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REDUCING NEPHROTOXICITY INDUCED BY TIO_2 NANOPARTICLES BY SOME MEDICINAL PLANTS

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Abstract

Titanium dioxide (TiO_2) nanoparticles (NPs) are used as a white pigment in the production of paints, plastics, foods, paper, cosmetics and toothpaste. Although the wide ranges of uses, there is not enough information on the effect of TiO_2NPs on animal and human health. Our study evaluated the ameliorative effects of Turmeric and/or Graviola extract against the titanium dioxide nanoparticles (TiO_2NPs) on rats for 1 month. The study was performed in 80 Adult male Albino rats and divided into eight groups. Rats were administered TiO_2 NPs by 600 mg/Kg body weight (BW) orally, Turmeric by 400 mg/kg BW orally and Graviola by 400 mg/kg BW orally. Administration of TiO_2NPs induced a highly significant increase in serum Creatinine levels, BUN levels and MDA in addition, TiO_2NPs rats exhibited anemia, thrombocytopenia, leukocytosis and significantly decrease in catalase. Also TiO_2NPs induced histological alterations in the kidney. But groups that were administrated with Turmeric and /or Graviola showed improvement when compared with control group. Our results indicate that Turmeric and/or Graviola effectively protect against TiO_2 nephrotoxicity.

Key words : TiO_2NPs, Graviola, Turmeric, Catalase, MDA and nephrotoxicity.

Introduction

Nanotechnology is a main important new technology in the field of molecular science that can be used in medical, agriculture, processing, military, cosmetics and manufacturing fields (Kisin ER, Murray AR *et al.,* 2007; Robertson TA, Sanchez WY, Roberts MS., 2010). The main basis of the nanotechnology has resulted in a lowering of particle sizes, which enhances cellular uptake efficiencies and gives novel physical characters that are potentially useful in biomedical research (Tholouli *et al.,* 2008; Stark, 2011; Hemmerich P.H. and von Mikecz A.H., 2013).

The huge number of products containing nanoparticles (NPs) and their wider applications results in increased released to the environment; NPs vary in their physical, chemical and toxicological properties with regards to the same material on a large scale. TiO_2NPs have two main forms of crystal structures, termed rutile and anatase. Both are toxic but the anatase NPs may result much more toxic than rutile NPs and these particles alternately associated with oxidizing mechanisms of an organism, which able to generate ROS (Gurr *et al.,* 2005) including oxidative stress, lipid peroxidation and DNA damage (Vamanu *et al.,* 2008; Falck *et al.,* 2009; Wang JX *et al.,* 2009; Ibrahim M.A., khalaf M.K. *et al.,* 2015).

In recent years, TiO_2NPs are particularly used in paints, plastics, papers, ink, cosmetics and skin care products especially in the case of 1-100 nm size (Nemmar *et al.,* 2008; Brunet *et al.,* 2009) Moreover, it has unique characteristics such as small size, large surface area per unit mass and high activity that NPs can rapidly enter the human body and then imposes high health risk on human Excellency (Oberdorster *et al.,* 2005; Warheit *et al.,* 2007). TiO_2 NPs containing products are easily pass through the human body by many means with different

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ways and may interrupt the body metabolism. Until now, most of the toxicological studies of TiO_2 NPs in mammalian models have concentrated on the toxicity by inhalation or dermal exposure. In toxicological researches of exposure to NPs, the alterations of some enzyme levels either by increasing or decreasing which directly reflects the damage in specific cells and organs (Moss OR, W VA., 2006; Shi H., *et al.,* 2013).

Antioxidants in food play a major role in the prevent and hindering of oxidative stress-related diseases/disorc (Pérez-Jiménez, J. arranz *et al.,* 2008). Turme (*Curcuma longa*), the bright yellow of the spice rainb is a powerful medicinal plant that has long been usec the Chinese and Indian medicine, (Warrier PK, 1994) an anti-inflammatory factor and to treat a lot of disord including jaundice, hepatitis, (Deshpande *et al.,* 19 flatulence, cystic fibrosis, menstrual distress, a rheumatoid arthritis. (Paranjape *et al.,* 2001) It a reduces cholesterol level, thus prevents cardiovascu disorders & has antioxidant effect against liver, kid diseases, and cancer (Bafna and Mishra, 2006; khajehdehi P., 2012; Khorsandi L., Mansouri E., *et al.,* 2016). Many studies detect the protective and the therapeutic effects of Cur in many forms of kidney injury, renal oxidative stress, lipid peroxidation (Kim BH *et al.,* 2016)

Graviola (*Annona muricata*) is a natural plant used in treatment of some diseases and disorders. Researches proved that the leaves extract has antihypertensive properties in rats. Moreover other properties and uses of *A. muricata* leaves mentioned by traditional folk medicine such as anti-cancer agent, hypoglycemic, anti-bacterial, anti-fungal and anti-mutagenic (Adeyemi *et al.,* 2008; Moghadamtousi S.Z., Fadaeinasab M., Nikazd S. *et al.,* 2015).

