TOXIC EFFECTS OF TITANIUM DIOXIDE NANO-PARTICLES IN ADULT MALE WISTAR RATS LIVER AND THE POSSIBLE PROTECTIVE ROLE OF BETA CAROTENE

Marwa A. Hasb Elnabi [1], Soheir A. Mohamed [1], Hend M. Ahmed [1], Hoda M. Elsayed [2], Marwa S. Hashim [3], Ahmed M. Said [1]

[1]Department of Forensic Medicine and Clinical Toxicology; [2]Department of Histology; [3]Department of Medical Biochemistry; Faculty of Medicine; Sohag University

Corresponding Author: Hend Mohammed Ahmed Salman
E-mail: hendzaied37@yahoo.com Telephone: +201011738674

ABSTRACT

Background: Titanium dioxide nanoparticles (Tio2NP) are important due to their various applications; sterilization, keeping rust away, and depigmentation. **Aim**: This study was designed to investigate the hepatic toxicity of sub-chronic oral exposure to Tio2 NP in adult male Wistar rats and to assess the possible protective effect of beta carotene (BC). **Material and methods:** Ninety adult male Wistar rats were divided into nine equal groups; Group I kept without any treatment (negative control), group II saline received (positive control), group III received BC (10mg/kg/day), groups IV, V, VI which were administrated with 30, 50 and 70mg/kg/day of Tio2NPs, group VII, VIII, IX which were administrated BC(10mg/kg/day) then 30, 50 and 70mg/kg/day of Tio2NPs for 60 days orally. Serum levels of AST and ALT were estimated after 30 days and at the end of the experiment. Furthermore, oxidative stress markers in liver tissue, including MDA and SOD were estimated. Histopathological examination of the liver tissues by light microscopy was also performed. **Results**: The results revealed a significant statistical increase in the levels of specific markers AST and ALT in TiO2 NPs treated groups in comparison to controls at the end of the study. There was a significant statistical decrease in the AST activity in protected groups by BC compared to 50 and 70 mg/kg administrated TiO2 NPs Tio2treated groups after 30 days of the study. Also, TiO2 NPs induced a significant elevation of MDA and a significant decrease in the antioxidant enzyme SOD in liver tissue, which was ameliorated by the administration of BC. Also, significant histopathological changes were detected in the form of numerous vacuolated hepatocytes, congestion in the portal vein, dilated congested sinusoids, numerous degenerated hepatocytes, and periportal inflammatory cell infiltration. These changes were improved by BC. **Conclusion**: It can be concluded that sub-chronic oral exposure to Tio2 NPs induces oxidative stress, which produces hepatotoxicity in the rat liver, and that of BC has a hepatoprotective and potential antioxidant role against its toxic effects. From the previous results, raising public awareness about the proper handling of TiO2 NPs materials and further studies about the usefulness of BC are recommended.

KEYWORDS: Titanium dioxide nanoparticles, hepatic toxicity, oxidative stress, male Wistar rats.

INTRODUCTION

Nanotechnology has been rapidly developing in recent years. It is concerned with the production and application of nanoparticles (NPs), which are particles with a size range of 1 to 100 nanometers **(Siddiqi et al., 2018).**

Titanium dioxide (TiO2) nanoparticles are widely utilized in a variety of applications, including plastics production, as an adjunct in pharmaceutical pill formulation, and as bleaching substances in the industry of paper, as well as in paints, sunscreens, cosmetics, and toothpaste manufacturing **(Weir et al., 2012).**

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. These NPs can enter the human body through a variety of ways, including inhalation, ingestion, cutaneous penetration, and injection **(Shi et al., 2013).**

TiO2NP has been shown to be harmful to humans and animals. The oral treatment of this nanoparticle to mice induces inflammation and impairs liver, kidney, and reproductive system function **(Zhao et al., 2013; Jia et al., 2017 and Jafari et al., 2018)**.

TiO2 NPs can infiltrate the cells of the liver and the liver is an important organ for detoxification. It has been proven that TiO2 NPs impair liver function and cause an attack of oxidative stress, resulting in liver toxicity. It has been proven that TiO2 NPs exposure can cause significant pathological abnormalities include severe liver damage manifested as steatosis, ballooning degeneration, necrotic changes, fibrosis and apoptosis **(Ma et al., 2009; Shakeel etal.2016)**.

Beta-Carotene (BC) is a known natural retinol precursor (vitamin A) that may be found in a variety of fruits and vegetables with possible antineoplastic and chemo-preventive properties. Beta carotene is an anti-oxidant preventing DNA damage by free-radical **(National Center for Biotechnology Information, 2020)**.

It has been proven by recent studies that b-carotene has potential preventive and protective activities against hepatic oxidative stress, inflammation, steatosis, fibrosis, and apoptosis **(Yilmaz et al., 2015).**

THE AIM OF THE WORK

The work aims at examining the toxic effects of sub-chronic oral exposure to titanium dioxide nanoparticles in adult male Wistar rats' livers and whether the use of beta carotene ameliorates these effects or not.

MATERIAL AND METHODS

Chemicals:

1. **Tio2NPs:** Titanium dioxide nanoparticle (less than 15 nm) powder was purchased from Nanotech chemicals (25 Ibrahim Abou Elnaga St., Ext. of Abbas El Akkad, Nasr City, 11765, Cairo, Egypt). The powder was dissolved in normal saline and dispersed by ultrasonic vibration and vortexing for 30 minutes before each dosing, and the suspension was physically shaken freshly.

2. **BC**: Sigma-Aldrich (Inc. PO Box 14508 Louis, MO 68178 USA) beta carotene (10 mg/kg).

Animals:

Ninety (90) adult male Wistar rats 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 for one week to the laboratory conditions.

Animals were supplemented with normal feed and water for the pellet. The experimental procedure occurred in agreement with the document on the handling and use of laboratory animals accepted by the ethical committee of Sohag University.

Experimental design:

We randomly divided the animals into 9 groups; each group contained 10 rats **(Redbook 2000, 2003).**

- **Group I (GI):** a group of negative controls that have not received any treatment.

- **Group II (GII):** a group of positive controls received normal saline for 60 days.

- **Group III (GIII):** received beta carotene in 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).**

- **Group IV (GIV):** received 30 mg/kg/day TiO2 NPs (approximately 0.25% of LD50) orally via gavage tube for

60 days. The LD50 of titanium dioxide nanoparticles (tio2 NPS**)** is greater than 12,000 mg/kg weight of the body after oral treatment **(Wang et al., 2007).**

- **Group V (GV):** was given 50 mg/kg/day TiO2 NPs (about 0.42% of LD50) orally through a gavage tube for 60 days.
- **Group VI (GVI):** received 70 mg/kg/day TiO2 NPs (approximately 0.58% of LD50) orally via gavage tube for 60 days.
- **Group VII (GVII):** received (10 mg/kg) BC 1 hour (1 h.) before TiO2 (30 mg/kg) for 60 days, taken orally through a gavage tube.
- **Group VIII (GVIII):** received (10 mg/kg) BC 1 h. before TiO2 (50 mg/kg) for 60 days, taken orally through a gavage tube.
- **Group IX (G IX):** received (10 mg/kg) BC 1 h. before TiO2 (70 mg/kg) for 60 days, taken orally through a gavage tube.

