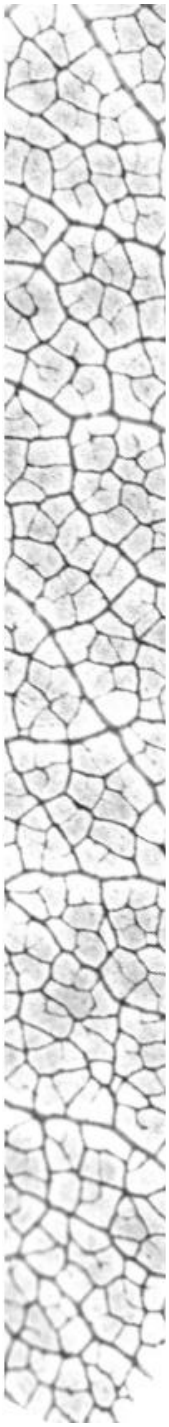

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Effect of Thymoquinone on some biochemical and hormonal indices and their protective effect on the genital organs of rats after cancer induction in Laboratory

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Abstract
The aim of this study is to demonstrate the importance of Thymoquinone as a treatment for cancer tumors and to indicate whether it has a negative effect on the male genital organs. For this purpose, 18 adult males of white rats were divided into three groups (A: control group, B: group treated with carcinogen (AOM) only, C: group treated with Thymoquinone after treatment with carcinogen (TQ+AOM)). Each group included six rats and induced colorectal cancer. By injecting Azoxymethane (AOM) once a week for groups B, C except group A was considered as a control group. The tumor was detected by examining the tissue in the AOM-injected groups compared to the control group and CEA concentration. Group treated by TQ obsorbed decreased in CEA concentration was response to the treatment used. The weights of the animals and genital organs were measured before and after the experiment where the carcinogen caused weight changes compared to the control group while the TQ did not show changes in the weight of animals and is evidence of resistance to this substance of oxidative stress due to carcinogens, also been conducted some tests for testicular functions such as measurement of the concentration of testosterone hormone, serum acid phosphatase and concentration of sperm living has been observed significant differences ($P < 0.05$) between these totals and for all tests. Amicroscopic examination of the sperm forms was performed notice the distortions in the sperm in AOM group, while TQ group these abnormalities were not observed. The histomicroscopic examination of the testicular, prostate gland and seminal vesicle by using light microscopy for all rats showed pathological changes in the group treated with AOM such as sperm cell degeneration , appearance of sperm ghosts, breakdown of the walls the Hyperplasia, and presence dermal infiltrating glandular cavities, infiltration Inflammatory cells around the Hyperplasia, while the TQ group showed no effect on these tissues. The current study aimed at showing the effect of TQ which is an antioxidant on the proliferative system and used as a treatment for tumors without causing any side effects on this tissue.

Keywords: Thymoquinone, cancer, azoxymethane

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INTRODUCTION

Thymoquinone (TQ) is a phytochemical compound found in the plant *Nigella sativa*.(Hashim *et al*.,1962). Several studies have demonstrated the pharmacologic effect of the black pill that has been included as an anticancer (Canonica *et al*. 1963, Badary *et al*. 2001) and antioxidant (Badary *et al*. 2003), as well as for the treatment of headaches, depression, topical pain treatment, joint stiffness (Sayed,1980) and treatment of bladder, kidney and diuretic diseases (Aboubasha *et al*. 1995).These pharmacological effects are due to the presence of TQ (Abduelah and Zaninal-abidin 2007, Jamal *et al*. 2008).

MATERIALS AND METHODS

The adult male rats were spragnedawely (18) rats, aged 3-4 months and weighed between 150-200 g, divided into three groups and each group of 6 rats injected the second and third groups with carcinogen Azoxymethane (AOM) 15 Mg / kg) once per week and for two weeks within the peritoneal cavity to develop colon cancer(Suaeyun *et al*. 2008), and then group III Thymoquinone orally (50 mg / kg) daily for two months

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and used these concentrations being far from the lethal dose, the second group was injected Only the carcinogen was considered the first group as a control group Only injected with normal saline solution.

Biochemical Tests

5 ml of rat blood was placed in non-anticoagulant tubes and placed in the centrifuge to obtain serum for tumor marker testing (CEA concentration) (Hernanto *et al*. 1994) and required biochemical tests such as testosterone hormone concentration test (Lenton *et al*. 1982), acid phosphatase activity (Tietz 1999) and measurement Concentration of live sperm in the serum (Sakamoto and Hashimoto 1986).