Materials and Methods

Experimental animals

Eighty healthy adult male Swiss albino rats, their weights 170±20.0 g, 6-8 weeks old, brought from the experimental animal house of the National Research Centre (NRC), Cairo University, Egypt. The animals were kept under typical environmental conditions on 12 hours light/dark cycle under a constant temperature of (25±1) ^{0}C, and a relative humidity, free access to food and water was permitted all the time.

Experimental design

All animals were randomized and divided into eight equal groups.

Characterization of Titanium Dioxide Nanoparticles

TiO_2NPs are typically available in the anatase or rutile crystal forms. Factors such as particle size, crystal form and aggregation potential affect their bioactivity. (Warheit D, *et al.,* 2007) Different crystal forms of the nanoparticle have previously been shown to elicit different toxicological responses. Fig.1 is a cartoon representation of rutile and anatase crystal forms of TiO_2 nanoparticles.

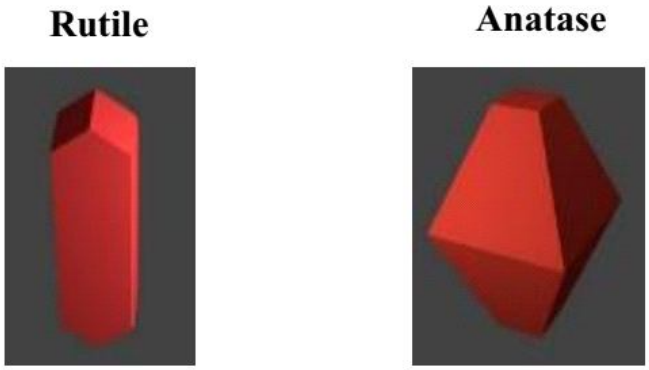

Fig.1: Schematic Representation of Rutile and Anatase Crystal shapes of Titanium Dioxide Nanoparticles.

Preparation of TiO_2 nanoparticle suspensions

We chose a commercially available form of TiO_2NPs (anatase) for the study to detect its toxicological results on rats. TiO_2NPs brought from Loba Chemie, Mumbai city, India. The NPS were UV sterilized and stock suspensions were kept in sterile Phosphate Buffer Saline (PBS) at pH 7.4, shaken for 2 minutes, sonicated for 30 min and stored in dark at 4ºC until use, and shaking well before gavaging (Joo NY, *et al.,* 2013).

X-Ray Diffraction (XRD)

XRD pattern of the investigated TiO_2 sample is illustrated in Fig. 2, the sample of TiO_2 revealed anatase form with excellent crystallinity. Also, it crystallized in the well-known tetragonal symmetry with 4 molecules per unit cell. The statistics were assessed and indexed with the ICDD (International Centre for Diffraction Data) card no 21-1272.

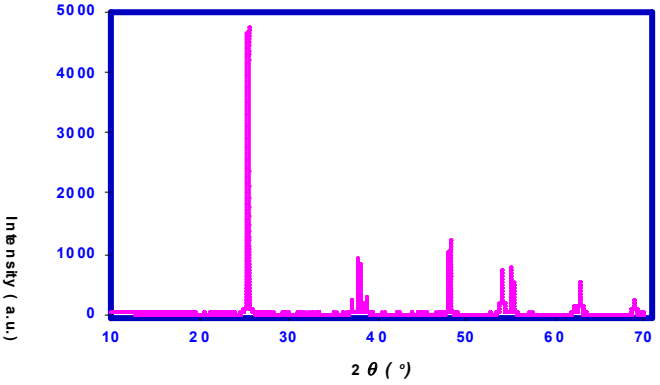

Fig. 2: XRD patterns of Spherical TiO_2 nanoparticles.

Field Emission Scanning Electron Microscopy (FESEM)

The particle size was calculated by Scherer's formula and it was found to be 69 nm The FESEM Fig. of TiO_2 nanoparticles is illustrated in Fig. 3, the TiO_2 particles appeared to be with homogenous distribution with a small degree of agglomeration.

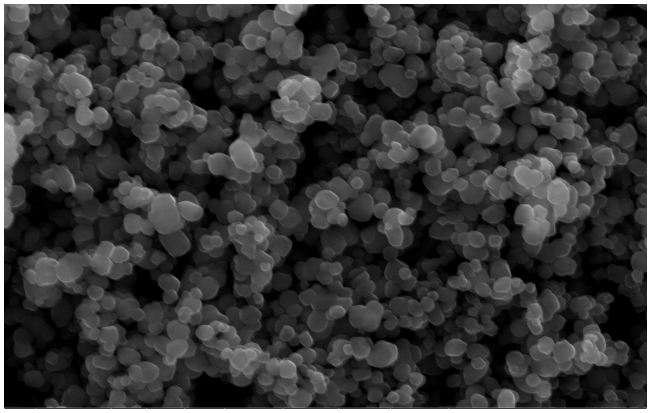

Fig. 3: FESEM image of TiO_2 nanoparticles.

