles, ranging in size from 1 to 100 nanometers, possess unique physical and chemical properties that enhance their ability to interact and deliver drugs. Objective: This study focuses on the therapeutic potential of Nigella sativa seed extract nanoparticles in treating testicular histological changes in anemic rats. The testicle is an oval organ involved in sperm formation and hormone production. It contains seminiferous tubules, Sertoli cells, and Leydig cells, which are responsible for sperm production and testosterone synthesis. Methods: The study included 36 male albino mice, aged between 8-12 weeks, and weighing 240-260 grams, from the Iraqi Center for Cancer Research and Medical Genetics. The animals were divided into six groups, each group having six animals. Results: The study showed that tartrazine at a concentration of 20 mg/kg: with Nigella sativa nanoparticles 50 mg/kg: an increase in weight. the body. While tartrazine at a concentration of 20 mg/kg with N.sativa nanoparticles at 75 mg/kg did not show significant differences compared with the control group. The results showed the Nigella sativa seed nanoparticles at a concentration of (50 mg/kg, 75 mg/kg): showed an increase in body weight, while tartrazine at a concentration of 20 mg/kg: a decrease in body weight, and no significant changes were observed in testicle weight in all treatments compared with the control group. Non-significant changes in testicle weight compare with Control group. The study showed that treatment with tartrazine 20 mg/kg showed noticeable histological changes in the histological structure of the testicle. Tartrazine with N.sativa nanoparticles (50 mg/kg, 75 mg/kg): No histological changes, indicating attenuation of tartrazine-induced damage. N.sativa nanoparticles (50 mg/kg, 75 mg/kg): demonstrated normal composition of testicular tissue, indicating no harmful effects on testicular tissue. has protective effects. Conclusion:The study aimed to investigate the potential of using nanoparticles extracted from Nigella sativa seeds to protect against damage caused by tartrazine in male albino rats.with significant therapeutic implications for the use of natural antioxidants in biomedical applications.

1. Introduction

Recently, nanoparticles have been given great attention in the field of medical research due to their unique properties and potential applications in various fields. Nanoparticles are small particles with dimensions from 1 to 100 nanometers, and this small size gives them distinct properties compared to other materials. Research has indicated the use of nanoparticles derived from the aqueous extract of Nigella sativa seeds, a medicinal plant known for its therapeutic benefits. In this research, we explore the effects of nanoparticles of the aqueous extract of Nigella sativa seeds on the histological structure of the testes, in male albino rats in which anemia was induced by. tetrazine (1).

The properties of nanoparticles make them useful for various applications. For example, it has a high surface-to-volume ratio, which allows for increased reactivity and improved drug delivery capabilities (2). This small size also helps it penetrate into cells and tissues, thus allowing the drug or treatment to reach easily (3). Its other distinguished properties include its stability, solubility, and cellular absorption (3). The testicle is an oval-shaped organ located inside the scrotum, and hanging outside the abdominal cavity. The outer layer of the testicle is called the tunica albuginea, which is a dense connective tissue that surrounds and protects the internal structures of the testicle. The testicle is divided into lobules, each lobe contains one to four coiled seminiferous tubules, which represent the functional units for sperm production. (4).

Spermatogenesis takes place within the seminiferous tubules, characterized by the presence of Sertoli cells, which line the tubules and which provide structural support and nutrition for the developing sperm cells. The seminiferous tubules are interspersed with Leydig cells, which are responsible for

the synthesis and secretion of the male sex hormone, testosterone (5).

The two most important functions of the testicle are hormone production and spermatogenesis. Spermatogenesis consists of a complex series of differentiation and cell divisions within the seminiferous tubules. Through successive divisions, stem cells located in the periphery of the tubules then undergo meiosis to form haploid sperm. These sperm undergo further maturation and differentiation to become sperm (6).

