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Protective Effect of Black Berry and Selenium on Titanium Dioxide Nanoparticles (TiO_2NPS) Induced Oxidative Stress, Hepatotoxicity, Cardiotoxicity, Genotoxicity and Histopathological Changes in Male Rats

Hounida A Attia[1,3] and Reham Z Hamza[1,2*]

[1]*Biology Department, Faculty of Science, Taif University, Taif 888, Saudi Arabia*
[2]*Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt*
[3]*Unit of Physiology and Biochemistry of Plant Responses to Abiotic Constraints, Biology Department, Faculty of Science, Tunis-El Manar University, Tunisia*

ABSTRACT

Titanium dioxide nanoparticles (TiO_2NPs) are excessively used and represent one of the top five most commonly used nanoparticles worldwide. Recently, various studies referred to their toxic potential on various organs using different treatment route. The present study was carried out to evaluate the potential protective role of blackberry and selenium (Se) against (TiO_2NPs)-induced oxidative stress and histological changes in liver and heart tissues of rats. Rats were divided into seven groups according to the treatment into control, Titanium dioxide treated group (TiO_2NPs) (500 mg/kg b.w.), Black berry (Bb) treated group (1.6 g/kg body), Selenium (Se) (0.5 mg/kg;), (TiO_2NPs)plus Bb and (TiO_2NPs)plus Se and the last group treated with combination of (TiO_2NPs) plus Bb and Se. All the Animals were treated orally for 30 successive days. (TiO_2NPs) increased alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT) activities and changed the levels of lipid profile as well as creatinine and uric acid levels. It marked decreased hepatic reduced glutathione (GSH) levels, antioxidant enzymes activities and increased the levels of lipid peroxidation, C-reactive protein and interleukins. Bb and Se prevented the (TiO_2NPs)-induced liver and heart injury as indicated by improving all the parameters previously illustrated. Histopathological results confirm the biochemical finding and the ameliorating effect of both Bb and Se on liver toxicities and heart failure. In conclusion, co-treatment of either Bb or Se possessed different protective mechanisms against (TiO_2NPs)-induced liver toxicity and heart damage. So, the intake of (TiO_2NPs) should be restricted due to its increased toxicity.

Keywords: Titanium dioxide (TiO_2NPs); Black berry (Bb); Selenium (Se); Antioxidant enzymes; Oxidative biomarkers; Hepatic effect; Heart damage

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INTRODUCTION

Nanotechnology means a new set of technologies that are used to develop nanoscale structures and devices with one dimension size 1–100 nm with special properties utilized in commercial applications [1]. Nanotechnology promises a great contribution to humanity, but without appropriate concept of risks, public confidence in this expanding field will diminish [2]. Concern has been raised about the effect of nanoparticles exposure on human health [3]. Titanium dioxide (TiO_2) is a widely used industrial nanomaterial that was used in various products including sunscreens and paints [4]. Therefore, Nano-TiO_2 (TiO_2NPs) risk assessment should be an integral part nowadays in our modern society.

Human exposure to TiO_2NPs may occur during both manufacturing and use. The major routes of TiO_2NP exposure that have toxicological relevance in the workplace are inhalation and dermal exposure. Oral exposure, as a nonmajor route, may occur from toothpaste, food colorants, and nutritional supplements that contain TiO_2NPs.

In a recent study by Weir et al. [5], they found that candies, sweets, and chewing gums contained the highest amount of TiO_2. One of the most important natural diets with antioxidant properties is blackberries. Berry fruits, wild or cultivated, have been proven to be a traditional and rich source of bioactive compounds, possessing important biological activities such as flavonoids (anthocyanin), some minerals (Na, K, Ca, Se, Zn and P), vitamins (vitamin A, complex, C and E), phenolic acids (galic, p-coumaric, caffeic, ferulic) and phenolic polymers (ellagic acids) [6]. The antioxidant capacity of these berries was related to their constituents, particularly total phenolics and anthocyanins [7]. These contents have antioxidants and can improve immunity, playing an antagonistic role of protective agent against toxic substances [8]. On the other hand, the induced oxidative stress and the alterations in antioxidant systems were normalized by the oral administration of blackberry juice (BBJ). Therefore, it can be concluded that blackberry administration could minimize the toxic effects of F indicating its free radical-scavenging and potent antioxidant activities [9].

Micronutrients are dietary minerals required by the human body in a very small quantity. They probably interact with xenobiotic at several sites like, during absorption and excretion, transport of metals in the body, binding to target proteins, metabolism and sequestration of toxic metals, and oxidative stress [10]. Besides this, they may also serve as required prosthetic groups in active sites or as co-enzymes for indispensable met-alloenzymes. Selenium (Se) is an essential trace element and a key component of important enzymes, including the antioxidant glutathione peroxidase (GPx) and the iodothyronine deiodinases [11]. A number of selenoenzymes play key roles in protecting against oxidative damage [12]. Accumulating evidence suggests that many selenoproteins, which contain Se in the form of amino acid selenocysteine, have important enzymatic functions associated with antioxidant activity [13]. Selenium is an essential dietary trace element and is always of research interest required for the maintenance of male fertility by way of testosterone biosynthesis, formation and normal development of spermatozoa [14]. There are many cellular enzymes were leakage into the serum due to change in the cell membrane permeability. Therefore, measuring serum activities of marker enzymes as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT) are very important in clinical diagnos is to assess the toxicity of any chemical on tissue [15]. Oxidative damage in the tissue takes place when there is an imbalance between prooxidants and antioxidants of the cell [16]. The level of lipid peroxidation is taken as evidence for oxidative stress [17].