FT-Raman Spectroscopy

The phase of TiO_2NPs was confirmed from FT-Raman analysis and the spectra are shown in Fig. 4, where active peaks near 144, 305, 510, 624 cm^{-1} are prominent for the anatase phase TiO_2NPs because of Eg, B1g, A1g and Eg vibrational changes respectively. Neither signal characteristics of brookite nor rutile phases of TiO_2 having Raman shifts in the range of 249- 826 cm^{-1} respectively, appear in the spectra.

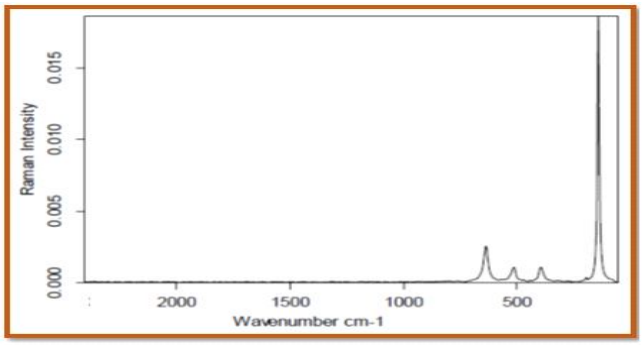

Fig. 4: FT-Raman spectra of TiO_2 nanoparticles.

3. Chemicals (Reagent)

Titanium dioxide nano particles (TiO_2)

TiO_2NPs (lot no. # SL53251301, CAS:13463-67-7, Mumbai, India) was obtained from LOBA Chemie, assay (cerimetric) min. 99.0%, Molecular weight 79.90 g/mol

Turmeric (Curcumin)

Rapid release gelatinous Capsules, (400 mg/Capsule) was obtained from Vitamin World, Inc. Ronkonkoma, NY 11779 U.S.A. and kept at temperature not exceeding 30°C until using and away from moisture. Each Capsule Contains: 400 mg turmeric (rhizome), preservative-free gelatin, Vegetable Cellulose, Silica and vegetable magnesium Stearate. Aliquots 400 mg/kg Turmeric were dissolved in distilled water and administered orally for 30 consecutive days to groups of Turmeric, Turmeric + Graviola, TiO_2+ Turmeric +Graviola rats

Graviola (Annona muricata)

Rapid release gelatinous veggie Capsules, (750 mg/ Capsule) was obtained from Maximum International Maximize, U.S.A. and kept at temperature not exceeding 30°C until using and away from moisture. Each Capsule Contains: 750 mg Graviola (leaf), preservative-free gelatin, Vegetable Cellulose, dicalcium phosphate, Silicon dioxide and vegetable magnesium Stearate. Aliquots 400 mg/kg Graviola were dissolved in distilled water and administered orally for 30 consecutive days to groups of Graviola, Turmeric+ Graviola, TiO_2+ Turmeric+ Graviola rats. Animal weights were measured and evaluated daily.

Reagents and laboratory wares

All reagents used in this study were analytical of the purest grades. All glasses and plastic wares were cleaned with detergent and acid, and washed with distilled water.

Dissection of animals

At the end of the study, the animals were fasted overnight and maintained under light ether anesthesia, immolated by cervical dislocation, and samples were collected from each animal into two tubes both with and without anticoagulants. And the Kidneys were dissected out.

Blood and tissue samples collection

Blood was drawn from all animals in each group and centrifuged at 3000 rpm for 10 minutes. Plasma and Serum samples were stored at 0°C until biochemical analysis in the same day. Kidney was removed, cleared from adhering connective tissue; the kidney was fixed in 10% formalin for histopathological examination. And the second kidney tissue was used for the analysis of antioxidant and oxidative stress parameters.

Preparation of kidney homogenate

Specimens from kidney tissue were weighted and homogenized for 10 sec. then being mixed with 1:9 cold phosphate buffer (PH 7.0) (Maldonado *et al.,* 2003), in an ice-containing medium. The homogenates were centrifuged at 19,000 rpm at 4°C for 30 min and the supernatants obtained were transferred into eppendorf tubes, and preserved at -80°C until using. The supernatant was used to measure the MDA level (Satoh K, 1978).

Biochemical, Antioxidant and Lipid peroxidation Analysis

The determination of creatinine was determined according to (Bartels H, 1971). And (Fabiny Dl and Ertingshausen G., 1971) Using reagent kits obtained from BioSystems Chemical Company (Spain). Creatinine in the sample reacts with picrate in alkaline medium forming a colored complex. The complex formation rate is measured in a short period to avoid interferences.