Preparation of the dose:

- The pure titanium dioxide nanoparticle 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 Tio2 NP for GIV and GVII, which were given Tio2 NP 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 Tio2 NP for GV and GVIII, which were given Tio2 NP dosing of 50 mg/kg/day, was 125 mg, prepared in 10 mL of normal saline. Each Wistar was given 1 mL of the prepared solution.
- The concentration of the solution of Tio2 NP for GVI and GIX, which were given Tio2 NP dosing of 70 mg/kg/day, was 175 mg prepared in 10 mL of normal saline. Each Wistar was given 1 mL of the prepared solution.
- he concentration of the solution of beta-carotene (100 mg) was combined with 2 mL of Tween-80 at the temperature of the room till a uniform paste was produced. Drop-by-drop, with forceful swirling, physiologic saline was added to a final concentration of 10 mg ß carotene per millilitre of the solution diluted in saline solution was 10 mg/Kg/day and vortexed before administration **(Orazizadeh, 2014)**.

Methods:

After 30 days, blood samples were taken by intra-cardiac blood sampling of 2 mL blood samples from three rats in each group into clean, dry blank tubes after anaesthetizing them with chloroform for 30 seconds to analyze aspartic aminotransferase (AST) and alanine aminotransferase (ALT) levels to detect early abnormalities.

After 60 days, blood samples were drawn from the cervical blood vessels before slaughtering and placed in clean, dry plain tubes to test liver function using the AST and ALT assays.

The serum was obtained after sampling by centrifugation (4000 rpm for 15 minutes), then transferred into sterile screw-capped polypropylene tubes and kept at -20 ^{0}C for biochemical analysis.

Then, the rats were sacrificed after being anaesthetized by inhalational ether and dissected to expose the liver. The samples of liver were collected from each animal.

- A phosphate buffer saline (PBS) solution with pH 7.4 and adding 0.16 mg/ml of heparin to it for the removal of any blood clots was used to perfuse parts of the liver. ١٠٠٠ mg of liver were homogenized in 5-10 ml of cold buffer (PH 7.5, 50 mM potassium phosphate). Then the homogenates were centrifuged for 15 minutes (4000 rpm). Supernatants were used to evaluate the levels of malondialdehyde (MDA) and superoxide dismutase (SOD).
- Formalin solution was used to fix the liver samples, which were then embedded in paraffin blocks for sectioning at a 4 micron thickness. Liver sections were processed to be stained using hematoxylin and eosin (H&E) and then viewed and photographed.
- **Statistical analysis:**

The results were analyzed using the Statistical Package for Social Science (SPSS) version 24 software. The Paired T-test was used to compare results after 30

days and after 60 days; the analysis of variance (ANOVA) test, accompanied by a post hoc test (Tukey's test), was used to compare means in more than two groups. Differences were considered significant at $P < 0.05$.

RESULTS

Biochemical parameters

After 30 days of the study

This work detected a highly significant difference ($P<0.001$) in the AST level mean value between the studied groups. There was a significant and highly significant statistical increase in AST levels in the 50 and 70 mg/kg treated TiO2 NPs groups in comparison to the control groups (GI, II, and III), as shown in **table (1).**

Table (1): One way ANOVA statistical analysis of AST after 30 days among comparative groups:

Groups	AST After 30 Days						ANOVA	
		Range		**Mean**	**±**	**SD**	**F**	**P-value**
GI	104	-	116	110.000	±	6.000	31.200	<0.001
GII	106	-	112	109.333	±	3.055		
GIII	106	-	114	110.000	±	4.000		
G IV	115	-	120	117.333	±	2.517		
G V	120	-	125	122.667	±	2.517		
GVI	140	-	150	145.333	±	5.033		
GVII	105	-	110	108.000	±	2.646		
G VIII	108	-	111	109.667	±	1.528		
G IX	107	-	115	110.667	±	4.041		

TUKEY'S Test

	GI	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**G VII**	**G VIII**
GII	1.000							
GIII	1.000	1.000						
GIV	0.335	0.241	0.335					
GV	0.013 (s)	0.008 (s)	0.013 (s)	0.709				
GVI	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)			
GVII	0.999	1.000	0.999	0.114	0.003 (s)	<0.001 (HS)		
GVIII	1.000	1.000	1.000	0.285	0.011 (s)	<0.001 (HS)	1.000	
GIX	1.000	1.000	1.000	0.450	0.021 (s)	<0.001 (HS)	0.992	1.000

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

Also, beta carotene might play a protective role as there was a significant and highly significant statistical decrease in the AST activity in the protected groups (G VII, VIII, and IX) compared to the Tio2treated groups (GV and VI).

The current study revealed a non-significant statistical difference ($P > 0.05$) among the studied groups as regards ALT level **(table 2).**

After 60 days of the study

Statistical analysis revealed a significant statistical difference ($P < 0.05$) in the AST level mean values between the studied groups. There was a significant statistical increase in AST levels in the 50 and 70 mg/kg treated TiO2 NPs in comparison to the control groups (GI, II, and III), as shown in **table 3**.

As regards ALT level, there was a statistically significant difference ($P < 0.05$) among the studied groups. **Table 4** shows that there was a statistically significant increase in ALT levels in the 30, 50, and 70 mg/kg TiO2 NPs groups compared to the control groups (GI and II).

Table (2): One way ANOVA statistical analysis of ALT after 30 days among comparative groups:

Groups	ALT After 30 Days						ANOVA	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
Group I	40	-	50	44.667	±	5.033	0.358	0.929
Group II	44	-	50	46.667	±	3.055		
Group III	40	-	50	44.000	±	5.292		
Group IV	40	-	50	45.000	±	5.000		
Group V	43	-	50	47.000	±	3.606		
Group VI	44	-	50	47.000	±	3.000		
Group VII	40	-	48	44.333	±	4.041		
Group VIII	46	-	50	47.333	±	2.309		
Group IX	45	-	50	47.000	±	2.646		

TUKEY'S Test

	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
II	0.999							
III	1.000	0.994						
IV	1.000	1.000	1.000					
V	0.998	1.000	0.987	0.999				
VI	0.998	1.000	0.987	0.999	1.000			
VII	1.000	0.998	1.000	1.000	0.994	0.994		
VIII	0.994	1.000	0.976	0.998	1.000	1.000	0.987	
IX	0.998	1.000	0.987	0.999	1.000	1.000	0.994	1.000

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

Table (3): One way ANOVA statistical analysis of AST after 60 days among comparative groups:

Groups	AST after 60 days						ANOVA	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
GI	120	-	157	139.000	±	18.520	5.181	0.001
GII	125	-	157	145.500	±	14.434		(s)
GIII	127	-	146	141.250	±	9.500		
GIV	143	-	203	172.750	±	25.695		
GV	165	-	211	186.400	±	19.204		
GVI	170	-	202	190.667	±	17.926		
GVII	138	-	170	149.500	±	14.640		
GVIII	160	-	170	165.333	±	5.033		
GIX	165	-	168	166.333	±	1.528		