Estimation of Carcinogenic Embryonic Antigen (CEA)

Colorectal cancer was detected by estimating the concentration of this test on rat serum using its Vidas CEA kit and its Vidas device based on the Enzyme immunoassay sandwish method (Hernanto *et al*. 1994).

Calculation of Sperm Content in the Epididymis

Sakamoto and Hashimoto (1986) method was used to count the sperm in the epididymis. The epididymis was separated and cut into small pieces in a petri dish. Then add 9.8 ml of neutral formalin and 1 microliter of the eosin dye (5%). Count the blood cells (Hemocytomerter) and placed on the stage of the microscope and fixed the lid of the slide and then placed a drop of the prepared solution nose in the middle stage of the slide near the edge of the lid cover and left the slide on the microscope for 5 minutes to ensure the stability of sperm on the squares and counted sperm in five boxes The number of sperm in 80 small squares, and then the total number of sperm in the epididymis Per milliliter.

Examination of Sperm Abnormalities

Remove the epididymis after placing it in 2 ml of 37 ° C saline solution, then take a small drop, put it on a glass slide, add a drop of the eosin dye and gently mix the drops for half a minute with a second slice angle. Pulled gently at a sharp angle on a second slide, after drying the swab was examined with oil lens 100 X (Al-saddi 1989).

Histological Study

After the weight of the rats before and after the experiment and taking the blood from them explained the group of rats injected with carcinogens two weeks after the injection to detect the incidence of cancer was also dissected animals after a week of the last dose of Thymoquinone and taken parts of the testes and bacterium and prostate gland and confirmed by 10% formalin For methodological study (Drury *et al*. 1967).

Statistical Analysis

Statistical analysis of the results of the experiment was carried out by using the Statistical Package for the Social Science, Version 20 (SPSS) (2008) to study the effect of experimental materials and to determine the mean differences between the averages at a probability level ($p < 0.05$) according to the Duncan test (Duncan 1955).

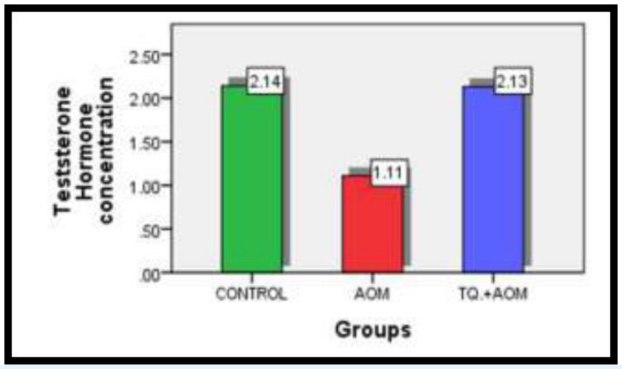

Fig. 1. Describe testosterone hormone concentration in serum for experimental groups

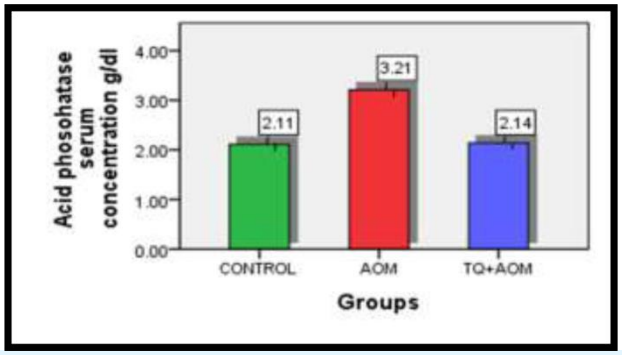

Fig. 2. Describe acid phosphotase concentration in serum for experimental groups

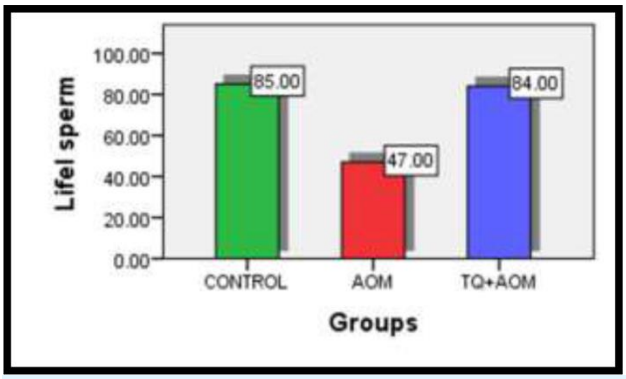

Fig. 3. Describe live sperm concentration for experimental groups

RESULTS AND DISCUSSION

Estimation Concentrations of CEA, Testosterone, and Acid Phosphatase Activity in Serum

