Original article

Prophylactic role of b-carotene against testicular toxic effects of three different doses of titanium dioxide nanoparticles in adult male Wistar rats

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Abstract:

Background: Titanium dioxide nanoparticles (TiO2NPs) are very important due to their usage in many products such as pigments, food, and cosmetics. **Aim:** The goal of this investigation was to explore if beta-carotene (BC) can protect adult male Wistar rats from the testicular toxicity of sub-chronic oral exposure to TiO2NPs**. Material and methods:** Nine equal groups of ten mature male Wistar rats were formed.; Group (1) (negative control), group (2) (positive control), group (3) received BC (10mg/kg/day), groups (4, 5, 6) which were administrated with 30, 50 and 70mg/kg/day of TiO2NPs, groups (7, 8, 9) which were administrated BC (10mg/kg/day)then (30, 50,70) mg/kg/day respectively of TiO2NPs for 60 days orally. Oxidative stress markers in testicular tissue, including malondialdehyde (MDA) and superoxide dismutase (SOD), were estimated. A histopathological examination of the testicular tissues by light microscopy was also performed. **Results:** The results demonstrated a statistically significant increase in MDA and a statistically significant drop in the antioxidant enzyme SOD in testicular tissue, both of which were alleviated by giving BC. Also, significant histopathological changes were detected in the form of degenerative changes in the seminiferous tubules. The interstitial tissue showed congestion and hemorrhage. These changes were improved by the administration of BC. **Conclusion:** It can be concluded that sub-chronic oral exposure to TiO2NPs induced oxidative stress and produces testicular toxicity in rats, and the administration of BC has a potential antioxidant role.

KEYWORDS: Titanium dioxide nanoparticles, testicular toxicity, oxidative stress, rats.

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I. Introduction:

Titanium dioxide nanoparticles (TiO2NPs) widespread application is due to their biocompatibility, corrosion resistance, low cost, whitening, photocatalysis, food additive (E171), and simplicity of generating nano-sized materials (Shakeel et al., 2016).

In recent years, the scientific community and consumer advocacy groups have expressed concern regarding the safety of E171. In Europe, the European Food Safety Authority (EFSA) found in 2016 that the use of this addition posed no risks (EFSA, 2016), according to a new update by the FDA. E171 can no longer be regarded safe when used as a food additive (EFSA. 2021).

TiO2NPs can also be found in soil as pollutants, causing harm to soil health and plant production (Chavan et al., 2020).

TiO2NPs had specie-specific effects on the growth of Gram-positive and Gram-negative bacteria, fungi, actinomycetes, and anaerobes, and particle size and light exposure have been postulated as variables in their toxicity (Hou et al., 2019 and Bhattacharjya et al., 2021).

Regardless of the diverse variety of uses and our daily interactions with these particles, there is a scarcity of data about human and animal health as well as environmental effects. Inhalation, ingestion, cutaneous penetration, and injection are all possible ways for these TiO2NPs to reach the human body (Shi et al., 2013).

Previous studies have indicated that TiO2NPs are hazardous to human organs and cause oxidative stress, according to recent research. They can be taken into the body through ingestion, inhalation, and dermal penetration, and are dispersed to major organ systems such as the lymph, brain, lung, liver, and kidney (Bermudez et al., 2004; Wang et al., 2007 and Li et al., 2010).

Many studies suggest that human male infertility has increased significantly over the past few decades (Jørgensen et al., 2006; Levine et al., 2017; and Agarwal et al., 2015).

As a result, the impact of TiO2NPs on male reproductive health has become an important subject of study. While several reports suggested

that some TiO2NPs might have protective effects on sperm cells (Afifi et al., 2015), other reports suggested that they compromise male fertility by interfering with spermatogenesis (Pinho et al., 2020).

Carotenoids are naturally occurring pigments found in plants, and are largely responsible for the vibrant colors of some fruits and vegetables. Beta-carotene is converted into vitamin A (retinol), which the body can utilize in a variety of ways, or it works as an antioxidant to protect cells from free radical damage (Orazizadeh et al., 2014).

It has been reported that increases in plasma carotenoid concentration have been linked to an increase in sperm motility and velocity (Almbro et al., 2011).

Beta-carotene has been shown to protect against toxicants such as fenvalerate, methotrexate, cadmium, and titanium in previous research (Orazizadeh et al., 2014).

The goal of this study was to investigate the protective effects of BC (10 mg/Kg/day) in the reduction of the sub-chronic oral exposure of testicular toxicity by administration of 3 different doses (30, 50, and 70) mg/Kg/day of TiO2NPs in adult male Wistar rats.

II. Material and methods

II.1. Chemicals:

1. TiO2NPs: Were purchased from Nanotech chemicals (25 Ibrahim Abou Elnaga St., Ext. of Abbas El Akkad, Nasr City, 11765, Cairo, Egypt) and provided as powder (less than 15 nm). Before each dosage, the powder was dissolved in normal saline, ultrasonically dispersed, and vortexed for 30 minutes, after which the solution was physically shaken.
2. BC: Sigma-Aldrich (Inc. PO Box 14508 St. Louis, MO 68178 United States) provided BC (10 mg/kg).

II.2. Animals:

Ninety Wistar rats, mature males, from Sohag University Experimental Animal House, weighing 200–250 gm., were utilized in this work and were kept at ambient temperature in cages of polypropylene, 21± 3 °C. At the start of the treatment protocol, rats were acclimatized of one-week duration to the laboratory condition.

The pellet was given to the animals along with a regular meal and water. The

procedure was carried out according to the ethics committee of Sohag University's protocol on the treatment and use of laboratory animals.