The testicle also plays an important role in the production of testosterone. Leydig cells, located in the interstitial spaces between the seminiferous tubules, synthesize and secrete testosterone in response to stimulation of luteinizing hormone (LH) secreted by the pituitary gland. Testosterone is necessary for the maintenance of secondary sexual characteristics in males, such as the voice, facial and body hair growth, and muscle growth. It also plays a role in sperm production, libido, and male reproductive function in general (7).This study aimed to determine the effect of nano-extract of Nigella sativa seeds in treating histological changes in the testes of treated male laboratory rats. It is also possible to mention some vitamins, such as vitamin C, which plays a protective role against some histotoxic effects in some organs of the body.(8)

2. Methodology

The study was conducted on 36 albino male mice obtained from the Iraqi Center for Cancer Research and Medical Genetics in Baghdad. Their ages ranged from eight to twelve weeks, and their weights ranged from 240 to 260 grams. Experimental animals were transported to the animal house and placed in plastic cages covered with metal mesh covers with dimensions of 20 × 30 × 50 cm. The floor was furnished with sawdust and cotton, and the bedding was replaced twice a week, in addition to regular cleaning and sterilization of the cages. The animals were left to acclimate in the animal house for fourteen days before starting the experiment. During the experiment period, the animals were under ideal laboratory conditions that were similar for all groups, such as temperature, lighting, and humidity. The animals were provided with healthy drinking water.

Nigella sativa were purchased from the markets in Najaf/Iraq. after which they were ground into powder in a milling machine.Preparation of the extract from the powder of the *Nigella sativa* ,This process was carried out under the conditions previously described (9) with some modifications. (400 ml) of water was added to (200 g) of *Nigella sativa* powder, and this process was done three times for six days in a dark incubator shaker, then filtered with filter paper. Then, the filtrate was collected and dried in a vacuum oven for seven days at a temperature of (40 °C).Then, the dried seed extract was dissolved by (4.5 g) in (150 ml) of distilled water. After that *Preparation of the* nano-extract of *Nigella sativa* nanoparticles ***was prepared*** according to the method of (9), then ground with an electric hand mill and kept in a clean box until use.

The histological study included the preparation of histological sections of the testes depending on (10), where the samples were washed with tap water to remove formalin (fixer), after which the following steps were performed(11).

3. Result and Discussion

The effect of treatment with Tartrazine, Tartrazine with *N.sativa* nanoparticles, and *N.sativa nanoparticles* on the total body weight (kg) in male albino rats.

The results of the current study showed that there was a significant difference in the body weight of the group treated with Tartrazine at a concentration of 20 mg/kg, as well as The group treated with Tartrazine at a concentration of 20 mg/kg and the nano-extract of Nigella sativa at a concentration of 50 mg/kg showed a decrease in body weight compared to the control group, while the results did not show significant differences in body weight in the groups treated with the nano-extract of Nigella sativa at a concentration of 75 mg/kg. With Tartrazine at a concentration of 20 mg/kg, as well as in groups treated with Nigella sativa nano extract at concentrations (50 and 75) mg/kg compared with

the control group table (1).

Table (1): The effect of treatment with Tartrazine, Tartrazine with N.sativa nanoparticles, and *N.sativa*

Total body weight/g	Mean±SD	P-value	
Control	289.0 ± 13.9		
Tartrazine 20 mg/kg	337.4 ± 2.182	0.0044**	Significant
Tartrazine with N.sativa nanoparticles 50 mg/kg	338.6 ± 2.731	0.0040**	Significant
Tartrazine with N.sativa nanoparticles 75 mg/kg	273.2 ± 3.917	0.1530	Non-Sign
N.sativa nanoparticles 50	277.4 ± 3.696	0.0411*	Non-Sign
N.sativa nanoparticles 75mg/kg	295.8 ± 4.488	0.3271	Non-Sign

nanoparticles on the total body weight (kg) in male albino rats.

The numbers represent the mean (average), while ± represents the standard error.

The effect of treatment with Tartrazine, Tartrazine with N.sativa nanoparticles, and N.sativa nanoparticles together Testicle weight (g) in male albino rats.

There was a no significant difference in total testicle weight compared to the control group in the Tartrazine 20mg/Kg, and There was a no significant difference in total testicle weight compared to the control group in the Tartrazine with N.sativa nanoparticles 50 mg/kg group. There was no significant difference in total testicle weight compared to the control group in the Tartrazine with N.sativa nanoparticles 75 mg/kg group. There was no significant difference in total testicle weight compared to the control group in the N.sativa nanoparticles 50 mg/kg group. The N.sativa nanoparticles 75 mg/kg group showed a no significant difference in total testicle weight compared to the control group table (2).