TUKEY'S Test

	GI	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**GVII**	**GVIII**
GII	1.000							
GIII	1.000	1.000						
GIV	0.196	0.346	0.189					
GV	0.013 (s)	0.023 (s)	0.010 (s)	0.937				
GVI	0.017 (s)	0.030 (s)	0.014 (s)	0.871	1.000			
GVII	0.994	1.000	0.998	0.547	0.053	0.061		
GVIII	0.571	0.799	0.599	0.999	0.700	0.618	0.930	
GIX	0.524	0.756	0.549	1.000	0.749	0.665	0.905	1.000

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

Table (4): One way ANOVA statistical analysis of ALT after 60 days among comparative groups:

Groups	ALT after 60 days						ANOVA	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
GI	44	-	53	49.000	±	4.583	4.127	0.003
GII	41	-	60	50.250	±	8.770		(s)
GIII	45	-	68	59.750	±	10.905		
GIV	60	-	75	67.250	±	6.602		
GV	60	-	75	68.000	±	5.701		
GVI	70	-	71	70.333	±	0.577		
GVII	53	-	64	60.000	±	4.830		
GVIII	55	-	65	60.000	±	5.000		
GXI	54	-	70	61.333	±	8.083		

TUKEY'S Test

	GI	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**GVII**	**GVIII**
GII	1.000							
GIII	0.532	0.590						
GIV	0.043(s)	0.041 (s)	0.827					
GV	0.022(s)	0.019 (s)	0.692	1.000				
GVI	0.021(s)	0.020 (s)	0.552	1.000	1.000			
GVII	0.503	0.558	1.000	0.851	0.724	0.582		
GVIII	0.587	0.651	1.000	0.896	0.802	0.660	1.000	
GXI	0.442	0.493	1.000	0.965	0.914	0.797	1.000	1.000

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

Table (5): Paired T-test statistical analysis of AST after 30 and 60 days of the study among comparative groups:

Groups	AST						Differences		Paired Test	
	After 30 Days			**After 60 Days**			**Mean**	**SD**	**T**	**P-value**
GI	110.000	±	6.000	139.000	±	18.520	-29.000	21.932	-2.290	0.149
GII	109.333	±	3.055	145.500	±	14.434	-36.000	14.933	-4.176	0.053
GIII	110.000	±	4.000	141.250	±	9.500	-36.000	4.000	-15.588	0.004(s)
GIV	117.333	±	2.517	172.750	±	25.695	-65.333	21.197	-5.338	0.033(s)
GV	122.667	±	2.517	186.400	±	19.204	-62.667	13.429	-8.083	0.015(s)
GVI	145.333	±	5.033	190.667	±	17.926	-45.333	13.317	-5.896	0.028(s)
GVII	108.000	±	2.646	149.500	±	14.640	-44.667	16.503	-4.688	0.043(s)
GVIII	109.667	±	1.528	165.333	±	5.033	-55.667	4.933	-19.546	0.003(s)
GIX	110.667	±	4.041	166.333	±	1.528	-55.667	5.033	-19.156	0.003(s)

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

Comparison after 30 and 60 days of work:

The findings revealed a statistically significant increase in AST levels at the end of the study period of more than 30 days in groups III, IV, V, VI, VII, VIII, and IX. On the other hand, there was no significant statistical change in groups I and II, as illustrated in **table (5)** and **figure (1)**. As shown in **table (6)** and **figure (2),** there was a significant statistical increase in ALT level at the end of the study more than a 30 day period in groups (I, IV, V, and VI). On the other hand, there was no statistically significant change in groups II, III, VII, VIII, and IX.

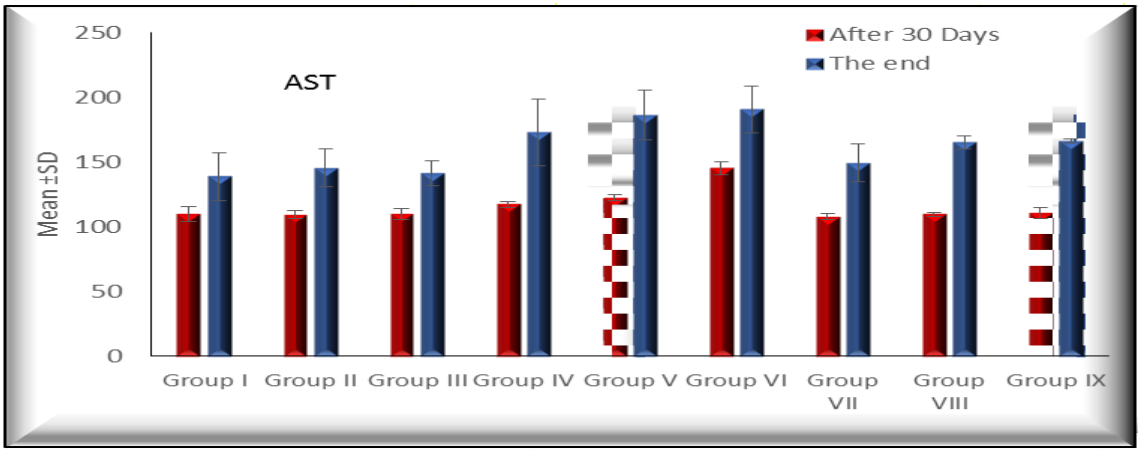

Figure (1): Paired T-test statistical analysis of AST after 30 and 60 days of the study among comparative groups.

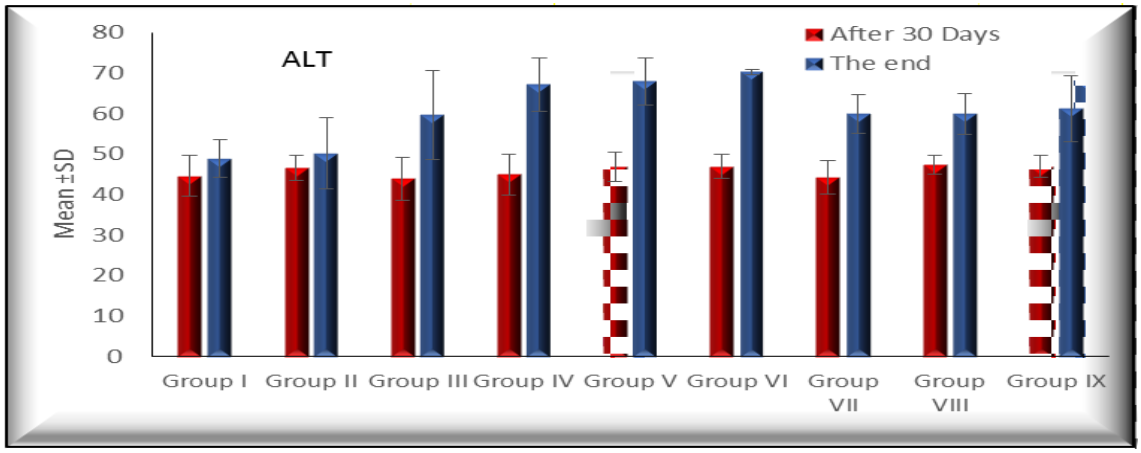

Figure (2): Paired T-test statistical analysis of ALT after 30 and 60 days of the study among comparative groups.