MATERIALS AND METHODS

TiO_2NPs and their Characterizations

The TiO_2NPs used in this study were a mixture of rutile and anatase forms purchased from Sigma Chemical Co., (St. Louis, MO, USA) in the form of odorless and white powder in the nanoscale range <100 nm using Brunauer-Emmett-Teller (BET)method and <50 nm using X-ray diffraction method with a purity of 99.5% and CAS number 13463-67-7. TiO_2NPs were ultrasonicated in deionized distilled water using the biologics ultrasonic homogenizer (Model 150VT) immediately prior to characterization and administration and the pH value of TiO2NPs suspensions was 6.8 and characterized using X-ray diffraction (XRD) to identify the crystal phase and the average crystallite size. Indeed, the particle size and morphology of TiO2NPs suspensions were detected using transmission electron microscopy (TEM) and the dispersion and aggregation status of these nanoparticles in water were determined by the dynamic light scattering (DLS) method using particle size distribution and zeta potential analyzer (Zeta sizer Nano ZS90, Malven Instruments, UK).

Animal Model

Animal experiments were carried out after following the European community Directive (86/609/EEC) and national rules on animal care that were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. The experimental animals were healthy; inbreed adult male Wistar albino rats, weighing approximately 150-200 g (12 weeks of age). The animals were maintained under standard laboratory conditions and were allowed to have food and water ad libitum (standard rat feed pellets). All the rats were housed under the condition of controlled temperature 26 ± 2°C with 12 h light and 12 h dark exposure.

Experimental Design

The Wistar rats were divided into seven groups (n=8). Group I was the control animals which were administrated distilled water as a vehicle. Group II was treated with titanium dioxide nanoparticles (Tio2NPs) (500 mg/kg) and

group III was treated Black berry (1.6 g/kg BW). Group IV was treated with selenium (0.5 mg/kg). Groups V and VI were treated with Tio_2NPs + Bb and Tio_2NPs + Se, respectively and the last group treated with combination of Tio_2NPs + Bb +Se. All the Animals were treated I.P for 30 successive days.

Sample Collection

The blood samples were collected from fasted animals overnight by retro-orbital puncture using blood capillary under ether anesthesia. Samples were incubated at room temperature for 10 minutes and centrifuged at 3000 r.p.m for 20 min. Serum was collected and kept at –80°C until biochemical investigation.

Preparation of Tissue Homogenates for Measurement of Redox State

Liver tissues (0.23 g) were used for the analysis of oxidative stress and antioxidant parameters. Tissues were perfused with a 50 mM of sodium phosphate buffer (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.4), 0.25 M sucrose and 0.1 mM ethylene diamine tetra-acetic acid (EDTA). Then, tissues were homogenized in 5 mL cold buffer/g tissue using a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 10,000 xg for 20 min at 4ºC for estimating enzymatic assays and was centrifuged at 2500 xg for lipid peroxidation level, and the resultant supernatant transferred into Eppendorf tubes that preserved in a deep freeze until being used.

Hepatic and Renal Functions Determination

Serum lactic dehydrogenase (LDH) activity (EC. 1.1.1.27) was determined by the method of King [18]. Aspartate and alanine aminotransferases (EC. 2.6.1.1, EC. 2.6.1.2) were assayed by the Reitman and Frankel [19]. Alkaline phosphatase (ALP) activity (EC. 3.1.3.1) was estimated according to Young et al. [20]. γ-GT activity (EC. 2.3.2.2) was determined by Orlowski and Meister [21]. Albumin level was determined according to a method of Bowers and Wong [22]. The total protein content was estimated by the method of Bradford [23]. The serum total cholesterol (TC) and triglycerides (TG) were determined by the method of Carr et al. [24]. Methods of Warnick et al. [25] was used to determine the high-density lipoprotein–cholesterol (HDL–c). Serum low-density lipoprotein–cholesterol (LDL–c) level was calculated according to Friedewald [26] formula: LDL-c = ¼ Total cholesterol levels – (Triglyceride concentration/5) – HDL-c concentration while VLDL-c = triglyceride/5. Levels of creatinine and uric acid were determined according to the methods of Fossati et al. [27], respectively. The level of urea was estimated according to the method of Patton and Crouch [28]. Bilirubin level was determined by using Sigma – Aldrich kit (MAK 126).

Inflammation Markers

The levels (IL-6 and TNF-α) were measured in liver homogenates by using a quantitative enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for rat (IL-6 and TNF-α). They were purchased from Immuno-Biological Laboratories Co., Ltd (IBL) the USA. Serum C-reactive protein (CRP) was determined according to the method reported by Wener et al. [29] using SEA821- Enzyme-linked Immunosorbent Assay Kit.