II.3. Experimental design:

We randomly divided the animals into 9 groups, each group contained 10 rats:

- Group 1 (G1): a control group that did not receive any treatment.
- Group2 (G2): the group of positive controls received normal saline for 60 days.
- Group3 (G3): was given BC at a dose of 10 mg/kg/day for 60 days. The dose of BC was chosen based on the findings of previous studies (Lyama et al., 1996; Matos et al., 2006 and Vardi et al., 2009).
- Group4 (G4): was given 30 mg/kg/day TiO2NPs (approximately 0.25% of LD50) orally via gavage tube for 60 days. The LD50 of TiO2NPs is greater than 12,000 mg kg-1 weight of the body after oral treatment (Wang et al., 2007).
- Group5 (G5): was given 50 mg/kg/day TiO2NPs (about 0.42% of LD50)**,** for 60 days, taken orally through a gavage tube.
- Group6 (G6): was treated with 70 mg/kg /day TiO2NPs (about 0.58% of LD50), for 60 days, taken orally through a gavage tube.
- Group 7 (G7) received (10 mg/kg) BC 1 hour before TiO2NPs (30 mg/kg) for 60 days via gavage tube.
- Group 8 (G8) received (10 mg/kg) BC 1 hour before TiO2NPs (50 mg/kg) for 60 days via gavage tube.
- Group 9 (G9) received (10 mg/kg) BC 1 hour before TiO2NPs (70 mg/kg) for 60 days via gavage tube.

II.4. Preparation of the dose:

- The Pure TiO2NPs powder was dissolved in a normal saline solution and was freshly prepared every day and vortexed for 30 minutes.
- The concentration of the solution of TiO2NPs for G4 and G7 which were given TiO2NPs dosing of 30 mg/kg/day, was 75 mg prepared in 10 ml of normal saline. Each Wistar was given 1 ml of the prepared solution.

- The concentration of the solution of TiO2NPs for G5 and G8 which were given TiO2NPs NP dosing of 50 mg/kg/day was 125 mg prepared in 10 ml normal saline each Wistar was given 1 ml of the prepared solution.
- The concentration of the solution of TiO2NPs for G6 and G9 which were given TiO2NPs dosing of 70 mg/kg/day was 175 mg prepared in 10 ml normal saline each Wistar was given 1 ml of the prepared solution.

The concentration of the solution of BC 100 mg combined with 2 mL of Tween-80 at the temperature of the room till uniform paste was produced. At the temperature of the room, physiologic saline was added dropwise to a final content of 10 mg BC per milliliter of the solution diluted in saline solution was 10 mg/Kg/ day and vortexed freshly before administration (Orazizadeh, 2014).

II.5. Methods:

After 60 days of the experiment, the rats were anaesthetized with inhalational ether and dissected to expose the testes. The rats were slaughtered. All animal groups had their autopsies performed.

- A phosphate-buffered saline solution (PBS) with pH 7.4 and adding 0.16 mg/ml of heparin to it for removal of any blood clots was used to perfuse parts of the testis. 1000 mg of testicular tissue was homogenized in 5-10 ml of cold buffer (PH 7.5, 50 mM potassium phosphate). The homogenates were then centrifuged for 15 minutes to separate the components (4000 rpm). Supernatants were used to evaluate the level of (MDA) and (SOD).

Testis preparation methods for SOD and MDA according to Khradmand et al., (2009)

III.5.A. Method of SOD activity:

We measured SOD activity by a kinetic method using the Biodiagnostic (Cat. No. SD 252) Kit.

Reagents:

- Phosphate Buffer (pH 8.5) 50 mM/L
- Nitroblue tetrazolium (NBT) at 1 mM/L
- NADH 1 mM/L
- Phenazine methosulphate (PMS) 0.05 mM/L
- Extraction Reagent

Preparation of solutions:

Reagent (1) was ready for use. ·

Reagent (2) reconstituted in 5 ml d. Water.

Reagent (3) reconstituted in 5 ml buffer.

Reagent (4) reconstituted in 5 ml d. Water.

- **Procedure**

a. R4 was diluted 1000 times immediately before use (10 µL + 10 ml distilled water) and, discarded after use. ·

b. Samples were diluted to give an inhibition percent between 30 and 60.

c. Working reagent: R1 + R2 + R3 were mixed in a ratio of (10+1+1 ml), immediately before use.

III.5.B. Method of MDA concentration:

We measured MDA concentration by a colorimetric method using the Biodiagnostic (Cat. No. MD 25 29) Kit.

Reagents:

- Standard 10 nmol / mL Chromogen (Thiobarbituric acid, Detergent, Stabilizer) 25 mmol / L.

Procedure:

a. The supernatant of tissue extract was brought out of the freezer.

b. Mixed well, then test tubes were covered with glass beads, heated in a boiling water bath for 30 minutes, cooled, and then added.

c. Mixed, read the absorbance of the sample against the blank and standard against distilled water at 534 nm.

Calculation:

Malondialdehyde in tissue = $\frac{absorbance\ of\ sample}{absorbance\ of\ standard}$ x $\frac{10}{gm\ tissue\ used}$ = nmol / g.tissue

II.6.Histopathology:

- Bouin's solution was used to fix the testicular samples, which were then embedded in paraffin blocks for sectioning at 4 micron thicknesses. Testicular sections were processed to be stained using hematoxylin and eosin (H&E), then viewed and photographed (Ellenburg et al., 2020).

II.7. Statistical analysis:

Data were analyzed using Statistical Package for Social Science (SPSS) version 24 software. Statistics were calculated by using the analysis of variance (ANOVA) test accompanied by a post hoc test (Tukey’s test) was used in

a comparison of the means in more than two groups. At $P < 0.05$, differences were considered a significant result.