Table (6): Paired T-test statistical analysis of ALT after 30 and 60 days of the study among comparative groups:

Groups	ALT						Differences		Paired Test	
	After 30 Days			**After 60 Days**			**Mean**	**SD**	**T**	**P-value**
GI	44.667	±	5.033	49.000	±	4.583	-4.333	1.528	-4.914	0.039(s)
GII	46.667	±	3.055	50.250	±	8.770	-2.000	10.440	-0.332	0.772
GIII	44.000	±	5.292	59.750	±	10.905	-13.000	16.703	-1.348	0.310
GIV	45.000	±	5.000	67.250	±	6.602	-23.333	2.887	-14.000	0.005(s)
GV	47.000	±	3.606	68.000	±	5.701	-23.000	6.928	-5.750	0.029(s)
GVI	47.000	±	3.000	70.333	±	0.577	-23.333	3.055	-13.229	0.006(s)
GVII	44.333	±	4.041	60.000	±	4.830	-15.000	8.185	-3.174	0.087
GVIII	47.333	±	2.309	60.000	±	5.000	-12.667	7.095	-3.092	0.091
GIX	47.000	±	2.646	61.333	±	8.083	-14.333	10.017	-2.478	0.131

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

Oxidative stress biomarkers in liver tissue:

MDA level:

A highly significant statistical difference was in the MDA level mean values in the liver tissue between the comparative groups, as shown in **table 7.** In the same set of studies, a highly significant elevation was seen in the MDA mean values in the Tio2groups (G IV, V, and VI) compared to the control rats (GI, II, and III). On the other hand, beta carotene might ameliorate these changes as a highly significant reduction was in the MDA mean values in BC groups (GVII, VIII and

IX) in comparison with the Tio2 groups (GIV, V, and VI).

SOD level:

This study revealed that a highly significant difference was in the SOD level mean values in the liver tissue between the comparative groups, as illustrated in **table 8.** Also, a highly significant statistical decrease was seen in the SOD mean values in the Tio2 groups (GIV, V, and VI) in comparison with the control groups (GI, II, and III). On the other hand, beta carotene might improve these changes as a highly significant and significant increase in the SOD mean values in BC groups (GVII, VIII, and IX) Compared to the Tio2 groups (GIV, V and VI).

Table (7): One way ANOVA statistical analysis of MDA among comparative groups at the end of the study:

Groups	MDA						ANOVA	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
GI	1.1	-	1.5	1.333	±	0.208	339.823	<0.001
GII	1.1	-	2.6	1.967	±	0.777		(HS)
GIII	1.4	-	2.4	1.900	±	0.500		
GIV	13.2	-	17.1	15.167	±	1.950		
GV	27.1	-	30.2	28.463	±	1.583		
GVI	51.2	-	60.2	55.733	±	4.500		
G VII	0.6	-	1.6	1.067	±	0.503		
GVIII	0.5	-	1.5	1.067	±	0.513		
GIX	1.8	-	2.8	2.200	±	0.529		

TUKEY'S Test

	GI	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**G VII**	**G VIII**
GII	1.000							
GIII	1.000	1.000						
GIV	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)					
GV	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)				
GVI	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)			
GVII	1.000	0.999	1.000	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)		
GVIII	1.000	0.999	1.000	<0.001 (HS)	<0.001 (HS)	<0.001HS)	1.000	
GIX	0.999	1.000	1.000	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.996	0.996

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

Histopathological findings:

Examination by a light microscope of H & E stained liver sections of control groups (I, II) revealed normal histological appearance **(Figure 3 A, B).** Examination of group III shows hepatic parenchyma that is more or less comparable to the control **(Figure 3 C, D).**

When compared to the control groups, histological alterations, particularly in higher dose TiO2 NPs groups in the shape of numerous vacuolated hepatocytes, congestion in the portal vein and central vein, dilated congested sinusoids, numerous degenerated hepatocytes, peri-portal inflammatory cell infiltration, and some apoptotic cells are seen. Histological observations were improved in protected groups by BC **Figures 4, 5 and 6.**

Table (8): One way ANOVA statistical analysis of SOD among comparative groups at the end of the study:

Groups	SOD						ANOVA	
	Range			**Mean**	**±**	**SD**	**F**	**P-value**
GI	183.26	-	199.56	191.020	±	8.178	25.549	<0.001
GII	190.98	-	193.37	192.463	±	1.295		(HS)
GIII	192.06	-	198.26	194.787	±	3.167		
GIV	156.67	-	170.98	164.793	±	7.349		
GV	159.13	-	170.98	164.057	±	6.172		
GVI	154.37	-	160.09	156.813	±	2.950		
GVII	190.34	-	198.09	194.597	±	3.931		
GVIII	185.67	-	195.08	190.330	±	4.706		
GIX	180.98	-	190.33	187.187	±	5.375		

TUKEY'S Test

	GI	**GII**	**GIII**	**GIV**	**GV**	**GVI**	**GVII**	**G VIII**
GII	1.000							
GIII	0.991	1.000						
GIV	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)					
GV	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	1.000				
GVI	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.640	0.741			
GVII	0.994	1.000	1.000	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)		
GVIII	1.000	1.000	0.975	<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	0.981	
GIX	0.990	0.937	0.693	0.001 (s)	0.001 (s)	<0.001 (HS)	0.719	0.997

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

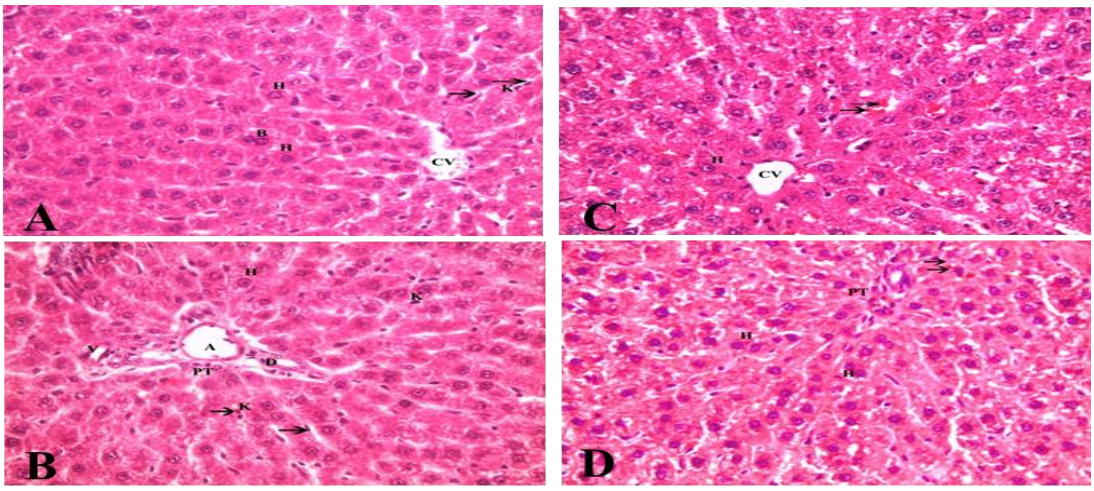

Figure (3): (A) (group I, II control**)** A photomicrograph of a liver section shows the central vein (CV), hepatocytes with vesicular nuclei, and acidophilic cytoplasm. Binucleated cells are observed (B), Note: Blood sinusoids (arrows), Kupffer cells (K). **(B)** (GI, GII), the portal area of a control rat shows the periportal area. The portal tract (PT) contains the portal artery (A), portal vein (V), and portal duct (D). Note: Hepatocytes (H), Kupffer cells (K), and blood sinusoids (arrows). **(C)** (groupIII control**)** showing central vein (CV), hepatocyte with vesicular nuclei, and acidophilic cytoplasm (H), note: congestion in few sinusoids (arrows). **(D)** (GIII) showing portal tract (PT), hepatocyte with vesicular nuclei, and acidophilic cytoplasm (H), note: congestion in a few sinusoids (arrows). **(H & E stain X400).**