Prooxidation and Antioxidant

In the liver tissues, lipid peroxidation was determined as described by Ohkawa et al. [30]. Superoxide dismutase (SOD) and catalase (CAT) were measured according to Marklund and Marklund [31] and Aebi [32], respectively. The activity of glutathione peroxidase (GPx) was estimated by the methods of Hafeman et al. [33]. Reduced glutathione (GSH) levels in the tissues were estimated by the method of Couri and Abdel-Rahman [34]. Myeloperoxidase (MPO) activity was measured by the method of Suzuki et al. [35]. Xanthine oxidase (XO) activity was assayed according to the method described by Litwack et al. [36].

Single Cell Gel Electrophoresis (SCGE) (Comet assay)

(Liver) pieces of the treated and control groups were placed into a small Petri dish with an ice-cold mincing solution (Ca^{2+}- and Mg^{2+}-free HBSS containing 20 mM EDTA and 10% DMSO). The viability of the cells of both examined organs was indirectly determined by analyzing the comet images after electrophoresis [37]. The samples were cut into smaller pieces, using a disposable microtome razor blade, and the solution was aspirated. Then, a fresh mincing solution was added and the samples were minced again to finer pieces. Resulting cell suspensions were collected and filtered (100 μm nylon meshes). All samples were stocked on ice in appropriate conditions to avoid light until the comet assay procedures. The comet assay was performed under alkaline conditions, according to a previously described standard protocol Collins and Dunsinka [38]. Briefly, an aliquot of 5 μl of each prepared cell suspension was mixed with 120 μl of 0.5% low melting point agarose at 37°C and layered onto conventional microscope slides, precoated with 1.5% normal melting point agarose. The slides were placed overnight in freshly prepared cold lysing

solution (1% Triton X-100, 2.5 mM NaCl, 0.1 mM Na_2EDTA, 10 mM Tris with 10% DMSO, pH 10.0) and then in a horizontal electrophoresis cube with alkaline electrophoresis solution (0.3 M NaOH, 1 mM Na_2EDTA, pH >13) at 4^oC for 20 min. The electrophoresis was performed at 25 V and 300 mA for 20 min. After electrophoresis, the slides were washed twice for 5 min in neutralizing buffer (0.4 M Tris-HCl, pH 7.5), fixed for 5 min in absolute alcohol, air-dried, and stored at room temperature. In order to evaluate extremely low molecular weight DNA diffusion, two slides from each animal were removed after lysis procedure, rinsed with a neutralizing solution, fixed and air-dried, and stored until analysis. Immediately before analysis, the DNA was stained with 50 µl of 20 µg/mL ethidium bromide. The slides were examined with a 40X objective lens with epi-illuminated fluorescence microscopy (Olympus-Bx60, excitation filter: 515-560 nm; barrier filter: 590 nm) attached to a color CCD video camera and connected to an image analysis system (Comet II, Perspective Instruments, UK). The Comets were analyzed by a visual scoring method and computerized image analysis. To quantify DNA damage, tail length (TL), tail DNA (%) (TDNA) and tail moment (TM) were analyzed using Comet Assay Project Software (CAPS), generally, 50-100 randomly selected cells are analyzed per sample.

Histological Evaluation

Parts of the liver and Heart were fixed in formalin (10%) and embedded in paraffin, sectioned as well as stained with hematoxylin and eosin [39]. The sections were examined by light microscope and photographed using a digital camera. The data in each group were reported based on the sum of the score histological criteria (overall score) using Kruskal-Wallis test SPSS (n=8).

Statistical Analysis

Data are expressed as a mean ± standard error (SE). All data were analyzed with the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina). Statistical significance between the different groups was evaluated by One Way-Analysis of Variance (ANOVA). When the groups showed significant difference then Tukey's multiple comparisons was followed and values of $P<0.05$ were considered as significant.

RESULTS

Biomarkers of Hepato-Renal Assessment

Serum ALT activities of TiO_2NPs groups were increased significantly when compared with control group (Table 1). Treatment of the rats with Se and/or Bb with TiO_2NPs decreased the activity of ALT significantly as compared with TiO_2NPs alone. The same observation has been noticed in the AST, ALP, LDH and γ-GT activities that elevated by treatment by TiO_2NPs and decreased by the treatment of the rats with Bb or Se combined with TiO_2NPs (Table 1). The total protein and albumin levels decreased in TiO_2NPs -treated rats depend on the dose but increased in the groups treated with the Bb, Se and TiO_2NPs (Table 1). The lipid profile (TG, TC, LDL-c, and VLDL-c) were increased (Table 2). Administration of either Bb and/or Se to the TiO_2NPs -treated groups restored all the parameters cited above. Urea, uric acid, creatinine, and bilirubin were measured to investigate the renal functions (Table 1). The significantly increased levels of all parameters in TiO_2NPs administration group were observed. The increase in urea level was noticed by increasing the doses as compared to control for TiO_2NPs. The uric acid levels were increased significantly in TiO_2NPs treated group as compared to control. Treatment of the rats with TiO_2NPs elevated the creatinine levels as compared to control, respectively. The total bilirubin was increased in dose-dependent manner as the effect of TiO_2NPs. Treatment the rats with Bb and/or Se and TiO_2NPs improved the level renal functions parameters significantly as compared with each its relative TiO_2NPs.