III. Results

III.1.Oxidative stress biomarkers in testicular tissue:

III.1.A.SOD level:

The present investigation found no statistically significant differences in the mean SOD enzyme values of testis tissues between- group (1) from one side and the protected groups (G7, 8), while there was a statistically significant decrease in mean values between-group (1) and the protected group (9) (P-value 0.032).

There were no significant changes among group (2) and the BC treated group (3) from one side and the protected groups (G7, 8, 9) from the other side. There was a statistically significant decrease in the mean values of SOD enzyme in testis tissues among control groups (1, 2) and BC treated group (3) on one hand and TiO2NPs treated groups (4,5,6) (P values < 0.001) from the other hand.

There were non-significant statistical changes in the mean values of SOD enzyme of testis tissues among the TiO2NPs (30 mg/kg) treated (G4) from one side and the other two TiO2NPs treated groups (50 and 70 mg/kg) (G5, 6) from the other side, while there were significant increases in the mean values of SOD enzyme of testis tissues between the TiO2NPs treated groups (G4, 5, 6) from one side and the protected group (G7, 8, 9) from the other side (P-values ≤ 0.001).

There were non-significant statistical changes in the mean values of SOD enzyme of testis tissues among the TiO2NPs (50 mg/kg) treated (G5) from one side and the other TiO2NPs treated groups (70 mg/kg) (G6) from the other side.

There were no statistically significant changes in the mean values of SOD enzyme of testis tissues among the protected group (G7) from one side with the other two protected groups (G8, 9) from the other side.

There were no statistically significant changes in the mean values of SOD enzyme of testis tissues among the protected group (G8) from one side with the other protected groups (G 9) from the other side table (1).

III.1.B. MDA level:

The present study showed non-significant statistical changes in the mean values of MDA of testis tissues among the negative control group (G1) from one side, the positive control group (G2), the BC treated group (G3), and the protected groups (G7, 8) from the other side, while there was a significant increase in the mean values of MDA of testis tissues among the control groups (G1, 2) and BC treated group (3) from one side, and TiO2NPs treated groups (G4, 5, 6) and the protected group (G9) from the other side (P-value < 0.001).

There was a significant statistical increase in the mean values of MDA of testis tissues among the TiO2NPs (30 mg/kg/day) treated (G4) from one side and the other TiO2NPs treated groups (70 mg/kg/day) (G6) (P-value < 0.001) and a significant decrease with the protected groups (G7, G8) from the other side (P value < 0.001).

There was a significant increase in the mean values of MDA of testis tissues among the TiO2NPs treated (G5) (50 mg/kg/day) from one side and the TiO2NPs treated group (G6) (70mg/kg/day) (P-value < 0.001) and highly significant decrease with the protected groups (G7, G8, 9) from the other side (P-value < 0.001).

There was a significant decrease in the mean values of MDA of testis tissues among the TiO2NPs treated (G6) (70 mg/kg) on one side and the protected groups (G7, G8, and G9) on the other side (P-value < 0.001).

There were no statistically significant changes in the mean values of MDA enzyme of testis tissues between the protected group (G7) from one side and the other protected groups (G8) from the other side, while there was a statistically significant increase between the protected group (G7) from one side and the other protected groups (G9) from the other side (P-value < 0.001). There was a statistically significant increase in the mean values of MDA of testis tissues between groups 8 and 9 (p value = 0.004) as shown in table (2)

III.2. Histopathological findings:

Light microscopic examination of H&E stained sections of the testis of the

negative control group (group 1) and positive control group (group 2) revealed a normal appearance of the testicular tissue. The testis is formed of seminiferous tubules separated by interstitial tissue. Tubules are lined with many layers of spermatogenic and Sertoli cells. Spermatogenic cells were organized into spermatogonia, primary spermatocytes, secondary spermatocytes, rounded spermatids, elongated spermatids, and sperm. The interstitial tissue contains Leydig cells, blood capillaries, and connective tissue cells (figure 1; A & B).