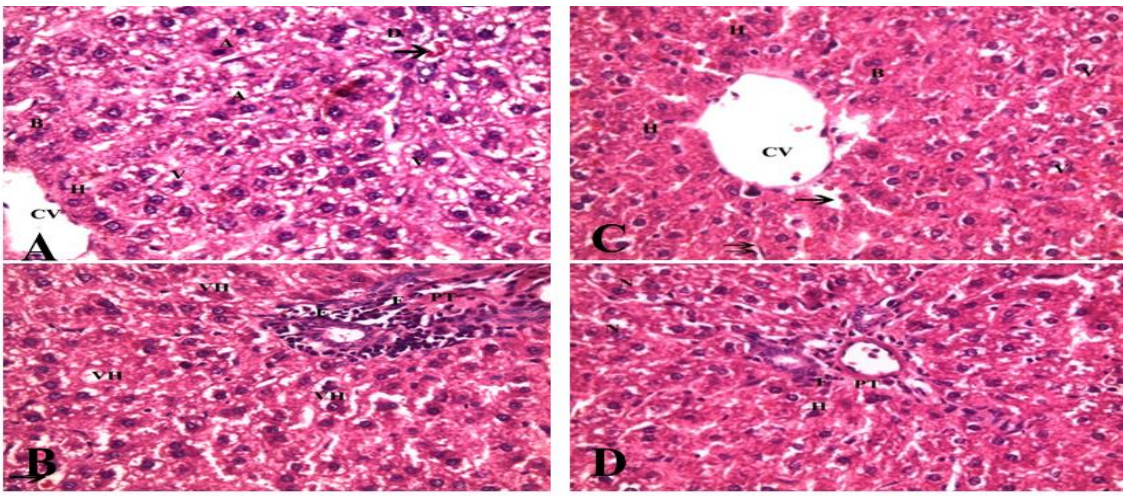

Figure (4): (A) (GIV of 30mg/kg TiO2 NPs) a photomicrograph of a liver section shows numerous vacuolated hepatocytes with pyknotic nuclei (v). Some hepatocytes have pyknotic nuclei and highly acidophilic cytoplasm (A), dilated congested sinusoids (arrow), some hepatocytes appear normal (H), other cells are binucleated (B), others have degenerated (D). **(B)** (GIV) showing periportal inflammatory infiltration (f), many vacuolated hepatocytes (VH), and some dilated, congested sinusoids (arrow). Note: portal tract (PT). **(C)** (GVII of 30mg/kg TiO2 NPs protected by BC**)** showing central vein (CV), majority of centrilobular cells are with a vesicular nucleus and acidophilic cytoplasm (H), some cells are binucleated (B) few cells have vacuolated cytoplasm, sinusoids appear more or less normal (double arrow) some sinusoids appear dilated (single arrow). **(D)** (GVII) showing decreased inflammatory cell infiltrations compared to the previous group (F), some hepatocytes appeared with vesicular nucleus (N), others with vacuolated cytoplasm (H), Note: Portal Tract (PT). **(H & E stain X400).**

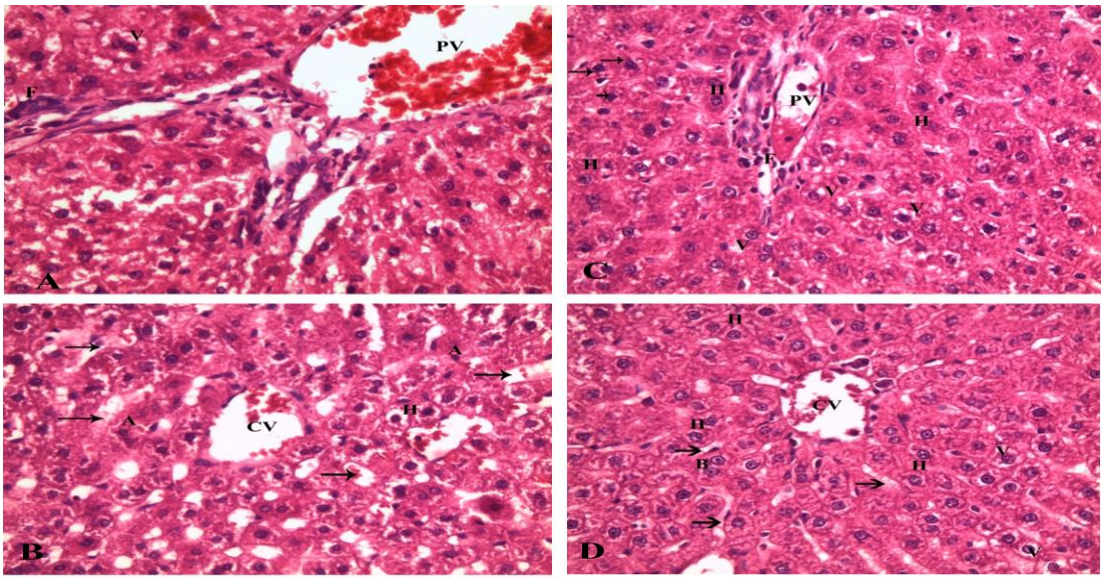

Figure (5): (A) (GV of 50mg/kg TiO2 NPs) A photomicrograph of a liver section shows congestion in the portal vein (PV), periportal inflammatory cell infiltration (F), and vacuolated hepatocyte (V). **(B)** (GV) showing congestion of the central vein (CV) dilated congested sinusoid (arrow) numerous apoptotic hepatocytes (A), some vacuolated hepatocyte (H). **(C)** (GVIII of 50mg/kg TiO2 NPs protected by BC) showing decreased congestion and inflammatory cells compared to the previous group (F). Most hepatocytes have a normal appearance (H), but there are still some apoptotic (arrow) and vacuolated cells (V). **(D)** (GVIII) shows most centrilobular hepatocytes appear normal with a vesicular nucleus and acidophilic cytoplasm (H), binucleated cells are frequently seen (B), and there is no congestion in sinusoids compared to the previous group. Some vacuolated hepatocytes are seen (V). Note: central vein (CV). **(H & E stain X400).**