Hepatic Antioxidant

Changes in serum hepatic antioxidant enzymes and thiol level in male rats treated with TiO_2NPs and Bb and/or Se were presented in Table 3. Serum MPO and XO activities were increased as the effect of TiO_2NPs while the thiol level decreased. They were improved when rats treated with Bb and/or Se and TiO_2NPs.

Treatment with TiO_2NPs alone caused an increase in C-reactive protein; these elevations were dose-dependent (Figure 1). The presence of Se or Bb in combination with TiO_2NPs modulated the elevation in C-reactive protein and maintained the levels closer to the normal values. Data presented in Figure 2 showed that treatment with TiO_2NPs caused a significant increase in the level of TNF-α. It was increased significantly as compared to control. However, the concurrent administration of Se and/or Bb to the rats treated with TiO_2NPs significantly decreased the levels of TNF-α as compared with its relative TiO_2NPs treatment group only. In dose-dependent manner, IL-6 levels increased in TiO_2NPs as compared to control, respectively (Figure 2). However, Se or Bb returned the IL-6 levels to almost the normal level when treated before the administration of TiO_2NPs. The results revealed an increase of LPO

in the liver of the TiO_2NPs -treated group in dose-dependent manner as evidenced by the enhanced malondialdehyde levels in the liver homogenates of adult rats (Figure 3). The Administration of Se or Bb combined with TiO_2NPs alleviated LPO significantly and modulated the MDA levels in the liver (Table 4). In the liver homogenates of TiO_2NPs -treated rats, SOD, CAT, and GPx activities decreased significantly, when compared to controls. Administration of Bb and/or Se with TiO_2NPs ameliorated the antioxidant enzymes group when compared to TiO_2NPs -group. A significant decrease of GSH and non-protein thiol (NPSH) levels in liver was evident in TiO_2NPs -group when compared to controls (Table 3).