Figs. 1, **2**, **4** showed a significant decrease in the level of hormone in the AOM group only compared to the control group and significant increase in the level of acid phosphatase in the AOM group only compared to the

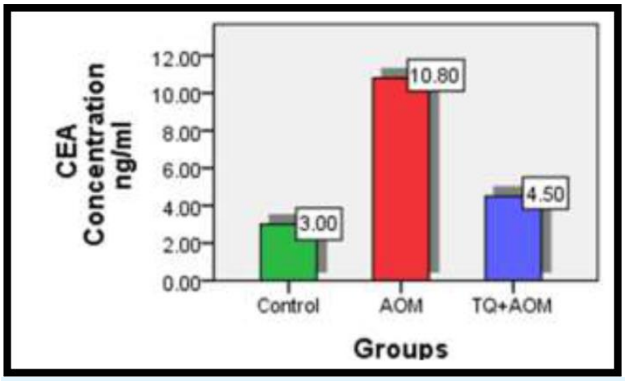

Fig. 4. Describe carcinogenic concentration for experimental groups

Table 1. Weights of animals and reproductive organs before and after the experiment

Groups	Animal weight		Weights of studied organs		
	After experimenting gram	Before experimenting gram	Prostate gram	Seminal vesicles gram	Testis gram
Control group	162 ± 2.89 a	223 ± 2.36 a	0.20± 0.07 d	0.19 ± 0.14 d	1.08 ± 0.10 d
Group treated with azoxymethane (AOM)	165 ± 1.25 a	195 ± 4.08 b	0.12 ± 0.04 f	0.14± 0.01 e	0.87 ± 0.05 e
Thymoquinone group (TQ)	166 ± 2.36 a	221 ±2.36 a	0.19 ± 0.01 e	0.25 ± 0.06 c	1.17± 0.03 c

*Values represent the mean ± standard deviation.
*The vertically different letters mean a significant difference at a significant level (P <0.05).
* Number of animals 6 per group.

control group while the group treatment with TQ did not show any significant differences compared with the control group This is an indication of the effect of the carcinogen on these conc. as result of its effect on the reproductive system (Kurus *et al*. 2017) and also significant increase in the CEA conc. in the AOM group only significance of te occurrence of the tumor while CEA conc. decreased in TQ group response to treatment (Hernand *et al*. 1994, Sturgeon *et al*. 2009)

Sperm Analysis

Concentration of live sperm

Fig. 3 shows a significant decrease in the concentration of live sperm in the AOM group and also non-significant increase in concentration in TQ group compared to the control group (Kurus *et al*. 2017).

Animal and Genitals Weights

The results showed a significant decrease in weight (P <0.05) in the AOM group compared with the TQ group and the control group as shown in **Table 1**. The weight loss was due to loss of skeletal muscle and fatty tissue as well as lack of nutrition during the treatment period due to the use of carcinogens, or as a result of the negative effect of the carcinogen which causes an increase in the production of free radicals and thus increases the fat peroxidation (Ameet *et al*. 2012). The group treated with TQ was observed an increase in weight compared to the AOM group (Jemal *et al*. 2008)

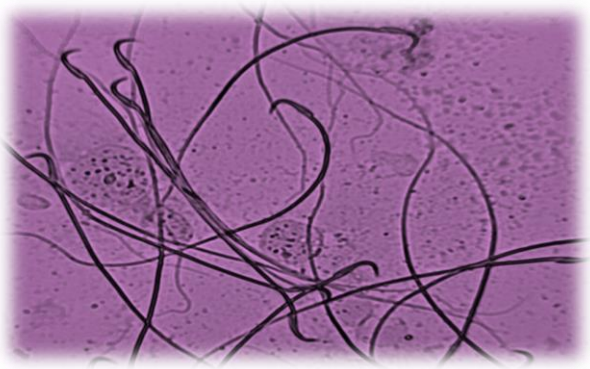

Image 1. A sperm control group describe the natural forms of sperm (E&N 400x)

Image 2. A sperm rats treated with carcinogenic group (AOM) shows abnormalities (Teratospermia) in the forms of some sperm where A-head is observed only sperm B-tail wrap (E&N 400x)

This TQ material is important in the process of stimulating the programmed death of cancer cells in infected tissues by regulating the gene expression of the programmed death genes, thus helping to kill the cancer cells (Baba et al 2015).