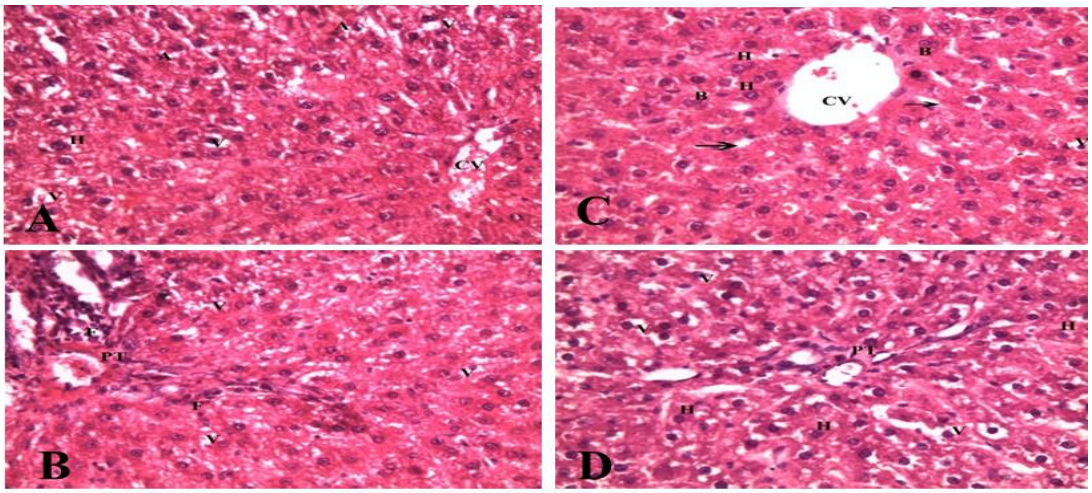

Figure (6): (A) (GVI of 70mg/kg TiO2 NPs) A photomicrograph of a liver section shows congestion in the central vein (CV), numerous vacuolated hepatocytes (V), some apoptotic cells are seen (A), some cells are degenerated (V), others are vacuolated (H). **(B)** (GVI) shows the periportal inflammatory cell infiltration (F), and numerous degenerated hepatocytes (V). **(C)** (GIX of 70mg/kg TiO2 NPs protected by BC) showing central vein (CV), hepatocyte with vesicular nuclei and acidophilic cytoplasm (H), binucleated cells are seen (B), sinusoids appear less congested compared to the previous group (arrow), some degenerated hepatocytes are seen (V). **(D)** (GIX) showing decreased inflammatory cells compared to the previous group, numerous vacuolated hepatocytes appear with vesicular nuclei and acidophilic cytoplasm (H), Note: portal tract (PT). **(H & E stain X400).**

DISCUSSION

The small size and huge surface area of nanoparticles (NPs) with an active group characterize them. These characteristics increase their chemical reactivity, allowing them to enter live cells. Some scientists and organizations have discussed the effects of NPs on humans and the environment **(Warheit et al., 2007).**

The liver is frequently the major target of xenobiotic toxicity. The detoxification of hazardous chemicals that enter the body is known to take place mostly in the liver. As a result, the liver can be used as an indicator of xenobiotic toxicity **(Wilson et al., 2016).**

The liver is a large organ that detoxifies dangerous substances in circulation. A wide range of substances, metals, and drugs have been demonstrated to alter the liver's structural and functional integrity. The extent of liver damage caused by TiO2 NPs is determined by the material's characteristics, dose, route, and length of exposure **(Vasantharaja et al., 2015)**.

The liver was found to be the primary target organ for Tio2 NP accumulation, followed by the spleen and lung. The liver could be the most vulnerable organ to the toxicity caused by ingested TiO2 NPs **(Geraets et al., 2014; Chen et al., 2019).**

After oral ingestion, **Wang et al. (2007)** mentioned that the aggregation of TiO2 nanoparticles occurs in the liver, spleen, kidneys, and lungs.

The liver is the primary location for biological metabolism, and it guards the body against external materials and xenobiotics, excretes the materials into bile, and, subsequently, the biliary organism is similarly subjected to NPs. Previous research has demonstrated that various toxic substances with different mechanisms, including induction of alcohol degeneration, membrane lipid peroxidation, suppression of protein formation, alteration of calcium homeostasis, and stimulation of receptor enzymes, induce the destruction of the liver cells (**Oberdorster et al., 2005).**

Previous research also found that the TiO2 NPs retention halftime in vivo was extended due to their tiny size and it was very difficult to clear on ingestion of 50 mg/kg and 100 mg/kg TiO2 NPs (**Vasantharaja et al., 2015).**

The present work was to examine liver biochemical and histological changes after ingestion of different oral TiO2 NPs doses and if BC has a protective role. BC, one of the carotenoids, is an organic substance abundant in plants and is the main Vitamin A precursor with multiple immune and antioxidant features. Beta-carotene has an antioxidant effect by diminishing singlet oxygen, peroxide radicals scavenging and immediately interacting with peroxy radicals, so protecting membrane lipids from free radical damage **(Bast et al., 1998).**

In our study, the biochemical parameters (AST, ALT) were measured at 30 days (midway monitoring) and at the end of the experiment **(Committee for Human Medicinal Products (CHMP), 2010).**

In the current study, it was found that there was a significant statistical increase in AST levels in the 50 and 70 mg/kg treated TiO2 NPs in comparison with the control groups, and there was a significant statistical elevation in ALT levels in the 30, 50, and 70 mg/kg treated TiO2 NPs groups in comparison with the control groups at the study end.

The results of the current study are consistent with those recorded by **Vasantharaja et al. (2015),** who revealed a

significant difference between AST and ALT levels within rats who received TiO2 NPs.

Also, **Wang et al. (2007)** mentioned that the significant variations of serum biochemical ALT/AST and LDH of the liver showing the obvious hepatotoxic effects were caused by acute TiO2 NPs oral ingestion (25 and 80 nm).

In harmony with our findings, **Vasantharaja et al. (2019)** mentioned that the elevation of levels of AST, ALT, ALP, and LDH in the liver visibly reveals that the liver is largely susceptible to the harmful effects of TiO2 NPs.

Similarly, **Shakeel et al. (2016)** showed that subcutaneous injection of TiO2 NPs induced significant elevated AST and ALT levels after exposure duration of 28 days in rats.

Vasantharaja et al. (2019) mentioned that discharge of specific marker enzymes from the liver into the blood has been estimated to be a sign of hepatic dysfunction as well as liver cell injury. An elevation in the activity of these enzymes in hepatic tissue indicates liver cell degeneration or injury and hence induces severe liver malfunction. As a result, cellular damage produced by hazardous chemicals is commonly associated with an increase in the permeability of the cell membrane **(Thangapandiyan et al., 2003).**

In toxicological terms, alterations in enzyme activity cause further damage to cells and organs. **(Casillas et al., 1983).**

For instance, AST, ALT, and ALP are excellent signals for liver problems when the animals are subjected to the toxicity of heavy metals **(Vinodhini et al., 2008).**

These enzymes are used to detect liver cell necrosis and as indicators of acute hepatic injury **(Jani et al., 1994; Wang et al., 2009; and Shakeel et al., 2016).**

On the contrary, **Jia et al. (2017)** studied the hepatotoxic effects of TiO2 NPs on mice that were administrated 5, 10, 50, 100, 150, and 200 mg/kg TiO2 NPs for an exposure duration of 14 days by intra-peritoneal injection and found that low TiO2 NPs doses (5, 10, 50, and 100 mg/kg) did not alter any blood biochemistry index, while high TiO2 NPs doses (150 to 200 mg/kg) increased levels of liver function biomarkers.