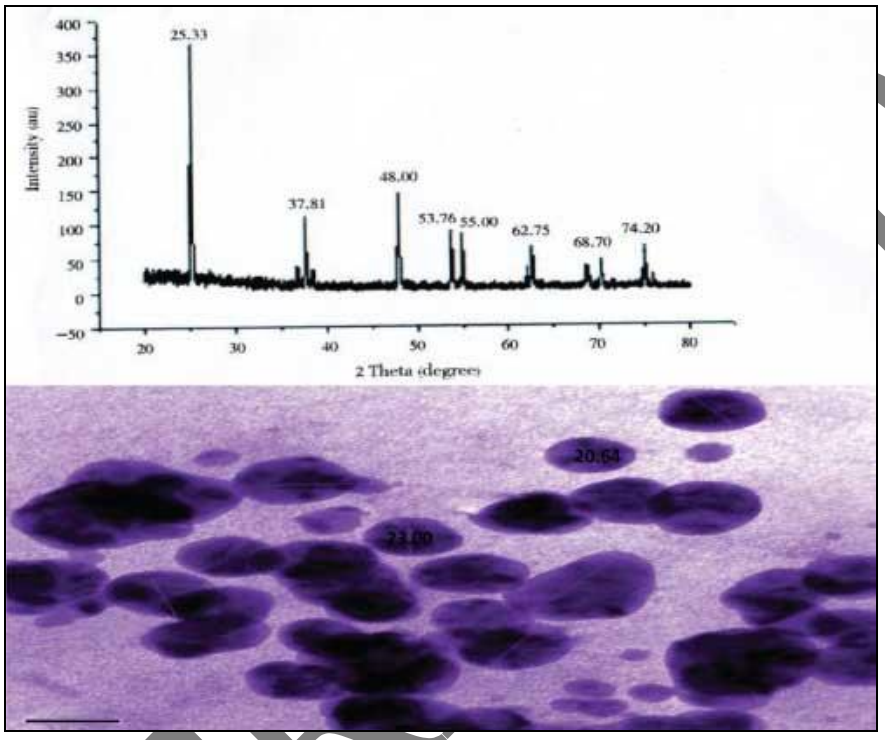

Figure 1: XRD images of biosynthesized TiO_2NP_S

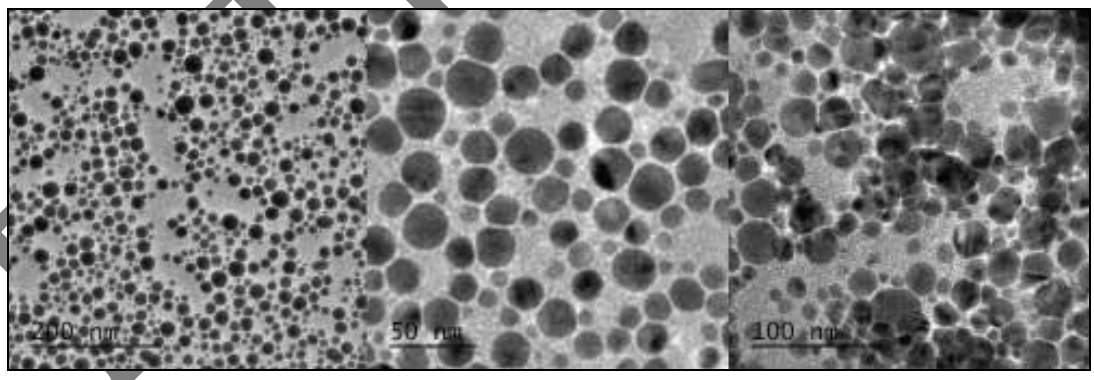

Figure 2: SEM images of biosynthesized TiO_2NP_S

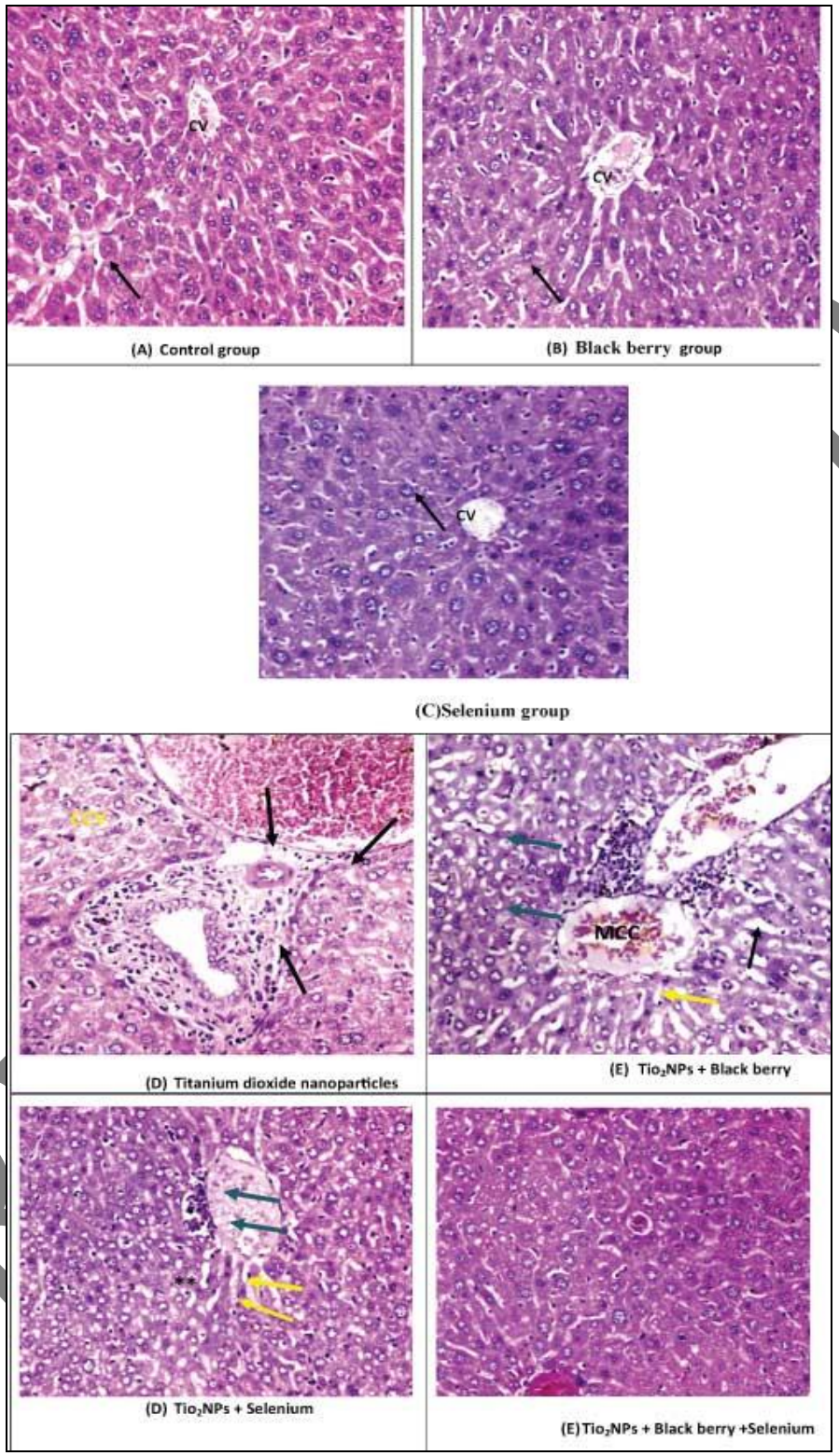

Figure 3: Histological observation

Histological Observation

Light microscopic examination indicated a normal structure of the liver in the controls and rats that treated with Se or Bb. Histopathological slides of the liver stained with hematoxylin and eosin in (A) control groups of rat showing

normal central vein (CV) with normal hepatocytes (Black arrow) and normal nuclei (400X) ; (B) cross section of rat liver treated with black berry (showing normal liver tissue with normal central vein (CV) surrounded by cords of polyhedral hepatocytes with central nuclei (Black arrow) and eosinophilic cytoplasm (400X); (C) cross section of rat liver treated with selenium showing normal liver tissue with normal central vein (CV) surrounded by cords of polyhedral hepatocytes with central nuclei (Black arrow) and eosinophilic cytoplasm (400X); (D) Titanium dioxide nanoparticles treated group (150 mg/kg) showing markedly dilated congested central vein (*) and aggregates of inflammatory cells in the portal tract (Black arrow) (400X); (E) Titanium dioxide nanoparticles combined with black berry showing slightly congested central vein (**) and few aggregated of inflammatory cells (Yellow arrow) with moderate fatty change (Green arrow) (400X).; (F): Titanium dioxide nanoparticles combined with selenium treated animals showing mildly congested central vein (mcv) and mild aggregates of inflammatory cells (Yellow arrow) with enlargement of nuclei size (Black arrow) with moderate fatty change (Green arrow) (400X). (G) Titanium dioxide nanoparticles combined with selenium and black berry treated animals showing mild aggregates of inflammatory cells with moderate fatty change (400X).