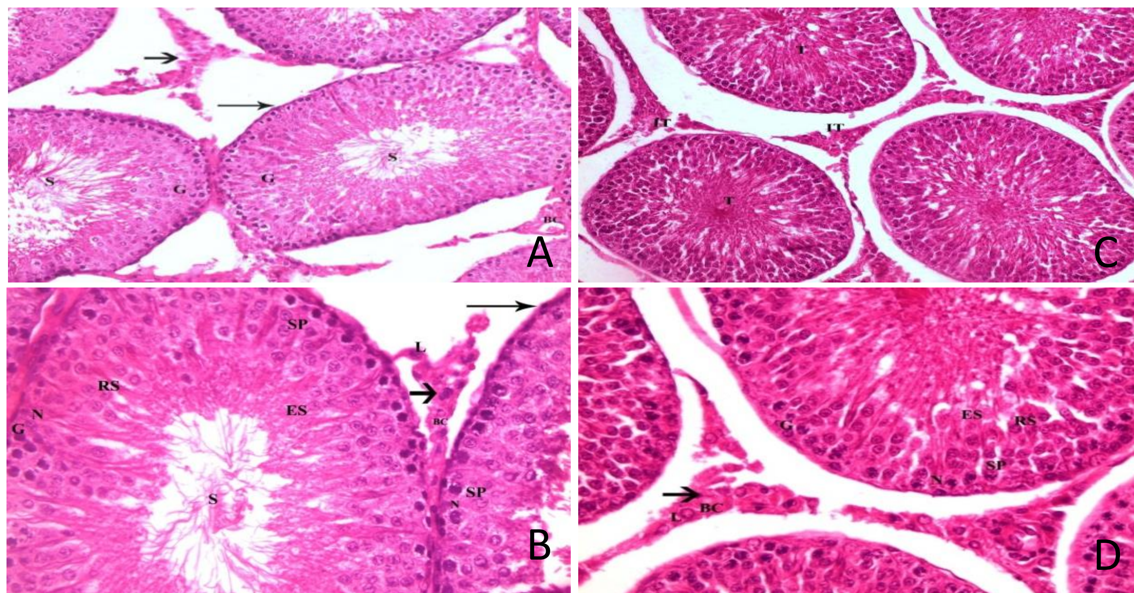

Figure (1): photomicrographs of a section in the testis of adult rat (A) section of the control groups 1, 2 showing a normal structure of the testicular tissue. H&E X 200. (B) Higher magnification showing seminiferous tubules with regular outlines (thin arrow), nucleus of Sertoli cell(N), primary spermatocyte(SP), rounded spermatocyte (RS), elongated spermatocytes (ES), and sperms (S). Interstitial tissue (short arrow) contains Leydig cells (L) H&E X 400**.** (C) A section in the testis, (D) Higher magnification of the same section of beta-carotene treated rat showing more or less similar to the control group H&E X 200.

Light microscopic examination of the BC-treated rats (group 3) revealed that the testicular tissue was more or less similar to that of the control group (figure 1; C & D). Light microscopic examination of the TiO2NPs treated rat group (4) revealed degenerative changes in the seminiferous tubules which appeared with irregular outlines. Germ cells appeared disorganized and some cells were detached from the basal lamina. Some germ cells had dense pyknotic nuclei and vacuolated cytoplasm. The interstitial tissue

showed congested blood capillaries. Interstitial haemorrhage was also noticed (figure 2; A & B).

Light microscopic examination of the TiO2NPs treated rat group (5) revealed degenerative changes in the seminiferous tubules which appeared edematous with irregular outlines. Germ cells appear disorganized and some are detached from the basal lamina. Some germ cells had dense pyknotic nuclei and some showed vacuolization. Interstitial tissue showed congested blood capillaries and there were increased areas of haemorrhage and acidophilic exudation (figure 2; C & D).

Light microscopic examination of the TiO2NPs treated rat group (6) revealed the same findings but with more hemorrhage and exudation (figure 2; E & F).

Light microscopic examination of the protected group (7) revealed that histological alterations were less prominent. Most tubules appeared more or less similar to those of the control group. A minor part of the tubules showed degenerative changes. The interstitial tissue, Leydig cells, and blood capillaries were similar to those in control group (figure 3; A & B).

Light microscopic examination of the protected group (8) revealed that histological alterations were less prominent. Some tubules appeared with a regular outline. The germinal epithelium was well organized, and the lumen of the tubules was filled with sperm. Other tubules appeared with irregular outlines and degeneration of germ cells. Interstitial tissue showed fewer hemorrhagic areas, and the blood vessels were less congested. Leydig cells showed vesicular nuclei (Figure 3; C & D).

Light microscopic examination of the protected group (9) revealed that some tubules appeared with a regular outline. The germinal epithelium was well organized, and the lumen of the tubules was filled with sperm. Other tubules appeared with irregular outlines and contained degenerated germ cells. Interstitial tissue showed fewer hemorrhagic areas and exudation. Blood vessels were less congested. Leydig cells appeared more or less similar to control groups (Figure 3; E & F).