According to the current study, BC might play a protective role as there was a significant and highly significant statistical decrease in the AST activity in the protected groups by BC compared to the Tio2treated groups after 30 days of the study.

The carotenoid BC is the most researched. BC is converted to vitamin A (retinol), which the body can utilize in many ways. Or it can protect cells from free radical damage by acting as an antioxidant **(Orazizadeh et al., 2014**).

The current work showed a highly significant statistical increase in the MDA mean values of the liver in the Tio2 NPs treated groups compared to the control groups and a highly significant statistical reduction was seen in the SOD mean values of the liver in the Tio2 NPs treated groups compared to the control groups.

The present study was reinforced by the results of **Liang et al. (2009),** who revealed the occurrence of oxidative stress and lipid peroxidation in the liver and kidneys after TiO2 NPs exposure. This was caused by the reduction of SOD activity and glutathione peroxidase and the elevation of MDA levels.

In harmony with us a study done by **Sherif et al. (2019),** rats treated with TiO2NPs

induced a significant increase in liver content of the lipid peroxidation marker, MDA, and a significant decrease in GSH level and the antioxidant activities of SOD and CAT as compared to control groups.

Our results were consistent with those recorded by **Jia et al. (2017),** who illustrated that administration of mice with 10 or 50 mg/kg TiO2 NPs for 60 days caused significant increases in the levels of MDA and decreases in the mRNA levels of SOD in the livers of mice.

The term oxidative stress refers to a severe imbalance between antioxidants and the production of reactive oxygen species, or ROS, which are important for a wide range of pathological situations such as cardiovascular disease, cancer, diabetes mellitus, inflammation, and enzymes in the cytoplasm of hepatic cells that are many times higher than those in extracellular fluid. The amount of these enzymes in the blood increases when the liver cells and membrane are destroyed or die, and this increase indicates liver damage **(Sies, 1997; Dambach et al., 2005).**

One of the most vital elements of NP toxicity is oxidative stress. TiO2 NPs promote the creation of ROS, which is one of the most important harmful processes observed in organisms. In experimental animals, liver damage related to oxidative stress caused by TiO2 NPs could be investigated by identifying lipid peroxidation indicators (LPO). TiO2 nanoparticles cause hepatic oxidative stress by raising LPO induced by free radicals **(Nel et al., 2006; Ma et al., 2010; Meena and Paulraj, 2012).**

Oxidative stress caused by ROS is considered a key factor in intracellular organ injury induced by TiO2 NP **(Long et al., 2007; Ma et al., 2010; Hou et al., 2019)**. Also, **Rajakumar et al. (2012)** proved the same results in hepatotoxicity caused by TiO2 NPs.

Molecular oxygen oxidation to form superoxide radicals may cause an increase in LPO levels. This reaction also produces H2O2, which causes the membrane unsaturated fatty acids peroxidation, resulting in the creation of malondialdehyde. The hydroxyl radical can cause LPO, a chain reaction of free radicals that causes membrane structure and function to be lost **(Kale et al., 1999; Sharma et al., 2014).**

Furthermore, LPO induces compromised functions, the structural integrity of the membrane, decreased volatility of the membrane, and inhibition of some membrane-bound enzymes, which may result in reduced ATP synthesis and elevated ROS formation in the liver **(Liang et al., 2009).**

Superoxide dismutase is a powerful antioxidant enzyme. That reduces oxidative damage. It is an oxy radical-handling enzyme that catalyzes the dis- mutation of the extremely reactive superoxide radical (O2−) to O2 and H2O2. Cellular oxidative destruction of cell membrane lipids is primarily utilized as a measure of oxidative stress in the recognition of lipid peroxidation. MDA is largely utilized as an indication of LPO **(Liang et al., 2009; Sharma et al., 2014).**

Malondialdehyde is one of the final products of unsaturated fatty acid oxidation in the cells and free radicals elevate the generation of MDA **(Negre-Salvayre et al., 2008).**

As a result, the liver could be a suitable target organ for TiO2 NPs; conversely, TiO2 nanoparticles' surface area increases quickly as particle diameter decreases. The huge surface area appears to be a cause of reactive oxygen species **(Wilson et al., 2002; Liang et al., 2009).**

In the current work, BC might play a protective role as a highly significant statistical reduction was in the MDA mean values in BC groups compared with the Tio2treated groups.

And a highly significant and significant statistical increase ($p < 0.001$) was in the SOD mean values in the BC groups compared to the Tio2 groups.

It is well recognized that BC has an antioxidant effect **(Iyama et al., 1996; Schweiggert et al., 2014; Orazizadeh et al., 2015).** Moreover, **Orazizadeh et al. (2014)** revealed that the spermatogenesis defects were improved by BC in mice that received TiO2 NPs. It has also been shown that beta carotene reduces oxidative stress and inhibits ethanol-caused cell death by preventing the expression of caspase-9 and caspase-3 **(Peng et al., 2010)**. Therefore, in our study, administration of BC alone led to an increase in antioxidant enzyme activities as noticed in studies carried out by **Bestwick and Milne, (2000) and Yang et al., (2004).**

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

BC's protective action could also be attributed to its antioxidant properties **(Peters et al., 2004; Matos et al., 2006; Vardi et al., 2009).**

Lin et al. (2013) reported that BC successfully protected mouse embryos against nicotine-induced teratogenicity via anti-oxidative, anti-apoptotic, and anti-inflammatory actions.

BC has numerous pharmacological and biological benefits, including antioxidant, radio-protective, cardiovascular protection, and anti-epilepsy properties **(Schweiggert et al., 2014).**

According to the current study and when compared to the control groups, histological changes, particularly in high dose TiO2 NPs groups in the shape of numerous vacuolated hepatocytes, congestion in the portal vein and central vein dilated congested sinusoids, numerous degenerated hepatocytes, peri-portal inflammatory cell infiltration, and some apoptotic cells are seen. Histological observations were improved in protected groups by BC.

The present results were similar to those published by **Donaldson et al. (2001), Duan et al. (2010), and Alarifi et al. (2013),** who mentioned that nanoparticles may alter the permeability of hepatocyte cell membranes and the endothelial lining of blood vessels, causing red cell and platelet sequestration, which could compromise circulation and increase thrombosis. The leakage of lysosomal hydrolytic enzymes that cause cytoplasmic degeneration can cause cellular degeneration **(Wang *et al.*, 2007).**

Previous research by **Abu-Dief et al. (2015) that** examined the histological changes of TiO2 NPs in the liver of adult male albino rats reported that in the treated group, histological changes in the morphology of the liver were seen. Both the portal and blood sinusoids experienced vasodilation and congestion. Some hepatocytes had ballooned and vacuolated cytoplasm with nuclear alterations, while others had hazy vacuolated cytoplasm, ambiguous cell borders, and a tiny condensed nucleus, indicating early signs of apoptosis.