Table (2): The effect of treatment with Tartrazine, Tartrazine with N.sativa nanoparticles, and N.sativa nanoparticles together Testicle weight (g) in male albino rats

Total body weight/g	Mean±SD	P-value	
Control	**0.3740 ± 0.038**		
Tartrazine 20 mg/kg	0.2960 ± 0.035	0.0880	Significant
Tartrazine with N.sativa nanoparticles 50 mg/kg	0.2600 ± 0.044	0.0040**	Non-Sign
Tartrazine with N.sativa nanoparticles 75 mg/kg	0.2520 ± 0.050	0.4040	Non-Sign
N.sativa nanoparticles 50 mg/kg	0.2840 ± 0.062	0.1272	Non-Sign
N.sativa nanoparticles 75mg/kg	0.2560 ± 0.079	0.3271	Non-Sign

The numbers represent the mean (average), while ± represents the standard error.

Effect of *N.sativa* nanoparticle and Tartrazine dye on Histological structur of testes in Male Albino Rats.

The current study showed the presence of histological changes in the histological structure of the testicles of the group treated with Tartrazine compared to the control group. These changes represented the interstitial spaces, an increased interstitial space with reduced interstitial cells, Increased Intracellular spaces in seminiferous tubule, degenerating cells have dark stained nuclei reduction of the number, disorganization, and vacuolation of most germinal epithelial cells, congested blood vessels and few Leydig cells.fig (1 and2).

The current study showed that there were no histological changes in the histological structure of the testicles of the group treated with tartrazan at a concentration of 20 mg/kg and the nano-extract of

Nigella sativa at a concentration of (50 and 75) mg/kg, respectively, compared with the control group fig (3 and 4).

The study showed histological sections in the testicles of male laboratory rats treated with the nano-extract of Nigella sativa at a concentration (50 , 75) mg/kg There were no histological changes compared to the group treated with physiological saline solution sa control group fig (5 and 6).

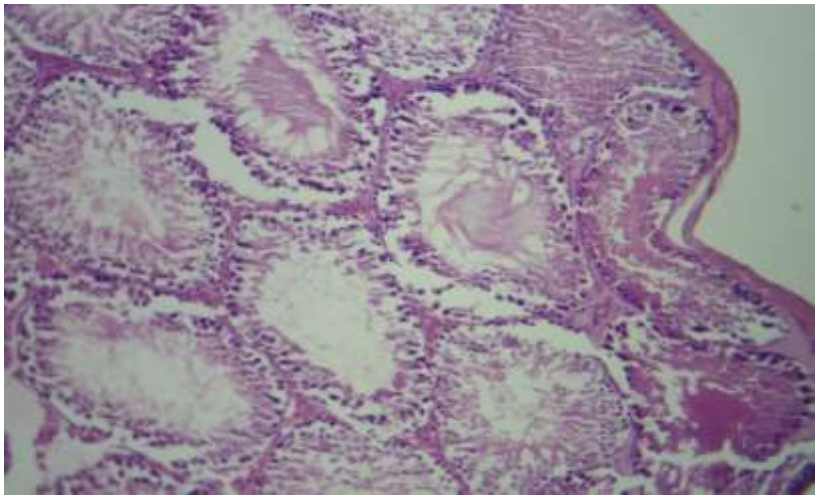

Figure (1): A histological section of the testis of a laboratory white albino rat in the group treated with Physiological normal saline, containing different types of germ cells; spermatogonia lying on the basement membrane, spermatocytes, spermatids spermatozoa and somatic Sertoli cells. The interstitial tissues found between seminiferous tubules contain interstitial cells; Leydig cells .H&E 400X