Levels of BUN in Serum were determined in accordance with the method provided by the Diamond Diagnostics kits. (Kaplan A.Urea. Kaplan a *et al.,* 1984, Tabacco A *et al.,* 1979 and Fawcett J K *et al.,* 1960).

Levels of CAT were assayed by the method of Aebi and Fossati (Aebi, H., 1984 and fossati, P., *et al.,* 1980). Briefly, Collect blood using an anticoagulant such as heparin Centrifuge the blood at 4000 r.p.m for 15 min. at 4^0C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at – 80^0C. The plasma stored will be stable for at least one month.

Levels of MDA were determined by Satoh's method (Satoh K, 1978). During dissection; perfuse the chosen tissue with a PBS Solution to eliminate any red blood cells and debris. Homogenate the tissue in 5 : 10 ml cold buffer per gram tissue, homogenate of kidney was made by 0.1 mol/L phosphate buffer then centrifuged at 4^0C, at 4000 rpm for 15 minutes, then take the supernatant for assaying and store on ice. In case of not processing on the same day, freeze the sample at – 80^0C and the sample will be stable for at least one month.

Histopathological Examination

For histological studies, animals were sacrificed and Kidneys were dissected out and fixed in 10% neutral formalin then stained by using Hematoxylan, Eosin.

Statistical Analysis

The data were examined by using one-way analysis of variance (ANOVA) followed by LSD computers to contrast various groups with each other and with the control. Outcomes and findings were stated as mean ± SD. The level of significance was stated as $P>0.05$ for insignificantly different, while $P<0.05$ was significantly different, while $P<0.01$ and $P<0.001$ were highly and very highly significantly different, respectively.

Results

Biochemistry

Kidney functions

The animals given Titanium alone exhibited highly significant elevation in both serum Creatinine and BUN levels in comparison with the control. Also, the Rats in Titanium treated groups BUN and Creatinine showed a significantly reduced when compared with Ti group and slightly increased when compared with control group. As shown in (Table 1).

Table 1: Kidney functions Concentrations in different experimental animals.

Groups	Creatinine mg/dL	Blood Urea mg/dL
Control	0.77±0.06	30.98±2.64
Turmeric	0.73±0.06	28.68±4.04
Graviola	0.72±0.04	30.66±3.20
Turmeric + Graviola	0.73±0.04	22.92±2.09
Titanium	0.97±0.07*	58.00±8.83**
Ti + Turmeric	0.89±0.08	37.66±9.65*
Ti+ Graviola	0.80±0.07	39.26±5.21*
Ti+ Turmeric+ Graviola	0.84±0.08	38.40±5.15*

The data were expressed as Mean+SD & * = $P<0.05$ and ** = $P<0.001$.

The antioxidants and oxidative stress analysis

In the table 2, the catalase (CAT) level was highly significant decreasing in Ti group But the CAT was significant increase in Turmeric and Graviola groups. Also the CAT level was highly significant increasing In group of (Turmeric + Graviola), while there were no significant differences in other groups when compared with control one.

The MDA was highly significant Increasing in Ti group while the MDA was significant increasing in Ti+Turmeric and Ti+Graviola groups. But In case of titanium+ Turmeric + Graviola group, there were marked improvement approximately near to the results of the control group.

Hematology

In the table 3, the hemoglobin was significant

Table 2: The antioxidants and oxidative stress in different experimental animals.

Groups	Catalase U/L	MDA nmol/ml
Control	218.30±21.97	1.49±0.25
Turmeric	334.04±42.05*	1.55±0.42
Graviola	320.16 ±0.34*	1.78±0.51
Turmeric + Graviola	496.41 ±16.02**	1.24±0.19
Titanium	87.12±18.11 **	5.63±0.84**
Ti + Turmeric	194.46 ±22.63	2.57±0.57*
Ti+ Graviola	209.12±14.60	2.52±0.75*
Ti+Turmeric+Graviola	291.73±56.12*	1.98±0.30*

The data were expressed as Mean±SD & * = $P<0.05$ and ** = $P<0.001$.

decreasing in Titanium group and in case of treatment with Turmeric or Graviola or both of them; there is no differences between the other groups when compared with control one. In case of the WBCs count, the highly significant increasing was in Titanium group and a significant increase in the titanium treated group when compared with control group. The highly significant decreasing in platelets count was recorded in Titanium group, but in titanium treated groups there is no change when compared with the control group.

Table 3: The hematological parameters changes in different experimental animals.

Groups	HBg/dl	TLC103 /cm	PLT103 /cm
Control	13.62±0.8	8.4±1.34	479.6±29.53
Turmeric	13.96±1.28	8.3±1.65	441.6±38.35
Graviola	13.58±1.07	9.14±1.35	469.4±42.22
Turmeric + Graviola	14.2±1.13	8.32±0.79	529.8±31.11*
Titanium	11.88±0.66*	18.08±3.22**	353.2±40.61**
Ti + Turmeric	13.8±0.96	11.94±2.84*	413.2±66.43
Ti+ Graviola	13.7±0.68	10.82±3.07	458.0±47.99
Ti + Turmeric + Graviola	14.54±0.87	12.18±2.66*	500.2±71.01

The data were expressed as Mean+SD & * = $P<0.05$ and ** = $P<0.001$.