Table 1: Changes in hepatic and renal functions in male rats treated with titanium dioxide (TiO_2NPS) Black berry extract (Bb) and/or Selenium (Se)

Parameters	Control	TiO_2NPS	Black berry	Selenium	TiO_2NPS + Bb	TiO_2NPS + Se	TiO_2NPS +Bb+ Se
ALT (U/L)	12.33 ± 0.32	159.88 ± 10.39^{a,b}	12.38 ± 0.12	11.38 ± 0.62	20.65 ± 2.698^{c}	21.88 ± 2.39	15.03 ± 1.36
AST (U/L)	15.36 ± 1.36	190.69 ± 11.69^{a}	11.85 ± 2.58	13.95 ± 1.36	22.39 ± 3.25^{c}	23.39 ± 4.25	19.66 ± 2.96
ALP (U/L)	60.59 ± 3.68	189.29 ± 8.69^{a,b}	55.06 ± 3.02	58.36 ± 4.23	50.36 ± 4.36^{c}	70.36 ± 5.36^{b}	66.84 ± 3.68
LDH (U/L)	143.56 ± 7.69	785.69 ± 9.65^{a,b}	144.95 ± 9.36	141.95 ± 9.36	202.69 ± 6.39^{c}	220.98 ± 5.36^{b}	163.03 ± 5.39
γ-GT U/L	5.36 ± 1.02	9.02 ± 2.36^{a}	5.30 ± 0.66	5.20 ± 1.06	6.48 ± 2.36^{c}	6.25 ± 1.69^{b}	5.96 ± 1.69
Total proteins (g/dL)	8.43 ± 1.36	4.32 ± 1.02^{a}	8.48 ± 1.09	8.68 ± 1.69	7.39 ± 1.69^{c}	6.25 ± 2.02^{b}	7.63 ± 1.26
Albumin (g/dL)	4.39 ± 0.65	2.98 ± 0.98^{a}	4.31 ± 0.29	4.61 ± 0.69	4.01 ± 0.96^{c}	3.02 ± 0.54^{b}	4.01 ± 0.69
Urea (g/dL)	20.36 ± 3.65	38.39 ± 6.25^{a}	19.34 ± 2.26	18.36 ± 3.23	24.23 ± 3.25^{c}	28.36 ± 3.25^{b}	22.59 ± 1.69
Uric acid (g/dL)	13.02 ± 2.02	28.36 ± 5.02^{a}	12.65 ± 1.02	13.65 ± 3.02	18.03 ± 2.62^{c}	20.36 ± 3.02^{b}	15.03 ± 1.69
Creatinine (g/dL)	0.52 ± 0.09	2.35 ± 0.45^{a}	0.58 ± 0.47	0.51 ± 0.07	0.67 ± 0.32^{c}	0.87 ± 0.16^{b}	0.79 ± 0.11
Total bilirubin (mg/dL)	1.25 ± 0.32	3.77 ± 0.35^{a}	1.41 ± 0.32	1.20 ± 0.22	1.56 ± 0.32^{c}	1.68 ± 0.65^{b}	1.51 ± 0.96

Values are expressed as means ± SE; n=8 for each treatment group.

Bb: Black berry extract; TiO_2NPs: Titanium dioxide; Se: Selenium; ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; LDH: Lactic dehydrogenase; γ-GT: Gamma glutamyl transferase. [a] significant difference as compared to control, [b] significant difference as compared to TiO_2NPs and [c] significant difference as compared to its relative group of TiO_2NPs ($P \leq 0.05$)

Table 2: Changes in lipid profile in male rats treated with titanium dioxide (TiO_2NPS) Black berry extract (Bb) and/or Selenium (Se)

Parameters	Control	TiO_2NPS	Black berry	Selenium	TiO_2NPS + Bb	TiO_2NPS + Se	TiO_2NPS +Bb+ Se
Triglycerides (mg/dl)	108.03 ± 4.25	188.09 ± 5.03^{a,b}	109.36 ± 3.65	110.69 ± 4.22	128.06 ± 6.06^{c}	130.09 ± 9.03^{b}	117.96 ± 4.69
Total Cholesterol (mg/dl)	60.25 ± 2.03	180.96 ± 9.35	58.36 ± 2.65	65.69 ± 3.65	79.06 ± 6.03^{c}	82.03 ± 3.06^{b}	71.52 ± 4.36
HDL-c	42.95 ± 5.36	26.06 ± 2.69^{a,b}	42.53 ± 2.98	41.03 ± 4.98	37.06 ± 4.23^{c}	36.98 ± 3.02^{b}	38.03 ± 3.69
LDL-c	19.06 ± 1.66	33.95 ± 3.28^{a,b}	19.18 ± 1.36	20.18 ± 2.36	25.69 ± 3.25^{c}	26.35 ± 3.02^{b}	22.56 ± 2.69
vLDL-c	21.60 ± 2.03	37.61 ± 5.03^{a}	20.13 ± 2.06	21.13 ± 2.06	25.62 ± 3.06^{c}	24.15 ± 4.35^{b}	22.09 ± 3.68

Values are expressed as means ± SE; n=8 for each treatment group.