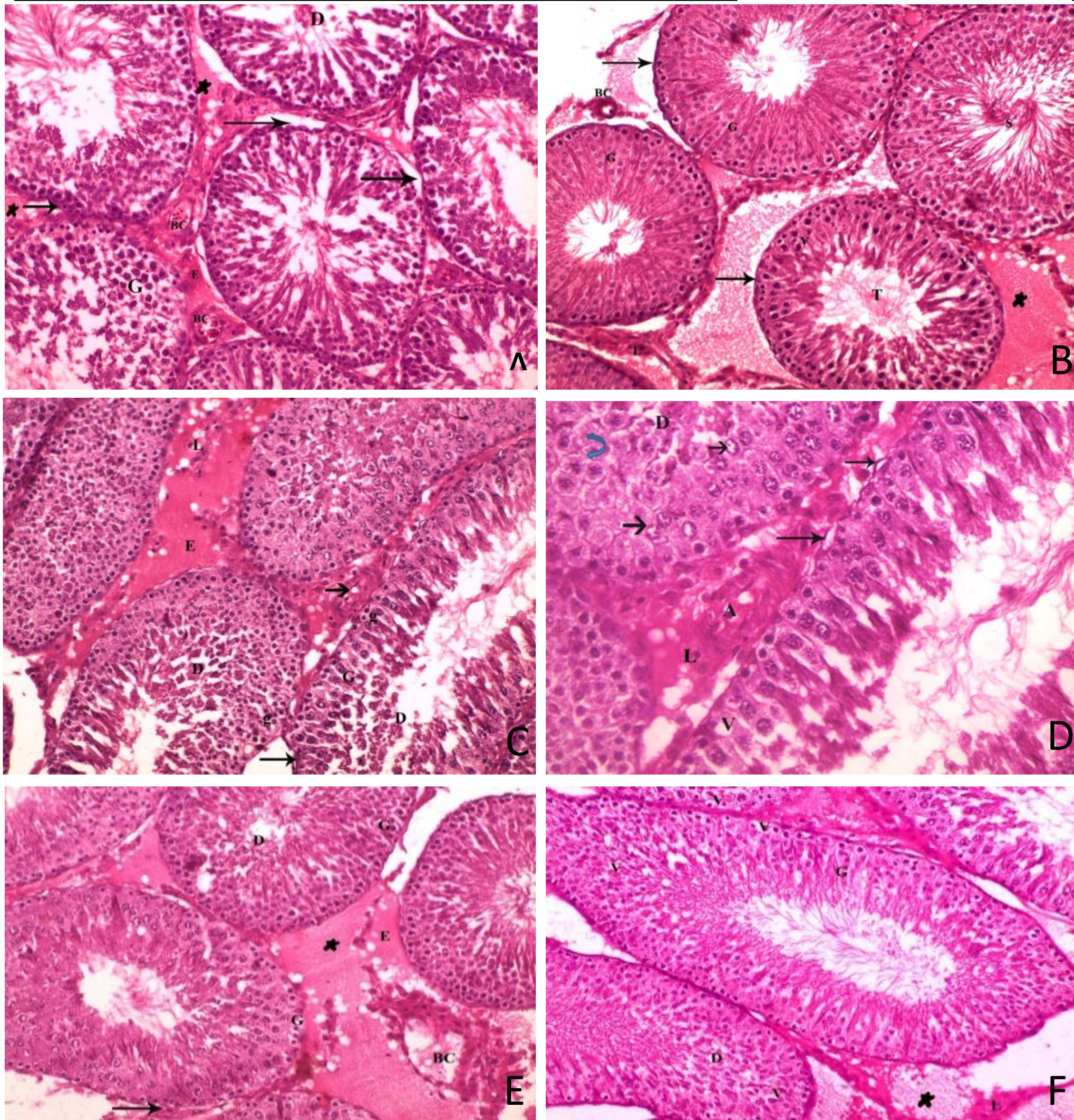

Figure (2): photomicrographs in the testes of TiO2NPs treated rats (A) A section of testis in group 4 (rats treated with 30 mg/kg /day TiO2NPs). (B) Higher magnification showing degenerated and disorganized germ cells (G) with wide spacing in between (irregular arrow), with vacuolization of germ cells (V), Note: congested blood capillaries (BC) in the interstitial tissue, acidophilic exudation (E) H&E X 400. (C) A section of a rat in group 5 (rats treated with 50 mg/kg/day TiO2NPs) (D) Higher magnification showing seminiferous tubules with irregular outlines (long thin arrows), germ cells showing chromatinolysis (short arrows), exfoliated germ cells (D) with abnormal chromatin distribution (blue curved arrow), vacuolated germ cells (V), Leydig cells with dense pyknotic nuclei (L), arteriole (A), H&E X 400. (E) A section of a rat in group 6 (rats treated with 70 mg/kg/day TiO2NPs) (F) Higher magnification showing germ cell degeneration and vacuolization (V), wide intercellular space , dislocated germ cells (thick arrow), interstitial tissue shows hemorrhage (star) and exudate (E), Leydig cell shows pyknotic nuclei (L) H&E X 400.