Histopathological observations.

Normal kidney control rats

There were no histological observations differences between experimental control and normal control. The kidney of normal rat is a bean-shaped organ, divided morphologically into an outer cortex and inner medulla. The functional unit of the kidney is the nephron. Each nephron is formed of Malpighian corpuscles and renal tubules; the latter consist of three major segments: a proximal convoluted tubule, loop of Henle and a distal convoluted tubule. The Malpighian corpuscles are spherical in shape and each consists of a tuft of blood capillaries known as glomerulus and a double-walled epithelial capsule known as Bowman's capsule Fig. 5a The urinary space of the Malpighian corpuscle continues into the lumen of the proximal tubule. The proximal convoluted tubules are lined with simple cuboidal or columnar epithelial cells. The proximal convoluted tubules are lined with simple cuboidal or columnar epithelial cells with acidophilic cytoplasm and exhibit brush borders at their apices. The nuclei of these cells are spherical and centrally located. The distal convoluted tubules are differentiated from the proximal convoluted tubules by the absence of brush borders, acquiring wider lumen and lined with simple cuboidal epithelia. The nuclei of these cells are spherical and centrally located Fig. 5a.

TiO_2 NPs group observations

Microscopic examination of kidney sections taken from rats of TiO_2 NPs group showed different signs of injury. Abnormal structures of glomeruli, such as being lobed with shrinkage of some glomeruli forming widen and irregular urinary space was also observed. Complete disappearance of some glomeruli and appearance of glomerular debris with ruptured Bowman's capsules which lead to formation of focal area of necrosis were seen. Also kidney exhibited severe and complete epithelial cell damage and desquamation (flattening epithelium) comparing to the control group and mononuclear leucocytes infiltration in the interstitial tissue of the renal tubules and in the glomerular tufts. Tubular necrosis, dilated tubules and fibrotic tissues were shown causing distribution in the architecture of the kidney. The epithelial cells were vacuolated and destructed near their brush borders. However the degenerated epithelial cells were sloughed as fragment and expelled into lumen of the renal tubules. Therefore; necrotic debris were observed in the tubule lumens as hyaline casts Fig. 5b and Fig. 5b*.

TiO_2 NPs + Turmeric group

TiO_2 NPs + Turmeric group showed that the Turmeric recovered the most abnormal injuries caused by Titanium NPs except some congestion but glomeruli, urinary space and convoluted tubules appeared normal Fig. 5c.

TiO_2 NPs + Graviola group

Microscopic examination of Kidney sections taken from rats treated with of TiO_2 NPs + Graviola group showed that the Graviola recovered some abnormal injuries caused by Titanium NPs except some congestion and Abnormal structures of glomeruli, such as being lobed with shrinkage of some glomeruli forming widen and irregular urinary space was also observed. Also kidney exhibited slightly epithelial cell damage and desquamation comparing to the control group with mononuclear leucocytes infiltration in the interstitial tissue of the renal tubules Fig. 5d

TiO_2 NPs +Turmeric + Graviola group

Microscopic examination of kidney sections taken from rats treated with of TiO_2 NPs + Turmeric + Graviola group showed that the Turmeric and Graviola recovered almost the most abnormal injuries caused by Titanium NPs and an approximate regain of normal appearance of the renal corpuscle and most of the renal tubules approximately Fig. 5e.

Explanation of the figures

Fig. 5a: Kidney section of control: showing The

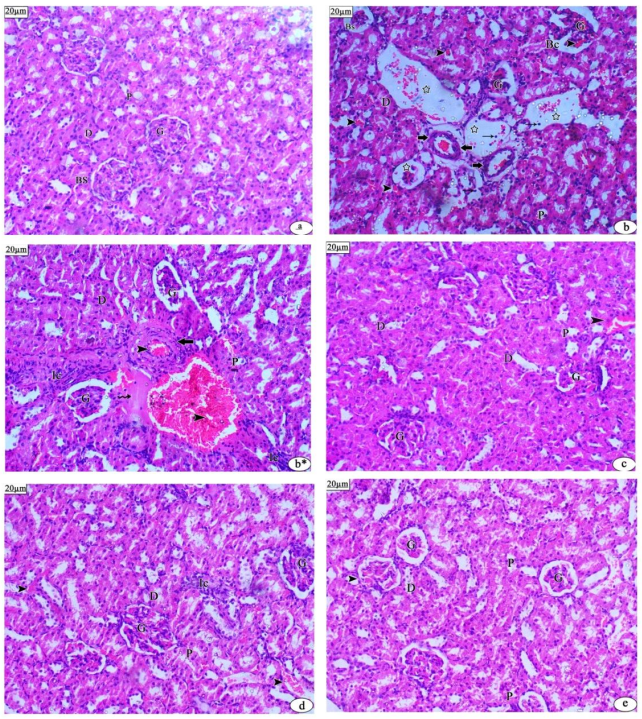

Fig. 5: Kidney sections of different animals groups.