Bb: Black berry extract; TiO_2NPs: Titanium dioxide; Se: Selenium; HDL-c: High density lipoprotein of cholesterol; LDLc: Low density lipoprotein of cholesterol; VLDL-c: volatile low density lipoprotein of cholesterol. [a] significant difference as compared to control, [b] significant difference as compared to TiO_2NPs and [c] significant difference as compared to its relative group of TiO_2NPs ($P \leq 0.05$)

Table 3: Changes in hepatic antioxidant enzymes and thiol level in male rats treated with titanium dioxide (TiO_2NPS) Black berry extract (Bb) and/or Selenium (Se)

Parameters	Control	TiO_2NPS	Black berry	Selenium	TiO_2NPS + Bb	TiO_2NPS + Se	TiO_2NPS +Bb+ Se
Hepatic myeloperoxidase (MPO) (nmol/min/mL)	17.06 ± 1.36	30.66 ± 3.62^{a,b}	17.06 ± 2.36^{a}	18.36 ± 2.30	24.85 ± 2.36^{c}	23.08 ± 3.02^{b}	19.06 ± 1.69^{c}
Hepatic xanthine oxidase (XO) (U/g)	15.03 ± 2.36	36.06 ± 4.36^{a,b}	14.69 ± 3.03^{a}	16.20 ± 1.36	22.36 ± 2.03^{c}	23.89 ± 3.08^{b}	18.63 ± 1.69^{c}
Hepatic thiol (μmol/g)	8.39 ± 2.03	10.69 ± 1.96^{a,b}	8.03 ± 1.03^{a}	7.33 ± 1.36	6.58 ± 1.69^{c}	6.20 ± 1.87^{b}	9.25 ± 1.69^{c}

Values are expressed as means ±SE; n=8 for each treatment group.

Bb: Black berry extract; TiO_2NPs: Titanium dioxide; Se: Selenium; MPO: Myloperoxidase; Xo: Xanthine oxidase.

[a]significant difference as compared to control, [b] significant difference as compared to TiO_2NPs and [c] significant difference as compared to its relative group of TiO_2NPs ($P \leq 0.05$)

Table 4: Changes in hepatic, spleen and cardiac antioxidant enzymes (MDA, CAT, GRx) in male rats treated with titanium dioxide (TiO_2NPS) Black berry extract (Bb) and/or Selenium (Se)

Parameters	MDA (nmol/g)	CAT (U/g)	GRx (mol/g)
	Liver	Liver	Liver
Control	2.03 ± 0.25	4.56 ± 1.02	5.39 ± 1.23
TiO_2NPS	15.39 ± 2.52	1.99 ± 0.65	1.35 ± 0.36
Black berry	2.02 ± 0.12	4.65 ± 1.36	5.44 ± 1.74
Selenium	2.10 ± 0.32	4.35 ± 1.22	5.41 ± 1.03
TiO_2NPS + Bb	12.08 ± 1.52	2.36 ± 1.02	2.32 ± 0.65
TiO_2NPS + Se	13.06 ± 2.03	2.69 ± 0.69	3.56 ± 0.48
TiO_2NPS +Bb+ Se	8.47 ± 1.36	3.96 ± 0.96	4.03 ± 0.98

Values are expressed as means ±SE; n=8 for each treatment group.

Bb: Black berry extract; TiO_2NPs: Titanium dioxide; Se: Selenium; MDA: Malondialdhyde; CAT: Catalase; GRx: Glutathione reduced.

[a] significant difference as compared to control, [b] significant difference as compared to TiO_2NPs and [c] significant difference as compared to its relative group of TiO_2NPs ($P \leq 0.05$)

DISCUSSION

The present study concerned on the biochemical and histopathological changes to prove that Bb and/or Se has an ameliorative effect on TiO_2NPS induced hepatic damage and cardiotoxicity. Increasing the activities of AST, ALT, ALP, LDH and γ-GT in serum indicated that damage has occurred in the membrane structure that could be due to the presence of reactive oxygen species (ROS) produced during the oxidative stress. These results are in agreement with Choudhary and Devi [40] that found the many xenobiotics change the biochemical parameters of rats. These elevations could be due to oxidative stress induced by TiO_2NPS and causing an imbalance in the membrane permeability. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver are indicators of tissue injury [41].

The decreasing levels of serum albumin and protein also suggest an adverse effect of ROS that produces during TiO_2NPS metabolism on the formation ability of the liver. Hypercholesterolemia has been considered a risk factor for hepatic injury as all the lipid profile parameters were changed. The liver is the main organ responsible for cholesterol metabolism [42]. The present study showed that TiO_2NPS induced changes in lipid metabolism, and could be involved in the development of hypercholesterolemia as reported by Prokić et al. [43]. Jang et al. [44] found that hypercholesterolemic atherosclerosis was associated with increases in ROS, which plays an importance role in the development of atherosclerosis and cardiovascular diseases as the effect of TiO_2NPS.