The results of the present study showed a significant decrease in sperm concentration in rats treated with AOM only compared to control group. It was also observed that there are abnormalities in the forms of some sperm (Teratospermia) as shown in **Image 2** compared to the control group as shown in **Image 1**. The AOM increase in oxidative processes due to the formation of reactive oxygen species is the main cause of the damage of the spermatid cells when exposed to the carcinogen this leads to apoptosis (Kurus *et al*. 2017) The results of the present study showed that there was a significant increase in the concentration of sperm in TQ group compared to AOM group only. As shown in **Image 3**, the sperm are sound from deformities, this is because TQ prevents thy types of oxygen reacting in the destruction of cancer cell in testis high efficiency thus confirming capcity TQ to reduce free redical and keep cells ,tissues intact thus increasing sperm concentration (Tufek *et al*. 2015)

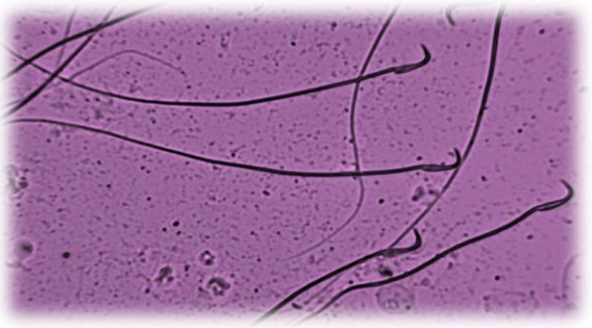

Image 3. A sperm group rats treated with carcinogenic with thymoqunin and observed sperm empty from distortions (E&N 400x)

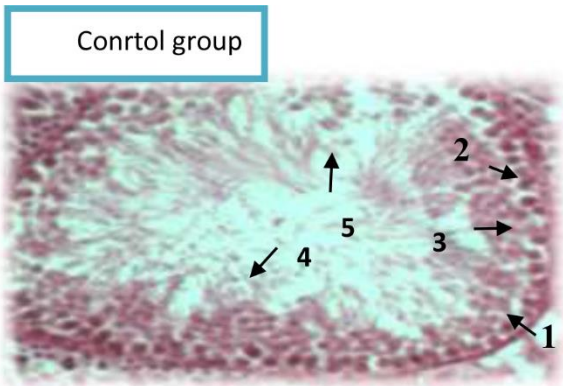

Image 4. Section in testis 1-basement membrane 2-spermatogonia cells 3-spermatocyte 4-spermatids 5-sperm (H&E) (100X)

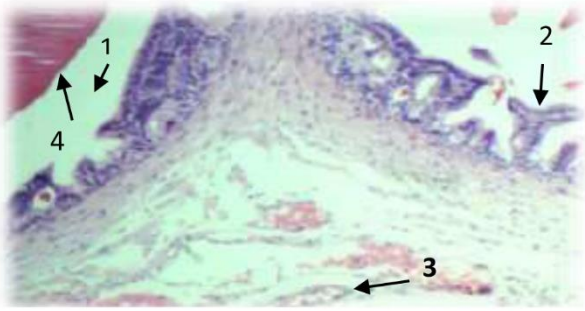

Image 5. Section in prostate gland 1-Prostatic hyperplasia2- Alveolar folds 3- Blood vessels in cell tissue4-gland filtered acid dye (H&E) (100X)

Histological Examination

The microscopic examination of the testis tissue in the AOM group showed only a thick capsule surrounding the testis tissue with appear congestion of all blood vessels under the capsule, and contained the seminiferous tubules as most of them appeared to rupture of the basement membrane and thicken with the enlargement of primary and secondary sperm and cells degeneration and the loss of of spermatids, degeneration of sperm cells and the appearance of sperm in a ghostly way. Compared to the control group **Image 7** compared to the control group (5). When the prostate gland was examined, one of the lobules of the