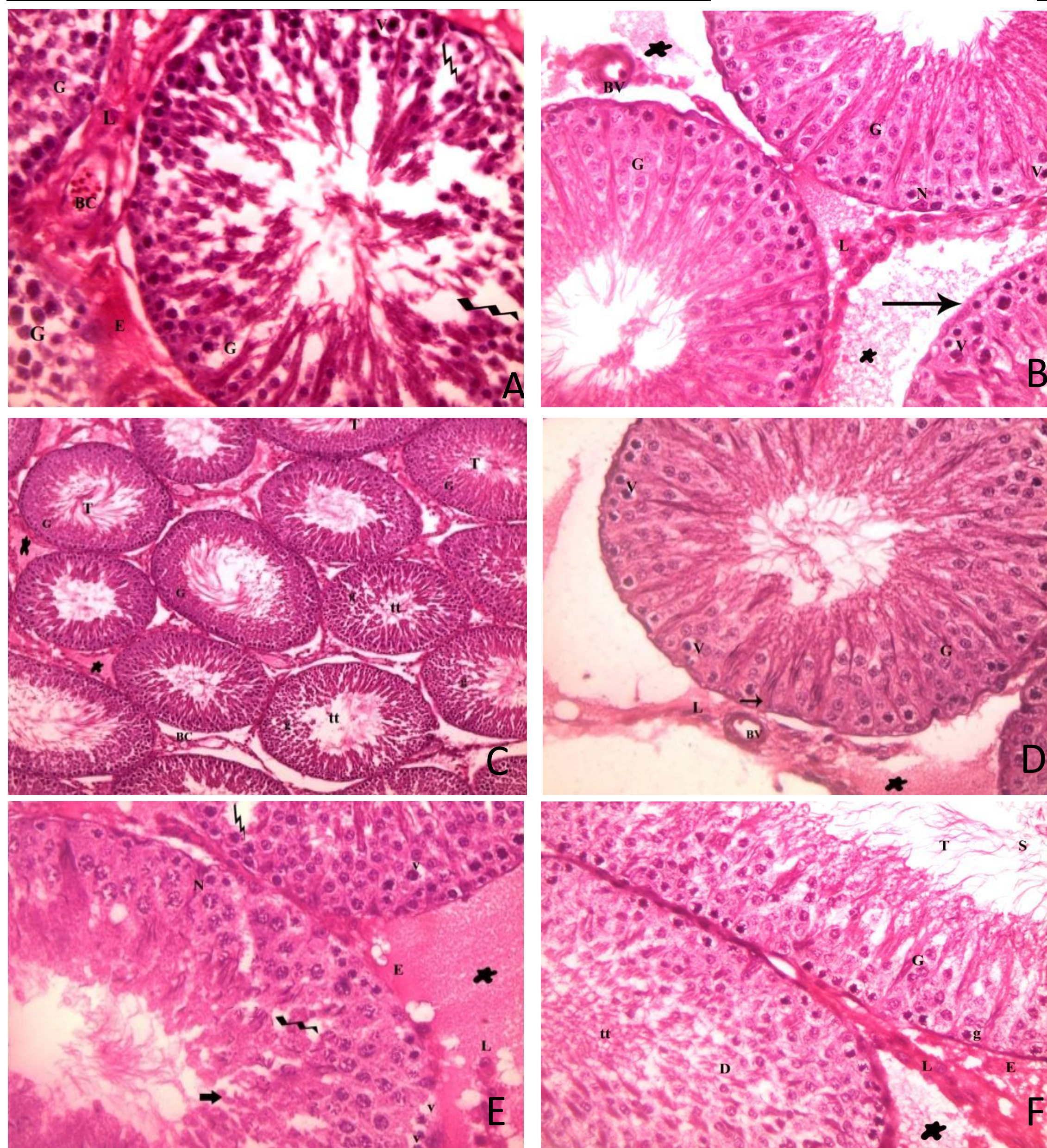

Figure (3): photomicrographs in the testes of beta-carotene protected rats. (A) A section of a rat in group 7 (rats treated with 10 mg/kg/day BC then 30 mg/kg /day TiO2NPs). (B) Higher magnification showing regular outlines of seminiferous tubules (arrow), germ cells are well organized (G), some germ cells appeared vacuolated (V), normal Leydig cells (L), interstitial tissue shows some hemorrhagic areas (stars), Sertoli cell nucleus appears more or less normal (N), H&E X 400. (C) A section of a rat in group 8 (rats treated with 10 mg/kg/day BC then 50 mg/kg /day TiO2NPs). (D) Higher magnification showing well-organized many layers of germ cells (G) and vacuolated germ cells (V), normal Leydig cells (L) with interstitial hemorrhage (star). H&E X 400. (E) A section of a rat in group 9 (rats treated with 10 mg/kg/day BC then 70 mg/kg /day TiO2NPs). (F) Higher magnification showing some well-organized seminiferous tubules (T) with many layers of germ cells (G) and sperms in the lumen (S). Other tubules (tt) show detached and vacuolated spermatogonia (g), and some germ cells are disorganized (D), normal Leydig cells (L) H&E X 400.

Table1: One way ANOVA statistical analysis of SOD among comparative groups.

Groups	SOD-T		ANOVA	
	Range	Mean ± SD	F	P-value
Group 1	357.06 - 370.76	362.690 ± 7.169	33.124	<0.001
Group 2	355.44 - 360.06	357.810 ± 2.312		
Group 3	306.01 - 345.98	329.027 ± 20.663		
Group 4	173.13 - 222.39	200.200 ± 24.990		
Group 5	170.04 - 256.96	205.817 ± 45.452		
Group 6	180.04 - 210.8	198.697 ± 16.394		
Group 7	310.08 - 328.37	318.143 ± 9.335		
Group 8	298.26 - 320.87	312.137 ± 12.151		
Group 9	298.04 - 305.05	300.920 ± 3.668		

TUKEY'S Test

	1	2	3	4	5	6	7	8
2	1.000							
3	0.540	0.716						
4	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)					
5	<0.001 (HS)	<0.001 (HS)	<0.001 HS)	1.000				
6	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	1.000	1.000			
7	0.217	0.341	0.999	<0.001 (HS)	<0.001 (HS)	<0.001 HS)		
8	0.116	0.194	0.978	<0.001 (HS)	<0.001 (HS)	<0.001 HS)	1.000	
9	0.032 (S)	0.057	0.740	<0.001 (HS)	0.001 (S)	<0.001 (HS)	0.976	0.999

P values:
> 0.05 Non significant (NS)
<0.05 Significant (S)
<0.001 highly significant (HS)
SD: Standard deviation

SOD-T: super oxide dismutase enzyme of testicular tissue.

Table2: One way ANOVA statistical analysis of MDA among comparative groups.

Groups	MDA-T						ANOVA	
	Range			Mean	±	SD	F	P-value
Group 1	1.5	-	1.8	1.667	±	0.153	88.515	<0.001
Group 2	1.6	-	1.9	1.767	±	0.153		
Group 3	1.3	-	2.1	1.767	±	0.416		
Group 4	6.1	-	10.8	8.167	±	2.401		
Group 5	10.2	-	10.7	10.467	±	0.252		
Group 6	14	-	15.2	14.567	±	0.603		
Group 7	0.9	-	1.3	1.133	±	0.208		
Group 8	2.1	-	3.1	2.500	±	0.529		
Group 9	5.4	-	6.5	5.933	±	0.551		

TUKEY'S Test

	1	2	3	4	5	6	7	8
2	1.000							
3	1.000	1.000						
4	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)					
5	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.092				
6	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.001 (HS)			
7	0.997	0.992	0.992	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)		
8	0.957	0.979	0.979	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.626	
9	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.109	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.004 (S)

P values
> 0.05 Non significant (NS)
<0.05 Significant (S)
<0.001 highly significant (HS)
SD: Standard deviation

MDA-T: malondialdehyde of testicular tissue.

IV. Discussion:

Titanium dioxide nano-particles are used in a variety of everyday products, including antifouling paints, household products, plastic goods, pharmaceuticals, cosmetics, sunscreens, pharmaceutical additives, and food colorants, and many new applications are in the works or in pilot production (Skočaj et al., 2011).

As the production of nano-sized TiO2NPs powder has increased, worries regarding the effects on individuals and the environment have grown (Klaine et al., 2008).

Color additives in food, toothpaste, and medicine capsules are all key sources of TiO2NPs entering the body via the oral route (FDA, 2002).

The goal of this study was to look at the long-term effects of TiO2NPs on the testes of adult male Wistar rats, as well as the potential protective impact of BC.