The significant increase of urea and creatinine could be due to the toxic effect of TiO_2NPS on kidney and that could cause renal dysfunction. These effects reduced glomerular filtration rate and retention of urea and creatinine as reported by Amin et al. [45]. Many pathological changes extending to cellular damage occur if the balance between oxidants and anti-oxidants cannot be maintained in tissues. Oxidative stress damage with free radical generation and overwhelming LPO are indication of TiO_2NPS toxicity. In the current study, MDA and C-reactive protein levels in ASP group were significantly increased and accompanied by an attendant decrease in the activities of antioxidant enzymes (SOD, CAT, GPx) as well as GSH and total protein levels in the liver tissues when compared with control group. The present study reports the hepatotoxic, cardiotoxic and genotoxic effect of TiO_2NPs. Moreover, exposure to TiO_2NPs produces mild to moderate change in the cytoarchitecture of liver and heart tissue. We hypothesized that those different toxic and genotoxic effects might lead to Alzheimer's disease incidence. From the present Comet assay, results report the genotoxic potential of TiO_2NPs on liver cells. Those results were in agreement with Landsiedel et al. [46]; they published a review that describes various knowledge about genotoxicity declare the evidence of TiO_2NPs genotoxicity represented by micronuclei development, as an indicative of chromosomal damage and DNA damage. Comet assay and the detection of in vitro mammalian chromosomal aberrations are the most commonly used test systems to assess genotoxicity. TiO_2NPs have the efficiency to lead to oxidative burst that can be represented by the immediate production and release of superoxide anions (O2-) that convert to multiple ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals, and peroxynitrites. The excess anions can diffuse from the plasma membrane and damage the proteins, lipids, and DNA of neighboring cells, especially hepatocytes, and might lead to hepatotoxicity. Therefore, TiO_2NPs exposure might lead to the incidence of the most common degenerative disorders [47]. Improving all the antioxidant parameters and decreasing LPO by Bb administration with TiO_2NPs in the present study could be related to the antioxidant properties of Bb. We can conclude that Bb administration could minimize the toxic effects of TiO_2NPs indicating its free radical-scavenging and potent antioxidant activities as reported before by Hassan and Abdel-Aziz [9] reported that the total pigment extract from blackberries exhibited strong antioxidant activity against LPO in a linoleic acid model system and scavenging capacities toward superoxide

anion radicals, generated by a pyrogallol autoxidation system or by an illuminating riboflavin system, hydroxyl radicals generated by Fenton reaction, and nitrite. The antioxidant activities and health benefits are related to Bb high anthocyanin and phenolic content. Anthocyanins and their derivatives protect against a variety of oxidants through a number of mechanisms. They can protect cell membrane lipids from oxidation, protect the amino acid tyrosine from the highly reactive oxidant peroxynitrite [48] and interfere with the dangerous hydroxyl radical-generating system – a major source of oxidants in the [49]. Also, it has been suggested that anthocyanins and non-anthocyanin phenolics in Bb act synergistically or additively in producing the observed beneficial effects through their ability to act as either antioxidants or prooxidants in some biological environments [50].

In fact, histological changes seen in the liver of rats treated with TiO_2NPs are characterized by extensive degenerative changes, varying from ballooning degeneration to complete cell necrosis and infiltration of mononuclear cells in hepatic lobules as seen essentially in dams [51]. These histological changes could be correlated with biochemical alterations, especially contents of thiobarbituric acid reactive substances in liver and heart that were significantly increased in adult rats.

Our study revealed that the effect of Se on normal control rat was significant improvement in testicular function. These results were in agreement with Brown and Arthur and Agarwal et al. [14,52] as they concluded that the administration of antioxidants such as Se to normal animals, not suffering from induced oxidative stress, also appears to improve general biochemical function. Se was a potent inducer of serum humoral immune response (IgG and IgM) in rats either alone or with high doses of SA. These results can be confirmed by the observation of Packer et al. [53] who noticed that Se played a role in the immunity function. In the meanwhile, TiO_2NPs induced the secretion of inflammatory cytokine, TNF- α which plays a role in activating T cells and rejecting tumor cells [54]. Thus, could be clarifying the ability of SA to induce TNF- α underlie its enhancement of natural immunity. Decreased TNF-α level after Se administration might have resulted into the modulation of above mentioned biochemical changes resulting into amelioration of hepatic architecture The present results showed that treatment with Se alone caused a significant decrease in the levels of serum TNF- α as compared with the TiO_2NPs.

Selenium is an important part of the enzyme GPx [55]. This enzyme destroys peroxides before they can damage body tissues. Antioxidants are known to reduce oxidative radical-induced reactions.

CONCLUSION

In conclusion, the results showed that TiO_2NPS causes Hepatic and cardiac damage with appeared genotoxicity and consequently affects their functions as indicated by remarkable biochemical as well as histological changes. Bb and Se greatly provided protection from these changes and decreased the injury induced by TiO_2NPS. Therefore, the intake of TiO_2NPS should be restricted and taken with Bb and/or Se when it is used in food or beverages to diminish its hazardous toxic effect.

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