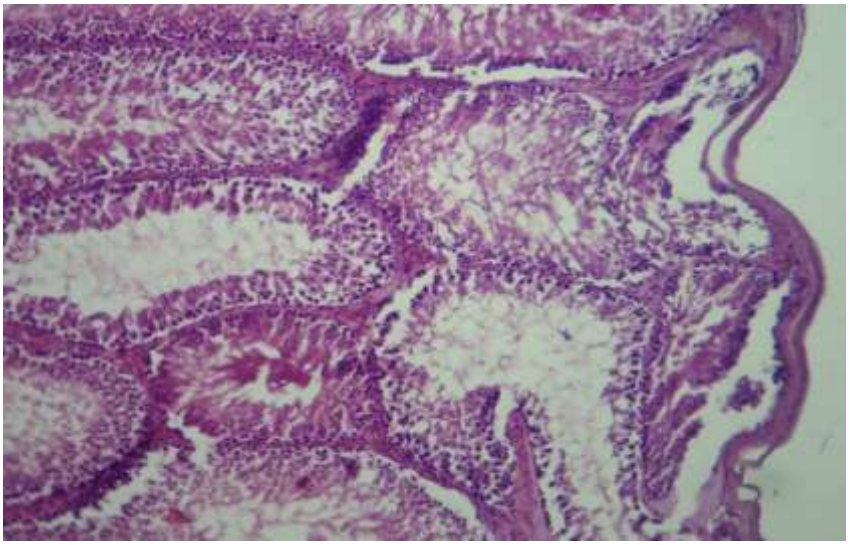

Figure (2): A histological section of the testis of a laboratory white albino rats in the group treated with Tartrazine dye at a concentration of 20 mg/kg , shows the interstitial spaces, an increased interstitial space with reduced interstitial cells, Increased Intracellular spaces in seminiferous tubule, degenerating cells have dark stained nuclei reduction of the number, disorganization, and vacuolation of most germinal epithelial cells, congested blood vessels and few Leydig cells .H&E 400X

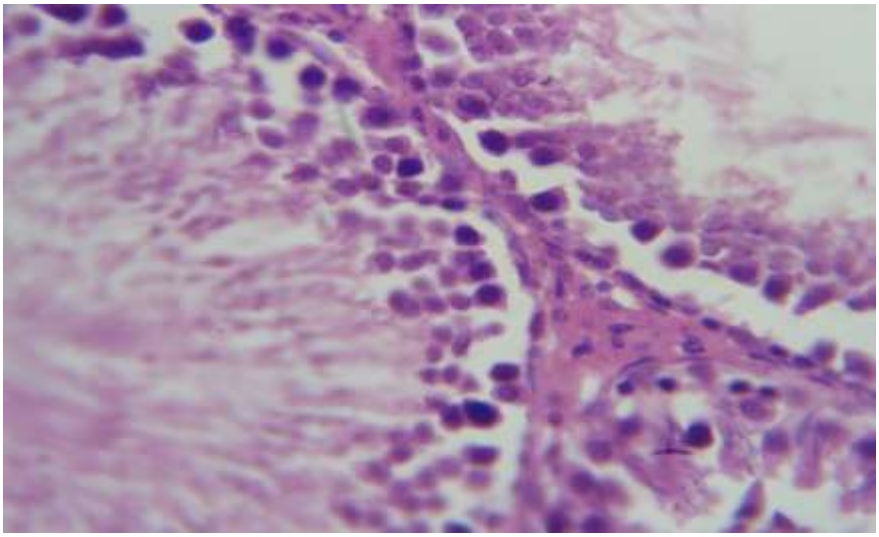

Figure (3): A histological section of the testis of a laboratory white albino rat in the group treated with Tartrazine dye at a concentration of 20 mg/kg and with treatment with nanoparticles of N.sativa s at a concentration of 50 mg/kg, it is noted that there are no histological changes in the structure of testis .H&E 400X

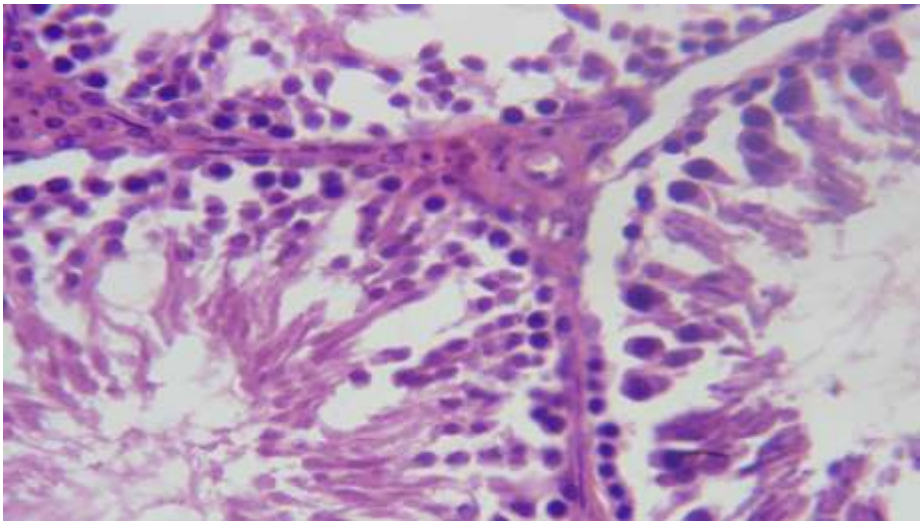

Figure (4): A histological section of the testis of a laboratory white albino rats in the group treated with Tartrazine dye at a concentration of 20 mg/kg and With treatment with nanoparticles of N.sativa s at a concentration of 75 mg/kg, It is noted that there are no histological changes in the structure of testis. H&E 400X