Johar et al. (2004) and Giray et al. (2011) mentioned that TiO2 NPs may react with

proteins and enzymes in the interstitial hepatic tissue, inhibiting the antioxidant protection mechanism and causing ROS production, which may mimic an inflammatory response.

Also, research by **Wang *et al.* (2007)** on rats found that TiO2 with a smaller size (5 – 10 nm) would be easier to enter liver cells and create an inflammatory process by producing some cytokines.

Our study revealed the protection of hepatocytes by beta carotene. These results are in agreement with **Yaqub et al. (2008),** which revealed NAC and BC either alone or in combination may have promising prophylactic or therapeutic activities in inflammatory liver diseases and also revealed recovery to normal architecture from necrosis.

Antioxidants can counteract the damage stemming from excessive ROS at the cellular level through the activation of some enzymatic or non-enzymatic pathways **Chang et al., (2005).**

Also, **Sains Malaysiana et al. (2022)** mentioned that through its antioxidant effect, oral intake of BC improves the hepatic structure and metabolism in mice (*mus musculus*) subjected to chronic ethanol use.

Mousah et al. (2016) support our study by clearing the protecting function of l-carnitine, vitamin A, and atorvastatin on acetaminophen-induced liver toxicity in rats.The study reported that the oral administration of l-carnitine, vitamin A, and atorvastatin daily for 7 days before and after the acetaminophen exposure hugely ameliorated acetaminophen-induced hepatic damage as illustrated in histopathology examination and biochemical parameters.

CONCLUSION

We can conclude that sub-chronic oral exposure to Tio2 NPs caused oxidative stress that produced hepatotoxic effects in the liver of the rats. According to histological and pathological findings, BC may protect against oxidative stress, so it can prevent pathological and biochemical changes expected by TiO2 NPs. BC has a hepato-protective and potential antioxidant role against TiO2 NPs' toxic effects.

RECOMMENDATIONS

- Raising public awareness about the proper handling of TiO2 NPs materials.
- Further studies about the usefulness of BC are recommended.
- Epidemiologic research will help to understand health outcomes associated with potentially hazardous substances. This research will serve as the foundation for quantitative risk projections to set safe levels for human health.

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الملخص العربي

الآثار السامة لجزيئات ثاني أكسيد التيتانيوم النانوية في كبد ذكورالجرذان الويستار البالغة والدور الوقائي المحتمل للبيتا كاروتين

مروة أحمد حسب النبى [١] سهير على محمد [١] هند محمد أحمد [١] هدى محمد السيد [٢] مروة شعبان هاشم [٣] أحمد محمد سعيد [١]

قسم الطب الشرعى والسموم الاكلينيكية [١], قسم الهستولوجى [٢] , قسم الكيمياء الحيوية [٣], كلية الطب البشرى , جامعة سوهاج , مصر

المقدمة: أصبحت جزيئات ثاني أكسيد التيتانيم النانوية مهمة للغاية لتطبيقاتها المختلفةمثل التعقيم ، وإزالة الصدأ و الصبغة.

الهدف: تم تصميم هذه الدراسة للتحقيق في السمية الكبدية الناتجة عن التعرض الفموي شبه المزمن لجزيئات ثانى أكسيد التيتانيم الناناوية في ذكور فئران ويستار البالغة ولتقييم التأثير الوقائي المحتمل لبيتا كاروتين.

طريقة البحث: تم تقسيم تسعين ذكور فئران ويستار البالغة إلى تسع مجموعات متساوية.

1- المجموعة الأولى (I) المراقبة السلبية.

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

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

4- المجموعة الرابعة (IV) مجموعةالجرعة المنخفضة والتي تم إعطاؤها ٣٠ مجم / كجم / يوم من الجزيئات الناناوية لثانى أكسيد التيتانيم.

٥- المجموعة الخامسة(V) مجموعة الجرعة المتوسطة والتي تم إعطاؤها ٥٠ مجم / كجم / يوم من الجزيئات الناناوية لثانى أكسيد التيتانيم.

٦- المجموعة السادسة (VI) مجموعة الجرعة المرتفعة والتي تم إعطاؤها ٧٠ مجم / كجم / يوم من من الجزيئات الناناوية لثانى أكسيد التيتانيم.

٧- المجموعات المحمية المجموعات السابعة والثامنة والتاسعة IX, VIII, VII والتى تم إعطاؤها قبلا البيتا كاروتين بجرعة (١٠ مجم / كجم / يوم) ثم ٣٠ ، ٥٠ و ٧٠ مجم / كجم / يوم على الترتيب من الجزيئات النانوية لثانى أكسيد التيتانيم لمدة ٦٠ يومًا عن طريق الفم. تم تقدير مستوى انزيمات الكبد ASTو ALT بعد ٣٠ يومًا وفي نهاية التجربة. علاوة على ذلك ، تم تقدير علامات الإجهاد التأكسدي في أنسجة الكبد بما في ذلك المالوندای ألدهايد و أنزيم السوبر أكسيد ديسميوتيز . كما تم إجراء فحص الأنسجة المرضية لأنسجة الكبد بالمجهر الضوئي.

النتائج: أظهرت النتائج زيادة ذات دلالة إحصائية في مستويات الانزيمات الكبدية AST و ALT في المجموعات المعالجة بالجزيئات النانوية لثانى أكسيد التيتانيم بالمقارنة مع مجموعة التحكم في نهاية الدراسة. كان هناك انخفاض إحصائي كبير في نشاط AST في المجموعات المحمية بواسطة البيتاكاروتين مقارنة بـ ٥٠ و ٧٠ مجم / كجم من مجموعات الجزيئات النانوية لثانى أكسيد التيتانيم المعالجة بعد ٣٠ يومًا من الدراسة. كما تسببت الجزيئات النانوية لثانى أكسيد التيتانيم في ارتفاع معنوي لالمالوندای ألدهايد وانخفاض معنوي في إنزيم مضادات الأكسدة سوبرأكسيد ديسميوتيز في أنسجة الكبد التي تم تحسينها عن طريق إعطاء البيتاكاروتين. أيضا ، تم الكشف عن تغيرات نسيجية مرضية كبيرة في شكل العديد من خلايا الكبد المفرغة ، واحتقان الوريد البابي ، واحتقان الجيوب الدموية المتوسعة ، والعديد من خلايا الكبد المتدهورة وتسلل الخلايا الالتهابية المحيطة بالمنطقةالبابية. تم تحسين هذه التغييرات بواسطة البيتاكاروتين.

الاستنتاج: بناء على ذلك نستنتج ان التعرض الفموي شبه المزمن لجزيئات النانوية لثانى أكسيد التيتانيم تسبب في الإجهاد التأكسدي الذي ينتج سمية كبدية في كبد الفئران وان إعطاء البيتاكاروتين له دور مضاد للأكسدة ومفيد للحماية من السمية الكبديةالتى تسببه الجزيئات النانوية. وتوصي هذه الدراسة بزيادة الوعي العام حول التعامل السليم مع مادة الجزيئات النانوية لثانى أكسيد التيتانيم وإجراء مزيد من الدراسات حول الفوائد الأخرى للبيتاكاروتين.