Malpighian corpuscles are spherical in shape and each consist of a tuft of blood capillaries, the glomerulus (G), and a double walled epithelial capsule, the Bowman's capsule, The urinary space (BS), proximal (P) tubule and the distal convoluted tubules (D). H&E X 200

Fig. 5b and 5b*: Kidney section of TiO_2 NPs Rats: showing most glomeruli (G) were shrinked, lobed and necrotic, some tubular cells show pyknotic nuclei and vacuolation, several congestion and interstitial hemorrhage (arrow head ➤), deposition of hyaline like material in some proximal tubules (zigzag arrow), mononuclear cell infiltration (IC), fibrosis (Thick arrow), exfoliation of some lining epithelial cells (arrow→), of several necrotic areas of glomeruli (star *), widding of bowman's space (BS). H&EX 200

Fig. 5c: Kidney section of TiO_2 NPs Rats treated with Turmeric: showing some blood congestion (➤), the glomeruli (G) appeared slightly swollen and the urinary space of the capsules got narrow was observed. H&E X 200

Fig. 5d: Kidney section of TiO_2 NPs Rats treated with Graviola: showing some blood congestion (➤), abnormal structures of some glomeruli (G), such as being lobed with shrinkage of some glomeruli forming widen and irregular urinary space, little mononuclear cell infiltration between degenerated tubules and renal corpuscle (IC), was also observed. H&E X 200.

Fig. 5e: Kidney section of TiO_2 NPs Rats treated with both of Turmeric and Graviola: showing an approximate regain of normal appearance. The renal corpuscle and most of the renal tubules approximately regained their normal appearance. H&E X 200.

Discussion

Nanotechnology is the latest technology which applied highly to gather basic sciences, agriculture, food resources, biotechnology and medicine (Weir A *et al.,* 2012). Nanoparticles pollution is cinsidered as a new problem recently (Liu S,Yang Z., 2013). Stability of dioxide metallic nanoparticles is very high in environment and food chain which causes continuation of their toxicity (Shi JW, ZhangF, ZhaoYL, Chai ZF., 2006). Because nanoparticles have high surface action, they have a perfect cabability to pass through organs. Nano-TiO_2 can be transported into several organs in the body through the blood and lymphatic circulatory systems. (Wang JX *et al.,* 2007).

Nanoparticles raise toxicity by interfering in membrane structure, oxidative stress, binding protein or DNA, leading to production of active oxygen (ROS) and cell death (Cohen-Naftaly M. *et al.,* 2011). In current study, toxicity of TiO_2NPs was investigated due to their recurrent application in industries (Weir A *et al.,* 2012).

Medicinal plants are traditionally used to treat human diseases. Turmeric (*Curcuma longa*) is one of the most commonly used herbal medicines. Curcumin (Cur) is the major and essential component of the Turmeric (Chattopadhyay I *et al.,* 2004). Many studies reveal the protective and the therapeutic effects of Cur in different forms of kidney injury, renal oxidative stress and lipid peroxidation (Kim BH *et al.,* 2016). Turmeric has several benefits such as antioxidant, anti-inflammatory, anti-hyperlipidemic and anticancer protective effects (Chattopadhyay I *et al.,* 2004).

Graviola (*A. muricata*) is a natural plant that has been used as a remedy for treatment of a variety of sickness. Studies have shown that the leaves of graviola have many properties documented by traditional uses include anti-cancerous, hypoglycemic, anti-bacterial, anti-fungal, anti-mutagenic among others (Adeyemi DO *et al.,* 2008).

The purpose of our study was to investigate the potential renal protective activity of Turmeric and/or Graviola against TiO_2NPs induced nephrotoxicity.

Kidneys are particularly susceptible to xenobiotics and renal excretion, it considered as an expected and possible elimination route for NPs in living organisms (Burns AA *et al.,* 2009). As the measure of plasma concentrations of creatinine and urea is usually a marker of kidney function and its other conditions (Ahamed *et*

al. 2010), so in the present study, in order to state any comment about nephrotoxicity, we investigated the above factors and also renal tissue structure.

According to the our results we found that oral administration of 400 mg/kg BW of Turmeric and/or Graviola for 30 consecutive days attenuate the nephrotoxicity caused by TiO_2 NPs. by decreasing kidney Functions (Urea, and creatinine).