Unnithan et al. (2011) demonstrated that fine nano-TiO2NPs (20 nm) at 40 mg/kg cause biochemical changes in Wistar rats, so we chose doses based on this (30, 50, and 70 mg/kg/day). The findings of this study demonstrated that taking TiO2NPs by mouth resulted in a higher significant decrease in mean values that occurred in the TiO2NPs treated groups (4, 5, 6) in SOD, which is explained by the depletion of dismutase enzyme compared to the control groups, and also the higher significant increase that occurred in the TiO2NPs treated groups (4, 5, 6) in MDA, the marker of lipid peroxidation compared to the control groups.

Malondialdehyde is a degradation product of bio membranes' polyunsaturated fatty acids, and its rise is due to considerable accumulation under strong oxidative stress. The MDA content serves as an indicator of the extent of lipid peroxidation and is an indirect reflection of the extent of cell damage (Wang et al., 2011).

It is unknown if TiO2NPs infiltrate Sertoli cells and causes oxidative stress and/or apoptosis, and the processes underlying this are unknown. This is significant because increased oxidative stress in the testicular environment has been shown to have significant effects on testicular physiology and sperm function after

exposure to nano-sized materials (Zhao et al., 2014).

Go in harmony with this study Meena et al. (2014) showed that in testis given larger dosages of TiO2NPs, intracellular antioxidant defenses, including CAT, GSH-Px, and SOD, were reduced, although lipid peroxidation levels considerably rose.

Zhao et al. (2014) found that exposing rats to TiO2NPs for 90 days caused oxidative stress in the testis, a decrease in reduced GSH and oxidized glutathione levels, suppression of SOD activity, and a modest increase in catalase activity.

Previous research has found a link between reactive oxygen species (ROS), lipid peroxidation, and DNA damage in response to toxicants (Ema et al., 2010 and Chaudhari et al., 2009).

Song et al. (2017) also revealed that SOD activity decreased when the mice were exposed to TiO2NPs. The SOD activity of mouse testes significantly decreased in the TiO2NPs treated groups, which suggested that the decomposition of O2- to H2O2 and O2 decreased in mouse testes. The MDA content of mice testes in the different treatment groups increased when the mice were exposed to TiO2NPs, this study is in agreement with ours.

Data from Jafari et al. (2019) revealed that the activity of CAT, SOD, and GPx in testis tissue was significantly decreased after chronic exposure to TiO2NPs nanoparticles.

According to Ahotupa and Huhtaniemi (1992), TiO2NPs induced spermatogenic damage, may be related to the generation of free radical products in the testicular tissue, which has a negative influence on spermatogenesis.

Noshy et al. (2015) explained this by showing that the recorded TiO2NPs induced a significant increase in testicular MDA level and a significant decrease in GSH content and CAT activity.

Orazizadeh et al. (2014) revealed BC pre-treatment for 10 days reduced the harmful effects of TNP on mouse spermatogenesis. The dose and pre-treatment time of BC employed in this investigation were chosen according to previous research.

Lyama et al. (1996) showed that after 10 days of oral treatment of BC, it accumulated in numerous tissues of mice and exhibited a protective effect against oxidative stress. Our study revealed that

BC protected rats had a higher SOD-T level and a decrease in MDA-T level. Thus, the protection of gametogenic activity in BC-treated rats could be the consequence of restoring the oxidative balance of testicular tissues, which could be the result of restoring testicular androgenesis (Sofikitis et al., 2008).

Thus, the protective effect of BC may also be due to its antioxidant effect (Vardi et al., 2009).

In Fenvalerate-induced alterations in oxidative stress, hemato-biochemical parameters, and semen quality in male rats, Almbro et al. (2011) found that vitamin E, BC alone, and/or in combination, improved semen quality.

Our histopathological results revealed that the TiO2NPs treated groups (G4, 5, 6) had an irregularity in the basement membrane with detached parts, disorganization of germ cells (some of them had pyknotic dense nuclei, some showed chromatinolysis, and some showed vacuolated cytoplasm), which means germ cell degeneration. Leydig cells showed pyknotic dense nuclei, congestion of interstitial blood capillaries, interstitial hemorrhage, and exudation, while the BC protected groups (G7, 8, 9) showed that most of the seminiferous tubules retained organization of the basement membrane with their lumen filled with sperm, some germ cells became more organized, interstitial haemorrhage decreased, and blood capillaries decreased congestion. Leydig cells become normal with vesicular nuclei.

Gao et al. (2013) reported that nanoparticles induced testicular injury and spermatogenesis inhibition in male mice, which may be related to changes in male sex hormone levels and testicular gene expression.

Braydich-Stolle et al. (2005) and Orazizadeh et al. (2014) revealed harmful activity of TiO2NPs on male germ cells, which coincided with pathological abnormalities in the testes, epididymis, prostate, and seminal vesicles. Gao et al. (2013) found that mice exposed to high doses of TiO2NPs had severe pathological alterations in their testes, including uncommon sperm, sperm breakages, Sertoli cell rarefaction, apoptosis, seminiferous tubule necrosis, and decreased germ layer thickness with sloughing and vacuolization. Meena et al. (2014) and Zhao et al. (2014) noticed that

after rat exposure to TiO2NPs vacuolization, disorganized and damaged seminiferous tubules with some inflammation in testicular cells; there was also a reduction in the number of spermatogonia, and Sertoli cell apoptosis or necrosis.

Elnagar et al. (2018) examination of the testis sections of the TiO2NPs nanoparticles treated group observed signs of inflammatory damage in the testicular tissue.

According to El Ghazzawy et al. (2011), intercellular spaces show advanced degenerative modifications that damage cell membrane integrity as a result of oxidative stress.