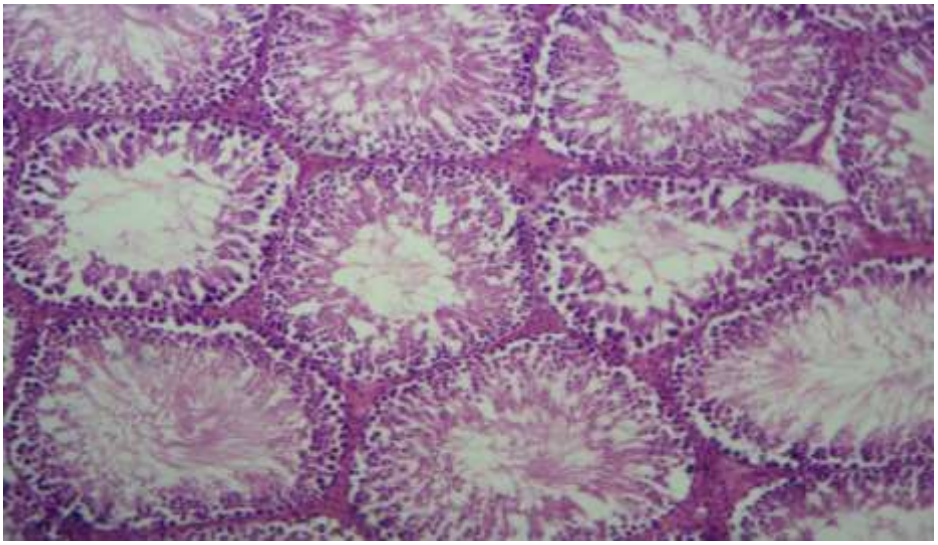

Figure (5): A histological section of the testis of a laboratory white albino rat in the group treated with Tartrazine dye at a concentration of 20 mg/kg and with treatment with nanoparticles of N.sativa s at a concentration of 100 mg/kg, it is noted that there are no histological changes in the structure of testis. H&E 400X

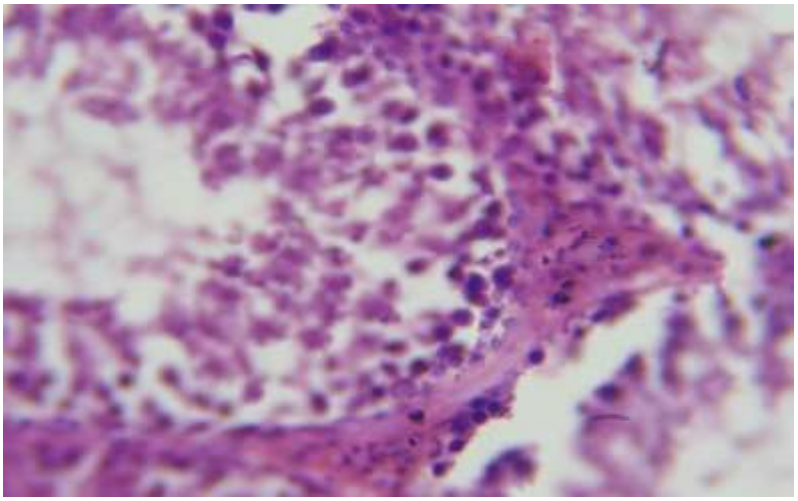

Figure (6): A histological section of the testis of a laboratory white albino rat in the group treated with nanoparticles of N.sativa s at a concentration of 50 mg/kg, it is noted that there are no histological changes in the structure of testis. H&E 400X

The study showed that the body weight of mice decreased to 260.4 ± 3.696 g from the control weight of 289.0 ± 13.9 g. This reduction is statistically significant with a P value of 0.0411. This indicates that at this dose, chemotherapy negatively affects the body weight of mice and the body weight of mice increased marginally to 295.8 ± 4.488 g, which is not statistically significant (P value 0.3271). This indicates that there is no significant effect on body weight when using different concentrations of black seed. Studies show that N.sativa (Nigella sativa) can affect body weight differently. Lower doses may result in weight loss due to appetite suppression resulting from concomitant doses of chemotherapy, while higher doses may increase weight by improving metabolic efficiency and nutrient absorption (12, 13). The body weight of mice increased significantly to 337.4 ± 2.182 g of control weight, with a P value of 0.0044. This indicates that black seed in high concentrations can reduce the effect of tartrazine and, as a result, increase body weight. Previous research has shown that tartrazine can lead to weight loss in mice, possibly due to metabolic changes due to increased food

consumption.(14, 15).