Our results revealed that serum creatinine and urea levels showed marked improvement in the treated titanium groups with turmeric and/or Graviola and lack of effect of TiO_2 nanoparticles on creatinine and urea excretion by kidney. Regulation of urea by kidneys is a vital part of the rat's body metabolism. In addition, to the role of urea as a carrier of waste nitrogen, it plays some interactions in the system of nephrons (Zhang *et al.,* 2012). Serum urea level showed improvement in the treated titanium groups with turmeric and/or Graviola when compared with the control group. But in TiO_2 NPs group a marked increase in serum urea and creatinine levels were recorded. In this regard, it can be observed the studies that nanoparticles of TiO_2 have raised the levels of urea (Tang *et al.,* 2010, Guo *et al.,* 2009) and creatinine (Tang *et al.,* 2010, Zhao *et al.,* 2010).

Our results in accordance with the results of (Morgan A *et al.,* 2017) he showed that administration of TiO_2NPs to rats lead to obvious elevation of renal parameters, depletion of renal antioxidant enzymes with noticeable elevation in MDA concentration. Our findings come in accordance with results reported by (Escárcega-Gonzálea CE *et al.,* 2016 and Fartkhooni FM *et al.,* 2016). Those increases might by due to over accumulation of TiO_2NPs in kidney.

In contrast, there are certain studies stated that nanoparticles of TiO_2 have decreased the level of urea (Liu *et al.,* 2009, Zhao *et al.,* 2010) and creatinine (Wang *et al.,* 2009).

Our results in agreement with (Kim BH *et al.,* 2016) who assessed the ameliorative effect of Curcumin (Turmeric) on renal Oxidative stress and lipid peroxidation in Type 2 Diabetic Nephropathy rats. He said that the Curcumin exhibits renoprotective characters by preventing renal lipid accumulation and oxidative stress.

Our results showed significant decreasing in Hemoglobin concentration and platelets count of TiO_2 NPs group, but in case of treatment with Turmeric and/ or Graviola; there was no differences when compared with control one. Our results revealed a highly significant increase In the WBCs count of TiO_2NPs group, and a significant increase in titanium treated groups (Ti+ Turmeric& Titanium +Graviola & Ti+ Turmeric+Graviola) when compared with control group.

Similar observations were obtained by (Usunobun Usunomena, 2014). He studied the protective pretreatment effects of *Annona muricata* ethanolic leaf extract (400 mg / kg body weight) for 7 days versus Dimethylnitrosamine. Results showed Increase in hematological parameters compared to DMN alone group.

Our results in agreement with USunobun Usunomena except WBCs decreased because he used DMN for induction the hepatotoxicity Where DMN reduced the immunity but in our study highly significant increasing was in Titanium group and a significant increase in the group (Ti+ Turmeric& Titanium +Graviola & Ti+ Turmeric+Graviola) when compared with control group.

According to the obtained results we found that oral administration of 600 mg/kg BW of TiO_2 NPs(69 nm) for 30 consecutive days result in severe oxidative stress, indicated by significant elevation of MDA the indicator for lipid peroxidation (LPO) and a significant reduction for CAT concentration.

(Schanen BC *et al.,* 2009) stated that as a result of Tio_2NPs photosensitivity, nano-TiO_2 can turn out to be a substance that produces reactive oxygen species (ROS) in the body, subsequent in free radical metabolic imbalances and an increases in ROS. Extreme ROS generate toxicity, leading to in the production of biofilms compounds that stimulate lipid peroxidation injury. When ROS is injurious, organisms use a lot of enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) to overcome ROS. $O_2^{\bullet-}$ is transformed to H_2O_2 and O_2 by SOD, while CAT and GSH-Px can eradicate H_2O_2 by converting it to H_2O and O_2 (Buege J A and Aust SD 1978; Beauchamp C and Fridovich I 1971). Extreme production of ROS disrupts the balance of the liver oxidant / antioxidant system, leading to lipid peroxidation and cell apoptosis (Liu HT *et al.,* 2010).

In the present investigation, the decreased CAT concentration and the elevated MDA levels designated that the production of oxidative stress upon TiO_2NPs toxicity. (Natarajan *et al.*2015) reported that, TiO_2NPs could stimulate oxidative stress and decreased the total antioxidant levels and therefore mediate LPO. (Jeon JM *et al.,* 2013) considered that part of the ROS generation might be owing to the catalytic natures of TiO_2NPs. The stimulation of oxidative stress in renal tissue up on TiO_2NPs intoxication had been reported by many studies (Gui SX *et al.,* 2011; Zhao JF *et al.,* 2010).

Our study revealed that titanium treated groups (Titanium + Turmeric & Titanium+ Graviola& Titanium+ Turmeric + Graviola) showed marked improvement

occurred in CAT enzyme and lipid peroxidation. The depletion of oxidative enzymes after TiO_2 NPs exposure was observed by (Salim A *et al.,* 2013). Though, the activity of the CAT enzyme was increased when treated with turmeric.