Fouad et al. (2009), used electron microscopy to examine testicular tissues exposed to ROS and measured inflammatory cytokines and found that ROS induces oxidative phosphorylation of cell membranes, resulting in interruption of the integrity of the intercellular junction complex.

Another study by Song et al. (2017) was conducted on 60 male mice divided randomly into four groups (n = 15). A solution of TiO2NPs was orally and intra-gastrically administered to three groups at dosages of 10, 50, or 100 mg/kg body weight (BW) per day for 28 days. This study revealed that TiO2NPs caused a reduction in germ cell number and led to spherospermia, interstitial glands, malalignment, and vacuolization in spermatogenic cells.

The morphology of testis tissues in the low dosage (10 mg kg-1 BW) group showed just spherospermia in the middle of tissues compared with that of the control. While the middle dosage (50 mg kg-1 BW) group causes spermatogenic cells in testis tissues to become disorderly and vacuolated.

According to Jafari et al. (2019), the TiO2NPs group had irregularly shaped seminiferous tubules, lumens with very few spermatozoa, and the interstitial space was wider with a lower number of Leydig cells.

Al-Doaiss et al., (2021) discovered that acute exposure to TiO2NPs at three different doses (126, 252, and 378 mg/kg bw) caused testicular changes such as spermatocyte degeneration, spermatid sloughing, and interstitial edema.

V. Conclusion :

It can be concluded that sub-chronic oral exposure to TiO2NPs caused oxidative

stress which produced dose dependent toxic effects in the testes of the rats. According to histological and pathological findings, BC may protect against oxidative stress, so it can prevent pathological changes expected by TiO2NPs. BC has a testicular-protective and potential antioxidant role against TiO2NPs' toxic effects.

VI. Recommendations:

- Administration of TiO2NPs by different routes should be revised more as nanoparticle properties may increase its toxicity.
- Further studies should be carried out on TiO2NPs in different doses and sizes to show their effects on DNA damage and genotoxicity and also study its effects on offspring.
- Further studies should be carried out on TiO2NPs' chronic toxic effects on testes regarding the quality of semen parameters in humans.
- Further experiments are needed to clarify the other benefits of BC on nanoparticle toxicity.

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الدور الوقائي للبيتا كاروتين ضد التأثيرات السامة للخصية لثلاث جرعات مختلفة من جزيئات ثاني أكسيد التيتانيوم النانوية في ذكور فئران ويستار البالغة

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المقدمة: أصبحت الجسيمات النانوية لثاني أكسيد التيتانيوم مهمة جدًا نظرًا لاستخدامها فى العديد من المنتجات مثل الأصباغ أو الطعام ومستحضرات التجميل. **الهدف:** تم تصميم هذه الدراسة لاستكشاف ما إذا كان البيتا كاروتين يمكن أن يقى ذكور فئران الويستار البالغة من سمية الخصية الناتجة عن التعرض الفموي شبه المزمن لـجزيئات ثانى أكسيد التيتانيم النانوية. **طريقة البحث**: تم تقسيم تسعين ذكور فئران ويستار البالغة إلى تسع مجموعات متساوية.

1- المجموعة الأولى (1)المراقبة السلبية.

2- المجوعة الثانية (2) (مجموعة المراقبة الايجابية) حيث تلقت محلول ملحي .

3- المجموعة الثالثة(3) تلقت (10مجم / كجم / يوم) من البيتا كاروتين .

4- المجموعة الرابعة (4) مجموعةالجرعة المنخفضة والتي تم إعطاؤها 30 مجم / كجم / يوم من الجزيئات النانـاوية لثانى أكسيد التيتانيم.

5- المجموعة الخامسة(5) مجموعة الجرعة المتوسطة والتي تم إعطاؤها 50 مجم / كجم / يوم من الجزيئات النانـاوية لثانى أكسيد التيتانيم.

6- المجموعة السادسة (6) مجموعة الجرعة المرتفعة والتي تم إعطاؤها 70 مجم / كجم / يوم من من الجزيئات النانـاوية لثانى أكسيد التيتانيم.

7- المجموعات المحمية المجموعات السابعة والثامنة والتاسعة 7 ,8 ,9 والتى تم إعطاؤها أولا البيتا كاروتين بجرعة (10 مجم / كجم / يوم) ثم 30 ، 50 و 70 مجم / كجم / يوم على الترتيب من الجزيئات النانوية لثانى أكسيد التيتانيم لمدة 60 يومًا عن طريق الفم. و فى نهاية التجربة تم تقدير علامات الإجهاد التأكسدي في أنسجة الخصية بما في ذلك المالونداى ألدهايد و أنزيم السوبر أكسيد ديسميوتيز . كما تم إجراء فحص الأنسجة المرضية لأنسجة الخصية بالمجهر الضوئي.

النتائج: أظهرت النتائج ارتفاعًا ذو دلالة احصائية في مستوى المالونداى ألدهايد وانخفاض ذو دلالة احصائية في إنزيم مضاد الأكسدة سوبرأكسيد ديسميوتيز في أنسجة الخصية والذي تم تحسينه عن طريق إعطاء البيتا كاروتين . كما تم الكشف عن تغيرات نسيجية مرضية في شكل تغيرات تنكسية في الأنابيب المنوية. أظهر النسيج الخلالي شعيرات دموية محتقنة. كما لوحظ نزيف خلالي. تم تحسين هذه التغييرات بواسطة البيتا

كاروتين.**الاستنتاج**: يمكن الاستنتاج أن التعرض الفموي شبه المزمن لـلـجزيئات النانوية لثانى أكسيد التيتانيم تسبب في الإجهاد التأكسدي الذي ينتج سمية الخصية في خصيتي الجرذان وإعطاء البيتا كاروتين له دور وقائي ومحتمل كمضاد للأكسدة ضد آثاره السامة.