The results showed that body weight increased significantly to 338.6 ± 2.731 g, with a P value of 0.0040. This indicates a significant synergistic effect of Nigella sativa nanoparticles and tartrazine, while at low concentrations the body weight decreased to 273.2 ± 3.917 g, which was statistically insignificant (P value 0.1530). This indicates that there is no significant effect on body weight at this dose when the two treatments are combined. The combined effects of N.sativa extract and tartrazine on body weight have not been extensively studied. However, the significant increase in body weight with combined treatment could be due to synergistic metabolic improvements (16).

The administration of tartrazine at 20 mg/kg resulted in a decrease in testicle weight (0.2960 ± 0.035) compared to the control group (0.3740 ± 0.038). Although this reduction was not statistically significant ($p = 0.0880$), it suggests a potential negative impact of tartrazine on testicle weight. Previous studies have shown that tartrazine, a synthetic food dye, can have adverse effects on health, including oxidative stress and damage to various organs, which may explain the observed reduction in testicle weight (17).

The study showed that N.sativa extract loaded with iron nanoparticles at different doses (50, 75 mg/kg) had varying effects on testicle weight. A slightly non-significant increase was observed compared to the control, with statistical significance ($P > 0.05$). N.sativa (Nigella sativa) has antioxidant properties and has been reported to reduce oxidative stress and improve organ weights in various studies(18)..

In groups treated with tartrazine and N.sativa nanoparticles at concentrations (50, 75 mg/kg), testicular weight decreased significantly compared to the control group. This suggests that N.sativa extract may mitigate some of the potential negative effects of tartrazine, consistent with previous studies and research indicating the protective effects of N.sativa against various toxins. (19).

The results of the current study are generally consistent with the results of previous research. Tartrazine has been shown to negatively affect testicular weight and induce oxidative stress, which may result in weight loss. N.sativa extract, especially when loaded onto nanoparticles, has shown potential protective effects due to its antioxidant properties. However, the lack of significant changes in testicular weight in this study, despite administration of tartrazine, suggests that the doses used or duration of treatment may not have been sufficient to produce measurable effects. Moreover, the potential protective effects of N.sativa extract in alleviating tartrazine-induced toxicity highlight the importance of further research to explore optimal dosing and treatment regimens. (20).

The results of microscopic examination of testicular tissue in the control group showed a normal tissue structure and no pathological changes. The seminiferous tubules contain different types of germ cells, including spermatocytes, spermatids, and spermatids, along with somatic Sertoli cells. The interstitial tissue between the seminiferous tubules contains interstitial cells, as shown in Figure 1. This is consistent with the results of standard histological studies on intact rat testes, confirming the normal underlying structure for comparison. (21).

The study showed a clear effect of tartrazine at a concentration of 20 mg/kg in causing noticeable histological changes in testicular tissue. The results showed increased interstitial spaces, decreased interstitial cells, increased intracellular spaces in seminiferous tubules, deterioration of cells with dark stained nuclei, and vacuolation of most germinal epithelial cells. In addition, there were crowded blood vessels and fewer Leydig cells, as shown in Figure 2. These changes indicate testicular toxicity and are consistent with previous studies that documented the adverse effects of tartrazine on testicular structure, which are likely due to oxidative stress and damage Cellular. (22).

In the combination treated groups with tartrazine (20 mg/kg) and N.sativa nanoparticles at concentrations of 50 mg/kg, 75 mg/kg no histological changes were observed in the structure of the testes (Figures 3-4 to 5-6). This indicates that N.sativa nanoparticles effectively ameliorated tartrazine-induced histological damage. The protective effect of N. sativa extract, especially its

antioxidant properties, has been well documented in the literature. Studies have shown that N.sativa extract can protect against various types of tissue damage by reducing oxidative stress and inflammation (23.

Treatment with N.sativa nanoparticles alone at concentrations of 50 mg/kg, 75 mg/kg showed no histological changes in the structure of the testes (Figures 5-6). This indicates that N.sativa nanoparticles do not adversely affect testicular histology at these doses. The absence of histological changes supports previous findings that N.sativa extract has no detrimental effects on testicular tissue and may even offer protective benefits (24).

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