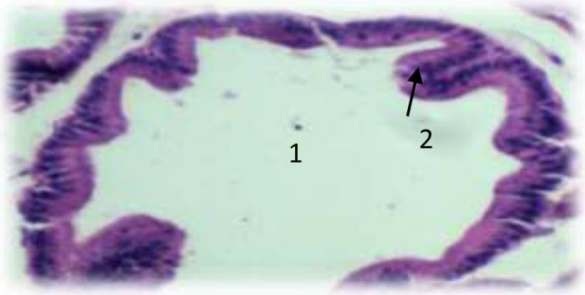

Image 6. section in seminal vesicle 1- seminal vesicle Hyperplasia 2- The folds of the gland lining the cells are stritifed columnar (H&E) (100X)

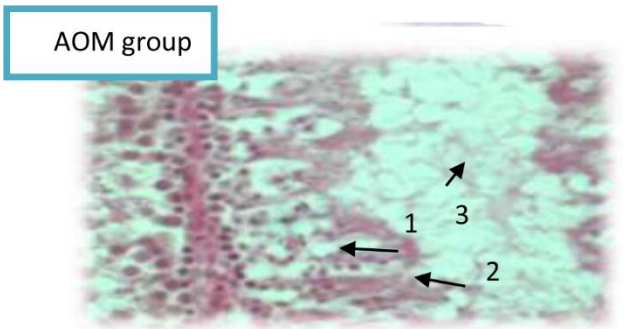

Image 7. Section in testis 1 - loss of spermatids 2 - degeneration of sperm cells 3 - the appearance of sperm in a ghostly way (H&E) (100X)

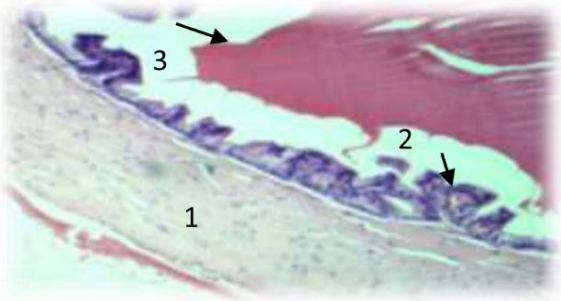

Image 8. Section in prostate gland 1- prostate gland capsule 2 - epithelial cells within the folds 3- gland filter (H&E)(100X)

gland appeared in a strong tissue of its epithelial cells so that it filled up and formed a large mass. Other Hyperplasia showed atrophy in the mucous folds, whereas in interstitial tissue there was a bloody congestion **Image 8** compared to the control group **Image 7**. In seminal vesicle sowed glands Hyperplasia, breakdown walls, dermal infiltrating glandular cavities, infiltration Inflammatory cells around the Hyperplasia, all glandular Hyperplasia appeared to be free of glandular filtered, which appeared clearly in the interstitial tissue, which had a homogeneous red color, and the basal membrane of most of these regions had ruptured **Image 9** compared to the control group **Image 5**. This indicates the AOM carcinogen effect on testis and reproductive tissues (Kurus *et al*. 2017).

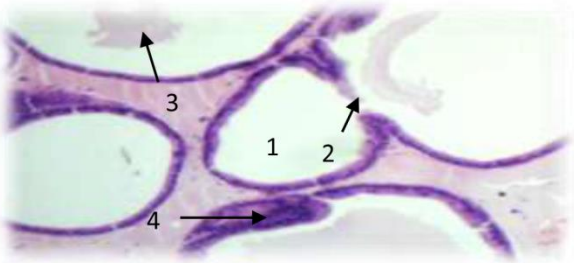

Image 9. Section seminal vesicle 1-glands Hyperplasia 2-breakdown walls 3-dermal infiltrating glandular cavities 4-infiltration Inflammatory cells around the Hyperplasia (H&E) (100X)

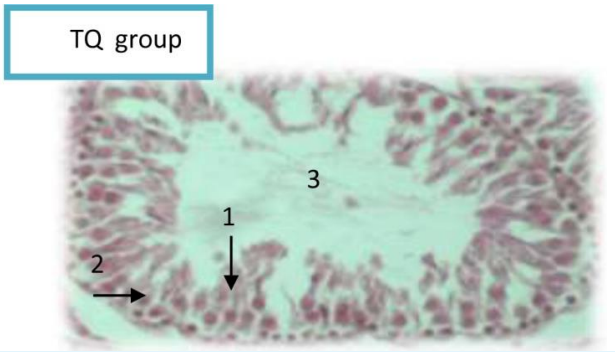

Image 10. Section in testis 1-primary and secondary spermatocyte hypertrophy 2-contraction of the basal membrane of the tubule 3- Rare sperm in the tubular cavity (H&E) (100X)