(Ciftci *et al.,* 2011) stated Turmeric possibly will prevent TCDD (2,3,7,8- tetra chlorodibenzo-P-dioxin) induced reactive oxygen species (ROS) due to its antioxidant character. Antioxidants existing in turmeric overcome free radicals thus fix the action of antioxidant enzymes. This means that the composites with antioxidant natures existing in turmeric protected antioxidant enzymes from exhaustion or synergized the action of these enzymes (Suhit *et al.,* 2010). Along with the obtained results we found that oral administration of TiO_2 NPs (69 nm) induced obvious nephrotoxicity marked by histopathological changes in kidney where TiO_2NPs causing several harms to rat kidney, showing several glomeruli (G) were shrunk, lobed and necrotic, some tubular cells show pyknotic nuclei and vacuolation, several congestion and interstitial hemorrhage, deposition of hyaline like material in some proximal tubules, mononuclear cell infiltration (IC), fibrosis, exfoliation of some lining epithelial cells, of several necrotic areas of glomeruli and widding of bowman's space were observed.

The great penetrative powers of nano-TiO_2 permit it to pass through several organs of the body. Upon accumulations in the internal organs, nano-TiO_2 creates toxicity, triggering organ damage. Ti^{4+} deposition increased in each organ, involving the brain, and particularly the liver, kidney and spleen. Administered nano-TiO_2 predominantly deposited in the livers of rats, with an accumulation of 69% afterward administration for 5 min and 80% after administration for 15 min (Huggins CB and Froehlich JP 1966). Furthermore, a pathological assessment by Chen *et al.,* demonstrated that nano-TiO_2 is accumulated in the liver, where it produces liver cell apoptosis, necrosis, and liver fibrosis, and in the kidney, where it triggers glomerular swelling. Nano-TiO_2 has also been shown to create toxicity in the liver and kidneys (Chen JY *et al.,* 2009).

Accumulating studies showed that the exposure to TiO_2NPs able to bring about many renal pathological alterations in the way of inflammation of the glomeruli, cell necrosis, degenerative changes and fibrosis (Fartkhooni FM *et al.,* 2016; Huang K *et al.,* 2015) which come in accordance with the current data.

In the histopathological studies, it was clearly found that the typical architecture of kidney tissue was observed in control, turmeric and Graviola groups. But In TiO_2NPs Group most glomeruli (G) were shrinked, lobed and necrotic, some tubular cells show pyknotic nuclei and vacuolation, several congestion and interstitial hemorrhage, deposition of hyaline like material in some proximal tubules, mononuclear cell infiltration, fibrosis, exfoliation of some lining epithelial cells and widding of bowman's space was observed.

Concerning the effect of nanoparticles of titanium dioxide in the kidney, (Wang *et al.,* 2007) supposed that nanoparticles of TiO_2 have been stored in the cells of kidney and began the pathological changes and nephron-like toxicity in the form of inflammation of the glomeruli of the kidney. And also particles of 25 nm TiO_2NPs can considerably increase the urea level of serum compared with the control group (Wang *et al.,* 2007). On the other hand, the investigations show that gold nanoparticles increase the quantity of urea, but urea level return to normal after certain time. This is due to the primary shock of kidney that step by step overcome and the renal function returned to normal (Zhang *et al.,* 2012).

According to (Borm *et al.,* 2006) stated that nanoparticles when pass into the blood, they may be eliminated by different mechanisms depending on the way of absorption and properties of their surface. The most mutual way to remove the nanoparticles is throughout the kidneys. This process contains filtration and purification of blood in the glomeruli of the kidney nephron (Borm *et al.,* 2006).

Recent studies show Oxidative stress can stimulate cell death by several signaling ways. The anti-inflammatory action of turmeric was described by (Hanai and Sugimoto, 2009). Turmeric's anti-inflammatory actions may be due to its capability to supress both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states (Tayyem *et al.,* 2006; Hanai and Sugimoto, 2009).

In the current study, titanium groups treated with 400mg/kg body weight of the Turmeric extract showed no significant histological changes. From the results, it is evident that Turmeric extract has significant anti-hepatotoxic, anti-nephrotoxic activity against TiO_2 NPs induced hepatotoxicity, nephrotoxicity in albino rats.

Moderate protection was observed with 400mg/kg BW dose of *A. muricata* leaf extract. There was no significant necrosis and normal structures of glomeruli (G), except some glomeruli being lobed and shrinked leading to forming widen and irregular urinary space. That mean Graviola (*A. muricata*) showed mild to moderate improvement in toxicity. Treatment with *A. muricata* leaf extract restored the renal architecture and protected the kidney tissue by preventing the toxic chemical reaction, oxidative stress, lipid peroxidation changes in the kidney tissues, (Koul IB, Kapil A, 1994; Handa SS, Sharma A 1990).

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