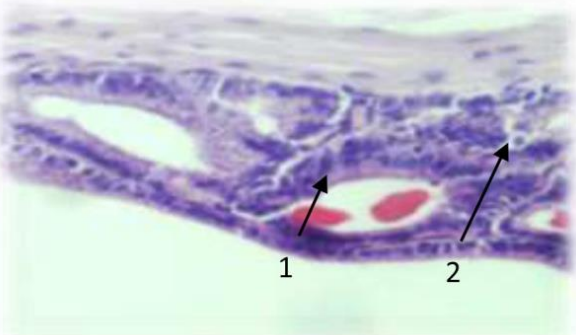

Image 11. Section in prostate gland 1-Glandular infiltration 2-cell lymphocytic infiltration (H&E) (100X)

When examining the testicular tissue of the TQ group, it was observed that the containment of wrapped seminiferous tubules in which show the enlargement of spermatocytes, especially primary and secondary spermatocytes are in the case of swelling with degeneration in spermatid with the appearance of a few numbers of sperm in the middle of the tubules **Image 10**. The condition does not include all the tissue has emerged numbers of germs naturally for different stages of evolution. As for the prostate, a number of lobules were found that contained smaller scales composed of very small spindle folds lined with cubic cells and surrounded by smooth muscle fibers with some tissue

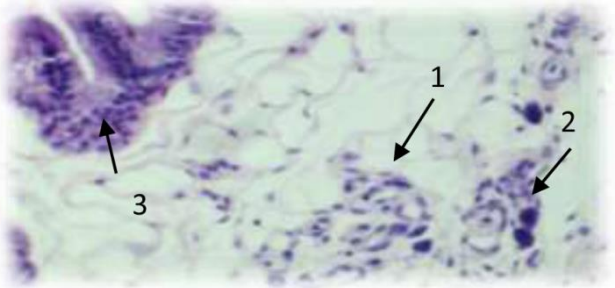

Image 12. Section seminal vesicle 1. massive numbers of inflammatory and phagocytic cells in the interstitial tissue 2-inflammatory cells and phagocytic 3- pseudostritified columnar cell (H&E) (100X)

fibers. The soft link containing inflammatory cells was exposed around the alveoli **Image 11**.

While the examination of the tissue of the seminal vesicle the presence of glandular glands with many folds lined with vertical cells and the application of false and the presence of red leaks in the inside of the gland, and the presence of blood vessels in the blood and the number of pharyngeal cells in the interstitial tissue have **Image 12**. This indicates that TQ has no effect on reproductive tissues and sexual hormons (Salahshoor *et al*. 2018)

Several studies have confirmed that TQ inhibits oxidative damage in various laboratory studies performed on mice (Yaman *et al*. 2010), because it is an antioxidant of fat in tissues (Mansour *et al*. 2002) and inhibits reactive oxygen species from destroying germ cells in the rat testicles with high efficiency. This supports our ability to reduce the level of free redicals and maintain the integrity of cells and tissues and thus increase the concentration of sperm. This supports our previous findings which demonstrated that TQ increases the concentration of sperm in the treated group with TQ compared to the treated group with carcinogen only. It also shows its great role in preserving the body tissues from the harmful effects of the carcinogen.

CONCLUSIONS

We conclude from the current study:

1- The study showed the important role (treatment and protective) of the two thymoquinone(TQ) that are considered antioxidants in reducing the stress caused by the carcinogen in the treated animals and led to the improvement of most of the disorders occurring in the body due to carcinogenic treatment .

2- The study showed the effect of carcinogen ((azoxymethane (AOM)) on the reproductive system of laboratory animals: For example, the genitals, such as the testes, seminal vesicles and prostate causing loss of spermatids, degeneration of sperm cells, glands Hyperplasia, breakdown walls, dermal infiltrating glandular cavities, infiltration Inflammatory cells around the Hyperplasia. prostate gland capsule. epithelial cells within the folds and gland filter.

3- The study showed the effect of carcinogen upon biochemical tests such as testosterone hormone concentration, acid phosphatase activity and t Concentration of live sperm.

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